# BEDOPS Documentation 

 Release 2.4.36Shane Neph, Alex Reynolds

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BEDOPS is an open-source command-line toolkit that performs highly efficient and scalable Boolean and other set operations, statistical calculations, archiving, conversion and other management of genomic data of arbitrary scale. Tasks can be easily split by chromosome for distributing whole-genome analyses across a computational cluster.
You can read more about BEDOPS and how it can be useful for your research in the Overview documentation, as well as in the original manuscript.

## CHAPTER 1

## Citation

If you use BEDOPS in your research, please cite the following manuscript:
Shane Neph, M. Scott Kuehn, Alex P. Reynolds, et al. BEDOPS: high-performance genomic feature operations. Bioinformatics (2012) 28 (14): 1919-1920. doi: 10.1093/bioinformatics/bts277

## CHAPTER 2

## Contents

### 2.1 Overview

### 2.1.1 About BEDOPS

BEDOPS is an open-source command-line toolkit that performs highly efficient and scalable Boolean and other set operations, statistical calculations, archiving, conversion and other management of genomic data of arbitrary scale.

The suite includes tools for set and statistical operations (bedops, bedmap and closest-features) and compression of large inputs into a novel lossless format (starch) that can provide greater space savings and faster data extractions than current alternatives. BEDOPS offers native support for this deep compression format, in addition to BED.

BEDOPS also offers logarithmic time search to per-chromosome regions in sorted BED data (in bedextract and core BEDOPS tools). This feature makes whole-genome analyses "embarassingly parallel", in that per-chromosome computations can be distributed onto separate work nodes, with results collated at the end in map-reduce fashion.

Sorting arbitrarily large BED files is easy with sort-bed, which easily scales beyond available system memory, as needed. We also offer portable conversion scripts that transform data in common genomic formats (SAM/BAM, GFF/GTF, PSL, WIG, and VCF) to sorted BED data that are ready to use with core BEDOPS utilities.

All of these tools are made to be glued together with common UNIX input and output streams. This helps make your pipeline design and maintenance easy, fast and flexible.

### 2.1.2 Why you should use BEDOPS

## BEDOPS tools are flexible

Our tools fit easily into analysis pipelines, allow practically unlimited inputs, and reduce I/O overhead through standard UNIX input and output streams:

```
$ bedops --intersect A.bed B.bed C.bed \
    | bedmap --echo --mean - D.bed \
```

Our bedops core tool offers numerous set operations of all kinds, including those in the slide below:


The bedmap core tool applies a wide variety of statistical and mapping operations to genomic inputs:


These and other tools send their results to the output stream, ready for consumption by processes downstream along your pipeline.

## BEDOPS tools are fast and efficient

BEDOPS tools take advantage of the information in a sorted BED file to use only what data are needed to perform the analysis. Our tools are agnostic about genomes: Run BEDOPS tools on genomes as small as Circovirus or as large as

## Polychaos dubium!

Independent tests comparing various kits show that BEDOPS offers the fastest operations with the lowest memory overhead:

## Independent tests with the BEDOPS, BEDTools and GROK toolkits



Ovaska, et al., Computational Biology and Bioinformatics, IEEE/ACM Transactions on (2013)

BEDOPS also introduces a novel and lossless compression format called Starch that reduces whole-genome BED datasets to $\mathbf{\sim 5 \%}$ of their original size (and BAM datasets to roughly $\mathbf{3 5 \%}$ of their original size), while adding useful metadata and random access, allowing instantaneous retrieval of any compressed chromosome:


## BEDOPS tools make your work embarrassingly easy to parallelize

BEDOPS tools introduce the -- chrom option to efficiently locate a specified chromosome within a sorted BED file, useful for "embarrassingly parallel" whole-genome analyses, where work can be logically divided by units of chromosome in a "map-reduce" fashion.

## BEDOPS tools are open, documented and supported

BEDOPS is available as GPL-licensed source code and precompiled binaries for Linux and Mac OS X. We offer support through online forums such as our own and Biostars and recipes showing BEDOPS tools in use for answering common research questions.

### 2.2 Installation

BEDOPS is available to users as pre-built binaries and source code.

### 2.2.1 Via pre-built packages

Pre-built binaries offer the easiest and fastest installation option for users of BEDOPS. At this time, we offer binaries for 64 -bit versions of Linux and OS X (Intel) platforms. 32-bit binaries can be built via source code by adjusting compile-time variables.

## Linux

1. Download the current 64-bit package for Linux from Github BEDOPS Releases.
2. Extract the package to a location of your choice. In the case of 64-bit Linux:
```
$ tar jxvf bedops_linux_x86_64-vx.y.z.tar.bz2
```

Replace $\mathrm{x}, \mathrm{y}$ and z with the version number of BEDOPS you have downloaded.
3. Copy the extracted binaries to a location of your choice which is in your environment's PATH, e.g. /usr/ local/bin:

```
$ cp bin/* /usr/local/bin
```

Change this destination folder, as needed.

## Mac OS X

1. Download the current Mac OS X package for BEDOPS from Github BEDOPS Releases.
2. Locate the installer package (usually located in $\sim / D o w n l o a d s ~-~ t h i s ~ w i l l ~ d e p e n d ~ o n ~ y o u r ~ w e b ~ b r o w s e r ~ c o n-~$ figuration):


> Name BEDOPS 2.2.0b.mpkg
> Kind Installer package
> Size 4.8 MB
> Created Wednesday, June 12, 2013 11:42 AM
> Modified Wednesday, June 12, 2013 11:42 AM
> Last opened Wednesday, June 12, 2013 11:42 AM
3. Double-click to open the installer package. It will look something like this:

4. Follow the instructions to install BEDOPS and library dependencies to your Mac. (If you are upgrading from a previous version, components will be overwritten or removed, as needed.)

### 2.2.2 Via source code

## Linux

Compilation of BEDOPS on Linux requires GCC 4.8 .2 (both gcc and g++ and related components) or greater, which includes support for $\mathrm{C}++11$ features required by core BEDOPS tools. Other tools may be required as described in the installation documentation that follows.

1. If you do not have GCC 4.8 .2 or greater installed (both gcc and $\mathrm{g}++$ ), first install these tools. You can check the state of your GCC installation with gcc --version and g++ --version, e.g.:
```
$ gcc --version
gcc (GCC) 4.8.2 20140120 (Red Hat 4.8.2-15)
```

If you lack a compiler or have a compiler that is older than 4.8.2, use your favorite package manager to install or upgrade the newer package. For example, in Ubuntu, you might run the following:

```
$ sudo apt-get install gcc-4.8
$ sudo apt-get install g++-4.8
$ sudo update-alternatives --install /usr/bin/gcc gcc /usr/bin/gcc-4.8 50
$ sudo update-alternatives --install /usr/bin/g++ g++ /usr/bin/g++-4.8 50
```

The specifics of this process will depend on your distribution and what you want to install. Please check with your system administration or support staff if you are unsure what your options are.

You may also need to install static libraries. For instance, in a CentOS- or RH-like environment:

```
$ sudo yum install libstdc++-static
$ sudo yum install glibc-static
```

In Ubuntu, you might instead do:

```
$ sudo apt-get install libc6-dev
$ sudo apt-get install build-essentials
```

2. Install a git client of your choice, if you do not already have one installed. Github offers an installation guide.

Alternatively, use apt-get or another package manager to install one, e.g. in Ubuntu:

```
$ sudo apt-get install git
```

And in CentOS:

```
$ sudo yum install git
```

3. Clone the BEDOPS Git repository in an appropriate local directory:
```
$ git clone https://github.com/bedops/bedops.git
```

4. Enter the top-level of the local copy of the BEDOPS repository and run make to begin the build process:
```
$ cd bedops
```

\$ make

Running make on its own will build so-called "typical" BEDOPS binaries, which make assumptions about line length for most usage scenarios.

Use make megarow or make float 128 to build support for longer-length rows, or BED data which requires statistical or measurement operations with bedmap with 128-bit precision floating point support.
If you want all build types, run make all.

Tip: BEDOPS supports parallel builds, which speeds up compilation considerably. If you are compiling on a multicore or multiprocessor workstation, edit the JPARALLEL variable in the top-level Makefile, or override it, specifying the number of cores or processors you wish to use to compile.
5. Once the build is complete, install compiled binaries and scripts to a local bin directory:

```
$ make install
```

If you ran make megarow or make float128, instead use make install_megarow or make install_float128, respectively, to install those binaries.

If you ran make all, use make install_all to install all binaries of the three types (typical, megarow, and float128) to the ./bin directory. You can use the switch-BEDOPS-binary-type script to switch symbolic links to one of the three binary types.
6. Copy the extracted binaries to a location of your choice that is in your environment's PATH, e.g. /usr/local/ bin:
\$ cp bin/* /usr/local/bin
Change this destination folder, as needed.

## Mac OS X

In Mac OS X, you have a few options to install BEDOPS via source code: Compile the code manually, or use the Bioconda or Homebrew package manager to manage installation.

Compilation of BEDOPS on Mac OS X requires Clang/LLVM 3.5 or greater, which includes support for $\mathrm{C}++11$ features required by core BEDOPS tools. Other tools may be required as described in the installation documentation that follows. GNU GCC is no longer required for compilation on OS X hosts.

## Manual compilation

1. If you do not have Clang/LLVM 3.5 or greater installed, first do so. You can check this with clang -v, e.g.:
```
$ clang -v
```

Apple LLVM version 8.0.0 (clang-800.0.42.1)
. . .

For Mac OS X users, we recommend installing Apple Xcode and its Command Line Tools, via the Preferences > Downloads option within Xcode. At the time of this writing, Xcode 8.2.1 (8C1002) includes the necessary command-line tools to compile BEDOPS.
2. Install a git client of your choice, if you do not already have one installed. Github offers an installation guide.
3. Clone the BEDOPS Git repository in an appropriate local directory:

```
$ git clone https://github.com/bedops/bedops.git
```

4. Run make in the top-level of the local copy of the BEDOPS repository:
```
$ cd bedops
$ make
```

Running make on its own will build so-called "typical" BEDOPS binaries, which make assumptions about line length for most usage scenarios.

Use make megarow or make float128 to build support for longer-length rows, or BED data which requires statistical or measurement operations with bedmap with 128-bit precision floating point support.

If you want all build types, run make all.

Tip: BEDOPS supports parallel builds, which speeds up compilation considerably. If you are compiling on a multicore or multiprocessor workstation, edit the JPARALLEL variable in the top-level Makefile, or override it, specifying the number of cores or processors you wish to use to compile.
5. Once the build is complete, install compiled binaries and scripts to a local bin folder:

```
$ make install
```

If you ran make megarow or make float 128 , instead use make install_megarow or make install_float128, respectively, to install those binaries.

If you ran make all, use make install_all to install all binaries of the three types (typical, megarow, and float128) to the ./bin directory.

You can use the switch-BEDOPS-binary-type script to switch symbolic links to one of the three binary types.
6. Copy the extracted binaries to a location of your choice that is in your environment's PATH, e.g. /usr/local/ bin:
\$ cp bin/*/usr/local/bin
Change this destination folder, as needed.

## Installation via Bioconda

Bioconda is a bioinformatics resource that extends the Conda package manager with scientific software packages, including BEDOPS. We aim to keep the recipe concurrent with the present release; occasionally, it may be a minor version behind.

What follows are steps taken from the Bioconda installation page. Use this guide for the most current set of instructions, which we briefly cover here:

1. Follow the instructions on Conda's website to install the Miniconda package, which installs the conda command-line tool.
2. If you have not already done so, add the Conda channels that Bioconda depends upon:
```
$ (conda config --add channels r)
$ conda config --add channels defaults
$ conda config --add channels conda-forge
$ conda config --add channels bioconda
```

3. Install the BEDOPS package:
```
$ conda install bedops
```

Other recipes are available for installation, as well.

## Installation via Homebrew

Homebrew is a popular package management toolkit for Mac OS X. It facilitates easy installation of common scientific and other packages. Homebrew can usually offer a version of BEDOPS concurrent with the present release; occasionally, it may be one or two minor versions behind.

1. If you do not have Clang/LLVM 3.5 or greater installed, first do so. You can check this with clang $-\mathrm{v}, e . g$. :
```
$ clang -v
Apple LLVM version 8.0.0 (clang-800.0.42.1)
...
```

For Mac OS X users, we recommend installing Apple Xcode and its Command Line Tools, via the Preferences > Downloads option within Xcode. At the time of this writing, Xcode 8.2.1 (8C1002) includes the necessary command-line tools to compile BEDOPS.
2. Follow the instructions listed on the Homebrew site to install the basic package manager components.
3. Run the following command:

```
$ brew install bedops
```


## Docker

Docker containers wrap up a piece of software (such as BEDOPS) in a complete, self-contained VM.
To set up a CentOS 7-based Docker container with BEDOPS binaries, you can use the following steps:

```
$ git clone https://github.com/bedops/bedops.git
$ cd bedops
$ make docker
$ docker run -i -t bedops
```

The following then generates a set of RPMs using the CentOS 7 image, which can run in CentOS 6 and Fedora 21 containers:

```
$ make rpm
```

Thanks go to Leo Comitale for his efforts here.

## Cygwin

1. Make sure you are running a 64-bit version of Cygwin. Compilation of BEDOPS on 32-bit versions of Cygwin is not supported.

To be sure, open up your Cywin installer application (separate from the Cygwin terminal application) and look for the $\mathbf{6 4}$ bit marker next to the setup application version number:


For instance, this Cygwin installer is version 2.831 and is 64-bit.
2. Check that you have GCC 4.8 .2 or greater installed. You can check this by opening the Cygwin terminal window (note that this is not the same as the Cygwin installer application) and typing gcc --version, e.g.:

```
$ gcc --version
gcc (GCC) 4.8.2
. . .
```

If you do not have gcc installed, then open the Cygwin (64-bit) installer application again, navigate through the current setup options, and then mark the GCC 4.8.* packages for installation:


If it helps, type in gcc into the search field to filter results to GCC-related packages. Make sure to mark the following packages for installation, at least:

- gcc-core
- gcc-debuginfo
- gcc-g++
- gec-tools-xyz
- libgcc1

Click "Next" to follow directives to install those and any other selected package items. Then run gcc --version as before, to ensure you have a working GCC setup.
3. Install a git client of your choice. You can compile one or use the precompiled git package available through the Cygwin (64-bit) installer:


If it helps, type in git into the search field to filter results to Git-related packages. Make sure to install the following package, at least:

- git

4. In a Cygwin terminal window, clone the BEDOPS Git repository to an appropriate local directory:
```
$ git clone https://github.com/bedops/bedops.git
```

4. Enter the top-level of the local copy of the BEDOPS repository and run make to begin the build process:
```
$ cd bedops
$ make
```

Tip: BEDOPS now supports parallel builds. If you are compiling on a multicore or multiprocessor workstation, use make $-j N$ where $N$ is 2,4 or however many cores or processors you have, in order to parallelize and speed up the build process.
5. Once the build is complete, install compiled binaries and scripts to a local bin folder:

```
$ make install
```

6. Copy the extracted binaries to a location of your choice that is in your environment's PATH, e.g. /usr/bin:
```
$ cp bin/* /usr/bin
```

Change this destination folder, as needed.

### 2.2.3 Building an OS X installer package for redistribution

1. Follow steps 1-3 and step 5 from the Via Source Code documentation.
2. Run make install_osx_packaging_bins in the top-level of the local copy of the BEDOPS repository:
```
$ make install_osx_packaging_bins
```

3. Install WhiteBox Packages.app, an application for building OS X installers, if not already installed.

On 10.13 hosts, it may be necessary to install a more recent development build of Packages . app via Packages Q\&A \#6.
4. Create a build directory to store the installer and open the BEDOPS.pkgproj file in the top-level of the local copy of the BEDOPS repository, in order to open the BEDOPS installer project, e.g.:

```
$ mkdir -p packaging/os_x/build && open packaging/os_x/BEDOPS.pkgproj
```

This will open up the installer project with the Packages.app application.
5. Within Packages.app, modify the project to include the current project version number or other desired changes, as applicable. Make sure the project is set up to build a "flat"-formatted (xar) package, not a bundle, otherwise the digital signing step will fail.
6. Run the Build > Build menu selection to construct the installer package, located in the packaging/ os_x/build subdirectory. Move this installer to the /tmp directory:

```
$ mv packaging/os_x/build/BEDOPS\ X.Y.Z.pkg /tmp/BEDOPS.X.Y.Z.unsigned.pkg
```

7. Find the Developer ID Installer name that will be used to digitally sign the installer pkg file, e.g.:
```
$ security find-certificate -a -c "Developer ID Installer" | grep "alis"
    "alis"<blob>="Developer ID Installer: Foo B. Baz (ABCD12345678)"
```

Here, the name is Developer ID Installer: Foo B. Baz.
(This certificate name is unique to the developer. If necessary, you may need to sign up for a Mac Developer Program account with Apple to set up required certificates.)
8. Sign the package installer, e.g.:
\$ productsign --timestamp --sign "Developer ID Installer: Foo B. Baz" /tmp/BEDOPS. $\hookrightarrow X . Y . Z . u n s i g n e d . p k g / t m p / B E D O P S . X . Y . Z . s i g n e d . p k g$
9. Compress the signed pkg file (via OS X zip, for instance) and publish via GitHub releases (see release preparation for information about publishing the installer).

### 2.3 Revision history

This page summarizes some of the more important changes between releases.

### 2.3.1 Current version

v2.4.36

Released: May 2, 2019

- bedmap
- Resolved an issue preventing use of a bash process substitution or Unix named pipe in the reference position: i.e., bedmap --options <(processToGenerateReferenceElements) map.bed and similar would issue incorrect output. Thanks to Wouter Meuleman and others for reports and test input.
- To avoid mapping problems, map elements should not contain spaces in the ID or subsequent non-interval fields. Use of the --ec can help identify problems in map input, at the cost of a longer runtime. The documentation is clarified to warn users about avoiding spaces in map input. Thanks to Wouter Meuleman for the report and providing test input.
- Added --unmapped-val <val> option, where <val> replaces the empty string output of $--e c h o-m a p *$ operations when there are no mapped elements. The $--m i n / m a x-e l e m e n t ~ o p e r a-~$ tors will give results as before (the empty string).
- :ref:General
- Reducedwarning: zero as null pointer constant [-Wzero-as-null-pointer-constant] compiler warnings via Clang.
- Begun work on a comprehensive test suite for BEDOPS applications. Tests are available via source distribution in $\$\{$ root $\} /$ tests and can be run by entering make in this directory.


### 2.3.2 Previous versions

v2.4.35

## Released: May 2, 2018

- starch
- When compressing records, if the last interval in the former chromosome is identical to the first interval of the next chromosome, then a test on the sort order of the remainder string of that interval is applied (incorrectly). This is patched to test that chromosome names are identical before applying sort order rules. Thanks to Andrew Nishida for the report and for providing test input.
v2.4.34

Released: April 26, 2018

- convert2bed
- In Issue 208 builds of convert2bed would exit with an error state when converting SAM input with newlinedelimited records longer than the 5 MB per-thread I/O buffer. The C2B_THREAD_IO_BUFFER_SIZE constant is now set to the suite-wide TOKENS_MAX_LENGTH value, which should make it easier to compile custom builds of BEDOPS that support very-long line lengths. Thanks to Erich Schwarz for the initial report.
- starchstrip
- When starchstrip is compiled with a C compiler, qsort uses a comparator that works correctly on the input chromosome list. When compiled with a C++ compiler (such as when building the larger BEDOPS toolkit), a different comparator is used that does not make variables of the correct type, and so the qsort result is garbage, leading to missing chromosomes. Thanks to Jemma Nelson for the initial report.
v2.4.33

Released: April 9, 2018

- convert2bed
- Resolved Issue 207 where a megarow build of convert2bed would raise segmentation faults when converting SAM input. This build uses constants that define a longer BED line length. Arrays ended up using more stack than available, resulting in segmentation faults. This issue could potentially affect conversion of any data with the megarow build of convert2bed. Arrays using megarow-constants were replaced with heap- or dynamically-allocated pointers. Thanks to Erich Schwarz for the initial report.


## v2.4.32

## Released: March 14, 2018

- New build type (128-bit precision floating point arithmetic, float128)
- A new build type adds support for long double or 128-bit floating point operations on measurement values in bedmap, such as is used with score operators like: $--\min ,--\max ,--m i n-e l e m e n t$, --max-element, --mean, and so on.
- This build includes support for measurements on values ranging from approximately $\pm 6.48 \mathrm{e} 4966$ to $\pm 6.48 \mathrm{e} 4966$ (subnormal), or $\pm 1.19 \mathrm{e}-4932$ to $\pm 1.19 \mathrm{e} 4932$ (normal), which enables bedmap to handle, for example, lower p-values without log- or other transformation preprocessing steps. The article on quadruple precision can be useful for technical review.
- For comparison, the current "non-float 128 " typical and megarow builds allow measurements on values from approximately $\pm 5 \mathrm{e} 324$ to $\pm 5 \mathrm{e} 324$ (subnormal) or $\pm 2.23 \mathrm{e}-308$ to $\pm 2.23 \mathrm{e} 308$ (normal). Please refer to the article on double precision for more technical detail.
- Please use make float128 \&\& make install_float128 to install this build type.
- This build type combines support for quadruple, 128-bit precision floats with the typical build type for handling "typical" BED4+ style line lengths. At this time, "megarow" support is not enabled with higher precision floats.
- This build will use more memory to store floating-point values with higher precision, and processing those data will require more computation time. It is recommended that this build be used only if analyses require a higher level of precision than what the double type allows.
- OS X (Darwin) megarow build
- Some applications packaged in the OS X installer or compiled via the OS X command-line developer toolkit lacked megarow build support, despite those flags being specified in the parent Makefile. These applications specifically were affected: bedextract, bedmap, and convert 2 bed . These binaries rely on wider suite-wide constants and data types that the megarow build variety specifies. The Darwinspecific Makefiles have been fixed to resolve this build issue, so that all OS X BEDOPS binaries should now be able to compile in the correct megarow-specific settings.
v2.4.31
Released: March 8, 2018
- User forum
- BEDOPS user forum moved domains from http://bedops.stamlab.org to https://bedops.altius.org
- Testing out administrator approval requirement for new forum accounts, to help try to reduce visits from spammers.
- Documentation
- Updated Homebrew installation instructions per issue 202 (thanks to user EricFromCanada).
- wig2bed
- Increased maximum length of chromosome name buffer to suite-wide TOKEN_CHR_MAX_LENGTH value, to reduce likelihood of segmentation faults (thanks to user ma-diroma).
- General
- Updated copyright dates in source and headers.


## v2.4.30

Released: November 25, 2017

- bedmap
- Errors are no longer reported when error checking is enabled and running in non-fast mode, when a fullynested element is detected. This follows up on issue 199.
- starch
- Previously, a chromosome record in a Starch archive would result in corrupted metadata, if the chromosome is larger than UINT32_MAX bytes ( $\sim 4.3 \mathrm{~GB}$ ) in size when compressed. This limitation is now removed, and a single chromosome (when compressed in a Starch archive) can be up to UINT64_MAX bytes in size.
- The starch binary does more stringent input checks for the character lengths of ID and remainder strings, which must be no larger than $2^{\text {ID_EXPONENT }}-1$ and $2^{\text {REST_EXPONENT }}-1$ characters in length. (These constants are specific to the build-time settings in the Makefile and in the app-wide constants.) This follows up on issue 195.
- starchcat
- Previously, a chromosome record in a Starch archive would result in corrupted metadata, if the chromosome is larger than UINT32_MAX bytes ( $\sim 4.3 \mathrm{~GB}$ ) in size when compressed. This limitation is now removed, and a single chromosome (when compressed in a Starch archive) can be up to UINT64_MAX bytes in size.
- More stringent memory management and stricter adherance to BEDOPS-wide constants, to help reduce likelihood of pointer access out of bounds and incidence of segfaults.
- unstarch
- The unstarch binary implements the character length constants of ID and remainder strings, specific to the build-time settings in the Makefile and in the app-wide constants. This follows up on issue 195.
- sort-bed
- Added --unique (-u) and --duplicates (-d) options to only print unique and duplicate in sorted output, to mimic the behavior of sort -u and uniq -d Unix tools. This follows up on issue 196.
- Switched compile-time, stack-allocated char arrays to runtime, heap-based pointers. Timing tests on shuffled FIMO datasets suggest the impact from having to allocate space for buffers at runtime is very minimal. Moving from stack to heap will help avoid segfaults from running into OS-level stack limits, when BEDOPS-level constants change the maximum line length to something larger than the stack.
- Revision testing
- Starch suite tests were updated for v2.2 archives created via v2.4.30 binaries (Linux, libc 2.22).
v2.4.29

Released: September 26, 2017

- bedmap
- Increased megarow build ID length up to $2^{18}$.
- Changed behavior of mapping to return mapped items in sort order provided in inputs. This follows up on issue 198.
- unstarch
- Changed behavior of --is-starch option to always return a successful exit code of 0 whether or not the input file is a Starch archive. It will now be up to the person running this option to test the 0 (false) or 1 (true) value printed to the standard output stream. This follows up on issue 197.
v2.4.28

Released: August 18, 2017

- bedmap
- Patched issue 191 where --wmean option was not recognized.
- bedextract
- Updated documentation with fixed usage statement.
- sort-bed
- Patched typo in update-sort-bed-starch-slurm.py script.
- Fixed bug with --max-mem on properly ordering things on fourth and subsequent columns, when the genomic intervals are the same.
- starch
- Updated Makefiles to remove lib on clean target and to help prevent ARCH variable from getting clobbered by third-party package managers.
- Build process
- Updated the OS X installer XML to resolve missing asset links.
- Updated the module_binaries target to copy over starchcluster_* and starch-diff assets for modules distributions.


## v2.4.27

## Released: July 17, 2017

This revision of BEDOPS includes significant performance improvements for core tools: bedops, bedmap, and closest-features. Performance tests were done with whole-genome TRANSFAC FIMO scans, with cache purges in between trials.
Pre-built binaries for Darwin and GNU/Linux targets include both the default typical and megarow builds of BEDOPS. The program names that you are accustomed to will remain as-is, but the binaries will exist as symbolic links pointing to the typical builds. These links can be repointed to the megarow builds by calling switch-BEDOPS-binary-type --megarow, which will set the usual BEDOPS binaries to link to the megarow builds. One can run switch-BEDOPS-binary-type --typical at any time to revert to the default (typical) builds.

The top-level Makefile includes some new variables for those who choose to build from source. The JPARALLEL variable sets the number of CPUs to use in parallel when compiling BEDOPS, which can speed compilation time dramatically. The MASSIVE_REST_EXP, MASSIVE_ID_EXP, and MASSIVE_CHROM_EXP are used when building the megarow to support any required row lengths (build using make megarow). These are the exponents (the $n$ in $2^{\mathrm{n}}$ ) for holding all characters after chromosome, start, and stop fields, the ID field (column 4, typically), and the chromosome field (column 1).

To simplify distribution and support, we have removed pre-built 32-bit program versions in this release. These can be built from source by specifying the correct ARCH value in the top-level Makefile. For OS X, our package installer now requires OS X version 10.10 or greater.

Application-level notes follow:

- bedops
- Performance of bedops tool improved, doing typical work in 76.5\% of the time of all previous versions.
- Performance of $-u /--$ everything has improved, doing the same work in only $\mathbf{5 5 . 6 \%}$ of the time of previous versions when given a large number of input files.
- The megarow build of this application handles input files with very long rows (4M+ characters). Such input might arise from conversion of very-long-read BAM files to BED via bam2bed, such as those that may come from Nanopore or PacBio MinION platforms. This build requires more runtime memory than the default (typical) build. Pertinent variables for megarow execution can be modified through the make system without changing source.
- bedmap
- Performance of bedmap tool improved, doing the same work in $\mathbf{8 6 . 7 \%}$ of the time of all previous versions.
- Automatically use --faster option when --exact is used as the overlap criterion, or if the input files are formatted as Starch archives, no fully-nested elements exist in the archives, and the overlap criterion supports --faster (such as --bp-ovr, --exact, and --range).
- The megarow build target handles input files with very long rows (4M+ characters). Such input might arise from conversion of very-long-read BAM files to BED via bam2bed, such as those that may come from Nanopore or PacBio MinION platforms. This build requires more runtime memory than the default (typical) build. Pertinent variables for megarow execution can be modified through the make system without changing source.
- New --min-memory option for use when the reference file has very large regions, and the map file has many small regions that fall within those larger regions. One example is when --range 100000 is used and the map file consists of whole-genome motif scan hits. Memory overhead can be reduced to that used by all previous versions, up to and including v2.4.26.
- Added --faster automatically when --exact is used, which is robust even when nested elements exist in inputs. Similarly, --faster is used automatically when inputs are Starch-formatted archives, none of which have nested elements (see unstarch --has-nested) when the overlap criterion allows for --faster.
- closest-features
- Performance of closest-features tool has been improved, doing the same work in $\mathbf{8 7 . 7 \%}$ of the time of all previous versions.
- The megarow build target is available to compile a version of the program that can handle input files with very long rows ( $4 \mathrm{M}+$ characters). This requires more runtime memory than the default build. Pertinent variables can be modified through the make system without editing source.
- convert2bed

Numerous internal changes, including giving line functors the ability to resize the destination (write) buffer in mid-stream, along with increased integration with BEDOPS-wide constants. Destination buffer resizing is particularly useful when converting very-long-read BAM files containing numerous D (deletion) operations, such as when used with the new --split-with-deletions option.

- psl2bed
* Migrated storage of PSL conversion state from stack to heap, which helps address segmentation faults on OS X (thanks to rmartson @ Biostars for the bug report).
- bam2bed and sam2bed
* Increased thread I/O heap buffer size to reduce likelihood of overflows while parsing reads from Nanopore and PacBio platforms.
* Added --split-with-deletions option to split spliced junctions by N and D CIGAR operations. The --split option now splits only on N operations.
* Added --reduced option to print first six columns of BED data to standard output.
- gff2bed
* Resolved issue parsing GFF input with \#\#FASTA directive.
- sort-bed
- The megarow build target is available to compile a version of the program that can handle input files with very long rows ( $4 \mathrm{M}+$ characters). This requires more runtime memory than the default build. The pertinent variables can be modified through the make system without changing source. This is useful for converting ultra-long reads from Nanopore and PacBio sequencing platforms to BED via bam2bed / convert2bed.
- starch
- Fixed a potential segmentation fault result with --header usage.
- Starch C++ API
- Fixed output from bedops -u (--everything, or multiset union) on two or more Starch archives, where the remainder string was not being cleared correctly.
- starch-diff
- Improved usage statement to clarify output (cf. Issue 180).
- Clang warnings
- Resolved compilation warnings for several binaries.


## v2.4.26

## Released: March 14, 2017

- starchstrip
- New utility to efficiently filter a Starch archive, including or excluding records by specified chromosome names, without doing expensive extraction and recompression. This follows up on internal discussion on the Altius Slack channel.
- starch-diff
- Fixed testing logic in starch-diff for certain archives. Thanks to Shane Neph for the report.
- starchcat
- Fixed possible condition where too many variables on the stack can cause a stack overload on some platforms, leading to a fatal segmentation fault. Improved logic for updating v2.1 to v2.2 Starch archives.
- Starch C++ API
- Patched gzip-backed Starch archive extraction issue. Thanks to Matt Maurano for the bug report.
- update-sort-bed-migrate-candidates
- Added detailed logging via --debug option.
- Added --bedops-root-dir option to allow specifying where all BEDOPS binaries are stored. This setting can be overruled on a per-binary basis by adding --bedextract-path, --sort-bed-path, etc.
- Added --non-recursive-search option to restrict search for BED and Starch candidates to the top-level of the specified parent directory --parent-dir option.
- Further simplification and customization of parameters sent to update-sort-bed-slurm and update-sort-bed-starch-slurm cluster scripts, as well as logging and variable name improvements to those two scripts.
- Thanks again to Matt Maurano for ongoing feedback and suggestions on functionality and fixes.
- gtf2bed
- Resolved segmentation fault with certain inputs, in follow-up to this BEDOPS Forum post. Thanks to zebasilio for the report and feedback.


## v2.4.25

Released: February 15, 2017

- convert2bed
- Patch for RepeatMasker inputs with blank lines that have no spaces. This follows up on Issue 173. Thanks to saketkc for the bug report.
- update-sort-bed-migrate-candidates

The update-sort-bed-migrate-candidates utility recursively searches into the specified directory for BED and Starch files which fail a sort-bed --check-sort test. Those files which fail this test can have their paths written to a text file for further downstream processing, or the end user can decide to apply an immediate resort on those files, either locally or via a SLURM-managed cluster. Grateful thanks to Matt Maurano for input and testing.

See update-sort-bed-migrate-candidates --help for more information, or review the sort-bed documentation.

- update-sort-bed-starch-slurm

This is an adjunct to the update-sort-bed-slurm utility, which resorts the provided Starch file and writes a new file. (The update-sort-bed-slurm utility only takes in BED files as input and writes BED as output.)
v2.4.24

Released: February 6, 2017

- starch-diff
- The starch-diff utility compares signatures of two or more v2.2+ Starch archives. This tool tests all chromosomes or one specified chromosome. It returns a zero exit code, if the signature(s) are identical, or a non-zero error exit code, if one or more signature(s) are dissimilar.
- update-sort-bed-slurm
- The update-sort-bed-slurm convenience utility provides a parallelized update of the sort order on BED files sorted with pre-v2.4.20 sort-bed, for users with a SLURM job scheduler and associated cluster. See update-sort-bed-slurm --help for more details.
- convert2bed
- Patched a memory leak in VCF conversion. Thanks to ehsueh for the bug report.


## v2.4.23

Released: January 30, 2017

- unstarch
- Fixed bug where missing signature from pre-v2.2 Starch archives would cause a fatal metadata error. Thanks to Shane Neph and Eric Rynes for the bug report.
- Improved logic reporting signature mismatches when input v2.2 archive lacks signature (e.g., for a v2.2 archive made with --omit-signature).
- starch and starchcat
- Added --omit-signature option to compress without creating a per-chromosome data integrity signature. While this reduces compression time, this eliminates the verification benefits of the data integrity signature.


## v2.4.22

Released: January 25, 2017

- convert2bed
- Fixed heap corruption in GFF conversion. Thanks to J. Miguel Mendez (ObjectiveTruth) for the bug report.


## v2.4.21

## Released: January 23, 2017

- bedmap
- New --wmean operation offers a weighted mean calculation. The "weight" is derived from the proportion of the reference element covered by overlapping map elements: i.e., a map element that covers more of the reference element has its signal given a larger weight or greater impact than another map element with a shorter overlap.
- Measurement values in bedmap did not allow + in the exponent (both - worked and no + for a positive value. Similarly, out in front of the number, + was previously not allowed. Shane Neph posted the report and the fix.
- The --min-element and --max-element operations in bedmap now process elements in unambiguous order. Former behavior is moved to the operations --min-element-rand and --max-element-rand, respectively.
- Fixed issue with use of --echo-overlap-size with --multidelim (cf. Issue 165). Shane Neph posted the fix. Thanks to Jeff Vierstra for the bug report!
- bedops
- Fixed issue with --chop where complement operation could potentially be included. Shane Neph posted the fix.
- The bedops --everything or bedops -u (union) operation now writes elements to standard output in unambiguous sort order. If any data are contained in fourth or subsequent fields, a lexicographical sort on that data is applied for resolving order of interval matches.
- sort-bed
- Improved sort times from replacing quicksort (std: :qsort) with inlined C++ std: :sort.
- Sorting of BED input now leads to unambiguous result when two or more elements have the same genomic interval (chromosome name and start and stop position), but different content in remaining columns (ID, score, etc.).
Formerly, elements with the same genomic interval that have different content in fourth and subsequent columns could be printed in a non-consistent ordering on repeated sorts. A deterministic sort order facilitates the use of data integrity functions on sorted BED and Starch data.
- starchcluster
- A SLURM-ready version of the starchcluster script was added to help SLURM job scheduler users with parallelizing the creation of Starch archives.
- Parallel bam2bed and bam2starch
- SLURM-ready versions of these scripts were added to help parallelize the conversion of BAM to BED files (bam2bed_slurm) or to Starch archives (bam2starch_slurm).
- unstarch
- Added --signature option to report the Base64-encoded SHA-1 data integrity signature of the Starchtransformed bytes of a specified chromosome, or to report the signature of the metadata string as well as the signatures of all chromosomes, if unspecified.
- Added --verify-signature option to compare the "expected" Base64-encoded SHA-1 data integrity signature stored within the archive's metadata with the "observed" data integrity signature generated from extracting the specified chromosome.
If the observed and expected signatures differ, then this suggests that the chromosome record may be corrupted in some way; unstarch will exit with a non-zero error code. If the signatures agree, the archive data should be intact and unstarch will exit with a helpful notice and a zero error code.
If no chromosome is specified, unstarch will loop through all chromosomes in the archive metadata, comparing observed and expected values for each chromosome record. Upon completion, error and progress messages will be reported to the standard error stream, and unstarch will exit with a zero error code, if all signatures match, or a non-zero exit state, if one or more signatures do not agree.
- The output from the --list option includes a signature column to report the data integrity signature of all Starch-transformed chromosome data.
- The output from the --list-json option includes a signature key in each chromosome record in the archive metadata, reporting the same information.
- The --is-starch option now quits with a non-zero exit code, if the specified input file is not a Starch archive.
- The --elements-max-string-length option reports the length of the longest string within the specified chromosome, or the longest string over all chromosomes (if no chromosome name is specified).
- starch
- Added --report-progress=N option to (optionally) report compression of the Nth element of the current chromosome to standard error stream.
- As a chromosome is compressed, the input Starch-transform bytes are continually run through a SHA-1 hash function. The resulting data integrity signature is stored as a Base64-encoded string in the output archive's metadata. Signatures can be compared between and within archives to help better ensure the data integrity of the archive.
- Fixed --header transform bug reported in Issue 161. Thanks to Shane Neph for the bug report!
- Added chromosome name and "remainder" order tests to STARCH2_transformHeaderlessBEDInput and STARCH2_transformHeaderedBEDInput functions.

Compression with starch ends with a fatal error, should any of the following comparison tests fail:

1. The chromosome names are not lexicographically ordered (e.g., chr1 records coming after chr2 records indicates the data are not correctly sorted).
2. The start position of an input element is less than the start position of a previous input element on the same chromosome (e.g., chr1:1000-1234 coming after chr1:2000-2345 is not correctly sorted).
3. The stop positions of two or more input elements are not in ascending order when their start positions are equal (e.g., chr1:1000-1234 coming after chr1:1000-2345 is not correctly sorted).
4. The start and stop positions of two or more input elements are equivalent, and their "remainders" (fourth and subsequent columns) are not in ascending order (e.g., chr1:1000-1234:id-0 coming after chr1:1000-1234:id-1 is not correctly sorted).
If the sort order of the input data is unknown or uncertain, simply use sort-bed to generate the correct ordering and pipe the output from that to starch, e.g. \$ cat elements.bed | sort-bed - | starch - > elements.starch.

- starchcat
- Added --report-progress $=\mathrm{N}$ option to (optionally) report compression of the $N$ th element of the current chromosome to standard error stream.
- As in starch, at the conclusion of compressing a chromosome made from one or more input Starch archives, the input Starch-transform bytes are continually run through a SHA-1 hash function. The resulting data integrity signature is stored as a Base64-encoded string in the chromosome's entry in the new archive's metadata.
- As in starch, if data should need to be extracted and recompressed, the output is written so that the order is unambiguous: ascending lexicographic ordering on chromosome names, numerical ordering on start positions, the same ordering on stop positions where start positions match, and ascending lexicographic ordering on the remainder of the BED element (fourth and subsequent columns, where present).
- convert2bed
- Improvements in support for BAM/SAM inputs with larger-sized reads, as would come from alignments made from data collected from third-generation sequencers. Simulated read datasets were generated using SimLoRD. Tests have been performed on simulated hg19 data up to 100 kb read lengths.

Improvements allow:

* conversion of dynamic number of CIGAR operations (up to system memory)
* conversion of dynamically-sized read fields (up to system memory and inter-thread buffer allocations)

These patches follow up on bug reports in Issue 157.

- Improvements in support for VCF inputs, to allow aribtrary-sized fields (up to system memory and interthread buffer allocations), which should reduce or eliminate segmentation faults from buffer overruns on fields larger than former stack defaults.
- Improvements in support for GFF inputs, to allow aribtrary-sized fields (up to system memory and interthread buffer allocations), which should reduce or eliminate segmentation faults from buffer overruns on fields larger than former stack defaults.
- Improvements in support for GTF inputs, to allow aribtrary-sized fields (up to system memory and interthread buffer allocations), which should reduce or eliminate segmentation faults from buffer overruns on fields larger than former stack defaults.
- Testing
- Our use of Travis CI to automate testing of builds now includes Clang on their OS X environment.
v2.4.20

Released: July 27, 2016

- convert2bed
- Increased memory allocation for maximum number of per-read CIGAR operations in BAM and SAM conversion to help improve stability. Thanks to Adam Freedman for the report!
- Improved reliability of gene ID parsing from GTF input, where gene_id field may be positioned at start, middle, or end of attributes string, or may be empty. Thanks to blaiseli for the report!
v2.4.19

Released: May 9, 2016

- convert2bed
- Fixed bug in BAM and SAM parallel conversion scripts (*_gnuParallel and *_sge) with inputs containing chromosome names without chr prefix. Thanks to Eric Haugen for the bug report!
- Starch C++ API
- Fixed bug with extraction of bzip2- and gzip-backed archives with all other non-primary Starch tools (all tools except starch, unstarch, starchcat, and sort-bed). Thanks to Eric Haugen for the bug report!
v2.4.18


## Released: April 28, 2016

- convert2bed
- Fixed compile warnings.
- Fixed bug in BAM and SAM conversion with optional field line overflow. Thanks to Jemma Nelson for the bug report!
- General documentation improvements
- Updated OS X Installer and Github release instructions
- Added thank-you to Feng Tian for bug report


## v2.4.17

## Released: April 26, 2016

- bam2bed and sam2bed
- Improved parsing of non-split BAM and SAM inputs.
- Docker container build target added for Debian
- Thanks to Leo Comitale (Poldo) for writing a Makefile target and spec for creating a BEDOPS Docker container for the Debian target.
- Starch C++ API
- Fixed bug with extraction of bzip2- and gzip-backed archives with all other non-primary Starch tools (all tools except starch, unstarch, starchcat, and sort-bed). Thanks to Feng Tian for reports.


## v2.4.16

Released: April 5, 2016

- bedmap
- Added new --echo-ref-row-id option to report reference row ID elements.
- Starch C++ API
- Fixed bug with extraction of archives made with starch --gzip (thanks to Brad Gulko for the bug report and Paul Verhoeven and Peter Weir for compile and testing assistance).
- General improvements
- Small improvements to build cleanup targets.
v2.4.15

Released: January 21, 2016

- Docker container build target added for CentOS 7
- Thanks to Leo Comitale (Poldo) for writing a Makefile target and spec for creating a BEDOPS Docker container for CentOS 7.
- convert2bed
- Fixed buffer overflows in convert 2bed to improve conversion reliability for VCF files (thanks to Jared Andrews and Kousik Kundu for bug reports).
- General improvements
- Improved OS X 10.11 build process.


## v2.4.14

Released: April 21, 2015

- convert2bed
- Fixed missing samtools variable references in cluster conversion scripts (thanks to Brad Gulko for the bug report).
- General suite-wide improvements
- Fixed exception error message for stdin check (thanks to Brad Gulko for the bug report).
v2.4.13


## Released: April 20, 2015

- bedops
- Resolved issue in using --ec with bedops when reading from stdin (thanks to Brad Gulko for the bug report).
- General suite-wide improvements
- Addressed inconsistency with constants defined for the suite at the extreme end of the limits we allow for coordinate values (thanks again to Brad Gulko for the report).


## v2.4.12

## Released: March 13, 2015

- bedops
- Checks have been added to determine if an integer argument is a file in the current working directory, before interpreting that argument as an overlap criterion for $-e$ and $-n$ options.
To reduce ambiguity, if an integer is used as a file input, bedops issues a warning of the interpretation and provides guidance on how to force that value to instead be used as an overlap specification, if desired (thanks to E. Rynes for the pointer).
- bedmap
- Added support for --prec / --sci with --min-element and --max-element operations (thanks to E. Rynes for the pointer).
- bedops I bedmap I closest-features
- Added support for bash process substitution/named pipes with specification of --chrom and/or --ec options (thanks to B. Gulko for the bug report).
- Fixed code that extracts gzip-backed Starch archives from bedops and other core tools (thanks again to B. Gulko for the bug report).
- convert2bed
- Switched matches and qSize fields in order of psl2bed output. Refer to documentation for new field order.
- Added null sentinel to GTF ID value.
- To help reduce the chance of buffer overflows, the convert2bed tool increases the maximum field length from 8191 to 24575 characters to allow parsing of inputs with longer field length, such as very long attributes from mosquito GFF3 data (thanks to T. Karginov for the bug report).
- Fixed bug in psl2bed where mat ches column value was truncated by one character. Updated unit tests. Thanks to M. Wirthlin for the bug report.


## v2.4.10

## Released: February 23, 2015

- starch
- In addition to checking chromosome interleaving, the starch tool now enforces sort-bed sort ordering on BED input and exits with an EINVAL POSIX error code if the data are not sorted correctly.
- convert2bed
- Added --zero-indexed option to wig2bed and wig2starch wrappers and convert $2 b e d$ binary, which converts WIG data that are zero-indexed without any coordinate adjustments. This is useful for WIG data sourced from the UCSC Kent tool bigWigToWig, where the bigWig data can potentially be sourced from 0-indexed BAM- or bedGraph-formatted data.
- If the WIG input contains any element with a start coordinate of 0 , the default use of wig2bed, wig2starch and convert2bed will exit early with an error condition, suggesting the use of --zero-indexed.
- Updated copyright date range of wrapper scripts


## v2.4.9

Released: February 17, 2015

- sort-bed
- Added support for --check-sort to report if input is sorted (or not)
- Starch
- Improved support for starch --header, where header contains tab-delimited fields
- Starch C++ API
- Fixed bug with starch --header functionality, such that BEDOPS core tools (bedops, etc.) would be unable to extract correct data from headered Starch archive


## v2.4.8

## Released: February 7, 2015

- Mac OS X packaging
- Installer signed with productsign to pass OS X Gatekeeper
- Linux packaging
- SHA1 hashes of each tarball are now part of the BEDOPS Releases description page, going forwards
- Updated copyright dates in source code


## v2.4.7

## Released: February 2, 2015

- convert2bed fixes and improvements
- Fixed --split support in psl2bed (thanks to Marco A.)
- Fixed compilation warning regarding comparison of signed and unsigned values
- Fixed corrupted psl2bed test inputs


## v2.4.6

## Released: January 30, 2015

- convert2bed fixes and improvements
- Added support for conversion of the GVF file format, including wrapper scripts and unit tests. Refer to the $g v f 2 b e d$ documentation for more information.
- Fixed bug in string copy of zero-length element attribute for $g f f 2 b e d$ and $g t f 2 b e d$ (GFF and GTF) formats
- General fixes and improvements
- Fixed possibly corrupt bzip2, Jansson and zlib tarballs (thanks to rekado, Shane N. and Richard S.)
- Fixed typo in bedextract documentation
- Fixed broken image in Overview
- Removed 19 MB _build intermediate result directory (which should improve overall git clone time considerably!)


## v2.4.5

Released: January 28, 2015

- convert2bed improvements
- Addition of RepeatMasker annotation output (.out) file conversion support, rmsk2bed and rmsk2starch wrappers, and unit tests


## v2.4.4

## Released: January 25, 2015

- Documentation improvements
- Implemented substantial style changes via A Better Sphinx Theme and various customizations. We also include responsive web style elements to help improve browsing on mobile devices.
- Fixes to typos in conversion and other documents.


## v2.4.3

## Released: December 18, 2014

- Compilation improvements
- Shane Neph put in a great deal of work to enable parallel builds (e.g., make -j N to build various targets in parallel). Depending on the end user's environment, this can speed up compilation time by a factor of 2,4 or more.
- Fixed numerous compilation warnings of debug builds of starch toolkit under RHEL6/GCC and OS X 10.10.1/LLVM.
- New bedops features
- Added --chop and --stagger options to "melt" inputs into contiguous or staggered disjoint regions of equivalent size.
- For less confusion, arguments for --element-of, --chop and other bedops operations that take numerical modifiers no longer require a leading hyphen character. For instance, --element-of 1 is now equivalent to the former usage of $--e l e m e n t-o f ~-1$.
- New bedmap features
- The --sweep-all option reads through the entire map file without early termination and can help deal with SIGPIPE errors. It adds to execution time, but the penalty is not as severe as with the use of $--e c$. Using --ec alone will enable error checking, but will now no longer read through the entire map file. The --ec option can be used in conjunction with --sweep-all, with the associated time penalties. (Another method for dealing with issue this is to override how SIGPIPE errors are caught by the interpreter (bash, Python, etc.) and retrapping them or ignoring them. However, it may not a good idea to do this as other situations may arise in production pipelines where it is ideal to trap and handle all I/O errors in a default manner.)
- New --echo-ref-size and --echo-ref-name operations report genomic length of reference element, and rename the reference element in chrom: start-end (useful for labeling rows for input for matrix2png or $R$ or other applications).
- bedextract
- Fixed upper bound bug that would cause incorrect output in some cases
- conversion scripts
- Brand new C99 binary called convert 2 bed , which wrapper scripts (bam2bed, etc.) now call. No more Python version dependencies, and the C-based rewrite offers massive performance improvements over old Python-based scripts.
- Added parallel_bam2starch script, which parallelizes creation of Starch archive from very large BAM files in SGE environments.
- Added bug fix for missing code in starchcluster.gnu_parallel script, where the final collation step was missing.
- The vcf2bed script now accepts the --do-not-split option, which prints one BED element for all alternate alleles.
- Starch archival format and compression/extraction tools
- Added duplicate- and nested-element flags in v2.1 of Starch metadata, which denote if a chromosome contains one or more duplicate and/or nested elements. BED files compressed with starch v2.5 or greater, or Starch archives updated with starchcat v2.5 or greater will include these values in the archive metadata. The unstarch extraction tool offers --has-duplicate and --has-nested options to retrieve these flag values for a specified chromosome (or for all chromosomes).
- Added --is-starch option to unstarch to test if specified input file is a Starch v1 or v2 archive.
- Added bug fix for compressing BED files with starch, where the archive would not include the last element of the BED input, if the BED input lacked a trailing newline. The compression tools now include a routine for capturing the last line, if there is no newline.
- Documentation improvements
- Remade some image assets throughout the documents to support Retina-grade displays


## v2.4.2

## Released: April 10, 2014

- conversion scripts
- Added support for sort-bed --tmpdir option to conversion scripts, to allow specification of alternative temporary directory for sorted results when used in conjunction with $--m a x-m e m$ option.
- Added support for GFF3 files which include a FASTA directive in $g f f 2 b e d$ and $g f f 2$ starch (thanks to Keith Hughitt).
- Extended support for Python-based conversion scripts to support use with Python v2.6.2 and forwards, except for sam2bed and sam2starch, which still require Python v2.7 or greater (and under Python3).
- Fixed --insertions option in vcf2bed to now report a single-base BED element (thanks to Matt Maurano).


## v2.4.1

Released: February 26, 2014

- bedmap
- Added --fraction-both and --exact (--fraction-both 1) to list of compatible overlap options with --faster.
- Added 5\% performance improvement with bedmap operations without --faster.
- Fixed scenario that can yield incorrect results (cf. Issue 43).
- sort-bed
- Added --tmpdir option to allow specification of an alternative temporary directory, when used in conjunction with --max-mem option. This is useful if the host operating system's standard temporary directory (e.g., /tmp on Linux or OS X) does not have sufficient space to hold intermediate results.
- All conversion scripts
- Improvements to error handling in Python-based conversion scripts, in the case where no input is specified.
- Fixed typos in $g f f 2 b e d$ and psl2bed documentation (cf. commit a091e18).
- OS X compilation improvements
- We have completed changes to the OS X build process for the remaining half of the BEDOPS binaries, which now allows direct, full compilation with Clang/LLVM (part of the Apple Xcode distribution).
All OS X BEDOPS binaries now use Apple's system-level C++ library, instead of GNU's libstdc++. It is no longer required (or recommended) to use GNU gcc to compile BEDOPS on OS X.

Compilation is faster and simpler, and we can reduce the size and complexity of Mac OS X builds and installer packages. By using Apple's C++ library, we also eliminate the likelihood of missing library errors.

In the longer term, this gets us closer to moving BEDOPS to using the CMake build system, to further abstract and simplify the build process.

- Cleaned up various compilation warnings found with clang / clang++ and GCC kits.


## v2.4.0

## Released: January 9, 2014

- bedmap
- Added new --echo-map-size and --echo-overlap-size options to calculate sizes of mapped elements and overlaps between mapped and reference elements.
- Improved performance for all --echo-map-* operations.
- Updated documentation.
- Major enhancements and fixes to sort-bed:
- Improved performance.
- Fixed memory leak.
- Added support for millions of distinct chromosomes.
- Improved internal estimation of memory usage with --max-mem option.
- Added support for compilation on Cygwin (64-bit). Refer to the installation documentation for build instructions.
- starchcat
- Fixed embarassing buffer overflow condition that caused segmentation faults on Ubuntu 13.
- All conversion scripts
- Python-based scripts no longer use temporary files, which reduces file I/O and improves performance. This change also reduces the need for large amounts of free space in a user's / tmp folder, particularly relevant for users converting multi-GB BAM files.
- We now test for ability to locate starch, sort-bed, wig2bed_bin and samtools in user environment, quitting with the appropriate error state if the dependencies cannot be found.
- Improved documentation. In particular, we have added descriptive tables to each script's documentation page which describe how columns map from original data input to BED output.
- bam2bed and sam2bed
* Added --custom-tags <value> command-line option to support a comma-separated list of custom tags (cf. Biostars discussion), i.e., tags which are not part of the original SAMtools specification.
* Added --keep-header option to preserve header and metadata as BED elements that use _header as the chromosome name. This now makes these conversion scripts fully "non-lossy".
- vcf2bed
* Added new --snvs, --insertions and --deletions options that filter VCF variants into three separate subcategories.
* Added --keep-header option to preserve header and metadata as BED elements that use _header as the chromosome name. This now makes these conversion scripts fully "non-lossy".
- gff2bed
* Added --keep-header option to preserve header and metadata as BED elements that use _header as the chromosome name. This now makes these conversion scripts fully "non-lossy".
- psl2bed
* Added --keep-header option to preserve header and metadata as BED elements that use _header as the chromosome name. This now makes these conversion scripts fully "non-lossy".
- wig2bed
* Added --keep-header option to wig2bed binary and wig2bed / wig2starch wrapper scripts, to preserve header and metadata as BED elements that use _header as the chromosome name. This now makes these conversion scripts fully "non-lossy".
- Added OS X uninstaller project to allow end user to more easily remove BEDOPS tools from this platform.
- Cleaned up various compilation warnings found with clang/clang++ and GCC kits.


## v2.3.0

## Released: October 2, 2013

- Migration of BEDOPS code and documentation from Google Code to Github.
- Due to changes with Google Code hosting policies at the end of the year, we have decided to change our process for distributing code, packages and documentation. While most of the work is done, we appreciate feedback on any problems you may encounter. Please email us at bedops@stamlab.org with details.
- Migration to Github should facilitate requests for code by those who are familiar with $g i t$ and want to fork our project to submit pull requests.
- bedops
- General --ec performance improvements.
- bedmap
- Adds support for the new --skip-unmapped option, which filters out reference elements which do not have mapped elements associated with them. See the end of the score operations section of the bedmap documentation for more detail.
- General --ec performance improvements.
- starch
- Fixed bug with starch where zero-byte BED input (i.e., an "empty set") created a truncated and unusable archive. We now put in a "dummy" chromosome for zero-byte input, which unstarch can now unpack.
This should simplify error handling with certain pipelines, specifically where set or other BEDOPS operations yield an "empty set" BED file that is subsequently compressed with starch.
- unstarch
- Can now unpack zero-byte ("empty set") compressed starch archive (see above).
- Changed unstarch --list option to print to stdout stream (this was previously sent to stderr).
- starch metadata library
- Fixed array overflow bug with BEDOPS tools that take starch archives as inputs, which affected use of archives as inputs to closest-features, bedops and bedmap.
- All conversion scripts
- Python scripts require v2.7+ or greater.
- Improved (more "Pythonic") error code handling.
- Disabled support for --max-mem sort parameter until sort-bed issue is resolved. Scripts will continue to sort, but they will be limited to available system memory. If you are processing files larger than system memory, please contact us at bedops@stamlab.org for details of a temporary workaround.
- gff2bed conversion script
- Resolved IndexError exceptions by fixing header support, bringing script in line with v1.21 GFF3 spec.
- bam2bed and sam2bed conversion scripts
- Rewritten bam2* and sam 2 * scripts from bash into Python (v2.7+ support).
- Improved BAM and SAM input validation against the v1.4 SAM spec.
- New --split option prints reads with N CIGAR operations as separated BED elements.
- New --all-reads option prints all reads, mapped and unmapped.
- bedextract
- Fixed stdin bug with bedextract.
- New documentation via readthedocs.org.
- Documentation is now part of the BEDOPS distribution, instead of being a separate download.
- We use readthedocs.org to host indexed and searchable HTML.
- PDF and eBook documents are also available for download.
- Documentation is refreshed and simplified, with new installation and compilation guides.
- OS X compilation improvements
- We have made changes to the OS X build process for half of the BEDOPS binaries, which allows direct compilation with Clang/LLVM (part of the Apple Xcode distribution). Those binaries now use Apple's system-level C++ library, instead of GNU's libstdc++.
This change means that we require Mac OS X 10.7 ("Lion") or greater-we do not support 10.6 at this time.
Compilation is faster and simpler, and we can reduce the size and complexity of Mac OS X builds and installer packages. By using Apple's C++ library, we also reduce the likelihood of missing library errors. When this process is completed for the remaining binaries, it will no longer be necessary to install GCC $4.7+$ (by way of MacPorts or other package managers) in order to build BEDOPS on OS X, nor will we have to bundle libstdc++ with the installer.


## v2.2.0b

- Fixed bug with OS X installer's post-installation scripts.


## v2.2.0

Released: May 22, 2013

- Updated packages
- Precompiled packages are now available for Linux (32- and 64-bit) and Mac OS X 10.6-10.8 (32- and 64-bit) hosts.
- Starch v2 test suite
- We have added a test suite for the Starch archive toolkit with the source download. Test inputs include randomized BED data generated from chromosome and bounds data stored on UCSC servers as well as static FIMO search results. Tests put starch, unstarch and starchcat through various usage scenarios. Please refer to the Starch-specific Makefiles and the test target and subfolder's README doc for more information.
- starchcat
- Resolves bug with --gzip option, allowing updates of gzip -backed v1.2 and v1.5 archives to the $v 2$ Starch format (either bzip2-or gzip-backed).
- unstarch
- Resolves bug with extraction of Starch archive made from BED files with four or more columns. A condition where the total length of additional columns exceeds a certain number of characters would result in extracted data in those columns being cut off. As an example, this could affect Starch archives made from the raw, uncut output of GTF- and GFF- conversion scripts.
- conversion scripts
- We have partially reverted wig2bed, providing a Bash shell wrapper to the original C binary. This preserves consistency of command-line options across the conversion suite, while making use of the C binary to recover performance lost from the Python-based v2.1 revision of wig2bed (which at this time is no longer supported). (Thanks to Matt Maurano for reporting this issue.)


## v2.1.1

Released: May 3, 2013

- bedmap
- Major performance improvements made in v2.1.1, such that current bedmap now operates as fast or faster than the v1.2.5 version of bedmap!
- bedops
- Resolves bug with --partition option.
- conversion scripts
- All v2.1.0 Python-based scripts now include fix for SIGPIPE handling, such that use of head or other common UNIX utilities to process buffered standard output no longer yields IOError exceptions. (Thanks to Matt Maurano for reporting this bug.)
- 32-bit Linux binary support
- Pre-built Linux binaries are now available for end users with 32-bit workstations.

Other issues fixed:

- Jansson tarball no longer includes already-compiled libraries that could potentially interfere with 32-bit builds.
- Minor changes to conversion script test suite to exit with useful error code on successful completion of test.


## v2.1.0

## Released: April 22, 2013

- bedops
- New --partition operator efficiently generates disjoint segments made from genomic boundaries of all overlapping inputs.
- conversion scripts
- All scripts now use sort-bed behind the scenes to output sorted BED output, ready for use with BEDOPS utilities. It is no longer necessary to pipe data to or otherwise post-process converted data with sort-bed.
- New psl2bed conversion script, converting PSL-formatted UCSC BLAT output to BED.
- New wig2bed conversion script written in Python.
- New $* 2$ starch conversion scripts offered for all $* 2$ bed scripts, which output Starch v2 archives.
- closest-features
- Replaced --shortest option name with --closest, for clarity. (Old scripts which use --shortest will continue to work with the deprecated option name for now. We advise editing pipelines, as needed.)
- starch
- Improved error checking for interleaved records. This also makes use of $* 2$ starch conversion scripts with the --do-not-sort option safer.
- Improved Mac OS X support
- New Mac OS X package installer makes installation of BEDOPS binaries and scripts very easy for OS X 10.6-10.8 hosts.
- Installer resolves fatal library errors seen by some end users of older OS X BEDOPS releases.


## v2.0.0b

Released: February 19, 2013

- Added starchcluster script variant which supports task distribution with GNU Parallel.
- Fixed minor problem with bam2bed and sam2bed conversion scripts.


## v2.0.0a

Released: February 7, 2013

- bedmap
- Takes in Starch-formatted archives as input, as well as raw BED (i.e., it is no longer required to extract a Starch archive to an intermediate, temporary file or named pipe before applying operations).
- New --chrom operator jumps to and operates on information for specified chromosome only.
- New --echo-map-id-uniq operator lists unique IDs from overlapping mapping elements.
- New --max-element and --min-element operators return the highest or lowest scoring overlapping map element.
- bedops
- Takes in Starch-formatted archives as input, as well as raw BED.
- New --chrom operator jumps to and operates on information for specified chromosome only.
- closest-features
- Takes in Starch-formatted archives as input, as well as raw BED.
- New --chrom operator jumps to and operates on information for specified chromosome only.
- sort-bed and bbms
- New --max-mem option to limit system memory on large BED inputs.
- Incorporated bboms functionality into sort-bed with use of --max-mem operator.
- starch, starchcat and unstarch
- New metadata enhancements to Starch-format archival and extraction, including: --note, --elements, --bases, --bases-uniq, --list-chromosomes, --archive-timestamp, --archive-type and --archive-version (see --help to starch, starchcat and unstarch binaries, or view the documentation for these applications for more detail).
- Adds 20-35\% performance boost to creating Starch archives with starch utility.
- New documentation with technical overview of the Starch format specification.
- conversion scripts
- New gt f2bed conversion script, converting GTF (v2.2) to BED.
- Scripts are now part of main download; it is no longer necessary to download the BEDOPS companion separately.


## v1.2.5b

## Released: January 14, 2013

- Adds support for Apple 32- and 64-bit Intel hardware running OS X 10.5 through 10.8.
- Adds README for companion download.
- Removes some obsolete code.


## v1.2.5

## Released: October 13, 2012

- Fixed unusual bug with unst arch, where an extra (and incorrect) line of BED data can potentially be extracted from an archive.
- Updated companion download with updated bam2bed and sam2bed conversion scripts to address 0-indexing error with previous revisions.


## v1.2.3

## Released: August 17, 2012

- Added --indicator option to bedmap.
- Assorted changes to conversion scripts and associated companion download.


### 2.4 Usage examples

The following examples demonstrate the use of BEDOPS in analyzing genomic data. Here, we provide source code and snippets of data to demonstrate "real-world" examples based on daily usage of these tools in the Stamatoyannopoulos lab.

### 2.4.1 Visualizing the relationship of SNPs and generic genomic features

We want to visualize how genome-wide association study single nucleotide repeats (GWAS SNPs) relate to other genomic features-in this case, these features are DNaseI-hypersensitive sites (DHSs). We could, instead, look at methylated regions, CpG islands, coding sequence or other genomic features. Normally, we might do this for all sites in the genome, but to reduce the file sizes we only look at a subset of data here and we have taken a subset of the real data for the purposes of demonstration.
Roughly speaking, we considered two classes of SNPs: those which are prostate-related (associated with PSA and prostate cancer) and some not (height). We have some BED files with positions of DNaseI-hypersensitive sites for various tissues: two from prostate (LNCaP and PrEC), the rest from other tissues (CACO2, HEPG2, K562, MCF7).

We will use BEDOPS tools to generate per-tissue DHS counts associated with our SNPs, using matrix2png to visualize results as a heatmap.

## BEDOPS tools in use

For this example, we use sort-bed to sort the input SNP data, and bedmap to count the number of single-base or greater overlaps between a SNP and a tissue-specific DHS. A modified version of this script uses loops and other shell features.

## Script

```
#!/bin/tcsh -efx
sort-bed GWAS_SNPs.bed > GWAS_SNPs.sorted.bed
bedmap --ec --delim "\t" --bp-ovr 1 --echo --count GWAS_SNPs.sorted.bed LNCaP_DHS.bed,
\hookrightarrow> SNP_DHS_matrix.bed
# add PrEC DHS overlap counts to matrix
bedmap --ec --delim "\t" --bp-ovr 1 --count GWAS_SNPs.sorted.bed PrEC_DHS.bed >
counts.txt
paste SNP_DHS_matrix.bed counts.txt > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
# add CACO2 DHS overlap counts to matrix
bedmap --ec --delim "\t" --bp-ovr 1 --count GWAS_SNPs.sorted.bed CACO2_DHS.bed > s
counts.txt
paste SNP_DHS_matrix.bed counts.txt > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
# add HEPG2 DHS overlap counts to matrix
bedmap --ec --delim "\t" --bp-ovr 1 --count GWAS_SNPs.sorted.bed HEPG2_DHS.bed >
counts.txt
paste SNP_DHS_matrix.bed counts.txt > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
```

```
# add K562 DHS overlap counts to matrix
bedmap --ec --delim "\t" --bp-ovr 1 --count GWAS_SNPs.sorted.bed K562_DHS.bed >
counts.txt
paste SNP_DHS_matrix.bed counts.txt > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
# add MCF7 DHS overlap counts to matrix
bedmap --ec --delim "\t" --bp-ovr 1 --count GWAS_SNPs.sorted.bed MCF7_DHS.bed > 
counts.txt
paste SNP_DHS_matrix.bed counts.txt > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
# cleanup and sort by disease trait
rm counts.txt
sort -k5d SNP_DHS_matrix.bed > new_SNP_DHS_matrix.bed
mv new_SNP_DHS_matrix.bed SNP_DHS_matrix.bed
# condense data fields into matrix2png form
awk '{print $1":"$2"-"$3"_"$4"_"$5"_"$6"\t"$7"\t"$8"\t"$9"\t"$10"\t"$11"\t"$12}' SNP_
\hookrightarrowDHS_matrix.bed > new_SNP_DHS_matrix.txt
mv new_SNP_DHS_matrix.txt SNP_DHS_matrix.txt
# add header
echo -e "O\tLNCaP\tPrEC\tCACO\tHEPG2\tK562\tMCF7" | cat - SNP_DHS_matrix.txt > new_
\hookrightarrowSNP_DHS_matrix.txt
mv new_SNP_DHS_matrix.txt SNP_DHS_matrix.txt
# make heatmap
matrix2png -r -c -g -size 16:16 -mincolor yellow -midcolor black -maxcolor red -data
\hookrightarrowSNP_DHS_matrix.txt > SNP_DHS_matrix.png
```


## Discussion

Each use of bedmap is identical: the options --ec --delim " \t" --bp-ovr 1 --count add several settings:

- Error checking/correction (--ec)
- Use of the tab character as a custom field delimiter (--delim " $\backslash$ t") to make results easier to parse with awk further downstream
- Manual specification of a single base-pair criteria for overlap (--bp-ovr 1, although this is the default)
- Using --count, counting the number of mapping elements (DHSs) which overlap a reference instance (a given SNP)

These results are calculated for each of the seven cell types and collated into matrix form to run through matrix2png. We show it here to give an idea of what kind of data bedmap generates, to help create these quantitative visualizations:
chr1:227797949-227797950_rs1390401_Height_Quantitative_traits chr10:81121695-81121696_rs2145998_Height_Quantitative_traits chr10:81139461-81139462_rs941873_Height_Quantitative_traits chr10:124165614-124165615_rs6585827_Height_Quantitative_traits chr10:126696871-126696872_rs4962416_Prostate_cancer_Cancer chr11:65336818-65336819_rs3782089_Height_Quantitative_traits chr11:75276177-75276178_rs606452_Height_Quantitative_traits chr11:75282051-75282052_rs634552_Height_Quantitative_traits chr11:85164750-85164751_rs10898392_Height_Quantitative_traits chr12:11855623-11855624_rs2187642_Height_Quantitative_traits chr12:11855772-11855773_rs2856321_Height_Quantitative_traits chr12:20758612-20758613_rs11611208_Height_Quantitative_traits chr12:20857466-20857467_rs10770705_Height_Quantitative_traits chr12:28534414-28534415_rs2638953_Height_Quantitative_traits chr12:53273903-53273904_rs902774_Prostate_cancer_Cancer chr12:53916714-53916715_rs12321906_Height_Quantitative_traits chr12:54041191-54041192_rs11170631_Height_Quantitative_traits chr12:66227256-66227257_rs7979673_Height_Quantitative_traits chr12:66358346-66358347_rs1042725_Height_Quantitative_traits chr12:66359751-66359752_rs8756_Height_Quantitative_traits chr12:66371879-66371880_rs7968682_Height_Quantitative_traits chr12:69827657-69827658_rs10748128_Height_Quantitative_traits chr12:69828680-69828681_rs11177669_Height_Quantitative_traits chr12:93976953-93976954_rs3825199_Height_Quantitative_traits chr12:93978503-93978504_rs11107116_Height_Quantitative_traits chr12:115094259-115094260_rs11067228_PSA_Serum_metabolites chr13:51111354-51111355_rs3116602_Height_Quantitative_traits chr17:47436748-47436749_rs7210100_Prostate_cancer_Cancer chr17:69108752-69108753_rs1859962_Prostate_cancer_Cancer chr18:20735407-20735408_rs4369779_Height_Quantitative_traits chr18:46991159-46991160_rs8099594_Height_Quantitative_traits chr19:2275078-2275079_rs2523178_Height_Quantitative_traits chr19:3428833-3428834_rs7507204_Height_Quantitative_traits chr19:38735612-38735613_rs8102476_Prostate_cancer_Cancer chr19:51361756-51361757_rs17632542_PSA_Serum_metabolites chr19:51364622-51364623_rs2735839_Prostate_cancer_Cancer chr2:56111308-56111309_rs3791675_Height_Quantitative_traits chr2:173311552-173311553_rs12621278_Prostate_cancer_Cancer chr2:233077063-233077064_rs7571816_Height_Quantitative_traits chr20:33975180-33975181_rs6088813_Height_Quantitative_traits chr22:23593050-23593051_rs5751614_Height_Quantitative_traits chr22:43518274-43518275_rs742134_Prostate_cancer_Cancer chr3:37996476-37996477_rs9311171_Prostate_cancer_Cancer chr3:87110673-87110674_rs2660753_Prostate_cancer_Cancer chr3:141102832-141102833_rs6763931_Prostate_cancer_Cancer chr3:141105569-141105570_rs724016_Height_Quantitative_traits chr5:1895828-1895829_rs12653946_Prostate_cancer_Cancer chr5:131699866-131699867_rs274546_Height_Quantitative_traits chr6:31118510-31118511_rs130067_Prostate_cancer_Cancer chr6:41536426-41536427_rs1983891_Prostate_cancer_Cancer 42 chr6:51666630-51666631_rs10498792_Prostate_cancer_Canefhapter 2. Contents chr6:105417977-105417978_rs314268_Height_Quantitative_traits

Rows are presented in sort-bed order. Cells in red show greatest relative number of counts, while yellow shows the least. Examining this heatmap, DHS elements appear to associate with prostate disease-related GWAS SNPs.
To make this clearer, here is the same result, with rows sorted by disease name:
chr 12:115094259-115094260_rs11067228_PSA_Serum_metabolites chr19:51361756-51361757_rs17632542_PSA_Serum_metabolites chr12:53273903-53273904_rs902774_Prostate_cancer_Cancer chr8:128413304-128413305_rs6983267_Prostate_cancer_Cancer chr2:173311552-173311553_rs12621278_Prostate_cancer_Cancer chr7:97816326-97816327_rs6465657_Prostate_cancer_Cancer chr6:41536426-41536427_rs1983891_Prostate_cancer_Cancer chr 10:126696871-126696872_rs4962416_Prostate_cancer_Cancer chr17:69108752-69108753_rs1859962_Prostate_cancer_Cancer chr19:51364622-51364623_rs2735839_Prostate_cancer_Cancer chr6:117210051-117210052_rs339331_Prostate_cancer_Cancer chr6:160833663-160833664_rs9364554_Prostate_cancer_Cancer chr7:27976562-27976563_rs10486567_Prostate_cancer_Cancer chr8:128103936-128103937_rs1456315_Prostate_cancer_Cancer chr3:141102832-141102833_rs6763931_Prostate_cancer_Cancer chr8:128093296-128093297_rs1016343_Prostate_cancer_Cancer chr8:23526462-23526463_rs1512268_Prostate_cancer_-Cancer chr 17:47436748-47436749_rs7210100_Prostate_cancer_Cancer chr 19:38735612-38735613_rs8102476_Prostate_cancer_Cancer chr22:43518274-43518275_rs742134_Prostate_cancer_Cancer chr3:37996476-37996477_rs9311171_Prostate_cancer_Cancer chr3:87110673-87110674_rs2660753_Prostate_cancer_Cancer chr5:1895828-1895829_rs12653946_Prostate_cancer_Cancer chr6:160581373-160581374_rs651164_Prostate_cancer_Cancer
chr6:31118510-31118511_rs130067_Prostate_cancer_Cancer chr6:51666630-51666631_rs10498792_Prostate_cancer_Cancer chr7:20994490-20994491_rs12155172_Prostate_cancer_Cancer chr8:128095155-128095156_rs13252298_Prostate_cancer_Cancer chr18:20735407-20735408_rs4369779_Height_Quantitative_traits chr2:233077063-233077064_rs7571816_Height_Quantitative_traits chr 13:51111354-51111355_rs3116602_Height_Quantitative_traits chr 18:46991159-46991160_rs8099594_Height_Quantitative_traits chr 3: 141105569-141105570_rs724016_Height_Quantitative_traits chr6:109742014-109742015_rs9487094_Height_Quantitative_traits chr9:95429119-95429120_rs9969804_Height_Quantitative_traits chr19:2275078-2275079_rs2523178_Height_Quantitative_traits chr 19:3428833-3428834_rs7507204_Height_Quantitative_traits chr 10:81139461-81139462_rs941873_Height_Quantitative_traits chr22:23593050-23593051_rs5751614_Height_Quantitative_traits chr6:105417977-105417978_rs314268_Height_Quantitative_traits chr 10:124165614-124165615_rs6585827_Height_Quantitative_traits chr 10:81121695-81121696_rs2145998_Height_Quantitative_traits chr11:65336818-65336819_rs3782089_Height_Quantitative_traits chr11:75276177-75276178_rs606452_Height_Quantitative_traits chr11:75282051-75282052_rs634552_Height_Quantitative_traits chr11:85164750-85164751_rs10898392_Height_Quantitative_traits chr12:11855623-11855624_rs2187642_Height_Quantitative_traits chr12:11855772-11855773_rs2856321_Height_Quantitative_traits chr12:20758612-20758613_rs11611208_Height_Quantitative_traits chr12:20857466-20857467_rs10770705_Height_Quantitative_traits chr1:227797949-227797950_rs1390401_Height_Quantitative_traits 44 chr 12:28534414-28534415_rs2638953_Height_Quantitative_traiCbapter 2. Contents chr12:53916714-53916715_rs12321906_Height_Quantitative_traits chr12:54041191-54041192_rs11170631_Height_Quantitative_traits chr 12:66227256-66227257_rs7979673_Height_Quantitative_traits

While there are some DHSs associated with non-disease SNPs, the majority accumulate with the prostate SNPs.

## Downloads

- The example script, after modification to use loops and other shell features.
- Data for this example are contained in a tarball (use tar -xzf to extract files).

The bedmap tool can operate directly on Starch-formatted archives. Alternatively, use the unstarch tool to decompress Starch data files to sorted BED format.

Note that these are not the full datasets that went into the original research, but snippets that should otherwise demonstrate the disease-DHS association phenomenon and the use of parts of the BEDOPS toolset.

### 2.4.2 Collapsing multiple BED files into a master list by signal

Given a list of five-column UCSC BED files, where scores are kept in the fifth column, we want to build a "master list" of non-overlapping elements from all the inputs. Elements that initially overlap are ranked by score, and the highest scoring element is added to the master list.

## BEDOPS tools in use

In the following example, we want to merge hotspot peaks for five fetal adrenal tissues, picking the highest scoring element where there are overlapping peaks. We'll use a mix of bedmap and its --max-element operation with bedops set operations to accomplish this.

## Script

```
#!/bin/bash
# author : Bob Thurman
beds=(fAdrenal-DS12528.dhs.bed
    fAdrenal-DS15123.dhs.bed
    fAdrenal-DS17319.dhs.bed
    fAdrenal-DS17677.dhs.bed
    fAdrenal-DS20343.dhs.bed)
out=fAdrenal.master.merge.bed
tmpd=/tmp/tmp$$
mkdir -p $tmpd
## First, union all the peaks together into a single file.
bedlist=""
for bed in ${beds[*]}
do
    bedlist="$bedlist $bed"
done
bedops -u $bedlist > $tmpd/tmp.bed
## The master list is constructed iteratively. For each pass through
## the loop, elements not yet in the master list are merged into
```

(continued from previous page)

```
## non-overlapping intervals that span the union (this is just bedops
## -m). Then for each merged interval, an original element of highest
## score within the interval is selected to go in the master list.
## Anything that overlaps the selected element is thrown out, and the
## process then repeats.
iters=1
solns=""
stop=0
while [ $stop == 0 ]
do
    echo "merge steps..."
    ## Condense the union into merged intervals. This klugey bit
    ## before and after the merging is because we don't want to merge
    ## regions that are simply adjacent but not overlapping
    bedops -m --range 0:-1 $tmpd/tmp.bed \
            | bedops -u --range 0:1 - \
            > $tmpd/tmpm.bed
    ## Grab the element with the highest score among all elements forming each
\hookrightarrowinterval.
    ## If multiple elements tie for the highest score, just grab one of them.
    ## Result is the current master list. Probably don't need to sort, but do it,
->anyway
    ## to be safe since we're not using --echo with bedmap call.
    bedmap --max-element $tmpd/tmpm.bed $tmpd/tmp.bed \
            | sort-bed - \
            > $tmpd/$iters.bed
    solns="$solns $tmpd/$iters.bed"
    echo "Adding `awk 'END { print NR }' $tmpd/$iters.bed` elements"
    ## Are there any elements that don't overlap the current master
    ## list? If so, add those in, and repeat. If not, we're done.
    bedops -n 1 $tmpd/tmp.bed $tmpd/$iters.bed \
        > $tmpd/tmp2.bed
    mv $tmpd/tmp2.bed $tmpd/tmp.bed
    if [ ! -s $tmpd/tmp.bed ]
    then
        stop=1
    fi
    ((iters++))
done
## final solution
bedops -u $solns \
    > $out
## Clean up
rm -r $tmpd
exit 0
```


## Discussion

A broad array of human cell tissue hotspot data for testing this example are available for public download from the UCSC Genome Browser:

- http://genome.ucsc.edu/cgi-bin/hgFileUi?db=hg19\&g=wgEncodeUwDnase

This includes hotspot data for DS12528, DS15123, DS17319, DS 17677 and DS20343 lines.

### 2.4.3 Measuring the frequency of signed distances between SNPs and nearest DHSes

In this example, we would like to find the signed distance between a single nucleotide repeat and the DNasehypersensitive site nearest to it, as measured in base pairs (bp).

## BEDOPS tools in use

To find nearest elements, we will use closest-features with the --dist, --closest, and --no-ref options.

## Script

SNPs are in a BED-formatted file called SNPs.bed sorted lexicographically with sort-bed. The DNasehypersensitive sites are stored in a sorted BED-formatted file called DHSs.bed. These two files are available in the Downloads section.

```
# author : Eric Rynes
closest-features --dist --closest --no-ref SNPs.bed DHSs.bed \
    | cut -f2 -d '|' \
    | grep -w -F -v -e "NA" \
    > answer.bed
```


## Discussion

The --dist option returns signed distances between input elements and reference elements, --closest chooses the single closest element, and --no-ref keeps SNP coordinates from being printed out.

The output from closest-features contains coordinates and the signed distance to the closest DHS, separated by the pipe (I) character. Such output might look something like this:

```
chr1 2513240 2513390 MCV-11 97.201400|25
```

This type of result is chopped up with the standard UNIX utility cut to get at the distances to the closest elements. Finally, we use grep -v to throw out any non-distance, denoted by NA. This can occur if there exists some chromosome in the SNP dataset that does not exist in the DHSs.

Thus, for every SNP, we have a corresponding distance to nearest DHS. As an example, from this data we could build a histogram showing the frequencies of distances-to-nearest-DHS.

## Downloads

- SNP elements
- DNase-hypersensitive elements

The closest-features tool can operate directly on Starch-formatted archives. Alternatively, use the unstarch tool to decompress Starch data files to sorted BED format.

### 2.4.4 Finding the subset of SNPs within DHSes

In this example, we would like to identify the set of SNPs that are within a DHS, printing out both the SNP element and the DHS it is contained within.

## BEDOPS tools in use

We use bedmap to answer this question, as it traverses a reference BED file (in this example, SNPs), and identifies overlapping elements from the mapping BED file (in this example, DHSs).

## Script

SNPs are in a BED-formatted file called SNPs.bed sorted lexicographically with sort-bed. The DNasehypersensitive sites are stored in a sorted BED-formatted file called DHSs.bed. These two files are available in the Downloads section.

```
bedmap --skip-unmapped --echo --echo-map SNPs.bed DHSs.bed \
```

    > subsetOfSNPsWithinAssociatedDHS.bed
    
## Discussion

The output of this bedmap statement might look something like this:

```
chr1 10799576 10799577 rs12.4.368 Systolic_blood_pressure
\hookrightarrowCardiovascular|chr1 10799460 10799610 MCV-1 9.18063
```

The output is delimited by pipe symbols ( 1 ), showing the reference element (SNP) and the mapped element (DHS).
If multiple elements are mapped onto a single reference element, the mapped elements are further separated by semicolons, by default.

## Downloads

- SNP elements
- DNase-hypersensitive elements

The bedmap tool can operate directly on Starch-formatted archives. Alternatively, use the unstarch tool to decompress Starch data files to sorted BED format.

### 2.4.5 Smoothing raw tag count data across the genome

In this example, we generate smoothed density signal by binning the genome into 20 bp intervals and counting the number of non-paired-end tag reads falling within 75 bp of each interval. A simple follow-on script marks up results to wig or bigWig format for loading into a track of a local UCSC Genome Browser.

## BEDOPS tools in use

For this script, we use bam2bed to convert a BAM file to BED, then we use bedmap to run a sliding density window over input genomic regions. Finally starch compresses the results.

## Script

```
#!/bin/tcsh -ef
# author : Richard Sandstrom
if ( $#argv != 5 ) then
    printf "Wrong number of arguments\n"
    printf "<bam-file> <out-file> <window-size> <step-size> <chromosome-file>\n"
    printf " where <chromosome-file> contains whole chromosome BED items for the\n"
    printf " genome, e.g., sort-bed formatted output from the UCSC hg19.chromInfog
\hookrightarrowtable.\n"
    exit -1
endif
# BAM file
set inBam = $argv[1]
# resulting density file
set outDensity = $argv[2]
# +/- window for counting read 5' ends
set window = $argv[3]
# step size across genome
set binI = $argv[4]
# chromosome file for organism of interest
set chromsfile = $argv[5]
set outDir = $outDensity:h
mkdir -p $outDir
set tmpDir = /tmp/`whoami`/scratch/$$
if ( -d $tmpDir ) then
    rm -rf $tmdDir
endif
mkdir -p $tmpDir
# clip tags to single 5' end base
bam2bed < $inBam \
        | awk '{if($6=="+"){s=$2; e=$2+1}else{s=$3-1; e=$3}print $1"\t"s"\t"e}' \
        | sort-bed --max-mem 2G - \
    >! $tmpDir/tags.bed
# create genome-wide bins and count how many tags fall within range of each
awk -v binI=$binI -v win=$window \
            '{ \
            i = 0; \
            for(i = $2; i <= $3-binI; i += binI) { print $1"\t"i"\t"i + binI } \
            # end of chrome may include a bin of size < binI \
            if ( i < $3) { print $1"\t"i"\t"$3; } \
            }' $chromsfile \
            | bedmap --faster --range $window --echo --count --delim "\t" - $tmpDir/tags.bed \
            | starch - \
    >! $outDensity
```

```
rm -rf $tmpDir
```

exit 0

### 2.4.6 Efficiently creating Starch-formatted archives with a cluster

In this example, we demonstrate how to use bedextract and starchcat to efficiently generate Starch-formatted archives from BED datasets.

## BEDOPS tools in use

For this script, we use bedextract to quickly build a list of chromosomes in an input BED dataset and extract records for each chromosome to separate files. We then use starch to compress each per-chromosome file and starchcat to concatenate per-chromosome Starch archives into one file.

## Script

Three versions of the starchcluster script are included with the source and package distributions of BEDOPS (see Installation for more detail).

One version makes use of an Oracle Grid Engine (or Sun Grid Engine) cluster environment to distribute perchromosome tasks, another script uses GNU Parallel to split the workload over cores or processors on the local host. Finally, we include a version that implements a SLURM -capable script.

## Discussion

The overview that follows applies to the Grid Engine-based version of the starchcluster script. However, the general algorithm is identical for the Grid Engine-, GNU Parallel-, and SLURM-based compression scripts.

## Splitting BED files

Whole-genome analyses are often "embarassingly parallel", in that per-chromosome computations can be placed onto separate work nodes of a computational cluster, with results collated at the end in "map-reduce" fashion.

If we want to filter any BED file to retrieve elements from a specific chromosome (say, to compress a BED file, one chromosome at a time), to arrange this kind of analysis, one trivial-but very slow-way to do this involves sequentially walking line by line through the file to parse and test each element. This can take a while to do.

However, just as BEDOPS tools use the information in sorted data to apply efficient set and statistical operations, we can use this same information to jump quickly through our data of interest.

Specifically, sorting allows us to perform a binary search:

1. We jump to the middle byte of the BED file, stream to the nearest element, then parse and test the chromosome name.
2. Either we have a match, or we jump to the middle of the remaining left or right half (decided by dictionary order), parse and test again.
3. We repeat steps 1 and 2 until we have matches that define the bounds of the target chromosome.


To indicate the kind of speed gain that the bedextract tool provides, in local testing, a naïve listing of chromosomes from a 36 GB BED input using UNIX cut and uniq utilities took approximately 20 minutes to complete on a typical Core 2 Duo-based Linux workstation. Retrieval of the same chromosome listing with bedextract --list-chr took only 2 seconds (cache flushed-no cheating!).

## Compressing BED subsets

Now we can very quickly demarcate where chromosomes start and stop in a BED file, we can apply starch on those subsets on separate cluster nodes.

## Stitching together compressed sets

Once we have per-chromosome Starch-formatted archives, we need some way to put them all together into one archive. This is where starchcat comes in, taking all the per-chromosome archives as inputs and creating a new archive as output.
The big picture view is like this:

## starchcluster: Parallelizing compression with bedextract and starchcat

Step 1: Build chromosome list
Run bedextract on input BED file


Step 3: Compress each per-chr file
Distribute starch compression tasks of per-chromosome files to computational cluster via job scheduler (e.g., Oracle/Sun Grid Engine or GNU Parallel)

## Step 4: Collate with starchcat

After all per-chromosome archives are ready, concatenate into one final Starch file with the starchcat utility:
starchcat *.starch > foo.starch

## Step 2: Split input file

Run bedextract on input BED file, looping over the list of chromosomes, extracting per-chromosome records:
bedextract chr1 foo.bed > chr1.bed bedextract chr10 foo.bed > chr10.bed bedextract chr11 foo.bed > chr11.bed


## Parallelization reduces BED compression times to the time taken to compress the largest chromosome!

As the figure notes, the compression time for a very large BED file is reduced roughly to the time taken to compress the largest chromosome in the original file. Parallelization of this process is an order of magnitude faster than compressing chromosomes in serial.

### 2.4.7 Working with many input files at once with bedops and bedmap

BEDOPS is designed to work with as many input files at once as you need, either through the bedops program, or through a combined use of that program with others in the suite.

## Discussion

Say we have five input BED files (A, B, C, D, E), and we need to identify those regions where any two (or more) of the input files ( $\{A, B\},\{A, C\},\{A, D\},\{A, E\},\{B, C\}, \ldots)$ overlap reciprocally by $30 \%$ or more.
One concrete application may be where we have multiple biological replicates, and we take any repeatable result (in two or more inputs, in this case) as true signal. Similarly, we might be interested in a problem like this if we have multiple related (or even unrelated) cell type samples and we want to be confident in peak calls for DNaseI sequencing of ChIP-seq experiments.

These sorts of problems often have efficient solutions in BEDOPS. Here, the solution is independent of how many inputs we start with, what overlap criteria we use, and whether the requirement calls for two or more files of overlap (or whether it is 4 or more files in the overlap, or 9 , or whatever).

Consider a case study of one such problem that utilizes both bedops and bedmap together to create an efficient solution:

```
$ bedops -u file1.bed file2.bed ... fileN.bed \
    | bedmap --echo --echo-map-id-uniq --fraction-both 0.5 - \
    | awk -F"|" '(split($2, a, ";") > 1)' \
    > answer.bed
```

Here, we pass in as many files as we have to bedops. The requirement of elements overlapping reciprocally is met by using --fraction-both, and the requirement that overlapping elements must come from two or more (distinct) files is satisfied by checking how many elements there are via the --echo-map-id-uniq operator.

The requirements for filel.bed through fileN.bed are that each is properly sorted (as expected for any BEDOPS input) and that their respective fourth-column ID fields identify the file. For example:

```
$ head -2 file1.bed
chr1 1 50 1 anything-else
chr1 230 400 1 whatever-you-like
$ head -2 file2.bed
chr1 23 78 2 other-fields
chr1 56 98 2 5.678 + peak-2
```

As a nice side-effect, answer. bed will show from which file each entry originated. If we don't want that extra information, we simply cut it out:

```
cut -f1-3,5- answer.bed >! my-final-answer.bed
```

There is also a column that shows exactly which files are part of the per-row intersection. If we don't want that information, then we just cut that:

```
cut -f1 -d'|' my-final-answer.bed
```

While this is just one example of how the tools can be used together to answer complicated questions efficiently, it demonstrates why it is worthwhile to learn about the relatively few core programs in BEDOPS.

If we look at what is required to answer this kind of question using other tool suites, we will quickly find that solutions do not scale to the number of files, nor with the requirement that overlaps must come from $k$ or more distinct input files. Even in the simplest case of just requiring the regions overlap in 2 of $n$ inputs, we must build on the order of $n^{2} / 2$
intermediate files (and sweep through the $n$ original inputs $n^{2}$ times as well). If our requirement is 3 of $n$ inputs, the polynomials increase accordingly.

The solution with BEDOPS is far more efficient than this and requires no intermediate results.

### 2.5 Performance

In this document, we compare the performance of our set operations and compression utilities with common alternatives. In-house performance measures include speed, memory usage, and compression efficiency on a dual-core machine with 18 GB of virtual memory. Additionally, we report independently-generated performance statistics collected by a research group that has recently released a similar analysis toolkit.

### 2.5.1 Test environment and data

Timed results were derived using actual running times (also known as wall-clock times), averaged over 3 runs. All timed tests were performed using a single 64-bit Linux machine with a dual-core 3 GHz Intel Xeon processor, 8 GB of physical RAM, and 18 GB of total virtual memory. All caches were purged in between sequential program runs to remove hardware biases.

Random subsamples of phyloP conservation for the human genome were used as inputs for testing whenever the full phyloP results were not used. The full phyloP results were downloaded from UCSC.

### 2.5.2 Set operations with bedops

In this section, we provide time and memory measurements of various bedops operations against analogous BEDTools utilities.

## Direct merge (sorted)

The performance of the mergeBed program (with the -i option) from the BEDTools suite (v2.12.0) was compared with that of the --merge option of our bedops utility.


As measured, the mergeBed program loads all data from a file into memory and creates an index before computing results, incurring longer run times and higher memory costs that can lead to failures. The bedops utility minimizes memory consumption by retaining only the information required to compute the next line of output.

## Complement and intersection

The complementBed (with -i and -g options) and intersectBed (with -u , -a , and -b options) programs from the BEDTools suite ( v 2.12 .0 ) also were compared to our bedops program.


## --intersect (sorted inputs)



> We calculate the intersection of two sorted input files with 200 k rows and up with BEDOPS bedops and BEDTools intersectBed (v2.12) with $-u,-a$ and $-b$ options.

The intersectBed operation fails at 51M rows, while bedops continues onwards, operating in linear time and constant memory.

Both BEDTools programs were unable to complete operations after 51 M elements with the allocated 18 GB of memory. The bedops program continued operating on the full dataset.

Important: It is our understanding that the BEDTools' intersect Bed program was modified to accept (optionally) sorted data for improved performance some time after these results were published.

A more recent study suggests bedops --intersect still offers better memory and running time performance characteristics than recent versions of BEDTools.

## Direct merge (unsorted)

In typical pipelines, where utilities are chained together to perform more complex operations, the performance and scalability gaps between BEDOPS and competitive tool suites widen. We show here the use of sort-bed on unsorted BED input, piping it to BEDOPS tools:

## --merge (unsorted inputs)



> We merge unsorted input files with 200 k rows and up with BEDOPS bedops and sort-bed, and BEDTools mergeBed (v2.12).

51M rows or more cannot be merged with mergeBed, while bedops continues onwards, operating in linear time and memory (up to 200M rows, at which point we apply sort-bed --maxmem 4G).

Time performance of bedops stays under that of mergeBed (BEDTools v2.12), while continuing past the point where mergeBed fails. Memory limitations of the system are easily overcome by using the --max-mem operator with sort-bed, allowing the --merge operation to continue unimpeded even with ever-larger unsorted BED inputs.

## Discussion

The bedops utility performs a wide range of set operations (merge, intersect, union, symmetric difference, and so forth). As with all main utilities in BEDOPS, the program requires sorted inputs and creates sorted results on output. As such, sorting is, at most, a one-time cost to operate on data any number of times in the most efficient way. Also, as shown in an independent study, BEDOPS also sorts data more efficiently than other tools. Further, our utility can sort BED inputs of any size.

Another important feature of bedops that separates it from the competition is its ability to work with any number of inputs at once. Every operation (union, difference, intersection, and so forth) accepts an arbitrary number of inputs, and each input can be of any size.

### 2.5.3 Compression characteristics of starch

The starch utility offers high-quality BED compression into a format with a smaller footprint than common alternatives. The format is designed to help manage data bloat in this genomic era. Further, the format actually enables improved access times to the vast majority of datasets, as compared with raw (uncompressed) and naively-compressed data.

Here, we provide two measures of this format's utility: comparing the compression efficiency of the bzip2-backed Starch format against common, "naive" bzip2-compression of UCSC BedGraph and WIG forms of BED data, and by comparing the time required to extract the records for any one chromosome from these formats as well as from a raw (uncompressed) BED file.

## Compression efficiency

After just 10K rows (roughly 300 kB of raw BED data storing phyloP conservation scores), compression into the Starch format begins to consistently outperform bzip2 compression of the same data stored in either variable-step WIG or UCSC BedGraph formats.

## Compression efficiency of starch



After just 10k rows (roughly 300 kB of raw BED data storing phyloP conservation scores), compression into the Starch format begins to consistently outperform bzip2 compression of the same data stored in either variable-step WIG or UCSC BedGraph formats.

For very large, raw BED datasets, the Starch format stores the original data in approximately $5 \%$ of the original input size. These improved compression results generalize to compressed versions of the fixedstep WIG format, as well.

For very large raw BED datasets, the Starch format stores the original data in approximately $5 \%$ of the original input size. These improved compression results generalize to compressed versions of the fixed-step WIG format, as well. For more information, refer to the Supplemental Data in our Bioinformatics paper.

## Extraction time

Data were sorted per sort-bed with chromosomes in lexicographical order. Extractions by chromosomes were significantly faster in general with the Starch format, even over raw (sequentially-processed) BED inputs:

## Extraction time with unstarch



Under the assumption that chromosomes create natural partitions of genomic data, random access to data within Starch archives uses a chromosome-indexing scheme. Extractions by chromosomes are significantly faster in general with the Starch format, even over uncompressed BED inputs.

This design improves data processing times for analyses focused on specific areas of whole-genome datasets, or where whole-genome experiments can be reduced to per-chromosome analyses that can be split and distributed on a computational cluster.

Under the assumption that chromosomes create very natural partitions of the data, the Starch format was designed using a chromosome-indexing scheme. This mechanism for random access further helps to improve data processing times
within a clustered environment. Again, for more information, refer to the Supplemental Data in our Bioinformatics paper.

Important: Our bedextract program similarly makes it possible to extract data quickly by chromosome in any properly sorted BED file. However, for large (or many) data sets, deep compression has serious benefit. In our lab, more than $99 \%$ of all files are not touched (even) on a monthly basis-and new results are generated every day. Why would we want to keep all of that data in fully-bloated BED form? The workhorse programs of BEDOPS accept inputs in Starch format directly, just as they do raw BED files, to help manage 'big data'.

### 2.5.4 Independent testing

## Genomic Region Operation Kit (GROK)

Ovaska, et al. independently developed a genomic analysis toolkit called Genomic Region Operation Kit (GROK), which is described in more detail in their publication in IEEE/ACM Transactions on Computational Biology and Bioinformatics.

In it, they compare the performance characteristics of their GROK toolkit with their analogs in the BEDTools and BEDOPS suites, which they summarize as follows:

## Results

Results of the benchmark analyses are shown in Table VII. GROK and BEDTools perform at comparable levels for speed and memory efficiency. In this benchmark BEDOPS is the fastest and least memory consuming method, which was expected due to performance optimized implementation of its operations ${ }^{9}$. The optimized performance of BEDOPS, however, entails stronger assumptions for the input than GROK and BEDTools, in particular the requirement for pre-sorting the input BED files.

Operational input was a 14 MB BED file containing annotations of human gene and exon coordinates, totaling $\sim 423 \mathrm{k}$ records. We summarize the results of operations on that input here:

Independent tests with the GROK toolkit



Ovaska, et al. released a similar genomic analysis toolkit called the Genomic Region Operation Kit (GROK). Their paper (Ovaska, et al., 2013) includes a comparison of the execution time and memory use of GROK with equivalent set operations in BEDTools and BEDOPS.

Operational input was a 14 MB BED file containing annotations of human gene and exon coordinates, totaling $\sim 423 \mathrm{k}$ records. Five operations are applied to this input with each toolkit.

As Ovaska, et al. show here, the BEDOPS toolkit provides substantially reduced execution time and memory consumption (even with recent improvements to two BEDTools operations). With the sole exception of the sort operation, all other operations consumed only 1 MB with bedops. As shown in this study, sorting with BEDOPS also outperformed analogous operations in other tool suites in terms of memory and running time.

Remember that with BEDOPS, sorting is, at most, a one-time cost to operate on data any number of times in the most efficient way. Since the programs in BEDOPS produce sorted outputs, you never need to sort results before using them in downstream analyses.

### 2.5.5 Worst-case memory performance

Non-sorting utilities operate efficiently with large inputs by keeping memory overhead low. The worst-case design scenario, however, causes the bedops or bedmap programs to load all data from a single chromosome from a single input file into memory. For bedops, the worst-case scenario applies only to the --element-of and --not-element-of options.
Fortunately, worst-case situations are conceptually easy to understand, and their underlying questions often require no windowing logic to answer, so simpler approaches can sometimes be used. Conceptually, any summary analysis over an entire chromosome triggers the worst-case scenario. For example, to determine the number of sequencing tags mapped to a given chromosome, bedmap loads all tag data for that one chromosome into memory, whereas a one-line awk statement can provide the answer with minimal memory overhead.

We note that the worst case memory performance of non-sorting BEDOPS utilities still improves upon the best case performance of current alternatives.

### 2.6 Reference

### 2.6.1 Set operations

## bedops

bedops is a core tool for finding relationships between two or more genomic datasets.
This is an important category of problems to solve. As examples, one might want to:

- Know how much overlap exists between the elements of two datasets, to quantitatively establish the degree to which they are similar.
- Merge or filter elements. For example, retrieving non-overlapping, "unique" elements from multiple BED files.
- Split elements from multiple BED files into disjoint subsets.

The bedops program offers several Boolean set and multiset operations, including union, subset, and difference, to assist investigators with answering these types of questions.

Importantly, bedops handles any number of any-size inputs at once when computing results in order to maximize efficiency. This use case has serious practical consequences for many genomic studies.

One can also use bedops to symmetrically or asymmetrically pad coordinates.

## Inputs and outputs

## Input

The bedops program reads sorted BED data and BEDOPS Starch-formatted archives as input.
Finally, bedops requires specification of a set operation (and, optionally, may include modifier options).
Support for common headers (including UCSC track headers) is offered through the --header option. Headers are stripped from output.

## Output

The bedops program returns sorted BED results to standard output. This output can be redirected to a file or piped to other utilities.

## Usage

The bedops program takes sorted BED-formatted data as input, either from a file or streamed from standard input. It will process any number of input files in parallel.
If your data are unsorted, use BEDOPS sort-bed to prepare data for bedops. You only need to sort once, as all BEDOPS tools read and write sorted BED data.

Because memory usage is very low, one can use sorted inputs of any size. Processing times generally follow a simple linear relationship with input sizes (e.g., as the input size doubles, the processing time doubles accordingly).

The --help option describes the set operation and other options available to the end user:

```
bedops
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Shane Neph & Scott Kuehn
            USAGE: bedops [process-flags] <operation> <File(s)>*
                Every input file must be sorted per the sort-bed utility.
                Each operation requires a minimum number of files as shown below.
                        There is no fixed maximum number of files that may be used.
                            Input files must have at least the first 3 columns of the BED specification.
                The program accepts BED and Starch file formats.
                May use '-' for a file to indicate reading from standard input (BED format_
\hookrightarrowonly).
            Process Flags:
                        --chrom <chromosome> Process data for given <chromosome> only.
            --ec Error check input files (slower).
            --header Accept headers (VCF, GFF, SAM, BED, WIG) in any input,
\hookrightarrowfile.
            --help Print this message and exit successfully.
            --help-<operation> Detailed help on <operation>.
                                An example is --help-c or --help-complement
            --range L:R Add 'L' bp to all start coordinates and 'R' bp to end
                coordinates. Either value may be + or - to grow or
                        shrink regions. With the -e/-n operations, the first
                                (reference) file is not padded, unlike all other_
\hookrightarrowfiles.
            --range S Pad or shink input file(s) coordinates symmetrically_
Gy S .
            --version Print program information.
    Operations: (choose one of)
            -c, --complement [-L] File1 [File]*
            -d, --difference ReferenceFile File2 [File]*
            -e, --element-of [number% | number] ReferenceFile File2 [File]*
                by default, -e 100% is used. 'bedops -e l' is also popular.
            -i, --intersect File1 File2 [File]*
            -m, --merge File1 [File]*
            -n, --not-element-of [number% | number] ReferenceFile File2 [File]*
                by default, -n 100% is used. 'bedops -n l' is also popular.
            -p, --partition Filel [File]*
            -s, --symmdiff File1 File2 [File]*
            -u, --everything File1 [File]*
            -w, --chop [bp] [--stagger [bp]] [-x] File1 [File]*
                    by default, -w 1 is used with no staggering.
    Example: bedops --range 10 -u filel.bed
    NOTE: Only operations -e|n|u preserve all columns (no flattening)
```

Note: Extended help is available for all operations in bedops. For example, the --help-symmdiff option in bedops gives detailed information on the --symmdiff operation.

## Operations

To demonstrate the various operations in bedops, we start with two simple datasets A and B, containing genomic elements on generic chromsome chrN:


These datasets can be sorted BED or Starch-formatted files or streams.

Note: The bedops tool can operate on two or more multiple inputs, but we show here the results of operations acting on just two or three sets, in order to help demonstrate the basic principles of applying set operations.

## Everything (-u, -everything)

The --everything option is equivalent to concatenating and sorting BED elements from multiple files, but works much faster:


As with all BEDOPS tools and operations, the output of this operation is sorted.

Note: The --everything option preserves all columns from all inputs. This is useful for multiset unions of datasets with additional ID, score or other metadata.

## Example

To demonstrate the use of --everything in performing a multiset union, we show three sorted sets First.bed, Second.bed and Third.bed and the result of their union with bedops:

```
$ more First.bed
chr1 100 200
chr2 150 300
chr2 200 250
chr3 100 150
```

```
$ more Second.bed
chr2 50 150
chr2 400 600
```

```
$ more Third.bed
chr3 150 350
```

\$ bedops --everything First.bed Second.bed Third.bed > Result.bed

| \$ more | Result.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr2 | 50 | 150 |
| chr2 | 150 | 300 |
| chr2 | 200 | 250 |
| chr2 | 400 | 600 |
| chr3 | 100 | 150 |
| chr3 | 150 | 350 |

This example uses three input sets, but you can specify two, four or even more sets with --everything to take their union.

## Element-of (-e, -element-of)

The --element-of operation shows the elements of the first ("reference") file that overlap elements in the second and subsequent "query" files by the specified length (in bases) or by percentage of length.

In the following example, we search for elements in the reference set $A$ which overlap elements in query set $B$ by at least one base:


Elements that are returned are always from the reference set (in this case, set A).

Note: The --element-of option preserves all columns from the first (reference) input.

## Example

The argument to --element-of is a value that species to degree of overlap for elements. The value is either integral for per-base overlap, or fractional for overlap measured by length.

Here is a demonstration of the use of --element-of 1 on two sorted sets First.bed and Second.bed, which looks for elements in the First set that overlap elements in the Second set by one or more bases:

| \$ more | First.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
$ bedops --element-of 1 First.bed Second.bed > Result.bed
```

| $\$$ more | Result.bed |  |
| :--- | :---: | :---: |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 400 | 475 |

One base is the least stringent (default) integral criterion. We can be more restrictive about our overlap requirement by increasing this value, say to 15 bases:

```
$ bedops --element-of 15 First.bed Second.bed > Result.bed
```

```
$ more Result.bed
chr1 100 200
```

Only this element from the First set overlaps one or more elements in the Second set by a total of fifteen or more bases.

We can also use percentage of overlap as our argument. Let's say that we only want elements from the First set, which overlap half their length or more of a qualifying element in the Second set:

```
$ bedops --element-of 50% First.bed Second.bed > Result.bed
```

```
$ more Result.bed
chr1 150 160
```

Note that -element-of is not a symmetric operation, as demonstrated by reversing the order of the reference and query set:


## Example

As we show here, by inverting the usual order of our sample sets First and Second, we retrieve elements from the Second set:

```
$ bedops --element-of 1 Second.bed First.bed > Result.bed
```

```
$ more Result.bed
chr1 120 125
chr1 150 155
chr1 150 160
chr1 460 470
```

While this operation is not symmetric with respect to ordering of input sets, --element-of (-e) does produce exactly everything that -- not $-e l e m e n t-o f(-n)$ does not, given the same overlap criterion and ordered input sets.

Note: We show usage examples with two files, but --element-of supports three or more input sets. For a more in-depth discussion of --element-of and how overlaps are determined with three or more input files, please review the BEDOPS forum discussion on this subject.

## Not-element-of (-n, -not-element-of)

The --not-element-of operation shows elements in the reference file which do not overlap elements in all other sets. For example:

## Inputs



## Example

We again use sorted sets First.bed and Second.bed to demonstrate --not-element-of, in order to look for elements in the First set that do not overlap elements in the Second set by one or more bases:

```
$ more First.bed
chr1 100 200
chr1 150 160
chr1 200 300
chr1 400 475
chr1 500 550
```

```
$ more Second.bed
chr1 120 125
chr1 150 155
```

|  |  |  | (continued from previous page) |
| :---: | :---: | :---: | :---: |
| chr1 | 150 | 160 |  |
| chrl | 460 | 470 |  |
| chrl | 490 | 500 |  |
| \$ bed | --not-element-of 1 First.bed Second.bed > Result.bed |  |  |
| \$ more Result.bed |  |  |  |
| chr1 | 200 | 300 |  |
| chr1 | 500 | 550 |  |

As with the --element-of $(-e)$ operator, the overlap criterion for $--n o t-e l e m e n t-o f(-n)$ can be specified either by length in bases, or by percentage of length.

Similarly, this operation is not symmetric - the order of inputs will specify the reference set, and thus the elements in the result (if any).

Note: The --not-element-of operatior preserves columns from the first (reference) dataset.

Note: The same caveat applies to use of --not-element-of $(-n)$ as with --element-of (-e), namely that the second and all subsequent input files are merged before the set operation is applied. Please review the BEDOPS forum discussion thread on this topic for more details.

## Complement (-c, -complement)

The --complement operation calculates the genomic regions in the gaps between the contiguous per-chromosome ranges defined by one or more inputs. The following example shows the use of two inputs:


Note this computed result will lack ID, score and other columnar data other than the first three columns that contain positional data. That is, computed elements will not come from any of the input sets, but are new elements created from the input set space.

## Example

To demonstrate --complement, we again use sorted sets First.bed and Second.bed, in order to compute the "gaps" between their inputs:

| \$ more | First.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| $\$$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
$ bedops --complement First.bed Second.bed > Result.bed
```

```
$ more Result.bed
chr1 300 400
chr1 475 490
```

As we see here, for a given chromosome, gaps are computed between the leftmost and rightmost edges of elements in the union of elements across all input sets.

Note: For a more in-depth discussion on using --complement with left and right bounds of input chromosomes, please review the BEDOPS forum discussion on this subject.

## Difference (-d, -difference)

The --difference operation calculates the genomic regions found within the first (reference) input file, excluding regions in all other input files:


## Example

To demonstrate --difference, we use sorted sets First.bed and Second.bed and compute the genomic space in First that excludes (or "subtracts") ranges from Second:

```
$ more First.bed
chr1 100 200
```

| chrr | 150 | 160 |
| :--- | :--- | :--- |
| chr1 | 200 | 300 |
| chr | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
bedops --difference First.bed Second.bed > Result.bed
```

```
$ more Result.bed
chr1 100 120
chr1 125 150
chr1 160 300
chr1 400 460
chr1 470 475
chr1 500 550
```

Note: As with --element-of and --not-element-of, this operation is not symmetric. While --not-element-of preserves all columns of elements found in the reference input and allows one to define overlaps, the --difference operator simply reports every genomic range as three-column BED, which does not overlap elements found in the second and subsequent input files by any amount.

## Symmetric difference (-s, -symmdiff)

The --symmdiff operation calculates the genomic range that is exclusive to each input, excluding any ranges shared across inputs:


## Example

To demonstrate --symmdiff, we use sorted sets First.bed and Second.bed and compute the genomic space that is unique to First and Second:

| $\$$ more | First.bed |  |
| :--- | :---: | :---: |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

\$ bedops --symmdiff First.bed Second.bed > Result.bed

| \$ more | Result.bed |  |
| :--- | :---: | :---: |
| chr1 | 100 | 120 |
| chr1 | 125 | 150 |
| chr1 | 160 | 300 |
| chr1 | 400 | 460 |
| chr1 | 470 | 475 |
| chr1 | 490 | 550 |

Tip: It has been observed that $--s y m m d i f f(-s)$ is the same as the union of --difference A B with --difference B A, but--symmdiff runs faster in practice.

Intersect (-i, -intersect)

The --intersect operation determines genomic regions common to all input sets:


## Example

To demonstrate --intersect, we use sorted sets First.bed and Second.bed and compute the genomic space that is common to both First and Second:

| $\$$ more | First.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
$ bedops --intersect First.bed Second.bed > Result.bed
```

| \$ more | Result.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |

Notice how this computed result is quite different from that of --element-of N , which functions more like a LEFT JOIN operation in SQL.

## Merge (-m, -merge)

The --merge operation flattens all disjoint, overlapping, and adjoining element regions into contiguous, disjoint regions:


## Example

To demonstrate --merge, we use sorted sets First.bed and Second.bed and compute the contiguous genomic space across both First and Second:

| \$ more | First.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :---: |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
$ bedops --merge First.bed Second.bed > Result.bed
```

```
$ more Result.bed
chr1 100 300
chr1 400 475
chr1 490 550
```

Tip: The preceding example shows use of -- merge $(-m)$ with two inputs, but the merge operation works just as well with one input, collapsing elements within the file that overlap or which are directly adjoining.

## Partition (-p, -partition)

The --partition operator splits all overlapping input regions into a set of disjoint segments. One or more input files may be provided; this option will segment regions from all inputs:


## Example

To demonstrate --partition, we use sorted sets First.bed and Second.bed and compute disjointed genomic regions across both First and Second:

| $\$$ more | First.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 200 |
| chr1 | 150 | 160 |
| chr1 | 200 | 300 |
| chr1 | 400 | 475 |
| chr1 | 500 | 550 |


| \$ more | Second.bed |  |
| :--- | :---: | :--- |
| chr1 | 120 | 125 |
| chr1 | 150 | 155 |
| chr1 | 150 | 160 |
| chr1 | 460 | 470 |
| chr1 | 490 | 500 |

```
$ bedops --partition First.bed Second.bed > Result.bed
```

| S more | Result.bed |  |
| :--- | :---: | :--- |
| chr1 | 100 | 120 |
| chr1 | 120 | 125 |
| chr1 | 125 | 150 |
| chr1 | 150 | 155 |
| chr1 | 155 | 160 |
| chr1 | 160 | 200 |
| chr1 | 200 | 300 |
| chr1 | 400 | 460 |
| chr1 | 460 | 470 |
| chr1 | 470 | 475 |
| chr1 | 490 | 500 |
| chr1 | 500 | 550 |

Notice that the result set of partitioned elements excludes any duplicates from input regions, thus enforcing the disjoint nature of the computed result.

Note: As with --merge, --complement and other "computing" operations, note the lack of ID, score and other columnar data in this computed result.

## Chop (-w, -chop)

The --chop operator merges all overlapping input regions and "chops" them up into a set of disjoint segments of identical length (with a default of one base). One or more input files may be provided; this option will segment regions from all inputs:


## Example

To demonstrate --chop, we use a sorted set called Regions.bed and compute a set of one-base genomic regions constructed from the merged input elements:

```
$ more Regions.bed
chr1 100 105
chr1 120 127
chr1 122 124
```

```
bedops --chop 1 Regions.bed > Result.bed
```

| \$ more | Result.bed |  |
| :--- | :---: | :---: |
| chr1 | 100 | 101 |
| chr1 | 101 | 102 |
| chr1 | 102 | 103 |
| chr1 | 103 | 104 |
| chr1 | 104 | 105 |
| chr1 | 120 | 121 |
| chr1 | 121 | 122 |
| chr1 | 122 | 123 |
| chr1 | 123 | 124 |
| chr1 | 124 | 125 |
| chr1 | 125 | 126 |
| chr1 | 126 | 127 |

Note: Overlapping and nested regions are merged into contiguous ranges before chopping. The end result contains unique, non-overlapping elements.

## Stagger (-stagger)

The --stagger operator works in conjunction with -chop. While --chop sets the size of each cut, the --stagger operator moves the start position of each cut by the specified number of bases, across each merged interval.


## Example

To demonstrate --stagger, we use a sorted set called Regions.bed and compute a set of one-base genomic regions constructed from the merged input elements, but move the start position across the merged regions by three
bases, before generating the next chop:

```
$ more Regions.bed
chr1 100 105
chr1 120 127
chr1 122 124
```

```
$ bedops --chop 1 --stagger 3 Regions.bed > Result.bed
```

```
$ more Result.bed
chr1 100 101
chr1 103 104
chr1 120 121
chr1 123 124
chr1 126 127
```

Note: Overlapping and nested regions are merged into contiguous ranges before chopping and staggering. The end result contains unique, non-overlapping elements.

## Exclude (-x)

Like -- stagger, $-x$ is a sub-option of the -chop operator, and it may be used with or without $--s t a g g e r$. This option will remove any remainder genomic chunk that is smaller than that specified with -- chop. For example, if you start with a 10 nt region and use - chop 4, the final segment would be 2 nt in length if -x is not specified. With $-x$, that last segment does not go to output. With $-x$, the chop operation produces output regions that are all the same size.

## Per-chromosome operations (-chrom)

All operations on inputs can be restricted to one chromosome, by adding the -- chrom <val> operator.

Note: This operator is highly useful for parallelization, where operations on large BED inputs can be split up by chromosome and pushed to separate nodes on a computational cluster. See the Efficiently creating Starch-formatted archives with a cluster documentation for a demonstration of this technique in action.

## Example

To demonstrate the use of -- chrom to restrict operations to a chromosome (such as chr3), we perform a perchromosome union of elements from three sorted sets First.bed, Second.bed and Third.bed, each with elements from multiple chromosomes:

| \$ more First.bed |  |  |
| :---: | :---: | :---: |
| chr1 | 100 | 200 |
| chr2 | 150 | 300 |
| chr2 | 200 | 250 |
| chr3 | 100 | 150 |

```
$ more Second.bed
chr2 50 150
chr2 400 600
```

```
$ more Third.bed
chr3 150 350
```

```
$ bedops --chrom chr3 --everything First.bed Second.bed Third.bed > Result.bed
```

```
$ more Result.bed
chr3 100 150
chr3 150 350
```


## Range (-range)

The --range operation works in conjunction with other operations.
When used with one value (--range S), this operation symmetrically pads all elements of input sets by the specified integral value $S$. When the specified value is positive, every genomic segment grows in size. An element will grow asymmetrically to prevent growth beyond base position 0 , if needed. Otherwise, when negative, elements shrink, and any element with zero (or less) length is discarded.

Alternatively, when used with two values (--range $L: R$ ), this operation asymmetrically pads elements, adding $L$ to each start coordinate, and adding $R$ to each stop coordinate. Negative values may be specified to grow or shrink the region, accordingly.

This option is immediately useful for adjusting the coordinate index of BED files. For example, to shift from 1-based to 0 -based coordinate indexing:

```
$ bedops --range -1:-1 --everything my1BasedCoordinates.bed > my0BasedCoordinates.bed
```

And, likewise, for 0-based to 1-based indexing:

```
$ bedops --range 1:1 --everything my0BasedCoordinates.bed > my1BasedCoordinates.bed
```

Note: The --range value is applied to inputs prior to the application of other operations (such as --intersect or --merge, etc.).

Padding elements with bedops is much more efficient that doing so with awk or some other script, and you do not need to go back and resort your data. Even symmetric padding can cause data to become unsorted in non-obvious ways. Using --range ensures that your data remain sorted and it works efficiently with any set operation.
Also, note that the --element-of and --not-element-of operations behave differently with --range, in that only the second and subsequent input files are padded.

## Starch support

The bedops application supports use of Starch-formatted archives as inputs, as well as text-based BED data. One or multiple inputs may be Starch archives.

Tip: By combining the --chrom operator with operations on Starch archives, the end user can achieve improved computing performance and disk space savings, particularly where bedops, bedmap and closest-features operations are applied with a computational cluster on separate chromosomes.

## Error checking (-ec)

Use the --ec option in conjunction with any aforementioned operation to do more stringent checking of the inputs' compliance to bedops requirements, including sorting checks, delimiter checks, among others.

To demonstrate, we can deliberately introduce a typo in dataset $A$, using the --ec option to try to catch it:

```
$ bedops --ec --everything BEDFileA
May use bedops --help for more help.
Error: in BEDFileA
First column should not have spaces. Consider 'chrl' vs. 'chrl '. These are\smile
cdifferent names.
See row: 3
```

The typo introduced was the addition of a space within the third line of dataset A.


#### Abstract

Note: Use of the --ec option will roughly double the running times of set operations, but it provides stringent error checking to ensure inputs and outputs are valid. --ec can help check problematic input and offers helpful hints for any needed corrections, when problems are detected.


## Tips

## Chaining operations

You can efficiently chain operations together, e.g.:
\$ bedops --range 50 --merge A $\mid$ bedops --intersect - B > answer.bed
In this example, elements from $A$ are padded 50 bases up- and downstream and merged, before intersecting with coordinates in B.

## Sorting inputs

For unsorted input, be sure to first use sort-bed to presort the data stream before using with bedops. Unsorted input will not work properly with BEDOPS tools.

Tip: If you will use an initially-unsorted file more than once, save the results of sorting. You only need to sort once! BEDOPS tools take in and export sorted data.

## bedextract

The bedextract utility performs three primary tasks, with the goal of doing them very quickly:

1. Lists all the chromosomes in a sorted input BED file.
2. Extracts all the elements in a sorted input BED file, for a given chromosome.
3. Finds elements of one BED file, which overlap elements in a second, reference BED file (when specific element criteria are satisfied).
One might ask why use this utility, when the first two tasks can already be performed with common UNIX text processing tools, such as cut, sort, uniq, and awk, and the third task can be performed with bedops with the --element-of 1 options?

The bedextract utility does the work of all those tools without streaming through an entire BED file, resulting in massive performance improvements. By using the hints provided by sorted BED input, the bedextract tool can jump around, seeking very quick answers to these questions about your data.

## How it works

Specifically, sorting with sort-bed allows us to perform a binary search:

1. We jump to the middle byte of the BED file, stream to the nearest element, then parse and test the chromosome name.
2. Either we have a match, or we jump to the middle of the remaining left or right half (decided by dictionary order), parse and test again.
3. We repeat steps 1 and 2 until we have matches that define the bounds of the target chromosome.


To indicate the kind of speed gain that the bedextract tool provides, in local testing, a naïve listing of chromosomes from a 36 GB BED input using UNIX cut and uniq utilities took approximately 20 minutes to complete on a typical Core 2 Duo-based Linux workstation. Retrieval of the same chromosome listing with bedextract --list-chr took only 2 seconds (cache flushed-no cheating!).

Tip: While listing chromosomes is perhaps a trivial task, 1200 seconds to 2 seconds is a $\mathbf{6 0 0}$-fold speedup. Similar improvements are gained from using --chrom and --faster options with other core BEDOPS tools like bedops and bedmap. If your data meet the criteria for using this approach-and a lot of genomic datasets do-we strongly encourage adding this to your toolkit.

## Inputs and outputs

## Input

Depending on specified options, bedextract requires one or two sorted BED files.

Note: It is critical that inputs are sorted as the information in a sorted file allows bedextract to do its work correctly. If your datasets are output from other BEDOPS tools, then they are already sorted!

## Output

Depending on specified options, the bedextract program will send a list of chromosomes or BED elements to standard output.

Tip: The use of UNIX-like standard streams allows easy downstream analysis or post-processing with other tools and scripts, including other BEDOPS utilities.

## Usage

The --help option describes the functionality available to the end user:

```
bedextract
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Shane Neph & Alex Reynolds
        Every input file must be sorted per sort-bed.
    USAGE:
        0) --help or --version Print requested info and exit successfully.
        1) --list-chr <input.bed> Print all unique chromosome names found in <input.
ubed>
        2) <chromosome> <input.bed> Retrieve all rows for chr8 with: bedextract chr8
<<input.bed>.
        3) <query.bed> <target> Grab elements from the <query.bed> that overlap
\hookrightarrowelements in <target>. Same as
\hookrightarrowthis option fails silently
\hookrightarrowelements. If no fully-nested
\hookrightarrowupon the performance of bedops.
\hookrightarrowithout fully-nested elements).
\hookrightarrowformat) comes from stdin.
```


## Listing chromosomes

Use the --list-chr option to quickly retrieve a listing of chromosomes from a given sorted BED input.
For example, the following lists the chromosomes in an example BED file of FIMO motif hits (see the Downloads section):

```
$ bedextract --list-chr motifs.bed
chr1
chr10
chr11
chr12
...
chr9
chrX
```

Note: The bedextract --list-chr operation only works on BED files. If you have a Starch file, use unstarch --list-chr to list its chromosomes.

## Retrieving elements from a specific chromosome

To quickly retrieve the subset of elements from a sorted BED file associated with a given chromosome, apply the second usage case and specify the chromosome as the argument.

For example, to retrieve chrx from the same motif sample:

```
$ bedextract chrX motifs.bed
chrX 6775077 6775092 +V_SPZ1_01 4.92705e-06 + + GTTGGAGGGAAGGGC
chrX 6775168 6775179 +V_ELF5_01 8.57585e-06 + TCAAGGAAGTA
chrX 67777906777799 +V_CKROX_Q2 8.90515e-06 + TCCCTCCCC
...
```

Note: The bedextract <chromosome> operation only works on BED files. If you have a Starch file, use unstarch <chromosome> to list the elements associated with that chromosome.

## Retrieving elements which overlap target elements

A common bedops query involves asking which elements overlap one or more bases between two BED datasets, which we will call here Query and Target.

One can already use bedops --element-of 1 to accomplish this task, but if certain specific criteria are met (which we will describe shortly) then a much faster result can often be obtained by instead using bedextract.

Three criteria make the use of bedextract in this mode very successful in practice, with potentially massive speed improvements:

1. Query is a huge file.
2. There are relatively few regions of interest in Target (say, roughly 30,000 or fewer).
3. There are no fully-nested elements in Query (but duplicate coordinates are fine).

Note: With some extra work, it is possible to use this mode of bedextract with a huge Query BED file that includes fully-nested elements. The technique requires that you create a merged version of Query and keep that result, Query-Index, around along with Query.

```
$ bedops -m Query > Query-Index
$ bedextract Query-Index Target \
    | bedextract Query - \
    | bedops --element-of 1 - Target \
    > answer.bed
```

Note: You may change the final overlap criterion to the bedops -element-of as you see fit for your final answer. Adding -range 10, for example, to bedops -m Query > Query-Index can often greatly reduce the size of Query-Index without much of an increase in the running time of this approach.

## What are nested elements?

For a precise definition of a nested element, refer to the documentation on nested elements.
For an example, we show the following sorted BED file:

| $\operatorname{chr} 1$ | 1 | 100 |
| :--- | :--- | :--- |
| $\operatorname{chr} 1$ | 100 | 200 |
| $\operatorname{chr} 1$ | 125 | 150 |
| chr1 | 150 | 1000 |

In this sorted dataset, the element chr1:125-150 is entirely nested within chr1:100-200:


Note: Fully-nested elements are not a problem for the other two bedextract features: 1) Listing all chromosomes, and 2) Retrieving all information for a single chromosome.

Fully-nested elements are only an issue for bedextract if they exist in the Query dataset. Results are not affected if the Target dataset contains nested elements. Overlapping (but not fully-nested) elements in the Query input file are fine, as are duplicated genomic positions.

Note: Our lab works with BED data of various types: cut-counts, hotspots, peaks, footprints, etc. These data generally
do not contain nested elements and so are amenable to use with bedextract for extracting overlapping elements.
However, other types of Query datasets can be problematic. FIMO search results, for example, might cause trouble, where the boundaries of one motif hit can be contained within another larger hit. Or paired-end sequence data, where tags are not of a fixed length. Be sure to consider the makeup of your BED data before using bedextract.

## Demonstration

To demonstrate this use of bedextract, for our Query dataset we will use the Map example from our bedmap documentation, which contains raw DNaseI hypersensitivity signal from a human K562 cell line (see the Downloads section for sample data):

| $\$$ cat | query.bed |  |  |  |
| :--- | ---: | ---: | :--- | :--- |
| chr21 | 33031165 | 33031185 | map-1 | 1.000000 |
| chr21 | 33031185 | 33031205 | map-2 | 3.000000 |
| chr21 | 33031205 | 33031225 | $\operatorname{map}-3$ | 3.000000 |
| chr21 | 33031225 | 33031245 | map-4 | 3.000000 |
| $\cdots$ |  |  |  |  |
| chr21 | 33032445 | 33032465 | map-65 | 5.000000 |
| chr21 | 33032465 | 33032485 | $\operatorname{map}-66$ | 6.000000 |

Our Target data is simply an ad-hoc BED region which overlaps part of the Query dataset, stored in a Starchformatted archive:

```
$ unstarch target.starch
chr21 33031600 33031700
```

We can now ask which elements of Query overlap the element in Target:

```
$ bedextract query.bed target.starch
chr21 33031585 33031605 map-22 26.000000
chr21 33031605 map-23 27.000000
chr21 33031625 33031645 map-24 29.000000
chr21 33031645 33031665 map-25 31.000000
chr21 33031665 33031685 map-26 31.000000
chr21 33031685 33031705 map-27 37.000000
```

Our Target dataset is a Starch-formatted file. Note that we can also use "-" to denote standard input for the Target dataset, as well as a regular BED- or Starch-formatted file. In other words, we can pipe target elements from another process to bedextract, e.g. we can query for an ad-hoc element as follows:

```
$ echo -e "chr21\t33031590\t33031600" | bedextract query.bed -
chr21 33031585 33031605 map-22 26.000000
```

Instead of an $a d$-hoc element as in this example, however, target elements could just as easily be piped in from upstream bedmap or bedops operations, or extracted elements from a Starch archive, etc.

Tip: The output of this particular use of bedextract is made up of elements from the Query dataset and is therefore sorted BED data, which can be piped to bedops, bedmap and other BEDOPS utilities for further downstream processing.

Note: Though bedextract only supports the overlap equivalent of bedops --element-of 1, other overlap criteria are efficiently supported by combining bedextract with bedops.

Specifically, we can quickly filter through just the results given by bedextract and implement other overlap criteria with bedops, e.g.:

```
$ bedextract query.bed target.bed | bedops -e 50% - target.bed
```


## Downloads

- Sample FIMO motifs
- Sample Query dataset: DHS signal
- Sample Target dataset: ad-hoc coordinates


## closest-features

The closest-features program efficiently associates nearest features between two sorted inputs, based upon genomic distance measures.
An application of this tool in our own research is finding the nearest DNase hypersensitive sites upstream and downstream from a given SNP, as well as signed distances. The closest-features program can report both results.
As another example of what one can do with this utility, we can identify the closest transcriptional start site for a given putative replication origin. Suppose we have a sorted BED file named TSS . bed that contains all transcriptional start sites of all genes in some genome. Further, suppose that we have a set of measurements showing probable replication origins for the same species in a sorted BED file named RepOrigins.bed. The following command gives the closest TSS to each origin:

```
$ closest-features --closest RepOrigins.bed TSS.bed
```

By default, the program will echo each entry from RepOrigins.bed, followed by the two closest elements in TSS.bed (the closest element to each side of the entry from RepOrigins.bed), with output columns separated by a pipe ( $\mid$ ). With the --shortest option, the echoed entry from RepOrigins.bed and only the single nearest element in TSS.bed will be part of the output.

## Inputs and outputs

## Input

The closest-features program takes two sorted BED files (a so-called reference file and a map file), as well as optional arguments for modifying behavior and outputs.

Alternatively, closest-features can accept Starch-formatted archives as inputs, with no need to extract archive data to intermediate BED files!

Support for common headers (such as UCSC track headers) is offered through the --header option. Headers are stripped from output.

## Output

The closest-features program returns summary data to standard output, which may include reference and nearest elements and distance values (depending on provided options).

## Usage

The --help option describes the various operations and options available to the end user:

```
closest-features
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Shane Neph & Scott Kuehn
USAGE: closest-features [Process-Flags] <input-file> <query-file>
    All input files must be sorted per sort-bed.
    The program accepts BED and Starch file formats
    May use '-' for a file to indicate reading from standard input (BED format only).
    For every element in <input-file>, determine the two elements from <query-file>>
\hookrightarrowfalling
            nearest to its left and right edges (See NOTES below). By default, echo the
\hookrightarrow<input-file>
            element, followed by those left and right elements found in <query-file>.
    Process Flags:
    --chrom <chromosome> : Process data for given <chromosome> only.
    --closest : Choose the closest element for output only. Ties go thes
\hookrightarrowleft element.
    --delim <delim> : Change output delimiter from '|' to <delim> between
\hookrightarrowcolumns (e.g. '\t')
    --dist : Print the signed distances to the <input-file> elements
\hookrightarrowas additional
@distance of 0.
    --ec : Error check all input files (slower).
    --header : Accept headers (VCF, GFF, SAM, BED, WIG) in any input,
\hookrightarrowfile.
    --help : Print this message and exit successfully.
    --no-overlaps : Overlapping elements from <query-file> will not be_
\hookrightarroweported.
    --no-ref : Do not echo elements from <input-file>.
    --version : Print program information.
    NOTES:
        If an element from <query-file> overlaps the <input-file> element, its distance
\hookrightarrowis zero.
            An overlapping element takes precedence over all non-overlapping elements.
\hookrightarrowThis is true
            even when the overlapping element's edge-to-edge distance to the <input-file>'s,
\hookrightarrowelement
            is greater than the edge-to-edge distance from a non-overlapping element.
            Overlapping elements may be ignored completely (no precedence) with --no-overlaps.
            Elements reported as closest to the left and right edges are never the same.
            When no qualifying element from <query-file> exists as a closest feature, 'NA' is,
\hookrightarrowreported.
```


## Per-chromosome operations (-chrom)

All operations on inputs can be restricted to one chromosome, by adding the --chrom <val> operator.

Tip: This option is highly useful for cluster-based work, where operations on large BED inputs can be split up by chromosome and pushed to separate cluster nodes.

To demonstrate the use of this option, we take two sample Starch-archived BED datasets A and B (refer to the Downloads section for sample inputs) which contain regions from multiple chromosomes:

| \$ unstarch A.starch |  |  |  |
| :--- | :---: | ---: | :--- |
| chr1 | 100 | 200 | id-001A |
| chr1 | 400 | 500 | id-002A |
| chr2 | 100 | 300 | id-003A |
|  |  |  |  |
| \$unstarch | B.starch |  |  |
| chr1 | 150 | 300 | id-001B |
| chr1 | 500 | 600 | id-002B |
| chr2 | 100 | 150 | id-003B |
| chr2 | 180 | 500 | id-004B |

Now we want to ask, what is the closest element from chr2 in A, to chr2 elements in B:

```
$ closest-features --chrom chr2 --closest A.starch B.starch
chr2 100 id-003A|chr2 100 150 id-003B
```

As we expect, element id-003A is closest to element id-003B between the two datasets.

## Error checking

For performance reasons, no error checking of input is done, by default. Add --ec for stringent error checking and debugging purposes.

Note: Using --ec will slow down analysis considerably. We recommend using this option to test and debug pipelines and then removing it for use in production.

## Downloads

- Sample dataset A
- Sample dataset B


## Nested elements

This page describes nested BED elements, their impact on the performance of BEDOPS tools, and how we can identify them beforehand.

## Definition

A nested element is defined as a BED element from a sorted BED file, where a genomic range is entirely enclosed by the previous element's range.

Loosely speaking, consider the following five overlap cases for pairings of generic, half-open intervals:


Of these five interval pairs, the fifth overlap pairing identifies a nested element, where the second interval is nested within the first.

More rigorously, we define two generic, half-open BED elements $A$ and $B$, both located on the same generic chromosome $N$, each with ranges $\left[a_{\text {start }}, a_{\text {stop }}\right)$ and $\left[b_{\text {start }}, b_{\text {stop }}\right)$, respectively.

These two elements $A$ and $B$ have the following relations:

1. $a_{\text {start }}<a_{\text {stop }}$
2. $b_{\text {start }}<b_{\text {stop }}$
3. $a_{\text {start }}<=b_{\text {start }}$
4. $a_{\text {stop }}<=b_{\text {stop }}$

Note: The third and fourth conditions place elements $A$ and $B$ into sort order, as applied by the sort-bed application.

If we further restrict these ranges: $a_{\text {start }}<b_{\text {start }}$ and $b_{\text {stop }}<a_{\text {stop }}$, then for the purposes of BEDOPS we call the element $B$ a nested element, one which is contained or nested within element $A$.

It can be useful to be able to identify nested elements in an input set. Here's a method that uses awk:

```
#!/usr/bin/env awk -f
{
    if (NR > 1) {
    currentChr = $1
    currentStart = $2
    currentStop = $3
    if ((previousStart < currentStart) && (previousStop > currentStop)) {
        print $0;
    }
    else {
```

```
        previousChr = currentChr
        previousStart = currentStart
        previousStop = currentStop
        }
    }
    else {
        previousChr = $1
        previousStart = $2
        previousStop = $3
    }
}
```

If this script is given the name getNestedElements. awk and is made executable, one could filter a BED file via
./getNestedElements.awk foo.bed > nested.bed, or similar.

## Example

A more concrete example of a sorted BED file which contains a nested element follows. Consider the following simple, sorted BED dataset:

| chr1 | 1 | 100 |
| :--- | :--- | :--- |
| chr1 | 100 | 200 |
| chr1 | 125 | 150 |
| chr1 | 150 | 1000 |

Here, the element chr1:125-150 is entirely nested within chr1:100-200:


## Why nested elements matter

BEDOPS bedmap and bedextract tools offer the --faster option to perform very fast retrieval of overlapping elements, so long as input datasets do not contain nested elements, as defined above.

To extract maximum performance out of the use of the BEDOPS toolkit, therefore, it is very useful to know if the input datasets contain such elements - if they do not, then we can apply this optimization.

Common datasets we work with do not contain nested elements: reads, peaks, footprints, and others. However, other datasets do, such as motif hits or paired-end sequencing reads.

How can we find out if our inputs have nested elements, before we start applying any operations?
The compression tool starch (v2.5 and greater) will look for these elements in an input BED file and store this condition as a flag in the output archive's metadata. This value can be retrieved in constant time with unstarch and other tools which make use of the Starch C++ API.

Additionally, the --ec (error-correction) option in bedmap will also report if inputs contain nested elements. This option doubles execution time, but when used in conjunction with the --faster option, the speed gains are more than recovered.

### 2.6.2 Statistics

## bedmap

The bedmap program is used to retrieve and process signal or other features over regions of interest in BED files (including DNase hypersensitive regions, SNPs, transcription factor binding sites, etc.), performing tasks such as: smoothing raw tag count signal in preparation for uploading to the UCSC Genome Browser, finding subsets of elements within a larger coordinate set, filtering multiple BED files by signal, finding multi-input overlap solutions, and much, much more.

## Inputs and outputs

## Input

The bedmap program takes in reference and mapping files and calculates statistics for each reference element. These calculations-operations-are applied to overlapping elements from the mapped file:

## Input: One or more BED or Starch files or streams; one or more operations



The bedmap program requires files in a relaxed variation of the BED format as described by UCSC's browser documentation. The chromosome field can be any non-empty string, the score field can be any valid numeric value, and information is unconstrained beyond the minimum number of columns required by the chosen options.

Note: Information is unconstrained, with one important exception: Map input (defined below) should not contain spaces in the ID or in subsequent fields. Running bedmap with --ec will identify problematic input.
An awk script can help with translating ID spaces to another non-whitespace delimiter, e.g., bedmap (--options...) reference.bed <(awk -vOFS="\t" -vFS="\t" '\{gsub(" ", "\%\%", \$4); print;\}' map.bed) and then $a w k-v O F S=" t "-v F S=" t "$ '\{gsub("\%\%",""); print;\}' result.bed to convert delimiters back to spaces.

Alternatively, bedmap can accept Starch-formatted archives of BED data as input-it is no longer necessary to extract Starch archive data to intermediate BED files!

Support for common headers (including UCSC browser track headers) is available with the --header option, although headers are stripped from output.

Most importantly, bedmap expects sorted inputs. You can use the BEDOPS sort-bed program to ensure your inputs are properly sorted.

Note: You only need to sort once, and only if your input data are unsorted, as all BEDOPS tools take in and export sorted BED data.

Operations are applied over map elements that overlap the coordinates of each reference element. You can use the default overlap criterion of one base, or define your own criteria using the overlap criteria operators.

Once you have overlapping elements, you can either perform numerical calculations on their scores or return identifiers or other non-score information. Additional modifier operators allow customization of how output is presented, to assist with downstream processing in a pipeline setting.

## Output

Depending on specified options, the bedmap program can send a variety of delimited information about the reference and mapped elements (as well as analytical results) to standard output. If the --echo option is used, the output will be at least a three-column BED file. The use of predictable delimiters (which are customizable) and the use of UNIX-like standard streams allows easy downstream analysis or post-processing with other tools and scripts.

## Usage

The --help option describes the various mapping and analytical operations and other options available to the end user:

```
bedmap
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Shane Neph & Scott Kuehn
USAGE: bedmap [process-flags] [overlap-option] <operation(s)...> <ref-file> [map-
\hookrightarrowfile]
            Any input file must be sorted per the sort-bed utility.
            The program accepts BED and Starch file formats.
            You may use '-' for a BED file to indicate the input comes from stdin.
            Traverse <ref-file>, while applying <operation(s)> on qualified, overlapping
\hookrightarrowelements from
            <map-file>. Output is one line for each line in <ref-file>, sent to standard\sqcup
\hookrightarrowoutput. There
            is no limit on the number of operations you can specify to compute in onem
@bedmap call.
            If <map-file> is omitted, the given file is treated as both the <ref-file> and
\hookrightarrow<map-file>.
            This usage is more efficient than specifying the same file twice.
            Arguments may be given in any order before the input file(s).
            Process Flags:
            --chrom <chromosome>
                    Jump to and process data for given <chromosome> only.
            --delim <delim> Change output delimiter from '|' to <delim> between,
\hookrightarrowcolumns (e.g. '\t').
            --ec Error check all input files (slower).
            --faster (advanced) Strong input assumptions are made. Compatible,
Gith:
\hookrightarrowoptions only.
            --header
Gfile.
            --help Print this message and exit successfully.
            --min-memory Minimize memory usage (slower).
            --multidelim <delim> Change delimiter of multi-value output columns from ';'`
๑to <delim>.
            --prec <int> Change the post-decimal precision of scores to <int>. 0
c<<<int>.
            --sci Use scientific notation for score outputs.
            --skip-unmapped Print no output for a row with no mapped elements.
            --sweep-all Ensure <map-file> is read completely (helps to prevent U
\hookrightarrowbroken pipes).
```

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```
    --unmapped-val <val> Print <val> on unmapped --echo-map* and --min/max-
\hookrightarrowelement* operations.
    The default is to print nothing.
    --unmapped-val <val> Use <val> in place of the empty string on unmapped --echo-
map* ops.
    --version Print program information.
    Overlap Options (At most, one may be selected. By default, --bp-ovr 1 is used):
        --------
            --bp-ovr <int> Require <int> bp overlap between elements of input,
\hookrightarrowfiles.
            --exact First 3 fields from <map-file> must be identical to
\hookrightarrow<ref-file>'s.
            --fraction-ref <val> The fraction of the element's size from <ref-file>
\hookrightarrowthat must overlap
    --fraction-map <val>
\hookrightarrowthat must overlap
must be true to
    --fraction-either <val> Either --fraction-ref <val> or --fraction-map <val>_
\leftrightarrow m u s t ~ b e ~ t r u e ~ t o ~
    --range <int>
    qualify as overlapping. Expect 0 < val <= 1.
    Grab <map-file> elements within <int> bp of <ref-file>
\hookrightarrow's element,
    where 0 <= int. --range 0 is an alias for --bp-ovre
\leftrightarrow 1 .
    Operations: (Any number of operations may be used any number of times.)
        SCORE:
            <ref-file> must have at least 3 columns and <map-file> 5 columns.
            --cv The result of --stdev divided by the result of --mean.
            --kth <val> Generalized median. Report the value, x, such that the
\hookrightarrowfraction <val>
sthan x,
\hookrightarrow< val <= 1.
    --mad <mult=1>
\hookrightarrow<map-file>.
clefault.
    --max
    --max-element
\hookrightarrowmap-file>.
            --max-element-rand
↔file>
    --mean The average score from overlapping elements in <map-file>.
    --median The median score from overlapping elements in <map-file>.
    --min The lowest score from overlapping elements in <map-file>.
    --min-element A (non-random) lowest-scoring and overlapping element in
\hookrightarrow<map-file>.
    --min-element-rand A random lowest-scoring and overlapping element in <map-
\hookrightarrowfile>.
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```

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```
    --stdev The square root of the result of --variance.
    --sum Accumulated scores from overlapping elements in <map-file>.
    --tmean <low> <hi> The mean score from overlapping elements in <map-file>,
\hookrightarrowafter
    ignoring the bottom <low> and top <hi> fractions of those
scores.
    --variance The variance of scores from overlapping elements in <map-
\hookrightarrowfile>.
    --wmean Weighted mean, scaled in proportion to the coverage of the
\hookrightarrow<ref-file>
    element by each overlapping <map-file> element.
    NON-SCORE:
        <ref-file> must have at least 3 columns.
        For --echo-map-id/echo-map-id-uniq, <map-file> must have at least 4 columns.
        For --echo-map-score, <map-file> must have at least 5 columns.
        For all others, <map-file> requires at least 3 columns.
    --bases The total number of overlapping bases from <map-file>.
    --bases-uniq The number of distinct bases from <ref-file>'s elementu
covered by
    --bases-uniq-f The fraction of distinct bases from <ref-file>'s element_
ccovered by
    --count The number of overlapping elements in <map-file>.
    --echo Print each line from <ref-file>.
    --echo-map List all overlapping elements from <map-file>.
    --echo-map-id List IDs from all overlapping <map-file> elements.
    --echo-map-id-uniq List unique IDs from overlapping <map-file> elements.
    --echo-map-range Print genomic range of overlapping elements from <map-file>.
    --echo-map-score List scores from overlapping <map-file> elements.
    --echo-map-size List the full length of every overlapping element.
    --echo-overlap-size List lengths of overlaps.
    --echo-ref-name Print the first 3 fields of <ref-file> using chrom:start-
end format.
    --echo-ref-row-id Print 'id-' followed by the line number of <ref-file>.
    --echo-ref-size Print the length of each line from <ref-file>.
    --indicator Print 1 if there exists an overlapping element in <map-file>
\hookrightarrow, O otherwise.
```


## Operations

To demonstrate the various operations in bedmap, we start with two simple, pre-sorted BED files that we label as Map and Reference (see the Downloads section for files you can use to follow along).

Our Map file is a snippet of real-world BED data derived from ENCODE experiments conducted by our lab: specifically, raw DNaseI hypersensitivity signal for the human K562 cell line (region chr21:33031165-33032485, assembly GRCh37/h19 and table wgEncodeUwDnaseK562RawRep1 from the UCSC Genome Browser).

This raw signal is the density of sequence tags which map within a 150 bp sliding window, at 20 bp steps across the genome-a smoothed picture of DNaseI hypersensitivity:

| chr21 | 33031165 | 33031185 | map-1 | 1.000000 |
| :--- | :--- | :--- | :--- | :--- |
| chr21 | 33031185 | 33031205 | map-2 | 3.000000 |
| chr21 | 33031205 | 33031225 | map-3 | 3.000000 |
| chr21 | 33031225 | 33031245 | map-4 | 3.000000 |
| chr21 | 33031245 | 33031265 | map-5 | 3.000000 |
| chr21 | 33031265 | 33031285 | map-6 | 5.000000 |
| chr21 | 33031285 | 33031305 | map-7 | 7.000000 |
| chr21 | 33031305 | 33031325 | map-8 | 7.000000 |
| chr21 | 33031325 | 33031345 | map-9 | 8.000000 |
| chr21 | 33031345 | 33031365 | map-10 | 14.000000 |
| chr21 | 33031365 | 33031385 | map-11 | 15.000000 |
| chr21 | 33031385 | 33031405 | map-12 | 17.000000 |
| chr21 | 33031405 | 33031425 | map-13 | 17.000000 |
| $\cdots$ |  | 33032445 |  | map-64 |
| chr21 | 33032425 | 5.000000 |  |  |
| chr21 | 33032445 | 33032465 | map-65 | 5.000000 |
| chr21 | 33032465 | 33032485 | map-66 | 6.000000 |

When visualized, the signal data has the following appearance:
DNasel hypersensitivity (all elements)


Note: Rectangles colored in grey represent each of the sixty-six map elements. The x-axis represents the start coordinate of the map element, while the $y$-axis denotes the tag density, or sum of tags over that element's 20 -base window.

Our sample Reference file is not as exciting. It is just three BED elements which span portions of this density file:

| chr21 | 33031200 | 33032400 | ref-1 |
| :--- | :--- | :--- | :--- |
| chr21 | 33031400 | 33031800 | ref-2 |
| chr21 | 33031900 | 33032000 | ref-3 |

These reference elements could be exons, promoter regions, etc. It doesn't matter for purposes of demonstration here, except to say that we can use bedmap to ask some questions about the Reference set.
Among them, what are the quantitative and qualitative features of the map elements that span over these three reference regions? For example, we might want to know the mean DNase hypersensitivity across each-the answer may have some biological significance.

It may help to first visualize the reference regions and the mapped elements associated with them. A default bedmap task will operate on the following set of mapped (red-colored) elements, for each reference element ref-1, -2 and -3.

Here we show elements from the Map set which overlap the ref-1 region chr21:33031200-33032400, colored in red:

DNasel hypersensitivity (ref-1 elements)


Likewise, here are elements of the Map set which overlap the ref-2 element chr21:33031400-33031800 and ref-3 element chr21:33031900-33032000, respectively, with the same coloring applied:

DNasel hypersensitivity (ref-2 elements)


DNasel hypersensitivity (ref-3 elements)


In these sample files, we provide the Map file with ID and score columns, and the Reference file with an ID column. These extra columns are not required by bedmap, but we can use the information in these columns in conjunction with the options provided by bedmap to identify matches, retrieve matched signals, and summarize data about signal across mapped elements.

## Overlap criteria

The default overlap criterion that bedmap uses is one base. That is, one or more bases of overlap between reference and mapping elements is sufficient for inclusion in operations. This value can be adjusted with the --bp-ovr option. The --range overlap option implicitly applies --bp-ovr 1 after symmetrically padding elements.

If a fractional overlap is desired, the --fraction-\{ref,map,both, either\} options provide the ability to filter on overlap by a specified percentage of the length of either or both the reference and mapping elements.

Finally, the --exact flag enforces exact matches between reference and mapping elements.

Note: The --exact option is an alias for--fraction-both 1.

Using --faster with --bp-ovr, --fraction-both, --exact or --range

The --faster modifier works with the --bp-ovr, --fraction-both and --exact (--fraction-both 1) overlap and --range specifiers to dramatically increase the performance of bedmap, when the following input restriction is met:

- No fully-nested elements in any input mapping file (duplicate elements and other overlapping elements are okay).

Note: The details of this restriction are explained in more detail in the nested element documentation.

This option also works with the --ec error checking flag, which indicates if the data contain nested elements. Using --ec carries its usual overhead, but as it only doubles the much-improved execution time, it may be worth using.

Tip: To give an idea of the speed improvement, a --range 100000 --echo --count operation on 8.4 million, non-nested mapping elements (DNaseI footprints across multiple cell types) took 2 minutes and 55 seconds without speed-up. By adding the --faster flag, the same calculation took 10 seconds. That is an $\mathbf{1 8}$-fold speed improvement.

One scenario where this option can provide great speed gains is where --range is used with a large numerical parameter. Another scenario where this option is very useful is where the reference file has large elements, and the mapping file is made up of many small elements-specifically, where a number of small elements overlap each big element from the reference file.

An example of a research application for our lab which benefits from this flag is where we perform statistical analysis of large numbers of small sequence tags that fall in hotspot regions.

If your data meet the non-nesting criteria, using --faster with --bp-ovr, --fraction-both, --exact or --range is highly recommended.

Note: Our lab works with BED data of various types: cut-counts, hotspots, peaks, footprints, etc. These data generally do not contain nested elements and so are amenable to use with bedmap's --faster flag for extracting overlapping elements.

However, other types of data can be problematic. FIMO search results, for example, may cause trouble, where the boundaries of one motif hit can be contained within another larger hit. Or paired-end sequence data, where tags are not of a fixed length.

Be sure to consider the makeup of your BED data before using --faster.

Tip: Using --ec with --faster will report if any nested elements exist in your data.

## Score operations

Score operators apply a numerical calculation on the values of the score column of mapping elements. Per UCSC specifications, bedmap assumes the score data are stored in the fifth column.

The variety of score operators include common statistical measures:

- mean (--mean)
- trimmed mean (--tmean)
- weighted mean (--wmean)
- standard deviation (--stdev)
- variance (--variance)
- coefficient of variance (--cv)
- median (--median)
- median absolute deviation (--mad)
- k-th order statistic (--kth)

One can also take the sum of scores (--sum), find the minimum or maximum score over a region ( --min and $--\max$, respectively), or retrieve the map element with the least or greatest signal over the reference region ( $--m i n-e l e m e n t$ and --max-element, respectively).

Note: Map input should not contain spaces in the ID or in subsequent fields. Running bedmap with --ec will identify problematic input. Spaces in these fields will cause problems with --min-element, --max-element, and other options that require parsing of the fourth and subsequent columns of the map input.

An awk script can help with translating ID spaces to another non-whitespace delimiter, e.g., bedmap (--options...) reference.bed <(awk -vOFS="\t" -vFS="\t" '\{gsub(" ", "\%\%", \$4); print; \}' map.bed) and then $a w k-v O F S=" t "-v F S=" t "$ '\{gsub("\%\%",""); print;\}' result.bed to convert delimiters back to spaces.

We will demonstrate some of these operators by applying them to the Reference and Map datasets (see the Downloads section for sample inputs).

As a reminder, the Map file contains regions of DNaseI-seq tag density. If we want the mean of the density across Reference elements, we use the --mean option:

```
$ bedmap --echo --mean reference.bed map.bed > mappedReferences.mean.bed
```

The --echo flag prints each Reference element, while the --mean flag calculates the mean signal of the Map elements which overlap the reference element:

```
$ more mappedReferences.mean.bed
chr21 33031200 33032400 ref-1|43.442623
chr21 33031400 33031800 ref-2|31.571429
chr21 33031900 33032000 ref-3|154.500000
```

This result tells us that the mean density across regions ref-1, ref-2 and ref-3 is $44.442623,31.571429$ and 154.5 , respectively.

Note: The pipe character ( 1 ) delimits the results of each specified option (with the exception of the so-called "multi" operators that return multiple results - this is discussed in the section on --echo flags). In the provided example, the delimiter divides the reference element from the mean score across the reference element.

Tip: Because we used the --echo flag in this example, we are guaranteed output that is at least three-column BED format and which is sorted, which can be useful for pipeline design, where results are piped downstream to bedmap, bedops and other BEDOPS and UNIX utilities.

If we simply want the mean values and don't care about the reference data, we can skip --echo:

```
$ bedmap --mean reference.bed map.bed
43.442623
31.571429
154.500000
```

While not very detailed, this single-column representation can be useful for those who use UNIX utilities like paste or need to do additional downstream calculations with R or other utilities, where the reference information is unnecessary (or, at least, more work to excise).

If a reference element does not overlap any map element, then a NAN is returned for any operation on that entry, e.g., we know that the $a d$ hoc element chr21:1000-2000 does not overlap any member of our Map dataset, and there is therefore no mean value that can be calculated for that element:

```
$ echo -e "chr21\t1000\t2000\tfoo-1" | bedmap --echo --mean - map.bed
chr21 1000 2000 foo-1|NAN
```

Tip: For this example, we use echo -e to send bedmap a sample reference coordinate by way of standard input. The bedmap program can process any BED data from the standard input stream, either as the reference or map data, by placing the dash character ( - ) where the file name would otherwise go.

In the example above, we sent bedmap a single reference element via standard input, but multiple lines of BED data can come from other upstream processes.

Using standard streams is useful for reducing file I/O and improving performance, especially in situations where one is using bedmap in the middle of an extended pipeline.

While bedmap returns a NAN if there are no mapped elements that associate with a reference element, we may want to filter these lines out. We can apply the --skip-unmapped option to leave out reference elements without mapped elements:

```
$ echo -e "chr21\t1000\t2000\tfoo-1" | bedmap --echo --mean --skip-unmapped - map.bed
$
```

Note: Some operations may yield a reference element with one or more mapped elements, which still return a NAN value by virtue of the calculation result. The --skip-unmapped operand will still allow these reference elements to be printed out!

For instance, consider the --variance operator, which requires two or more map elements to calculate a variance. Where there is only one mapped element associated with the reference element, a --variance calculation will
return a NAN. In this case, --skip-unmapped will still print this element, even though the result is NAN.
Given the following statement:

```
$ bedmap --skip-unmapped --variance file1 file2
```

This is functionally equivalent to the following statement:

```
$ bedmap --indicator --variance --delim "\t" file1 file2 | awk '($1==1) {print $2}'
```

The --indicator operand calculates whether there are any mapped elements (or none)—see the indicator section for more detail. The awk statement then prints results which have one or more mapped elements, effectively filtering unmapped references.

It should therefore be more convenient to use --skip-unmapped where unmapped reference elements are not needed.

Another option is to retrieve the mapping element with the highest or lowest score within the reference region, using the --max-element or --min-element operators, respectively.

Going back to our sample Reference and Map data, we can search for the highest scoring mapping elements across the three reference elements:

| \$ bedmap --echo --max-element --prec 0 reference.bed map.bed |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| chr21 33031200 | 33032400 | ref-1\|chr21 | 33031885 | 33031905 | - |
| $\hookrightarrow$ map-37 165 |  |  |  |  |  |
| chr21 33031400 | 33031800 | ref-2\|chr21 | 33031785 | 33031805 | - |
| $\hookrightarrow$ map-32 82 |  |  |  |  |  |
| chr21 33031900 | 33032000 | ref-3\|chr21 | 33031885 | 33031905 | - |
| $\hookrightarrow \operatorname{map}-37165$ |  |  |  |  |  |

Over reference elements ref-1 and ref-3, the mapping element map-37 has the highest score. Over reference element ref -2 , the highest scoring mapping element is map-32.
Likewise, we can repeat this operation, but look for the lowest scoring elements, instead:

| \$ bedmap --echo --min-element --prec 0 reference.bed map.bed |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| chr21 33031200 | 33032400 | ref-1\|chr21 | 33032265 | 33032285 | - |
| $\rightarrow \operatorname{map}-562$ |  |  |  |  |  |
| chr21 33031400 | 33031800 | ref-2\|chr21 | 33031525 | 33031545 | $\checkmark$ |
| $\leftrightarrow$ map-19 13 |  |  |  |  |  |
| chr21 33031900 | 33032000 | ref-3\|chr21 | 33031985 | 33032005 | - |
| $\hookrightarrow \quad \operatorname{map}-42138$ |  |  |  |  |  |

Note: Where there are ties in score values, using --max-element or --min-element now selects the lexicographically smallest element amongst the set of tied elements. This generally means that the first element in the lexicographic ordering of the ID fields (fourth column) will determine the selection.

A random selection process was used for $--m a x-e l e m e n t$ and $--m i n-e l e m e n t ~ i n ~ v e r s i o n s ~ 2.4 .20 ~ a n d ~ p r e v i o u s . ~$ If you wish to randomly sample a maximum- or minimum-scoring element from amongst tied elements (say, to reproduce the procedure of prior analyses), you may use the $--m a x-e l e m e n t-r a n d$ or $--m i n-e l e m e n t-r a n d$ options, respectively.

We can also perform multiple score operations, which are summarized on one line, e.g., to show the mean, standard deviation, and minimum and maximum signal over each Reference element, we simply add the requisite options in series:

```
$ bedmap --echo --mean --stdev --min --max reference.bed map.bed
chr21 33031200 33032400 ref-1|43.442623|50.874527|2.000000|165.000000
chr21 33031400 33031800 ref-2|31.571429|19.638155|13.000000|82.000000
chr21 33031900 33032000 ref-3|154.500000|9.311283|138.000000|165.000000
```

Multiple score-operational results are ordered identically with the command-line options. The section on formatting score output demonstrates how one can change the precision and general format of numerical score results.

## Non-score operations

Sometimes it is useful to get summary or non-score statistics about the map elements. This category of operators returns information from the ID column of mapping elements, or can return counts and base overlap totals.

Note: As with score data, we follow the UCSC convention for the BED format and retrieve ID data from the fourth column.

## Echo

The ID, score and coordinate columns of the reference and map files are very useful for reading and debugging results, or reporting a more detailed mapping.

We can use the --echo, --echo-map, --echo-map-id, --echo-map-id-uniq, --echo-map-score, --echo-map-range, --echo-map-size, --echo-overlap-size, --echo-ref-name, --echo-ref-row-id, and echo-ref-size flags to tell bedmap to report additional details about the reference and map elements.

The --echo flag reports each reference element. We have already seen the application of --echo in earlier examples. The option helps to clearly associate results from other chosen operations with specific reference elements. Additionally, --echo enables the output from bedmap to be used as input to additional BEDOPS utilities, including bedmap itself.

The --echo-map flag gathers overlapping mapped elements for every reference element. The option is useful for debugging and detailed downstream processing needs. This is the most general operation in bedmap in that overlapping elements are returned in full detail, for every reference element. While results are well-defined and easily parsed, the output can be very large and difficult to read.

As an example of using the --echo-map-id operator in a biological context, we examine a FIMO analysis that returns a subset of transcription factor binding sites in BED format, with TRANSFAC motif names listed in the ID column:

| chr1 | 45341614534177 -V_GRE_C | $4.20586 \mathrm{e}-06$ | - | CGTACACACAGTTCTT |
| :---: | :---: | :---: | :---: | :---: |
| chr1 | 4534192.4 .364205 -V_STAT_Q6 | $2.21622 \mathrm{e}-06$ | - | AGCACTTCTGGGA |
| chr1 | 45342094534223 +V_HNF4_Q6_01 | $6.93604 e-06$ | + | GGACCAGAGTCCAC |
| chr1 | 4962522.4 .362540 -V_GCNF_01 | $9.4497 e-06$ | - | CCCAAGGTCAAGATAAAG |
| chr1 | 49625294962539 +V_NUR77_Q5 | $8.43564 e-06$ | + | TTGACCTTGG |

This input is available from the Downloads section as the Motifs dataset.
We will treat this as a map file, asking which motif IDs are associated with a region of interest (chr1:4534150-4534300). To do this using bedmap, we use the--echo-map-id option to summarize the IDs of mapped elements:

```
$ echo -e "chr1\t4534150\t4534300\tref-1" | bedmap --echo --echo-map-id - motifs.bed
chr1 4534150 4534300 ref-1|-V_GRE_C;-V_STAT_Q6;+V_HNF4_Q6_01
```

Note: To expand on the types of questions one can answer with bedmap in this context, in conjunction with the --count operator (described below), one can quantify predicted transcription factor binding sites by sliding a reference window across the entire genome.

One could determine, for example, where predicted sites are most prevalent and investigate the distribution of factors or other genomic features at or around these dense regions.

The --echo-map-id-uniq operator works exactly like--echo-map-id, except that duplicate IDs are removed from the result. For example, we can pull all the motifs hits from a wide region on chr2:

```
$ echo -e "chr2\t1000\t10000000\tref-1" | bedmap --echo --echo-map-id - motifs.bed
chr2 1000 10000000 ref-1|+V_OCT1_05;+V_OCT_C;-V_CACD_01;+V_IRF_Q6;-V_
\hookrightarrowBLIMP1_Q6;-V_IRF2_01;-V_IRF_Q6_01;+V_SMAD_Q6_01;-V_TATA_01;-V_TATA_C;-V_CEBP_01;-V_
\hookrightarrowHNF 6_Q6;+V_MTF1_Q4;+V_MYOD_Q6_01;-V_KROX_Q6;+V_EGR1_01;-V_SP1SP3_Q4; +V_EGR_Q6;+V_
\hookrightarrowSP1_Q6;-V_SP1_Q2_01;-V_CKROX_Q2;+V_SP1_Q6_01;-V_SREBP1_Q5;+V_VDR_Q3;-V_DMRT1_01;-V_
\hookrightarrowDMRT7_01;+V_DMRT1_01;-V_DMRT1_01;-V_DMRT7_01;+V_DMRT1_01;-V_DMRT1_01;-V_DMRT7_01
```

However, some hits (e.g., -V_DMRT7_01) show up two or more times. If we want a non-redundant list, we replace --echo-map-id with --echo-map-id-uniq:

```
$ echo -e "chr2\t1000\t10000000\tref-1" | bedmap --echo --echo-map-id-uniq - motifs.
\hookrightarrowbed
chr2 1000 10000000 ref-1|+V_DMRT1_01;+V_EGR1_01;+V_EGR_Q6;+V_IRF_Q6;+V_
\hookrightarrowMTF1_Q4;+V_MYOD_Q6_01;+V_OCT1_05;+V_OCT_C;+V_SMAD_Q6_01;+V_SP1_Q6;+V_SP1_Q6_01;+V_
\hookrightarrowVDR_Q3;-V_BLIMP1_Q6;-V_CACD_01;-V_CEBP_01;-V_CKROX_Q2;-V_DMRT1_01;-V_DMRT7_01;-V_
\hookrightarrowHNF6_Q6;-V_IRF2_01;-V_IRF_Q6_01;-V_KROX_Q6;-V_SP1SP3_Q4;-V_SP1_Q2_01;-V_SREBP1_Q5;-
\hookrightarrowV_TATA_01;-V_TATA_C
```

The --echo-map-score flag works in a similar fashion to --echo-map-id, reporting scores instead of IDs. The formatting score output section demonstrates how one can use--echo-map-score to summarize score data from mapped elements.

Note: Both the --echo-map-id and --echo-map-score flags use the semi-colon (;) as a default delimiter, which may be changed with the $--m u l t i d e l i m$ option (see the Delimiters section for more information on this and other modifier operators).

The --echo-map-range flag tells bedmap to report the genomic range of overlapping mapped elements. If we apply this flag to the Reference and Map datasets (see Downloads), we get the following result:

| \$ bedmap | --echo | --echo-map-range reference.bed map.bed |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| chr21 | 33031200 | 33032400 | ref-1\|chr21 | 33031185 | 33032405 |
| chr21 | 33031400 | 33031800 | ref-2\|chr21 33031385 | 33031805 |  |
| chr21 | 33031900 | 33032000 | ref-3\|chr21 | 33031885 | 33032005 |

Note: The --echo-map-range option produces three-column BED results that are not always guaranteed to be sorted. The --echo operation is independent, and it produces reference elements in proper BEDOPS order, as shown. If the results of the --echo-map-range option will be used directly as BED coordinates in downstream BEDOPS analyses (i.e., no --echo operator), first pipe them to sort-bed to ensure proper sort order.

Note: Map input should not contain spaces in the ID or in subsequent fields. Running bedmap with --ec will identify problematic input. Spaces in these fields will cause problems with --echo-map-id,--echo-map-id-uniq, and other options that require parsing of the fourth and subsequent columns of the map input.
An awk script can help with translating ID spaces to another non-whitespace delimiter, e.g., bedmap (--options...) reference.bed < (awk -vOFS="\t" -vFS="\t" '\{gsub(" ", "\%\%", \$4); print; \}' map.bed) and then $a w k-v O F S=" t "-v F S=" t "$ '\{gsub("\%\%"," "); print; \}' result.bed to convert delimiters back to spaces.

The --echo-ref-size flag reports the difference between the stop and start coordinates of the reference element. The --echo-ref-name flag produces a converted format for the first 3 BED fields, A:B-C, where A is the chromosome name, B is the start coordinate, and C is the stop coordinate for that reference element.

The --echo-ref-row-id flag prints the prefix id- with the line number of the reference element.
Finally, the --echo-map-size flag reports the difference between the stop and start coordinates of each mapped element, while the --echo-overlap-size flag reports the length of the overlap between the reference element and each mapped element.

## Element and overlap statistics

Looking back at the Map and Reference datasets, let's say we want to count the number of elements in Map that overlap a given Reference element, as well as the extent of that overlap as measured by the total number of overlapping bases from mapped elements. For this, we use the --count and --bases flags, respectively:

```
$ bedmap --echo --count --bases reference.bed map.bed
chr21 33031200 33032400 ref-1|61|1200
chr21 33031400 33031800 ref-2|21|400
chr21 33031900 33032000 ref-3|6|100
```

This result tells us that there are 61 elements in Map that overlap ref-1, and 1200 total bases from the 61 elements overlap bases of ref-1. Similarly, 21 elements overlap ref-2, and 400 total bases from the 21 elements overlap bases of ref-2, etc.

The --bases operator works on Map elements. If, instead, we want to quantify the degree to which Reference elements overlap Map, we can use the --bases-uniq and --bases-uniq-f flags to count the number of bases and, respectively, the fraction of total bases within Reference which are covered by overlapping elements in Map.

This last example uses Motifs elements and all of the options: --bases, --bases-uniq and --bases-uniq-f, to illustrate their different behaviors:

```
$ echo -e "chrl\t4534161\t4962550\tadhoc-1" | bedmap --echo --bases --bases-uniq --
\hookrightarrowbases-uniq-f - motifs.bed
chr1 4534161 4962550 adhoc-1|169|71|0.000166
```


## Indicator

If we simply want to know if a reference element overlaps one or more map elements, we can use the --indicator operator, which returns a 1 or 0 value, depending on whether there is or is not an overlap, respectively. For example:

```
$ bedmap --echo --indicator reference.bed map.bed
chr21 33031200 33032400 ref-1|1
chr21 33031400 33031800 ref-2|1
chr21 33031900 33032000 ref-3|1
```

All three of our reference elements have mapped elements associated with them. If we, instead, test a reference element that we know ahead of time does not contain overlapping map elements, we get a 0 result, as we expect:

```
$ echo -e "chr21\t1000\t2000\tfoo-1" | bedmap --echo --indicator - map.bed
chr21 1000 2000 foo-1|0
```

Note: The --indicator option is equivalent to testing if the result from --count is equal to or greater than 0 :

```
$ bedmap --count foo bar | awk '{ print ($1 > 0 ? "1" : "0") }' -
```

This option eliminates the need for piping bedmap results to awk.

## Modifiers

These options can modify the coordinates used for generating the set of mapped regions, as well as alter the presentation of results. These modifiers can be useful, depending on how bedmap is used in your own workflow.

## Range

The --range option uses --bp-ovr 1 (i.e., one base of overlap) after internally and symmetrically padding reference coordinates by a specified positive integer value. The larger reference elements are used to determine overlapping mapped elements, prior to applying chosen operations.

Tip: To change the coordinates of a BED file on output (symmetrically or asymmetrically), see the --range option applied with bedops -everything.

As an example, we look again at element ref-3 from the Reference dataset and where it overlaps with Map:
chr21 $33031900 \quad 33032000 \quad$ ref-3


If we want to apply an operation on 100 bp upstream and downstream of this and the other reference elements, we can use the --range option:

```
$ bedmap --echo --echo-map-id --range 100 reference.bed map.bed > mappedReference.
spadded.bed
```

Any operation will now be applied to a broader set of mapped elements, as visualized here with a "padded" version of ref-3:

DNasel hypersensitivity (ref-3 elements)


We can compare mean densities, in order to see the effect of using --range. Here is the mean density across the original, unpadded ref-3:

```
$ bedmap --echo --mean reference.bed map.bed
..
chr21 33031900 33032000 ref-3|154.500000
```

And here is the mean density across the padded ref-3:

```
$ bedmap --echo --range 100 --mean reference.bed map.bed
...
chr21 33031900 33032000 ref-3|117.750000
```

Looking at the visualizations above, we would expect the mean density to be lower, as the expanded reference region includes map elements with lower tag density, which pushes down the overall mean.

Note: The --range option is classified as an overlap option (like --fraction-map or --exact) that implicitly uses --bp-ovr 1 after padding reference elements. As shown above, the extended padding is an internal operation and it is not reflected in the output with the --echo option. Real padding can be added by using bedops --range 100 --everything reference.bed and piping results to bedmap.

Note: Because --range is an internal operation, some statistical operations like --bases and --bases-uniq do not work as one might expect.
As an example, we might want to count the number of overlapping, unique bases between a 1000 -base window around a reference element and a set of mapped elements. The following command will not work:

```
$ bedmap --echo --range 1000 --bases-uniq reference.bed map.bed
```

Instead, use bedops to build the window, piping it into a downstream bedmap command. The result of this operation can be piped into the core utility paste with the original reference set, in order to associate reference elements with the windowed operation result:

```
$ bedops --range 1000 --everything reference.bed \
    | bedmap --bases-uniq - map.bed \
    | paste reference.bed -
```

To extend this demonstration even further, let's say we are interested in calculations of unique base counts across 1 , 2.5 and 5 kilobase windows around reference elements. We can build a matrix-like result through a judicious use of UNIX pipes that progressively expand windows:

```
$ bedops --range 1000 --everything reference.bed \
    | bedmap --echo --bases-uniq - map.bed \
    | bedops --range 1500 --everything - \
    | bedmap --echo --bases-uniq - map.bed \
    | bedops --range 2500 --everything - \
    | bedmap --echo --bases-uniq - map.bed \
    | cut -f2- -d'|' \
    | paste reference.bed - \
    | tr '|' '\t'
```

To explain how this works, we first build a 1 kilobase window around reference elements with bedops and pipe these windows to bedmap, which does two things:

1. Use --echo to print the windowed element.
2. Use--bases-uniq to print the number of uniquely-mapped bases across the window.

In turn, this result is passed to the second bedops operation, which expands the 1-kilobase window from bedmap by another 1.5 kilobases. This creates a window that is now 2.5 kilobases around the original reference element. We pipe this to the second bedmap operation, which prints the 2.5 kb window and the number of bases across that window.

In the third and last round of operations, we expand the 2.5 kb window by another 2.5 kb , creating a 5000-base window around the original reference element. We repeat the same mapping operation.
At this point, each line of the output consists of a windowed reference element, and pipe characters (the default bedmap delimiter) which separate the unique base counts across the $1,2.5$ and 5 kilobase windows. The final cut, paste and $t r$ operations strip out the windows, paste in the original reference elements and replace default delimiters with tab characters, creating a matrix-like output.

To make this analysis run quickly, use the --faster modifier on each of the bedmap, if the data allow it. See the following section for more details on where and how --faster can be used.

## Using --faster with --range

The --faster modifier works with the --bp-ovr, --fraction-both and --exact (--fraction-both 1) overlap and --range specifiers to dramatically increase the performance of bedmap, when the following input restriction is met:

- No fully-nested elements in any input mapping file (duplicate elements and other overlapping elements are okay).

Note: The details of this restriction are explained in more detail in the nested element documentation.

This option also works with the --ec error checking flag, which indicates if the data contain nested elements. Using --ec carries its usual overhead, but as it only doubles the much-improved execution time, it may be worth using.

Tip: To give an idea of the speed improvement, a --range 100000 --echo --count operation on 8.4 million, non-nested mapping elements (DNaseI footprints across multiple cell types) took 2 minutes and 55 seconds without speed-up. By adding the --faster flag, the same calculation took 10 seconds. That is an $\mathbf{1 8}$-fold speed improvement.

One scenario where this option can provide great speed gains is where --range is used with a large numerical parameter. Another scenario where this option is very useful is where the reference file has large elements, and the mapping file is made up of many small elements-specifically, where a number of small elements overlap each big element from the reference file.

An example of a research application for our lab which benefits from this flag is where we perform statistical analysis of large numbers of small sequence tags that fall in hotspot regions.

If your data meet the non-nesting criteria, using --faster with --bp-ovr, --exact or --range is highly recommended.

Note: Our lab works with BED data of various types: cut-counts, hotspots, peaks, footprints, etc. These data generally do not contain nested elements and so are amenable to use with bedmap's --faster flag for extracting overlapping elements.

However, other types of data can be problematic. FIMO search results (motif hits), for example, may cause trouble, where the boundaries of one motif hit can be contained within another larger hit. Or paired-end sequence data, where tags are not of a fixed length.

Be sure to consider the makeup of your BED data before using --faster.

Tip: Using --ec with --faster will report if any nested elements exist in your data. Using --ec carries its usual overhead, but as it only doubles the much-improved execution time, it may be worth using.

## Formatting score output

The --prec and --sci process flags are useful for controlling the arithmetic precision and notation of score output, when used with the --echo-map-score, --sum, --mean and other numerical score operators. This will also format results from the non-score operator --bases-uniq-f.

To demonstrate their use, we revisit the Motifs dataset, which includes $p$-values reporting the statistical significance of putative transcription factor binding sites:

| chr1 | 45341614534177 -V_GRE_C | $4.20586 e-06$ | - | CGTACACACAGTTCTT |
| :---: | :---: | :---: | :---: | :---: |
| chr1 | 4534192.4.364205-V_STAT_Q6 | $2.21622 \mathrm{e}-06$ | - | AGCACTTCTGGGA |
| chr1 | 45342094534223 +V_HNF 4_Q6_01 | $6.93604 e-06$ | + | GGACCAGAGTCCAC |
| chrl | 4962522.4 .362540 -V_GCNF_01 | $9.4497 e-06$ | - | CCCAAGGTCAAGATAAAG |
| chr1 | 49625294962539 +V_NUR77_Q5 | $8.43564 e-06$ | + | TTGACCTTGG |

Let's say we want a list of motifs and associated $p$-values mapped to a coordinate range of interest (chr1:4534150-4534300). In order to conserve space, however, we only want two significant figures for the score data. So we use --prec 2 to try to reformat the score output:

```
$ echo -e "chr1\t4534150\t4534300\tref-1" \
    | bedmap --prec 2 --echo --echo-map-id --echo-map-score - motifs.bed \
    > motifsForRef1.bed
```

Here is the output:

```
chr1 4534150 4534300 ref-1|-V_GRE_C;-V_STAT_Q6;+V_HNF4_Q6_01|0.00;0.00;0.00
```

It looks like our $p$-values were rounded down to zeroes, which is not what we want. But we remember that the binding site $p$-values are listed in scientific notation, and so we add the $--s c i$ flag to preserve the format of the score data in scientific notation:

```
$ echo -e "chr1\t4534150\t4534300\tref-1" \
    | bedmap --prec 2 --sci --echo --echo-map-id --echo-map-score - motifs.bed \
    > correctedMotifsForRef1.bed
```

Here is the corrected output:

```
chr1 4534150 4534300 ref-1|-V_GRE_C;-V_STAT_Q6;+V_HNF4_Q6_01|4.21e-06;2.22e-06;6.
\hookrightarrow94e-06
```

Rounding of the mantissa is done to the precision specified in --prec.
Obviously, the --sci flag is useful for very small or large score data. You probably wouldn't use --sci with most integer signal (e.g., raw tag counts or most discrete measurements).

## Delimiters

As shown in the examples above, the pipe (।) and semi-colon (;) characters are used to split operational and echo-ed results, respectively. The -- delim and -- multidelim flags change these delimiters to characters of your choice, which let you pick what makes most sense for your custom post-processing or other downstream pipelining work (for instance, in our lab--delim " $\backslash \mathrm{t}$ " is a popular alternative to the default | character).

As an example, the following bedmap result is obtained from using the --echo, --echo-map-id, --echo-map-score and --max options on the Motifs dataset:

```
chr1 4534150 4534300 ref-1|-V_GRE_C;-V_STAT_Q6;+V_HNF4_Q6_01|4.21e-06;2.22e-06;6.
\hookrightarrow94e-06|6.94e-06
```

For this result, the bedmap program organizes data using the default set of delimiters:

```
[reference-line] | [map-IDs] | [map-scores] | [maximum-map-score]
```

Here, you can use the --delim option to replace the pipe character with an alternative delimiter.
Within the map-IDs and map-scores subgroups, individual results are split further by semi-colon:

```
[id-1] ; [id-2] ; ... ; [id-N]
```

```
[score-1] ; [score-2] ; ... ; [score-N]
```

You can use the $--m u l t i d e l i m$ option to replace the semi-colon with another delimiter, e.g.:

```
$ echo -e "chr1\t4534150\t4534300\tref-1" | bedmap --multidelim '$' --echo --echo-map-
\hookrightarrowid - motifs.bed
chr1 4534150 4534300 ref-1|-V_GRE_C$-V_STAT_Q6$+V_HNF4_Q6_01
```

Note: Grouped results derived with the --echo-map, --echo-map-id, and --echo-map-score options are listed in identical order. In other words, ID results line up at the same position as their score result counterparts when
both --echo-map-id and --echo-map-score are chosen together. The same applies to the --echo-map option.

## I/O event handling

During normal use of bedmap, the application will usually terminate when it is determined that no more map data needs to be processed. This improves performance by limiting execution time to only that which is required to do actual work. However, closing early can trigger SIGPIPE or broken pipe errors that can cause batch scripts that use the standard input stream to pass data to bedmap to terminate early with an error state (even though there is often no functional problem from this early termination of bedmap).

When adding --ec, bedmap will go into error checking mode and read through the entire map dataset.
One method for dealing with this is to override how SIGPIPE errors are caught by the interpreter (bash, Python, etc.) and retrapping them or ignoring them. However, it may not a good idea to do this as other situations may arise in production pipelines where it is ideal to trap and handle all I/O errors in a default manner.

Until now, we have proposed using the --ec (error checking) option in bedmap as one way to prevent raising SIGPIPE events when chaining commands via pipes, by forcing all inputs to be read entirely. Early pipe termination can cause scripts to stop processing when certain flags are set (for example, when -e is used with tcsh). This hidden behavior of $--e c$ has been replaced with the explicit option $--s w e e p-a l l$.

The --ec and --sweep-all options work independently, and --ec no longer has the --sweep-all side-effect. These options may be used in conjunction. The --sweep-all option can add significant execution time in cases where early termination is possible.

## Per-chromosome operations (-chrom)

All operations on inputs described so far can be restricted to one chromosome, by adding the --chrom <val> operator. This is highly useful for cluster-based work, where operations on large BED inputs can be split up by chromosome and pushed to separate cluster nodes.

Here, we use the --echo and --echo-map-id operators on our Motifs dataset, but we limit operations to those on elements on chromosome chr2:

```
$ echo -e "chr2\t1000000\t5000000\tref-1" | bedmap --chrom chr2 --echo --echo-map-id -
m motifs.bed
chr2 1000000 5000000 ref-1|+V_OCT1_05;+V_OCT_C;-V_CACD_01;+V_IRF_Q6;-V_BLIMP1_Q6;-
\hookrightarrowV_IRF2_01;-V_IRF_Q6_01
```

If the reference elements are not on the specified chromosome provided to --chrom, then no output is generated. In the following example, our reference element is on chr2, but we ask for operations to be limited to chr3, yielding an empty set:

```
$ echo -e "chr2\t1000000\t5000000\tref-1" | bedmap --chrom chr3 --echo --echo-map-id -
-> motifs.bed
$
```


## Starch support

The bedmap application supports use of Starch-formatted archives as inputs, as well as text-based BED data. One or multiple inputs may be Starch archives.

For example, we can repeat the overlapping-motif example from the Echo section, using a Starch archive made from the regions in Motifs:

```
$ echo -e "chrl\t4534150\t4534300\tref-1" | bedmap --echo --echo-map-id - motifs.bed.
ustarch
chr1 4534150 4534300 ref-1|-V_GRE_C;-V_STAT_Q6;+V_HNF4_Q6_01
```

By combining the --chrom operator with operations on Starch archives, the end user can achieve improved computing performance and disk space savings, particularly where bedops, bedmap and closest-features operations are applied with a computational cluster on separate chromosomes.

## Error checking

The bedmap program does not perform error checking by default, but it offers an --ec option for comprehensive checks.

Note: Use of the --ec option will roughly double the running time, but it provides stringent error checking to ensure all inputs are valid. --ec can help check problematic input and offers helpful hints for any needed corrections, when problems are detected.

## Endlines

The bedmap program expects endlines ( $\backslash \mathrm{n}$ ) appropriate to Linux and Mac OS X operating systems. Microsoft Windows uses different characters for endlines. In UNIX-like environments, you can quickly check to see if your file contains the native endlines with this command:

```
$ head myData.bed | cat -et
```

The appropriate endlines will show up as a \$ character at the end of each line. See the dos 2 unix program (sometimes called fromdos) to convert newlines from files saved on Microsoft Windows. The unix2dos (or todos) program can convert files in the other direction, if needed.

## Downloads

- Sample Reference dataset: reference elements
- Sample Map dataset: map elements
- Sample Motifs dataset: motif elements


### 2.6.3 File management

## Sorting

sort-bed

The sort-bed utility sorts BED files of any size, even larger than system memory. BED files that are in lexicographic-chromosome order allow BEDOPS utilities to work efficiently with data from any species without software modifications. Further, sorted files can be traversed very quickly.

Sorted BED order is defined first by lexicographic chromosome order, then ascending integer start coordinate order, and finally by ascending integer end coordinate order. To make the sort order unambiguous, a lexicographical sort is applied on fourth and subsequent columns, where present in the input BED dataset.
Other utilities in the BEDOPS suite require data in sorted order as described. You only need to sort once: BEDOPS utilities all read and write data in sorted order.

## Migrating older BED and Starch files

The utility update-sort-bed-migrate-candidates recursively locates BED and pre-v2.4.20 Starch files in the specified parent directory, tests if they require re-sorting to conform to the updated, post-v2.4.20 'sort-bed' order, and offers actions to log candidate files, or immediately apply a resort action that is performed locally or via a SLURM-managed cluster.
The convenience utilities update-sort-bed-slurm and update-sort-bed-starch-slurm update the sort order of BED or Starch files sorted with pre-v2.4.20 sort-bed via a SLURM-based cluster. See update-sort-bed-slurm --help or update-sort-bed-starch-slurm --help for more details. These utilities can be used standalone or in conjunction with the update-sort-bed-migrate-candidates utility.

## Inputs and outputs

## Input

The sort-bed utility requires one or more three-column BED file(s). Support for common headers (such as UCSC BED track headers) is included, although headers will be stripped from the output.

## Output

The sort-bed utility sends sorted BED data to standard output, which can be redirected to a file or piped to other utilities, including core BEDOPS utilities like bedops and bedmap. Sort order is defined by a lexicographical sort on chromosome name, a numerical sort on start coordinates, a numerical sort on stop coordinates where there are start matches, and finally a lexicographical sort on the remainder of the BED element (if additional columns are present). Additional options may be specified to print only unique or duplicate elements, or check the sort order of input.

## Usage

The --help option is fairly basic, but describes the usage:

```
sort-bed
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Scott Kuehn
USAGE: sort-bed [--help] [--version] [--check-sort] [--max-mem <val>] [--tmpdir <path>
\hookrightarrow] [--unique] [--duplicates] <file1.bed> <file2.bed> <...>
        Sort BED file(s).
        May use '-' to indicate stdin.
        Results are sent to stdout.
        <val> for --max-mem may be 8G, 8000M, or 8000000000 to specify 8 GB of memory.
```

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```
    --tmpdir is useful only with --max-mem.
    --unique can be used to print only unique BED elements (similar to "sort -u").
    --duplicates can be used to print only duplicated or repeated elements_
\hookrightarrow(similar to "uniq -d").
```

A simple example of using sort-bed would be:

```
$ sort-bed unsortedData.bed > sortedData.bed
```

The sort-bed program efficiently sorts BED inputs. By default, all input records are read into system memory and sorted. If your BED dataset is larger than available system memory, use the --max-mem option to limit the amount of memory sort-bed uses to do its work:

```
$ sort-bed --max-mem 2G reallyHugeUnsortedData.bed > reallyHugeSortedData.bed
```

This option allows sort-bed to scale to input of any size.
The --tmpdir option allows specification of an alternative temporary directory, when used in conjunction with $--m a x-m e m$ option. This is useful if the host operating system's standard temporary directory (e.g., /tmp on Linux or OS X) does not have sufficient space to hold intermediate results.

For example, to use the current working directory to store temporary data, one could use the \$PWD environment variable:

```
$ sort-bed --max-mem 2G --tmpdir $PWD reallyHugeUnsortedData.bed >
```

$\hookrightarrow$ reallyHugeSortedData.bed

Use of the --check-sort option returns a message if the input is sorted, or not.
The --unique and --duplicates options print only unique or duplicated elements in sorted output, respectively. These options mimic sort $-u$ and uniq -d commands, respectively.

## Compression

## starch

With high-throughput sequencing generating large amounts of genomic data, archiving can be a critical part of an analysis toolkit. BEDOPS includes the starch utility to provide a method for efficient and lossless compression of UCSC BED-formatted data into the Starch v2 format.

Starch v2 archives can be extracted with unstarch to recover the original BED input, or processed as inputs to bedops and bedmap, where set operations and element calculations can be performed directly and without the need for intermediate file extraction.

The starch utility includes large file support on 64-bit operating systems, enabling compression of more than 2 GB of data (a common restriction on 32-bit systems).

Data can be stored with one of two open-source backend compression methods, either bzip2 or gzip, providing the end user with a reasonable tradeoff between speed and storage performance that can be useful for working with constrained storage situations or slower hardware.

## Inputs and outputs

## Input

As with other BEDOPS utilities, starch takes in sorted BED data as input. You can use sort-bed to sort BED data, piping it into starch as standard input (see Example section below).

Note: While more than three columns may be specified, most of the space savings in the Starch format are derived from from a pre-processing step on the coordinates. Therefore, minimizing or removing unnecessary columnar data from the fourth column on (e.g., with cut $-f 1-3$ or similar) can help improve compression efficiency considerably.

## Output

This utility outputs a Starch v2-formatted archive file.

## Requirements

The starch tool requires data in a relaxed variation of the BED format as described by UCSC's browser documentation. BED data should be sorted before compression, i.e. with BEDOPS sort-bed.

At a minimum, three columns are required to specify the chromosome name and start and stop positions. Additional columns may be specified, containing up to 128 kB of data per row (including tab delimiters).

## Usage

Use the --help option to list all options:

```
starch
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    binary version: 2.4.36 (typical) (creates archive version: 2.2.0)
    authors: Alex Reynolds and Shane Neph
USAGE: starch [ --note="foo bar..." ]
    [ --bzip2 | --gzip ]
    [ --omit-signature ]
    [ --report-progress=N ]
    [ --header ] [ <unique-tag> ] <bed-file>
    * BED input must be sorted lexicographically (e.g., using BEDOPS sort-bed).
    * Please use '-' to indicate reading BED data from standard input.
    * Output must be directed to a regular file.
    * The bzip2 compression type makes smaller archives, while gzip extracts
        faster.
    Process Flags
    ------------------------------------------------------------------------------------
    --note="foo bar..." Append note to output archive metadata (optional).
    --bzip2 | --gzip Specify backend compression type (optional, default
        is bzip2).
    --omit-signature Skip generating per-chromosome data integrity signature
        (optional, default is to generate signature).
```

```
--report-progress=N Report compression progress every N elements per
    chromosome to standard error stream (optional)
--header Support BED input with custom UCSC track, SAM or VCF
    headers, or generic comments (optional).
    Optional. Specify unique identifier for transformed
    data.
    --version Show binary version.
    --help Show this usage message.
```


## Options

## Backend compression type

Use the --bzip2 or --gzip operators to use the bzip2 or gzip compression algorithms on transformed BED data. By default, starch uses the bzip2 method.

## Note

Use the -- note $=$ "xyz . . ." option to add a custom string that describes the archive. This data can be retrieved with unstarch --note.

Tip: Examples of usage might include a description of the experiment associated with the data, a URL to a UCSC Genome Browser session, or a bar code or other unique identifier for internal lab or LIMS use.

Note: The only limitation on the length of a note is the command-line shell's maximum argument length parameter (as found on most UNIX systems with the command get conf ARG_MAX) minus the length of the non- --note=". . . " command components. On most desktop systems, this value will be approximately 256 kB .

## Per-chromosome data integrity signature

By default, a data integrity signature is generated for each chromosome. This can be used to verify if chromosome streams from two or more Starch archives are identical, or used to test the integrity of a chromosome, to identify potential data corruption.

Generating this signature adds to the computational cost of compression, or an integrity signature may not be useful for all archives. Add the --omit-signature option, if the compression time is too high or the data integrity signature is not needed.

## Compression progress

To optionally track the progress of compression, use the --report-progress $=\mathrm{N}$ option, specifying a positive integer N to report the compression of the $N$-th element for the current chromosome. The report is printed to the
standard error stream.

Note: For instance, specifying a value of 1 reports the compression of every input element of all chromosomes, while a value of 1000 would report the compression of every 1000 th element of the current chromosome.

## Headers

Add the --header flag if the BED data being compressed contain extra header data that are exported from a UCSC Genome Browser session.

Note: If the BED data contain custom headers and --header is not specified, starch will be unable to read chromosome data correctly and exit with an error state.

## Unique tag

Adding a <unique-tag> string replaces portions of the filename key in the archive's stream metadata.

Note: This feature is largely obsolete and included for legacy support. It is better to use the -- note="xyz..." option to add identifiers or other custom data.

## Example

To compress unsorted BED data (or data of unknown sort order), we feed starch a sorted stream, using the hyphen (-) to specify standard input:

```
$ sort-bed unsorted.bed | starch - > sorted.starch
```

This creates the file sorted. starch, which uses the bzip2 algorithm to compress transformed BED data from a sorted permutation of data in unsorted.bed. No note or custom tag data is added.

It is possible to speed up the compression of a BED file by using a cluster. Start by reviewing our starchcluster script.

## unstarch

With high-throughput sequencing generating large amounts of genomic data, archiving can be a critical part of an analysis toolkit. BEDOPS includes the unst arch utility to recover original BED input and whole-file or per-chromosome data attributes from archives created with starch (these can be v1.x or $v 2 . x$ archives).
The unstarch utility includes large file support on 64-bit operating systems, enabling extraction of more than 2 GB of data (a common restriction on 32-bit systems).
Starch data can be stored with one of two open-source backend compression methods, either bzip2 or gzip. The unstarch utility will transparently extract data, without the end user needing to specify the backend type.

## Inputs and outputs

## Input

The unstarch utility takes in a Starch v1.x or v2.x archive as input.

## Output

The typical output of unstarch is sorted BED data, which is sent to standard output.
Specifying certain options will instead send archive metadata to standard output, either in text or JSON format, or export whole-file or per-chromosome attributes (also to standard output).

## Requirements

The metadata of a Starch v2.x archive must pass an integrity check before unstarch can extract data. Any manual changes to the metadata will cause extraction to fail.

## Usage

Use the --help option to list all options:

```
unstarch
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    binary version: 2.4.36 (typical) (extracts archive version: 2.2.0 or older)
    authors: Alex Reynolds and Shane Neph
USAGE: unstarch [ <chromosome> ] [ --elements |
    --elements-max-string-length |
    --bases | --bases-uniq |
    --has-duplicates | --has-nested | --list |
    --list-json | --list-chromosomes |
    --archive-timestamp | --note |
    --archive-version | --is-starch |
    --signature | --verify-signature ]
    <starch-file>
    Modifiers
    -----------------------------------------------------------------------------------------
    <chromosome> Optional. Either unarchives chromosome-
        specific records from the starch archive
        file or restricts action of operator to
        chromosome (e.g., chrl, chry, etc.).
    Process Flags
    --elements Show total element count for archive. If
        <chromosome> is specified, the result
        shows the element count for the
        chromosome.
    --elements-max-string-length Show the maximum string length over all
        elements in <chromosome>, if specified.
```

| --bases, <br> --bases-uniq | ```Show total and unique base counts, respectively, for archive. If <chromosome> is specified, the count is specific to the chromosome, if available.``` |
| :---: | :---: |
| --has-duplicate-as-string, <br> --has-duplicate | Show whether there is one or more duplicate elements in the specified chromosome, either as a numerical (1/0) or string (true/false) value. If no <chromosome> is specified, the value given indicates if there is one or more duplicate elements across all chromosome records. |
| --has-nested-as-string, <br> --has-nested | Show whether there is one ore more nested elements in the specified chromosome, either as a numerical (1/0) or string (true/false) value. If no <chromosome> is specified, the value given indicates if there is one or more nested elements across all chromosome records. |
| --list | List archive metadata (output is in text format). If chromosome is specified, the attributes of the given chromosome are shown. |
| $\begin{aligned} & \text {--list-json, } \\ & \text {--list-json-no-trailing-newline } \end{aligned}$ | List archive metadata (output is in JSON format) |
| --list-chr, <br> --list-chromosomes | List all or specified chromosome in starch archive (like "bedextract --listchr"). If <chromosome> is specified but is not in the output list, nothing is returned. |
| --note | Show descriptive note, if available. |
| --signature | Display the Base64-encoded SHA-1 data integrity signature for specified <chromosome>, or the signatures of the metadata and all available chromosomes, if the <chromosome> is unspecified. |
| --verify-signature | Verify data integrity of specified <chromosome>, or the integrity of all available chromosomes, if the <chromosome> is unspecified. |


|  | (continued from |
| :---: | :---: |
| --archive-timestamp | Show archive creation timestamp (ISO 8601 format). |
| --archive-type | Show archive compression type. |
| --archive-version | Show archive version. |
| --is-starch | Test if <starch-file> is a valid archive and print $0 / 1$ (false/true) to standard output. Unstarch will also return a nonzero error code if the input file is not a valid archive. |
| --version | Show binary version. |
| --help | Show this usage message. |

## Extraction

Specify a specific chromosome to extract data only from that chromosome. This is optional; if a chromosome is not specified, data are extracted from all chromosomes in the archive.

```
$ unstarch chr12 example.starch
```

...

## Archive attributes

Archive attributes are described in greater depth in the Starch specification page. We provide an overview here of the major points.

## Metadata

Use the --list-json or --list options to export the archive metadata as a JSON- or table-formatted text string, sent to standard output:

```
$ unstarch --list-json example.starch
{
    "archive": {
        "type": "starch",
        "customUCSCHeaders": false,
        "creationTimestamp": "2014-05-01T14:09:29-0700",
        "version": {
            "major": 2,
            "minor": 2,
            "revision": 0
        },
        "compressionFormat": 0
    },
    "streams": [
        {
```

```
        "chromosome": "chr1",
        "filename": "chr1.pid31740.fiddlehead.regulomecorp.com",
        "size": "88330",
        "uncompressedLineCount": 10753,
        "nonUniqueBaseCount": 549829,
        "uniqueBaseCount": 548452,
        "duplicateElementExists": false,
        "nestedElementExists": false,
        "signature": "XtnjojM1LyuMnZI4CIneSzgLI5Q="
        "uncompressedLineMaxStringLength": 371
        },
        ...
    ]
}
```

The--list-chr (or--list-chromosomes) option exports a list of chromosomes stored in the Starch archive.

## Note

Using --note will export any note stored with the archive, when created.

Tip: One can use starchcat to add a new note to an existing Starch archive.

## Timestamp

The --archive-timestamp option will report the archive's creation date and time as an ISO 8601 -formatted string.

## Compression type

The --archive-type option will report the compression type of the archive, either bzip2 or gzip:

```
$ unstarch --archive-type example.starch
unstarch
archive compression type: bzip2
```


## Version

The --version option reports the Starch archive version. This value is different from the version of the starch binary used to create the archive.

## Whole-file or per-chromosome attributes

## Data integrity

For a specified chromosome, the --signature operator reports the very nearly unique "signature" or message digest generated from hashing the extracted, post-transform bytes within the chromosome stream with the SHA-1
hash function, followed with an encoding step with the Base64 scheme to turn it into a human-readable string.
If no chromosome is specified, this operator reports the encoded SHA-1 digests of the archive metadata and the signatures of each chromosome stream.
Signatures can be used to compare chromosome streams between two or more archives.
Further, use of the --verify-signature option with a chromosome name will compare the signature stored in the metadata (the "expected" signature) with an "observed" value generated from extracting the bytes of the chromosome record and hashing them, and then Base64-encoding the result.

If the observed and expected signatures or digests are identical, this validates or verifies the integrity of the chromosome record. A mismatch would result in a non-zero exit state and suggest potential data corruption and the need for further investigation.

## Elements

The --elements operator reports the number of BED elements that were compressed into the chromosome stream, if specified. If no chromosome is specified, the sum of elements over all chromosomes is reported.

Tip: This option is equivalent to a wc -1 (line count) operation performed on BED elements that match the given chromosome, but is much, much faster as data are precomputed and stored with the archive, retrieved from the metadata in $\mathrm{O}(1)$ time.

The --elements-max-string-length operator reports the maximum string length of BED elements over the specified chromosome, or the maximum string length over all chromosomes, if no chromosome name is specified.

## Bases

The --bases and --bases-uniq flags return the overall and unique base counts for a specified chromosome, or the sum of counts over all chromosomes, if no one chromosome is specified.

## Duplicate element(s)

The --has-duplicate operator reports whether the chromosome stream contains one or more duplicate elements, printing a 0 if the chromosome does not contain a duplicate element, and a 1 if the chromosome does contain a duplicate.

Note: A duplicate element exists if there are two or more BED elements where the chromosome name and start and stop positions are identical. Id, score, strand and any other optional columns are ignored when determining if a duplicate element is present.

Tip: To get a string value of true or false in place of 1 and 0 , use the --has-duplicate-as-string operator, instead.

Note: If the chromosome name argument to unstarch is omitted, or set to all, the --has-duplicate and --has-duplicate-as-string operators will return a result for all chromosomes (if any one chromosome has
one or more duplicate elements, the return value is 1 or true, respectively). If the chromosome name is provided and the archive does not contain metadata for the given chromosome, these operators will return a 0 or false result.

## Nested element(s)

The--has-nested operator reports whether the chromosome stream contains one or more nested elements, printing a 0 if the chromosome does not contain a nested element, and a 1 if the chromosome does contain a nested element.

Note: The definition of a nested element relies on coordinates and is explained in the documentation for nested elements. Id, score, strand and any other optional columns are ignored when determining if a nested element is present.

Tip: To get a string value of $t r u e$ or false in place of 1 and 0 , use the --has-nested-as-string operator, instead.

Note: If the chromosome name argument to unstarch is omitted, or set to all, the --has-nested and --has-nested-as-string operators will return a result for all chromosomes (if any one chromosome has one or more nested elements, the return value is 1 or true, respectively). If the chromosome name is provided and the archive does not contain metadata for the given chromosome, these operators will return a 0 or false result.

## Example

To extract a generic Starch file input to a BED file:

```
$ unstarch example.starch > example.bed
```

This creates the sorted file example.bed, containing BED data from extracting example.starch. This can be a bzip2 or gzip-formatted Starch archive—unstarch knows how to extract either type transparently.

To list the chromosomes in a Starch v2 archive, use the --list-chr (or--list-chromosomes) option:

```
$ unstarch --list-chr example.starch
chr1
chr10
chr11
chr11_gl000202_random
chr12
chr13
chr14
chr15
chr16
chr17
...
```

To show the number of BED elements in chromosome chr13, use the --elements operator:

```
$ unstarch chr13 --elements example.starch
10753
```

To find the number of unique bases in chromosome chr8:

```
$ unstarch chr8 --bases-uniq example.starch
545822
```

To report if the chromosome chr14 contains at least one duplicate BED element:

```
$ unstarch chr14 --has-duplicate-as-string example.starch
true
```

To show when the archive was created:

```
$ unstarch --archive-timestamp example.starch
2014-05-01T14:09:29-0700
```

To get the SHA-1 message digest, or "signature" of chromosome chr8, use the --signature operator:

```
$ unstarch chr8 --signature example.starch
nZI4CIneSzgLI5QXtnjojM1LyuM=
```

The signature is written to the standard output stream.
To verify the data integrity of the same chromosome, use --verify-signature:

```
$ unstarch chr8 --verify-signature example.starch
PROGRESS: Expected and observed data integrity signatures match for chromosome [chr8]
```

Any output from--verify-signature is written to the standard error stream.

Note: Some option calls will not work with legacy v1.x or v2.0 archives. For instance, to get a result for nested or duplicate elements, you need to input a v2.1 (or greater) archive. If you have a v1.x or v2.0 archive, use the starchcat utility to upgrade an older archive to a Starch v2.2 file, which will recalculate the metadata and make all current attributes available.

## starchcat

The starchcat utility efficiently merges per-chromosome records contained within one or more BEDOPS Starchformatted archives. This is an equivalent operation to bedops --everything or bedops -u (a multiset union), but inputs are starch archives rather than uncompressed BED files.

As a further advantage to using this over bedops, in the case where a starch input contains BED elements exclusive to one chromosome, this utility will directly and quickly copy over compressed elements to a new archive, avoiding the need for costly and wasteful extraction and re-compression.

In the general case, where two or more starch inputs contain BED elements from the same chromosome, a sorted merge is performed and the stream reprocessed into a Starch-formatted archive.

## Parallelization

Those with access to a computational cluster such as an Oracle/Sun Grid Engine or a group of hosts running SSH services should find starchcat highly useful, as this facilitates:

- Much faster compression of an entire genome of BED data, using nodes of a computational cluster to compress separate chromosomes, followed by a collation step with starchcat (see the Efficiently creating Starch-formatted archives with a cluster documentation).
- Extraction, manipulation and reintegration of a starch -ed chromosome into a larger starch archive
- Refreshing metadata or re-compressing the data within a lone starch archive.

To demonstrate the first application of this utility, we have packaged a helper script with the BEDOPS suite called starchcluster, which archives data much faster than starch alone. By distributing work across the nodes of a computational cluster, the upper bound on compression time is almost entirely determined by the largest chromosome, reducing compression time by an order of magnitude.

## Inputs and outputs

## Input

The input to starchcat consists of one or more BEDOPS Starch-formatted archive files.

Note: If a single archive is provided as input, it may be reprocessed with specified options. When two or more archives are specified, the output will be the equivalent of a multiset union of the inputs.

Note: This utility does not accept standard input.

## Output

The starchcat tool outputs a starch -formatted archive to standard output, which is usually redirected to a file.
Additionally, an optional compression flag specifies if the final starch output should be compressed with either the bzip2 or gzip method (the default being bzip2).

Note: If starch inputs use a different backend compression method, the input stream is re-compressed before integrated into the larger archive. This will incur extra processing overhead.

## Usage

Use the --help option to list all options:

```
starchcat
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36 (typical)
    authors: Alex Reynolds and Shane Neph
USAGE: starchcat [ --note="..." ]
    [ --bzip2 | --gzip ]
    [ --omit-signature ]
    [ --report-progress=N ] <starch-file-1> [<starch-file-2> ...]
    * At least one lexicographically-sorted, headerless starch archive is
        required.
    * While two or more inputs make sense for a multiset union operation, you
```

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```
    can starchcat one file in order to update its metadata, recompress it
    with a different backend method, or add a note annotation.
* Compressed data are sent to standard output. Use the '>' operator to
    redirect to a file.
Process Flags
--note="foo bar..." Append note to output archive metadata (optional).
--bzip2 | --gzip Specify backend compression type (optional, default
        is bzip2).
--omit-signature Skip generating per-chromosome data integrity signature
        (optional, default is to generate signature).
--report-progress=N Report compression progress every N elements per
        chromosome to standard error stream (optional)
--version Show binary version.
--help Show this usage message.
```


## Per-chromosome data integrity signature

By default, a data integrity signature is generated for each chromosome. This can be used to verify if chromosome streams from two or more Starch archives are identical, or used to test the integrity of a chromosome, to identify potential data corruption.

Generating this signature adds to the computational cost of compression, or an integrity signature may not be useful for all archives. Add the --omit-signature option, if the compression time is too high or the data integrity signature is not needed.

## Example

Let's say we have a set of 23 starch archives, one for each chromosome of the human genome: chr1. starch, chr2.starch, and so on, to chrY.starch. (To simplify this example, we leave out mitochondrial, random, pseudo- and other chromosomes.) We would like to build a new starch archive from these 23 separate files:
\$ starchcat chr1.starch chr2.starch ... chrY.starch > humanGenome.starch

The starchcat utility parses the metadata from each of the 23 inputs, determines what data to either simple copy or reprocess, and then it performs the merge. Cleanup is performed afterwards, as necessary, and the output is a brand new starch file, written to humanGenome. starch.

Note: No filtering or processing is performed on extracted BED elements, before they are written to the final output. Thus, it is possible for duplicate BED elements to occur. It would be easy to use the --signature option to validate the expected content of a new Starch archive.

However, the final archive is sorted per sort-bed ordering, so that data extracted from this archive will be ready for use with BEDOPS utilities.

Note: When input archives contain data on disjoint chromosomes, use of starchcat is very efficient as data are simply copied, instead of extracted and re-compressed.

## Starch (v2.x) specification

This document describes the specification for a "Starch v2.x"-formatted archive, which is created by the starch and starchcat utilities and extracted with the unstarch utility.

## Archive structure

A Starch v2.x archive is divided up into six portions:

| ca5cade5 | chr1 | -* | chrn | \{...\} | 000000 | HUzESr... | +-7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| magic bytes | chromosome streams |  |  | metadata | metadata offset | checksum | padding |
| 4 bytes | compressed, variable length |  |  | variable length | 20 bytes | 28 bytes | 80 bytes |

Each portion is explained below.

## Magic bytes

## magic bytes

## 4 bytes

We use four unsigned char bytes ca5cade 5 to identify the file as a Starch $\mathrm{v} 2 . \mathrm{x}$ archive. BEDOPS utilities and applications which process Starch archives search for these magic bytes at the start of the file to identify it as a v2.x archive.
If the file does not have these bytes, it may still be a legacy (v1, v1.2 or v1.5) Starch archive, which is identified and processed by other means not described in this document.

## Chromosome streams

## chr1



## chromosome streams

## compressed, variable length

These variable-length data streams contain compressed, transformed BED data separated by chromosome.
Transformation is performed on BED input to remove redundancy in the coordinate data provided in the second and third columns ("start" and "stop" coordinates). Data in any additional columns are left unchanged. Transformed data are highly reduced and compressed further with open-source bzip2 or gzip libraries.

Starch v2 streams extracted with unstarch, bedops, bedmap or closest-features are uncompressed with the requisite backend compression library calls and then reverse-transformed to recover the original BED input.

## Metadata

The archive metadata is made up of data, offset and hash components, each with different characteristics as described below.

## Data

## $\{.$.

## metadata

## variable length

This variable-length portion of the archive is a JSON -formatted ASCII string that describes the Starch archive contents. We choose JSON as it provides a human-readable structure, allows easier extensibility for future revisions of BEDOPS and is a common format in web services, facilitating usage with web- and command-line-based bioinformatics pipelines.

The format of a typical Starch v2 JSON object is made up of two key-value pairs, one for archive and the second for streams, which we describe in greater detail below.

## Archive

The archive key scheme is described below:

```
{
    "archive": {
        "type": "starch",
        "customUCSCHeaders": (Boolean),
        "creationTimestamp": (string),
        "version": { "major": 2, "minor": 2, "revision": 0 },
        "compressionFormat": (unsigned integer),
        "note": (string, optional)
    },
    ...
}
```

At this time, the type key will specify starch.
The customUCSCHeaders value is either true or false. If $t r u e$, the --header option was provided to starch when the archive was created, and the archive may likely contain UCSC headers commonly encountered with UCSC Genome Browser data downloads. Archives created with starchcat do not support UCSC headers (i.e., this value is false in archives created with starchcat).

The creationTimestamp value is an ISO 8601 string that specifies the creation date and time of the archive. Most scripting and programming languages can parse ISO 8601 -formatted date strings with little or no extra work.

The version is a triplet of integer values specifying the version of the archive. For a v2.x archive, the major version will be set to 2 . Major, minor and revision values need not necessarily be the identical to the version of the starch binary used to create the archive.

At this time (November 2016), we offer v2.0, v2.1, and v2.2 archives: Each version makes different stream metadata fields available.

The compressionFormat key specifies the backend compression format used for the chromosome streams contained within the archive. We currently use 0 to specify bzip2 and 1 to specify gzip. No other backend formats are available at this time.

The note key is an optional string that can contain information if the --note="abc. . . " option is provided to starch when the archive is created. If this option is not specified at creation time, this key will not be present in the metadata.

## Streams

The streams key scheme contains an array of objects, each describing the attributes of an individually-compressed chromosome stream, sorted on chromosome name:

```
{
    "streams": [
    {
        "chromosome": (string),
        "filename": (string),
        "size": (unsigned integer),
        "uncompressedLineCount": (unsigned integer),
        "nonUniqueBaseCount": (unsigned integer),
        "uniqueBaseCount": (unsigned integer),
        "duplicateElementExists": (Boolean),
        "nestedElementExists": (Boolean),
        "signature": (string),
        "uncompressedLineMaxStringLength": (integer)
    },
```

```
    ]
```

\}

The chromosome key specifies the name of the chromosome associated with the compressed regions. For example, this might be chr1, chrX, etc.

The filename key is a string that concatenates the chromosome name, process ID and host strings (unless a unique-tag value is given to starch when creating an archive, which would replace the process ID and host values). It is a holdover from a procedure for creating legacy archives and exists for backwards-compatibility.

The size key specifies the byte-size of the compressed stream and exists for calculating offsets within the archive where a chromosome stream begins (and ends). In this way, unstarch and other Starch-capable applications can extract data only from a desired chromosome, without wasteful processing of the remainder of the archive.
The uncompressedLineCount key specifies the number of BED elements that were compressed into the chromosome stream. This is a precomputed equivalent to the result of a wc -l (line count) operation performed on BED elements that match the given chromosome, without needing to stream through the entire file.

The nonUniqueBaseCount key specifies the sum of non-unique bases across all BED elements compressed into the chromosome stream. Non-uniqueness allows multiple counting of bases in elements which overlap.

The uniqueBaseCount key specifies the sum of unique bases across all BED elements compressed into the chromosome stream. Uniqueness takes into account overlapping elements and therefore only counts bases once.

The duplicateElementExists key specifies if there is a duplicate BED element somewhere within the compressed chromosome stream. A duplicate element is defined by matching chromosome name and start and stop coordinates; id, score, strand and other optional information are ignored when determining if a duplicate element exists.

The nestedElementExists key specifies if there is a nested BED element somewhere within the compressed chromosome stream. Refer to BEDOPS documentation to see how nested elements are defined.

The signature key, available in v2.2 archives, specifies the Base64-encoded SHA-1 data integrity signature generated from the transformed chromosome stream (not the raw BED data, but the reduced or transformed form that is compressed). This can be used to compare the transformed bytes for chromosomes from different archives, or to validate the genomic data in a Starch archive by chromosome, or in entirety. (Note that, if --omit-signature was used to create a v2.2 archive, this key will not be present in the stream object.)

The uncompressedLineMaxStringLength key, available in v2.2 archives, specifies the maximum string length over all records in the chromosome stream.

## Offset

## 0000000000123456

## metadata offset

## 20 bytes

The metadata offset is a 20-byte long, zero-padded string that specifies the number of bytes into the file where the JSON-formatted metadata string is stored.

The unstarch utility and the newer versions of bedops and bedmap applications use this offset to jump to the correct point in the file where the metadata can be read into memory and processed into an internal data structure.

## Hash

## 

## checksum

## 28 bytes

The metadata hash is a 28 -byte long, Base64 -encoded SHA-1 hash of the bytes that make up the JSON-formatted metadata string.

This data is used to validate the integrity of the metadata: Any change to the metadata (e.g., data corruption that changes stream offset values, or the data integrity signatures for each chromosome stream) causes unstarch and other Starch utilities and applications to exit early with a fatal, informative error.

## Padding



## padding <br> 80 bytes

The remainder of the file is made up of 80 bytes of padding, which are unused at this time.

## starch-diff

This tool allows the end user to quickly compare the compressed chromosomes in two or more v2.2+ Starch archives.

## Inputs and outputs

## Input

The starch-diff utility takes in two or more Starch v2.2+ archives as input. The end user may also add -chr <chr> to compare one chromosome directly; otherwise, all chromosomes in specified archives are compared.

## Output

The typical output of starch-diff is a message indicating the archives' chromosome(s) are identical or dissimilar.
In addition, if the chromosomes are identical, starch-diff exits with a zero status code. Likewise, if any chromosomes are dissimilar, starch-diff exits with a non-zero status code.

## Requirements

If the user passes in a pre-v 2.2 archive, the utility will exit with a fatal error.

## Usage

Use the --help option to list all options:

```
starch-diff
    citation: http://bioinformatics.oxfordjournals.org/content/28/14/1919.abstract
    version: 2.4.36
    authors: Alex Reynolds and Shane Neph
    $ starch-diff [ --chr <chr> ] starch-file-1 starch-file-2 [ starch-file-3 ... ]
    The 'starch-diff' utility compares the signatures of two or more specified
    Starch v2.2+ archives for all chromosomes, or for a specified chromosome.
```


## Data conversion

Wrapper scripts around the convert2bed utility quickly convert a variety of common genomic data types to BED with no loss of information. In using these tools, you can easily prepare data from these formats for use with core BEDOPS tools, whether VCF, GFF/GTF/GVF, BAM/SAM, PSL (Blat), RepeatMasker annotation output, etc.

Some other formats not covered here can be converted with, for instance, the UCSC Kent toolset (e.g., altGraphX, bigWig, bigBed, etc.). Just remember to use the sort-bed utility to prepare BED output from external programs for use with BEDOPS core tools.

## convert2bed

The convert2bed binary converts common binary and text genomic formats (BAM, GFF, GTF, GVF, PSL, RepeatMasker annotation output (OUT), SAM, VCF and WIG) to unsorted or sorted, extended BED or BEDOPS Starch (compressed BED) with additional per-format options.
Convenience wrapper bash scripts are provided for each of these input formats, which convert standard input to unsorted or sorted BED, or to BEDOPS Starch (compressed BED). Scripts expose format-specific convert2bed options.

We also provide bam2bed_sge, bam2bed_gnuParallel, bam2starch_sge and bam2starch_gnuParallel convenience scripts, which parallelize the conversion of indexed BAM to BED or to BEDOPS Starch via a Sun Grid Engine-based computational cluster or local GNU Parallel installation.

## Dependencies

Conversion of BAM and SAM input is dependent upon the installation of SAMtools and convert2bed. All $* 2$ starch wrapper scripts are further dependent on the installation of the starch binary, part of a typical BEDOPS installation.

## Source

The convert2bed conversion tool is part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

Generally, to convert data in format xyz to sorted BED:

```
$ convert2bed -i xyz < input.xyz > output.bed
```

Add the -o starch option to write a BEDOPS Starch file, which stores compressed BED data and feature metadata:

```
$ convert2bed -i xyz -o starch < input.xyz > output.starch
```

Wrappers are available for each of the supported formats to convert to BED or Starch, e.g.:

```
$ bam2bed < reads.bam > reads.bed
$ bam2starch < reads.bam > reads.starch
```

Tip: Format-specific options are available for each wrapper; use --help with a wrapper script or --help-bam, $--h e l p-g f f$ etc. with convert 2 bed to get a format-specific description of the conversion procedure and options.

## Example

Please review documentation for each wrapper script to see format-specific examples of their use.

## bam2bed

The bam2bed script converts 0-based, half-open [start-1, end) Binary (Sequence) Alignment/Map (BAM) to sorted, 0-based, half-open [start-1, end) UCSC BED data.

For convenience, we also offer bam2 starch, which performs the extra step of creating a Starch-formatted archive.
The bam2bed script is "non-lossy" (with the use of specific options, described below). Other toolkits tend to throw out information from the original BAM input upon conversion; bam2bed can retain everything, facilitating reuse of converted data and conversion to other formats.

Tip: Doing the extra step of creating a Starch-formatted archive can save a lot of space relative to the original BAM format, up to $33 \%$ of the original BAM dataset, while offering per-chromosome random access.

## Dependencies

The bam2bed wrapper script is dependent upon the installation of SAMtools and convert2bed. The bam2starch wrapper script is further dependent on the installation of the starch binary, part of a typical BEDOPS installation.

## Source

The bam2bed and bam2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The bam2bed script parses BAM data from standard input and prints sorted BED to standard output. The bam2starch script uses an extra step to parse BAM to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a BAM file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Tip: If you work with RNA-seq data, you may use the --split option to process reads with N-CIGAR operations, splitting them into separate BED elements.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If sorting converted data larger than system memory, use the -- max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., $--\max -\operatorname{mem} 2 \mathrm{G}$ or similar. See -- help for more details.

## Example

To demonstrate these scripts, we use a sample binary input called foo.bam (see the Downloads section to grab this file).

We can convert it to sorted BED data in the following manner (omitting standard error messages):

(continues on next page)


Note that we strip the header section from the output. If we want to keep this, the use of the --keep-header option will preserve the BAM file's header, turning it into BED elements that use _header as a chromosome name.

Here's an example:


With this option, the bam2bed and bam2starch scripts are completely "non-lossy" (with the exception of unmapped reads; see note below). Use of awk or other scripting tools can munge these data back into a SAM-formatted file.

Note: The provided scripts strip out unmapped reads from the BAM file. We believe this makes sense under most circumstances. Add the --all-reads option if you need unmapped and mapped reads.

## Column mapping

In this section, we describe how non-header BAM data (converted to SAM columns) are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| SAM field | BED column index | BED field |
| :--- | :--- | :--- |
| RNAME | 1 | chromosome |
| POS -1 | 2 | start |
| POS + length(CIGAR) -1 | 3 | stop |
| QNAME | 4 | id |
| MAPQ | 5 | score |
| $16 \&$ FLAG | 6 | strand |

The remaining SAM-converted columns are mapped as-is, in same order, to adjacent BED columns:

| SAM field | BED column index | BED field |
| :--- | :--- | :--- |
| FLAG | 7 |  |
| CIGAR | 8 |  |
| RNEXT | 9 |  |
| PNEXT | 10 |  |
| TLEN | 11 |  |
| SEQ | 12 |  |
| QUAL | 13 |  |

Because we have mapped all columns, we can translate converted BED data back to headered or headerless SAM reads with a simple awk statement (or other script) that reverts back to 1-based coordinates and permutes columns to SAM-based ordering.

## Downloads

- Sample BAM dataset: foo.bam


## Parallel bam2bed

The bam2bed_slurm, bam2bed_sge, and bam2bed_gnuParallel scripts use a SLURM, Sun or Oracle Grid Engine (SGE/OGE), or GNU Parallel job scheduler, respectively, to parallelize the work of bam2bed, which converts an indexed, 0-based, half-open [start-1, end) Binary (Sequence) Alignment/Map (BAM) file to a sorted, 0based, half-open [start-1, end) UCSC BED dataset.

This script splits the indexed BAM file by chromosome name. Each chromosome of BAM records is converted to a BED-formatted dataset with bam2bed (via convert2bed). Once all per-chromosome BED files are made, they are collated into one final BED file with a multiset union performed with bedops -everything.

## Dependencies

This shell script is dependent upon a working computational grid that is managed with SLURM 16.05.0, Sun Grid Engine 6.1 u 5 (or higher), or installation of GNU Parallel v20130922 or greater.

## Source

The bam2bed_slurm, bam2bed_sge, and bam2bed_gnuParallel conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

Note: Please review and edit the contents of the relevant script before use with your data, particularly if you use a Sun or Oracle Grid Engine environment and make use of the SGE version of this script. Customization may be required to match your SLURM, SGE/OGE or GNU Parallel installation and environment, as well as the nature of your BAM data.

At minimum, use of this script with an SGE/OGE computational cluster will require editing of the queue parameter, possible adjustments to qsub options, and may also require adjustments to paths to working BEDOPS and Samtools binaries. If you use the module system, running module add samtools will take care of this dependency.
You will also need to make sure your BAM data are indexed. There must be a second BAI file with the same prefix name as the BAM file you wish to compress, located in the same working directory. If this index file is not present, the script will exit early with an error.

You may also wish to review other parameters available with the bam2bed script, applying them in this script as needed (see the bam2bed documentation for more details).

## Parallel bam2starch

The bam2starch_slurm, bam2starch_sge, and bam2starch_gnuParallel scripts use a SLURM, Sun or Oracle Grid Engine (SGE/OGE), or GNU Parallel job scheduler, respectively, to parallelize the work of bam2starch, which converts an indexed, 0-based, half-open [start-1, end) Binary (Sequence) Alignment/Map (BAM) file to a sorted, 0-based, half-open [start-1, end) UCSC BED dataset, and thence converts this to a Starch-formatted archive.

This script splits the indexed BAM file by chromosome name. Each chromosome of BAM records is converted to a Starch-formatted archive with bam2starch (via convert2bed). Once all per-chromosome archives are made, they are collated into one final Starch archive with starchcat.

Tip: A Starch-formatted archive can save a great deal of space relative to the original BAM format, up to 33\% of the original BAM dataset, while offering per-chromosome random access. Further, use of a computational grid practically reduces the total compression time to that of the largest chromosome (e.g., chr1 or similar), an order of magnitude reduction over bam2starch alone.

## Dependencies

This shell script is dependent upon a working computational grid that is managed with SLURM 16.05.0, Sun Grid Engine 6.1u5 (or higher), or installation of GNU Parallel v20130922 or greater.

## Source

The bam2starch_slurm, bam2starch_sge, and bam2starch_gnuParallel conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

Note: Please review and edit the contents of this script before use with your data. Customization may be required to match your SLURM, SGE/OGE, or GNU Parallel installation and environment, as well as the nature of your BAM data.

At minimum, use of this script with an SGE/OGE computational cluster will require editing of the queue parameter, possible adjustments to qsub options, and may require adjustments to paths to working BEDOPS binaries.

You will also need to make sure your BAM data are indexed. There must be a second BAI file with the same prefix name as the BAM file you wish to compress, located in the same working directory. If this index file is not present, the script will exit early with an error.

You may also wish to review other parameters available with the bam2starch script, applying them in this script as needed (see the bam2bed documentation for more details).

## gff2bed

The gff2bed script converts 1-based, closed [start, end] General Feature Format v3 (GFF3) to sorted, 0based, half-open [start-1, end) extended BED-formatted data.

For convenience, we also offer $g f f 2 s t a r c h$, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The $g f f 2$ bed script requires convert2bed. The $g f f 2$ starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the GFF3 specification. A GFF3-format validator is available here to ensure your input follows specification.

Tip: Conversion of data which are GFF-like, but which do not follow the specification can cause parsing issues. If you run into problems, please check that your input follows the GFF3 specification. Tools such as the GFF3 Online Validator are useful for this task.

## Source

The $g f f 2$ bed and $g f f 2$ starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The $g f f 2$ bed script parses GFF3 from standard input and prints sorted BED to standard output. The gff2starch script uses an extra step to parse GFF to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a GFF file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the $--\mathrm{do}-\mathrm{not}-\mathrm{sort}$ option. Run the script with the - -help option for more details.

Tip: If sorting converted data larger than system memory, use the -- max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., --max-mem 2G or similar. See --help for more details.

## Example

To demonstrate these scripts, we use a sample GFF input called foo.gff (see the Downloads section to grab this file).

| \#\#gff-version 3 |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| chr1 | Canada | exon | 1300 | 1300 | . | + | . | ID=exon00001; score=1 |
| chr1 | USA | exon | 1050 | 1500 | . | - | 0 | ID=exon00002; Ontology_ |
| $\rightarrow$ ¢erm="GO:0046703"; Ontology_term="GO:0046704" |  |  |  |  |  |  |  |  |
| chr1 | Canada | exon | 3000 | 3902 | . | ? | 2 | ID=exon00003; score=4; |
| $\rightarrow$ Name=foo |  |  |  |  |  |  |  |  |
| chr1 | . | exon | 5000 | 5500 | . | . | . | ID=exon00004; Gap=M8 |
| $\rightarrow$ D3 | I1 M6 |  |  |  |  |  |  |  |
| chr1 | . | exon | 7000 | 9000 | 10 | + | 1 | ID=exon00005; Dbxref= |
| $\hookrightarrow " N C B I \_g i: 10727410 "$ |  |  |  |  |  |  |  |  |

We can convert it to sorted BED data in the following manner:

| \$ gff2bed < foo.gff3 |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| chr1 | 1049 | 1500 | exon00002 | . | - | USA | exon | 0 | - |
| $\hookrightarrow I D=e x o n 00002$; Ontology_term="GO:0046703"; Ontology_term="GO:0046704" |  |  |  |  |  |  |  |  |  |
| chr1 | 1299 | 1300 | exon00001 | . | + | Canada | exon | - | - |
| $\hookrightarrow I D=e x o n 00001$; score=1; zeroLengthInsertion=True |  |  |  |  |  |  |  |  |  |
| chr1 | 2999 | 3902 | exon00003 | . | $?$ | Canada | exon | 2 | - |
| $\hookrightarrow I D=e x o n 00003$; score $=4$; Name=foo |  |  |  |  |  |  |  |  |  |
| chr1 | 4999 | 5500 | exon00004 | - | - | . | exon | - | - |
| $\hookrightarrow I D=e x o n 00004 ; G a p=M 8$ D3 M6 I1 M6 |  |  |  |  |  |  |  |  |  |
| chr1 | 6999 | 9000 | exon00005 | 10 | $+$ | - | exon | 1 | - |
| $\hookrightarrow I D=e x o n 00005$; Dbxref="NCBI_gi:10727410" |  |  |  |  |  |  |  |  |  |

The default usage strips the leading pragma, or header (\#\#gff-version 3), but adding the --keep-header option will preserve this as a BED element that uses _header as a chromosome name:


Note: Zero-length insertion elements are given an extra attribute called zeroLengthInsertion which lets a BED-to-GFF or other parser know that the element will require conversion back to a right-closed element [a, b], where a and b are equal.

Note: Note the conversion from 1- to 0-based coordinate indexing, in the transition from GFF3 to BED. BEDOPS supports operations on input with any coordinate indexing, but the coordinate change made here is believed to be convenient for most end users.

## Column mapping

In this section, we describe how GFF3 columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| GFF3 field | BED column index | BED field |
| :--- | :--- | :--- |
| seqid | 1 | chromosome |
| start | 2 | start |
| end | 3 | stop |
| ID (via attributes) | 4 | id |
| score | 5 | score |
| strand | 6 | strand |

The remaining columns are mapped as follows:

| GFF3 field | BED column index | BED field |
| :--- | :--- | :--- |
| source | 7 |  |
| type | 8 |  |
| phase | 9 |  |
| attributes | 10 |  |

If we encounter zero-length insertion elements (which are defined where the start and stop GFF3 field values are equivalent), the start coordinate is decremented to convert to 0-based, half-open indexing, and a zero_length_insertion attribute is added to the attributes GFF3 field value.

## Downloads

- Sample GFF dataset: foo.gff
gtf2bed

The gtf2bed script converts 1-based, closed [start, end] Gene Transfer Format v2.2 (GTF2.2) to sorted, 0based, half-open [start-1, end) extended BED-formatted data.

For convenience, we also offer gtf2starch, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The $g t f 2$ bed script requires convert2bed. The $g t f 2$ starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the GTF 2.2 specification. A GTF-format validator is available here to ensure your input follows specification.

Tip: Conversion of data which are GTF-like, but which do not follow the specification can cause parsing issues. If you run into problems, please check that your input follows the GTF specification.

## Source

The gtf2bed and gtf2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The gt f 2 bed script parses GTF from standard input and prints sorted BED to standard output. The gtf2starch script uses an extra step to parse GTF to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If sorting converted data larger than system memory, use the -- max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., $--\max -\mathrm{mem} 2 \mathrm{G}$ or similar. See --help for more details.

## Example

To demonstrate these scripts, we use a sample GTF input called foo.gtf (see the Downloads section to grab this file).


We can convert it to sorted BED data in the following manner:

```
$ gtf2bed < foo.gtf
chr20 9874840 9874841 ZNF366 . protein_coding exon . . . gene_
\hookrightarrowid "ENSBTAG00000020601"; transcript_id "ENSBTAT0000002.4.36"; gene_name "ZNF366";
\hookrightarrowzero_length_insertion "True";
chr20 9873503 9874841 ZNF366 . + protein_coding CDS 0 0 gene_
\hookrightarrowid "ENSBTAG00000020601"; transcript_id "ENSBTAT0000002.4.36"; gene_name "ZNF366";
chr20 9877487 9877679 ENSBTAG00000020601 + . protein_coding exon -
\hookrightarrow . gene_id "ENSBTAG00000020601"; transcript_id "ENSBTAT0000002.4.36";
```

Tip: After, say, performing set or statistical operations with bedops, bedmap etc., converting data back to GTF is accomplished through an awk statement that re-orders columns and shifts the coordinate index:

```
$ awk '{print $1"\t"$7"\t"$8"\t"($2+1)"\t"$3"\t"$5"\t"$6"\t"$9"\t"(substr($0, index(
\hookrightarrow$0,$10)))}' foo_subset.bed > foo_subset.gtf
```

Note: Zero-length insertion elements are given an extra attribute called zero_length_insertion which lets a BED-to-GTF or other parser know that the element will require conversion back to a right-closed element [a,b], where a and b are equal.

Note: Note the conversion from 1- to 0 -based coordinate indexing, in the transition from GTF to BED. BEDOPS supports operations on input with any coordinate indexing, but the coordinate change made here is believed to be convenient for most end users.

## Column mapping

In this section, we describe how GTF2.2 columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| GFF2.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| seqname | 1 | chromosome |
| start | 2 | start |
| end | 3 | stop |
| gene_id | 4 | id |
| score | 5 | score |
| strand | 6 | strand |

The remaining columns are mapped as follows:

| GFF2.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| source | 7 |  |
| feature | 8 |  |
| frame | 9 |  |
| attributes | 10 |  |

If present in the GTF2.2 input, the following column is also mapped:

| GFF2.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| comments | 11 |  |

If we encounter zero-length insertion elements (which are defined where the start and stop GFF3 field values are equivalent), the start coordinate is decremented to convert to 0 -based, half-open indexing, and a zero_length_insertion attribute is added to the attributes GTF2.2 field value.

## Downloads

- Sample GTF dataset: foo.gtf


## gvf2bed

The gvf2bed script converts 1-based, closed [start, end] Genome Variation Format (GVF, a type of General Feature Format v3 or GFF3) to sorted, 0-based, half-open [start-1, end) extended BED-formatted data.

For convenience, we also offer gvf2starch, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The gvf2bed script requires convert2bed. The gvf2starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the GFF3 specification. A GFF3-format validator is available here to ensure your input follows specification.

Tip: Conversion of data which are GFF-like, but which do not follow the specification can cause parsing issues. If you run into problems, please check that your input follows the GFF3 specification. Tools such as the GFF3 Online Validator are useful for this task.

## Source

The gvf2bed and gvf2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The gvf2bed script parses GVF from standard input and prints sorted BED to standard output. The gvf2starch script uses an extra step to parse GVF to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.
The header data of a GVF file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If sorting converted data larger than system memory, use the --max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., --max-mem 2G or similar. See --help for more details.

## Example

To demonstrate these scripts, we use a sample GVF input called foo.gvf (see the Downloads section to grab this file).

```
##gvf-version 1.07
##feature-ontology http://www.sequenceontology.org/resources/obo_files/current_
\hookrightarrowrelease.obo
##multi-individual NA19240,NA18507,NA12878,NA19238
##genome-build NCBI B36.3
```

```
##sequence-region chr16 1 88827254
chr16 dbSNP SNV 49291360 49291360 + . . . ND=ID_2;
\hookrightarrowVariant_seq=C,G;Individual=0,1,2,3;Genotype=0:1,0:0,1:1,0:1;
chr16 dbSNP SNV 49302125 49302125 + . . ND=ID_3;
\hookrightarrowVariant_seq=C,T; Individual=0,1,3;Genotype=0:1,2:2,0:2;
chr16 dbSNP SNV 49302365 49302365 + . . ND=ID_4;
\hookrightarrowVariant_seq=G; Individual=0,1;Genotype=0:0,0:0;
chr16 dbSNP SNV 49302700 49302700 + . . ND=ID_5;
\hookrightarrowVariant_seq=C,T; Individual=2,3;Genotype=0:1,0:0;
chr16 dbSNP SNV 49303084 49303084 + . . ND=ID_6;
\hookrightarrowVariant_seq=T,G,A;Individual=3;Genotype=1, 2:;
chr16 dbSNP SNV 49303427 49303427 + . . ND=ID_8;
\hookrightarrowVariant_seq=T;Individual=0;Genotype=0:0;
chr16 dbSNP SNV 49303596 49303596 + . . ND=ID_9;
\hookrightarrowVariant_seq=A,G,T;Individual=0,1,3;Genotype=1:2,3:3,1:3;
```

We can convert it to sorted BED data in the following manner:


As shown, the default usage strips the leading pragmas (\#\#gvf-version 1.07 , etc.), but adding the --keep-header option will preserve pragmas as BED elements that use _header as a chromosome name:

(continues on next page)


Note: Zero-length insertion elements are given an extra attribute called zero_length_insertion which lets a BED-to-GVF or other parser know that the element will require conversion back to a right-closed element [a, b], where $a$ and $b$ are equal.

Note: Note the conversion from 1- to 0-based coordinate indexing, in the transition from GVF to BED. BEDOPS supports operations on input with any coordinate indexing, but the coordinate change made here is believed to be convenient for most end users.

## Column mapping

In this section, we describe how GVF columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| GVF field | BED column index | BED field |
| :--- | :--- | :--- |
| seqid | 1 | chromosome |
| start | 2 | start |
| end | 3 | stop |
| ID (via attributes) | 4 | id |
| score | 5 | score |
| strand | 6 | strand |

The remaining columns are mapped as follows:

| GVF field | BED column index | BED field |
| :--- | :--- | :--- |
| source | 7 |  |
| type | 8 |  |
| phase | 9 |  |
| attributes | 10 |  |

When we encounter zero-length insertion elements (which are defined where the start and stop GVF field values are equivalent), the start coordinate is decremented to convert to 0-based, half-open indexing, and a zero_length_insertion attribute is added to the attributes field value.

## Downloads

- Sample GVF dataset: foo.gvf


## psl2bed

The psl2bed script converts 0-based, half-open [start-1, end) Pattern Space Layout (PSL) to sorted, 0-based, half-open [start-1, end) extended BED-formatted data.

For convenience, we also offer psl2starch, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The psl2bed script requires convert2bed. The psl2starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the PSL specification.

Tip: Conversion of data which are PSL-like, but which do not follow the specification can cause parsing issues. If you run into problems, please check that your input follows the PSL specification.

## Source

The psl2bed and psl2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The psl2bed script parses PSL from standard input and prints sorted BED to standard output. The psl2starch script uses an extra step to parse GFF to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a headered PSL file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

If your data contains a record with multiple blocks (block count is greater than one, and the tStarts field has multiple target start positions), you can use the --split option to print that record to separate BED elements, each with a start position defined by tStarts and a length defined by the associated value in the blockSizes string.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If you are sorting data larger than system memory, use the $--m a x-m e m$ option to limit sort memory usage to a reasonable fraction of available memory, e.g., --max-mem 2G or similar. See --help for more details.

## Example

To demonstrate these scripts, we use a sample PSL input called foo.psl (see the Downloads section to grab this file).


We can convert it to sorted BED data in the following manner:



As you see here, the header data of a headered PSL file is discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Here is a demonstration of conversion of the same headered input, adding the --keep -header option:

(continues on next page)


With this option, the psl2bed and psl2starch scripts are completely "non-lossy". Use of awk or other scripting tools can munge these data back into a PSL-formatted file.

This example PSL file contains one record with a block count of 2 . If we were to add the $--s p l i t$ option, this record would be split into two separate BED elements that have start positions 32933028 and 32933032 , with lengths 4 and 31, respectively. These elements fall within the genomic range already defined by the $t$ Start and $t$ End fields (32933028 and 32933063 ).

Note: The psl2bed and psl2starch scripts work with headered or headerless PSL data.

Note: By default, the psl2bed and psl2starch scripts assume that PSL data do not need splitting. If you expect your data to contain multiple blocks, add the --split option.

## Column mapping

In this section, we describe how PSL columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| PSL field | BED column index | BED field |
| :--- | :--- | :--- |
| tName | 1 | chromosome |
| tStart $(*)$ | 2 | start |
| tEnd $\left(^{*}\right)$ | 3 | stop |
| qName | 4 | id |
| matches | 5 | score |
| strand | 6 | strand |

The remaining PSL columns are mapped, in order, to the remaining columns of the BED output:

| PSL field | BED column index | BED field |
| :--- | :--- | :--- |
| qSize | 7 |  |
| misMatches | 8 |  |
| repMatches | 9 |  |
| nCount | 10 |  |
| qNumInsert | 11 |  |
| qBaseInsert | 12 |  |
| tNumInsert | 13 |  |
| tBaseInsert | 14 |  |
| qStart | 15 |  |
| qEnd | 16 |  |
| tSize | 17 |  |
| blockCount | 18 |  |
| blockSizes | 19 |  |
| qStarts | 20 |  |
| tStarts | 21 |  |

This is a lossless mapping. Because we have mapped all columns, we can translate converted BED data back to headerless PSL with a simple awk statement that permutes columns to PSL-based ordering:

```
$ awk 'BEGIN { OFS = "\t" } \
    { \
        print $5" "$8" "$9" "$10" "$11" "$12" "$13" "$14" "$6" "$4" "$7" "$15" "$16" "$1"
\hookrightarrow"$17" "$2" "$3" "$18" "$19" "$20" "$21 }' converted.bed > original.psl
```

In the case where the --split option is added, the $t$ Start and $t$ End fields are replaced with each of the values in the larger $t$ Starts string, added to the respective values in the larger blockSizes string. This is still a lossless conversion, but modifications to the awk script printed above would be required to rebuild the original PSL.

## Downloads

- Sample PSL dataset: foo.psl


## rmsk2bed

The rmsk2bed script converts 1-based, closed [start, end] RepeatMasker annotation output (OUT) to sorted, 0 -based, half-open [start-1, end) extended BED-formatted data.
For convenience, we also offer rmsk2starch, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The rmsk 2 bed script requires convert2bed. The rmsk2starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the RepeatMasker annotation output specification, outlined here: http://www.repeatmasker.org/webrepeatmaskerhelp.html.

## Source

The rmsk 2 bed and rmsk2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The rmsk2bed script parses RepeatMasker annotation output from standard input and prints sorted BED to standard output. The rmsk2starch script uses an extra step to parse RepeatMasker annotation output to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.
The header data of a RepeatMasker annotation output file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the $--\mathrm{do}-$ not-sort option. Run the script with the -- help option for more details.

Tip: If sorting converted data larger than system memory, use the -- max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., $--\max -\mathrm{mem} 2 \mathrm{G}$ or similar. See -- help for more details.

## Example

To demonstrate these scripts, we use a sample RepeatMasker annotation output input called foo. out (see the Downloads section to grab this file).

| SW perc | perc | erc | query | position in query |  |  |  | matching repeat |  | position in |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \hookrightarrow \text { repeat } \\ & \text { score div. } \\ & \hookrightarrow \text { (left) ID } \end{aligned}$ | l. | ns. | sequence | begin | end | (left) |  | repeat | class/family | begin end |
|  | $\begin{aligned} & 6.2 \\ & 20 \end{aligned}$ | 0.0 | HSU08988 | $6563$ | $6781$ | (22462) | C | MER 7 A | DNA/MER2_type | (0) ـ |
| $\begin{array}{cc} 12279 & 10.5 \\ \hookrightarrow 2418 & 1486 \end{array}$ | $\begin{array}{r} 2.1 \\ 19 \end{array}$ | 1.7 | HSU08988 | 6782 | 7718 | (21525) | C | Tigger1 | DNA/MER2_type | $(0)$ |
| $\begin{array}{rr} 1769 & 12.9 \\ \leftrightarrow 317 & 1 \end{array}$ | $\begin{aligned} & 6.6 \\ & 17 \end{aligned}$ | 1.9 | HSU08988 | 7719 | 8022 | (21221) | C | AluSx | SINE/Alu | (0) - |
| $\begin{array}{\|cc} 12279 & 10.5 \\ \rightarrow 1486 & 818 \end{array}$ | $\begin{array}{r} 2.1 \\ 19 \end{array}$ | 1.7 | HSU08988 | 8023 | 8694 | (20549) | C | Tigger1 | DNA/MER2_type | (932) $\smile$ |
| $\begin{array}{rr} 2335 & 11.1 \\ \rightarrow 305 & 1 \end{array}$ | $\begin{aligned} & 0.3 \\ & 18 \end{aligned}$ | 0.7 | HSU08988 | 8695 | 9000 | (20243) | C | AluSg | SINE/Alu | (5) - |
| 1227910.5 | 2.1 | 1.7 | HSU08988 | 9001 | 9695 | (19548) | C | Tigger1 | DNA/MER2_type | $(1600)=$ |
| $\rightarrow 818 \quad 2$ |  |  |  |  |  |  |  |  |  |  |
| 72121.2 | 1.4 | 0.0 | HSU08988 | 9696 | 9816 | (19427) | C | MER7A | DNA/MER2_type | (224) |
| $\hookrightarrow 122 \quad 2$ |  |  |  |  |  |  |  |  |  |  |

We can convert it to sorted BED data in the following manner:


Note: Use bedops -merge to merge elements, e.g.: rmsk2bed < foo.out | bedops --merge - > merged_repeatmasker_elements.bed

As shown above, we strip the header element, but adding the -- keep-header option will preserve this header as a BED element that uses _header as a chromosome name:


Note: Note the conversion from 1- to 0-based coordinate indexing, in the transition from RepeatMasker annotation output to BED. BEDOPS supports operations on input with any coordinate indexing, but the coordinate change made here is believed to be convenient for most end users.

## Column mapping

In this section, we describe how RepeatMasker annotation columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| RepeatMasker annotation field | BED column index | BED field |
| :--- | :--- | :--- |
| Query sequence | 1 | chromosome |
| Query start | 2 | start |
| Query end | 3 | stop |
| Repeat name | 4 | id |
| Smith-Waterman score | 5 | score |
| Strand | 6 | strand |

The remaining columns are mapped as follows:

| RepeatMasker annotation field | BED column index | BED field |
| :--- | :--- | :--- |
| Percentage, substitutions | 7 |  |
| Percentage, deleted bases | 8 |  |
| Percentage, inserted bases | 9 |  |
| Bases in query, past match | 10 |  |
| Repeat class | 11 |  |
| Bases in complement of the repeat consensus sequence | 12 |  |
| Match start | 13 |  |
| Match end | 14 |  |
| Unique ID | 15 |  |
| Higher-scoring match (optional) | 16 |  |

## Downloads

- Sample RepeatMasker annotation dataset: foo. out


## sam2bed

The sam2bed script converts 1-based, closed [start, end] Sequence Alignment/Map (SAM) to sorted, 0-based, half-open [start-1, end) UCSC BED data.

For convenience, we also offer sam2 starch, which performs the extra step of creating a Starch-formatted archive.
The sam2bed script is "non-lossy" (with the use of specific options, described below). Other toolkits tend to throw out information from the original SAM input upon conversion; sam2bed retains everything, facilitating reuse of converted data and conversion to other formats.

Tip: Doing the extra step of creating a Starch-formatted archive can save a lot of space relative to the original SAM format, up to $33 \%$ of the original SAM dataset, while offering per-chromosome random access.

## Dependencies

The sam2bed wrapper script is dependent upon the installation of SAMtools and convert2bed. The sam2starch wrapper script is further dependent on the installation of the starch binary, part of a typical BEDOPS installation.

## Source

The sam2bed and sam2starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The sam2bed script parses SAM data from standard input and prints sorted BED to standard output. The sam2starch script uses an extra step to parse SAM to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a SAM file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Tip: If you work with RNA-seq data, you can use the --split option to process reads with N-CIGAR operations, splitting them into separate BED elements.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If sorting converted data larger than system memory, use the $--m a x-m e m$ option to limit sort memory usage to a reasonable fraction of available memory, e.g., --max-mem 2G or similar. See --help for more details.

## Example

To demonstrate these scripts, we use a sample binary input called foo. sam (see the Downloads section to grab this file).

```
@HD VN:1.0 SO:coordinate
@SQ SN:seq1 LN:5000
@SQ SN:seq2 LN:5000
@CO Example of SAM/BAM file format.
B7_591:4:96:693:509 73 seq1 1 09 06M 0
@ CACTAGTGGCTCATTGTAAATGTGTGGTTTAACTCG <<<<<<<<<<<<<<<<<<<;<<<<<<<<<<<5<<<<<<; :<;77 u 
\hookrightarrowMF:i:18 Aq:i:73 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
```



```
@ CTAGTGGCTCATTGTAAATGTGTGGTTTAACTCGT 
\hookrightarrowMF:i:18 Aq:i:66 NM:i:0 UQ:i:0 HO:i:1 H1:i:0
EAS51_64:8:5:734:57 137 seq1 5 99 35M 0 0 0 0
@ AGTGGCTCATTGTAAATGTGTGGTTTAACTCGTCC 
\hookrightarrowMF:i:18 Aq:i:66 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
. . .
```

We can convert it to sorted BED data in the following manner (omitting standard error messages):

```
$ sam2bed < foo.sam
seq1 
\hookrightarrow<<<<<;:<;7 MF:i:18 Aq:i:73 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
```



```
\hookrightarrow<<3/:<6): MF:i:18 Aq:i:66 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
seq1 4 39 EAS51_64:8:5:734:57 99 +
\hookrightarrow 0 AGTGGCTCATTGTAAATGTGTGGTTTAACTCGTCC <<<<<<<<<<<< 年71<<<;<;;<7;<
\hookrightarrow<3;); 3*8/5 MF:i:18 Aq:i:66 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
```



```
\hookrightarrow',+-,),''*, MF:i:130 Aq:i:63 NM:i:5 UQ:i:38 H0:i:0 H1:i:0
...
```

Note also that we strip the header section from the output. If we want to keep this, the use of the --keep-header option will preserve the BAM file's header, turning it into BED elements that use _header as a chromosome name.

Here's an example:

```
$ sam2bed --keep-header < foo.sam
lllll
    CACTAGTGGCTCATTGTAAATGTGTGGTTTAACTCG }<<<<<<<<<<<<<<<<<;<<<<<<<<<<< 
0 0 CACTAGTGGCTCATTGTAAATGTGTGGTTTAACTCG <<<
```



```
\hookrightarrow 0 CTAGTGGCTCATTGTAAATGTGTGGTTTAACTCGT <<<<<<<<<<<<< < < < < < 655 << 7 <<<<:9
\hookrightarrow<<3/:<6): MF:i:18 Aq:i:66 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
```



```
4 0 0 AGTGGCTCATTGTAAATGTGTGGTTTAACTCGTCC <<<<<<<<<<<<7;71<<;<;;<7;<
\hookrightarrow<3;);3*8/5 MF:i:18 Aq:i:66 NM:i:0 UQ:i:0 H0:i:1 H1:i:0
```



```
4 0 0 GTGGCTCATTGTAATTTTTTGTTTTAACTCTTCTCT (-&---------)--) -),'--)---
\hookrightarrow',+-,),''*, MF:i:130 Aq:i:63 NM:i:5 UQ:i:38 H0:i:0 H1:i:0
...
```

With this option, the sam2bed and sam2starch scripts are completely "non-lossy" (with the exception of unmapped reads; see note below). Use of awk or other scripting tools can munge these data back into a SAM-formatted file.

Note: The provided scripts strip out unmapped reads from the SAM file. We believe this makes sense under most circumstances. Add the --all-reads option if you need unmapped and mapped reads.

Note: Note the conversion from 1- to 0-based coordinates. While BEDOPS fully supports 0-and 1-based coordinates, the coordinate change in BED is believed to be convenient to most end users.

## Column mapping

In this section, we describe how SAM columns are mapped to BED columns. We start with the first six UCSC BED columns as follows:

| SAM field | BED column index | BED field |
| :--- | :--- | :--- |
| RNAME | 1 | chromosome |
| POS - 1 | 2 | start |
| POS + length(CIGAR) - 1 | 3 | stop |
| QNAME | 4 | id |
| MAPQ | 5 | score |
| $16 \&$ FLAG | 6 | strand |

The remaining SAM columns are mapped as-is, in same order, to adjacent BED columns:

| SAM field | BED column index | BED field |
| :--- | :--- | :--- |
| FLAG | 7 |  |
| CIGAR | 8 |  |
| RNEXT | 9 |  |
| PNEXT | 10 |  |
| TLEN | 11 |  |
| SEQ | 12 |  |
| QUAL | 13 |  |

Because we have mapped all columns, we can translate converted BED data back to headered or headerless SAM reads with a simple awk statement (or other script) that reverts back to 1-based coordinates and permutes columns to SAM-based ordering.

## Downloads

- Sample SAM dataset: foo.sam


## vcf2bed

The vcf2bed script converts 1-based, closed [start, end] Variant Call Format v4.2 (VCF) to sorted, 0-based, half-open [start-1, start) extended BED data.


#### Abstract

Note: Note that this script converts from [start, end] to [start-1, start). Unless the --snvs, --insertions or --deletions options are added, we perform the equivalent of a single-base insertion to make BED output that is guaranteed to work with BEDOPS, regardless of what the actual variant may be, to allow operations to be performed. The converted output contains additional columns which allow reconstruction of the original VCF data and associated variant parameters.


For convenience, we also offer vcf2starch, which performs the extra step of creating a Starch-formatted archive.

## Dependencies

The vcf2bed script requires convert2bed. The vcf2starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

This script is also dependent on input that follows the VCF v4.2 specification.

Tip: Conversion of data which are VCF-like, but which do not follow the specification can cause parsing issues. If you run into problems, please check that your input follows the VCF specification using validation tools, such as those packaged with VCFTools.

## Source

The vcf2bed and vcf 2 starch conversion scripts are part of the binary and source downloads of BEDOPS. See the Installation documentation for more details.

## Usage

The vcf 2 bed script parses VCF from standard input and prints sorted BED to standard output. The vcf2starch script uses an extra step to parse VCF to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a VCF file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Note: By default, multiple BED annotations are printed if there are multiple alternate alleles in a variant call. Use the
 for the variant call.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If the converted BED output looks truncated or incomplete, and you are converting a VCF file that is larger than the capacity of your /tmp folder, then use the --sort-tmpdir option to specify an alternative directory to store intermediate sort results. Or, if you do not want to sort the converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If you are sorting data larger than system memory, use the $--m a x-m e m$ option to limit sort memory usage to a reasonable fraction of available memory, e.g., --max-mem 2G or similar. See --help for more details.

## Customized variant handling

By default, the vcf2bed script translates all variants to single-base positions in the resulting BED output. Depending on the category of variant you are interested in, however, you may want more specific categories handled differently.

Based on the VCF v4.2 specification, we also provide three custom options for filtering input for each of the three types of variants listed: --snvs, --insertions and --deletions. In each case, we use the length of the reference and alternate alleles to determine which type of variant is being handled.

In addition, using any of these three custom options automatically results in processing of mixed variant records for a microsatellite, where present. For instance, the following record contains a mixture of a deletion and insertion variant (GTC -> G and GTC -> GTCT, respectively):


When using --snvs, --insertions or --deletions, this record is split into two distinct BED records and filtered depending on which custom option was chosen. The --insertions option would only export the singlebase position of the insertion in this mixed variant, while --deletions would show the deletion.
In this way, you can control what kinds of variants are translated into BED outputs-most importantly, there is also no confusion about what the length of the BED element signifies.

## Example

To demonstrate these scripts, we use a sample VCF input called foo.vcf (see the Downloads section to grab this file).

Note: This data is also publicly available from the Broad Institute.

```
##fileformat=VCFv4.0
##FILTER=<ID=LowQual,Description="QUAL < 50.0">
##FORMAT=<ID=AD,Number=., Type=Integer,Description="Allelic depths for the ref and alt,
\hookrightarrowalleles in the order listed">
##FORMAT=<ID=DP, Number=1, Type=Integer,Description="Read Depth (only filtered reads,
\hookrightarrowused for calling)">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=PL,Number=3,Type=Float,Description="Normalized, Phred-scaled likelihoods,
for AA,AB,BB genotypes where A=ref and B=alt; not applicable if site is not,
\hookrightarrowbiallelic">
##INFO=<ID=AC,Number=., Type=Integer,Description="Allele count in genotypes, for each
\hookrightarrowALT allele, in the same order as listed">
##INFO=<ID=AF,Number=., Type=Float,Description="Allele Frequency, for each ALT allele,u
Gin the same order as listed">
##INFO=<ID=AN, Number=1, Type=Integer,Description="Total number of alleles in called,
\hookrightarrowgenotypes">
##INFO=<ID=DB,Number=0, Type=Flag,Description="dbSNP Membership">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=DS,Number=0, Type=Flag,Description="Were any of the samples downsampled?">
##INFO=<ID=Dels,Number=1,Type=Float,Description="Fraction of Reads Containing_
\hookrightarrowSpanning Deletions">
##INFO=<ID=HRun,Number=1, Type=Integer,Description="Largest Contiguous Homopolymer Run,
\hookrightarrowOf Variant Allele In Either Direction">
##INFO=<ID=HaplotypeScore,Number=1,Type=Float,Description="Consistency of the site,
\hookrightarrowwith two (and only two) segregating haplotypes">
##INFO=<ID=MQ,Number=1,Type=Float,Description="RMS Mapping Quality">
##INFO=<ID=MQO,Number=1,Type=Integer,Description="Total Mapping Quality Zero Reads">
##INFO=<ID=QD,Number=1, Type=Float,Description="Variant Confidence/Quality by Depth">
##INFO=<ID=SB,Number=1,Type=Float,Description="Strand Bias">
##INFO=<ID=VQSLOD,Number=1, Type=Float,Description="loglO-scaled probability of,
\hookrightarrowvariant being true under the trained gaussian mixture model">
##UnifiedGenotyperv2="analysis_type=UnifiedGenotyperv2 input_file=[TEXT CLIPPED FOR
\hookrightarrowCLARITY] "
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA12878
(continues on next page)
```



We can convert VCF to sorted BED data in the following manner:


As you see here, the header data of the VCF file is discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

Here we use --keep-header with our example dataset:

```
$ vcf2bed --keep-header < foo.vcf
_header 1 2 ##FILTER=<ID=LOwQual,Description="QUAL < 50.0">
_header 2 3 ##FORMAT=<ID=AD,Number=.,Type=Integer,Description="Allelic
\hookrightarrowdepths for the ref and alt alleles in the order listed">
_header 3 4 ##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth
\hookrightarrow(only filtered reads used for calling)">
_header 4 5 ##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype
\hookrightarrowQuality">
_header 5 6 ##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
_header 6 7 ##FORMAT=<ID=PL,Number=3,Type=Float,Description="Normalized, 
\hookrightarrowPhred-scaled likelihoods for AA,AB,BB genotypes where A=ref and B=alt; not 
\hookrightarrowapplicable if site is not biallelic">
_header 7 8 ##INFO=<ID=AC,Number=.,Type=Integer,Description="Allele count 
\hookrightarrowin genotypes, for each ALT allele, in the same order as listed">
_header 8 % ##INFO=<ID=AF,Number=.,Type=Float,Description="Allele_
\hookrightarrowFrequency, for each ALT allele, in the same order as listed">
_header 9 10 ##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number,
\hookrightarrowOf alleles in called genotypes">
_header 10 11 ##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP Membership
\hookrightarrow">
```

(continues on next page)


With this option, the vcf 2 * scripts are completely "non-lossy". Use of awk or other scripting tools can munge these data back into a VCF-formatted file.

Note: Note the conversion from 1- to 0-based coordinate indexing, in the transition from VCF to BED. While BEDOPS supports 0 - and 1 -based coordinate indexing, the coordinate change made here is believed to be convenient for most end users.

## Column mapping

In this section, we describe how VCF v4.2 columns are mapped to BED columns. We start with the first five UCSC BED columns as follows:

| VCF v4.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| \#CHROM | 1 | chromosome |
| POS -1 | 2 | start |
| POS $\left(^{*}\right)$ | 3 | stop |
| ID | 4 | id |
| QUAL | 5 | score |

The remaining columns are mapped as follows:

| VCF v4.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| REF | 6 |  |
| ALT | 7 |  |
| FILTER | 8 |  |
| INFO | 9 |  |

If present in the VCF v4.2 input, the following columns are also mapped:

| VCF v4.2 field | BED column index | BED field |
| :--- | :--- | :--- |
| FORMAT | 10 |  |
| Sample ID 1 | 11 |  |
| Sample ID 2 | 12 |  |
| $\ldots$ | 13,14, etc. |  |

When using --deletions, the stop value of the BED output is determined by the length difference between ALT and REF alleles. Use of --insertions or --snvs yields a one-base BED element.
If the ALT field contains more than one allele, multiple BED records will be printed. Use the --do-not-split option if you only want one BED record per variant call.
The "meta-information" (starting with \#\#) and "header" lines (starting with \#) are discarded, unless the --keep-headers options is specified.

## Downloads

- Sample VCF dataset: foo.vcf


## wig2bed

The wig2bed script converts both variable - and fixed -step, 1-based, closed [start, end] UCSC Wiggle format (WIG) to sorted, 0 -based, half-open [start-1, end) extended BED data.

In the case where WIG data are sourced from bigWigToWig or other tools that generate 0 -based, half-open [start-1, end) WIG, a --zero-indexed option is provided to generate coordinate output without any reindexing.
For convenience, we also offer wig2starch, which performs the extra step of creating a Starch-formatted archive.
The utility also supports multiple embedded WIG sections in a single file, which are output to the BED file with modified ID fields, using the --multisplit option.

## Source

The wig2bed script requires convert2bed. The wig2starch script requires starch. Both dependencies are part of a typical BEDOPS installation.

## Usage

The wig2bed script parses WIG from standard input and prints sorted BED to standard output. The wig2 starch script uses an extra step to parse WIG to a compressed BEDOPS Starch-formatted archive, which is also directed to standard output.

The header data of a WIG file is usually discarded, unless you add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

If the input data contain WIG elements with a start position of 0 , the default use of wig2bed and wig2starch will exit early with an EINVAL error. Add the --zero-indexed option to denote that the input WIG data are zero-indexed, and re-run the conversion tool to print unmodified output coordinates.

Tip: If your WIG input is potentially zero-indexed, e.g., if derived from bigWigToWig, where the bigWig data are themselves sourced from BAM- or bedGraph-formatted data, then it is recommended to use the --zero-indexed option as a safety measure.

If your data contain multiple WIG sections, use the --multisplit <basename> option to split sections out to BED elements with modified ID fields. This option can be used in conjunction with the $--k e e p-h e a d e r ~ o p t i o n ~ t o ~$ preserve metadata.

Tip: By default, all conversion scripts now output sorted BED data ready for use with BEDOPS utilities. If you do not want to sort converted output, use the --do-not-sort option. Run the script with the --help option for more details.

Tip: If sorting converted data larger than system memory, use the --max-mem option to limit sort memory usage to a reasonable fraction of available memory, e.g., $--\max -\mathrm{mem} 2 \mathrm{G}$ or similar. See -- help for more details.

## Example

To demonstrate these scripts, we use a sample multi-section WIG input called foo.wig (see the Downloads section to grab this file). We can convert WIG to sorted BED data in the following manner:

```
$ wig2bed < foo.wig
chr1 147971108 147971158 id-1 -0.590000
chr1 147971146 147971196 id-2 0.120000
chr1 147971184 147971234 id-3 0.110000
chr1 147971222 147971272 id-4 -0.760000
```

Note: Even though our WIG input foo.wig has multiple sections, we can omit the use of --multisplit, because conversion and sorting puts everything into one sorted BED file. However, the header data of the WIG file is discarded.

If we want to preserve the header data, we can add the --keep-header option. In this case, BED elements are created from these data, using the chromosome name _header to denote content. Line numbers are specified in the start and stop coordinates, and unmodified header data are placed in the fourth column (ID field).

In the case of the sample input foo.wig, we will also need to add the $--m u l t i s p l i t$ option, as header BED elements from each section will otherwise be collated in a non-sensical way. Adding --multisplit ensures that header data are converted and stored in separate BED files.

To demonstrate, we next repeat the above conversion, adding the --keep-header and --multisplit options:

```
$ wig2bed --multisplit bar --keep-header < foo.wig > foo.bed
```

Conversion of this two-section WIG input results in output with modified ID fields to denote their section association:


Note: Note the conversion from 1- to 0-based coordinate indexing, in the transition from WIG to BED. While BEDOPS supports 0- and 1-based coordinate indexing, the coordinate change made here is believed to be convenient for most end users.

In the case where the WIG data contain elements that have a start position of 0 , the default use of wig2bed and wig2starch will exit early with an EINVAL error. Add the --zero-indexed option to denote that the WIG input is zero-indexed and re-run to convert without any coordinate shift.

Note: Multiple WIG sections in the input file are merged together by the default wig2bed behavior. When using the $--m u l t i s p l i t$ option, each WIG section instead receives its own ID prefix.

## Downloads

- Sample WIG dataset: foo.wig


### 2.7 Summary

These tables summarize BEDOPS utilities by option, file inputs and BED column requirements.

### 2.7.1 Set operation and statistical utilities

## bedextract

- Efficiently extracts features from BED input.
- BEDOPS bedextract documentation.

| option | description | min. <br> file <br> in- <br> puts | max. <br> file inputs | min. BED columns |
| :---: | :---: | :---: | :---: | :---: |
| --list-c | cRrint every chromosome found in input . bed | 1 | 1 | 3 |
| <chromo | sReterieve all rows for specified chromosome, e.g. bedextract chr8 input.bed | 1 | 1 | 3 |
| $\begin{aligned} & \text { <query> } \\ & \text { <referer } \end{aligned}$ | Grab elements of query that overlap elements in reference. Same as nbedops -e -1 query reference, except that this option fails when query contains fully-nested BED elements. May use - to indicate stdin for reference only. | 2 | 2 | 3 |

## bedmap

- Maps source signals from map-file onto qualified target regions from ref-file. Calculates an output for every ref-file element.
- BEDOPS bedmap documentation.



## description

Reports the total number of bases from map-file that overlap the ref-file 's element.
Reports the number of distinct bases from ref-file 's element overlapped by elements in ma Reports the fraction of distinct bases from ref-file 's element elements in map-file.
Require <int> bases of overlap between elements of input files.
Process data for given <chromosome> only.
Reports the number of overlapping elements in map-file.
Reports the Coefficient of Variation: the result of --st dev divided by the result of --mean. Error-check all input files (slower).
Echo each line from ref-file.
Reports the overlapping elements found in map-file.
Reports the IDs (4th column) from overlapping map-file elements.
List unique IDs from overlapping map-file elements.
Reports the genomic range of overlapping elements from map-file.
Reports the scores (5th column) from overlapping map-file elements.
Calculates difference between start and stop coordinates (or size) of each mapped element.
Calculates size of overlap between each mapped element and its reference element.
Reports the first 3 fields of ref-file element in chrom:start-end format.
Reports the length of the ref-file element.
(Advanced) Strong input assumptions are made. Review documents before use. Compatible wit The fraction of the element's size from ref-file that must overlap the element in map-file The fraction of the element's size from map-file that must overlap the element in ref-file
Both--fraction-ref <val> and --fraction-map <val> must be true to qualify as Both--fraction-ref <val> and --fraction-map <val> must be true to qualify as Shorthand for--fraction-both 1. First three fields from map-file must be identical to Reports the presence of one or more overlapping elements in map-file as a binary value ( 0 or Reports the value at the $k$ th fraction. A generalized median-like calculation, where --kth 0 . Reports the 'median absolute deviation' of overlapping elements in map-file, multiplied by Reports the highest score from overlapping elements in map-file.
The lexicographically "smallest" element with the highest score from overlapping elements in $m$ A randomly-chosed element with the highest score from overlapping elements in map-file. I

| option | description |
| :--- | :--- |
| -- mean | Reports the average score from overlapping elements in map-file. |
| -- median | Reports the median score from overlapping elements in map-file. |
| -- min | Reports the lowest score from overlapping elements in map-file. |
| -- min-element | The lexicographically "smallest" element with the lowest score from overlapping elements in ma |
| -- min-element-rand | A randomly-chosed element with the lowest score from overlapping elements in map-file. If |
| -- skip-unmapped | Omits printing reference elements which do not associate with any mapped elements. |
| -- stdev | Reports the square root of the result of --variance. |
| -- sum | Reports the accumulated value from scores of overlapping elements in map-file. |
| -- sweep-all | Reads through entire map-file dataset to avoid early termination that may cause SIGPIPE or |
| -- tmean <low> <hi> | Reports the mean score from overlapping elements in map-file, after ignoring the bottom $<l$ |
| -- variance | Reports the variance of scores from overlapping elements in map-file. |

## bedops

- Offers set and multiset operations for files in BED format.
- BEDOPS bedops documentation.

| option | description | min. <br> file inputs | max. <br> file inputs | min. BED columns |
| :---: | :---: | :---: | :---: | :---: |
| --chrom <br> <chromo | Process data for given chromosome only. some> | 1 | No imposed limit | 3 |
| $\begin{aligned} & -- \text { compl } \\ & -\mathrm{c} \end{aligned}$ | enkeprorts the intervening intervals between the input coordinate segments. | 1 | No <br> im- <br> posed <br> limit | 3 |
| $\begin{aligned} & \text {--chop, } \\ & - \text { w } \end{aligned}$ | Breaks up merged regions into fixed-size chunks, optionally anchored on start coordinates a fixed distance apart. | 1 | No imposed limit | 3 |
| $\begin{aligned} & \text {--diffef } \\ & -\mathrm{d} \end{aligned}$ | Reeperts the intervals found in the first file that are not present in any other input file. | 2 | No <br> im- <br> posed <br> limit | 3 |
| --ec | Error-check input files (slower). | 1 | No <br> im- <br> posed <br> limit | 3 |
| $\begin{aligned} & \text {--eleme } \\ & -e \end{aligned}$ | htReports rows from the first file that overlap, by a specified percentage or number of base pairs, the merged segments from all other input files. | 2 | No <br> im- <br> posed <br> limit | 3 |
| --headef | f Accept headers (VCF, GFF, SAM, BED, WIG) in any input file. | 1 | No imposed limit | 3 |
| $\begin{aligned} & \text {--inter } \\ & -i \end{aligned}$ | \$ Repports the intervals common to all input files. | 2 | No imposed limit | 3 |
| $\begin{aligned} & -- \text { merge, } \\ & -\mathrm{m} \end{aligned}$ | Reports intervals from all input files, after merging overlapping and adjoining segments. | 1 | No <br> im- <br> posed <br> limit | 3 |
| $\begin{aligned} & \text {--not-e } \\ & -n \end{aligned}$ | Reqports exactly everything that --element-of does not, given the same overlap criterion. | 2 | No <br> im- <br> posed <br> limit | 3 |
| $\begin{aligned} & -- \text { partit } \\ & -p \end{aligned}$ | iRepports all disjoint intervals from all input files. Overlapping segments are cut up into pieces at all segment boundaries. | 1 | No imposed limit | 3 |
| $\begin{aligned} & \text {--range } \\ & \mathrm{L}: \mathrm{R} \end{aligned}$ | Add L bases to all start coordinates and R base to end coordinates. Either value may be positive or negative to grow or shrink regions, respectively. With the -e or -n operation, the first (reference) file is not padded, unlike all other files. | 1 | No <br> im- <br> posed <br> limit | 3 |
| $\begin{aligned} & \text {--range } \\ & \mathrm{S} \end{aligned}$ | Pad input file(s) coordinates symmetrically by S bases. This is shorthand for --range $-S: S$. | 1 | No imposed | 3 |
| 2.7. Summ | nary |  | posed limit | 163 |
| $\begin{aligned} & \text {--symmd } \\ & -s \end{aligned}$ | frepports the intervals found in exactly one input file. | 2 | No imposed | 3 |

closest-features

- For every element in input-file, find those elements in query-file nearest to its left and right edges.
- BEDOPS closest-features documentation.

| option | description | min. file inputs | max. file inputs | min. <br> BED <br> columns |
| :---: | :---: | :---: | :---: | :---: |
| (no option) | NA | 2 | 2 | 3 |
| $\begin{aligned} & \text {--chrom } \\ & \text { <chromosome } \end{aligned}$ | Process data for given <chromosome> only. | 2 | 2 | 3 |
| --dist | Output includes the signed distances between the input-file element and the closest elements in query-file. | 2 | 2 | 3 |
| --ec | Error-check all input files (slower). | 2 | 2 | 3 |
| --no-overlap | ©o not consider elements that overlap. Overlapping elements, otherwise, have highest precedence. | 2 | 2 | 3 |
| --no-ref | Do not echo elements from input-file. | 2 | 2 | 3 |
| --closest | Choose the nearest element from query-file only. Ties go to the leftmost closest element. | 2 | 2 | 3 |

### 2.7.2 Sorting

sort-bed

- Sorts input BED file(s) into the order required by other utilities. Loads all input data into memory.
- BEDOPS sort-bed documentation.

| option | description | min. <br> file <br> in- <br> puts | max. <br> file <br> in- <br> puts | min. <br> BED <br> columns |
| :--- | :--- | :--- | :--- | :--- |
| (no op- <br> tion) | NA | 1 | 1000 | 3 |
| -- max-mexival> specifies the maximum memory usage for the sort-bed process, <br> <val> <br> which is useful for very large BED inputs. For example, --max-mem may <br> be 8G, 8000M, or 8000000000 to specify 8 GB of memory. | 1 | 1000 | 3 |  |
| --uniquiReport unique elements (those which only occur once) in output. | 1 | 1000 | 3 |  |
| -- duplid Repers duplicate elements (those which occur 2+ times) in output. | 1 | 1000 | 3 |  |

### 2.7.3 Compression and extraction

starch

- Lossless compression of any BED file.
- BEDOPS starch documentation.

| option | description | min. file inputs | max. file inputs | min. BED columns |
| :---: | :---: | :---: | :---: | :---: |
| (no option) | NA | 1 | 1 | 3 |
| $\begin{aligned} & -- \text { bzip2 } \\ & \text { or }- \text { gzip } \end{aligned}$ | The internal compression method. The default --bzip2 method favors storage efficiency, while --gzip favors compression and extraction time performance. | 1 | 1 | 3 |
| $\begin{aligned} & \text {--note="f } \\ & \text { bar... " } \end{aligned}$ | o Append note to output archive metadata (optional). | 1 | 1 | 3 |
| --report-p |  | 1 | 1 | 3 |

unstarch

- Extraction of a starch archive or attributes.
- BEDOPS unstarch documentation.

| option | description | min. <br> file <br> in- <br> puts | max. <br> file inputs | min. BED column |
| :---: | :---: | :---: | :---: | :---: |
| (no option) | NA | 1 | 1 | NA |
| --archive-type | Show archive's compression type (either bzip2 or gzip). | 1 | 1 | NA |
| --archive-version | Show archive version (at this time, either 1.x or 2.x). | 1 | 1 | NA |
| --archive-timestamp | Show archive creation timestamp (ISO 8601 format). | 1 | 1 | NA |
| --bases <chromosome> | Show total, non-unique base counts for optional <chromosome> (omitting <chromosome> shows total non-unique base count). | 1 | 1 | NA |
| --bases-uniq <br> <chromosome> | Show unique base counts for optional <chromosome> (omitting <chromosome> shows total, unique base count). | 1 | 1 | NA |
| <chromosome> | Decompress information for a single <chromosome> only. | 1 | 1 | NA |
| --duplicatesExist or --duplicatesExistAsStr with <chromosome> | Report if optional <chromosome> or chromoisognes contain duplicate elements as $0 / 1$ numbers or false/true strings | 1 | 1 | NA |
| --elements <br> <chromosome> | Show element count for optional <chromosome> (omitting <chromosome> shows total element count). | 1 | 1 | NA |
| --elements-max-string- | 1Shoost alement maximum string length for optional <chromosome> (omitting <chromosome> shows maximum string length over all chromosomes). | 1 | 1 | NA |
| --is-starch | Test if the <starch-file> is a valid starch archive, returning $0 / 1$ for a false/true result | 1 | 1 | NA |
| --list or--list-json | Print the metadata for a starch file, either in tabular form or with JSON formatting. | 1 | 1 | NA |
| --list-chr or <br> --list-chromosomes  | List all chromosomes in starch archive (similar to bedextract --list-chr). | 1 | 1 | NA |
| ```--nestedsExist or --nestedsExistAsString with <chromosome>``` | Report if optional <chromosome> or chromosomes contain nested elements as $0 / 1$ numbers or false/true strings | 1 | 1 | NA |
| --note | Show descriptive note (if originally added to archive). | 1 | 1 | NA |
| --signature with <chromosome> | Show SHA-1 signature of specified chromosome (Base64-encoded) or all signatures if chromosome is not specified. | 1 | 1 | NA |
| --verify-signature <br> with <chromosome> | Compare SHA-1 signature of specified chromosome with signature that is stored in the archive metadata, reporting error is mismatched. | 1 | 1 | NA |

## starchcat

- Merge multiple starch archive inputs into one starch archive output.
- BEDOPS starchcat documentation.

| option | description | min. file inputs | max. file inputs | min. BED columns |
| :---: | :---: | :---: | :---: | :---: |
| (no option) | NA | 1 | No imposed limit | NA |
| $\begin{aligned} & \text {--bzip2 } \\ & \text { or --gzip } \end{aligned}$ | The internal compression method. The default --bzip2 method favors storage efficiency, while --gzip favors compression and extraction time performance. | 1 | No imposed limit | NA |
| $\begin{aligned} & \text {--note="f } \\ & \text { bar... " } \end{aligned}$ | odppend note to output archive metadata (optional). | 1 | No imposed <br> limit | NA |
| --report- | p-Hagte 9 cogress to standard error stream for every N input elements. | 1 | No imposed limit | NA |

starchstrip

- Extract or filter a starch archive by one or more specified chromosome names.
- BEDOPS starchstrip documentation.

| option | description | min. file inputs | max. file inputs | min. BED columns |
| :---: | :---: | :---: | :---: | :---: |
| (no option) | NA | 1 | No imposed limit | NA |
| --include or <br> --exclude with <br> <chromosomes>  | Writes output with inclusion or exclusion of specified chromosome name records (comma-delimited string). | NA | No imposed limit | NA |

### 2.8 Release

This document attempts to enumerate steps to get from a development branch to a final release, with all associated packages and documentation changes.

### 2.8.1 Preparation

Preparing a major, minor or maintenance release of BEDOPS from a development branch involves several steps, which we outline here:

1. Review the Github issues list
a. Close out open documentation or feature issues, making necessary pushes to the current development branch.
b. If any issues can't be closed out, rename the assigned version tag to the next anticipated release version (e.g., v2.4.36 to $v 2 p 5 p 0$, etc.)
2. Pull the most recent commit for the development branch to a local folder on build hosts (Linux with sufficiently old kernel, current OS X, etc.).
a. Follow the Installation (via source code) documentation to build BEDOPS for the given platform.
1) For Linux, we build a 64-bit version. It may help to use VirtualBox or a similar virtualization host to set up and run different (and consistent) versions of Linux build hosts.
2) For Mac OS X, we currently build the Mac target with whatever the modern Xcode and current OS X release happens to be (currently, command-line tools that ship with Xcode 9 and OS X High Sierra/10.13). If things work correctly, build flags generate 64-bit binaries that should run on 10.10 and newer OS releases.
b. For all platforms, run test suites for various tools and conversion scripts; tests should pass on supported platforms. If not, add an Issue ticket, fix it, close it and start over with the build/test process.
c. If things work properly, make a bzip2-compressed tarball from the compiled binaries.

The naming scheme we currently use for Linux packages is as follows:
bedops_linux_x86_64-vX.Y.Z.tar.bz2 (64-bit)
Run shasum -a 256 on the tarball to get its SHA256 hash (store this SHA256 hash in a file for later retrieval).

For the OS X Installer, use productsign per OS X Installer documentation to digitally sign the package. Compress the Installer with the Finder or $z i p$ :

```
BEDOPS.X.Y.Z.pkg.zip
```

The X.Y.Z scheme should follow the development branch name, e.g. 2.4.36, etc.
3. Collect tarballs and zipped Installer in one location for later addition with web browser, via BEDOPS Github web site.

### 2.8.2 Release

1. Merge BEDOPS development branch into master branch:
```
$ git checkout master
$ git pull origin master
$ git merge vXpYpz
$ git push origin master
```

Ideally, whatever steps are used to merge the development branch into the master branch should preserve the overall commit history.

As before, the $X . Y . Z$ scheme should follow the development branch name, e.g. 2.4.36, etc.
2. Add a new release via the Github site. Or click on the Draft a new release button from the Github Releases page.

Fill out the resulting form, as described below:
a. Tag version should be of the form $v X . Y . Z$ (using the "semantic versioning" naming scheme triggers Github to set up useful and automatic package features).
Tags should be applied to the master branch, since we pushed the development branch up to the master branch.
b. Release title can be of the form BEDOPS $v$ X.Y.Z.
c. Describe this release can be populated with the following Markdown-formatted boilerplate:

```
Downloads are available at the bottom of this page. Please read the [BEDOPS vX.Y.
\hookrightarrow Z ~ r e v i s i o n ~ h i s t o r y ] ~ ( h t t p : / / b e d o p s . r e a d t h e d o c s . i o / e n / l a t e s t / c o n t e n t / r e v i s i o n - ~
\hookrightarrowistory.html#vX-Y-Z), which summarizes new features and fixes in this release.
------
### Linux
**bedops_linux_x86_64-vX.Y.Z.tar.bz2** (64-bit, SHA256: ``abcd1234``)
This package of BEDOPS vX.Y.Z binaries is for Linux 64-bit (glibc v2.17) hosts.u
\hookrightarrowhose who require 32-bit or pre-2.17 glibc-compiled binaries will need to build
\hookrightarrowinaries from source code; please read [$2.2. Via source-code] (http://bedops.
\hookrightarroweadthedocs.io/en/latest/content/installation.html#via-source-code) of the,
GEDOPS Installation document for details.
For installation instructions, please read [$2.1.1. Linux] (http://bedops.
\hookrightarrowreadthedocs.io/en/latest/content/installation.html#linux) of the BEDOPS
\hookrightarrownstallation document.
------
### Mac OS X
**BEDOPS.X.Y.Z.pkg.zip**
This package of BEDOPS vX.Y.Z is a digitally-signed installer for 64-bit binaries,
\hookrightarrowthat run under OS X (10.10 - 10.13) on Intel-based Macs.
For installation instructions, please read [$2.1.2. Mac OS X] (http://bedops.
\hookrightarrowreadthedocs.io/en/latest/content/installation.html#mac-os-x) of the BEDOPSG
|nstallation document.
```

d. Attach per-platform binaries to this release by dragging each of them into the field underneath the description text. It can take a few moments for the web browser to upload each binary into the release page, so be patient. There should be at least two binary packages: one for Linux 64-bit, and one for Mac OS X.
e. Click the Publish Release button.
3. After at least $5-10$ minutes from pushing the development branch to the master branch, check the BEDOPS documentation site to ensure that the "latest" or default documenation shown is for the new version.

If not, take a look at the build page to manually trigger document rebuilds, or examine error logs, if necessary.
4. Update the Github bedops/bedops master README.md file to note the current version number, if necessary.
5. Push fixes to any documentation errors in the master branch.

Note: We should aim to fix typos and other errors as soon after a new release as possible, because then shortly afterwards we can simply pull a new development branch off the current state of the master branch with minimal commit losses.

Tip: If we push any subsequent changes to the master branch, it's not the end of the world. However, it is recommended that the version tag is pushed forwards to the latest commit:

```
$ git tag -f -a vX.Y.Z -m 'pushed current version tag forwards to latest commit'
$ git push -f --tags
•••
```

This way, anyone who downloads source via GitHub will get the "freshest" code, with all the typo fixes and so forth.
6. Visit the BEDOPS documentation administration site to disable documentation for the development branch.

Specifically, click on the versions tab to deactivate the old development branch. (Likewise, when adding a new development branch, add an active link here, so that edits to the documentation folder in the new development branch are available.)
7. Update a local fork of homebrew-science with details for the BEDOPS formula. Submit pull request to homebrew-science folks.
a. After establishing a local fork, add the upstream remote so that you can fetch/pull updated formulas from Homebrew (if this is already done, this step can be skipped):

```
$ git remote add upstream git://github.com/homebrew/homebrew-science.git
```

b. Fetch and pull data to the master branch from the upstream remote:

```
$ git checkout master
$ git fetch
$ git pull upstream master
...
```

c. Make a branch of the master entitled bedops-vXpYpZ and check it out:

```
$ git branch bedops-vXpYpZ
$ git checkout bedops-vXpYpz
```

d. Edit changes to bedops.rb formula. Change the version number in the tarball download and remove the sha1 line (you'll replace this later on).
e. Test the new formula. Add the --build-from-source option to skip the per-platform bottle code:

```
$ brew install ./bedops.rb --build-from-source
```

f. If the installation is successful, there will be a SHA1 validation code that you can copy and paste into the formula with the shal header (see step $d$-basically, you are updating the line you removed in that step).
g. Add, commit and push the updated formula to the bedops-vXpYpZ branch:

```
$ git add bedops.rb
$ git commit -am 'BEDOPS X.Y.Z'
$ git push origin bedops-vXpYpZ
```

h. Visit the homebrew-science site and initiate a pull request from your local fork's newly pushed branch (there will be a big green button at the top of the GitHub site that asks you to start this pull request).
i. Wait for success or failure; the homebrew-science people will indicate if there are any problems, usually within 48-72 hours.
8. Consider closing out or deleting the development branch, as well as setting up the next development branch.

### 2.8.3 Celebrate

At this point, we can email links to Linux packages to IT for updating the cluster BEDOPS module and make announcements on websites, mailing lists, etc.

