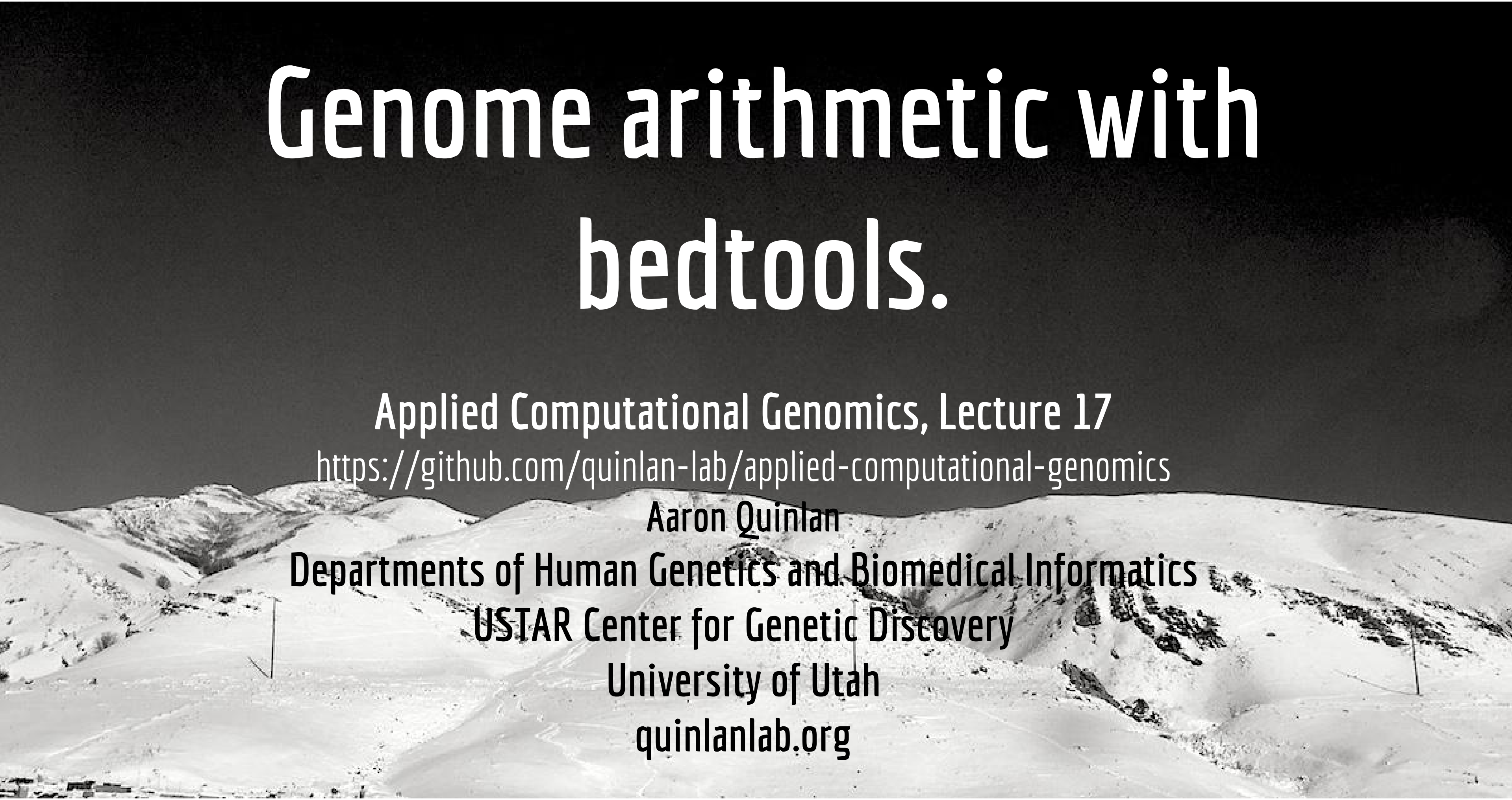

Genome arithmetic with bedtools.

Applied Computational Genomics, Lecture 17

https://github.com/quinlan-lab/applied-computational-genomics

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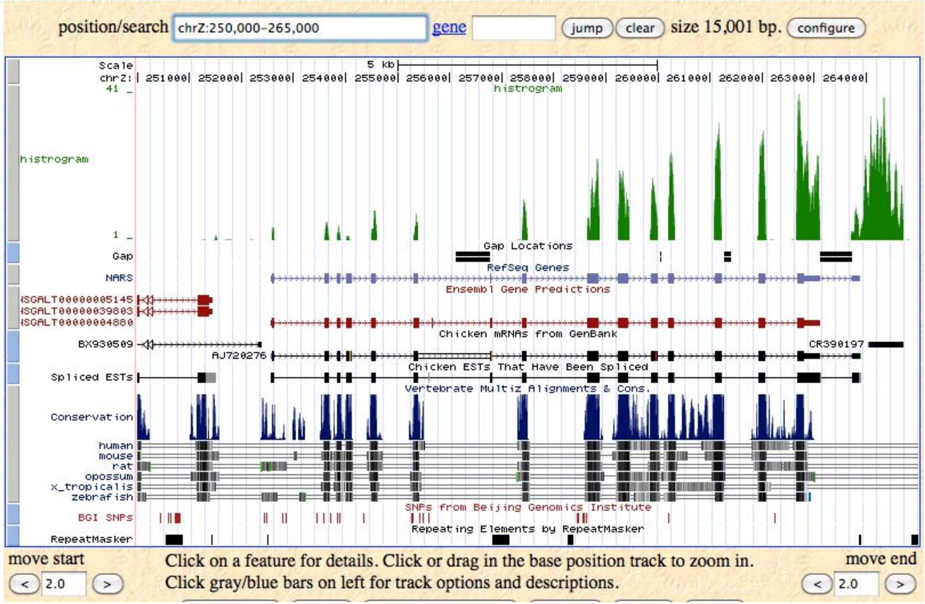
What is a genome interval?

- Genes: exons, introns, UTRs, promoters (BED, GFF, GTF
- Conservation (BEDGRAPH)
- Genetic variation (VCF)
- Sequence alignments (BAM)
- Transcription factor binding sites (BED, BEDGRAPH)
- CpG islands (BED)
- Segmental duplications (BED)
- Chromatin annotations (BED)
- Gene expression data (WIG, BIGWIG, BEDGRAPH)
- Your own observations: put them in context

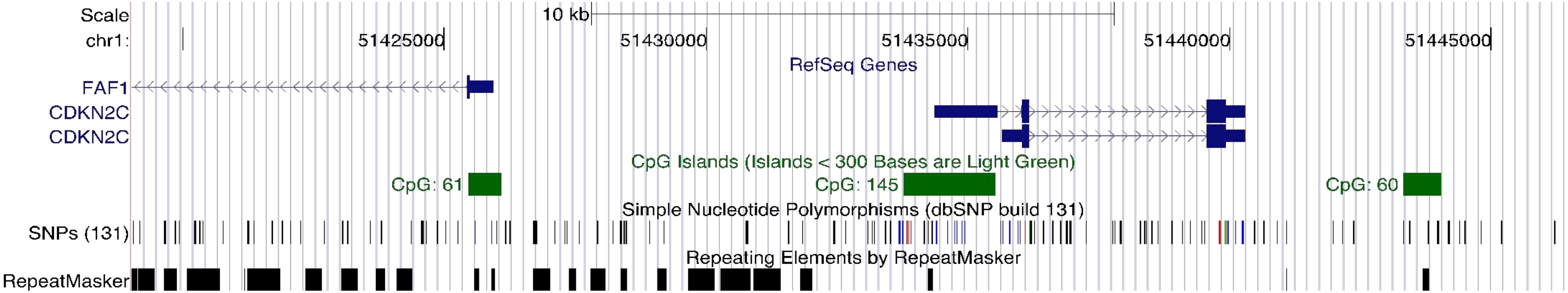


position/search chrZ:250,000-265,000 gene jump clear size 15,001 bp. configure

Scale
5 kb
chrZ: 251000| 252000| 253000| 254000| 255000| 256000| 257000| 258000| 259000| 260000| 261000| 262000| 263000| 264000|
41
histrogram
histrogram
1
Gap Locations
Gap
RefSeq Genes
NARS
Ensembl Gene Predictions
ENSGALT00000005145
ENSGALT00000039803
ENSGALT00000004880
Chicken mRNAs from GenBank
BX930509
AJ720276
CR390197
Chicken ESTs That Have Been Spliced
Spliced ESTs
Vertebrate Multiz Alignments & Cons.
Conservation
human
mouse
rat
opossum
x_tropicalis
zebrafish
SNPs from Beijing Genomics Institute
BGI SNPs
Repeating Elements by RepeatMasker
RepeatMasker
move start
< 2.0 >
move end
< 2.0 >

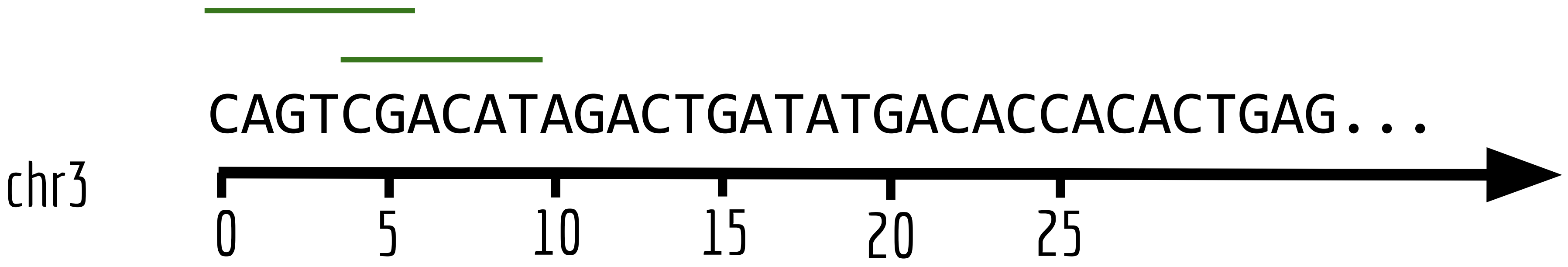
Click on a feature for details. Click or drag in the base position track to zoom in.
Click gray/blue bars on left for track options and descriptions.

Genome intervals


Scale
10 kb
chr1:
51425000
51430000
51435000
51440000
51445000
RefSeq Genes
FAF1
CDKN2C
CDKN2C
CpG Islands (Islands < 300 Bases are Light Green)
CpG: 61
CpG: 145
CpG: 60
Simple Nucleotide Polymorphisms (dbSNP build 131)
SNPs (131)
Repeating Elements by RepeatMasker
RepeatMasker

Genome arithmetic: the method of comparing, contrast and gain insight among multiple genome interval files

Genome arithmetic depends upon the genome coordinate system



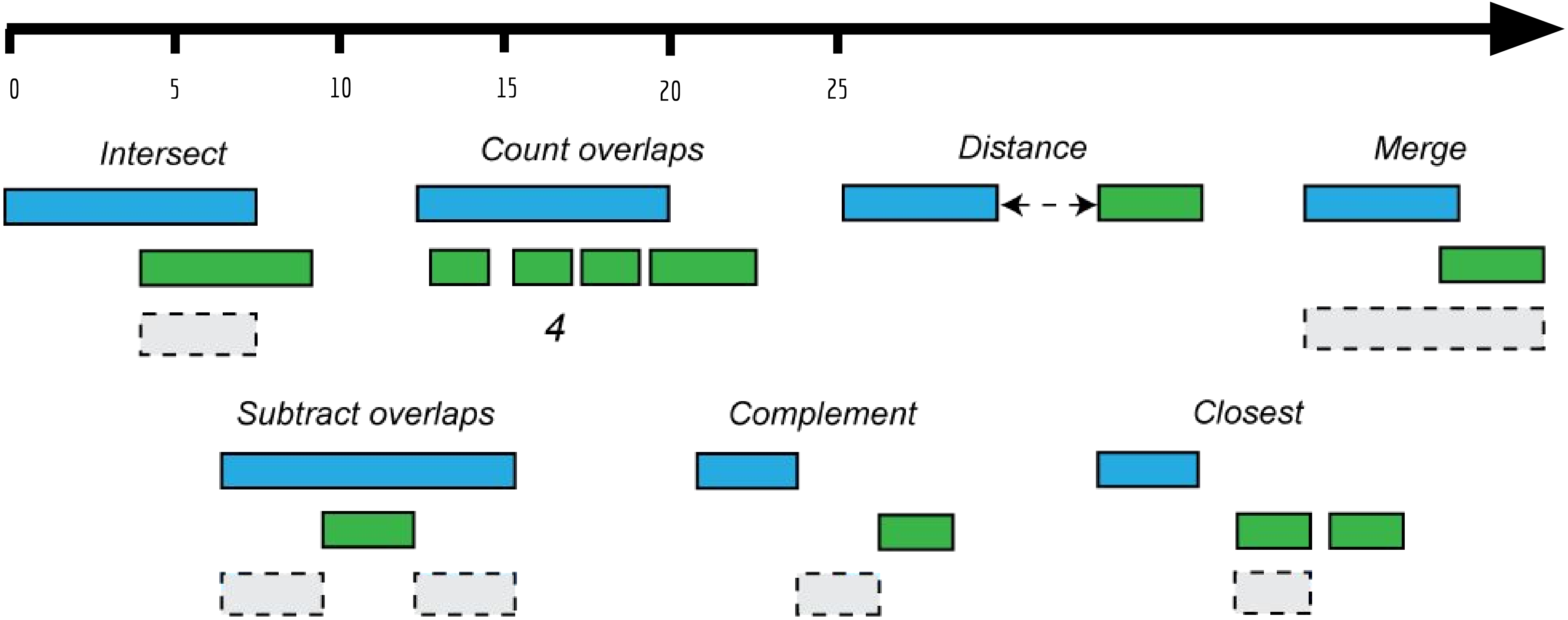
CAGTCGACATAGACTGATATGACACCACACTGAG...

chr3

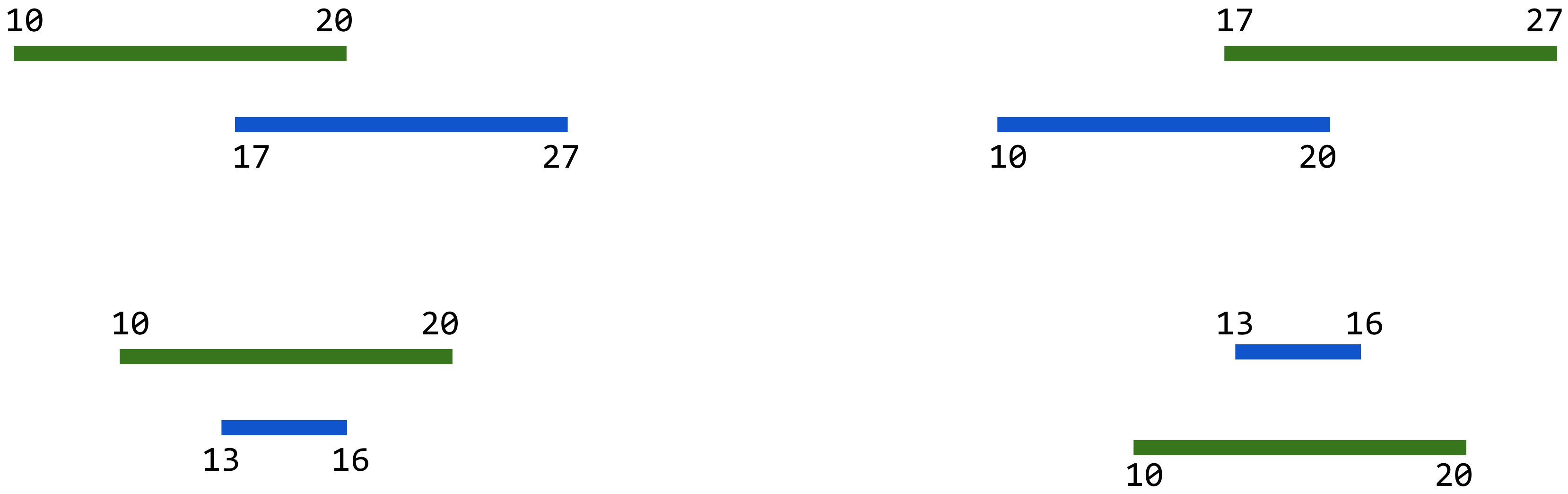
0 5 10 15 20 25

```
chr3    0    7
chr3    4    10
```

Genome arithmetic operations

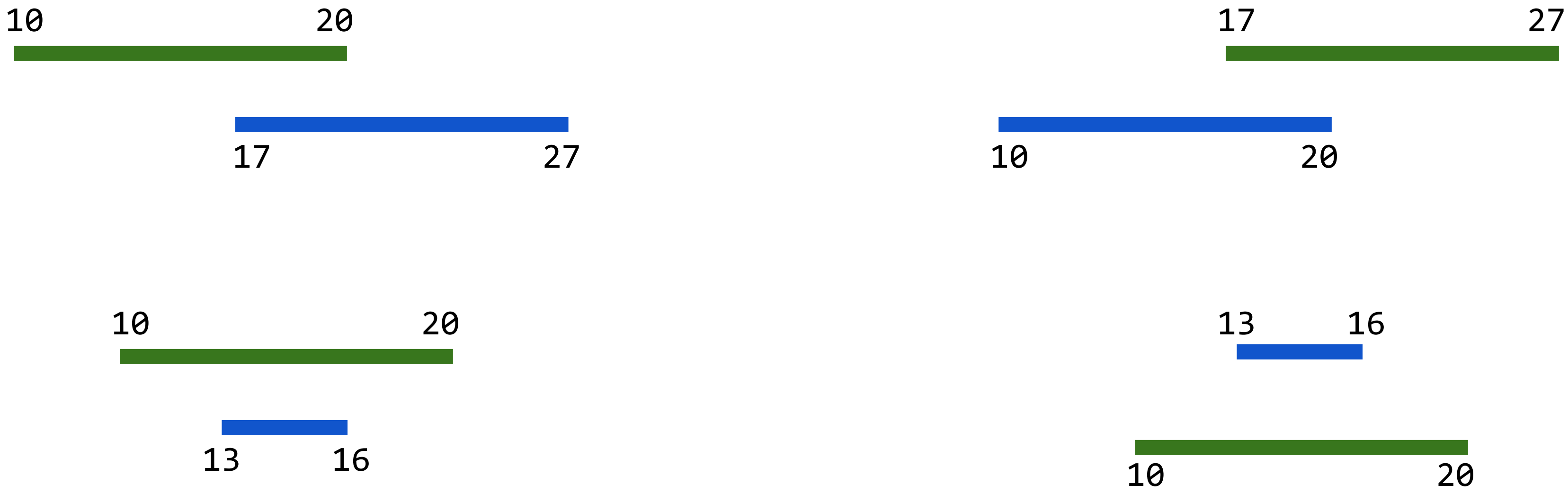


Do two intervals intersect (overlap)?



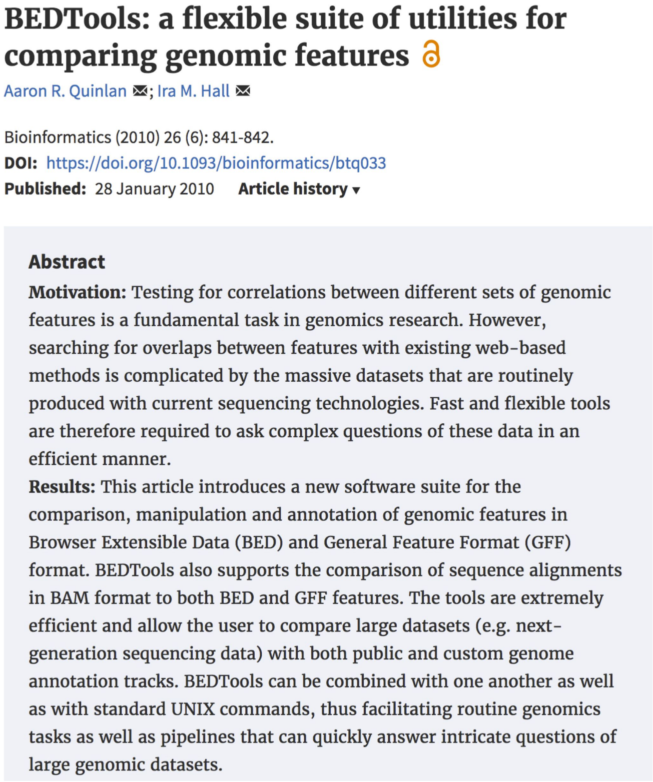
```
if ((a.start <= b.start and a.end >= b.start) or
    (b.start <= a.start and b.end >= a.start) or
    (a.start <= b.start and a.end >= b.end)   or
    (b.start <= a.start and b.end >= a.end))
{
  INTERSECTION!!!
}
else NADA!!!
```

Do two intervals intersect (overlap)? A simpler way.



```
I = min(a.end, b.end) - max(a.start, b.start)

if I > 0, intersection,
if I <= 0, distance between the intervals

= min(20, 27) - max(10, 17)
= 20-17 = 3
```

Bedtools: a swiss army knife for genome analysis



BEDTools: a flexible suite of utilities for comparing genomic features

Aaron R. Quinlan ✉; Ira M. Hall ✉

Bioinformatics (2010) 26 (6): 841-842.
DOI: https://doi.org/10.1093/bioinformatics/btq033
Published: 28 January 2010 **Article history ▾**

Abstract

Motivation: Testing for correlations between different sets of genomic features is a fundamental task in genomics research. However, searching for overlaps between features with existing web-based methods is complicated by the massive datasets that are routinely produced with current sequencing technologies. Fast and flexible tools are therefore required to ask complex questions of these data in an efficient manner.

Results: This article introduces a new software suite for the comparison, manipulation and annotation of genomic features in Browser Extensible Data (BED) and General Feature Format (GFF) format. BEDTools also supports the comparison of sequence alignments in BAM format to both BED and GFF features. The tools are extremely efficient and allow the user to compare large datasets (e.g. next-generation sequencing data) with both public and custom genome annotation tracks. BEDTools can be combined with one another as well as with standard UNIX commands, thus facilitating routine genomics tasks as well as pipelines that can quickly answer intricate questions of large genomic datasets.

Papers:

https://doi.org/10.1093/bioinformatics/btq033
DOI: 10.1002/0471250953.bi1112s47

Documentation:

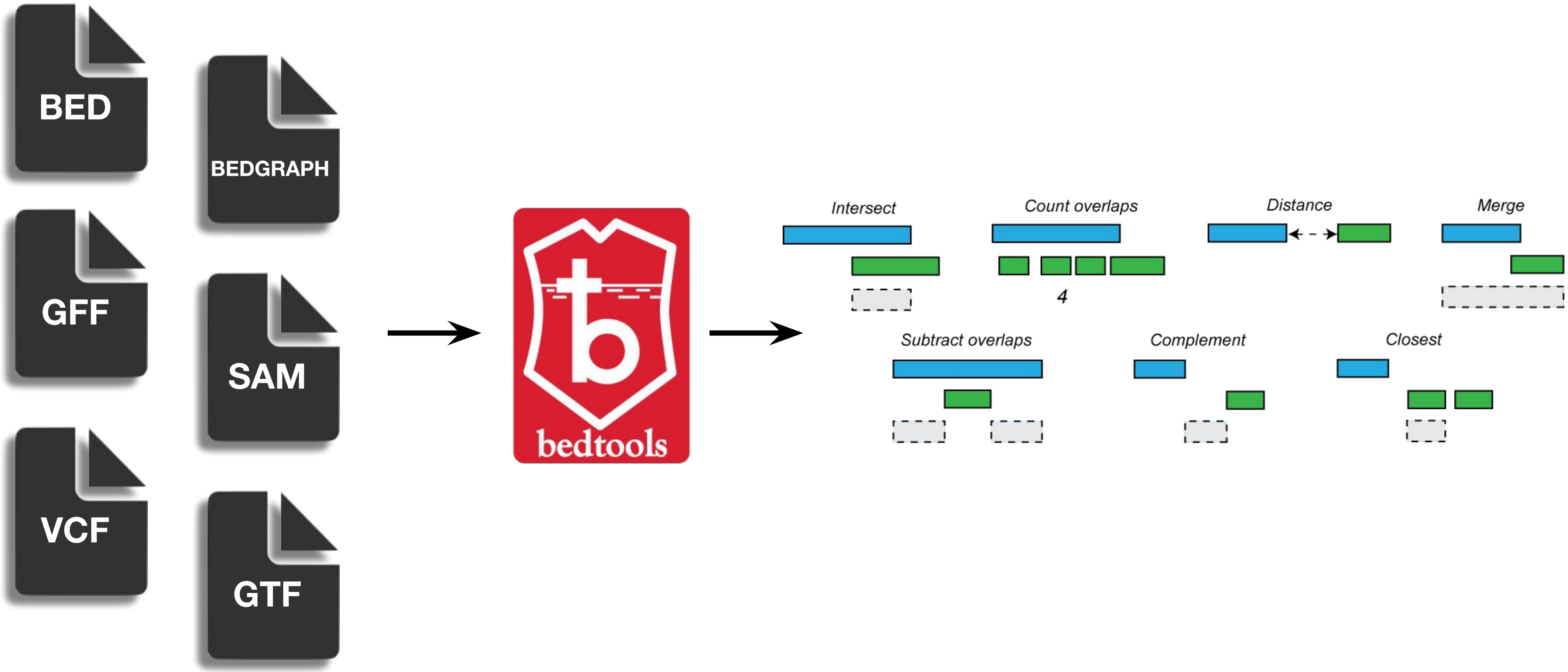
http://bedtools.readthedocs.io/en/latest/

Code:

https://github.com/arq5x/bedtools2

http://bedtools.readthedocs.io/en/latest/

Supports most interval formats & handles diff. coordinate systems



BED

BEDGRAPH

GFF

SAM

VCF

GTF

bedtools

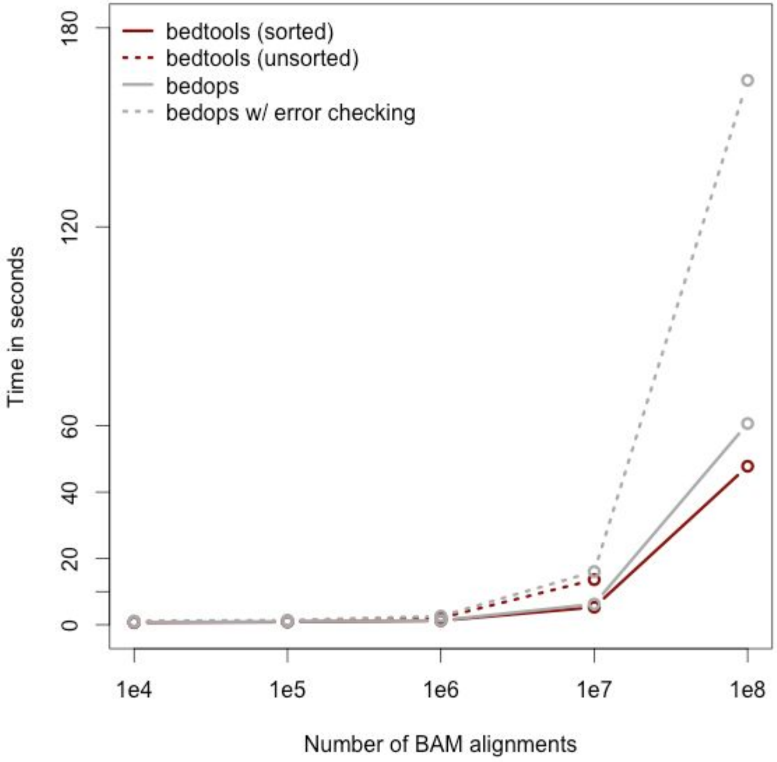
http://bedtools.readthedocs.io/en/latest/

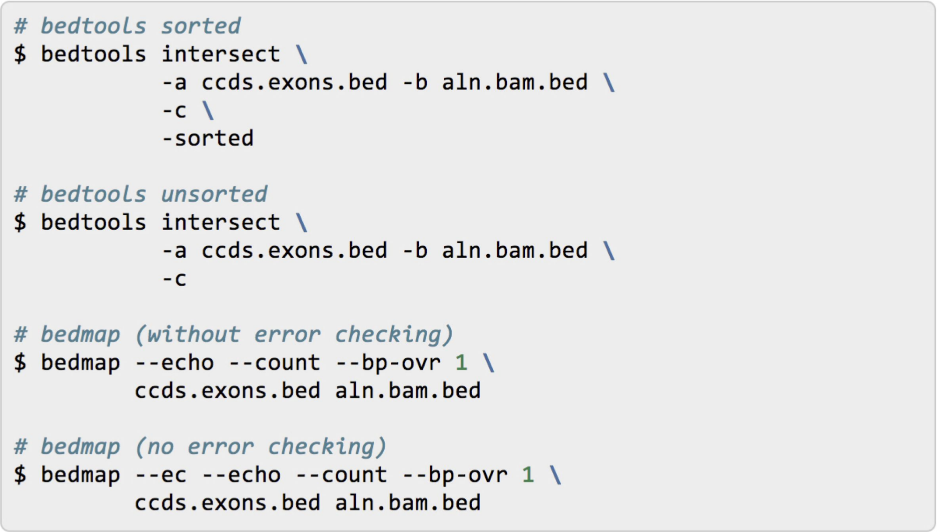
Bedtools: example analyses

- Closest gene to a ChIP-seq peak.
- Is my latest discovery novel?
- Is there strand bias in my data?
- How many genes does this mutation affect?
- Where did I fail to collect sequence coverage?
- Is my favorite feature significantly correlated with some other feature?
- What is the density of variants in ”windows” along the genome?

http://bedtools.readthedocs.io/en/latest/

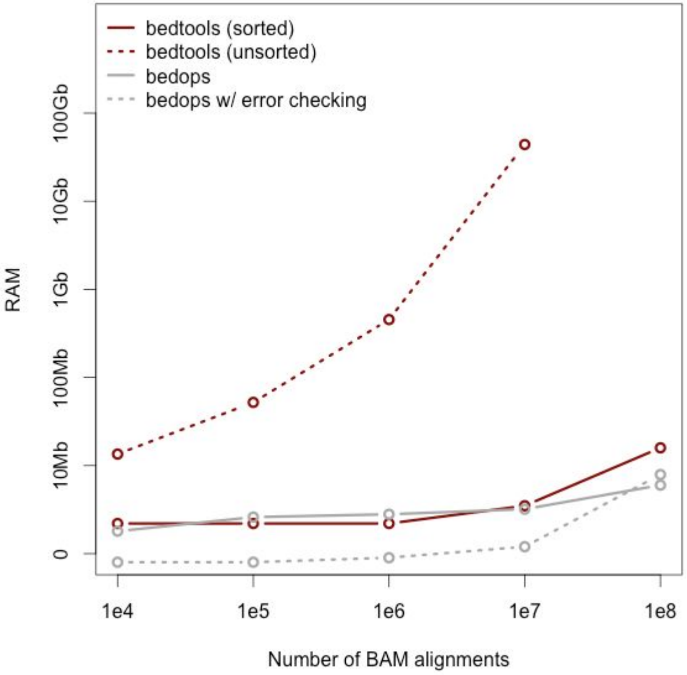
Bedtools is fairly fast.

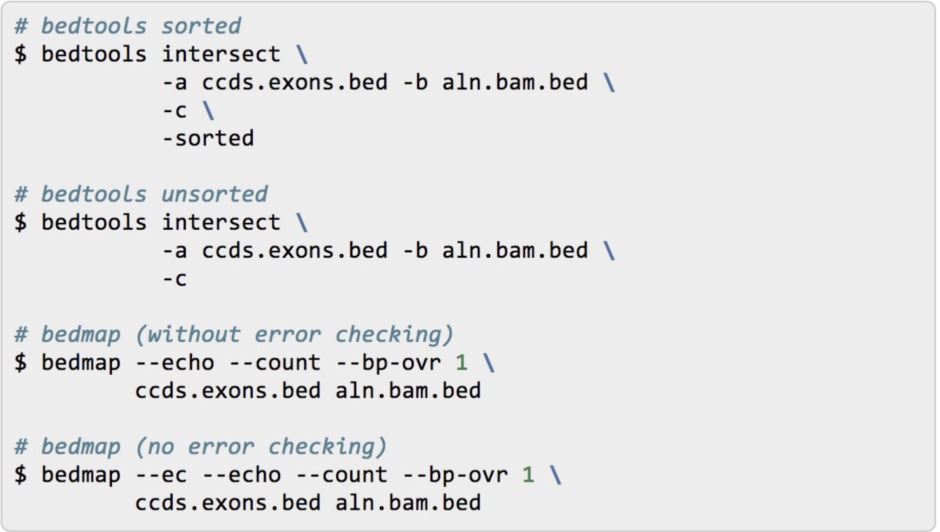




```
# bedtools sorted
$ bedtools intersect \
           -a ccds.exons.bed -b aln.bam.bed \
           -c \
           -sorted

# bedtools unsorted
$ bedtools intersect \
           -a ccds.exons.bed -b aln.bam.bed \
           -c

# bedmap (without error checking)
$ bedmap --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed

# bedmap (no error checking)
$ bedmap --ec --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed
```

And doesn’t use (too) much memory when files are ”genome sorted”.





```
# bedtools sorted
$ bedtools intersect \
           -a ccds.exons.bed -b aln.bam.bed \
           -c \
           -sorted

# bedtools unsorted
$ bedtools intersect \
           -a ccds.exons.bed -b aln.bam.bed \
           -c

# bedmap (without error checking)
$ bedmap --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed

# bedmap (no error checking)
$ bedmap --ec --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed
```

Sort chromosomes lexicographically.

Then sort numerically by start coordinate

```
sort -k1,1 -k2,2n myfile.bed > myfile.sorted.bed
```

The ”chromsweep” algorithm

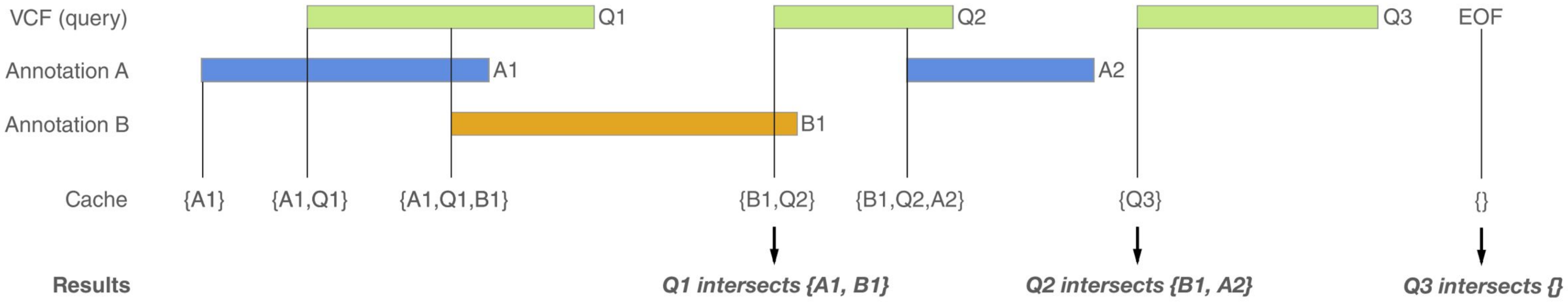
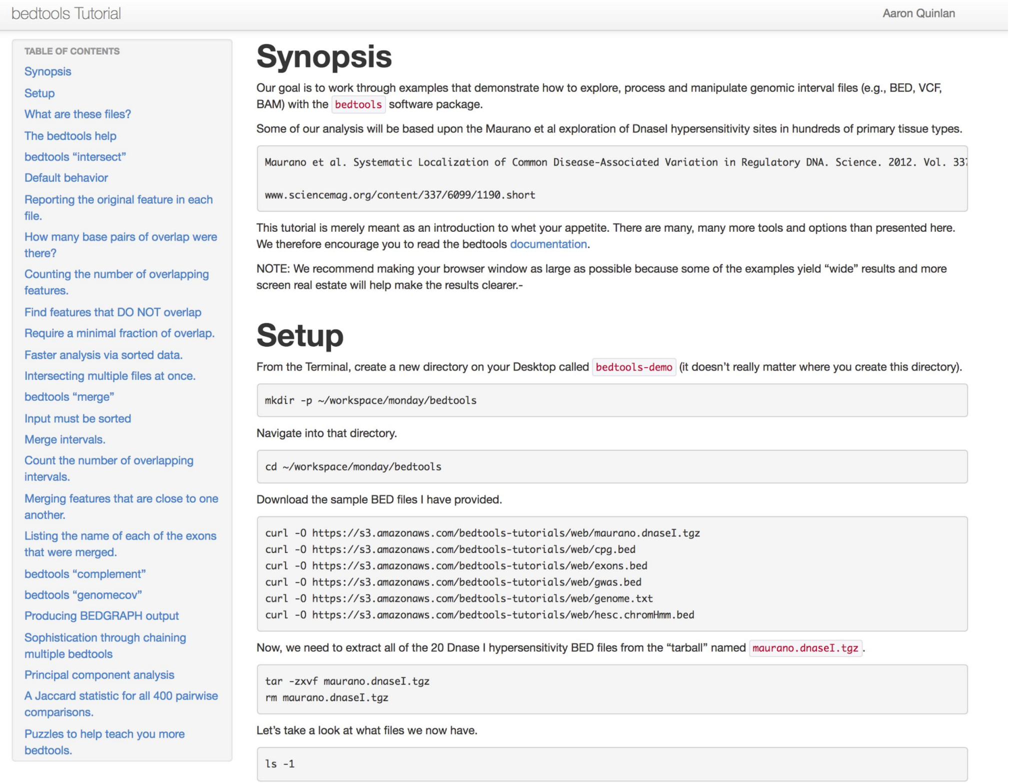


Fig. 2 Overview of the chrom-sweep interval intersection algorithm. The chrom-sweep algorithm sweeps from *left* to *right* as it progresses along each chromosome. *Green* intervals from the query VCF in the *first row* are annotated by annotation files A (*blue*) and B (*orange*) in the *second* and *third rows*, respectively. The cache row indicates which intervals are currently in the cache at each point in the progression of the sweeping algorithm. Intervals enter the cache in order of their chromosomal start position. First *A1* enters the cache followed by *Q1*. Since *Q1* intersects *A1*, they are associated, as are *Q1* and *B1* when *B1* enters the cache. Each time a new query interval enters the cache, any interval it does not intersect is ejected. Therefore, when *Q2* enters the cache, *Q1* and *A1* are ejected. Since *Q1* is a query interval, it is sent to be reported as output. Proceeding to the right, *A2* and then *Q3* enter the cache; the latter is a query interval and so the intervals that do not overlap it—*B1*, *Q2*, and *A2*—are ejected from the cache with the query interval, *Q2*, which is sent to the caller. Finally, as we reach the end of the incoming intervals, we clear out the final *Q3* interval and finalize the output for this chromosome. EOF: End of File

Let’s work through the bedtools tutorial.



bedtools Tutorial

Aaron Quinlan

Synopsis

Our goal is to work through examples that demonstrate how to explore, process and manipulate genomic interval files (e.g., BED, VCF, BAM) with the `bedtools` software package.

Some of our analysis will be based upon the Maurano et al exploration of DnaseI hypersensitivity sites in hundreds of primary tissue types.

```
Maurano et al. Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. Science. 2012. Vol. 33

www.sciencemag.org/content/337/6099/1190.short
```

This tutorial is merely meant as an introduction to whet your appetite. There are many, many more tools and options than presented here. We therefore encourage you to read the bedtools documentation.

NOTE: We recommend making your browser window as large as possible because some of the examples yield “wide” results and more screen real estate will help make the results clearer.-

Setup

From the Terminal, create a new directory on your Desktop called `bedtools-demo` (it doesn’t really matter where you create this directory).

```
mkdir -p ~/workspace/monday/bedtools
```

Navigate into that directory.

```
cd ~/workspace/monday/bedtools
```

Download the sample BED files I have provided.

```
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/maurano.dnaseI.tgz
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/cpg.bed
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/exons.bed
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/gwas.bed
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/genome.txt
curl -O https://s3.amazonaws.com/bedtools-tutorials/web/hesc.chromHmm.bed
```

Now, we need to extract all of the 20 Dnase I hypersensitivity BED files from the “tarball” named `maurano.dnaseI.tgz`.

```
tar -zxvf maurano.dnaseI.tgz
rm maurano.dnaseI.tgz
```

Let’s take a look at what files we now have.

```
ls -1
```

Connect to malibu.

```
mkdir bedtools-tutorial
cd bedtools-tutorial
```

http://quinlanlab.org/tutorials/bedtools/bedtools.html

Homework #6.

1. Finish the bedtools tutorial on your own **before class on Thursday**.
2. Answer the 10 puzzles at the bottom of: http://quinlanlab.org/tutorials/bedtools/bedtools.html

Due March 21

Submit your answers as a txt file named LASTNAME.uNID.HW6.TXT to the following Google Drive location (just drag and drop to this location):
https://drive.google.com/drive/folders/0B5Jmsvw39gJkbGdfeHZqTzlxbGc?usp=sharing