# Useful software utilities for computational genomics

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# Overview

- Search and download genomic datasets: GEOquery, GEOsearch and GEOmetadb, SRAdb

- Genomic coordinate systems

- Manipulate genomic range data with bedtools or bedops

- UCSC utils: http://hgdownload.soe.ucsc.edu/admin/exe/

- GenomicRanges and GRange container objects

- Annotation with ChIPpeakAnno

- Visualizing ChIP-seq data with ChIPseeker and Rtracklayer

- Gene set enrichment with chipenrich

- Getting annotation with UCSC Table browser

# Search and download genomic data

- **GEOsearch** provides a Bioconductor package and a webapp *https://zhiji.shinyapps.io/GEOsearch* for searching NCBI GEO (Gene Expression Omnibus) database for genomics datasets

- Comprehensive search by expanding **Gene Symbol** aliases and synonyms

- GEO Series (GSE) to Sample (GSM) mapping

- **GEOmetadb** is an SQLite database which stores GEO metadata and annotations. Provides fast search access to GEO annotation and dataset information.

- **GEOquery** is an interface to GEO platform, sample, series and dataset information

- **SRAdb** enables searching a local sqlite database for SRA (NCBI Sequence Read Archive) annotation

# Genomic Coordinate Systems

- There are two major coordinate systems in genomics.

- **Base coordinate system** anchors genomic feature to nucleotide positions while the **Interbase coordinate system** anchor genomic feature between nucleotide positions.

- Most genome annotation portals (e.g. **NCBI or Ensembl**), bioinformatics software (e.g. BLAST) and annotation file formats (e.g. **SAM, VCF, GFF and Wiggle**) use the base coordinate system, which represents a feature starting at the first nucleotide as **position 1**

- Other systems (e.g. **UCSC, Chado, DAS2**) and formats **(BAM, BCFv2, BED, and PSL)** use the interbase coordinate system, whereby a feature starting at the first nucleotide is represented as **position 0**

# Genomic Coordinate Systems

- The UCSC genome browser uses both systems and refer to the base coordinate system as "**one-based, fully-closed**" (used in the UCSC genome browser display) and interbase coordinate system as "**zero-based, half-open**" (used in their tools and fi formats).
- The interbase coordinate system is also referred t as "space-based".

There are several advantage for using the interbase coordinate system including:
1. the ability to represent features that occur between nucleotides (like a splice site),
2. simpler arithmetic for computing the length of features (length=end-start) and overlaps (max(start1,start2), min(end1,end2))
3. more rational conversion of coordinates from the positive to the negative strand

http://bergmanlab.genetics.uga.edu/?s=coordinate
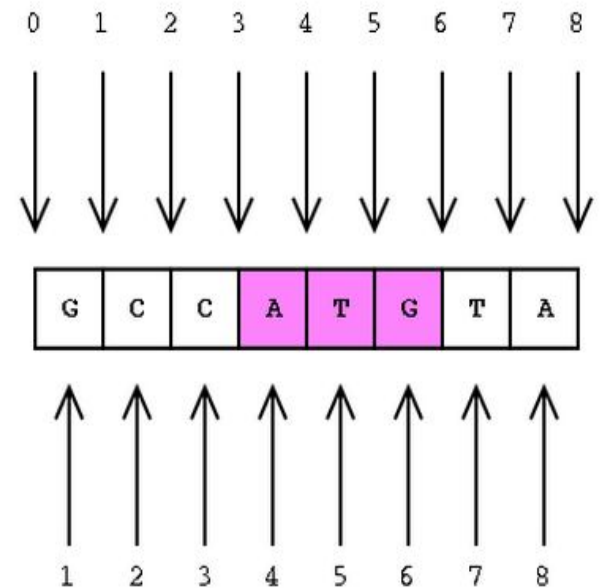
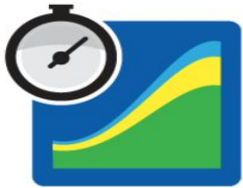# Genomic interval data with bedtools
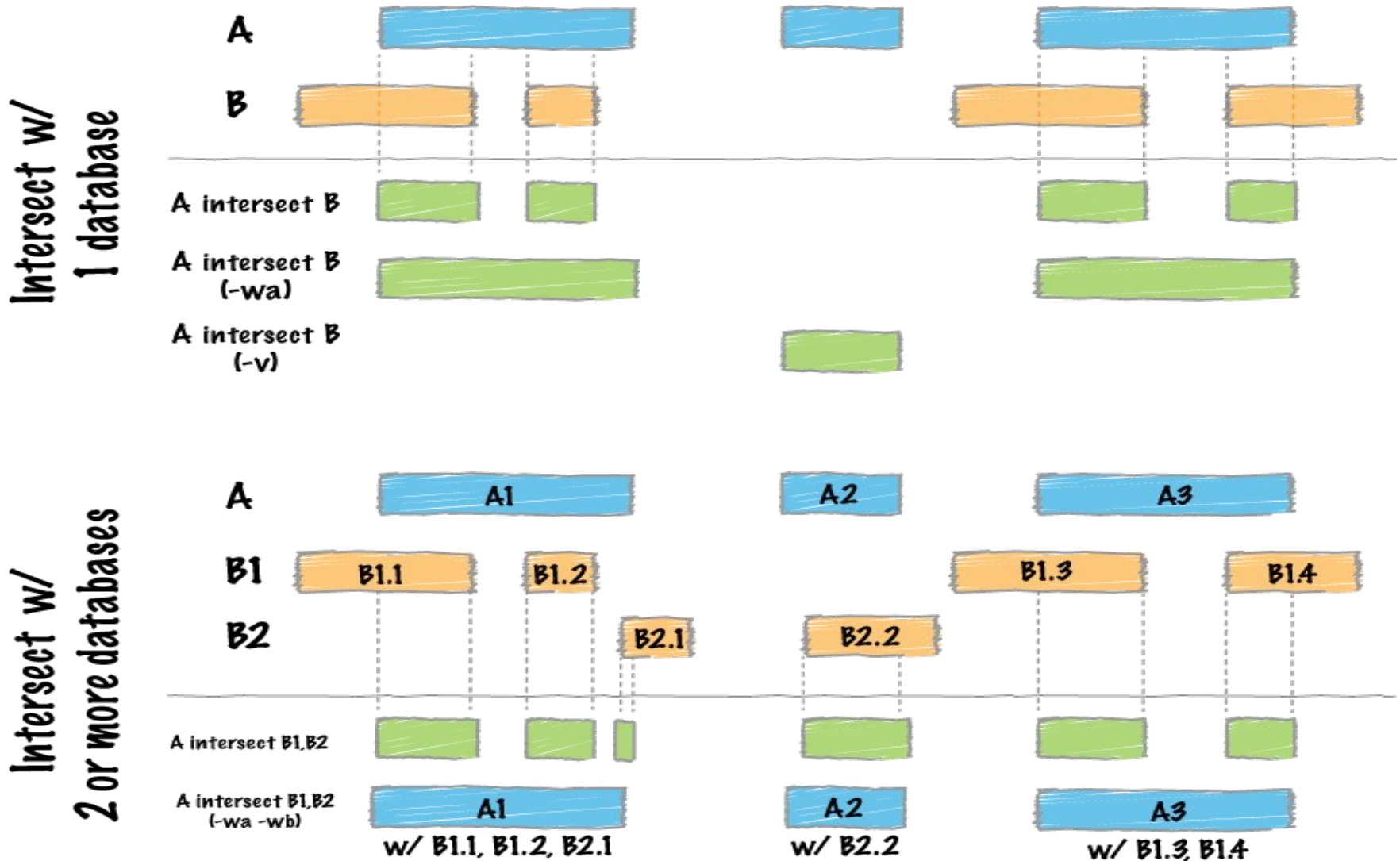
# bedtools

- [Bedtools documentation](#)
- The **bedtools** utilities are a swiss-army knife of tools for a wide-range of genomics analysis tasks.
- The most widely-used tools enable *genome arithmetic*: that is, set theory on the genome.
- For example, **bedtools** allows one to *intersect, merge, count, complement,* and *shuffle* genomic intervals from multiple files in widely-used genomic file formats such as BAM, BED, GFF/GTF, VCF.
- While each individual tool is designed to do a relatively simple task (e.g., *intersect* two interval files), quite sophisticated analyses can be conducted by chaining multiple bedtools operations on the UNIX command line.
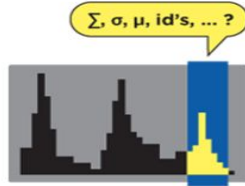
# bedops

## SET OPERATIONS

- **bedops** - apply set operations on any number of BED inputs
- **bedextract** - efficiently extract BED features
- **closest-features** - matches nearest features between BED files

## STATISTICS

- **bedmap** - map overlapping BED elements onto target regions and optionally compute any number of common statistical operations

## FILE MANAGEMENT

- **sort-bed** - apply lexicographical sort to BED data
- **starch** and **unstarch** - compress and extract BED data
- **starchcat** - merge compressed archives
- **starchstrip** - filter archive by chromosomes
- Conversion tools - convert common genomic formats to BED

## PERFORMANCE

- Parallel **bam2bed** and **bam2starch** - parallelized conversion and compression of BAM data
- Set operations with **bedops**
- Compression characteristics of **starch**
- Independent testing

## OTHER

- Table summary of **BEDOPS** toolkit
- Starch v2.2 format specification
- About nested elements
- Revision history
- Github release instructions
- Github repository

## SUPPORT RESOURCES

- How to install **BEDOPS**
- Usage examples of **BEDOPS** tools in action
- **BEDOPS** user forum
- **BEDOPS** discusssion mailing list

# UCSC utilities

- Useful UCSC utilities
  - Dusters -DNA and Protein (removes non sequence and format characters from fasta files)
  - LiftOver tool convert between genome build coordinates.
  - bedCoverage  analyses coverage by bed files - chromosome by chromosome and genome-wide usage: bedCoverage *database bedFile*
  - bedGraphToBigWig converts a bedGraph file to bigWig format. usage:

    bedGraphToBigWig *in.bedGraph chrom.sizes out.bw*
  - bigWigToWig Convert bigWig to wig.  This will keep more of the same structure of the original wig than bigWigToBedGraph does, but still will break up large stepped sections into smaller ones. Usage bigWigToWig *in.bigWig out.wig*

# Introduction to GenomicRanges

- GRanges objects
  - The GRanges() constructor
  - GRanges accessors
  - Vector operations on GRanges objects
  - Range-based operations on GRanges objects
- GRangesList objects
  - The GRangesList() constructor
  - GRangesList accessors
  - Vector operations on GRangesList objects
  - List operations on GRangesList objects
  - Range-based operations on GRangesList objects

# The GRanges class

- **GRanges** container stores a set of genomic ranges (a.k.a. genomic regions or genomic intervals).
- Each genomic range is described by **chromosome name**, **start**, **end**, and **strand**.
- **start** and **end** are both **1-based** positions relative to the 5' end of the plus strand of the chromosome, even when the range is on the minus strand.
- **start** and **end** are both considered to be included in the interval (except when the range is empty).
- The width of the range is the number of genomic positions included in it. So *width = end - start + 1*.
- **end** is always >= **start**, except for empty ranges (a.k.a. zero-width ranges) where end = start - 1. Note that the start is always the leftmost position and the end the rightmost, even when the range is on the minus strand.
- Note that a TSS is at the end of the range associated with a transcript located on the minus strand.

# ChIPpeakAnno

- Enables annotation of peaks to genomic features (Genes, TSSs, Promoters, Enhancers, CpG islands, Repeats  etc) and custom annotation
- Can perform overlap analysis
- Identify bidirectional promoters
- Combine with chromosomal conformation capture to find peaks associated with enhancer interactions
- Combine profiles from many TFs
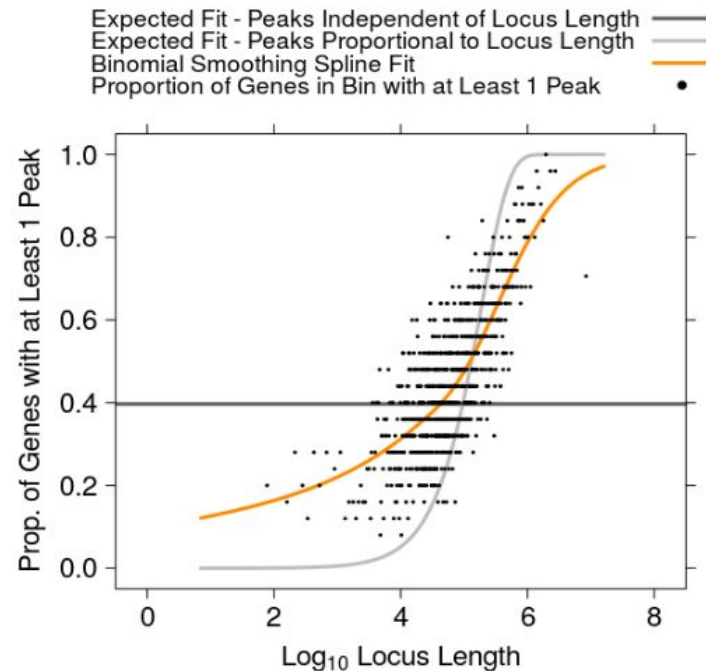- Visualize binding patterns

# ChIPseeker

- Provides methods to identify overlap with annotated genomic features, other ChIP-seq datasets and biological replicates.
- Useful for detecting co-operative binding of different factors.
- Contains a variety of visualization options
- Peak Annotation is performed by ***annotatePeak***. User can define TSS (transcription start site) region, by default TSS is defined from -3kb to +3kb.
- Peaks are annotated with the following features:
  - Promoter
  - 5' UTR
  - 3' UTR
  - Exon
  - Intron
  - Downstream
  - Intergenic
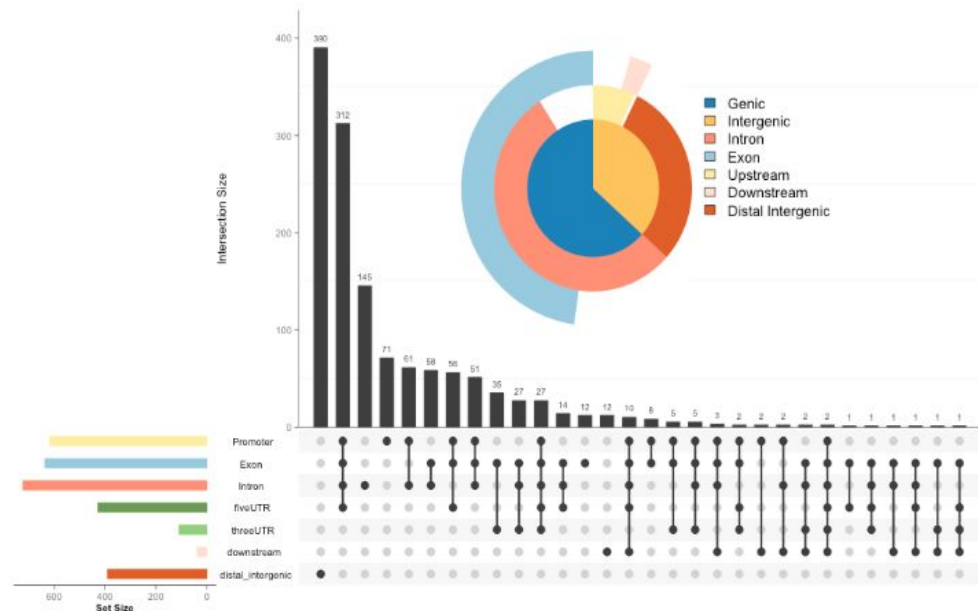
# chipenrich

- Gene set enrichment analysis for ChIP-seq.
- Takes into account length or coverage biases (length of a gene's regulatory region affects the probability that a peak will be assigned to it, the number of peaks that will be assigned to it, or the proportion of it covered by peaks.)
  - Broadenrich is designed for use with broad peaks.
  - chipenrich is designed for use with 1,000s or 10,000s of narrow peaks.
  - Polyenrich is also designed for narrow peaks, but where there are 100,000s of narrow peaks.
- Built in locus definitions, gene sets and mappability.
- Enrichment methods with FP minimization

Expected Fit - Peaks Independent of Locus Length
Expected Fit - Peaks Proportional to Locus Length
Binomial Smoothing Spline Fit
Proportion of Genes in Bin with at Least 1 Peak

# UCSC Table Browser

- Search for genes and annotation

- Setup and filters

- Join tables

- Retrieve sequences

- Intersecting tracks

- Export to external resources

# UCSC Table browser interface

# UCSC Table browser usage

- Retrieve the DNA sequence data or annotation data underlying Genome Browser tracks for the entire genome, a specified coordinate range, or a set of accessions

- Apply a filter to set constraints on field values included in the output

- Generate a custom track and automatically add it to your session so that it can be graphically displayed in the Genome Browser

- Conduct both structured and free-from SQL queries on the data

- Combine queries on multiple tables or custom tracks through an intersection or union and generate a single set of output data

- Display basic statistics calculated over a selected data set

- Display the schema for table and list all other tables in the database connected to the table

- Organize the output data into several different formats for use in other applications, spreadsheets, or databases

# Rtracklayer

- The rtracklayer package is an interface (or layer ) between R and genome browsers
- Its main purpose is the visualization of genomic annotation tracks, whether generated through experimental data analysis performed in R or loaded from an external data source.
- The features of rtracklayer may be divided into two categories:
  - The import/export of track data
  - The control and querying of external genome browser sessions and views.
- The basic track data structure in Bioconductor is the *GRanges* class, defined in the *GenomicRanges* package.
- All positions in a GRanges should be 1-based.

- rtracklayer supports the import and export of tracks from and to files in various formats.
- With rtracklayer, the user may start a genome browser session, create and manipulate genomic views, and import/export tracks and sequences to and from a browser.

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