Lab Assignment 3: Sequence Comparison

2024 02 02

Obtained from 2024 MTEs

Part 1: Finding the difference between hg38 and CHM13

Part 1: Compare hg38 to CHM13

- hg38 is a chimeric reference from a few individuals
  - Maintained and developed by the Genome Research Consortium
  - Many gaps and satellite contigs
- CHM13 is from the CHM13 cell line that expresses human telomerase reverse transcriptase (hTERT) (retains 46,XX karyotype)
  - Developed by the T2T consortium

Cold Spring Harbor Laboratory

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doi: https://doi.org/10.1101/2021.05.26.445798

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0 0 0 0 85 0 3418

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Part 1: Compare hg38 to CHM13

- Copy the finding_gaps_template.py script to your directory
  - /storage1/fs1/workshops/Active/BIO5488/assignments/assignment3/finding_gaps_template.py

- Complete the script to do the following:
  - Count nucleotide frequencies
  - Count dinucleotide frequencies
  - Count and gaps and gap sizes
  - Plot gap size distribution

Part 2: Align Reads to Chr22 and mark Duplicates

Step 1: Create Indices for hg38 and CHM13

- Create an index for both assemblies of chr22
- Example command in assignment

```
asouthard-smith@genomics:~$ bowtie2-build
No input sequence or sequence file specified!
Bowtie 2 version 2.3.4.1 by Ben Langmead (langmea@cs.jhu.edu, www.cs.jhu.edu/~langmea)
Usage: bowtie2-build [options]* <reference_in> <bt2_index_base>
    reference_in            comma-separated list of files with ref sequences
    bt2_index_base          write bt2 data to files with this dir/basename
*** Bowtie 2 indexes work only with v2 (not v1).  Likewise for v1 indexes. ***
Options:
    -f                      reference files are Fasta (default)
    -c                      reference sequences given on cmd line (as
                            <reference_in>)
    --large-index           force generated index to be 'large', even if ref
                            has fewer than 4 billion nucleotides
    -a/--noauto             disable automatic -p/--bmax/--dcv memory-fitting
    -p/--packed             use packed strings internally; slower, less memory
    --bmax <int>            max bucket sz for blockwise suffix-array builder
    --bmaxdivn <int>        max bucket sz as divisor of ref len (default: 4)
    --dcv <int>             diff-cover period for blockwise (default: 1024)
    --nodc                  disable diff-cover (algorithm becomes quadratic)
    -r/--noref              don't build .3/.4 index files
    -3/--justref            just build .3/.4 index files
    -o/--offrate <int>      SA is sampled every 2^<int> BWT chars (default: 5)
    -t/--ftabchars <int>    # of chars consumed in initial lookup (default: 10)
    --threads <int>         # of threads
    --seed <int>            seed for random number generator
    -q/--quiet              verbose output (for debugging)
    -h/--help               print detailed description of tool and its options
    --usage                 print this usage message
    --version               print version information and quit
asouthard-smith@genomics:~$
```

Step 2: Align with bowtie2

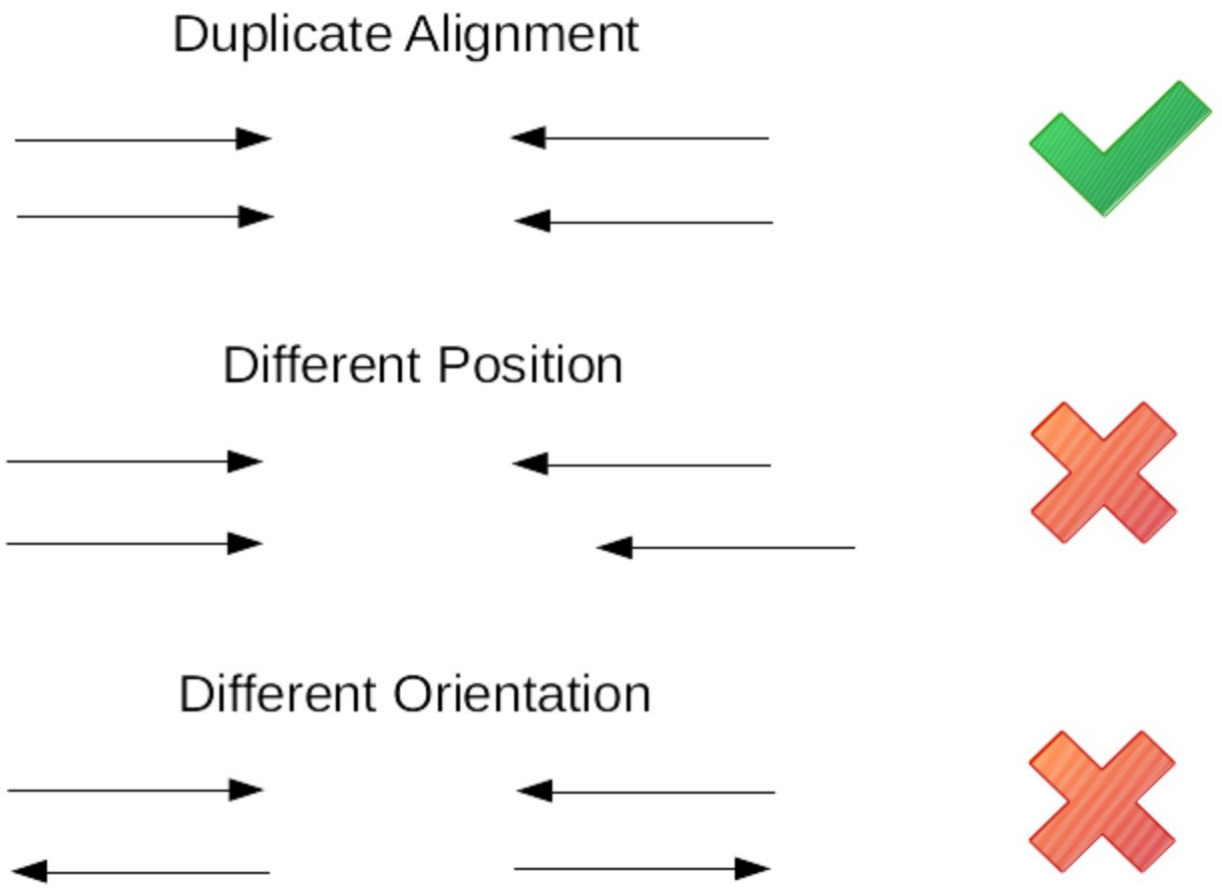
- Reads are in:
  /storage1/fs1/workshops/Active/BIO5488/assignments/assignment3/test-500k.fq
- The fastq file is unpaired
- Save both the alignment and report output files

http://bowtie-bio.sourceforge.net/bowtie2/manual.shtml

```
asouthard-smith@genomics:~$ bowtie2
No index, query, or output file specified!
Bowtie 2 version 2.3.4.1 by Ben Langmead (langmea@cs.jhu.edu, www.cs.jhu.edu/~langmea)
Usage:
  bowtie2 [options]* -x <bt2-idx> {-1 <m1> -2 <m2> | -U <r> | --interleaved <i>} [-S <sam>]

  <bt2-idx>  Index filename prefix (minus trailing .X.bt2).
             NOTE: Bowtie 1 and Bowtie 2 indexes are not compatible.
  <m1>       Files with #1 mates, paired with files in <m2>.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <m2>       Files with #2 mates, paired with files in <m1>.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <r>        Files with unpaired reads.
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <i>        Files with interleaved paired-end FASTQ reads
             Could be gzip'ed (extension: .gz) or bzip2'ed (extension: .bz2).
  <sam>      File for SAM output (default: stdout)

  <m1>, <m2>, <r> can be comma-separated lists (no whitespace) and can be
  specified many times.  E.g. '-U file1.fq,file2.fq -U file3.fq'.

Options (defaults in parentheses):

 Input:
  -q                 query input files are FASTQ .fq/.fastq (default)
  --tab5             query input files are TAB5 .tab5
  --tab6             query input files are TAB6 .tab6
  --qseq             query input files are in Illumina's qseq format
  -f                 query input files are (multi-)FASTA .fa/.mfa
  -r                 query input files are raw one-sequence-per-line
  -F k:<int>,i:<int> query input files are continuous FASTA where reads
                     are substrings (k-mers) extracted from a FASTA file <s>
                     and aligned at offsets 1, 1+i, 1+2i ... end of reference
  -c                 <m1>, <m2>, <r> are sequences themselves, not files
  -s/--skip <int>    skip the first <int> reads/pairs in the input (none)
  -u/--upto <int>    stop after first <int> reads/pairs (no limit)
  -5/--trim5 <int>   trim <int> bases from 5'/left end of reads (0)
  -3/--trim3 <int>   trim <int> bases from 3'/right end of reads (0)
  --phred33          qualities are Phred+33 (default)
  --phred64          qualities are Phred+64
  --int-quals        qualities encoded as space-delimited integers

 Presets:                 Same as:
  For --end-to-end:
   --very-fast           -D 5 -R 1 -N 0 -L 22 -i S,0,2.50
   --fast                -D 10 -R 2 -N 0 -L 22 -i S,0,2.50
   --sensitive           -D 15 -R 2 -N 0 -L 22 -i S,1,1.15 (default)
   --very-sensitive      -D 20 -R 3 -N 0 -L 20 -i S,1,0.50

  For --local:
   --very-fast-local     -D 5 -R 1 -N 0 -L 25 -i S,1,2.00
   --fast-local          -D 10 -R 2 -N 0 -L 22 -i S,1,1.75
   --sensitive-local     -D 15 -R 2 -N 0 -L 20 -i S,1,0.75 (default)
   --very-sensitive-local -D 20 -R 3 -N 0 -L 20 -i S,1,0.50

 Alignment:
```

Step 3: Remove Duplicates with Samtools

- Duplicate reads are measured from the position of the 5’ end of the read
- Duplicates are the result of non-uniform amplification and hybridization rates during library construction & sequencing

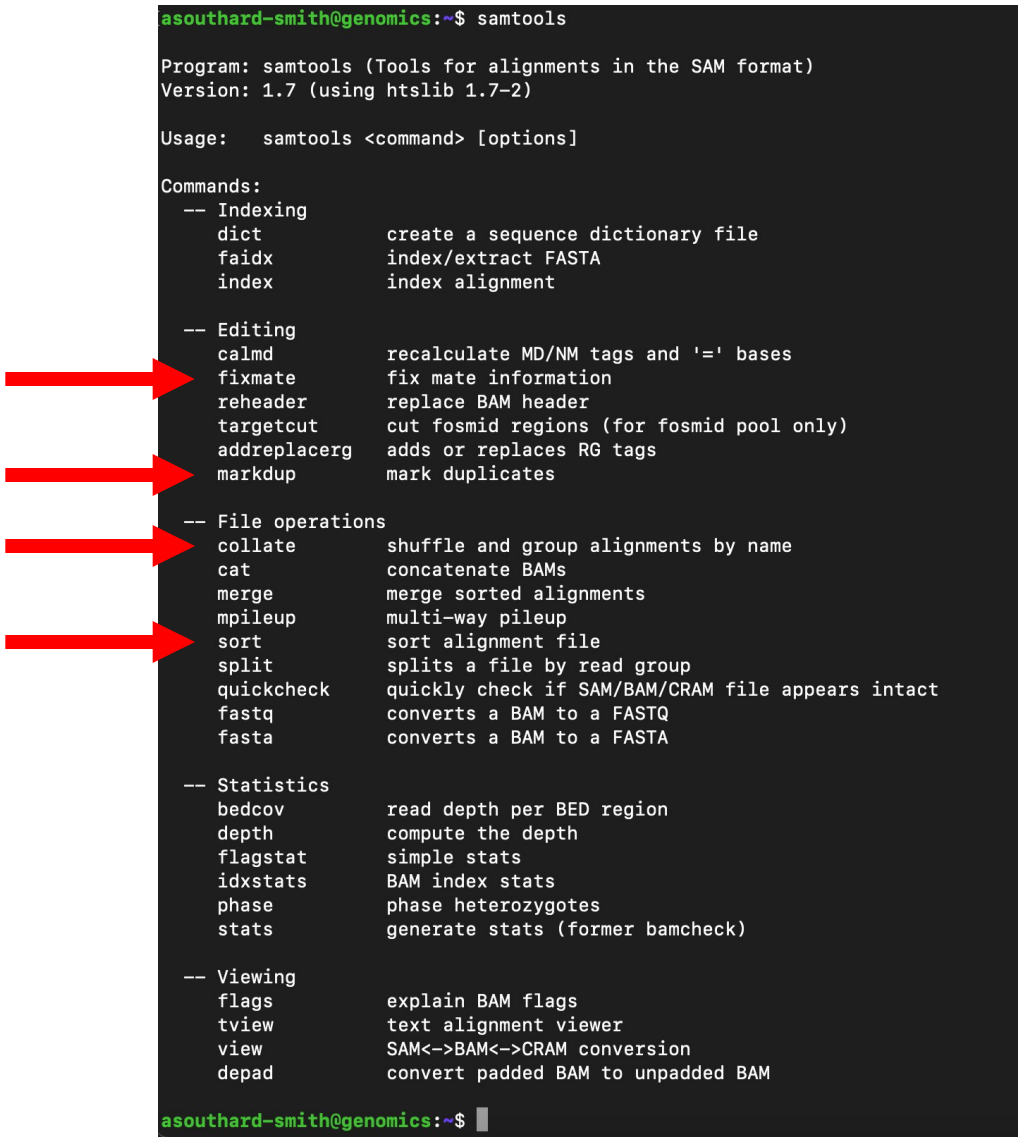


https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-016-1097-3

http://www.htsli b.org/algorithms/duplicate.html

Step 3: Remove Duplicates with Samtools

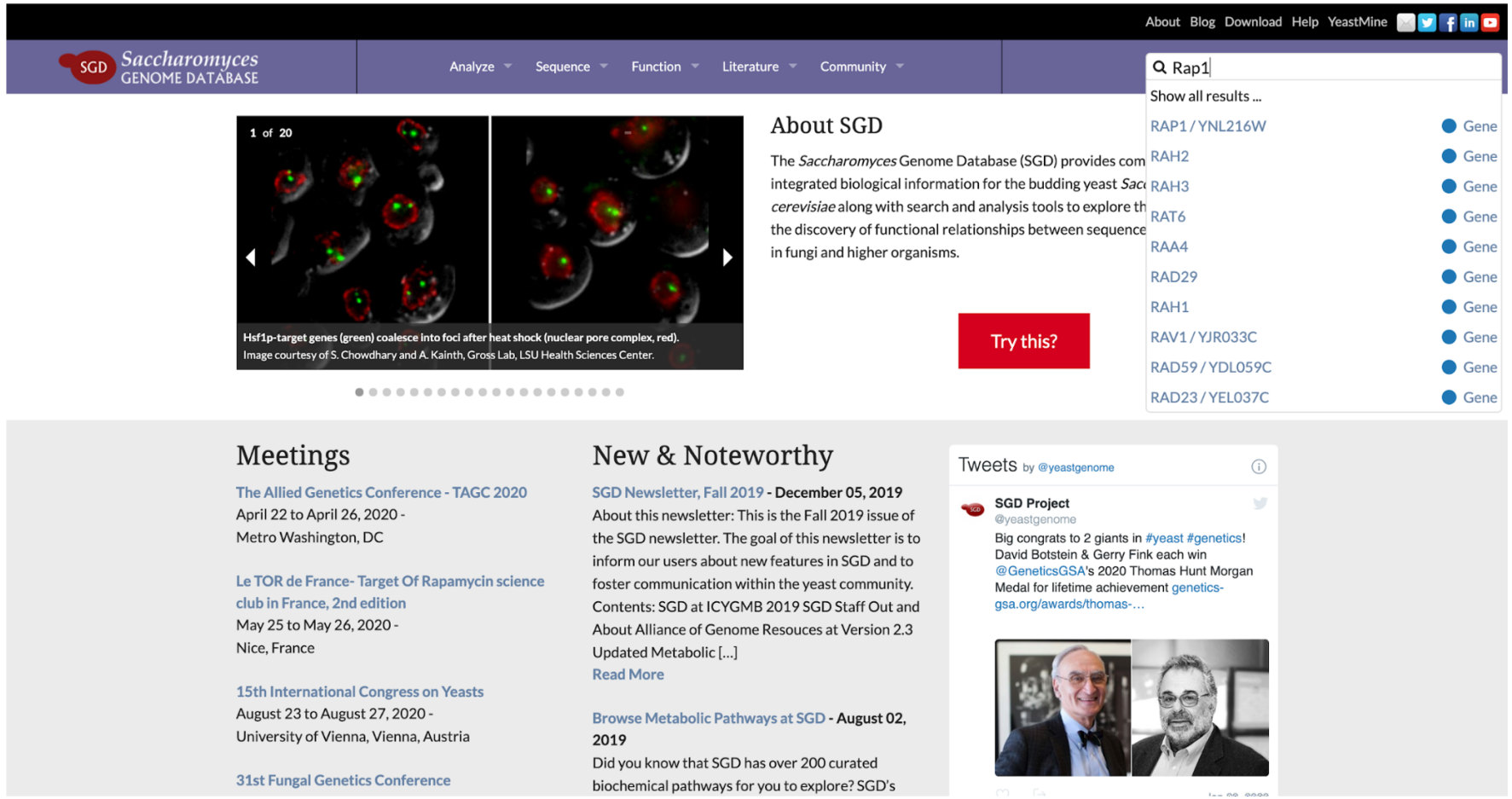
- 4 stages:
  - Collate
  - Fixmate
  - Sort
  - MarkDuplicates
- markdup output should be saved to a file
- markdup report will be output to terminal



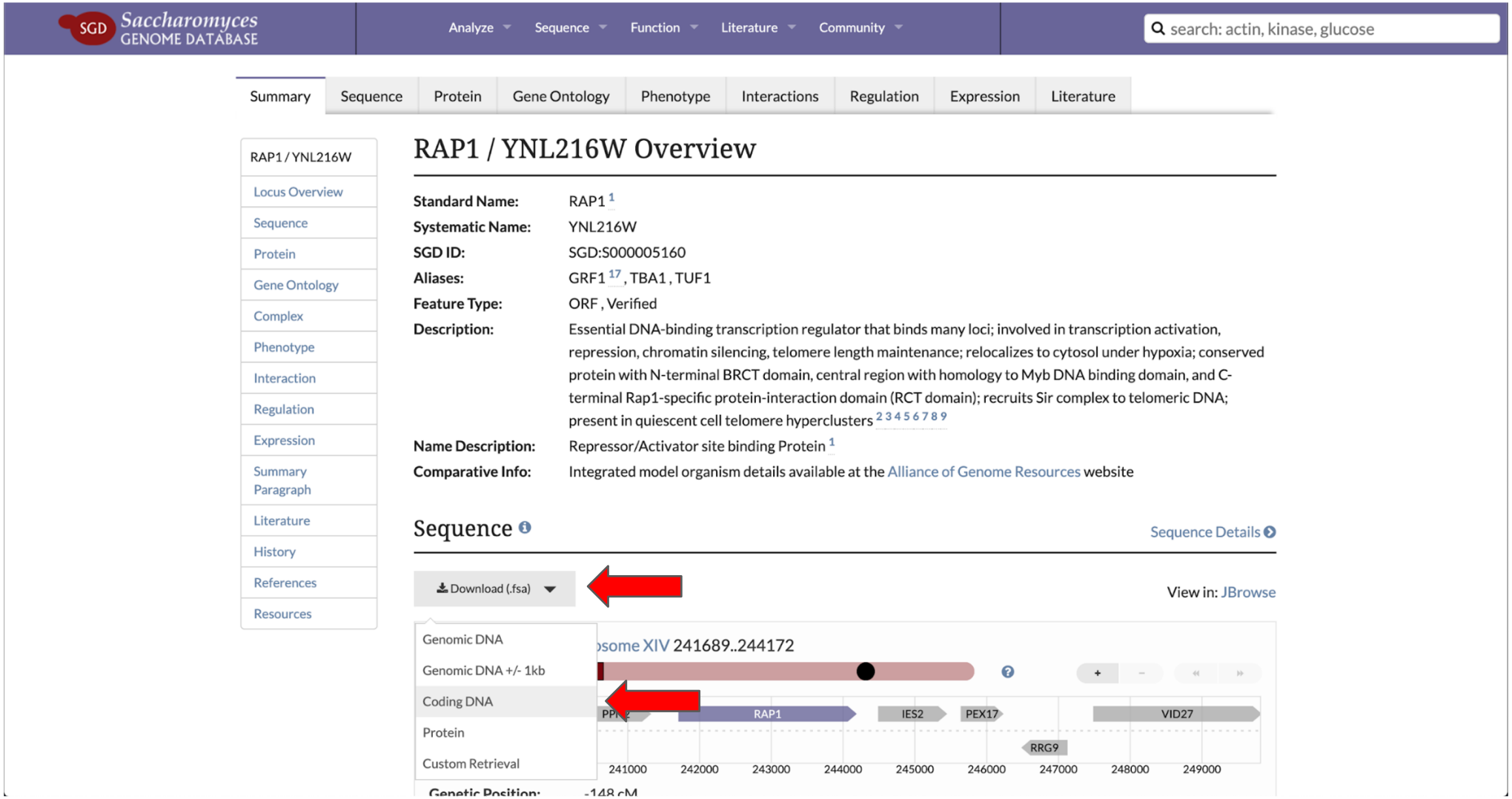
Part 3: Running BLAST

Step 1: Obtain Gene Sequence

- Obtain **coding** sequence for *RAP1* from yeastgenome.org

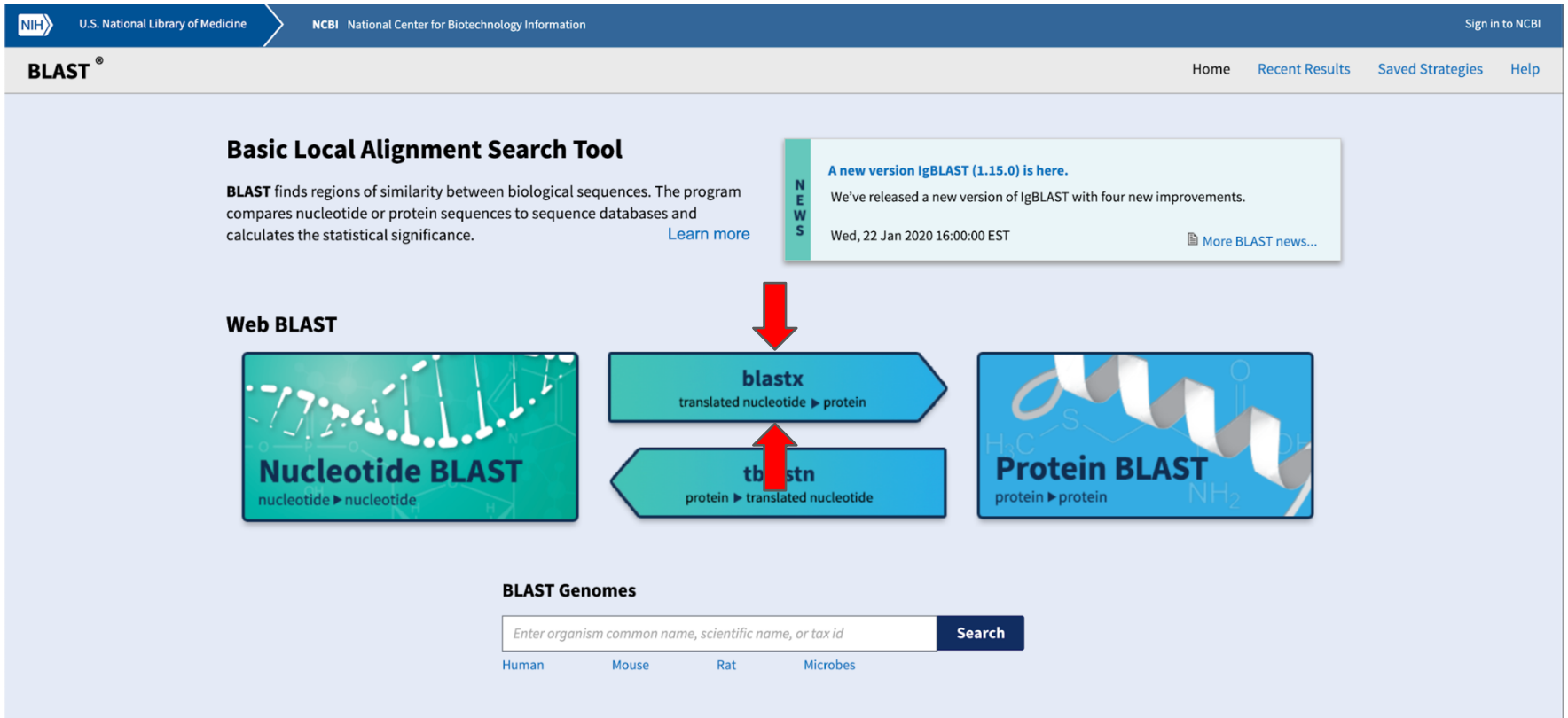


Step 1: Obtain Gene Sequence

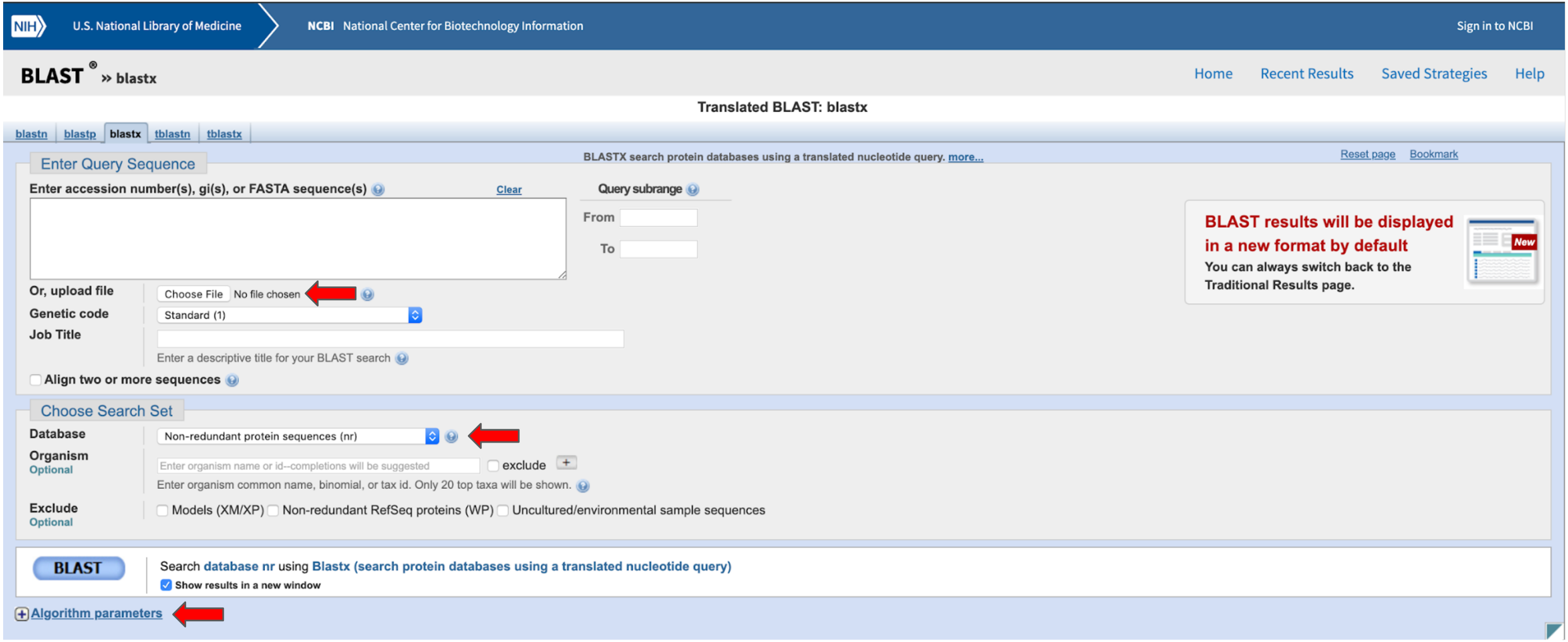


Step 2: Run BLASTx Web Tool

- Compare BLOSUM62 vs. BLOSUM80
- Compare default existence (11), extension (1) penalties vs existence of 7, extension of 2



Step 2: Run BLASTx Web Tool



Step 2: Run BLASTx Web Tool

Things to turn in:

- README.txt
- finding_gaps.py script
- hg38-chr22_gap_distribution.png
- Bowtie2 Alignment files
- Bowtie2 Report files
- Samtools duplicates removed sam file
- Extra credit items:
  - Additional gap size distribution plot
  - Python script for tallying duplicates from the bowtie2 aligned sam file.