RNA-Seq: Aligner, Output (SAM), and Visualization

BCB 5250 Introduction to Bioinformatics II

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RNA-Seq Tools for:

- Data Quality Control
- Read Mapping
- Differential Gene Expression

Data Quality Control

Quality assessment

- FastQC, MultiQC

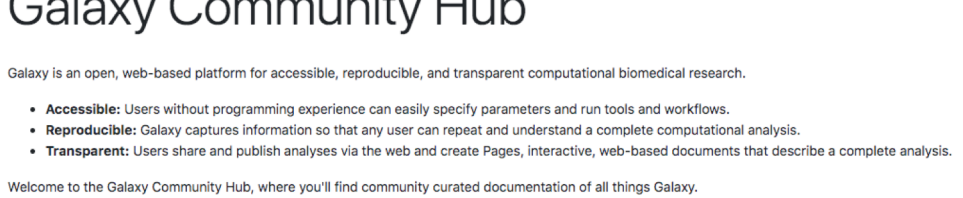
Trim and filtering:

- FASTX Tool Kit: http://hannonlab.cshl.edu/fastx_toolkit/
- Trimmomatic: http://www.usadellab.org/cms/?page=trimmomatic
- Tim Galore: http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
- fastp: https://github.com/OpenGene/fastp
- BBTools – BBDuk: https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/
- Cutadapt: https://cutadapt.readthedocs.io/en/stable/

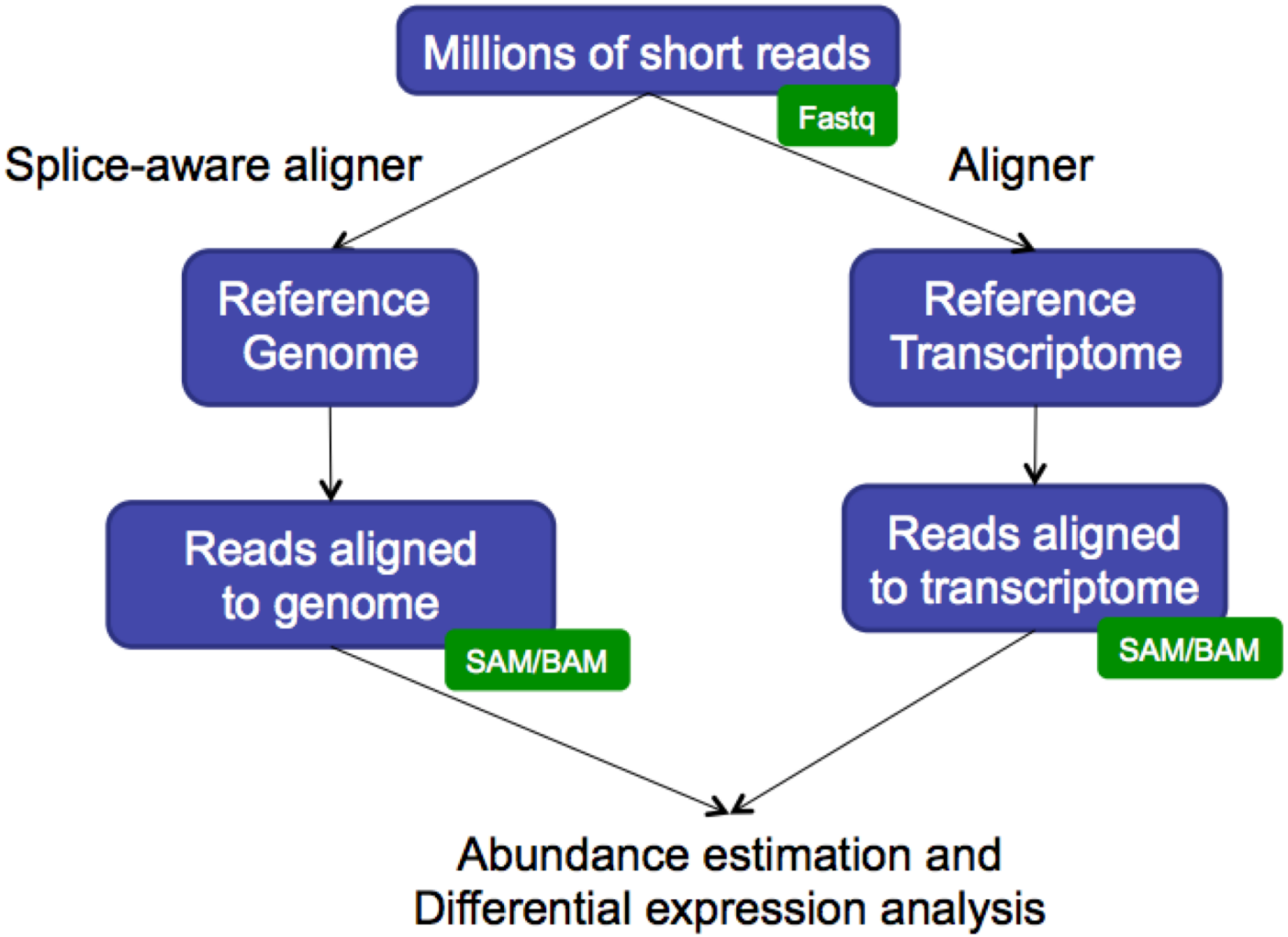
Error correction:

- BBTools – Tadpole: https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/tadpole-guide/
- https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-017-1784-8
- For long sequencing, check the recently published papers and archive such as http://www.pacb.com/asset_tags/error-correction/

https://galaxyproject.org/



Mapping Reads



Aligner

- Short Read Mapper
  - Bowtie2: http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
  - BWA: http://bio-bwa.sourceforge.net/
- Splice Aligner
  - TopHat2: https://ccb.jhu.edu/software/tophat/index.shtml
  - HiSAT2: https://daehwankimlab.github.io/hisat2/
  - STAR: https://github.com/alexdobin/STAR

Mapping

- Input
  - Fastq files
  - Index of genome/transcriptome
  - Annotation file (optional for some, but required for others)
- Output
  - SAM (text) / BAM (binary) alignment files
    - SAMtools – SAM/BAM file manipulation (http://samtools.sourceforge.net/) (http://www.htslib.org/)
    - Picard-tools – SAM/BAM file manipulation (https://broadinstitute.github.io/picard/)
  - Summary statistics (per read library)
    - reads with unique alignment
    - reads with multiple alignments
    - reads with no alignment
    - reads properly paired (for paired-end libraries)

Most Aligner

Most aligners have modules for below two steps:

1. Generating genome indexes files.
2. Mapping reads to the genome.

Why?

Let us test bowtie2 aligner

1. Open manual (http://bowtie-bio.sourceforge.net/bowtie2/manual.shtml#getting-started-with-bowtie-2-lambda-phage-example)
2. Let us work "Getting Started"
   - Copy the genome to your lab directory and index a reference genome
     - /public/ahnt/courses/bcb5250/rna_seq_lab/bowtie2_example/reference/lambda_virus.fa
   - Aligning example reads
     - /public/ahnt/courses/bcb5250/rna_seq_lab/bowtie2_example/reads/reads_1.fq
   - Paired-end example
     - reads_1.fq, reads_2.fq
   - From the paired-end aligning output, let us test SAMtools/BCFtools downstream analysis
     - Convert SAM to BAM
     - Sort
     - To generate variant calls in VCF format
     - Then to view the variants

SAM (BAM) Format

- Sequence Alignment/Map format
  - Universal standard
  - Human-readable (SAM) and compact & binary (BAM) forms
- Structure
  - Header
    - version, sort order, reference sequences, read groups, program/processing history
  - Alignment records

Check header

$ samtools view -H -S XXX.sam

[samopen] SAM header is present: 1 sequences.

@HD VN:1.0 SO:unsorted

@SQ SN:genome LN:451226

@PG ID:bowtie2 PN:bowtie2 VN:2.3.4.3 CL:"/usr/local/miniconda/bin/bowtie2-align-s --wrapper basic-0 -x ../GENOME_data/genome -S Sp_ds.sam -p 12 -t -1 Sp_ds.left.fq.gz -2 Sp_ds.right.fq.gz"

Check alignment

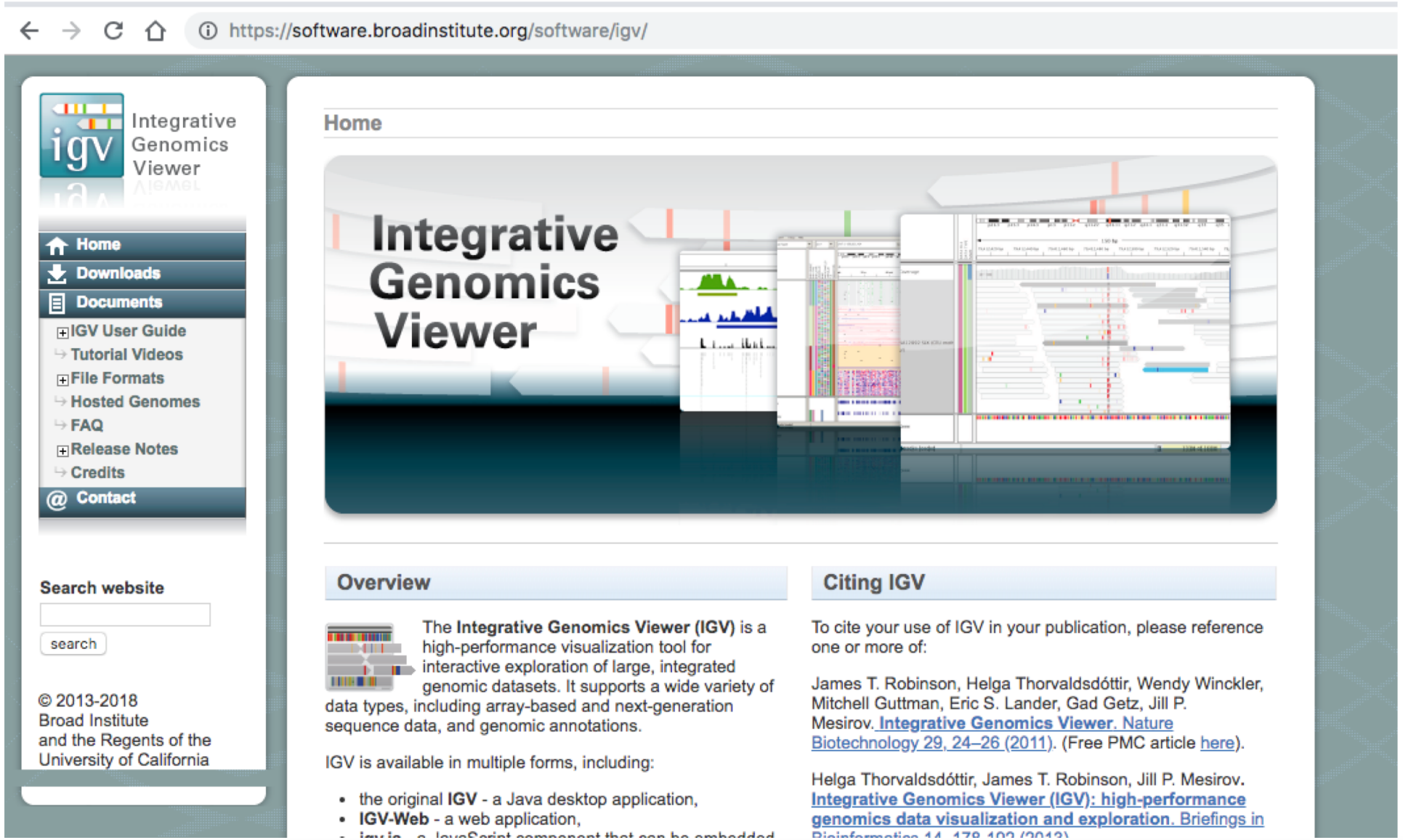
$ samtools view -S XXX.sam | more

```
61G9EAAXX100520:5:100:10001:20086       83      genome  59809   42      68M     =       59622   -255    GGTTTTAAAATTGTGCATGTTTATAGTTACCTTCATTTATGCTGGAAGAAATGAAATGAATTTT
TGTT    :CBE?EGFDFAEFEEEE?EFEGGGGGGGDFGGGGGFDDGGGGGGGGGGGGGGEGGGGGGGGEGGGGGGG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10001:20086       163     genome  59622   42      68M     =       59809   255     GTGTTAAATACTTGGAGGAGAACTTTGGCGCTCTCAAGGTTAAATTATCCGATGCCACTGTTAA
GGAA    GFGGGGGGGGGGGGGDGGFGGGGGGGGGGGGGGGGGGGFDGGGGGGGGDGGBFGFFEDGGBEEEBEDG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10002:14745       99      genome  400042  42      68M     =       400185  211     GGTAGTAGTAGCAGCAGCATTTGTGGAAGCTGAAGTAGGCAAGGTGCTGGGAGTAGATTCAACA
GCCT    EEEEEEFFCFFGGGFGFFEFGFFDEECEDCDCC?C=A;C@BAAA9CAACCC?C6A=36?8<?:?5<=@        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10002:14745       147     genome  400185  42      68M     =       400042  -211    CAGTCTCAGTGGTGGCAGGATAGTGATAGGCGGCGGACTTGCTCTTAGTACTAACATCAGTAGG
TTCA    GDEFBGBEGFFGF?GFFGFDGGFGGGGFGGEGGGGGFGGGGGEFGGGGEFGGGFGGGGGGGGGGGEGGG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10007:9987        83      genome  130215  42      68M     =       130091  -192    GAGTCTTGTGGTTGCAAGAGCAGCGCTGTTGATAACTGCAAGTGCTCTTCTTGCAAATGTGCTT
CGAA    CDBCDCCBDDDCEEEDEEEEFEGCFFGFGFFGGGGGGGGGGEEGEGGGGFFGGGGGGGGGGGGGGGG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10007:9987        163     genome  130091  42      68M     =       130215  192     ATCGACTTTCAACAACTCATATCACCCTAACAAACTTTCAACGATGGAACACACTACCCAATGT
AAGA    GGGGGGGGGGGGGGGGFGGGGGGGGGGGFDGGFGGGGGGGFGFGGGEGGEGFGGGGGFGEGDFFFGFE        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10008:6997        99      genome  400469  42      68M     =       400602  201     GTGGCAGAGCTATAGTCGAGGTTGGCGGGTTGAGGAGGATGAGAGCGTTTAGCGTTAAGGTCTT
GAGT    GGGFGGGGGGGGGDGFEGEGFCFFFEEEE:B@@AA=AA=???=:?ACACBABAC?B<=AA:????###        AS:i:-5 XN:i:0  XM:i:1  XO:i:0  XG:i:0  NM:i:1  MD:Z:0A67       YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10008:6997        147     genome  400602  42      68M     =       400469  -201    TGGTTTCATTGCGCTTCTCAGCGGCCTTGACGGCATCATAGGCTTGAGTGGCACAACGAGTGAT
TTCC    EECFEEDCGEEEGCGFDDEEEEGGCDEFFEFGGDFGGGGGGGGGGGGGGGGGGFGGGGGGGGGGGFGGG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:-5 YT:Z:CP
61G9EAAXX100520:5:100:10022:7766        153     genome  160514  42      68M     =       160514  0       ATGGTACTGAGTCTTTGCGGAGTAATTTCATTTCGTAATACTGATGCTCTAAGTTTCGGGTTAT
TTTT    ?FFDECFEDBEEFBFEGBDGGEFGGFFEDFGDDGGGGGGBGGGGEGGGGGGDGGEGGGGGGGGGGGGE        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YT:Z:UP
61G9EAAXX100520:5:100:10022:7766        101     genome  160514  0       *       =       160514  0       CCATGATTTAAGAAAATTTCCAGCGATTTCCGCAAACAGTACATTCACAAAACGTTGTCATAGG
CTCA    GFFGGGEGGGGGGEFDGGGFGGGGGDGGGDEEGFAGDFGDGEDGFBFEFEEFEF?E=EEC?ECADFF:        YT:Z:UP
61G9EAAXX100520:5:100:10038:8120        99      genome  166132  42      68M     =       166425  361     GGGGCATGTAACATTGGTAGCATAATCGCAACCAGAGCTAGAGCAGTTGAAACGTCCAATTTCG
GCAA    GGGGGEGGGGGGGGGGDGEGGFGGGGGGGGFDEGFFBDFFEE@FE@ECEEDD?DD@DDC@CCCD=CCBB        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:-4 YT:Z:CP
61G9EAAXX100520:5:100:10038:8120        147     genome  166425  42      68M     =       166132  -361    TACCGGAGTTAGCTTCAGGCTTAGTCGAATCAATTAAACGAATGCCATGGGGGAACGAAGGATA
TACA    ECDDFEEBDECGEBFEDEGEFGGFGGEGFFEFFGGFEDGGGGFFGFGEGGGGGGEG:GFGGGGGGFGG        AS:i:-4 XN:i:0  XM:i:1  XO:i:0  XG:i:0  NM:i:1  MD:Z:56A11      YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10040:13843       99      genome  166901  42      68M     =       166913  80      GAAGAGATGGGTTGAGGCAAAACAGCCAAAACAGCAATACCTACACAACTAGGGTTACCGTTTC
CAAC    GFGGGGGGGGGFGGGFGGDGFGGGGFDFFFGFFGFEEFEFEGGFDFEEEBEEEDBFFDDFCCDCCCDC        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
61G9EAAXX100520:5:100:10040:13843       147     genome  166913  42      68M     =       166901  -80     TGAGGCAAAACAGCCAAAACAGCAATACCTACACAACTAGGGTTACCGTTTCCAACTCCAAAGA
ATAC    DDEFDEEEEDGEEEGEEEGGEEEGERGEEDGEGGEGEGGGGDGEEGGGDGGGGGEGEDGGGGGGGGGG        AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:68 YS:i:0  YT:Z:CP
```

Samtools

- http://www.htslib.org/
- https://samtools.github.io/hts-specs/SAMv1.pdf

IGV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James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. Integrative Genomics Viewer. Nature Biotechnology 29, 24–26 (2011). (Free PMC article here).
Helga Thorvaldsdóttir, James T. Robinson, Jill P. Mesirov. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Briefings in

IGV

- Load Genome
- Load sorted BAM alignments (you need index file)