Visualization and Variant Calling

GSND 5340Q, BMDA

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Visualization of Aligned Sequencing Data

Options for data visualization

- UCSC Genome Browser
- IGB (http://bioviz.org/igb/)
- IGV (http://www.broadinstitute.org/igv/)
- SAMtools

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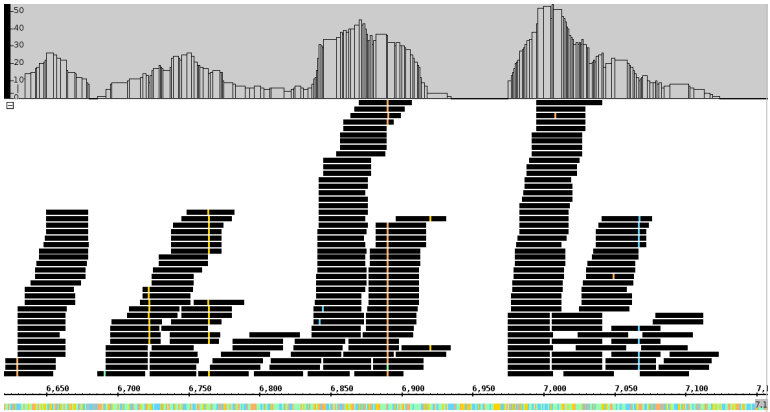
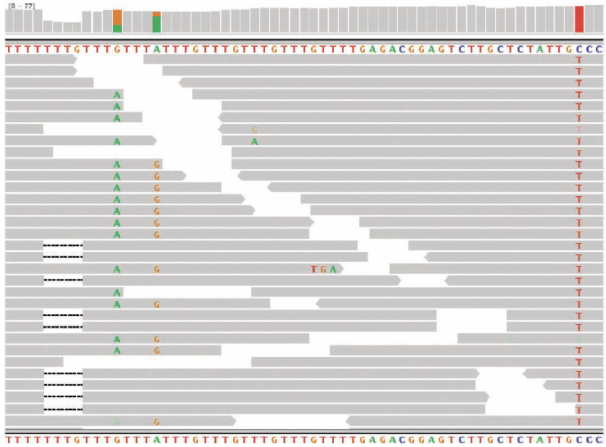
IGB Example

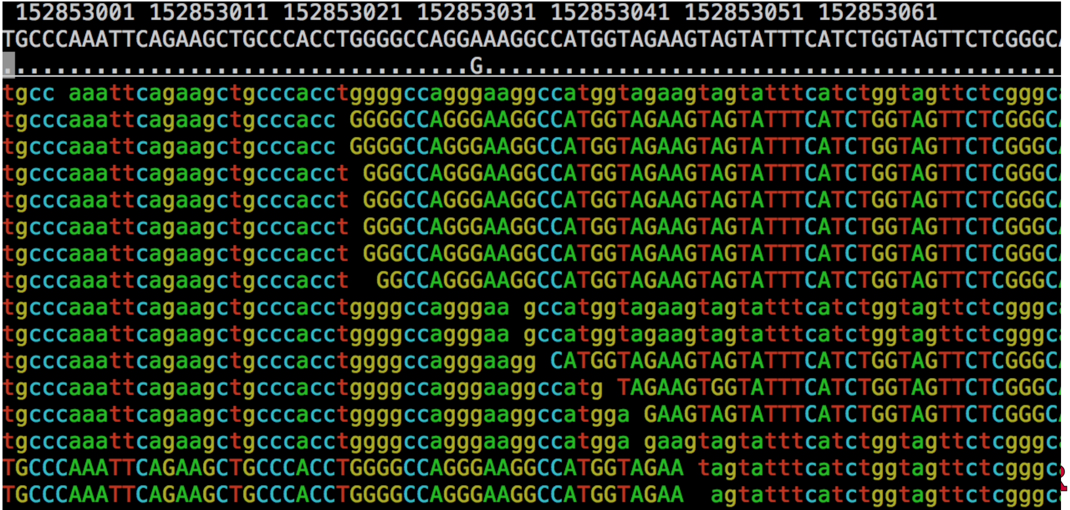

50
40
30
20
10
6,650
6,700
6,750
6,800
6,850
6,900
6,950
7,000
7,050
7,100

IGV Example



DePristo et al. *Nature Genetics 2011; 43: 491-8.*

SAMtools



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SAMtools Example (Amarel Desktop)

```
# Load SAMtools
module load samtools

# Convert SAM file to BAM format:
samtools view -bS myalignments.sam > myalignments.bam

# Sort the BAM file:
samtools sort myalignments.bam -o myalignments.sorted.bam

# Index the BAM file:
samtools index myalignments.sorted.bam

# View BAM file in SAMtools:
samtools tview myalignments.sorted.bam genomefile.fa
```

SAMtools Example

SAMtools commands:

- The one command to remember: '?'
- g: go to a specific location (i.e. chrX:152852988 or chrX_149913753:2939235)
- m,b: mapping quality, base quality
- n: color by nucleotide
- '.': dot/base view
- r: read name
- q: quit SAMtools

SAM/BAM/CRAM Format – Conversions

```
# Convert SAM to BAM
samtools view -bS in.sam > out.bam

# Convert BAM to SAM
samtools view -ho out.sam in.bam

# Convert BAM to fastq
samtools bam2fq in.bam > out.fastq
```

RSamtools

`Rsamtools` is a very useful (although somewhat limited) version of Samtools available in R:

```
# Install Rsamtools
BiocManager::install("Rsamtools")

# Convert SAM to BAM
asBam(in.sam, out.bam)
```

You can also index and sort .bam files, as well as extract alignments from a .bam file (very useful!).

Other Data Formats

Standard format for keeping tables

field1	field2	field3	...
...	...	...	...

Fields (columns) separated by a character on each line:

- Comma (or Character) Separated Vector (CSV)
- Tab Separated Vector (TSV)
- Some interpreters take any space (space or tab) as a separator (such as `awk`, `cut`).
- Some have column name as first row (header), some don't

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Genomic regions

- A region is defined by three required fields:
  - sequence name (e.g. chromosome)
  - start coordinate
  - end coordinate
- Define regions of interest: introns, exons, genes, etc.
- Additional information saved as fields after the first three.
- Three standard tab-separated formats: BED, GFF, GTF No headers

BED Format

Mandatory fields:

1. chrom - Name of the chromosome/scaffold/reference sequence
2. chromStart - 0-based starting position of the feature on chrom
3. chromEnd - Ending position of the feature in the chromosome or scaffold.

The chromEnd base is not included in the display of the feature.

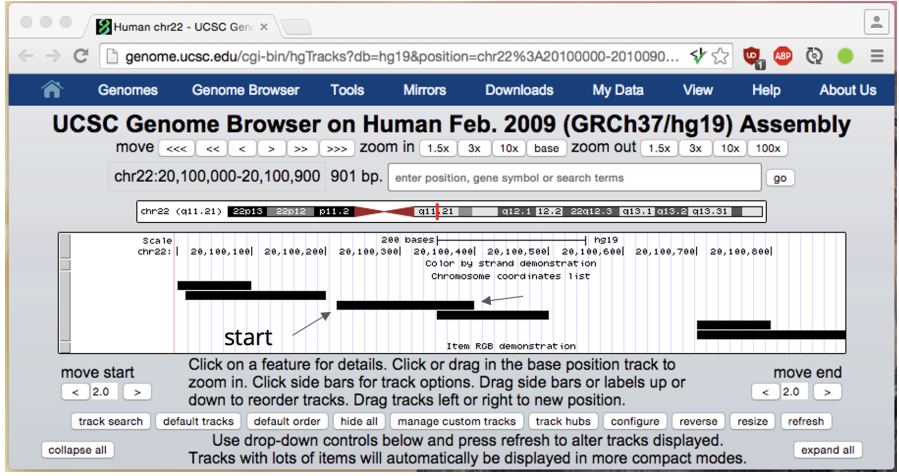
For example, the first 100 bases of a chromosome are defined as:

- chromStart=0
- chromEnd=100
- span the bases numbered 0-99

BED Format

chr1	11873	14409	uc001aaa.3	0	+	11873	11873
chr1	11873	14409	uc010nxr.1	0	+	11873	11873
chr1	11873	14409	uc010nxq.1	0	+	12189	13639
chr1	14361	16765	uc009vis.3	0	-	14361	14361
chr1	14361	19759	uc009vit.3	0	-	14361	14361
chr1	14361	19759	uc009viu.3	0	-	14361	14361
chr1	14361	19759	uc001aae.4	0	-	14361	14361
chr1	14361	29370	uc001aah.4	0	-	14361	14361
chr1	14361	29370	uc009vir.3	0	-	14361	14361
chr1	14361	29370	uc009viq.3	0	-	14361	14361
chr1	14361	29370	uc001aac.4	0	-	14361	14361
chr1	14406	29370	uc009viv.2	0	-	14406	14406
chr1	14406	29370	uc009viw.2	0	-	14406	14406
chr1	15602	29370	uc009vix.2	0	-	15602	15602
chr1	15795	18061	uc009vjd.2	0	-	15795	15795
chr1	16606	29370	uc009viy.2	0	-	16606	16606
chr1	16606	29370	uc009viz.2	0	-	16606	16606
chr1	16857	17751	uc009vjc.1	0	-	16857	16857
chr1	16857	19759	uc001aai.1	0	-	16857	16857

BED Format


Human chr22 - UCSC Gen
genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&position=chr22%3A20100000-2010090...
Genomes
Genome Browser
Tools
Mirrors
Downloads
My Data
View
Help
About Us
UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move
zoom in
zoom out
chr22:20,100,000-20,100,900
901 bp.
enter position, gene symbol or search terms
go
chr22 (q11.21)
Scale
chr22:
200 bases
hg19
20,100,100| 20,100,200| 20,100,300| 20,100,400| 20,100,500| 20,100,600| 20,100,700| 20,100,800|
Color by strand demonstration
Chromosome coordinates list
start
Item RGB demonstration
move start
move end
Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.
track search
default tracks
default order
hide all
manage custom tracks
track hubs
configure
reverse
resize
refresh
Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.
collapse all
expand all

BED Format

Optional fields:

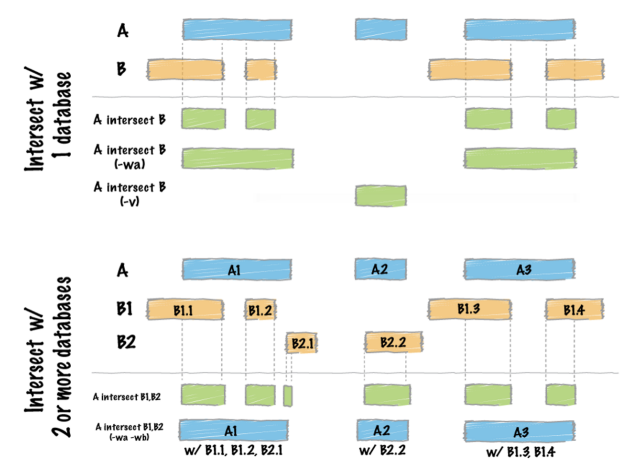
4. Name
5. Score
6. Strand
7. 7-12. Display options (thick starts and end, color, blocks...) to control the view on the genome browser

bedtools

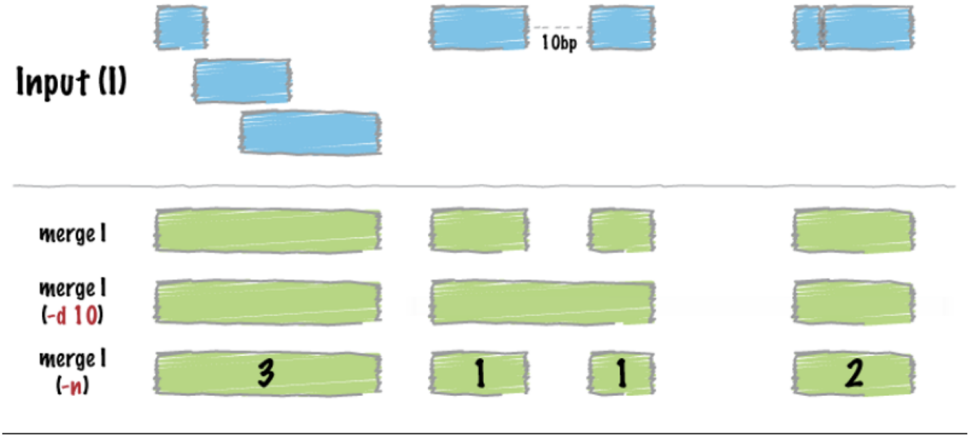
http://bedtools.readthedocs.io/

- sort (sort bed files)
- Intersect (get intersections of bed files)
- merge
- coverage
- overlap
- substract
- ...

bedtools intersect



bedtools merge



GFF - General features

9 mandatory fields, tab separated:

1. seqname - The name of the sequence. Must be a chromosome or scaffold.
2. source - The program that generated this feature.
3. feature - The name of this type of feature (e.g. gene, exon, etc).
4. start - The starting position of the feature in the sequence (1-based)
5. end - The ending position of the feature (inclusive).
6. score - A score between 0 and 1000.
7. strand - Valid entries include "+", "-", or "." (for don't know/don't care).
8. frame - If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be ".".
9. group - All lines with the same group are linked together into a single item

Gene Information

GTF (Gene Transfer Format, GTF2.2)

- Extension to GFF2, backwards compatible
- First eight GTF fields are the same as GFF
- *feature field* is the same as GFF, has controlled vocabulary:
  - *gene, transcript, exon, CDS, 5UTR, 3UTR, inter, inter_CNS, and intron_CNS, etc*
- group field expanded into a list of attributes (i.e. key/value pairs)

The attribute list must begin with the one mandatory attribute: - gene_id value - A globally unique identifier for the genomic source of the sequence

GTF format

```
##description: evidence-based annotation of the human genome (GRCh38), version 27 (Ensembl 90)
##provider: GENCODE
##contact: gencode-help@sanger.ac.uk
##format: gtf
##date: 2017-08-01
chr1    HAVANA  gene    923928  944581  .       +       .       gene_id "ENSG00000187634.11"; gene_type
"protein_coding"; gene_name "SAMD11"; level 2; havana_gene "OTTHUMG00000040719.10";
```

- seqname: chr1
- source: HAVANA
- feature: gene
- start: 923928
- end: 944581
- score: . (no score)
- strand: +
- frame: . (not coding feature)
- attributes:
  - gene_id: ENSG00000187634.11
  - gene_type: protein_coding
  - gene_name: SAMD11
  - level: 2
  - havana_gene: OTTHUMG00000040719.10

GFF/GTF encodes relationships

- Features are hierarchical, e.g.:
  - A gene has 1 or more transcripts
  - A transcript has 1 or more exons
  - An exon is a coding sequence (CDS)
- Relationships encoded in attributes

```
##description: evidence-based annotation of the human genome (GRCh38), version 27 (Ensembl 90)
##provider: GENCODE
##contact: gencode-help@sanger.ac.uk
##format: gtf
##date: 2017-08-01
chr1    HAVANA  gene        923928  944581  .       +       .       gene_id "ENSG00000187634.11"; gene_type
"protein_coding"; gene_name "SAMD11"; level 2; havana_gene "OTTHUMG00000040719.10";

chr1    HAVANA  transcript  923928  939291  .       +       .       gene_id "ENSG00000187634.11";
transcript_id "ENST00000420190.6"; gene_type "protein_coding"; gene_name "SAMD11"; transcript_type
"protein_coding"; transcript_name "SAMD11-203";

chr1    HAVANA  exon        923928  924948  .       +       .       gene_id "ENSG00000187634.11";
transcript_id "ENST00000420190.6"; gene_type "protein_coding"; gene_name "SAMD11"; transcript_type
"protein_coding"; transcript_name "SAMD11-203"; exon_number 1; exon_id "ENSE00001637883.2";

chr1    HAVANA  CDS         924432  924948  .       +       0       gene_id "ENSG00000187634.11";
transcript_id "ENST00000420190.6"; gene_type "protein_coding"; gene_name "SAMD11"; transcript_type
"protein_coding"; transcript_name "SAMD11-203"; exon_number 1; exon_id "ENSE00001637883.2";
```

SNP and Variant Calling

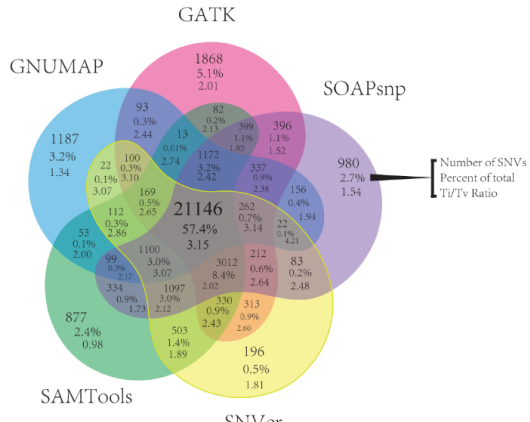
Methods for SNP Calling

Methods for SNP calling:

- Mapper/callers: MAQ, SOAPsnp, GNUMAP, Crossbow (Bowtie)
- Callers: SAMtools (mpileup), GATK (HaplotypeCaller, Mutect2), FreeBayes, others

Inconsistencies Among Aligners

Low concordance of variant-calling pipelines (O'Rawe, *Genome Med*, 2013)



SAMtools Example

Multiple sample SNP calling:

```
samtools mpileup -f genomefile.fa \
  myalignments.sorted.bam > myalignments.vcf
```

VCF files

8 fixed columns: #CHROM, POS, ID, REF, ALT, QUAL, FILTER, INFO, FORMAT
Additional columns, one for each sample, with sample ID

(a) VCF example

Header

```
##fileformat=VCFv4.1
##fileDate=20110413
##source=VCFtools
##reference=file:///refs/human_NCBI36.fasta
##contig=<ID=1,length=249250621,md5=1b22b98cdeb4a9304cb5d48026a85128,species="Homo Sapiens">
##contig=<ID=X,length=155270560,md5=7e0e2e580297b7764e31dbc80c2540dd,species="Homo Sapiens">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
```

Body

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	SAMPLE1	SAMPLE2
1	1	.	ACG	A,AT	40	PASS	.	GT:DP	1/1:13	2/2:29
1	2	.	C	T,CT	.	PASS	H2;AA=T	GT	0|1	2/2
1	5	rs12	A	G	67	PASS	.	GT:DP	1|0:16	2/2:20
X	100	.	T	<DEL>	.	PASS	SVTYPE=DEL;END=299	GT:GQ:DP	1:12:.	0/0:20:36

Danecek et al. Bioinformatics 2011

Complete Variant Calling Pipeline (Outdated!)}

Our analysis pipeline consisted of the following:

- Align the FASTQ files to genome
- Convert SAM file to BAM, add read group info
- Filter the reads based on quality (BAMTools)
- Samtools to sort and index, and use Picard to mark duplicates
- GATK calibration, realignment, variant calling (HaplotypeCaller, Mutect2)
- Filter the called variants (GATK filtersnps and filterindels).
- Annotation of SNPs (snpEff, condel)
- Filter by frequency (thousand genomes, TCGA, etc.)
- Downstream analysis (rare variants, pedigree, pathway level, etc)

Downstream Annotation and Analysis (Outdated!)

Downstream Annotation Tools (old list):

- snpEff (http://snpeff.sourceforge.net/)
- Condel (http://bg.upf.edu/condel/home)
- SIFT http://sift.jcvi.org/
- Polyphen 2 http://genetics.bwh.harvard.edu/pph2/
- http://mutationassessor.org/
- Ensembl variant effect predictor (http://www.ensembl.org/info/docs/variation/vep/index.html)
- Thousand Genomes variant frequency (e.g. 1% threshold) and Exome Sequence Project variant frequency (e.g. 1%).

Session info

```
sessionInfo()
```

```
## R version 4.4.2 (2024-10-31)
## Platform: aarch64-apple-darwin20
## Running under: macOS Sequoia 15.4.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib;  LAPACK version 3.12.0
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/Denver
## tzcode source: internal
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] digest_0.6.37     fastmap_1.2.0     xfun_0.52         Matrix_1.7-3
##  [5] lattice_0.22-7    reticulate_1.42.0 knitr_1.50        htmltools_0.5.8.1
##  [9] png_0.1-8         rmarkdown_2.29    cli_3.6.5         grid_4.4.2
## [13] compiler_4.4.2    rstudioapi_0.17.1 tools_4.4.2       evaluate_1.0.3
## [17] Rcpp_1.0.14       yaml_2.3.10       jsonlite_2.0.0    rlang_1.1.6
```