Basic file formats

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File formats

- *FASTA* - sequence data
- *FASTQ* - sequence data including quality (**SRA**)
- GTF/GFF, GFF3 - Gene feature & transfer format
- *SAM*/**BAM** - aligned/mapped data (**CRAM**)
- *VCF*/**BCF** - variant calling

Text format
Binary format

FASTA

- Represents a sequence - DNA/RNA/protein
- Two lines:
  - header starts '>'
  - Second line contains the sequence

header

```
>NM_001199335.1 Danio rerio toll-like receptor 21 (tlr21), mRNA
CTAGACGAAGCTCTCACAGAGTGAAAGGCTTTGTAATGTTGGACTCAGTTTATAGGAATT
ACATTTAAAAGTGGAACTGAAAAACATGGCACACTCTGCATGCCACAAACTGATACTAAA
GGCCACATTCATCTGTCTCATAAAACTTGCCTGCAGCTACAGTTTCAGAAGTTGCATAGA
GATCCCAGATTCTAATCATACAATCTTTACATGTGTTAAAAGTTATGAACGAGACATAAC
TGCGATTGTGAGTGATGTATTTCCCACTGCATTAAATCTTACAATCTCTCACA
```

Genomes

Transcriptomes

FASTQ

- Represents a sequence along with quality information
- Four lines:
  - header starts '@'
  - Second line contains the sequence
  - Quality header starts with '+'
  - Fourth line contains the quality for each base in the second line

FASTQ quality encoding

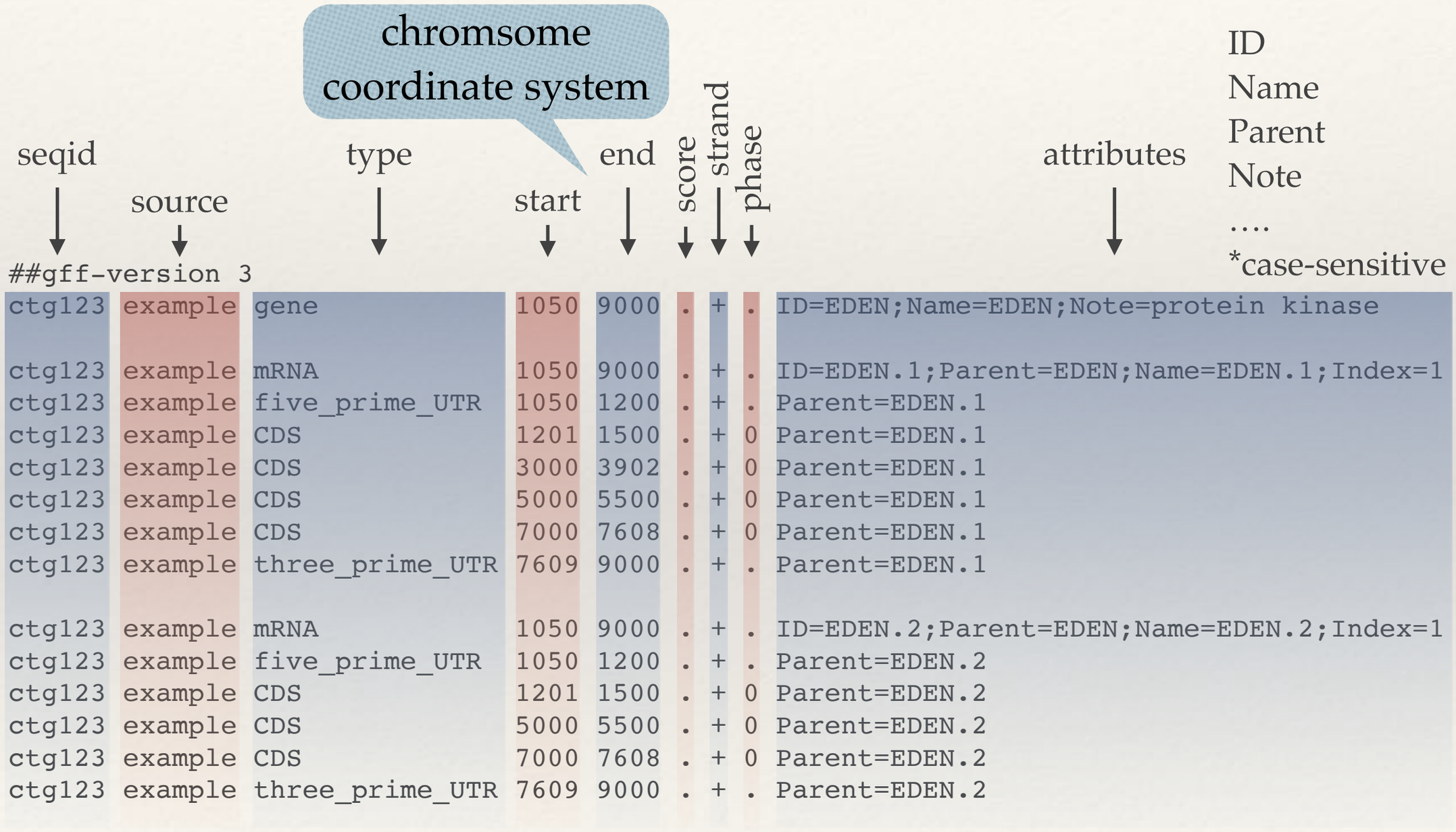
```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................
  .........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
  ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
  ................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                     |
 33                        59   64       73                            104                   126
  0........................26...31.......40
                          -5....0........9.............................40
                               0........9.............................40
                                  3.....9.............................41
  0.2......................26...31........41

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 41)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

https://en.wikipedia.org/wiki/FASTQ_format

GFF/GTF

- GFF: General Feature Format
  - Current format is based on version 3 and called GFF3
  - format consists of one line per feature
  - each containing 9 columns of data, plus optional track definition lines.
- GTF: General Transfer Format is identical to GFF version 2.5.

GFF3



http://gmod.org/wiki/GFF3

BED format

- Browser Extensible Data
- 3-12 fields; but generally contains 6 or more

chromsome coordinate system

chromosome # | chromStart | chromEnd | name | score | strand

```
chr7    127471196  127472363  Pos1  0  +
Chr7    127472363  127473530  Pos2  0  +
chr7    127473530  127474697  Pos3  0  +
chr7    127474697  127475864  Pos4  0  +
chr7    127475864  127477031  Neg1  0  -
chr7    127477031  127478198  Neg2  0  -
```

There are far more annotation formats but will stop here!!!

Chromosome coordinate systems

- 0-based

  ACTGACTG

  012345678

- 1-based

  ACTGACTG

  123456789

To represent the TGAC:

0-based inclusive: 2-5
1-based inclusive: 3-6
1-based exclusive: 3-7

Ensembl: 1-based inclusive

UCSC: 0-based start
1-based end
1-based for display

SAM, VCF, GFF, GTF: 1-based

BAM, BCF: 0-based

BED: 0-based exclusive

Most tools are aware of these differences

https://arnaudceol.wordpress.com/2014/09/18/chromosome-coordinate-systems-0-based-1-based/

SAM/BAM

- SAM - Sequence Alignment/Map format
- BAM - same as/similar to SAM but in binary format
  - Most softwares and tools prefer BAM

- Header

  (access using samtools view -H)

- Alignment records

  (access using samtools view; use -h to include Header)

http://samtools.github.io/hts-specs/SAMv1.pdf

SAM/BAM: header

```
@HD  VN:1.0GO:none  SO:coordinate
@SQ  SN:chrM  LN:16571
@SQ  SN:chr1  LN:249250621
...
@SQ  SN:chrX  LN:155270560
@SQ  SN:chrY  LN:59373566
...
...
@RG  ID:86-191PL:ILLUMINA LB:IL500 SM:86-191-1
@RG  ID:BsK010PL:ILLUMINA LB:IL501 SM:BsK010-1
...
@RG  ID:SDH023PL:ILLUMINA LB:IL508 SM:SDH023
@PG  ID:GATK IndelRealigner  VN:2.0-39-gd091f72CL:knownAlleles=[]
targetIntervals=tmp.intervals.list LODThresholdForCleaning=5.0
consensusDeterminationModel=USE_READS entropyThreshold=0.15
maxReadsInMemory=150000 maxIsizeForMovement=3000 maxPositionalMoveAllowed=200
maxConsensuses=30 maxReadsForConsensuses=120 maxReadsForRealignment=20000
noOriginalAlignmentTags=false nWayOut=null generate_nWayOut_md5s=false
check_early=false noPGTag=false keepPGTags=false indelsFileForDebugging=null
statisticsFileForDebugging=null SNPsFileForDebugging=null
@PG  ID:bwaPN:bwaVN:0.6.2-r126
```

← sort order

← Reference sequence names with length information

← Read group with platform, library and sample information

↙ Program analysis history

http://samtools.github.io/hts-specs/SAMv1.pdf

SAM/BAM: alignment records

1				2	3		4	5	6	7	8	9
HW-ST605:127:B0568ABXX:2:1201:10933:3739				147	chr1		27675	60	101M	=	27588	-188

10 TCATTTTATGGCCCCTTCTTCCTATATCTGGTAGCTTTTAAATGATGACCATGTAGATAATCTTTATTGTCCCTCTTTCAGCAGAC

11 =7;:;<=??<=BCCEFFEJFCEGGEFFDF?E@E@ADCACB>CCDCBACDCDDDAB@@BCADDCBC@BCBB8@ABCCCDCBDA@>:/

12 RG:Z:86-191 . . . . . .

Col	Field	Type	Regexp/Range	Brief Description
1	QNAM	String	[!-?A-~]{1,254}	Query template NAME
2	FLAG	Int	[0,2^{16}-1]	bitwise FLAG
3	RNAME	String	* | [!-()+-<>-~][!-~]*	Reference sequence NAME
4	POS	Int	[0,2^{31}-1]	1-based leftmost mapping POSition
5	MAPQ	Int	[0,2^{8}-1]	MAPping Quality
6	CIGAR	String	* | ([0-9]+	CIGAR string
7	RNEXT	String	* | = | [!-()+-<>-~][!-~]*	Ref. name of the mate/next read
8	PNEXT	Int	[0,2^{31}-1]	Position of the mate/next read
9	TLEN	Int	[-2^{31}+1,2^{31}-1]	observed Template LENght
10	SEQ	String	* | [A-Za-z=.]+	sequence SEQuence
11	QUAL	String	[!-~]+	ACSII of Phred-scaled base QUALity+33

http://samtools.github.io/hts-specs/SAMv1.pdf

SAM/BAM

- To check FLAG info: https://broadinstitute.github.io/picard/explain-flags.html

- BAM/SAM files are produced by alignment/mapping softwares and other tools.
- QC of BAM/SAM files - Picard, Qualimap
- Manipulating and extracting information from varied file formats - SAMtools, BEDtools, BCFtools

VCF/BCF

- Variant call format
- BCF - same as/similar to VCF but in binary format
  - Most softwares and tools prefer BCF or VCF in zip format
  - header and records

header

```
##fileformat=VCFv4.2
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=xxxx,species="Homo sapiens",taxonomy=x>
...
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
...
##FILTER=<ID=q10,Description="Quality below 10">
...
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
…
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001 NA00002 NA00003
```

https://samtools.github.io/hts-specs/VCFv4.2.pdf

VCF/BCF: records

```
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001
20 14370 rs6054257 G A 29 PASS NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP:HQ 0|0:48:1:51,51

20 17330 . T A 3 q10 NS=3;DP=11;AF=0.017 GT:GQ:DP:HQ 0|0:49:3:58,50

20 1110696 rs6040355 A G,T 67 PASS NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27
```

	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	SAMPLEs
SNP	20	3	.	C	G	.	PASS	DP=10		
Deletion	20	2	.	TC	C	.	PASS	DP=10		
Insertion	20	2	.	TC	TCA	.	PASS	DP=10		
Alleles	20	2	.	TC	TG,T	.	PASS	DP=10		
Alleles	20	2	.	TCG	TG,T,TCA	.	PASS	DP=10		

+ Structural variants

http://vcftools.sourceforge.net/VCF-poster.pdf

Qualimap

- GUI, command line based
  - examines sequencing alignment data in SAM/BAM format
- requires Java, R
- Uses:
  - FastQC, Picard, SAMTools, NOISeq, Repitools and JProfiler
  - BAM QC
  - RNA-seq QC
  - Counts QC
  - Multi-sample BAM QC

http://qualimap.bioinfo.cipf.es/

SAMtools/BCFtools

- SAMtools
  - manipulating SAM/BAM
- BCFtools
  - manipulating VCF/BCF
- Part of HTSlib tool kit

```
-- indexing
     faidx       index/extract FASTA
     index       index alignment
-- editing
     calmd       recalculate MD/NM tags and '=' bases
     fixmate     fix mate information
     reheader    replace BAM header
     rmdup       remove PCR duplicates
     targetcut   cut fosmid regions (for fosmid pool only)
-- file operations
     bamshuf     shuffle and group alignments by name
     cat         concatenate BAMs
     merge       merge sorted alignments
     mpileup     multi-way pileup
     sort        sort alignment file
     split       splits a file by read group
     bam2fq      converts a BAM to a FASTQ
-- stats
     bedcov      read depth per BED region
     depth       compute the depth
     flagstat    simple stats
     idxstats    BAM index stats
     phase       phase heterozygotes
     stats       generate stats (former bamcheck)
-- viewing
     flags       explain BAM flags
     tview       text alignment viewer
     view        SAM<->BAM<->CRAM conversion
```

http://www.htslib.org/

BEDtools

- Awesome tool to compare dataset in BED/GFF/VCF and BAM formats
- Very well documented and Highly recommended!!

- Example:

  http://bedtools.readthedocs.io/en/latest/content/tools/intersect.html

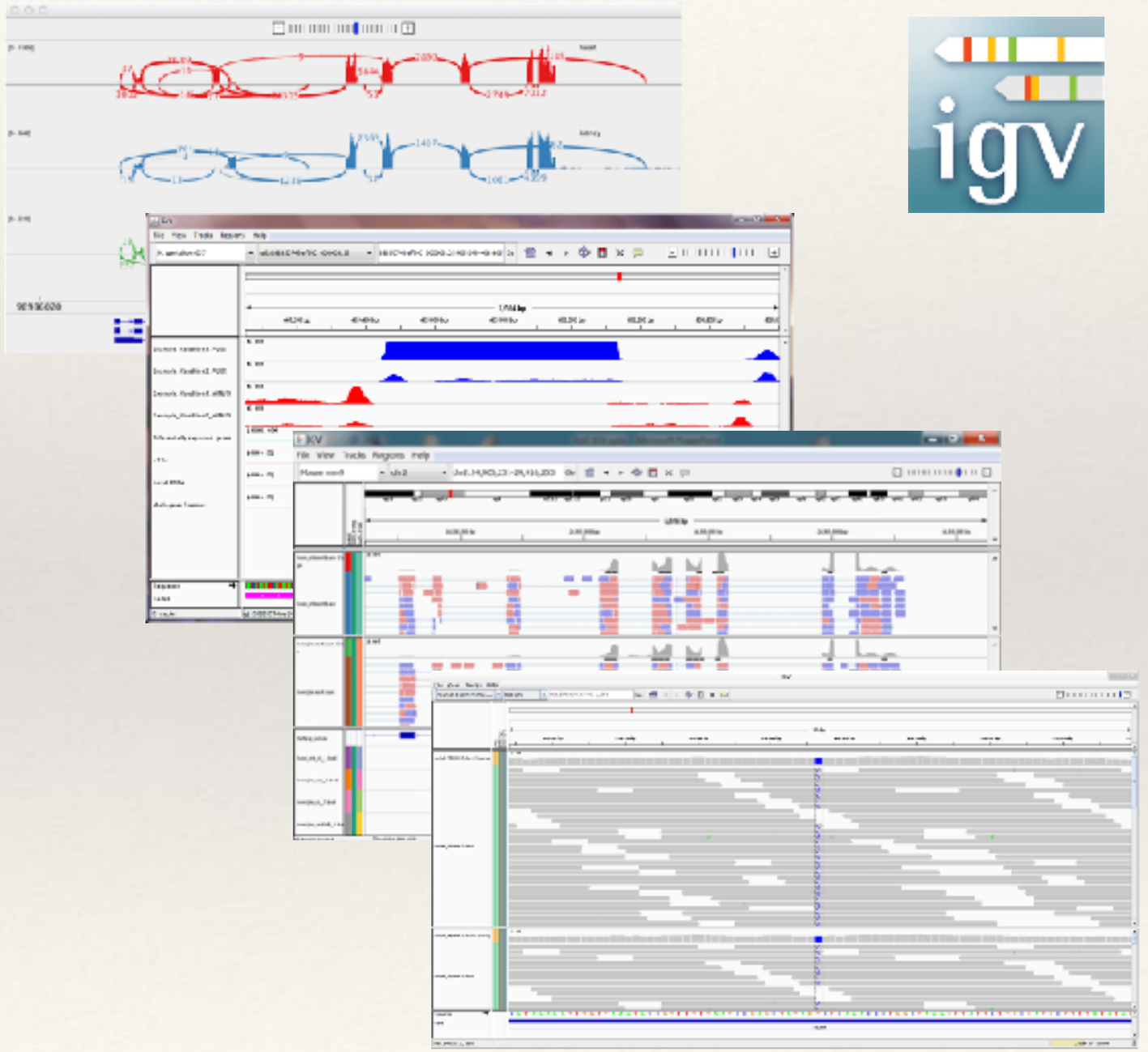
http://bedtools.readthedocs.io/en/latest/

IGV - Integrative Genomics Viewer

- View BAM, VCF, GTF files and more
- Ensembl browser

  http://ensembl.org/
- UCSC browser

  http://genome.ucsc.edu/



http://software.broadinstitute.org/software/igv/

Further reading

- GFF3, GTF - http://gmod.org/wiki/GFF3
- SAM/BAM format - http://samtools.github.io/hts-specs/SAMv1.pdf
- VCF/BCF format - https://samtools.github.io/hts-specs/VCFv4.2.pdf

  - http://vcftools.sourceforge.net/VCF-poster.pdf
- IGV - http://software.broadinstitute.org/software/igv/

Databases

- Ensembl - http://ensembl.org/
- UCSC - http://genome.ucsc.edu/
- NCBI - http://ncbi.nlm.nih.gov/
  - RefSeq - https://www.ncbi.nlm.nih.gov/refseq/

There are too many databases containing specific information!!!