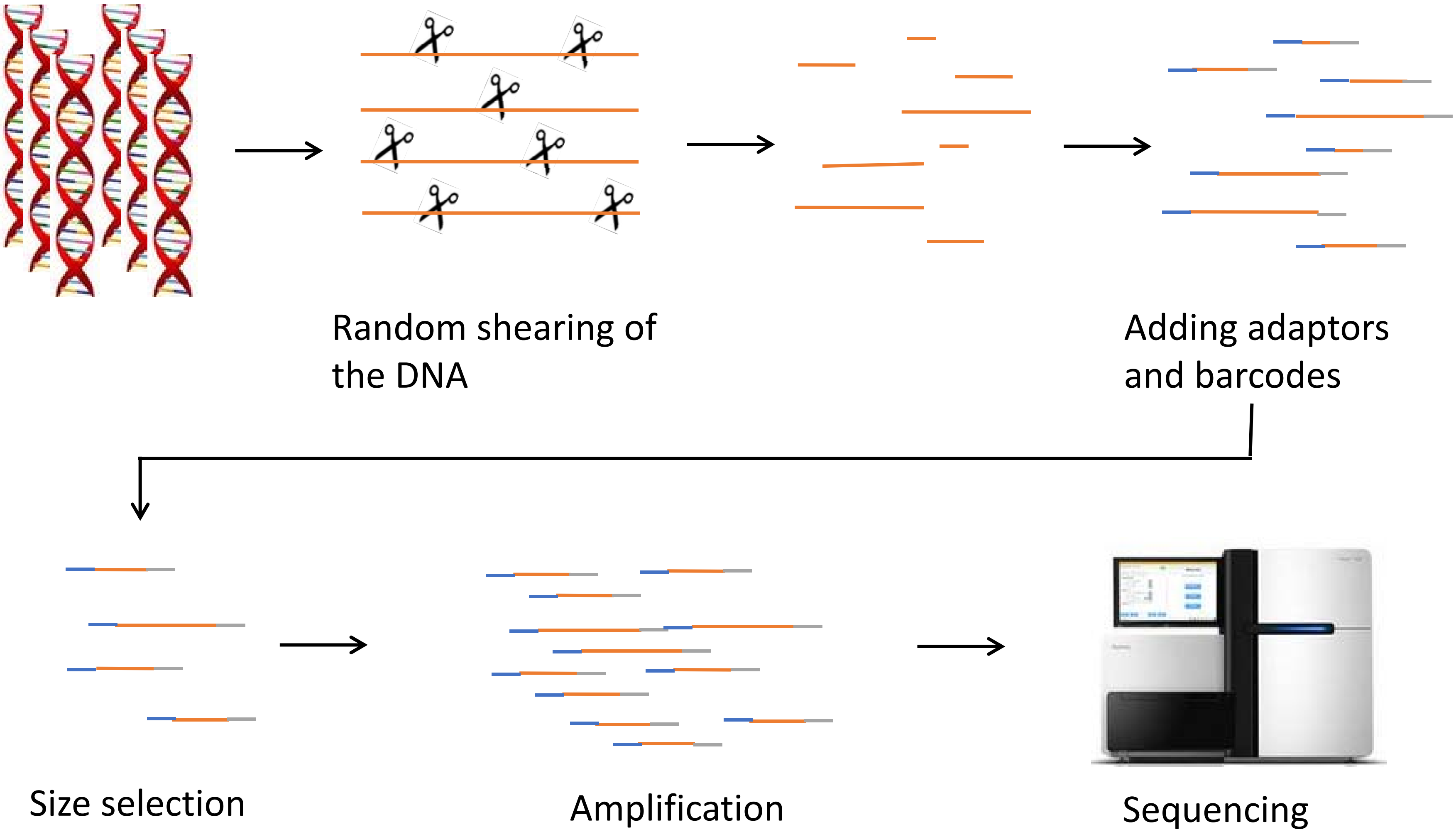
NGS data formats and variant calling

2019 Dragon Star Bioinformatics Course (Day 1)

Sample Preparation



Random shearing of the DNA

Adding adaptors and barcodes

Size selection

Amplification

Sequencing

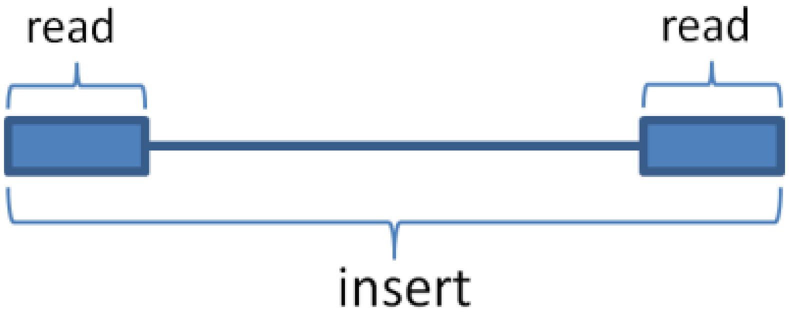
Basic Concepts in NGS

Insert – the DNA fragment that is used for sequencing

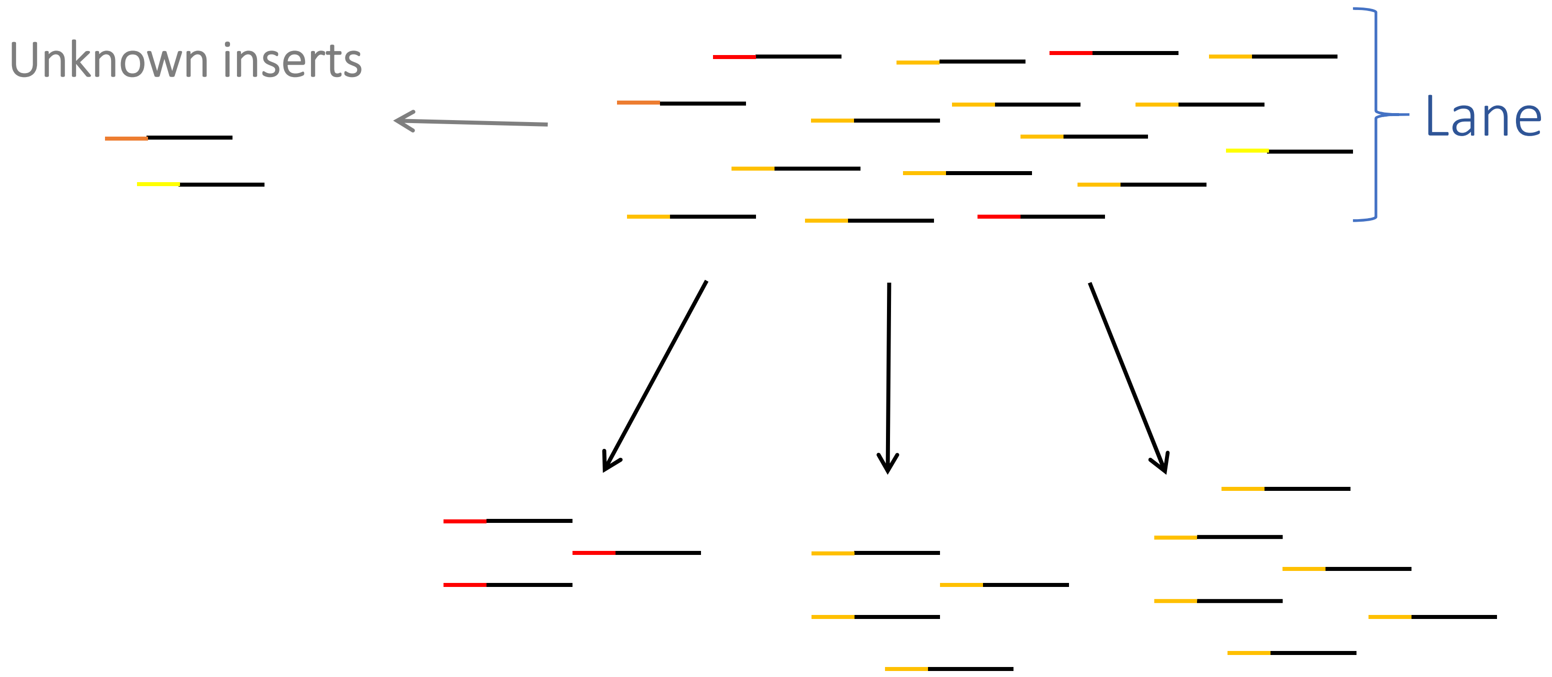
Read – the part of the insert that is sequenced

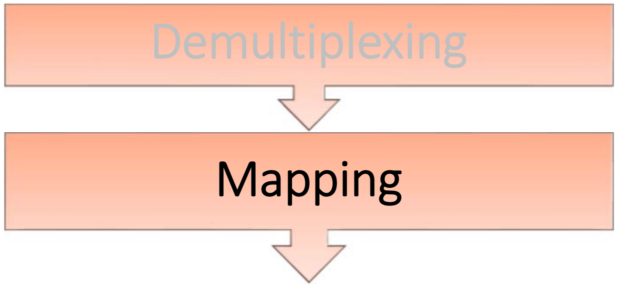
Single End – a sequencing procedure by which the insert is sequenced from one end only

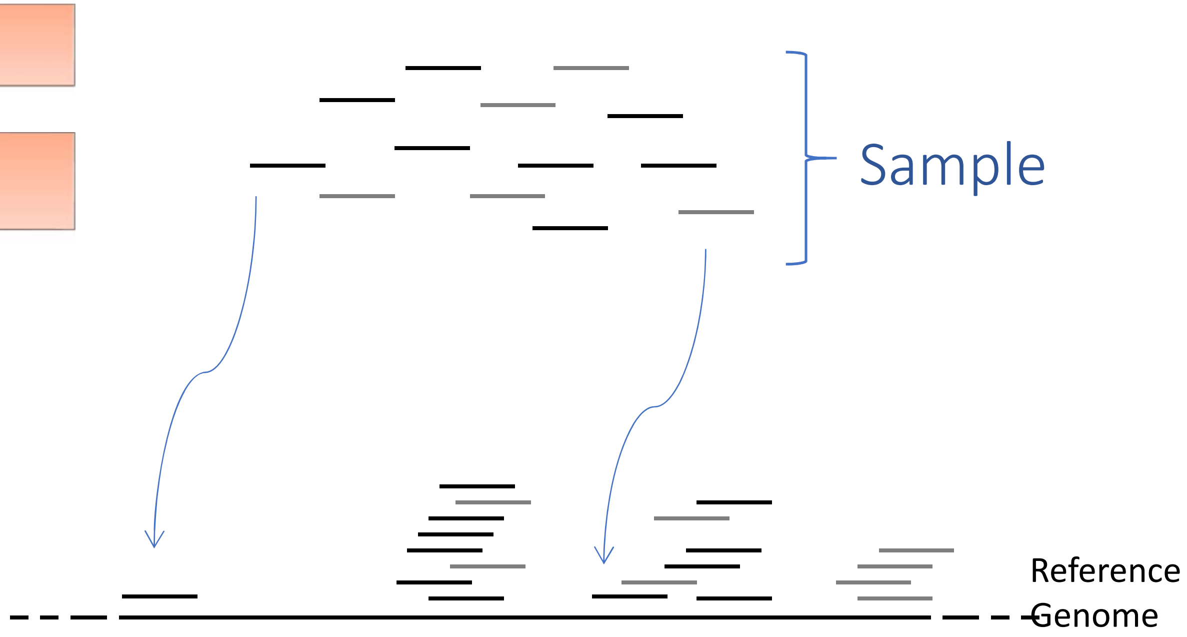
Paired End – a sequencing procedure by which the insert is sequenced from both ends



Demultiplexing



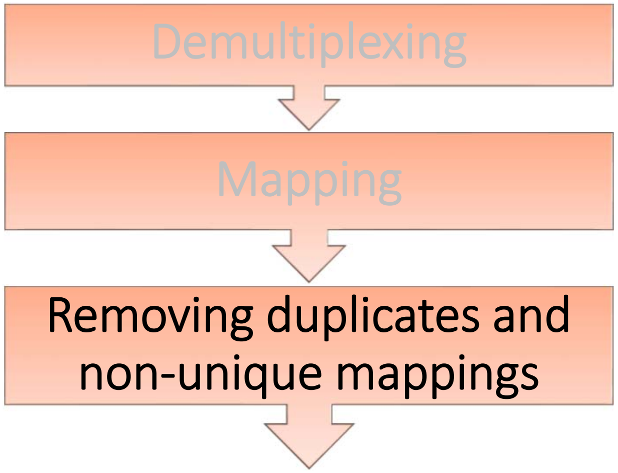




Example of mapping parameters:

- Number of mismatches per read
- Scores for mismatch or gaps

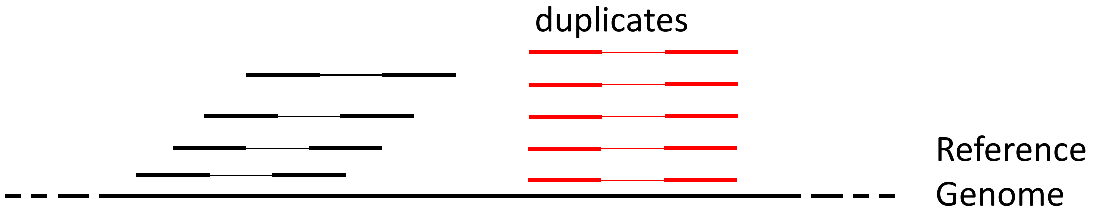
Mapping parameters affect the rest of the analysis

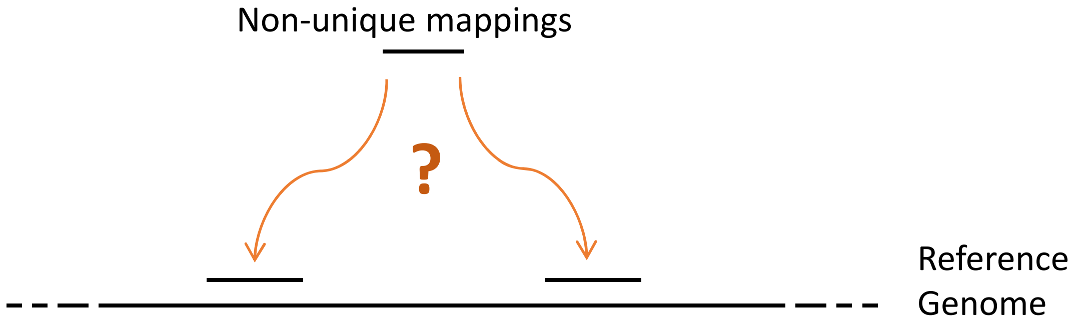


Demultiplexing

Mapping

Removing duplicates and non-unique mappings





$$\boldsymbol{average\ coverage} = \frac{read\ length\ \cdot\ number\ of\ reads\ \cdot\ \%\ uniquely\ mapped\ reads}{genome\ size}$$

NGS – high-throughput, but

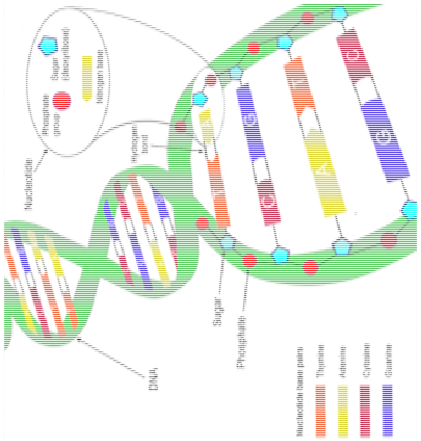
- Shorter reads
  - Sanger sequencing: up to ~1Kb
  - NGS technologies: typically 30-300bp
  - Implication: a lot of computational tasks e.g, assembly, read alignment, haplotyping, detection of SNPs, CNVs, indels etc.
  - Higher per-base sequencing error rate
    - Sanger sequencing: < 0.001%
    - NGS: 0.5-1%
    - Implication: Need redundant sequencing of each base to distinguish sequencing errors from true polymorphisms

Now How do NGS Data Look Like?

- What do you want them to look like?

Fantasy Land

DNA



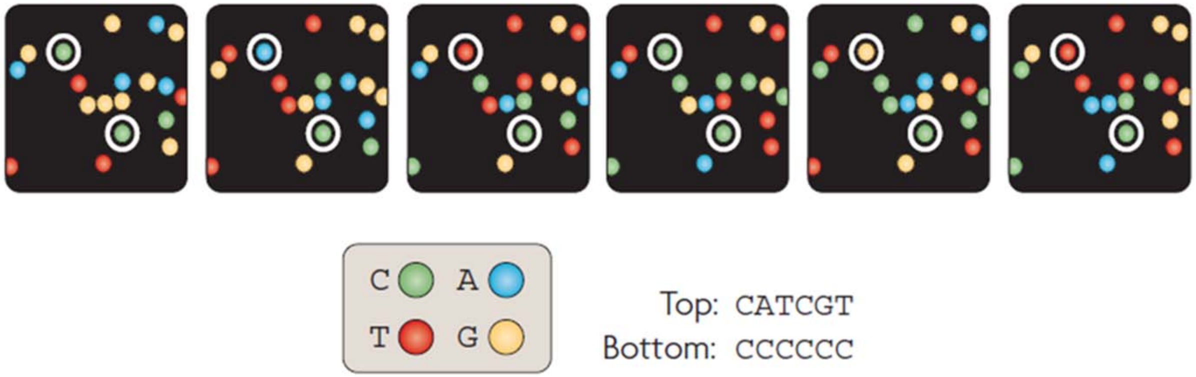
```
Chr1 haplo1: agttataagat...
Chr1 haplo2: agttattagat...
Chr2 haplo1: cctagctggat...
Chr2 haplo2: ccaagctcgat...
Chr3 haplo1: agctctgagcg...
Chr3 haplo2: agctctgagcg...
Chr4 haplo1: atcgttcgatc...
Chr4 haplo2: atcgatcgaac...
              etc...
```

3 billion bases from the beginning of chromosome 1 to the end of the last sex chromosome (2x) in haplotypes

https://mammothmemory.net/biology/dna-genetics-and-inheritance/dna-base-pairing/dna-double-helix.html

The Raw Raw Data

- Typically: images



- The first step is to call nucleotides at each base of each read: **base calling**, which is NOT 100% accurate
  - Typically base calling is done by the sequencer itself, and we start analysis after base calling (for example, fastq format file)

How do the Data Really Look Like: fastq

```
@IL27_748:3:286:254:231/1
GTGGAATAATGACCATGACGAAGAGGATGACAGTCC
+
BBBDCDED4DEAECEFEF2DC/>>@&*/C6208'<*
@IL27_748:3:285:138:811/1
AAGTGGATTACTACCTACAGAGAGTCAGTAAGAGAG
+
BB3D2D<D>D7DE0+19242?=57?=4%'6%.2.'(
@IL27_748:3:142:204:780/1
AGAAAAAGAAAGAGAGAGACAGACAGACAGAGAAAG
+
26B3C8<DDD>AAA0FCF7DCA012A?(;2?AC(=/
@IL27_748:3:23:252:759/1
TTTTAGATGAAGTTATTTCCTTTACTACCGTAGGCC
+
BB0D;DED>;>CEC:2EFA@69CDC3?@'%=585=‘
…
```

Millions of short reads from unknown genetic locations

How do the Data Really Look Like: fastq

```
@IL27_748:3:286:254:231/1
GTGGAATAATGACCATGACGAAGAGGATGACAGTCC
+
BBBDCDED4DEAECEFEF2DC/>>@&*/C6208'<*
```

Read 1

```
@IL27_748:3:285:138:811/1
AAGTGGATTACTACCTACAGAGAGTCAGTAAGAGAG
+
BB3D2D<D>D7DE0+19242?=57?=4%'6%.2.'(
```

Read 2

```
@IL27_748:3:142:204:780/1
AGAAAAAGAAAGAGAGAGACAGACAGACAGAGAAAG
+
26B3C8<DDD>AAA0FCF7DCA012A?(;2?AC(=/
```

Read 3

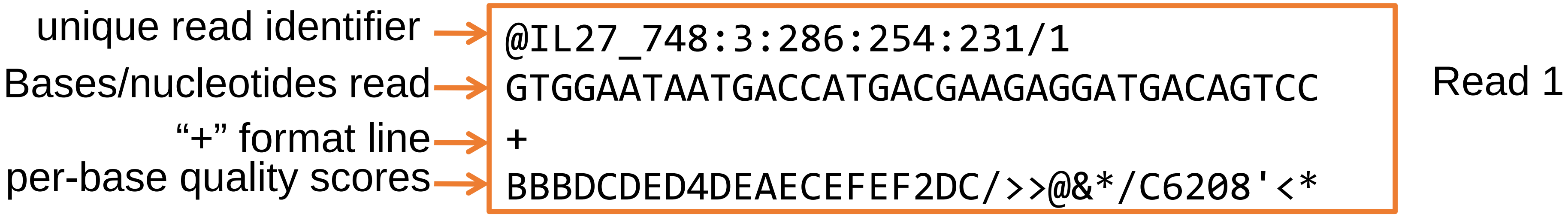
```
@IL27_748:3:23:252:759/1
TTTTAGATGAAGTTATTTCCTTTACTACCGTAGGCC
+
BB0D;DED>;>CEC:2EFA@69CDC3?@'%=585=‘
```

Read 4

…

Millions of short reads from unknown genetic locations

How do the Data Really Look Like: fastq



unique read identifier

Bases/nucleotides read

“+” format line

per-base quality scores

Read 1

```
@IL27_748:3:286:254:231/1
GTGGAATAATGACCATGACGAAGAGGATGACAGTCC
+
BBBDCDED4DEAECEFEF2DC/>>@&*/C6208'<*
@IL27_748:3:285:138:811/1
AAGTGGATTACTACCTACAGAGAGTCAGTAAGAGAG
+
BB3D2D<D>D7DE0+19242?=57?=4%'6%.2.'(
@IL27_748:3:142:204:780/1
AGAAAAAGAAAGAGAGAGACAGACAGACAGAGAAAG
+
26B3C8<DDD>AAA0FCF7DCA012A?(;2?AC(=/
@IL27_748:3:23:252:759/1
TTTTAGATGAAGTTATTTCCTTTACTACCGTAGGCC
+
BB0D;DED>;>CEC:2EFA@69CDC3?@'%=585=‘
…
```

Millions of short reads from unknown genetic locations

Base Qualities

Short Read Sequence

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

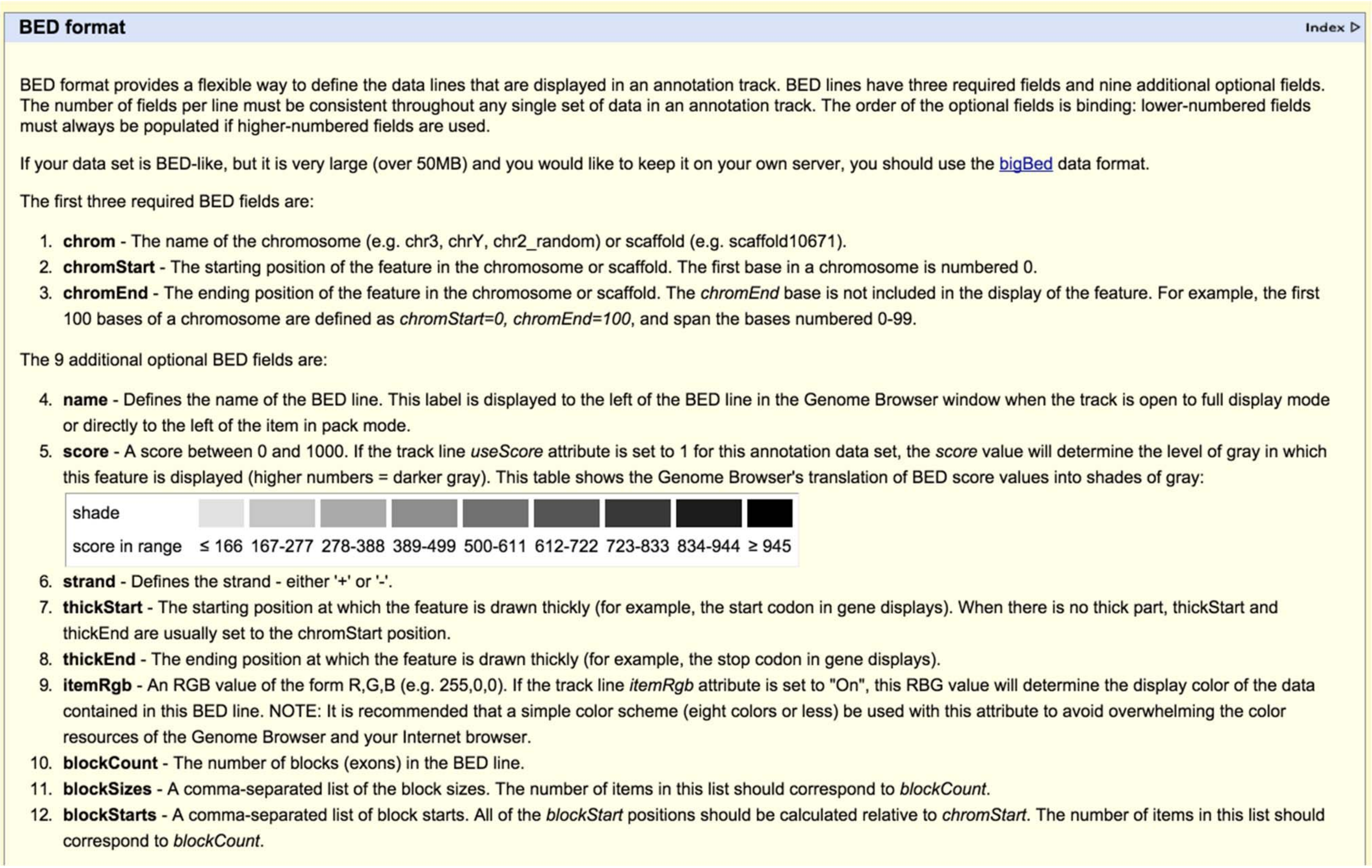
Short Read Base Qualities

30.30.28.28.29.27.30.29.28.25.24.26.27.24.24.23.20.21.22.10.25.25.20.20.18.17.16.15.14.14.13.12.10

- Each base is typically associated with a quality value
- Measured on a "Phred" scale, which was introduced by Phil Green for his Phred sequence analysis tool

$$BQ = -10log_{10}(\varepsilon)\ \ where\ \epsilon\ is\ the\ probability\ of\ an\ error$$

BED format



BED format

BED format provides a flexible way to define the data lines that are displayed in an annotation track. BED lines have three required fields and nine additional optional fields. The number of fields per line must be consistent throughout any single set of data in an annotation track. The order of the optional fields is binding: lower-numbered fields must always be populated if higher-numbered fields are used.

If your data set is BED-like, but it is very large (over 50MB) and you would like to keep it on your own server, you should use the bigBed data format.

The first three required BED fields are:

1. **chrom** - The name of the chromosome (e.g. chr3, chrY, chr2_random) or scaffold (e.g. scaffold10671).
2. **chromStart** - The starting position of the feature in the chromosome or scaffold. The first base in a chromosome is numbered 0.
3. **chromEnd** - The 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*, and span the bases numbered 0-99.

The 9 additional optional BED fields are:

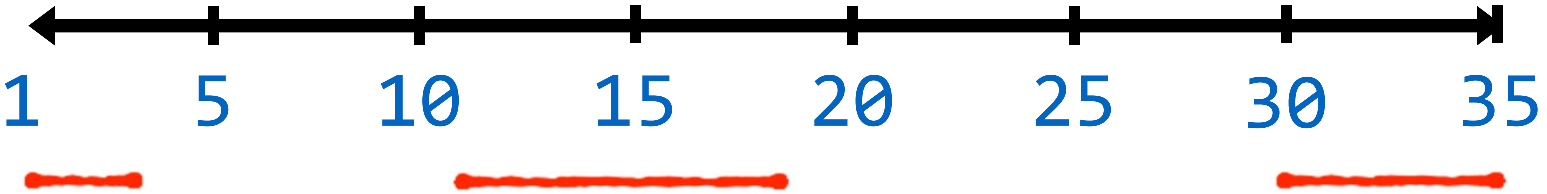
4. **name** - Defines the name of the BED line. This label is displayed to the left of the BED line in the Genome Browser window when the track is open to full display mode or directly to the left of the item in pack mode.
5. **score** - A score between 0 and 1000. If the track line *useScore* attribute is set to 1 for this annotation data set, the *score* value will determine the level of gray in which this feature is displayed (higher numbers = darker gray). This table shows the Genome Browser's translation of BED score values into shades of gray:

shade	░	░	▒	▒	▓	▓	▓	█	█
score in range	≤ 166	167-277	278-388	389-499	500-611	612-722	723-833	834-944	≥ 945

6. **strand** - Defines the strand - either '+' or '-'.
7. **thickStart** - The starting position at which the feature is drawn thickly (for example, the start codon in gene displays). When there is no thick part, thickStart and thickEnd are usually set to the chromStart position.
8. **thickEnd** - The ending position at which the feature is drawn thickly (for example, the stop codon in gene displays).
9. **itemRgb** - An RGB value of the form R,G,B (e.g. 255,0,0). If the track line *itemRgb* attribute is set to "On", this RBG value will determine the display color of the data contained in this BED line. NOTE: It is recommended that a simple color scheme (eight colors or less) be used with this attribute to avoid overwhelming the color resources of the Genome Browser and your Internet browser.
10. **blockCount** - The number of blocks (exons) in the BED line.
11. **blockSizes** - A comma-separated list of the block sizes. The number of items in this list should correspond to *blockCount*.
12. **blockStarts** - A comma-separated list of block starts. All of the *blockStart* positions should be calculated relative to *chromStart*. The number of items in this list should correspond to *blockCount*.

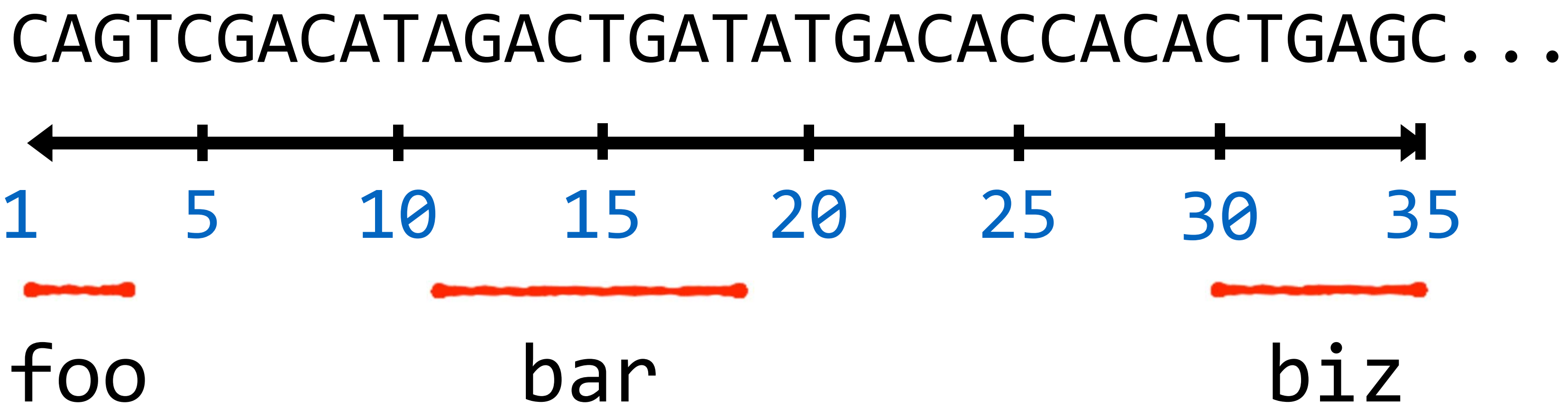
Minimal BED format. So-called BED3 format.

CAGTCGACATAGACTGATATGACACCACACTGAGC...



```
chr1   0    3
chr1   11   18
chr1   29   35
```

BED format supports “labels”



CAGTCGACATAGACTGATATGACACCACACTGAGC...

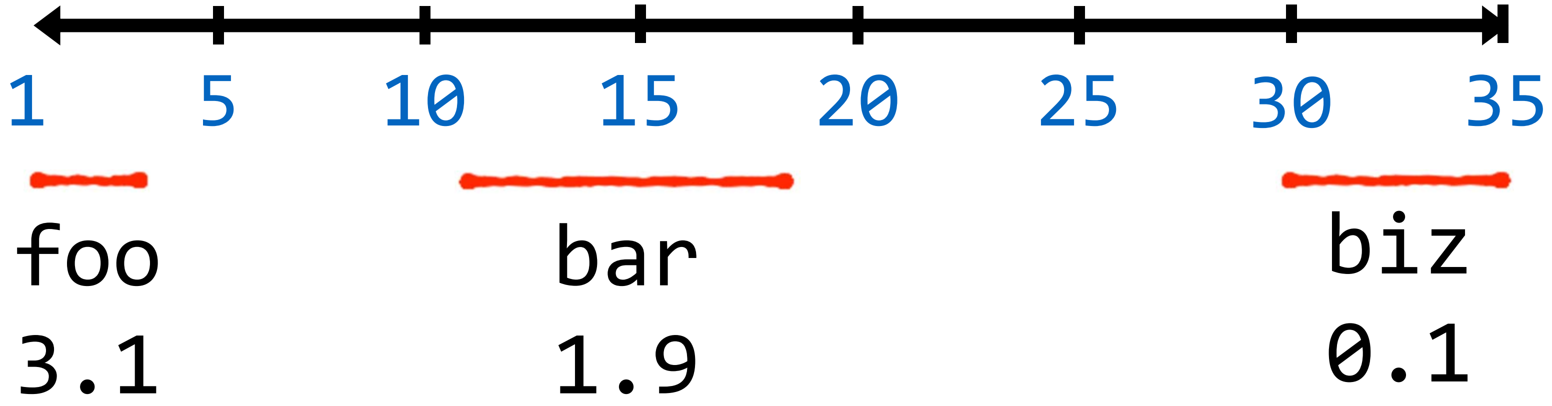
1 5 10 15 20 25 30 35

foo bar biz

```
chr1    0     3     foo
chr1    11    18    bar
chr1    29    35    biz
```

And scores

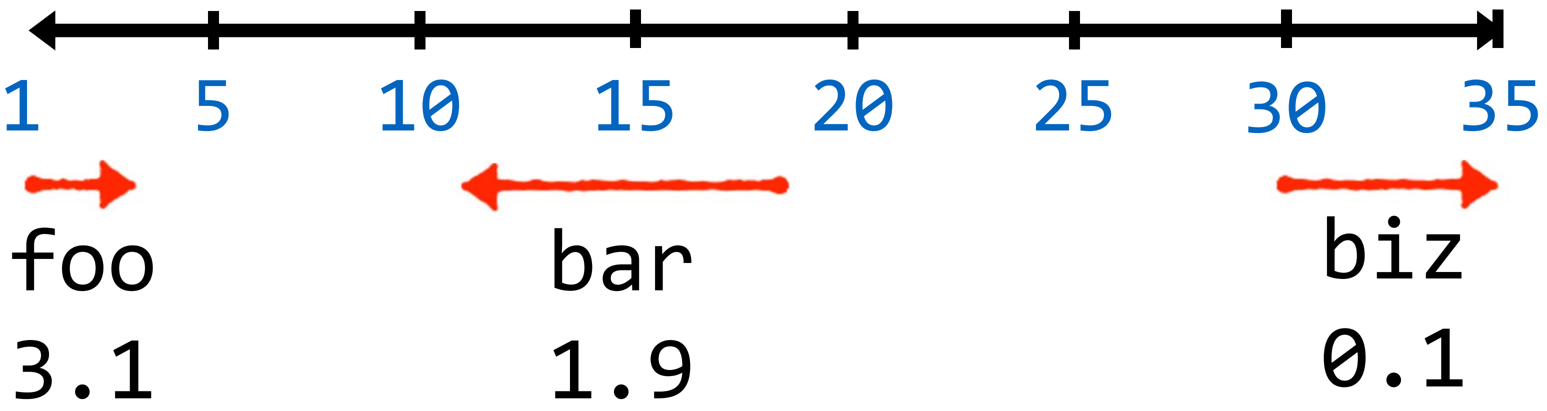
CAGTCGACATAGACTGATATGACACCACACTGAGC...



```
chr1    0     3     foo    3.1
chr1    11    18    bar    1.9
chr1    29    35    biz    0.1
```

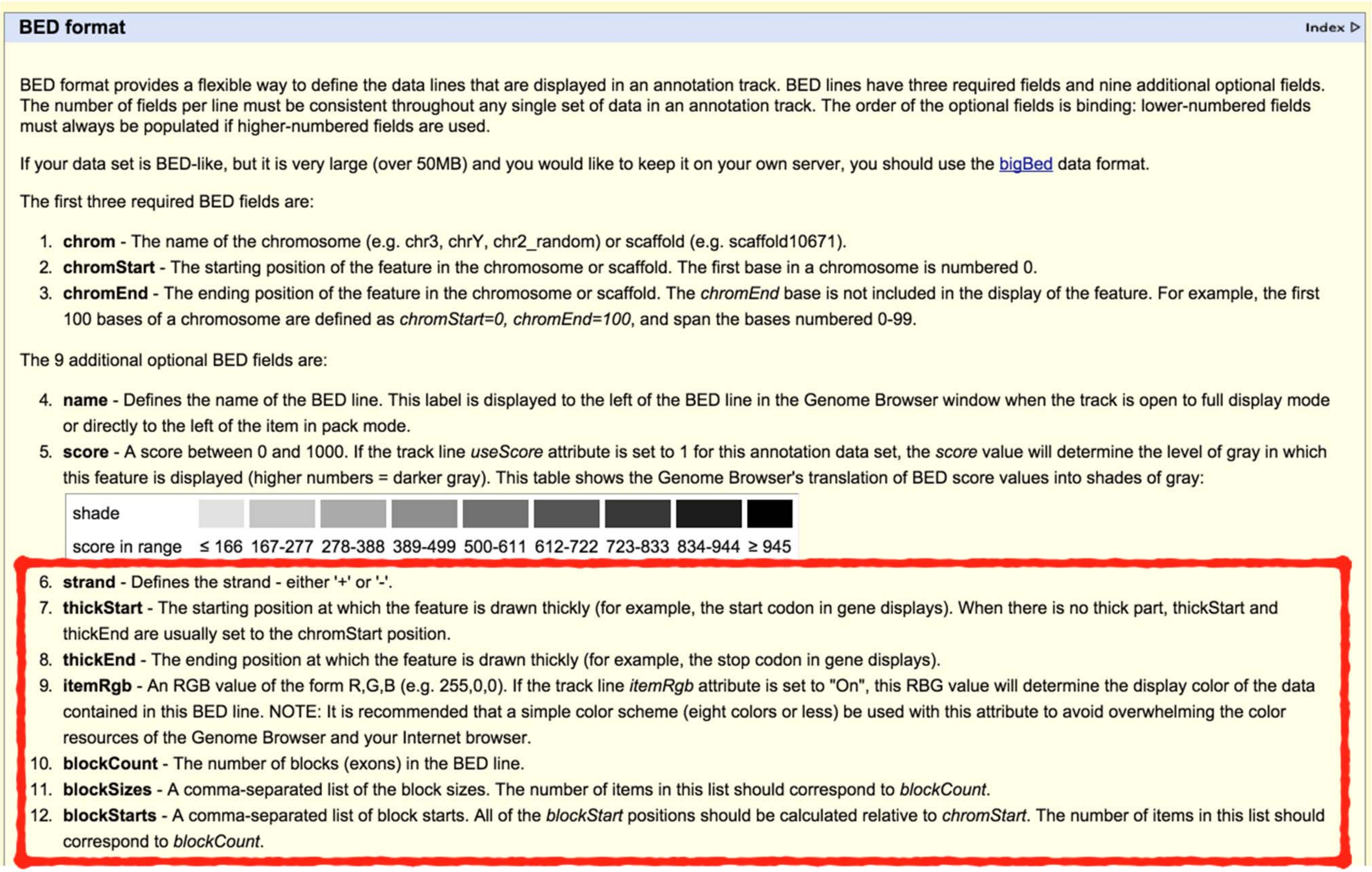
And strands. This is so-called BED6 format.

CAGTCGACATAGACTGATATGACACCACACTGAGC...



```
chr1    0    3     foo    3.1    +
chr1    11   18    bar    1.9    -
chr1    29   35    biz    0.1    +
```

And more! BED12 format



BED format Index ▷

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3. **chromEnd** - The 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*, and span the bases numbered 0-99.

The 9 additional optional BED fields are:

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5. **score** - A score between 0 and 1000. If the track line *useScore* attribute is set to 1 for this annotation data set, the *score* value will determine the level of gray in which this feature is displayed (higher numbers = darker gray). This table shows the Genome Browser's translation of BED score values into shades of gray:

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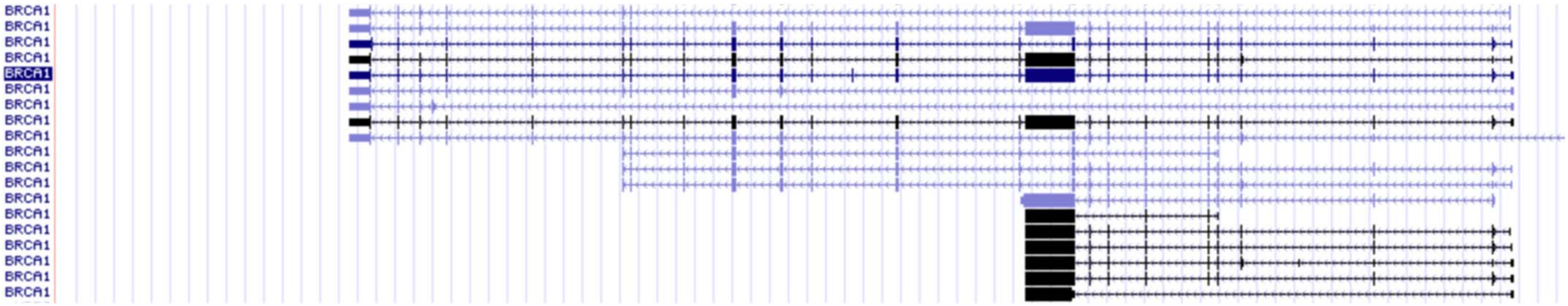
6. **strand** - Defines the strand - either '+' or '-'.
7. **thickStart** - The starting position at which the feature is drawn thickly (for example, the start codon in gene displays). When there is no thick part, thickStart and thickEnd are usually set to the chromStart position.
8. **thickEnd** - The ending position at which the feature is drawn thickly (for example, the stop codon in gene displays).
9. **itemRgb** - An RGB value of the form R,G,B (e.g. 255,0,0). If the track line *itemRgb* attribute is set to "On", this RBG value will determine the display color of the data contained in this BED line. NOTE: It is recommended that a simple color scheme (eight colors or less) be used with this attribute to avoid overwhelming the color resources of the Genome Browser and your Internet browser.
10. **blockCount** - The number of blocks (exons) in the BED line.
11. **blockSizes** - A comma-separated list of the block sizes. The number of items in this list should correspond to *blockCount*.
12. **blockStarts** - A comma-separated list of block starts. All of the *blockStart* positions should be calculated relative to *chromStart*. The number of items in this list should correspond to *blockCount*.

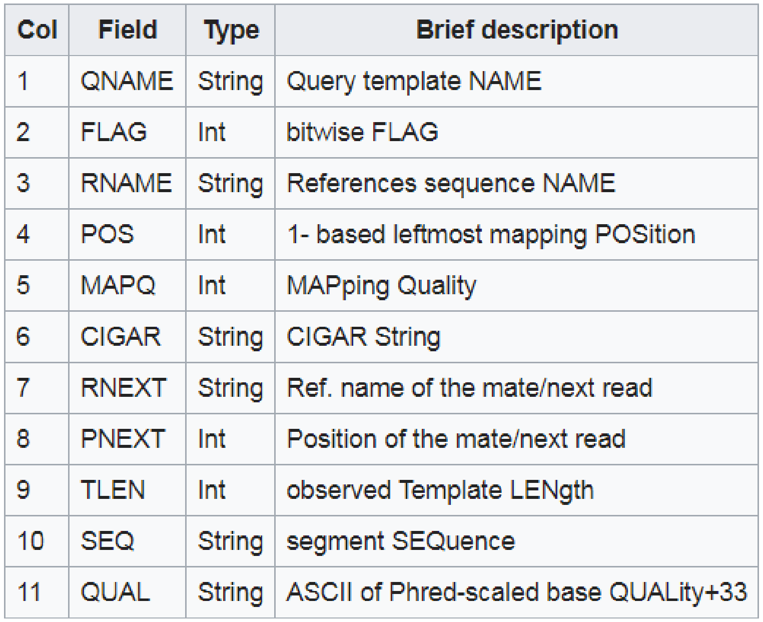
BED12 example



```
chr17   41196311        41277340        uc010whm.2      0       -       41197694        41277202        0       8       1508,61,74,55,84,41,78,142,     0,3348,4826,6768,12757,19038,19579,80887,
chr17   41196311        41277340        uc002icp.4      0       -       41197694        41258496        0       23      1508,61,74,55,84,41,78,88,311,191,127,172,89,3426,77,46,103,140,89,56,54,99,142,
0,3348,4826,6768,12757,19038,19579,23313,26633,30036,32193,38109,46649,47140,51551,52949,55480,59827,60573,62183,71431,79722,80887,
chr17   41196311        41277468        uc002icu.3      0       -       41197800        41276113        0       22      1508,61,55,84,41,78,88,311,191,124,172,89,117,77,46,106,140,89,78,54,99,175,
0,3348,6768,12757,19038,19579,23313,26633,30036,32193,38109,46649,50449,51551,52949,55480,59827,60573,62161,71431,79722,80982,
chr17   41196311        41277468        uc010cyx.3      0       -       41197694        41258543        0       22      1508,61,74,55,84,41,78,88,311,191,127,172,89,3426,77,46,106,140,89,78,99,175,
0,3348,4826,6768,12757,19038,19579,23313,26633,30036,32193,38109,46649,47140,51551,52949,55480,59827,60573,62161,79722,80982,
chr17   41196311        41277500        uc002ict.3      0       -       41197694        41276113        0       24      1508,61,74,55,84,41,78,88,311,191,124,66,172,89,3426,77,46,106,140,89,78,54,99,213,
0,3348,4826,6768,12757,19038,19579,23313,26633,30036,32193,35039,38109,46649,47140,51551,52949,55480,59827,60573,62161,71431,79722,80976,
chr17   41196311        41277500        uc010whn.2      0       -       41197694        41226495        0       11      1508,61,74,55,84,41,78,88,311,191,213,  0,3348,4826,6768,12757,19038,19579,23313,26633,30036,80976,
chr17   41196311        41277500        uc010who.3      0       -       41197694        41202109        0       5       1508,61,74,129,213,     0,3348,4826,5767,80976,
chr17   41196311        41277500        uc002icq.3      0       -       41197694        41276113        0       23      1508,61,74,55,84,41,78,88,311,191,127,172,89,3426,77,46,106,140,89,78,54,99,213,
0,3348,4826,6768,12757,19038,19579,23313,26633,30036,32193,38109,46649,47140,51551,52949,55480,59827,60573,62161,71431,79722,80976,
chr17   41196311        41322420        uc010whp.2      0       -       41197694        41258543        0       22      1508,61,74,55,84,41,78,88,311,191,124,172,89,117,77,46,106,140,89,78,54,278,
0,3348,4826,6768,12757,19038,19579,23313,26633,30036,32193,38109,46649,50449,51551,52949,55480,59827,60573,62161,71431,125831,
chr17   41215349        41256973        uc010whq.1      0       -       41215349        41256198        0       12      41,78,88,311,191,127,172,89,117,106,140,89,
0,541,4275,7595,10998,13155,19071,27611,31411,36442,40789,41535,
chr17   41215349        41277468        uc002idc.1      0       -       41215349        41276113        0       18      41,78,88,311,191,127,172,89,117,77,46,103,140,89,78,54,99,175,
0,541,4275,7595,10998,13155,19071,27611,31411,32513,33911,36442,40789,41535,43123,52393,60684,61944,
chr17   41215349        41277468        uc010whr.1      0       -       41215349        41258543        0       17      41,78,88,311,191,127,172,89,117,77,46,106,140,89,78,99,175,
0,541,4275,7595,10998,13155,19071,27611,31411,32513,33911,36442,40789,41535,43123,60684,61944,
chr17   41243116        41276132        uc002idd.3      0       -       41243347        41276113        0       9       3761,77,46,106,140,89,78,54,99, 0,4746,6144,8675,13022,13768,15356,24626,32917,
chr17   41243451        41256973        uc002ide.1      0       -       41243452        41256198        0       4       3426,103,140,89,        0,8340,12687,13433,
chr17   41243451        41277340        uc010cyy.1      0       -       41243452        41276113        0       10      3426,77,46,106,140,89,78,54,99,142,     0,4411,5809,8340,12687,13433,15021,24291,32582,33747,
chr17   41243451        41277468        uc010whs.1      0       -       41243452        41276113        0       10      3426,77,46,106,140,89,78,54,99,175,     0,4411,5809,8340,12687,13433,15021,24291,32582,33842,
chr17   41243451        41277500        uc010cyz.2      0       -       41243452        41258543        0       11      3426,77,46,106,140,89,78,116,54,99,213, 0,4411,5809,8340,12687,13433,15021,19030,24291,32582,33836,
chr17   41243451        41277500        uc010cza.2      0       -       41243452        41276113        0       9       3426,77,46,106,140,89,54,99,213,        0,4411,5809,8340,12687,13433,24291,32582,33836,
chr17   41243451        41277500        uc010wht.1      0       -       41243452        41246659        0       2       3426,213,       0,33836,
chr17   41277599        41292342        uc002idf.3      0       +       41277599        41277599        0       4       188,63,182,1669,        0,5625,7373,13074,
chr17   41277599        41292342        uc010czb.2      0       +       41277599        41277599        0       2       188,1669,       0,13074,
chr17   41277599        41297125        uc002idg.3      0       +       41277599        41277599        0       5       188,63,266,468,381,     0,5625,13074,14233,19145,
chr17   41277599        41305688        uc002idh.3      0       +       41277599        41277599        0       8       188,63,125,182,205,266,120,70,  0,5625,7016,7373,12573,13074,16874,28019,
```

BAM/SAM format

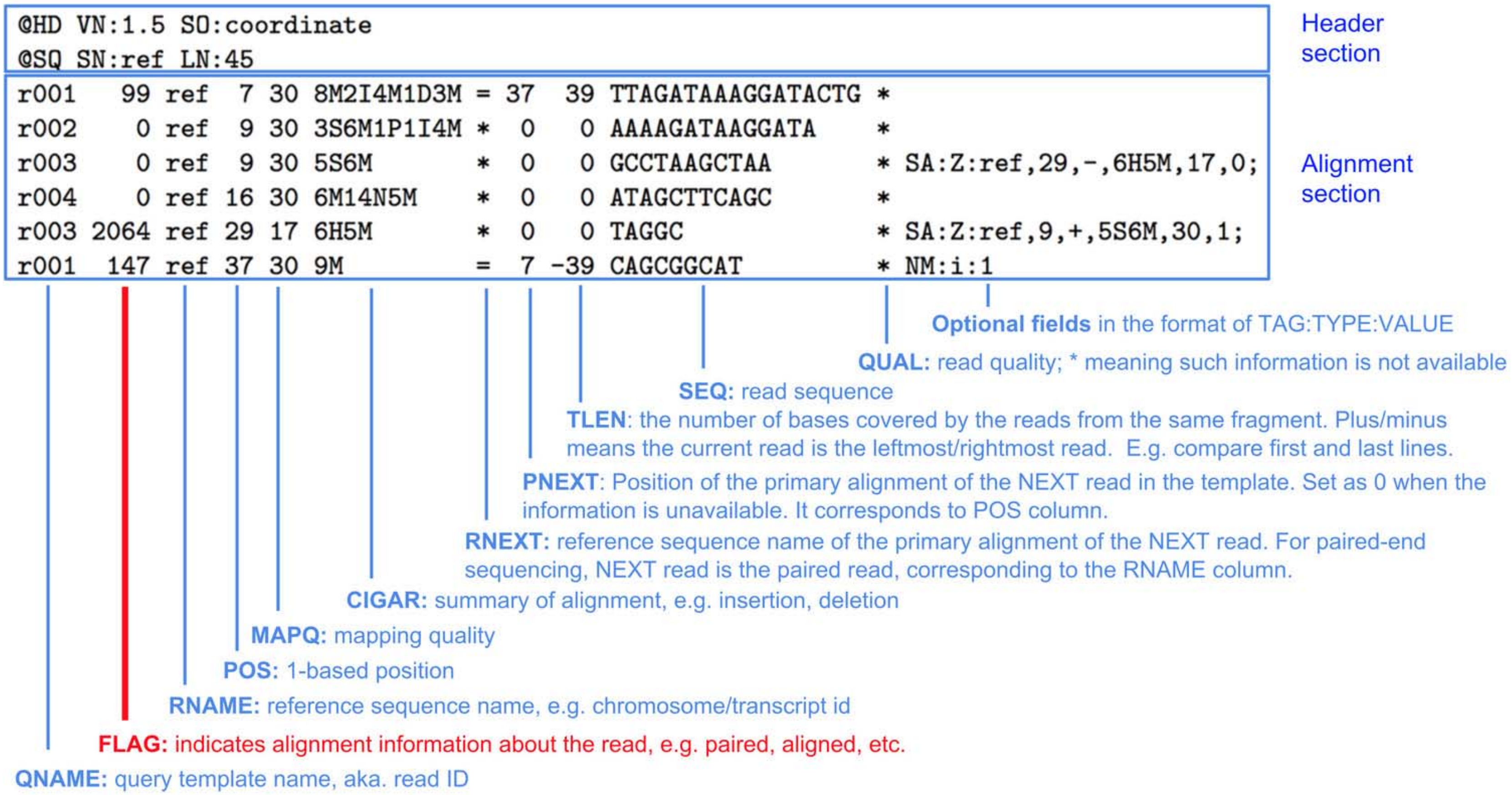
- SAM: Sequence Alignment/Map format (tab-delimited text file).
- BAM: The binary equivalent of a SAM file, which stores the same data in a compressed binary representation



Col	Field	Type	Brief description
1	QNAME	String	Query template NAME
2	FLAG	Int	bitwise FLAG
3	RNAME	String	References sequence NAME
4	POS	Int	1- based leftmost mapping POSition
5	MAPQ	Int	MAPping Quality
6	CIGAR	String	CIGAR String
7	RNEXT	String	Ref. name of the mate/next read
8	PNEXT	Int	Position of the mate/next read
9	TLEN	Int	observed Template LENgth
10	SEQ	String	segment SEQuence
11	QUAL	String	ASCII of Phred-scaled base QUALity+33

Li et al, Bioinformatics, 2009

Example of a SAM file



```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

Header section

Alignment section

Optional fields in the format of TAG:TYPE:VALUE

QUAL: read quality; * meaning such information is not available

SEQ: read sequence

TLEN: the number of bases covered by the reads from the same fragment. Plus/minus means the current read is the leftmost/rightmost read. E.g. compare first and last lines.

PNEXT: Position of the primary alignment of the NEXT read in the template. Set as 0 when the information is unavailable. It corresponds to POS column.

RNEXT: reference sequence name of the primary alignment of the NEXT read. For paired-end sequencing, NEXT read is the paired read, corresponding to the RNAME column.

CIGAR: summary of alignment, e.g. insertion, deletion

MAPQ: mapping quality

POS: 1-based position

RNAME: reference sequence name, e.g. chromosome/transcript id

FLAG: indicates alignment information about the read, e.g. paired, aligned, etc.

QNAME: query template name, aka. read ID

https://www.samformat.info/sam-format-flag

CRAM

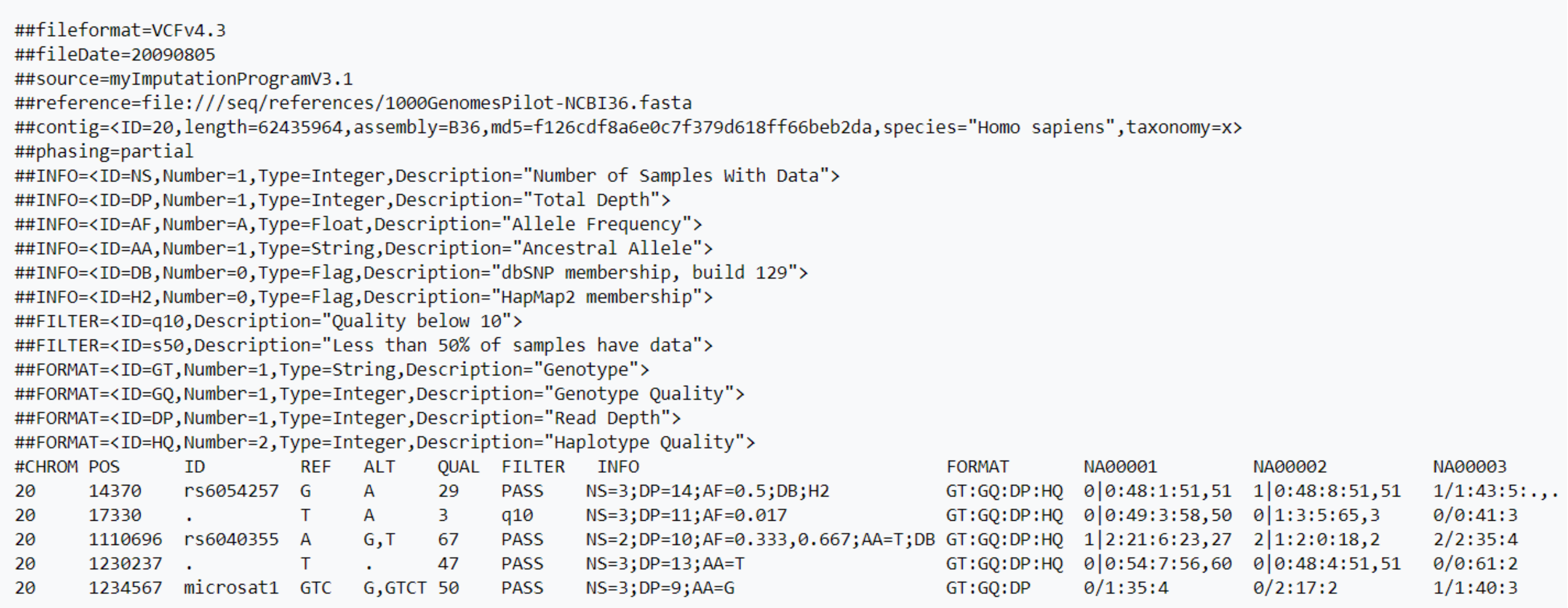
- CRAM was designed to be an efficient reference-based alternative to SAM/BAM file formats
- Better lossless compression than BAM, but also allow for controlled loss of BAM data
- Typically used for large-scale population-based genome/exome sequencing project (for example, CRAMs has ~50TB for 50K exomes in UK Biobank).

https://samtools.github.io/hts-specs/CRAMv3.pdf

VCF file format

- Variant Call Format, established > 10 years ago and is now a gold standard for describing variants.
- One locus per line, and it may contain more than one mutations, but most lines contain on variant only.
- Additional header lines starts with “#” to explain the meaning of the various tags in the file

Example of a VCF file



```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##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">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS      ID         REF   ALT    QUAL  FILTER   INFO                             FORMAT       NA00001         NA00002          NA00003
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  1|0:48:8:51,51   1/1:43:5:.,.
20     17330    .          T     A      3     q10     NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ  0|0:49:3:58,50  0|1:3:5:65,3     0/0:41:3
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  2|1:2:0:18,2     2/2:35:4
20     1230237  .          T     .      47    PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51   0/0:61:2
20     1234567  microsat1  GTC   G,GTCT 50    PASS    NS=3;DP=9;AA=G                    GT:GQ:DP     0/1:35:4        0/2:17:2         1/1:40:3
```

As of June 2019, the latest version is 4.3

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

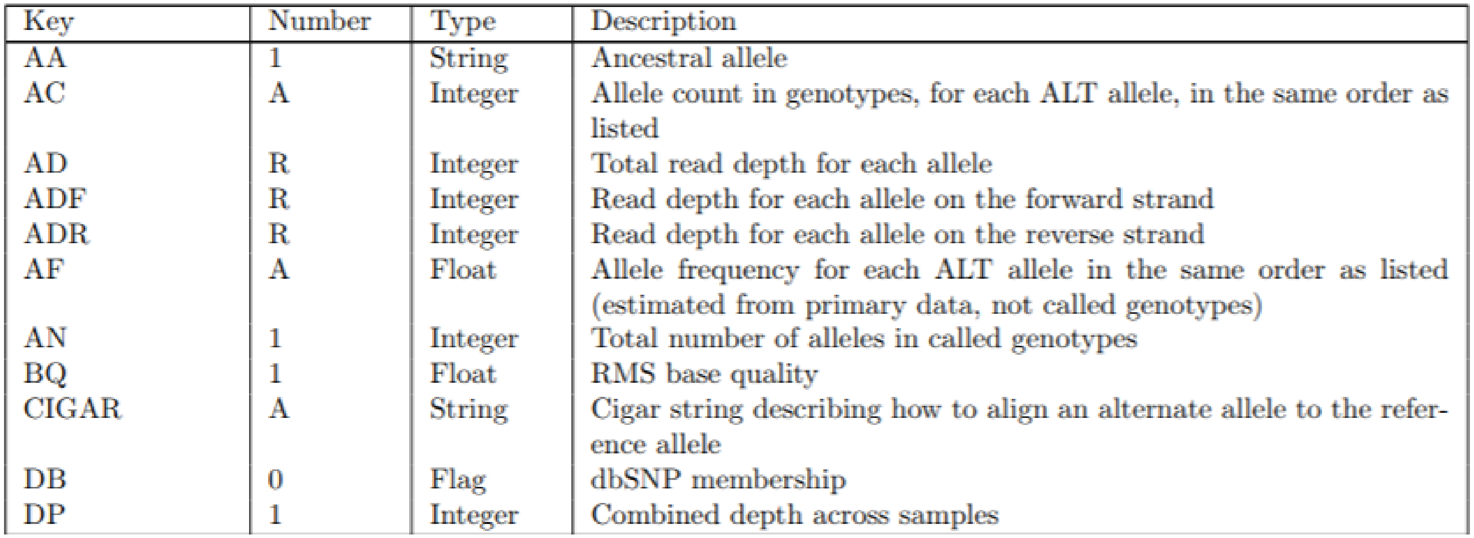
The header line of a VCF file

- The header line names the 8 fixed, mandatory columns. These columns are as follows:
  - #CHROM POS ID REF ALT QUAL FILTER INFO

- If genotype data is present in the file, these are followed by a FORMAT column header, then an arbitrary number of sample IDs

The INFO line of a VCF file

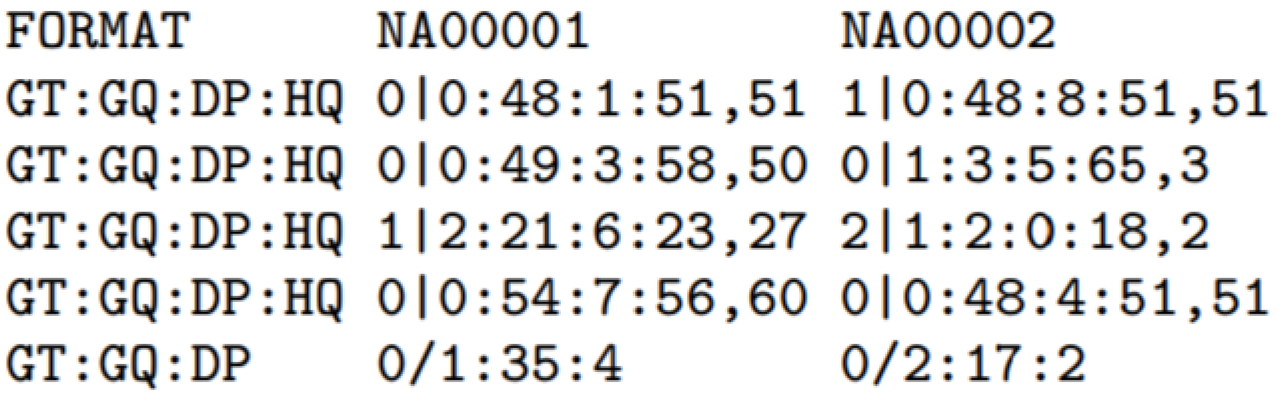
- INFO fields are encoded as a semicolon-separated series of short keys with optional values in the format: key[=data[,data]].
- Some keys are reserved:



Key	Number	Type	Description
AA	1	String	Ancestral allele
AC	A	Integer	Allele count in genotypes, for each ALT allele, in the same order as listed
AD	R	Integer	Total read depth for each allele
ADF	R	Integer	Read depth for each allele on the forward strand
ADR	R	Integer	Read depth for each allele on the reverse strand
AF	A	Float	Allele frequency for each ALT allele in the same order as listed (estimated from primary data, not called genotypes)
AN	1	Integer	Total number of alleles in called genotypes
BQ	1	Float	RMS base quality
CIGAR	A	String	Cigar string describing how to align an alternate allele to the reference allele
DB	0	Flag	dbSNP membership
DP	1	Integer	Combined depth across samples

The genotypes in a VCF file

- A FORMAT field is given specifying the data types and order
- This is followed by one data block per sample, with the colon-separated data corresponding to the types specified in the format.

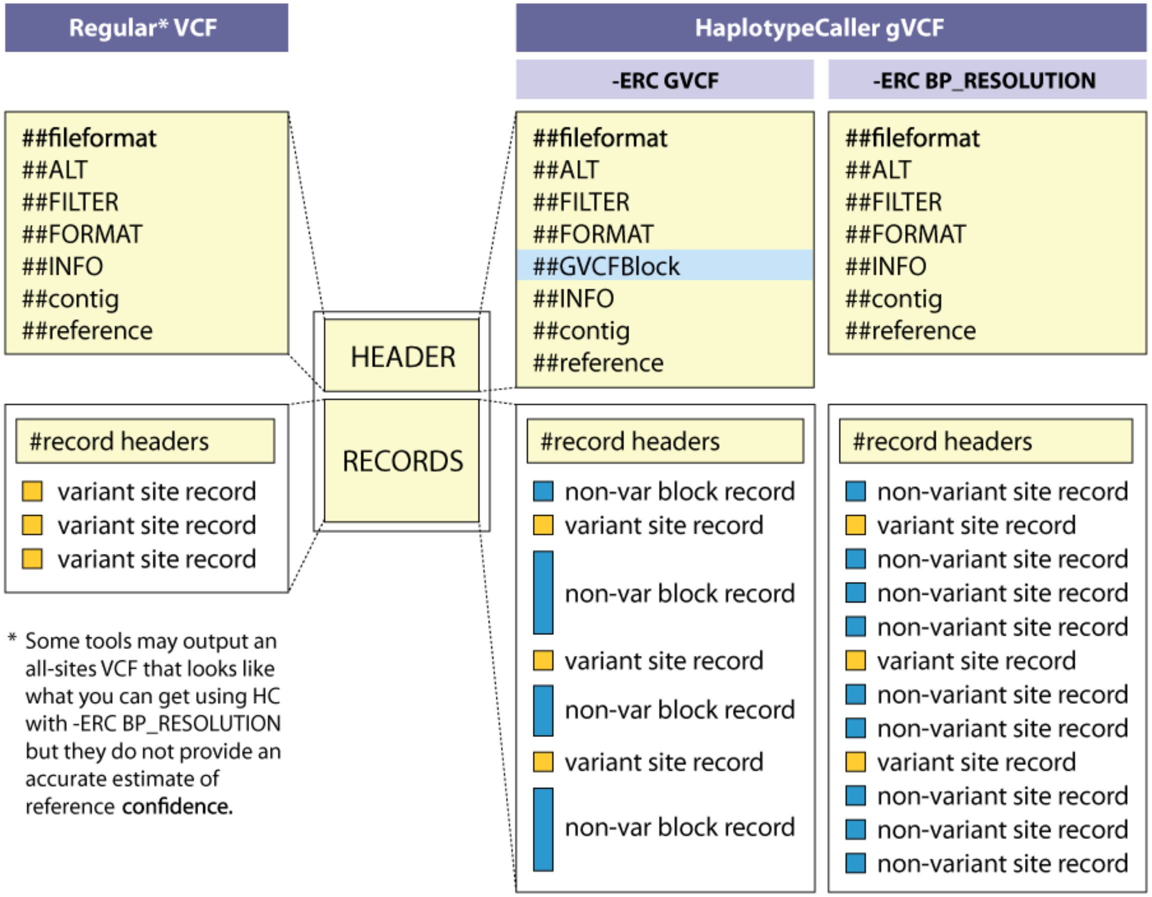


```
FORMAT      NA00001         NA00002
GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51
GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3
GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2
GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51
GT:GQ:DP    0/1:35:4        0/2:17:2
```

gVCF format

- gVCF (Genomic VCF): the basic format specification is the same as for a regular VCF, but gVCF contains extra information.
- gVCF was developed to store sequencing information for both variant and non-variant positions, which is required for human clinical applications.

VCF versus gVCF



Regular* VCF

##fileformat
##ALT
##FILTER
##FORMAT
##INFO
##contig
##reference

#record headers
🟨 variant site record
🟨 variant site record
🟨 variant site record

HEADER

RECORDS

HaplotypeCaller gVCF

-ERC GVCF

##fileformat
##ALT
##FILTER
##FORMAT
##GVCFBlock
##INFO
##contig
##reference

#record headers
🟦 non-var block record
🟨 variant site record
🟦 non-var block record
🟨 variant site record
🟦 non-var block record
🟨 variant site record
🟦 non-var block record

-ERC BP_RESOLUTION

##fileformat
##ALT
##FILTER
##FORMAT
##INFO
##contig
##reference

#record headers
🟦 non-variant site record
🟨 variant site record
🟦 non-variant site record
🟦 non-variant site record
🟦 non-variant site record
🟨 variant site record
🟦 non-variant site record
🟦 non-variant site record
🟨 variant site record
🟦 non-variant site record
🟦 non-variant site record
🟦 non-variant site record

* Some tools may output an all-sites VCF that looks like what you can get using HC with -ERC BP_RESOLUTION but they do not provide an accurate estimate of reference **confidence.**

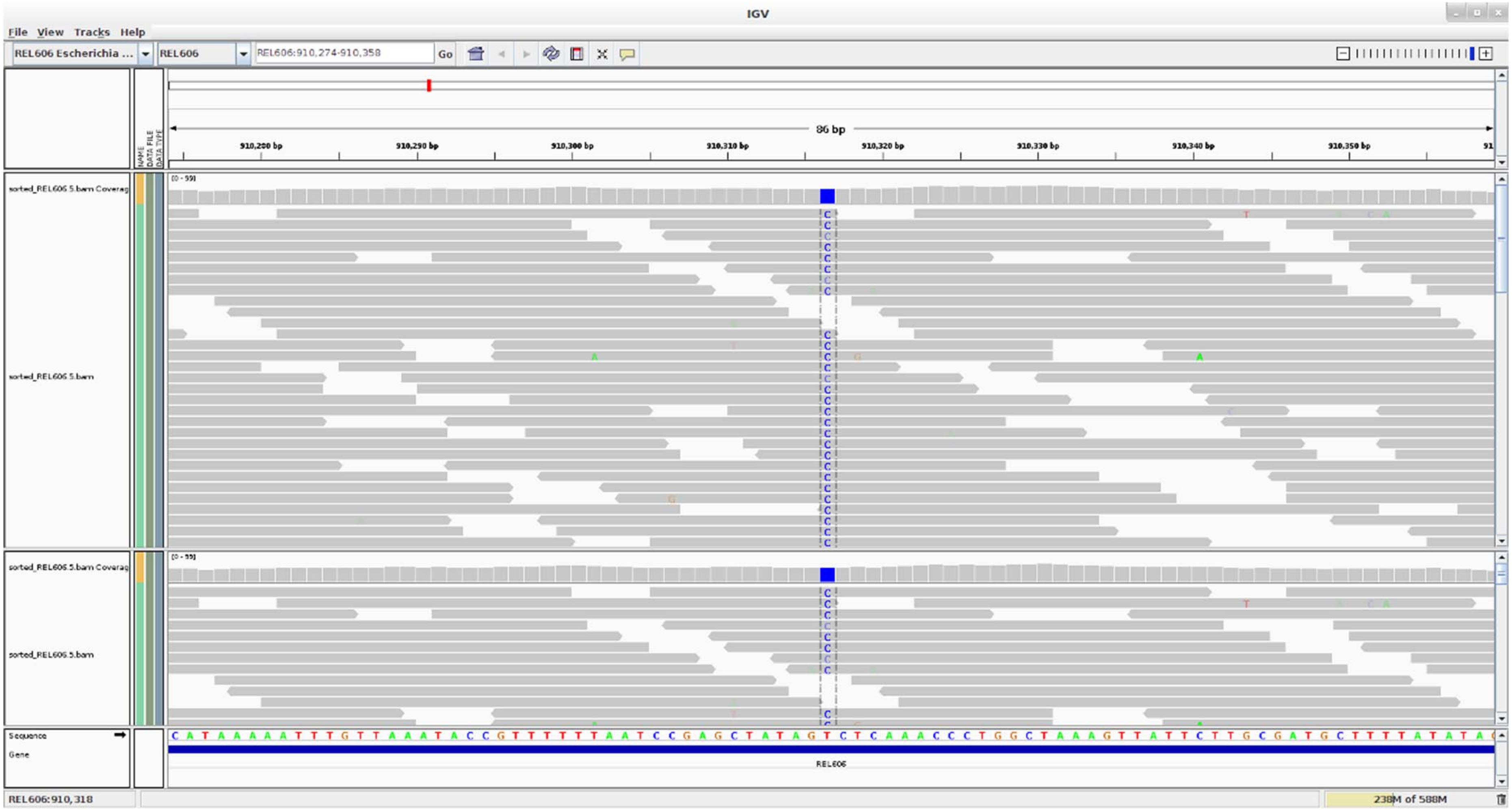
https://software.broadinstitute.org/gatk/documentation/article.php?id=4017

Visualization of genomic data

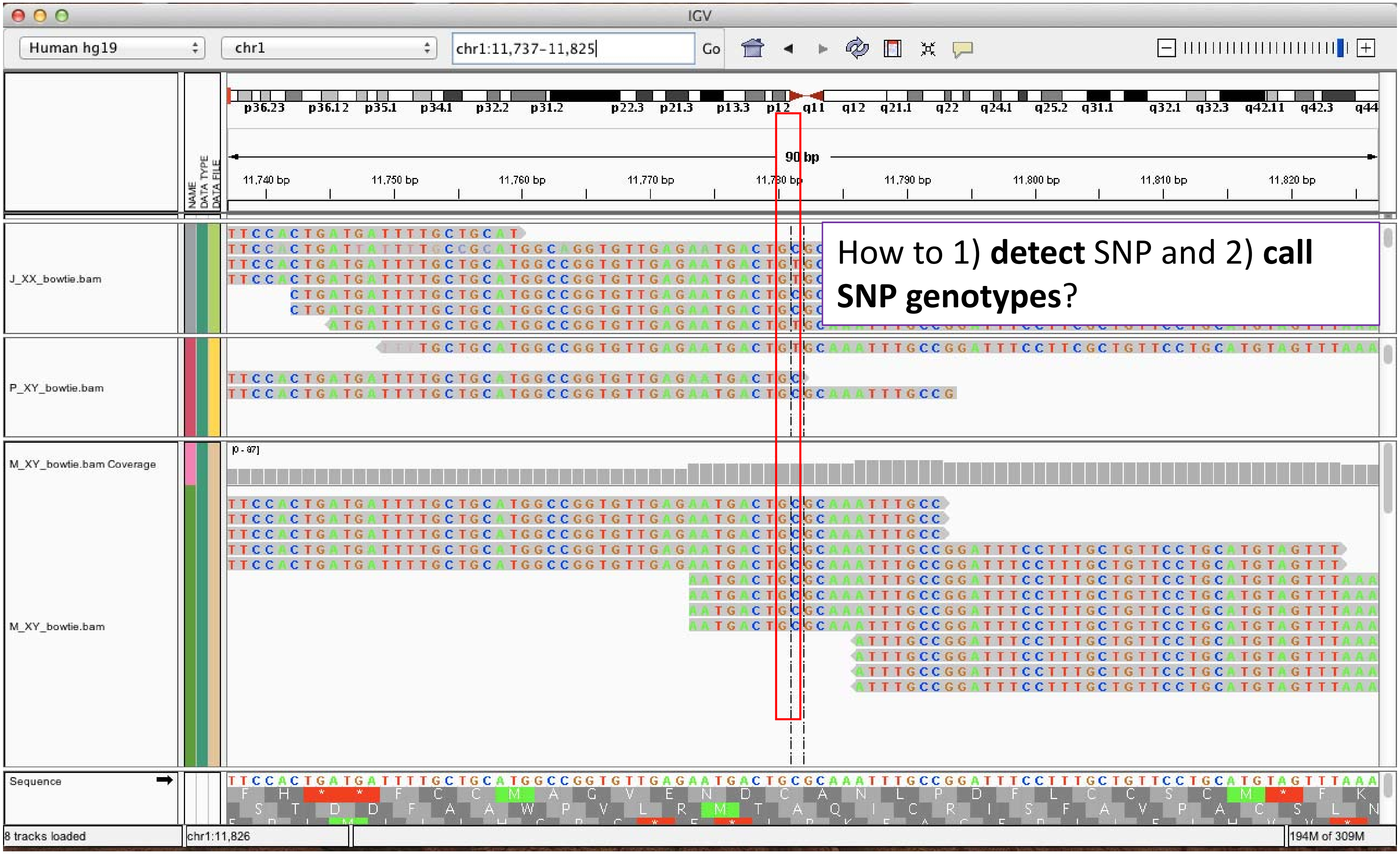
- Integrative Genomics Viewer (IGV) is a high-performance visualization tool for interactive exploration of large, integrated genomic datasets.

https://software.broadinstitute.org/software/igv/

Visualization: IGV Viewer



Visualization: IGV Viewer



How to 1) **detect** SNP and 2) **call SNP genotypes**?

Calling a single nucleotide variant

FDR calculation: What is the expected number of such sites (3 errors of the same) given an average depth of 30x?

N_errors / base = 30x0.005 / 3 = 0.05;

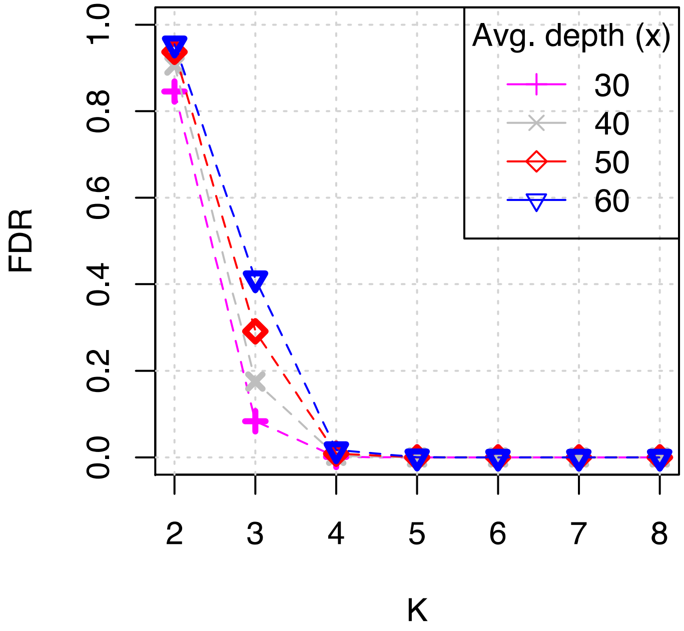
#errors/site ~ Poisson(0.05) [for a particular Ref-Alt error]

E(# sites with >=3 errors) ~ [1- ppois(2, 0.05)] * 3 * N

Coding regions ~ 30Mbp, number of variants in total ~ 20,000

E(false positive | K=3) = 1800 → FDR ~ 9%

K=4 → FDR ~ 0.1%



K-allele algorithm:

- Map reads to the reference genome
- Pick a depth threshold ***K***, (e.g. *K*=4)
- Candidate variant sites: if $\boldsymbol{n_1 \geq K}$
- Additional filters {testing a different null model (there is a SNV)}:
    - Strand bias
    - Mapping bias
    - Quality bias

SNV calling: improvement from K-allele

Problems with K-allele method:

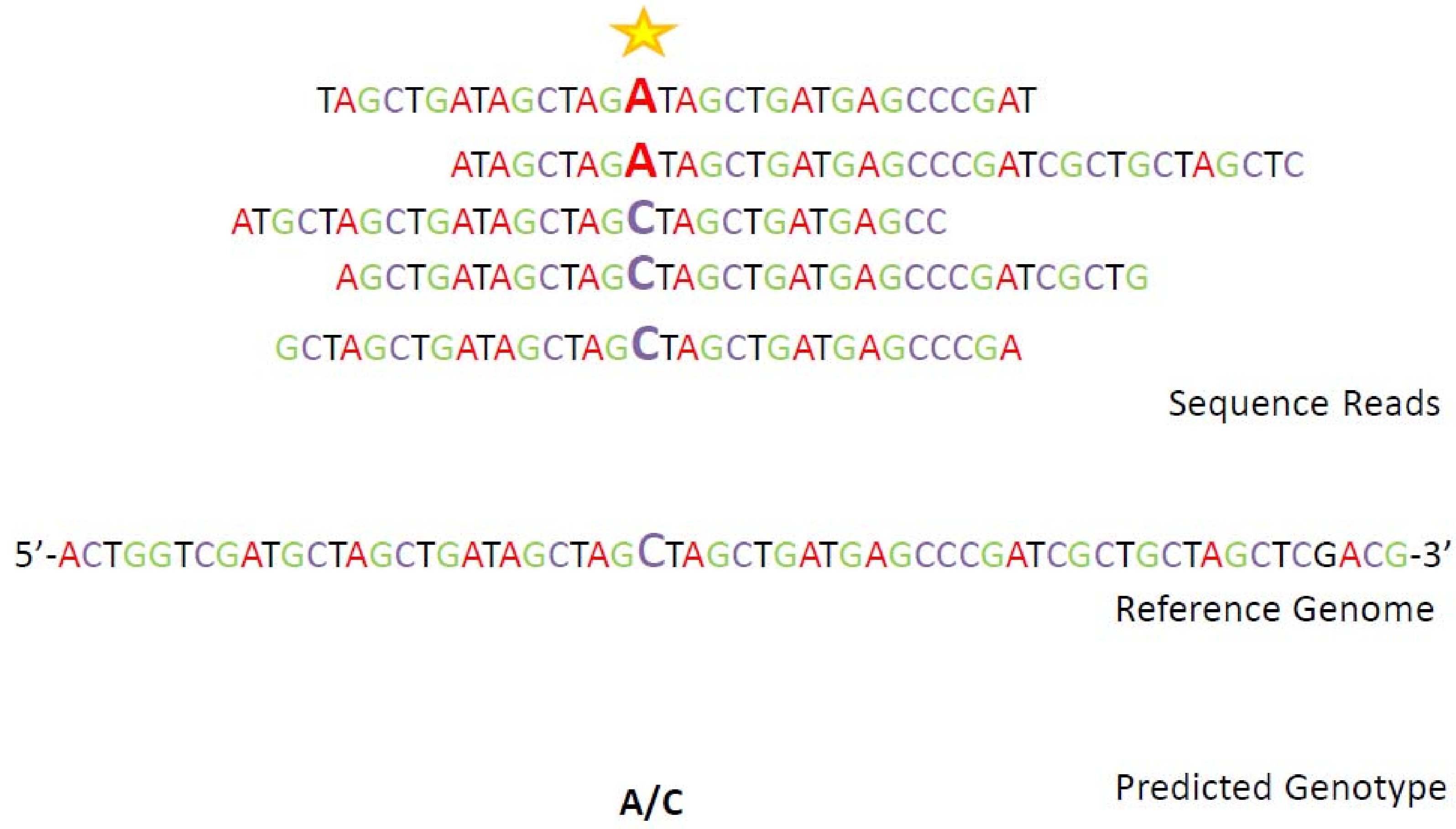
1. Errors are not independent
2. Hard to set threshold when ***average depth of coverage*** varies across samples and batches.

Solutions:

- Factor in context-dependency
- Full likelihood calculation
    - Genotype likelihood and posterior
- Leverage genetic priors:
    - Diploid genome,
    - known polymorphisms

General strategy for variant calling

- Reads piled up at each base of interest
- With per-base qualities and mapping quality



TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

A/C

Predicted Genotype

NGS Data

Sequence Reads

★

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 1.0

P(reads|A/C, read mapped)= 1.0

P(reads|C/C, read mapped)= 1.0

Possible Genotypes

NGS Data

★

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= P(C observed|A/A, read mapped)

P(reads|A/C, read mapped)= P(C observed|A/C, read mapped)

P(reads|C/C, read mapped)= P(C observed|C/C, read mapped)

Possible Genotypes

NGS Data

★

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 0.01

P(reads|A/C, read mapped)= 0.50

P(reads|C/C, read mapped)= 0.99

Possible Genotypes

NGS Data

★

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

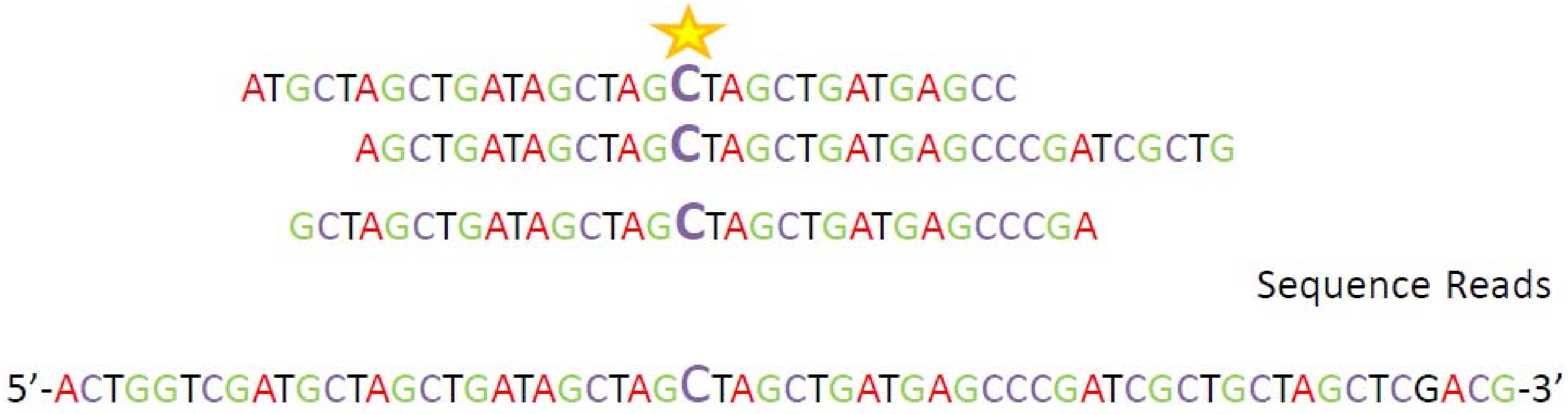
P(reads|A/A, read mapped)= 0.0001

P(reads|A/C , read mapped)= 0.25

P(reads|C/C , read mapped)= 0.98

Possible Genotypes

NGS Data



ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A , read mapped)= 0.000001

P(reads|A/C , read mapped)= 0.125

P(reads|C/C , read mapped)= 0.97

Possible Genotypes

NGS Data

★

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A , read mapped)= 0.00000099

P(reads|A/C , read mapped)= 0.0625

P(reads|C/C , read mapped)= 0.0097

Possible Genotypes

NGS Data

```
               TAGCTGATAGCTAGATAGCTGATGAGCCCGAT
                     ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC
           ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC
                AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG
             GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA
```

Sequence Reads

```
5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’
```

Reference Genome

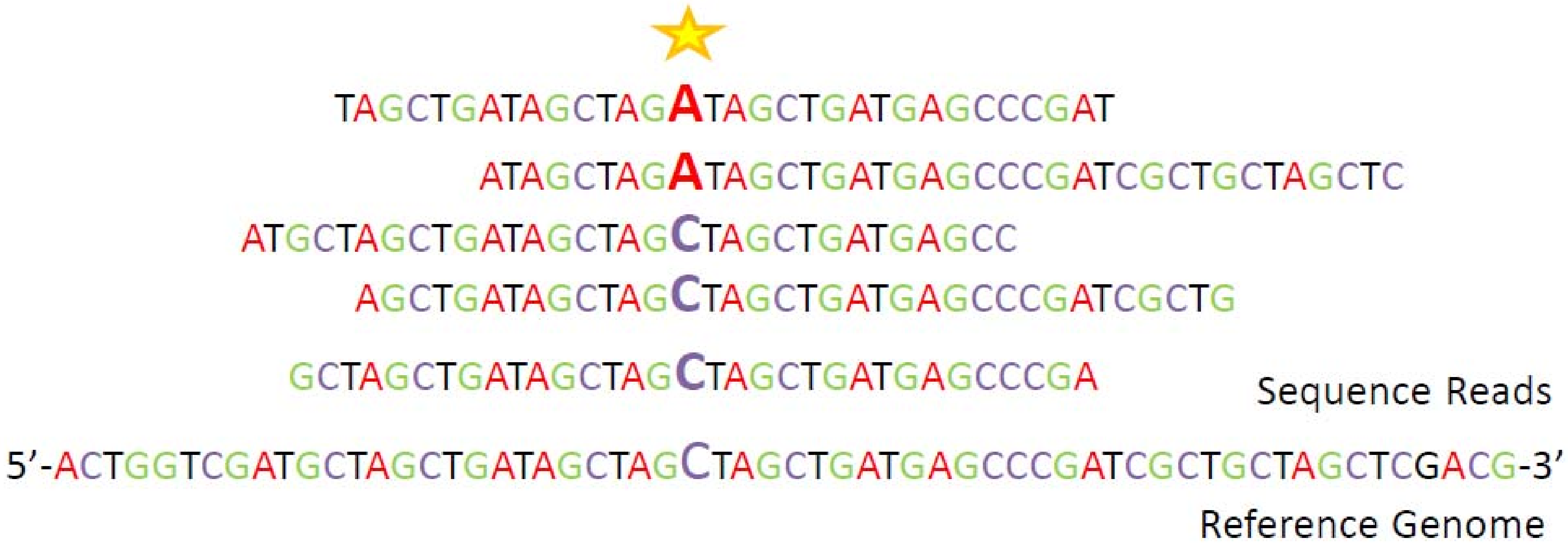
P(reads|A/A , read mapped)= 0.00000098

P(reads|A/C , read mapped)= 0.03125

P(reads|C/C , read mapped)= 0.000097

Possible Genotypes

NGS Data



TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5'-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3'

Reference Genome

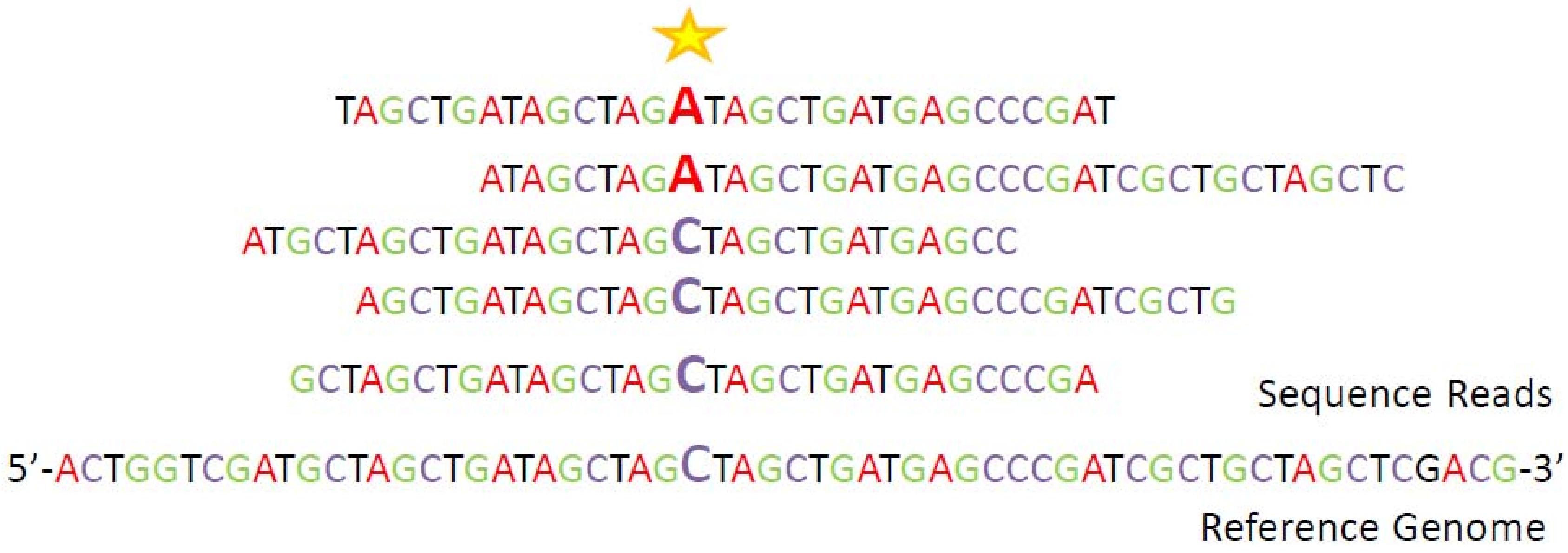
P(reads|A/A, read mapped)= 0.00000098

P(reads|A/C, read mapped)= 0.03125

P(reads|C/C, read mapped)= 0.000097

Combine these likelihoods with a prior incorporating information from other individuals and flanking sites to assign a genotype.

NGS Data



TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

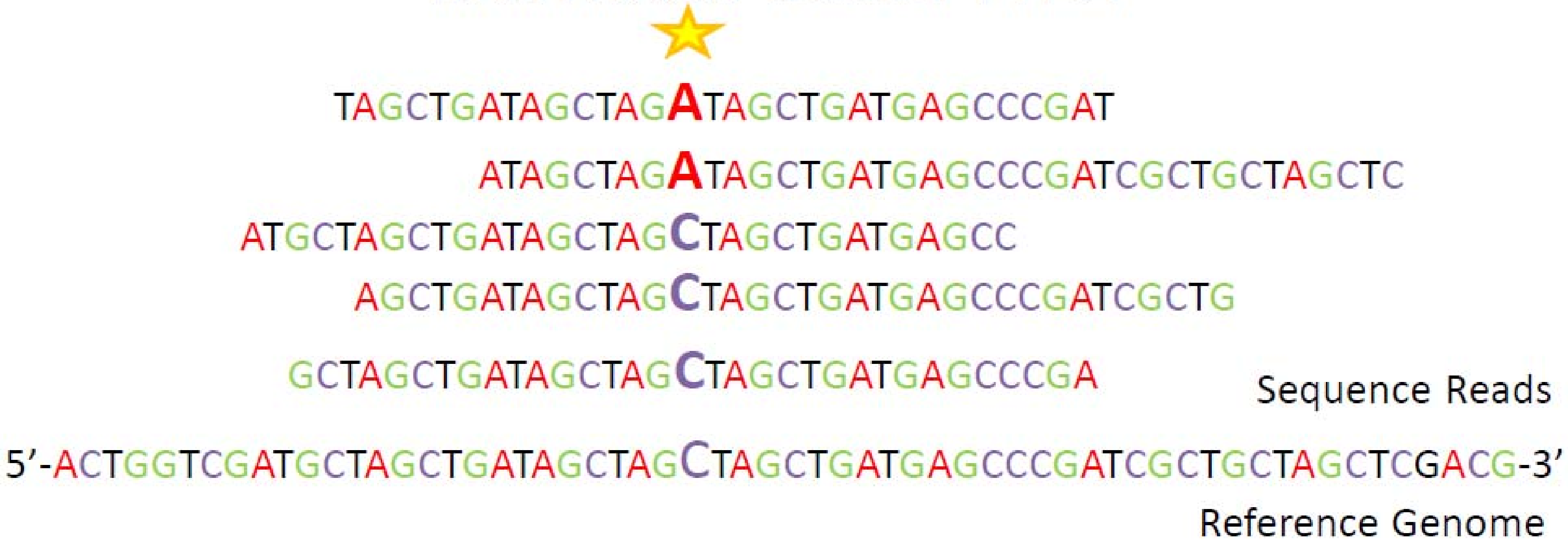
$$P(Genotype|reads) = \frac{P(reads|Genotype)Prior(Genotype)}{\sum_G P(reads|G)Prior(G)}$$

Combine these likelihoods with a prior incorporating information from other individuals and flanking sites to assign a genotype.

Ingredients in the Prior

- Most sites don’t vary
  - P(non-reference base) ~ 0.001
- When a site does vary, it is usually heterozygous
  - P(non-reference heterozygote) ~ 0.001 * 2/3
  - P(non-reference homozygote) ~ 0.001 * 1/3
- Mutation model
  - Transitions account for most variants (C⟷T or A⟷G)
  - Transversions account for minority of variants

From Sequence to Genotype: Individual Based Prior



TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5'-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3'

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.00034	Posterior(A/A) = <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.00066	Posterior(A/C) = 0.175
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.99900	Posterior(C/C) = 0.825

Individual Based Prior: Every site has 1/1000 probability of varying.

From Sequence to Genotype: Individual Based Prior

TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.00034	**Posterior(A/A) =** <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.00066	**Posterior(A/C) =** 0.175
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.99900	**Posterior(C/C) =** 0.825

Individual Based Prior: Every site has 1/1000 probability of varying.

Calling SNP Genotype for One Person at a Time

- Idea is simple
  - Calculate from observed data P(reads | genotype)
  - Impose a prior on P(genotype)
  - Get posterior probability P(genotype | reads)

- Issues
  - Choice of a prior
  - P(reads | genotype) involves per-base errors that are very likely to be corrected and/or not well calibrated, reads that are mapped with different level of confidence

More on Prior

- **Individual Based Prior**
  - Assumes all sites have an equal probability of showing polymorphism
  - Specifically, assumption is that about 1/1000 bases differ from reference
  - If reads were error free and sampling Poisson …
  - … 14x coverage would allow for 99.8% genotype accuracy
  - … 30x coverage of the genome needed to allow for errors and clustering

From Sequence to Genotype: Population Based Prior

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.04	Posterior(A/A) = <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.32	Posterior(A/C) = 0.999
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.64	Posterior(C/C) = <.001

Population Based Prior: Use frequency information from examining others at the same site.

In the example above, we estimated P(A) = 0.20

From Sequence To Genotype: Population Based Prior

```
               TAGCTGATAGCTAGATAGCTGATGAGCCCGAT
                     ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC
           ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC
                AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

             GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA
```

Sequence Reads

```
5'-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3'
```

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.04	**Posterior(A/A) =** <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.32	**Posterior(A/C) =** 0.999
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.64	**Posterior(C/C) =** <.001

Population Based Prior: Use frequency information from examining others at the same site.
In the example above, we estimated P(A) = 0.20

More on Prior

- **Population Based Prior**
  - Uses frequency information obtained from examining other individuals
  - Calling very rare polymorphisms still requires 20-30x coverage of the genome
  - Calling common polymorphisms requires much less data