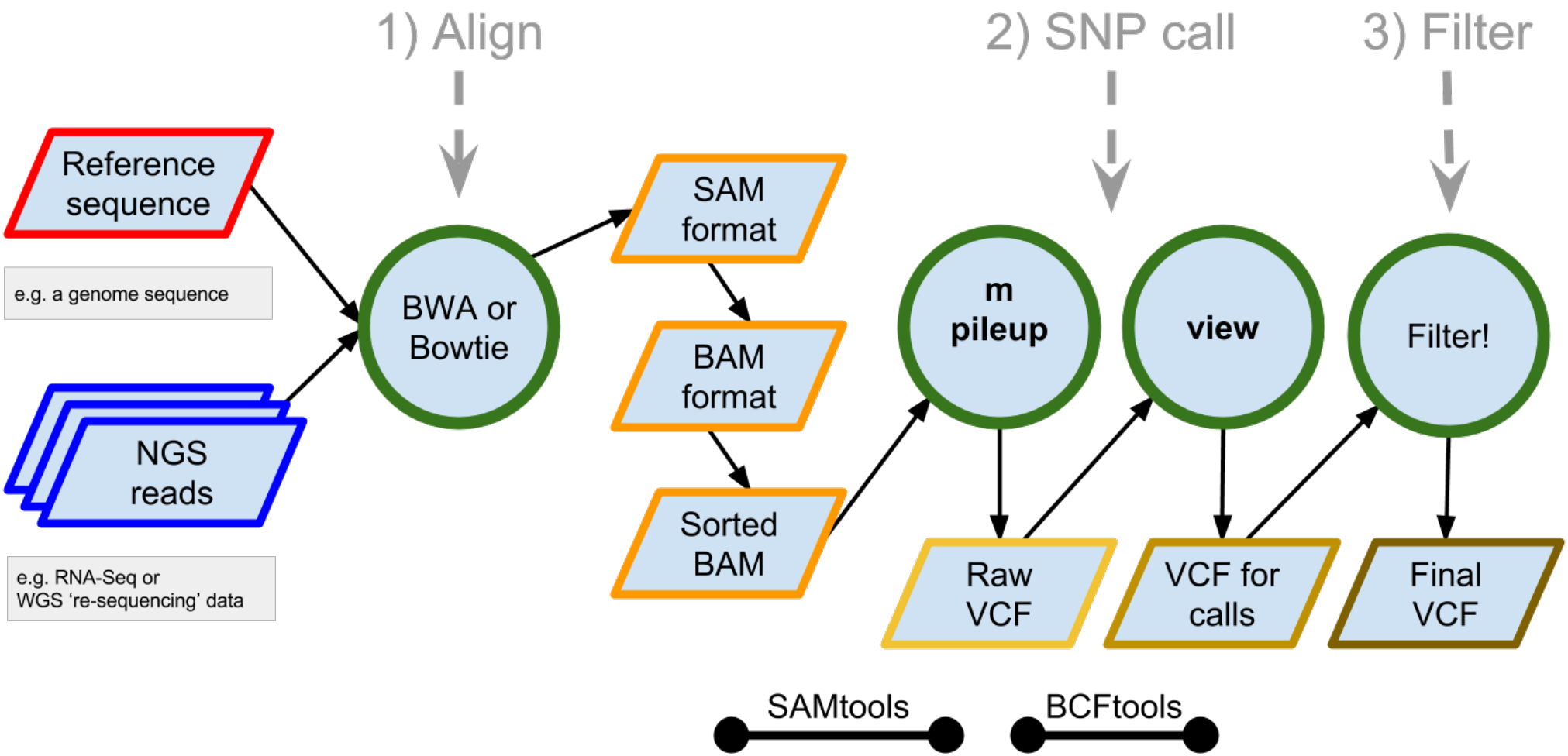
A simple SNP calling pipeline

dbolser@ebi.ac.uk

EMBL-EBI

Pipeline overview

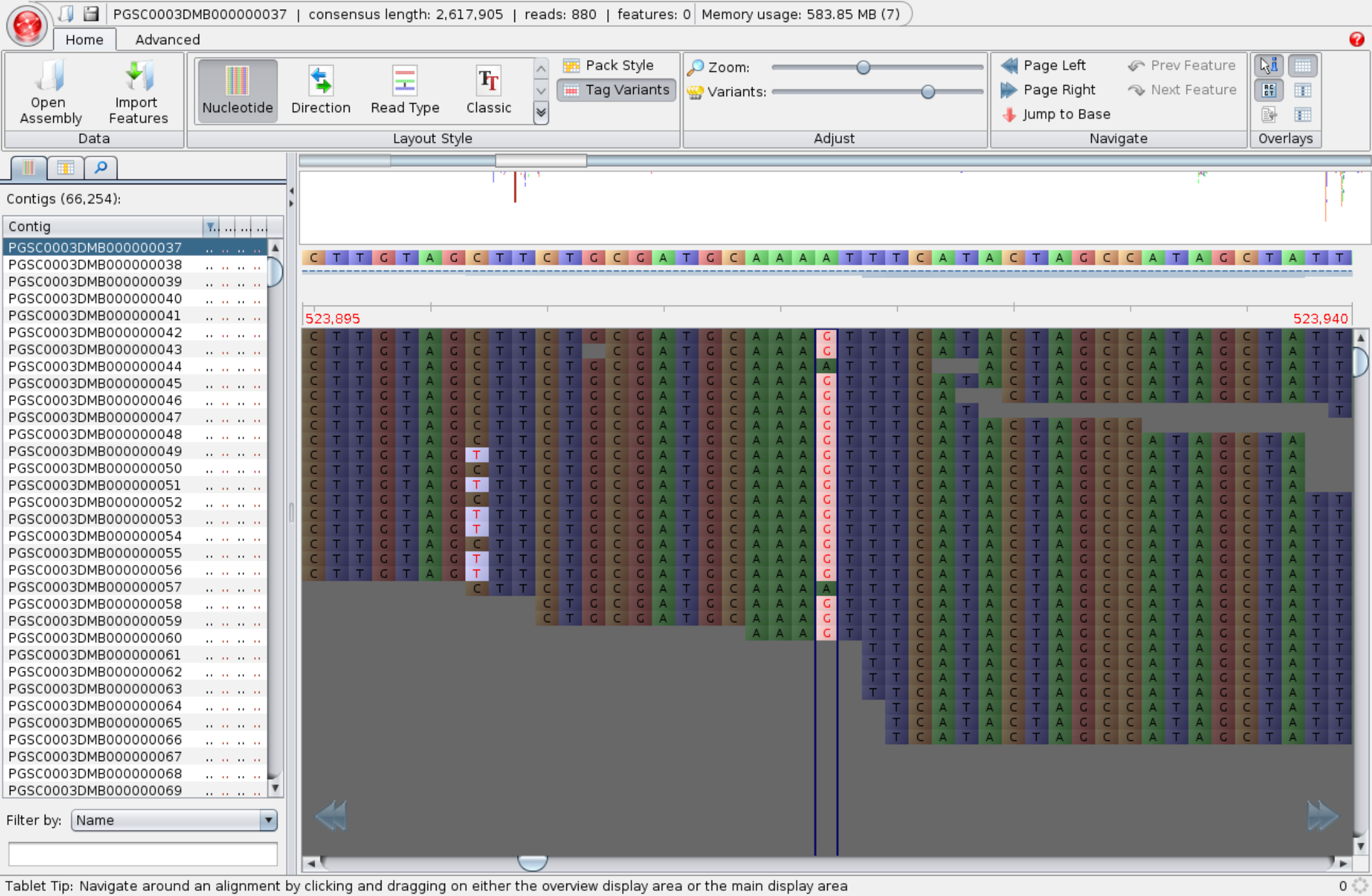


1) Align reads to reference (using BWA)

1. Index the reference (genome) sequence
   - `bwa index my.fasta`
   - `# The various index files are output in the CWD`

2. Perform the alignment
   - `bwa aln [opts] my.fasta my.fastq > my.sai`

3. Output results in SAM format (single end)
   - `bwa samse my.fasta my.sai my.fastq > my.sam`


PGSC0003DMB000000037 | consensus length: 2,617,905 | reads: 880 | features: 0 | Memory usage: 583.85 MB (7)
Home
Advanced
Open Assembly
Import Features
Data
Nucleotide
Direction
Read Type
Classic
Pack Style
Tag Variants
Layout Style
Zoom:
Variants:
Adjust
Page Left
Page Right
Jump to Base
Prev Feature
Next Feature
Navigate
Overlays
Contigs (66,254):
Contig
PGSC0003DMB000000037
PGSC0003DMB000000038
PGSC0003DMB000000039
PGSC0003DMB000000040
PGSC0003DMB000000041
PGSC0003DMB000000042
PGSC0003DMB000000043
PGSC0003DMB000000044
PGSC0003DMB000000045
PGSC0003DMB000000046
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PGSC0003DMB000000060
PGSC0003DMB000000061
PGSC0003DMB000000062
PGSC0003DMB000000063
PGSC0003DMB000000064
PGSC0003DMB000000065
PGSC0003DMB000000066
PGSC0003DMB000000067
PGSC0003DMB000000068
PGSC0003DMB000000069
Filter by:
Name
523,895
523,940
Tablet Tip: Navigate around an alignment by clicking and dragging on either the overview display area or the main display area
0

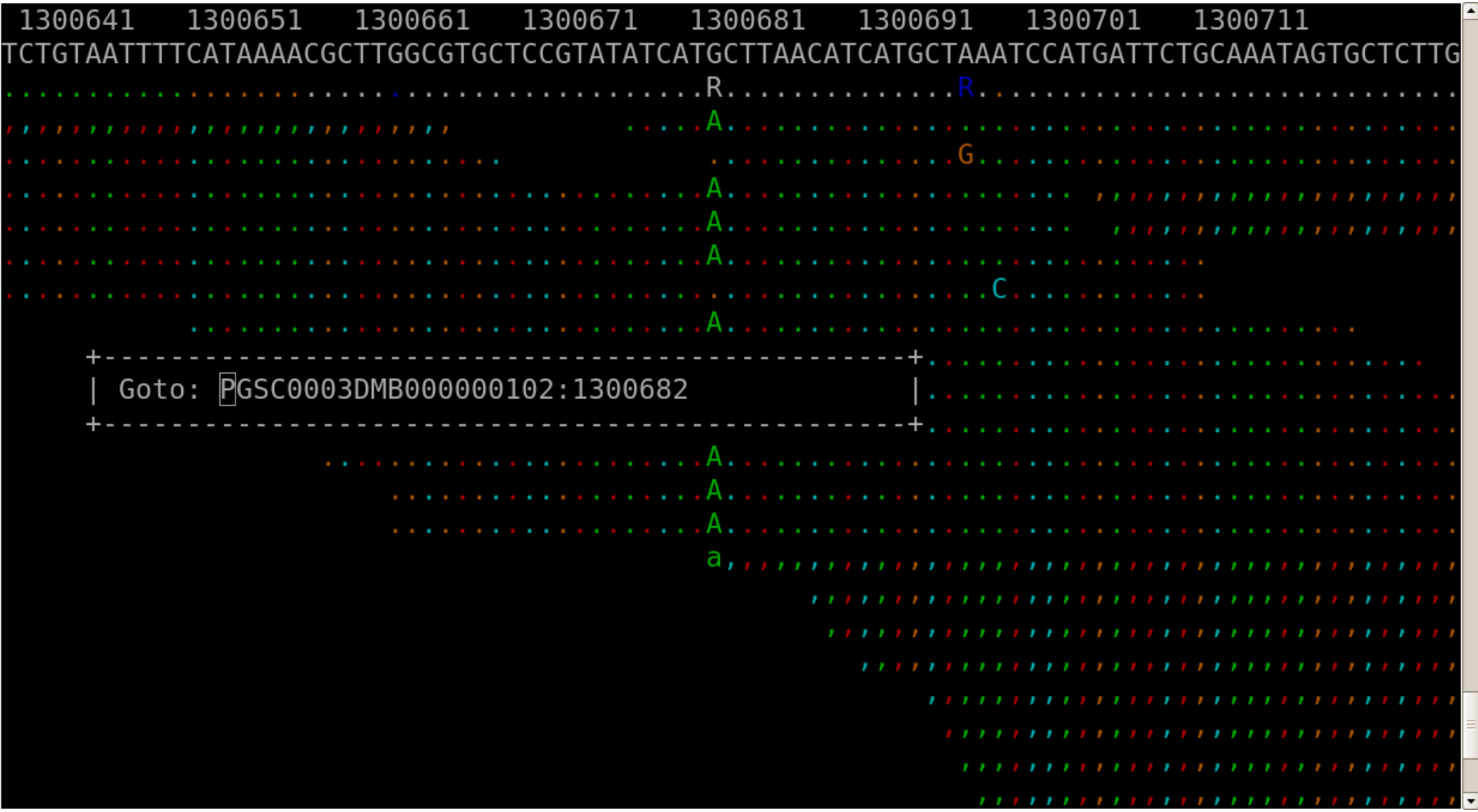
Ambiguity codes

https://wikipedia.org/wiki/Nucleic_acid_notation

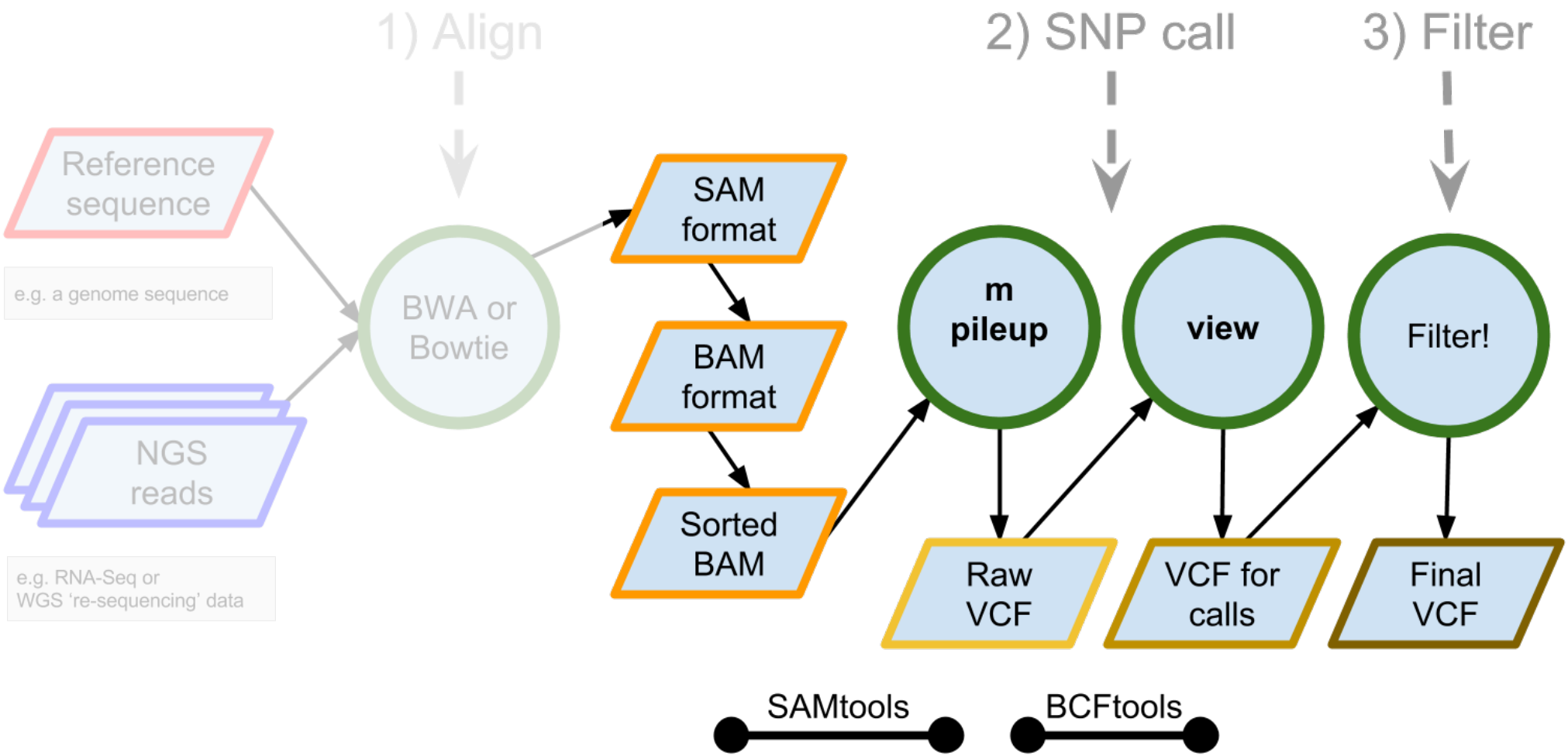
Nomenclature Committee of the International Union of Biochemistry (NC-IUB) (1984). "Nomenclature for Incompletely Specified Bases in Nucleic Acid Sequences". Retrieved 2008-02-04.

Symbol[2]	Description	Bases represented				
A	**A**denine	A				1
C	**C**ytosine		C			
G	**G**uanine			G		
T	**T**hymine				T	
U	**U**racil				U	
W	**W**eak	A			T	2
S	**S**trong		C	G		
M	a**M**ino	A	C			
K	**K**eto			G	T	
R	pu**R**ine	A		G		
Y	p**Y**rimidine		C		T	
B	not A (**B** comes after A)		C	G	T	3
D	not C (**D** comes after C)	A		G	T	
H	not G (**H** comes after G)	A	C		T	
V	not T (**V** comes after T and U)	A	C	G		
N or **-**	a**N**y base (not a gap)	A	C	G	T	4


235661 235671 235681 235691 235701 235711 235721
CAGCTGAGGAAATACCAGCTTTCCATTTTCACTGGCTGAGAGTTACCCTTATTTATTTTTGCTTGTTGAGAATATACATATGGTTAA
Goto: PGSC0003DMB000000454:235697


1300641 1300651 1300661 1300671 1300681 1300691 1300701 1300711
TCTGTAATTTTCATAAAACGCTTGGCGTGCTCCGTATATCATGCTTAACATCATGCTAAATCCATGATTCTGCAAATAGTGCTCTTG
Goto: PGSC0003DMB000000102:1300682

Alignment is done! Next, SNP calling!!



First... convert alignments (using SAMtools)

1. Convert SAM to BAM for sorting
   - `samtools view -S -b my.sam > my.bam`

2. Sort BAM for SNP calling
   - `samtools sort my.bam my-sorted`

Alignments are both:

- compressed for long term storage and
- sorted for variant discovery.

2) Call SNPs (using SAMtools)

1. Index the genome assembly (again!)
   - `samtools faidx my.fasta`

2. Run 'mpileup' to generate VCF format
   - `samtools mpileup -g -f my.fasta my-sorted-1.bam my-sorted-2.bam my-sorted-n.bam > my-raw.bcf`

NB: All we did so far (roughly) is to perform a format conversion from BAM to VCF!

2) Call SNPs (using bcftools)

3. Call SNPs...
   - `bcftools view -bvcg my-raw.bcf > my-var.bcf`

Again...

- `samtools mpileup`
  - Collects summary information in the input BAMs, computes the likelihood of data given each possible genotype and stores the likelihoods in the BCF format.

- `bcftools view`
  - Applies the prior and does the actual calling.

3) Filter SNPs

1. Filter SNPs
   - `bcftools view my.var.bcf |`
     `vcfutils.pl varFilter - > my.var-final.vcf`

Now...

Your turn!

Options for BWA

For details, see http://bio-bwa.sourceforge.net/bwa.shtml

Options for SAMtools

For details, see http://samtools.sourceforge.net/samtools.shtml

Options for vcfutils.pl varFilter

```
Usage:   vcfutils.pl varFilter [options] <in.vcf>

Options: -Q INT    minimum RMS mapping quality for SNPs [10]
         -d INT    minimum read depth [2]
         -D INT    maximum read depth [10000000]
         -a INT    minimum number of alternate bases [2]
         -w INT    SNP within INT bp around a gap to be filtered [3]
         -W INT    window size for filtering adjacent gaps [10]
         -1 FLOAT  min P-value for strand bias (given PV4) [0.0001]
         -2 FLOAT  min P-value for baseQ bias [1e-100]
         -3 FLOAT  min P-value for mapQ bias [0]
         -4 FLOAT  min P-value for end distance bias [0.0001]
         -e FLOAT  min P-value for HWE (plus F<0) [0.0001]
         -p        print filtered variants

Note: Some of the filters rely on annotations generated by SAMtools/BCFtools.
```

Glossary of file formats

Sequence data formats:

- FASTA:
  Simple format for DNA or peptide sequences.

- FASTQ:
  Stores sequences and sequence **quality** information together.

Alignment data formats

- SAM / BAM
  Sequence Alignment/Map

Variation data

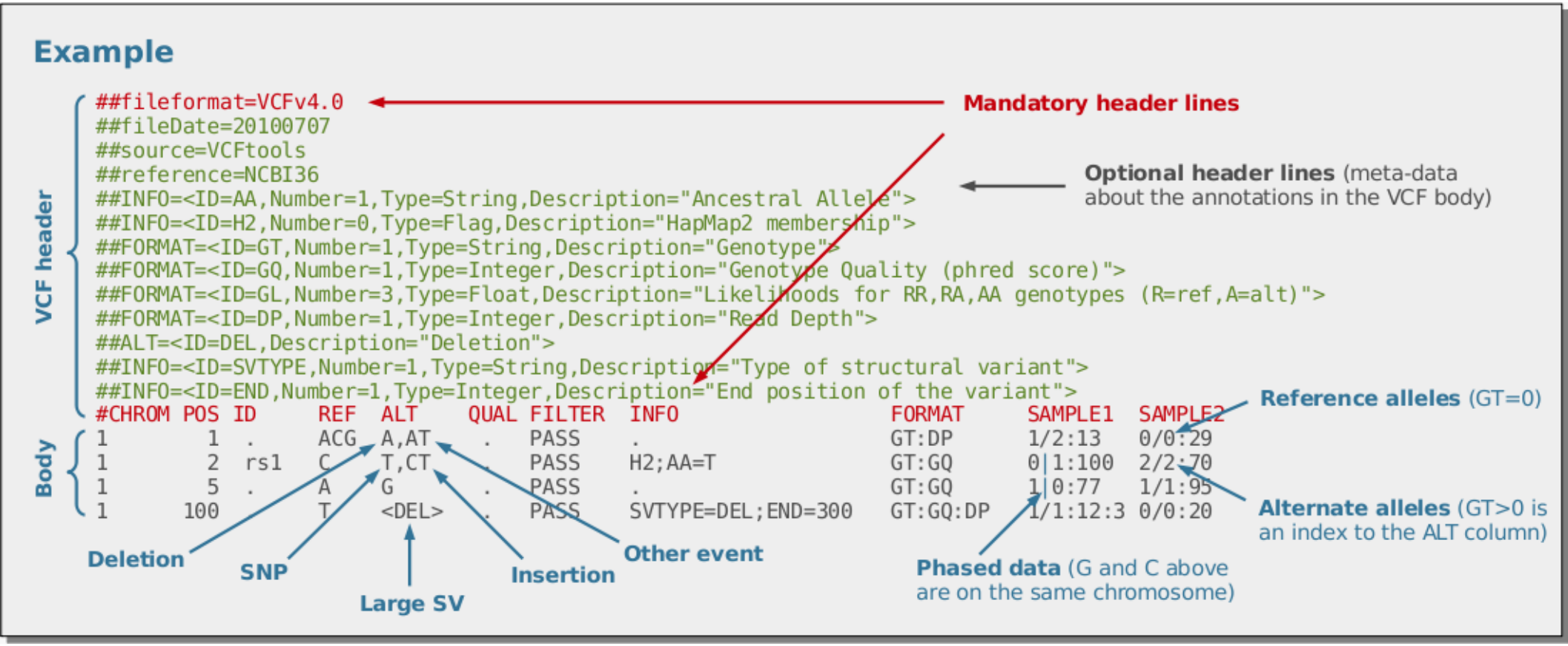
- VCF / BCF
  Variant Call Format

http://www.ebi.ac.uk/ena/about/read-file-formats

VCF format

- A standard format for sequence variation: SNPs, indels and structural variants.
- Compressed and indexed.
- Developed for the 1000 Genomes Project.
- VCFtools for VCF like SAMtools for SAM.
- Specification and tools available from http://vcftools.sourceforge.net

Example


VCF header
Body
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##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 (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A=alt)">
##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">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT SAMPLE1 SAMPLE2
1 1 . ACG A,AT . PASS . GT:DP 1/2:13 0/0:29
1 2 rs1 C T,CT . PASS H2;AA=T GT:GQ 0|1:100 2/2:70
1 5 . A G . PASS . GT:GQ 1|0:77 1/1:95
1 100 T <DEL> . PASS SVTYPE=DEL;END=300 GT:GQ:DP 1/1:12:3 0/0:20
Mandatory header lines
Optional header lines (meta-data about the annotations in the VCF body)
Reference alleles (GT=0)
Alternate alleles (GT>0 is an index to the ALT column)
Deletion
SNP
Large SV
Insertion
Other event
Phased data (G and C above are on the same chromosome)