Variant Calling Workflow

Answers to questions

1. ***What does "SO:coordinate" in the "@HD" tag on the first line of the bam file mean?***

SO stand for “sort order”

Coordinate means that the reads in the bam file are sorted in ascending order by sequence name (i.e. chromosome) and position.

2. ***What does "SN:2" and "LN:243199373" in the "@SQ tag mean?***

SN:2 means that sequence name is “2”. We have selected chromosome 2 as reference because the data is selected on chromosome 2.

LN:243199373 means that the length of the reference sequence is 243199373 bp. This is the length of chromosome 2.

3. ***What is encoded in the @RG tag?***

Information about read groups.

4. ***What is the leftmost mapping position of the first read in the bamfile?***

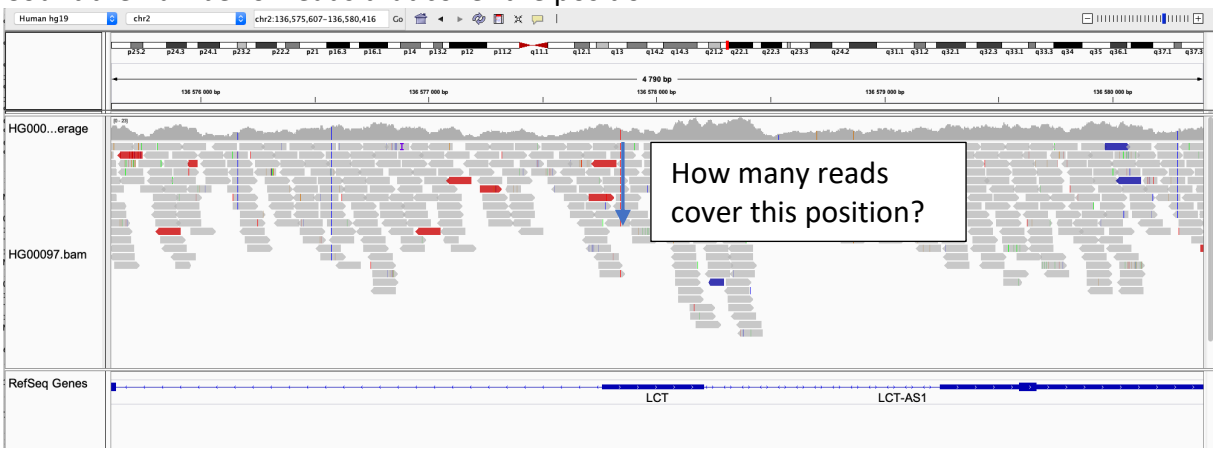
Chromosome 2, position 3843448

5. ***What is the read length?***

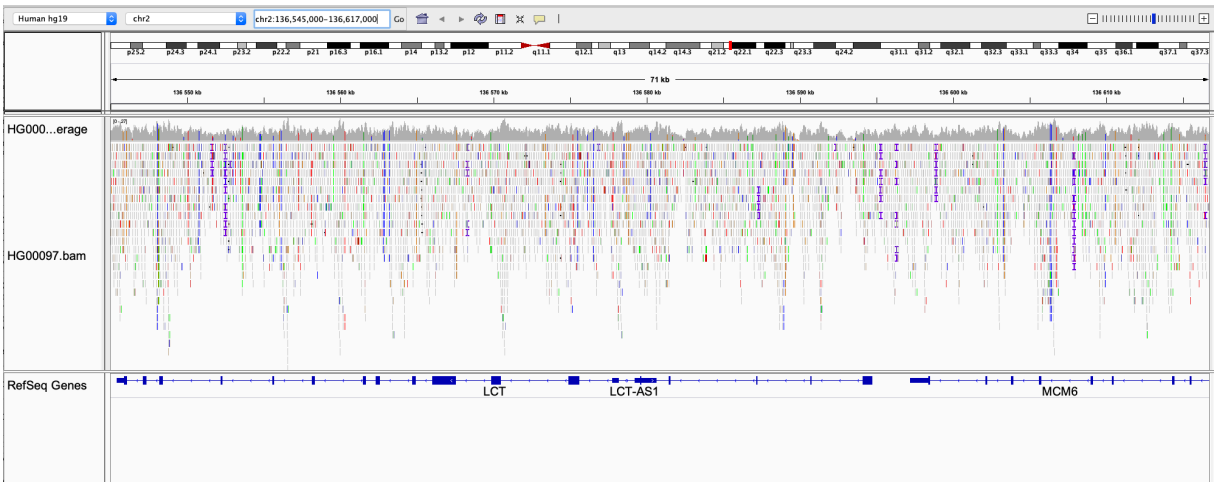
101 bp

6. ***How can you estimate the coverage in IGV?***

Count the number of reads that cover the position.



7. Which genes are located within the region chr2:136545000-136617000?



LCT, LCT-AS1 and MCM6

8. What column of the VCF file contains genotype information for the sample HG00097?

The 10th column with header "HG00097"

```
#CHROM    POS       ID        REF       ALT       QUAL      FILTER
          INFO      FORMAT    HG00097
```

9. What does GT in the FORMAT column of the data lines mean?

Genotype

```
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
```

10. What genotype does the sample HG00097 have at position 2: 136545844?

1/1
This individual has the alternative allele on both copies of chromosome 2.

11. What does AD in the FORMAT column of the data lines mean?

Number of reads that match the reference allele and the alternative alleles, respectively.

```
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic
depths for the ref and alt alleles in the order listed">
```

12. What is the allelic depths for the reference and alternative alleles in sample HG00097 at position 2: 136545844?

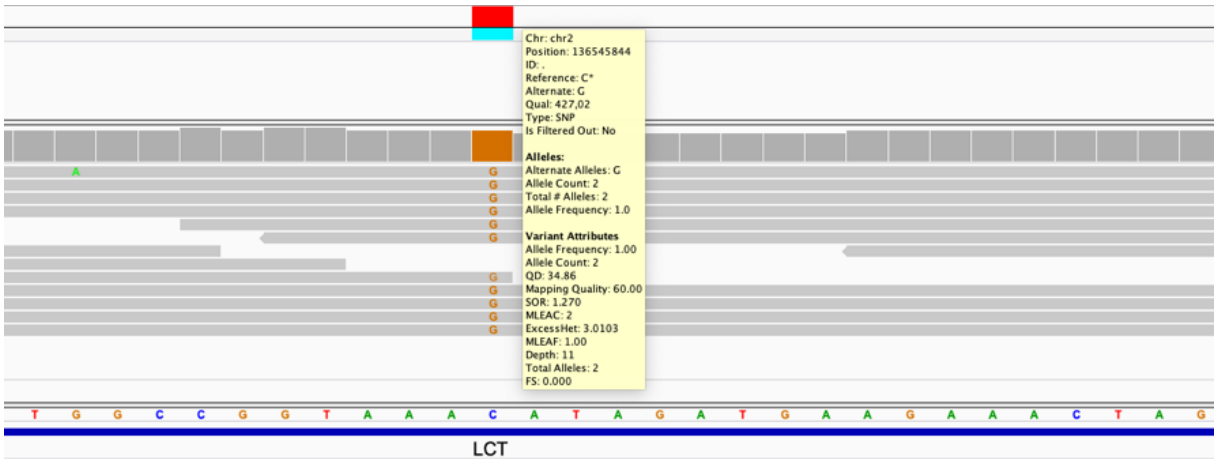
0 reads match the reference allele and 11 reads match the alternative allele.

```
2          136545844          .          C          G          427.02
           .
           AC=2;AF=1.00;AN=2;DP=11;ExcessHet=3.0103;FS=0.000;M
LEAC=2;MLEAF=1.00;MQ=60.00;QD=34.86;SOR=1.270
           GT:AD:DP:GQ:PL     1/1:0,11:11:33:441,33,0
```

13. How many genetic variants was detected in the sample?

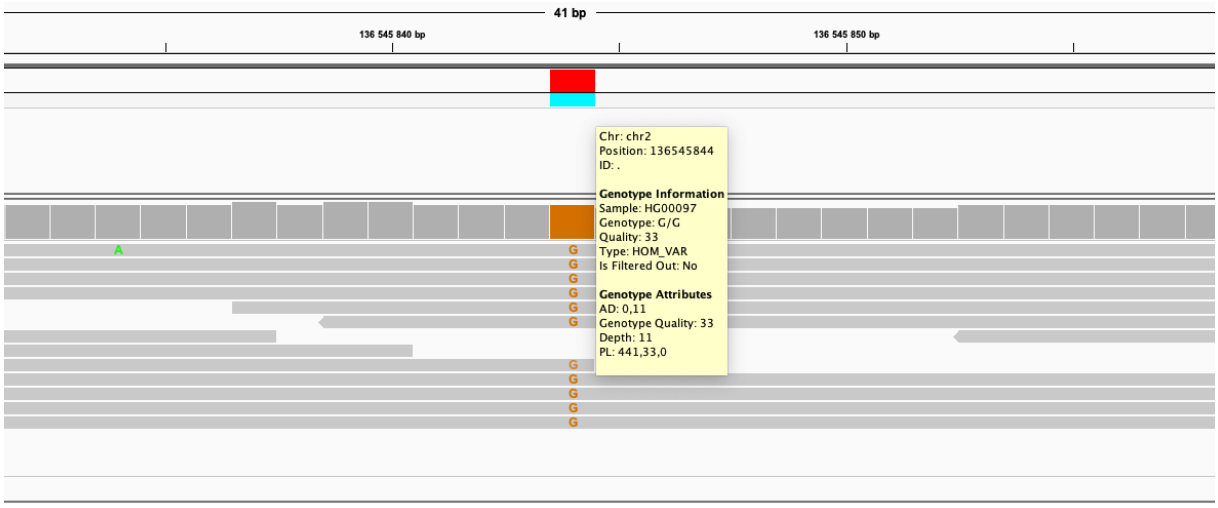
The linux command
grep -v "#" HG00097.vcf | wc -l
extracts all lines in that don't start with "#", and then counts these lines.
206 variants

14. Hoover the mouse over the upper row of the vcf track. What is the reference and alternative alleles of the variant at position 2:136545844?



Referenec allele = C
Alternative allele = G

15. Hoover the mouse over the lower row of the vcf track and look under "Genotype Information". What genotype does HG00097 have at position 2:136545844? Is this the same as you found by looking directly in the vcf file in question 10?



Genotype = G/G
Yes, this is the same genotype as can be seen directly in the vcf file, but in the vcf file it is encoded as 1/1 which means two copies of the alternative allele.

16. Look in the bam track and count the number of reads that have "G" and "C", respectively, at position 2:136545844. How is this information captured under "Genotype Attributes"? (Hoover the mouse over the lower row of the vcf track to find the "Genotype Attributes")

0 reads have "C" which is the reference allele, 11 reads have "G" which is the alternative allele for this variant. This information is captured as "AD=0,11" under Genotype Attributes.

17. How many data lines do the cohort.g.vcf file have? You can use the linux command `grep -v "#" cohort.g.vcf` to extract all lines in "cohort.g.vcf" that don't start with "#", then `|`, and then `wc -l` to count those lines.

```
grep –v "^#" cohort.g.vcf | wc –l
```

This returns 313376 lines

18. How many data lines do the cohort.vcf file have?

```
grep –v "^#" cohort.vcf | wc –l
```

This returns 718 lines

19. Explain the difference in number of data lines?
Cohort.g.vcf contains information about every position in the analyzed region (although some positions are merged into blocks), cohort.vcf contain information about sites where genetic variants was detected.

20. Look at the header line of the cohort.vcf file. What columns does it have?

```
grep "#CHROM" cohort.vcf
```

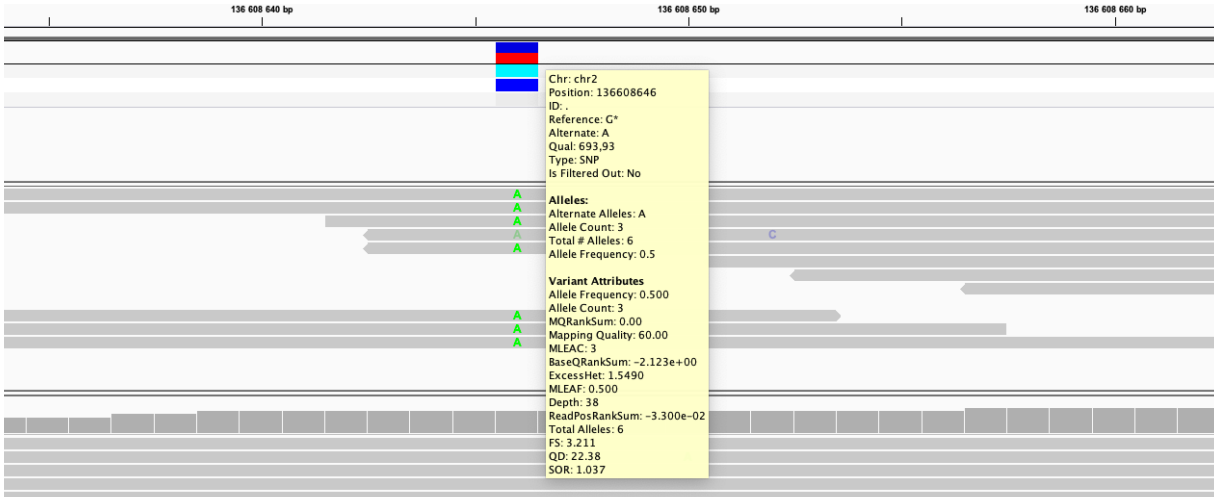
gives:

```
#CHROM  POS ID  REF ALT QUAL    FILTER  INFO    FORMAT
    HG00097 HG00100 HG00101
```

21. What is encoded in the last three columns of the data lines?

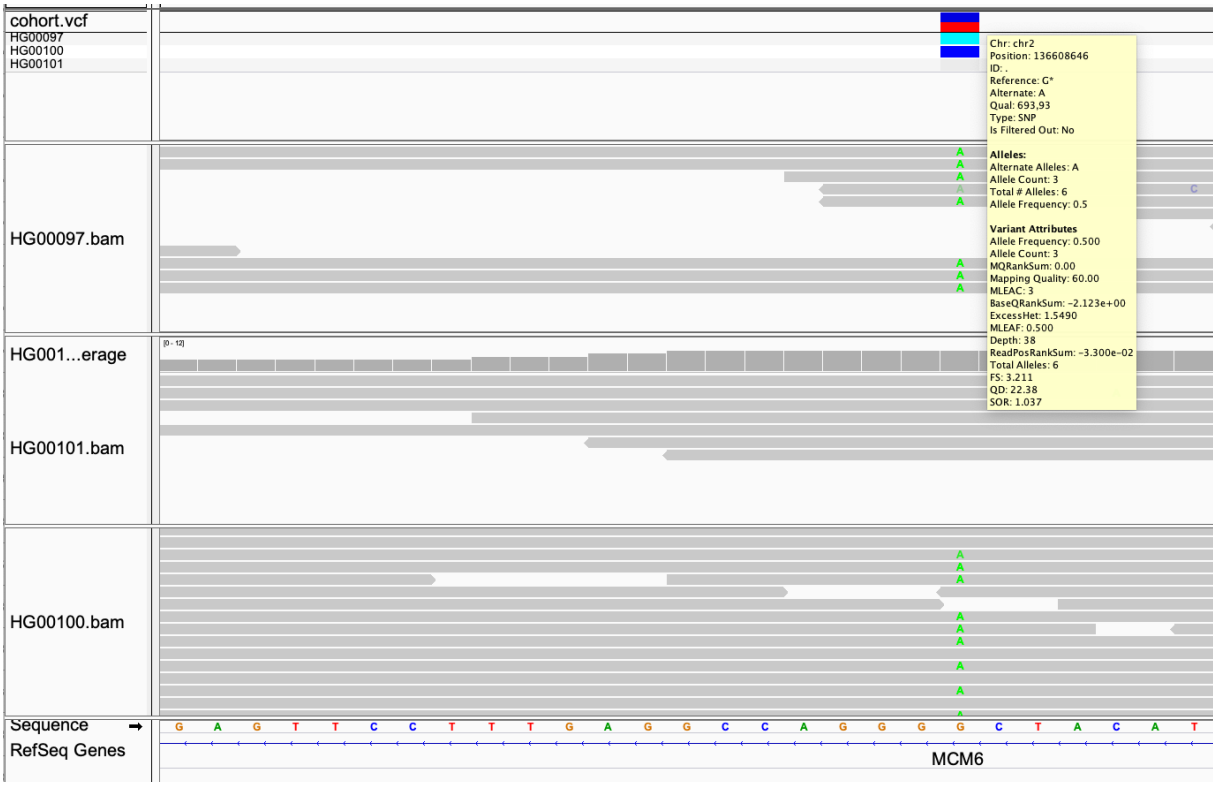
Genotypes and genotype attributes of the samples HG00097, HG00100 and HG00101

22. What is the reference and alternative alleles at chr2:136608646?



Reference allele = G
Alternative allele = A

23. What genotype do the three samples have at chr2:136608646? Note how genotypes are color coded in IGV (it is possible to modify the color coding but lest stick with the default settings in this lab).



HG00097 is A/A
HG00100 is A/G
HG00101 is G/G

Homozygote genotypes for the alternative allele are colored in light blue
Heterozygote genotypes are colored in dark blue
Homozygote genotypes for the reference allele are colored in light grey.

24. Should any of the individuals avoid drinking milk?

Yes, HG00101 is homozygote for the G/G allele, and therefore do not have new transcription factor binding site that functions as an enhancer that upregulates *LCT* in adulthood.

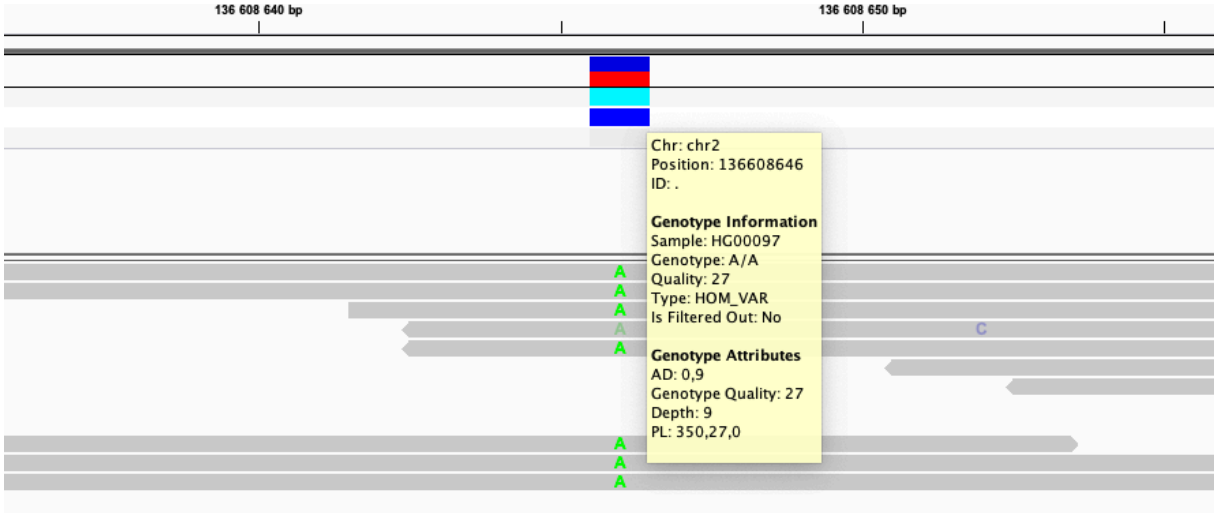
25. Now let's compare the data shown in IGV with the data in the VCF file. Extract the row for the chr2:136608646 variant in the cohort.vcf file, for example using `grep '136608646' cohort.vcf`. What columns of the vcf file contain the information shown in the upper part of the vcf track in IGV?

```
grep '136608646' cohort.vcf
```

results in

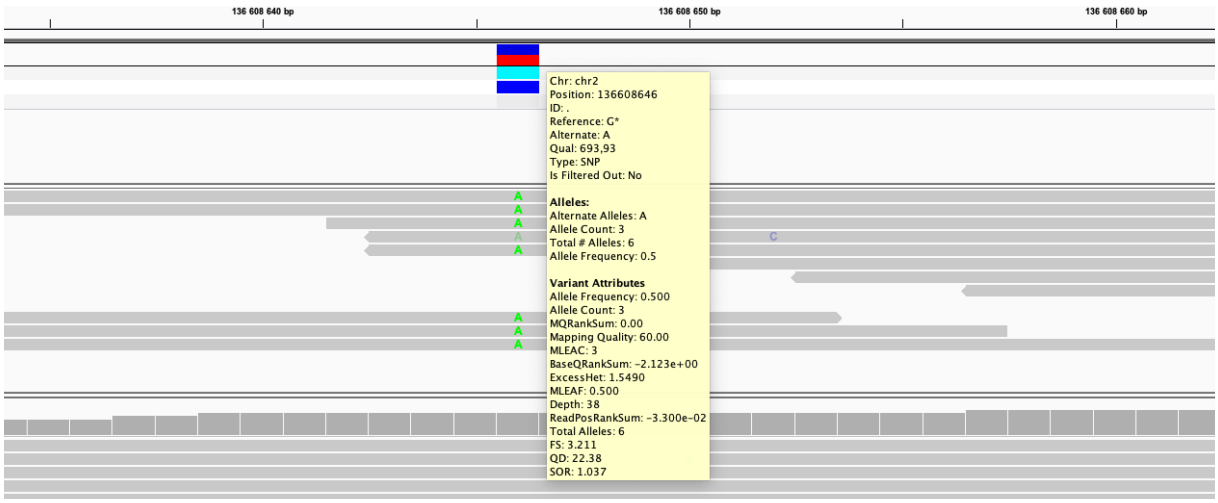
```
2136608646  .          G          A          693.93     .
 AC=3;AF=0.500;AN=6;BaseQRankSum=-
2.123e+00;DP=38;ExcessHet=1.5490;FS=3.211;MLEAC=3;MLEAF=0.500;MQ=60.00;MQ
RankSum=0.00;QD=22.38;ReadPosRankSum=-3.300e-02;SOR=1.037
 GT:AD:DP:GQ:PL        1/1:0,9:9:27:350,27,0 0/1:11,11:22:99:360,0,405
 0/0:7,0:7:21:0,21,239
```

Columns 1-8 of the VCF file is shown in the upper part of the vcf track in IGV.



26. What columns of the vcf file contain the information shown in the lower part of the vcf track?

The sample columns (column 10 and forward) are shown in the lower part of the vcf track, which are called genotype tracks. There is one genotype track for each sample column in the VCF file.



27. Zoom out so that you can see the MCM6 and LCT genes. Is the variant at chr2:136608646 locate within the LCT gene?

No, its located in an exon of MCM6 which acts as enhancer for LCT.

28. How many variants are present in the cohort.filtered.vcf file?

```
grep –v "^#" cohort.filtered.vcf | wc –l
```

716 variants

29. How many variants are present in the cohort.filtered.vcf file?

```
grep –v '^#' cohort.filtered.vcf | grep 'PASS' | wc –l
```

711 variants

30. at the variants that did not pass the filters using `grep -v 'PASS' cohort.filtered.vcf`. Try to understand why these variants didn't pass the filter.

```
grep –v 'PASS' cohort.filtered.vcf | grep 'PASS' | wc –l
```

```
#CHROM      POS   ID    REF   ALT   QUAL  FILTER      INFO  FORMAT           HG00097
HG00100     HG00101
2     136164548   .     C     G     129.26      MQfilter
      AC=1;AF=0.167;AN=6;BaseQRankSum=0.431;DP=32;ExcessHet=3.0103;FS=4.771;MLEAC=1;MLEA
F=0.167;MQ=34.64;MQRankSum=0.431;QD=21.54;ReadPosRankSum=-4.310e-01;SOR=3.258
      GT:AD:DP:GQ:PL    0/0:15,0:15:36:0,36,540 0/0:11,0:11:33:0,33,444
      0/1:2,4:6:51:138,0,51
2     136174478   .     G     A     120.26      MQfilter
      AC=1;AF=0.167;AN=6;BaseQRankSum=-
1.981e+00;DP=33;ExcessHet=3.0103;FS=2.430;MLEAC=1;MLEAF=0.167;MQ=38.80;MQRankSum=0.524;Q
D=12.03;ReadPosRankSum=-8.160e-01;SOR=1.60GT:AD:DP:GQ:PL  0/0:12,0:12:33:0,33,495
      0/0:11,0:11:33:0,33,420 0/1:6,4:10:99:129,0,205
2     136174552   .     C     T     42.41 MQfilter
      AC=1;AF=0.167;AN=6;BaseQRankSum=0.792;DP=20;ExcessHet=3.0103;FS=0.000;MLEAC=1;MLEA
F=0.167;MQ=36.93;MQRankSum=-7.920e-01;QD=6.06;ReadPosRankSum=-
1.068e+00;SOR=0.446GT:AD:DP:GQ:PL  0/0:5,0:5:9:0,9,135    0/0:8,0:8:24:0,24,316
      0/1:5,2:7:51:51,0,148
2     136269833   .     A     T     30.37 QDfilter    AC=1;AF=0.167;AN=6;BaseQRankSum=-
7.530e-
01;DP=25;ExcessHet=3.0103;FS=0.000;MLEAC=1;MLEAF=0.167;MQ=60.00;MQRankSum=0.00;QD=1.90;R
eadPosRankSum=1.19;SOR=1.445 GT:AD:DP:GQ:PGT:PID:PL:PS    0/0:5,0:5:15:.:.:0,15,187
      0|1:14,2:16:39:0|1:136269833_A_T:39,0,617:136269833 0/0:4,0:4:12:.:.:0,12,172
2       136269844     .           A           T           30.37        QDfilter
        AC=1;AF=0.167;AN=6;BaseQRankSum=-9.720e-
01;DP=26;ExcessHet=3.0103;FS=0.000;MLEAC=1;MLEAF=0.167;MQ=60.00;MQRankSum=0.00;QD=1.90;R
eadPosRankSum=-5.570e-01;SOR=1.445 GT:AD:DP:GQ:PGT:PID:PL:PS  0/0:5,0:5:12:.:.:0,12,180
        0|1:14,2:16:39:0|1:136269833_A_T:39,0,617:136269833   0/0:5,0:5:15:.:.:0,15,214
```

Reasons for not passing filter:

##FILTER=<ID=MQfilter,Description="MQ < 40.0">
##INFO=<ID=MQ,Number=1,Type=Float,Description="RMS Mapping Quality">

##FILTER=<ID=QDfilter,Description="QD < 2.0">
##INFO=<ID=QD,Number=1,Type=Float,Description="Variant Confidence/Quality by Depth">