Tutorial 4 – Samtools & Bcftools

02 OCTOBER 2020

MICB405

What is Samtools

"Swiss army knife" of Sam file manipulation

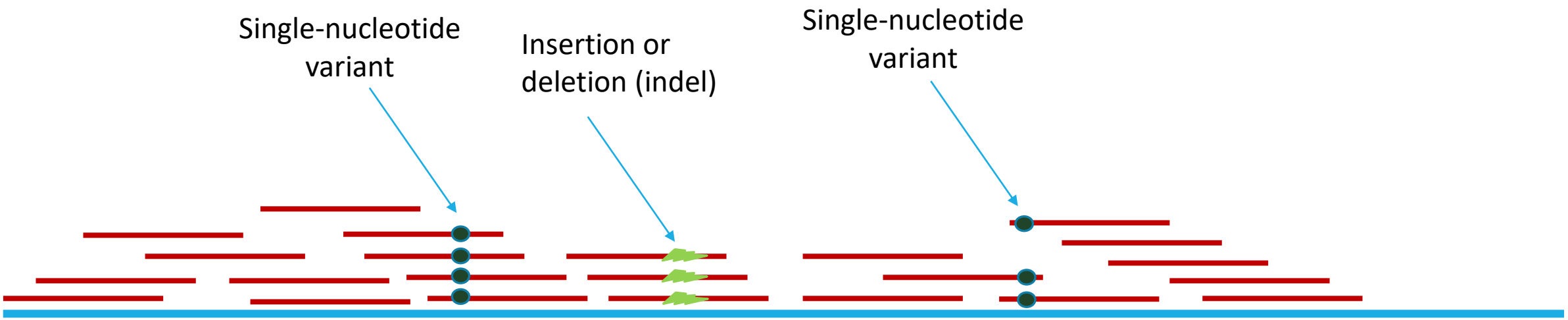
Can be used for various project-specific tasks such as filtering out unnecessary reads, and organizing reads

Here, we will be going over a Samtools & Bcftools (more later) workflow for calling variants

Calling Variants

Before genomic data can be analyzed, it must be organized in a way that compactly represents differences between it and its reference

SAM files show sequence assemblies, but do not explicitly describe variation between the sequence of DNA



Let’s find them!

```
ahauduc_mb20@orca01:~/alignments$ samtools view bhinzii.bam | head -n 2
M01783:4:000000000-A4CKG:1:1101:1673:14600      89      NZ_CP012076.1   366929  37      250M    =       366929  0       G
TGTCGAAAGTATGGCTGATGGCCCGGGCCAGGGGCGGACTGTCGATGACCAGCCCGAGCTCGGTGTTCAGGTGGGCCGAGCGCGGGTCGAAATTGAAGGAGCCCACGAACACGCGGTGGT
CGTCCACGGCGAAGGTCTTGGCATGCAGGCTGGAGCCCGAGCTGCCGAAGGGGCCCAGGCCGCGGTGGCGCTGGACCTCGTCGCCGGCCCGGCGCATCTCGAATAGCTGCACGCCGCTGG
CCAGCAAGG       ?BFEFFFFFFBFAB:/FFFB@@@@-EAFFFF@@F@BFFB-B@FFFFFFFB?@@;@BFB?;<?FFFFFFFFFFF@FA-=B@@@@;@@EEFFFFBFFFFFFFFA;?
EFFFFF?@@=-EE?@@@AFB?@A-GBEFGGGGGGHFGFBHGHGHGHGFEC-C??CGCC@CC?EFHHCGGF/AEGCBCCCAGC/EE/E1FGFE//C@E@/>/?>A///EA/A1/C020GFG
DCF1000A0A1AF1CB>11D@1A?AA      XT:A:U  NM:i:0  SM:i:37 AM:i:0  X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:250
M01783:4:000000000-A4CKG:1:1101:1673:14600      181     NZ_CP012076.1   366929  0       *       =       366929  0       G
TGTCGAAAGTATGGCTGATGGCCCGGGCCAGGGGCGGACTGTCGATGACCAGCCCGAGCTCGGTGTTCAGGTGGGCCGAGCGCGGGTCGAAATTGAAGGAGCCCACGAACACGCGGTGGT
CGTCCACGGCGAAGGTCTTGGCATGCAGGCTGGAGCCCGAGCTGCCGAAGGGGCCCAGGCCGCGGTGGCGCTGGACCTCGTCGCCGGCCCGGCGCATCTCGAATAGCTGCACGCCGCTGG
CCAGCAAGG       ?BFEFFFFFFBFAB:/FFFB@@@@-EAFFFF@@F@BFFB-B@FFFFFFFB?@@;@BFB?;<?FFFFFFFFFFF@FA-=B@@@@;@@EEFFFFBFFFFFFFFA;?
EFFFFF?@@=-EE?@@@AFB?@A-GBEFGGGGGGHFGFBHGHGHGHGFEC-C??CGCC@CC?EFHHCGGF/AEGCBCCCAGC/EE/E1FGFE//C@E@/>/?>A///EA/A1/C020GFG
DCF1000A0A1AF1CB>11D@1A?AA
```

Review of **SAM**

Review of alignment with **BWA**

```
#!/bin/bash

bwa aln -t 8 bhinzii.fa \
/projects/micb405/data/bordetella/F01_R1.fastq >
bhinzii.r1.sai

bwa aln -t 8 bhinzii.fa \
/projects/micb405/data/bordetella/F01_R2.fastq >
bhinzii.r2.sai

bwa sampe bhinzii.fa \
          bhinzii.r1.sai \
          bhinzii.r2.sai \
          /projects/micb405/data/bordetella/F01_R1.fastq \
          /projects/micb405/data/bordetella/F01_R1.fastq \
          > bhinzii.sam
```

Converting a **SAM** file to a **BAM** file

```
samtools view –b -h
~/alignments/bhinzii.sam \
> ~/alignments/bhinzii.bam
```

The possibilities are endless…

```
samtools view -b -h -F 4
~/alignments/B_hinzii.F01.bam \
> ~/alignments/B_hinzii.F01.no.unaligned.bam
```

The possibilities are endless…

```
samtools view -b -h -f 4
~/alignments/B_hinzii.F01.bam \
> ~/alignments/B_hinzii.F01.no.unaligned.bam
```

Let’s take a look at the help page

Sort by *alignment* position

```
samtools sort
~/alignments/B_hinzii.F01.bam \
-o ~/alignments/B_hinzii.F01.sorted.bam
```

(You need to specify output with –o since sort cannot keep all temporary files in memory using standard output)

Remove (PCR) duplicates

```
samtools rmdup \
~/alignments/B_hinzii.F01.sorted.bam \
~/alignments/B_hinzii.F01.sorted.rmdup.bam
```

(Here you don’t specify -o and you just separate the name of the output file with a space)

Indexing…

```
samtools index \
~/alignments/B_hinzii.F01.sorted.rmdup.bam
```

… and calling (within directory)

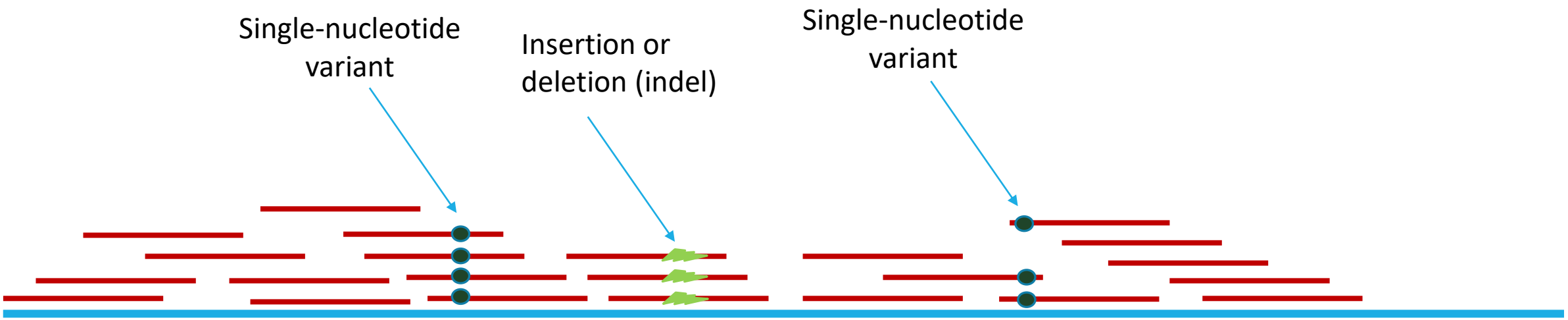
```
bcftools mpileup --fasta-ref bhinzii.fa \
bhinzii.sorted.rmdup.bam \
| bcftools call -mv - > bhinzii.raw.vcf
```

```
ahauduc_mb20@orca01:~/alignments$ samtools view bhinzii.bam | head -n 2
M01783:4:000000000-A4CKG:1:1101:1673:14600      89      NZ_CP012076.1   366929  37      250M    =       366929  0       G
TGTCGAAAGTATGGCTGATGGCCCGGGCCAGGGGCGGACTGTCGATGACCAGCCCGAGCTCGGTGTTCAGGTGGGCCGAGCGCGGGTCGAAATTGAAGGAGCCCACGAACACGCGGTGGT
CGTCCACGGCGAAGGTCTTGGCATGCAGGCTGGAGCCCGAGCTGCCGAAGGGGCCCAGGCCGCGGTGGCGCTGGACCTCGTCGCCGGCCCGGCGCATCTCGAATAGCTGCACGCCGCTGG
CCAGCAAGG       ?BFEFFFFFFBFAB:/FFFB@@@@-EAFFFF@@F@BFFB-B@FFFFFFFB?@@;@BFB?;<?FFFFFFFFFFF@FA-=B@@@@;@@EEFFFFBFFFFFFFFA;?
EFFFFF?@@=-EE?@@@AFB?@A-GBEFGGGGGGHFGFBHGHGHGHGFEC-C??CGCC@CC?EFHHCGGF/AEGCBCCCAGC/EE/E1FGFE//C@E@/>/?>A///EA/A1/C020GFG
DCF1000A0A1AF1CB>11D@1A?AA      XT:A:U  NM:i:0  SM:i:37 AM:i:0  X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:250
M01783:4:000000000-A4CKG:1:1101:1673:14600      181     NZ_CP012076.1   366929  0       *       =       366929  0       G
TGTCGAAAGTATGGCTGATGGCCCGGGCCAGGGGCGGACTGTCGATGACCAGCCCGAGCTCGGTGTTCAGGTGGGCCGAGCGCGGGTCGAAATTGAAGGAGCCCACGAACACGCGGTGGT
CGTCCACGGCGAAGGTCTTGGCATGCAGGCTGGAGCCCGAGCTGCCGAAGGGGCCCAGGCCGCGGTGGCGCTGGACCTCGTCGCCGGCCCGGCGCATCTCGAATAGCTGCACGCCGCTGG
CCAGCAAGG       ?BFEFFFFFFBFAB:/FFFB@@@@-EAFFFF@@F@BFFB-B@FFFFFFFB?@@;@BFB?;<?FFFFFFFFFFF@FA-=B@@@@;@@EEFFFFBFFFFFFFFA;?
EFFFFF?@@=-EE?@@@AFB?@A-GBEFGGGGGGHFGFBHGHGHGHGFEC-C??CGCC@CC?EFHHCGGF/AEGCBCCCAGC/EE/E1FGFE//C@E@/>/?>A///EA/A1/C020GFG
DCF1000A0A1AF1CB>11D@1A?AA
```

SAM

```
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  bhinzii.sorted.rmdup.bam
NZ_CP012076.1   62084   .       T       C       15.8048 .       DP=252;VDB=0.0256773;SGB=-0.693139;RPB=0.298514;MQB=0.0143494;MQSB=5.37752e-08;BQB=2.10992e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=72,14,21,15;MQ=54     GT:PL   0/1:51,0,255
NZ_CP012076.1   72730   .       A       C       18.7483 .       DP=248;VDB=0.0862903;SGB=-0.693136;RPB=0.712867;MQB=0.000563025;MQSB=0.66238;BQB=5.39485e-11;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=55,30,16,19;MQ=54       GT:PL   0/1:54,0,255
NZ_CP012076.1   72884   .       A       C       6.97062 .       DP=249;VDB=0.00330069;SGB=-0.693146;RPB=0.000306521;MQB=6.14155e-05;MQSB=1.35848e-15;BQB=1.55976e-12;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=75,20,13,30;MQ=49       GT:PL   0/1:41,0,255
NZ_CP012076.1   180497  .       T       G       77      .       DP=156;VDB=0.961403;SGB=-0.692067;RPB=0.0987007;MQB=0.167746;MQSB=0.12665;BQB=0.197143;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=11,3,17,3;MQ=46       GT:PL   0/1:110,0,156
NZ_CP012076.1   180632  .       A       C       18.6198 .       DP=262;VDB=0.0277801;SGB=-0.693097;RPB=0.245373;MQB=0.771275;MQSB=0.585838;BQB=5.66673e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=77,7,20,10;MQ=54  GT:PL   0/1:54,0,255
NZ_CP012076.1   208525  .       G       C       4.91571 .       DP=250;VDB=0.0177041;SGB=-0.693054;RPB=0.883359;MQB=0.953977;MQSB=0.999366;BQB=2.8855e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=63,28,11,17;MQ=58  GT:PL   0/1:39,0,255
NZ_CP012076.1   208530  .       G       C       29.7766 .       DP=243;VDB=0.121142;SGB=-0.693079;RPB=0.708874;MQB=0.931274;MQSB=0.977573;BQB=6.36885e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=56,29,11,18;MQ=58  GT:PL   0/1:65,0,255
NZ_CP012076.1   208536  .       T       C       7.25462 .       DP=245;VDB=0.0230065;SGB=-0.693127;RPB=0.650458;MQB=0.980013;MQSB=0.99841;BQB=3.21071e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=53,29,12,21;MQ=58  GT:PL   0/1:42,0,255
NZ_CP012076.1   208539  .       G       C       18.6457 .       DP=246;VDB=0.134747;SGB=-0.693021;RPB=0.566774;MQB=0.999733;MQSB=0.969107;BQB=5.74972e-06;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=55,33,9,18;MQ=58   GT:PL   0/1:54,0,255
NZ_CP012076.1   213780  .       C       T       35.7076 .       DP=161;VDB=0.0105084;SGB=-0.693147;RPB=0.813717;MQB=0.00148234;MQSB=0.0616543;BQB=1.28835e-06;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=52,15,2,51;MQ=38       GT:PL   0/1:69,0,255
NZ_CP012076.1   227404  .       G       C       14.722  .       DP=248;VDB=0.159881;SGB=-0.693079;RPB=0.999774;MQB=1;MQSB=1;BQB=5.36873e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=54,38,6,23;MQ=60 GT:PL   0/1:50,0,255
NZ_CP012076.1   246361  .       A       C       3.68653 .       DP=249;VDB=0.0422406;SGB=-0.693143;RPB=0.931854;MQB=0.428684;MQSB=0.953899;BQB=1.49487e-11;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=39,48,14,24;MQ=58 GT:PL   0/1:37,0,255
NZ_CP012076.1   280905  .       A       C       45.5341 .       DP=203;VDB=0.147976;SGB=-0.693141;RPB=0.65553;MQB=0.0243711;MQSB=0.00300644;BQB=1.15583e-08;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=6,41,14,23;MQ=49 GT:PL   0/1:80,0,255
NZ_CP012076.1   280922  .       C       G       14.8967 .       DP=204;VDB=0.0419789;SGB=-0.692831;RPB=0.999864;MQB=0.0787541;MQSB=0.867229;BQB=1.08842e-06;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=12,41,3,21;MQ=53 GT:PL   0/1:50,0,255
NZ_CP012076.1   280941  .       T       C       10.9475 .       DP=224;VDB=0.189371;SGB=-0.693054;RPB=0.443031;MQB=0.368929;MQSB=0.626774;BQB=6.79775e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=21,31,6,22;MQ=56   GT:PL   0/1:46,0,255
NZ_CP012076.1   281024  .       G       C       15.1545 .       DP=250;VDB=0.000501562;SGB=-0.693097;RPB=0.0797368;MQB=0.875695;MQSB=0.463424;BQB=8.48096e-06;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=47,23,14,16;MQ=54      GT:PL   0/1:50,0,255
NZ_CP012076.1   281043  .       A       G       4.3431  .       DP=250;VDB=1.62214e-05;SGB=-0.693132;RPB=0.0170843;MQB=0.493851;MQSB=0.479649;BQB=1.6574e-11;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=52,20,16,18;MQ=54       GT:PL   0/1:38,0,255
NZ_CP012076.1   285334  .       A       C       6.72407 .       DP=254;VDB=0.00854758;SGB=-0.693132;RPB=0.894356;MQB=0.805458;MQSB=0.993685;BQB=5.55742e-08;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=30,33,13,21;MQ=56       GT:PL   0/1:41,0,255
NZ_CP012076.1   295704  .       G       C       29.3095 .       DP=216;VDB=0.001811;SGB=-0.693147;RPB=0.311041;MQB=0.000220568;MQSB=2.21587e-05;BQB=8.45161e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=29,46,44,3;MQ=41      GT:PL   0/1:63,0,255
NZ_CP012076.1   346097  .       A       C       39.9683 .       DP=246;VDB=0.00164725;SGB=-0.693147;RPB=0.892335;MQB=0.000430366;MQSB=9.91059e-09;BQB=1.82972e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=38,45,32,15;MQ=44  GT:PL   0/1:74,0,255
NZ_CP012076.1   353202  .       G       C       12.8597 .       DP=249;VDB=0.0634319;SGB=-0.693139;RPB=0.257128;MQB=0.0854744;MQSB=0.204499;BQB=1.19043e-11;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=21,66,17,19;MQ=56       GT:PL   0/1:48,0,255
NZ_CP012076.1   367189  .       T       C       4.80995 .       DP=250;VDB=2.8507e-05;SGB=-0.69311;RPB=0.586195;MQB=0.953372;MQSB=0.699266;BQB=7.10746e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=30,46,11,20;MQ=58 GT:PL   0/1:39,0,255
NZ_CP012076.1   444652  .       GTTTTTTTTTGCC   GTTTTTTTTGCC    25.4901 .       INDEL;IDV=142;IMF=0.572581;DP=248;VDB=0.665273;SGB=-0.693147;MQSB=0.960082;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=40,88,29,91;MQ=59 GT:PL   0/1:55,0,4
NZ_CP012076.1   553283  .       G       A       29.7041 .       DP=249;VDB=2.40206e-05;SGB=-0.693147;RPB=0.0834371;MQB=2.685e-07;MQSB=5.35822e-11;BQB=1.32048e-13;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=68,25,16,43;MQ=50  GT:PL   0/1:64,0,255
NZ_CP012076.1   553314  .       T       C       24.9486 .       DP=250;VDB=0.0341296;SGB=-0.693143;RPB=0.575889;MQB=0.0147348;MQSB=7.42463e-08;BQB=1.25666e-09;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=64,15,19,19;MQ=53      GT:PL   0/1:60,0,255
NZ_CP012076.1   665128  .       C       G       19.7163 .       DP=252;VDB=0.0179823;SGB=-0.693136;RPB=0.00519041;MQB=5.29875e-05;MQSB=0.000261027;BQB=3.06327e-11;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=33,30,7,28;MQ=49  GT:PL   0/1:54,0,255
NZ_CP012076.1   666034  .       T       G       25.7317 .       DP=248;VDB=0.00661159;SGB=-0.693054;RPB=0.0894573;MQB=0.18299;MQSB=0.274747;BQB=2.51206e-07;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=22,47,10,18;MQ=56       GT:PL   0/1:61,0,255
NZ_CP012076.1   717216  .       A       C       8.05014 .       DP=252;VDB=0.12407;SGB=-0.69312;RPB=0.539357;MQB=0.803192;MQSB=0.765549;BQB=1.73233e-08;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=44,33,16,16;MQ=58     GT:PL   0/1:43,0,255
NZ_CP012076.1   762441  .       G       A       45.7888 .       DP=193;VDB=0.662894;SGB=-0.693145;RPB=0.485692;MQB=5.09488e-05;MQSB=3.61198e-07;BQB=2.11327e-05;MQ0F=0;ICB=1;HOB=0.5;AC=1;AN=2;DP4=31,14,6,34;MQ=42     GT:PL   0/1:79,0,255
```

VCF



Now we can easily view these!