H3ABioNet

Pan African Bioinformatics Network for H3Africa

WELLCOME GENOME CAMPUS

CONNECTING SCIENCE

ADVANCED COURSES+ SCIENTIFIC CONFERENCES

Next Generation Sequencing Bioinformatics Course 2021

Pathogen Variant Calling

Variant calling

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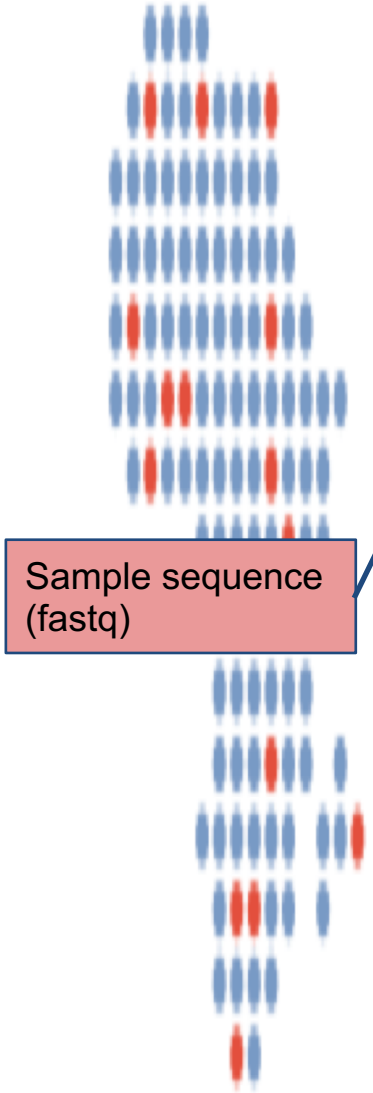
Overview



Overview

- Question: Investigating resistance in *M. tuberculosis*
- Next steps:
  - Where do errors come from?
  - How are variants called
  - Variant filtering
  - Investigate VCF files

Overview

What does variant calling do?

- Variant calling identifies small differences (variants) between a sample and a reference
- Resolution dependent on the read length & algorithm
- Most tools are limited to detecting events shorter than the length of a short-read sequencing alignment.
- SNPs (single-nucleotide polymorphisms), Indels (insertions and deletions), MNPs (multi-nucleotide polymorphisms)

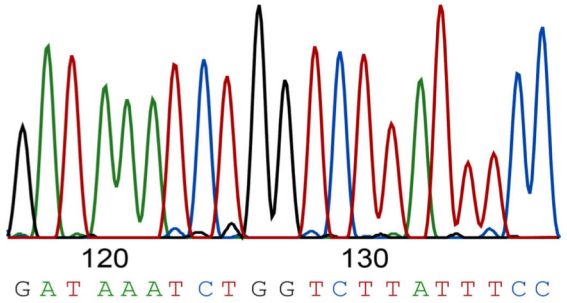
What does it not do?

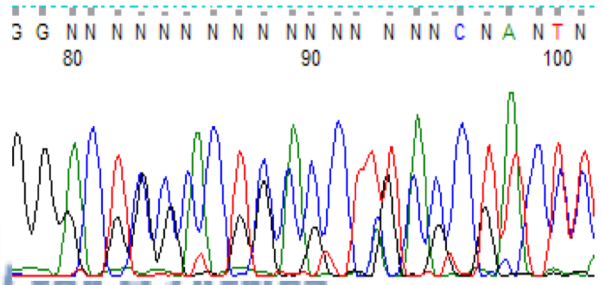
- Identification of large structural events
  - Chromosomal rearrangements, large deletions, gene duplications
- Often variants are incorrectly called at structural breakpoints

QC - Base calling errors

Where do errors come from?

- Uncertainty from the base call
  - Normally quality falls off towards the end of the read
  - Low quality, wrong base
- PCR errors
  - Errors early in the PCR reaction
  - Bridge PCR (Illumina / Solexa solid phase amplification) or emulsion PCR
  - High quality, wrong base
- PCR vs PCR free sequencing
  - Nanopore sequencing = no PCR
- Platform specific error profiles





Variant calling

Goal:

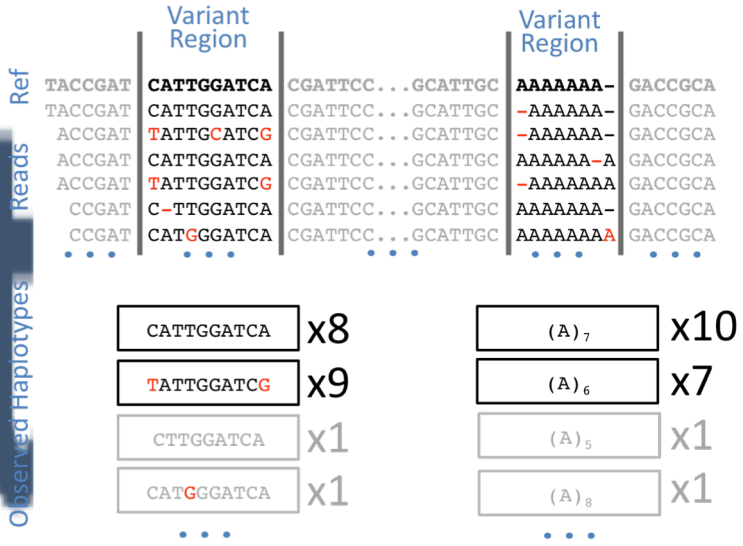
Given an alignment (bam), identify positions where reads support an alternate allele at a position compared to the reference sequence.

Tools:

- Freebayes
- SAMtools mpileup / bcftools
- GATK

Variant calling

- Freebayes
  - Uses a Bayesian approach to find variants
- GATK
  - Built for human genomes
  - Widely used, constantly improved
- SAMtools mpileup
  - bcftools mpileup generates genotype likelihoods
  - bcftools call makes the calls
- How to choose
  - Look at what other researchers are using for the organism
  - Use what you have support for / your team has experience with (but stay up to date)
  - Read papers to stay up to date



Source: https://github.com/freebayes/freebayes

Term definition: A haplotype is a group of genes within an organism that was inherited together from a single parent.

QC - Variant filtering

- What can you filter on?
  - Depth, ploidy, quality
- Depth
  - Why can high coverage be bad?
  - Could indicate a region with a lot of multi-mapping reads from a gene duplication
- Ploidy
  - In haploid organisms, things are simpler
  - Many tools built for diploid, still output genotype information (A/A or A/C or C/C)
- Quality
  - QUAL column: phred-scaled probability the ALT is wrong
  - GQ (Genotype quality) in the format column. Defined as -10log_10p(genotype call is wrong)
  - Examples:
    - 1 / 1 000 chance the genotype call is wrong
      - -10log_10(0.001) = 30
    - GQ = 12
      - 10 ^ (-12 / 10) = Probability of 0.063095 that the call is incorrect

QC - Variant filtering

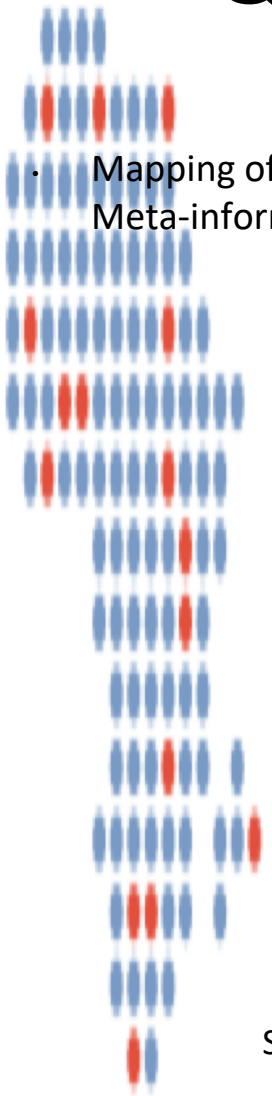
- Challenges
  - Duplicate genes
    - Sequenced genome had two copies of a region
    - Region appears only once in the reference genome
  - Missing genes
    - If a gene is missing in a sample but present in the reference, no variants will be called.
  - Closely related reference genome NB

QC - Variant filtering

- Tools
  - gatk VariantFiltration
  - vcftools
  - bcftools filter / view
  - bcftools query
    - Useful to test filtering and report general quality
  - A useful cheat sheet for BCF:
    - https://gist.github.com/elowy01/93922762e131d7abd3c7e8e166a74a0b
    - By: Ernesto Lowy

QC - Anatomy of a VCF file

- Mapping of info in the Meta-information lines



```
##fileformat=VCFv4.0
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=1000GenomesPilot-NCBI36
##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=.,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      N
A00001        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 GTCT    G,GTACT 50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP
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Source: https://www.internationalgenome.org/wiki/Analysis/vcf4.0/

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##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">
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0/1:35:4       0/2:17:2       1/1:40:3
```

Source: https://www.internationalgenome.org/wiki/Analysis/vcf4.0/

QC - Anatomy of a VCF file

- Multiple samples in the same VCF

```
##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
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```

Source: https://www.internationalgenome.org/wiki/Analysis/vcf4.0/

QC - Variant filtering

- bcftools filter -i 'type="snp" && QUAL>=50 && FORMAT/DP>5 && MQ>=30' -g10 -G10 MD001_variants.vcf -o MD001_SNPs_filtered_try1.vcf
- QUAL = Base quality score column
- DP = Read depth
- MQ = Mapping quality