Variant calling using GATK

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June 28, 2023

https://www.melbournebioinformatics.org.au/tutorials/tutorials/variant_calling_gatk1/variant_calling_gatk1/

Workshop overview

1. Objectives

2. Introduction to genetic variants
   - Why we study variants?
   - Types of genetic variants

3. GATK
   - Overview
   - Best practices pipeline for variant calling

4. Resources and tools
   - Resources
   - Databases
   - Tools
   - Help

5. Workshop

1. Objectives

- We aim to cover:
  - Perform QC of sequencing data
  - Align raw reads to reference sequences
  - Perform alignment metric and generating a QC report
  - Prepare alignment data for variant calling
  - Identify simple variants using GATK HaplotypeCaller
  - Visualise simple variant data (VCF files)
  - Perform basic variant filtering

2. Introduction to genetic variants

- There are approximately 3 billion base pairs in the human genome.
- Humans share 99.5% of DNA with other humans.
- A **variant** is a difference between similar genomes.
- In most cases – this means a difference between DNA sequences compared to a **reference genome**.
- In this context - a variant is described by its location (genomic coordinates) and genetic change.

e.g. chr2 9834 A→G

2. Introduction to genetic variants

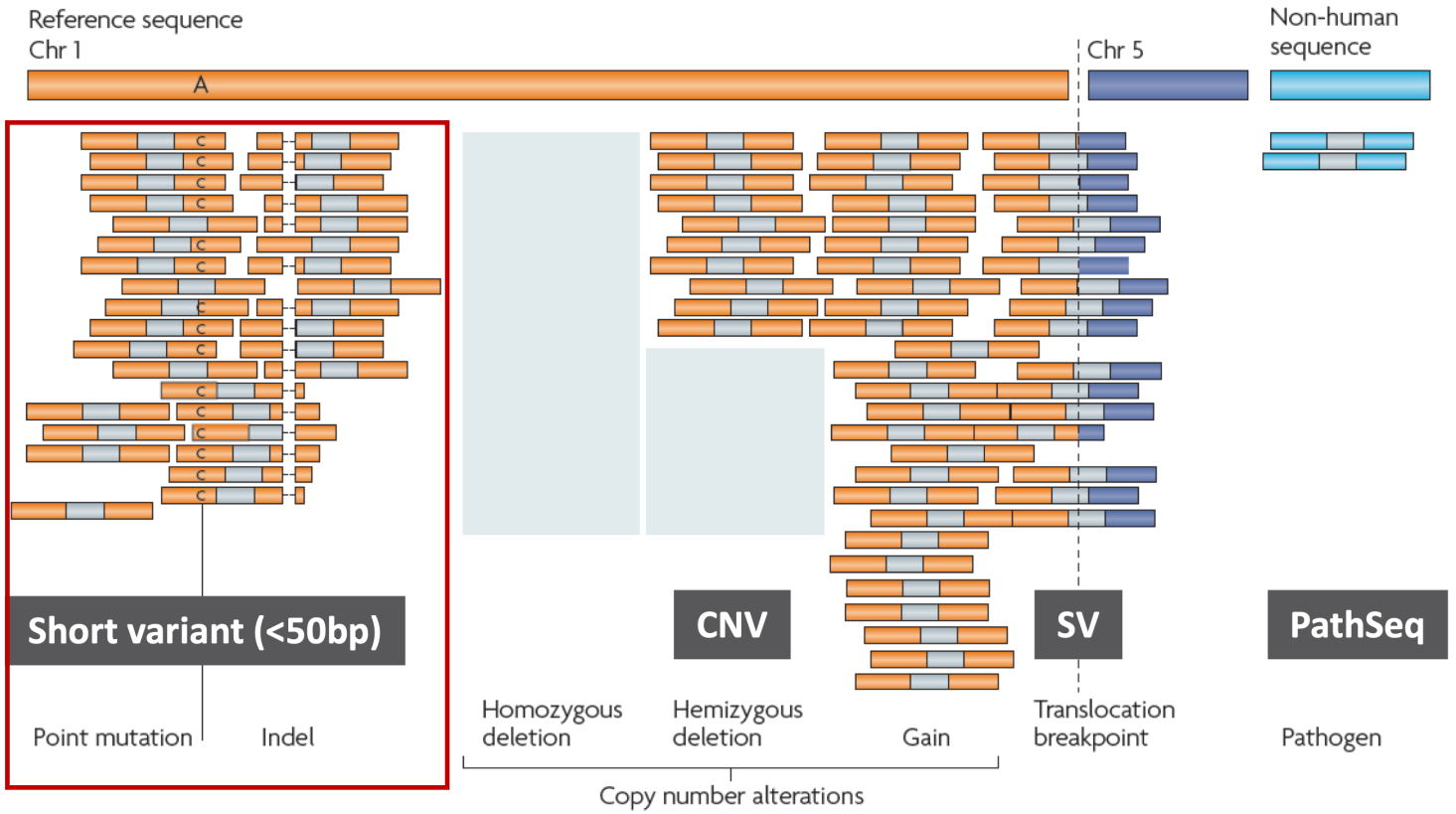
There is high degree of similarity but the human genome is large ~3 billion nucleotides.

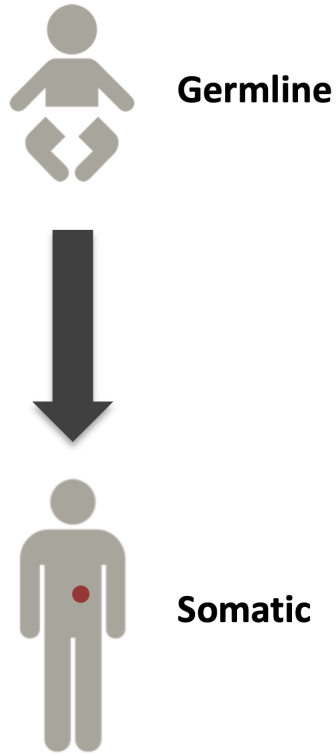
This results in approximately 4-5 million variants between any individual and the reference genome.

These, seemingly small number of variations likely explains a significant proportion of phenotypic diversity among humans.

2. Types of genetic variants

Types of genetic variants:





Focus of this workshop:
Calling short germline variants

3. GATK overview

Pre-processing	Variant discovery	Refinement
• Prepare analysis ready bam files	• Variant discovery	• Use metadata from variant calling to improve genotype accuracy

- Genome Analysis Toolkit (GATK): software package to analyze high-throughput sequencing data

3. GATK overview

- Download available from

- https://github.com/broadinstitute/gatk/releases
- Tutorial version: GATK 4.2.0.0
- Current version: GATK 4.4.0.0

- Explore GATK website - gatk.broadinstitute.org
  - Tool index – provides tools usage instructions
  - Technical documentation – provides details on for example Algorithms
  - Forum – provides access to Q&As and community discussions

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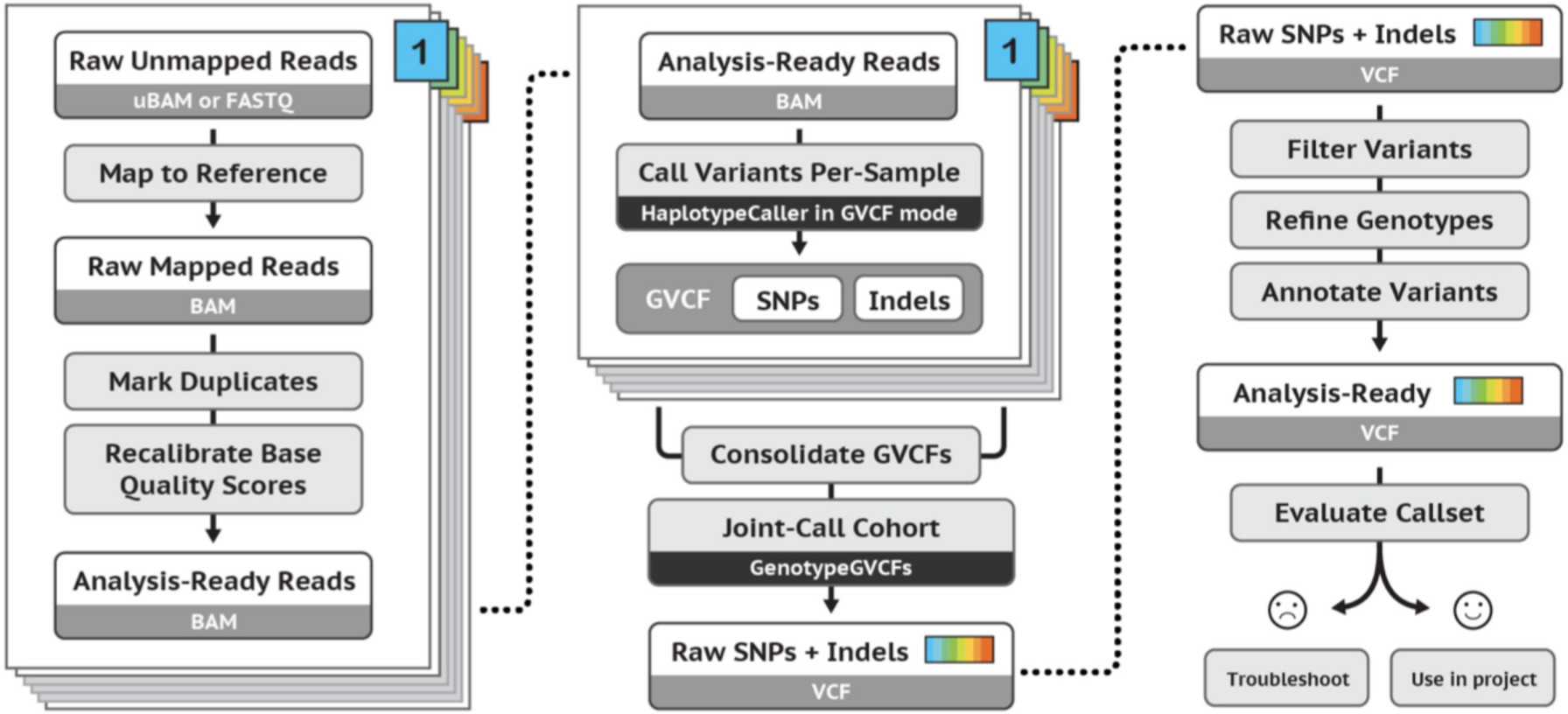
3. GATK Best practices pipeline

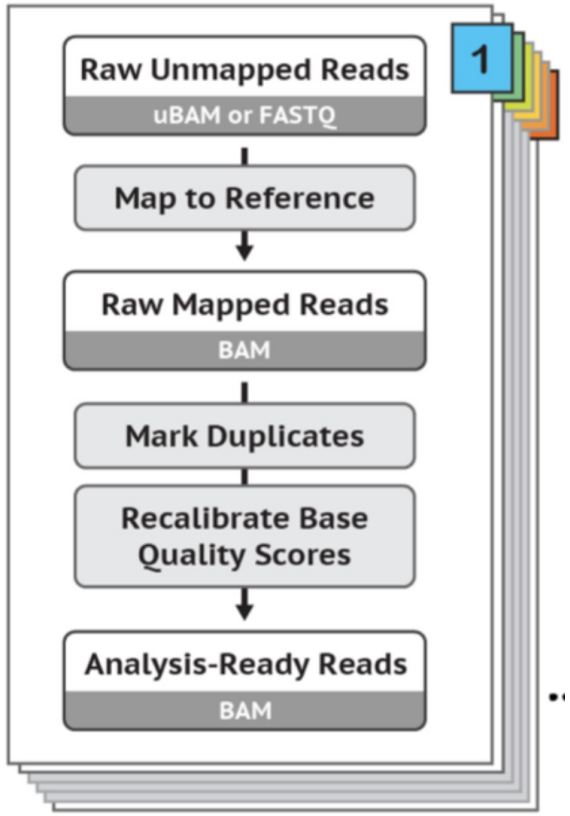


3.1 Pre-processing

3.2 Variant discovery

3.3 Refinement

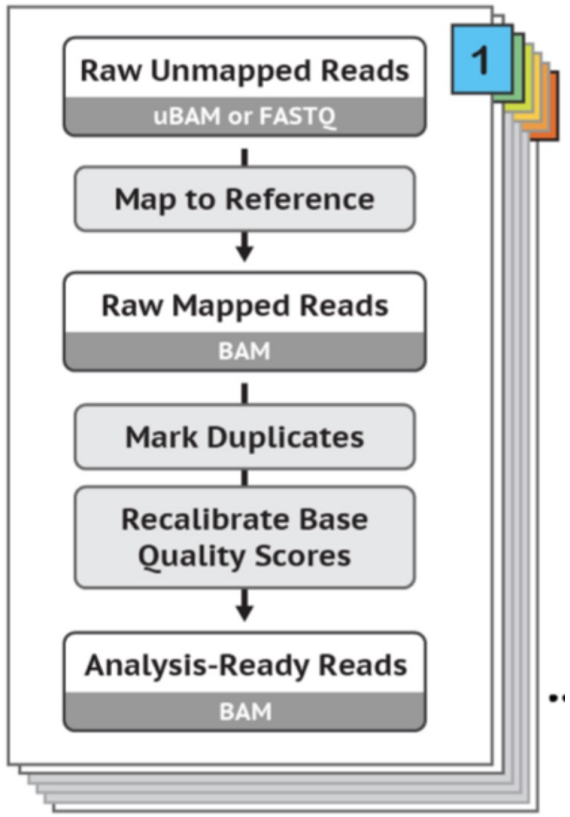
3.1 Pre-processing



Pre-processing

- A sequencing experiment results in a large volume of sequencing reads
- Reads are not mapped to a reference
- Reads can contain errors and technical artifacts
- e.g. a molecule sequenced multiple times will result in duplicate reads
- We need to filter and prepare the reads and the alignment data – ready for variant calling

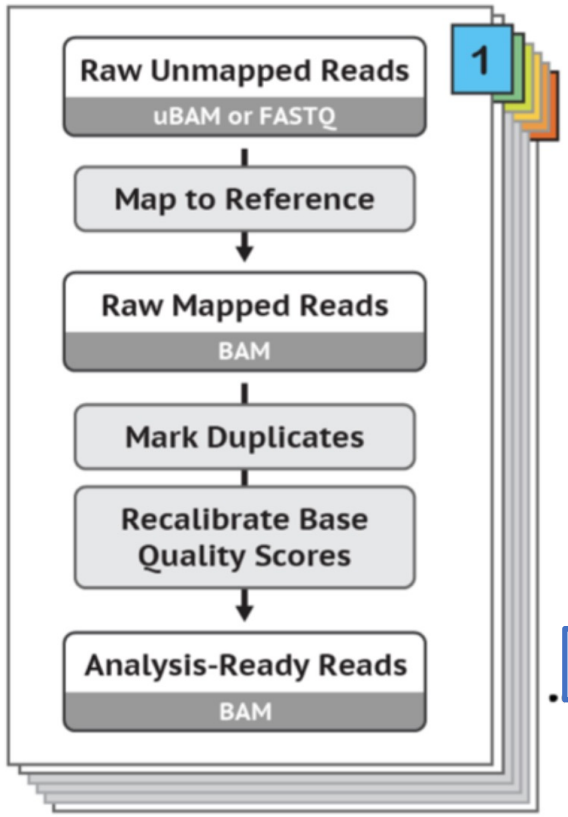
3.1 Map to reference



Pre-processing

- **BWA-MEM**
- `bwa mem -M -t 4 -R "@RG\tID:SRR622461.7\tSM:NA12878\tLB:ERR194147\tPL:ILLUMINA" <reference> sample_1.fastq sample_2.fastq > alignment.sam`
- -M: inserts a tag to the alignment if non-primary alignment (required by GATK)
- -R: read group
- -t: threads or number of cpus
- <reference>: path to reference genome in fasta format and the BWA index files

3.1 Map to reference



Pre-processing

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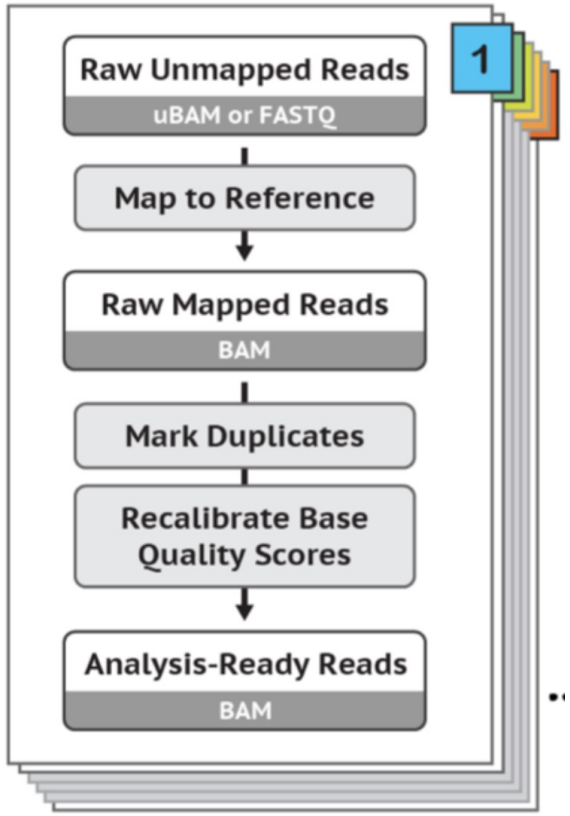
- -R: read group contains information such as the sample name, library and flow cell.
- Refers to a set of reads generated from a single sequencing run in particular machine

```
@RG     ID:SRR622461.7 SM:NA12878     LB:ERR194147    PL:ILLUMINA
```

3.1 Map to reference



Pre-processing

- Output is a SAM/BAM file.
- SAM file specifications: https://samtools.github.io/hts-specs/SAMv1.pdf

Header

```
@HD    VN:1.5  SO:coordinate
@RG    ID:SRR622461.7  SM:NA12878    LB:ERR194147    PL:ILLUMINA
@PG    ID:bwa  PN:bwa  VN:0.7.17-r1188 CL:bwa mem -M -t 4 -R
```

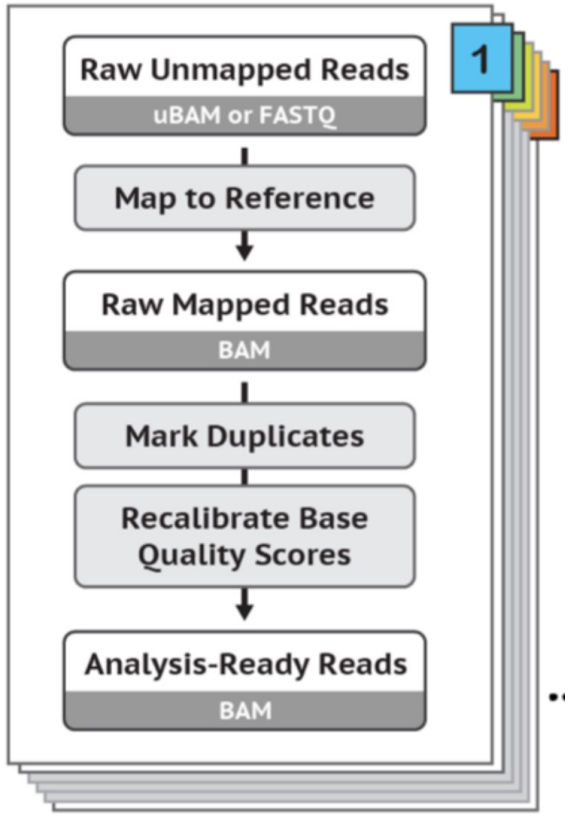
Alignment

position CIGAR read flags/ metadata

```
ERR194147.45  163  chr18 6576006 99 101M = 6578028 317  CATTTCT... <B<<BBBBB...  NM:i:0 MD:Z:101...
```

read name flag mapping quality mate information PHRED quality

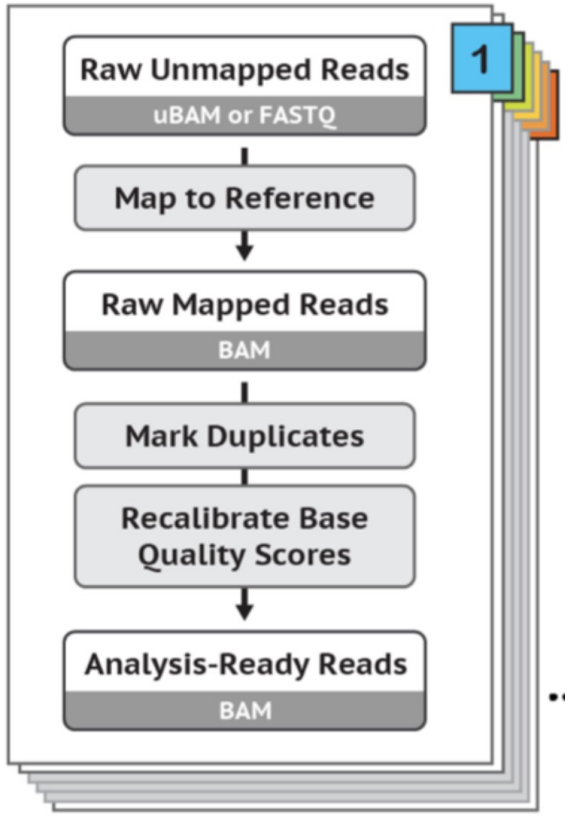
3.1 Mark duplicates



Pre-processing

- **Mark Duplicates**
- Identify reads that are non-independent measurement of sequence fragment
  - Same template of DNA sampled multiple times
  - PCR duplicates
- High sequence identify
- Align to same reference position

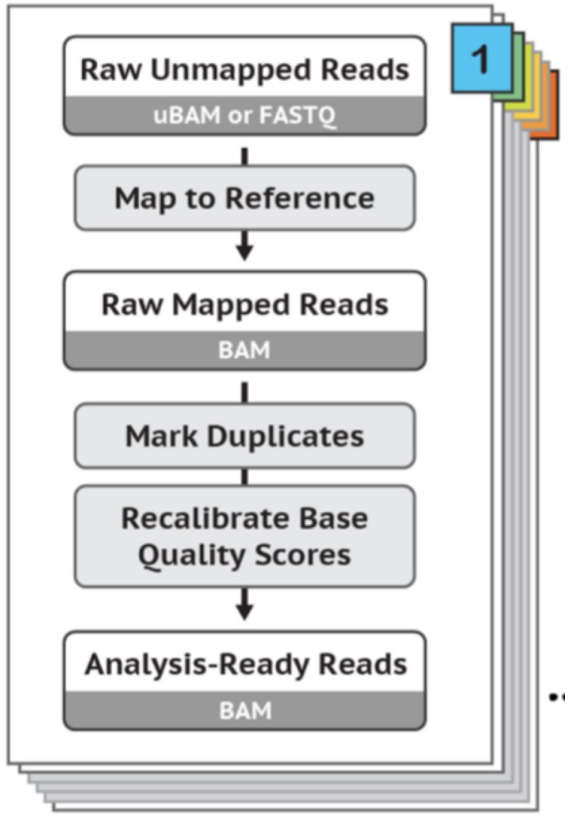
3.1 Mark duplicates



Pre-processing

- **Mark Duplicates**
- `gatk MarkDuplicates -I sample.bam -O sample.dedup.bam -M sample.dedup.metrics.txt`
- Recommended to be performed on reads per library or lane
- SAM flags are used to mark reads as duplicates
- Downstream GATK tools depend on these flags to assess support for variants and alleles

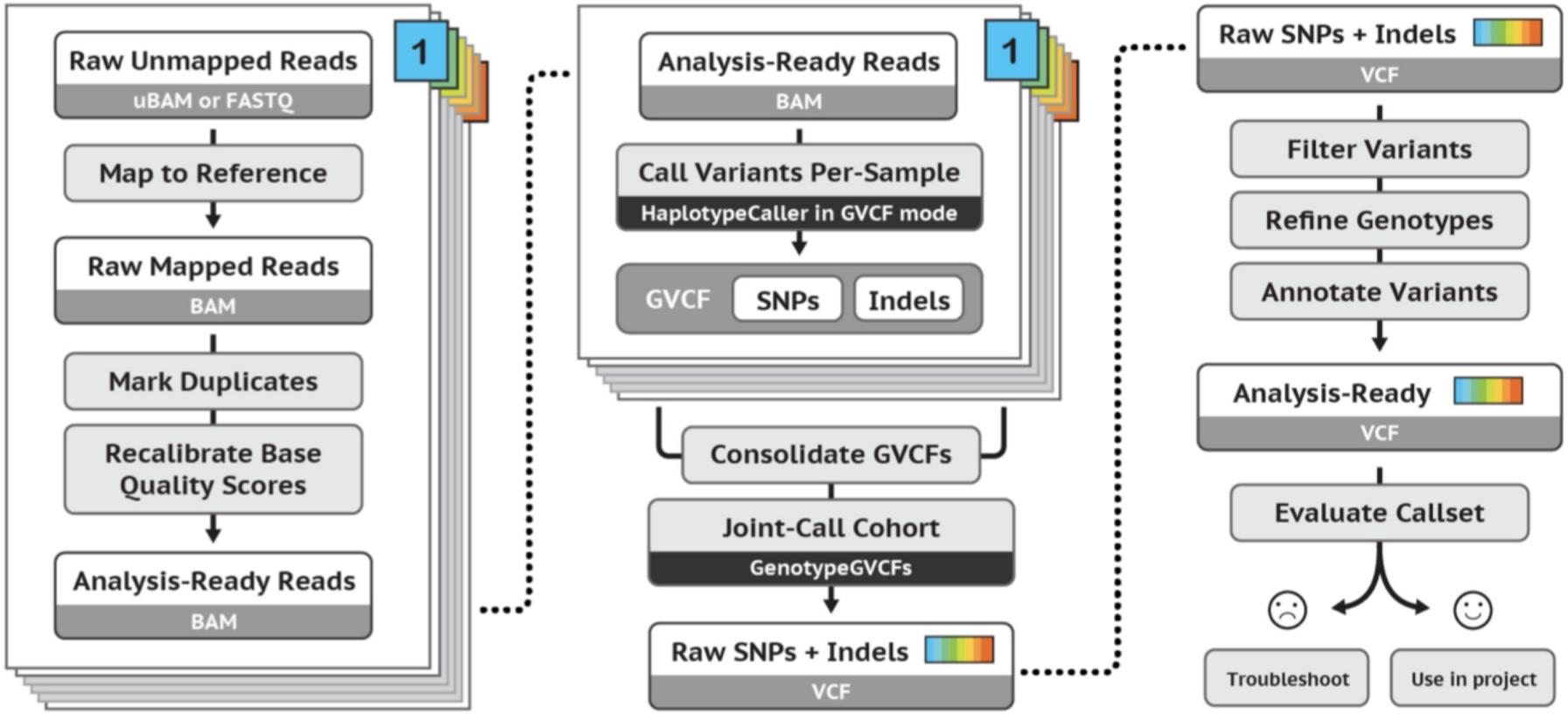
3.1 Base recalibration



Pre-processing

- **Base recalibration**
- `gatk tools BaseRecalibrator and ApplyRecalibration`
- Performed per-sample to detect and correct for patterns of systematic errors in base quality scores.
- Evidenced by calculating metrics based on known variant locations
- Important for building reliable evidence for downstream analysis.

3. GATK Best practices pipeline

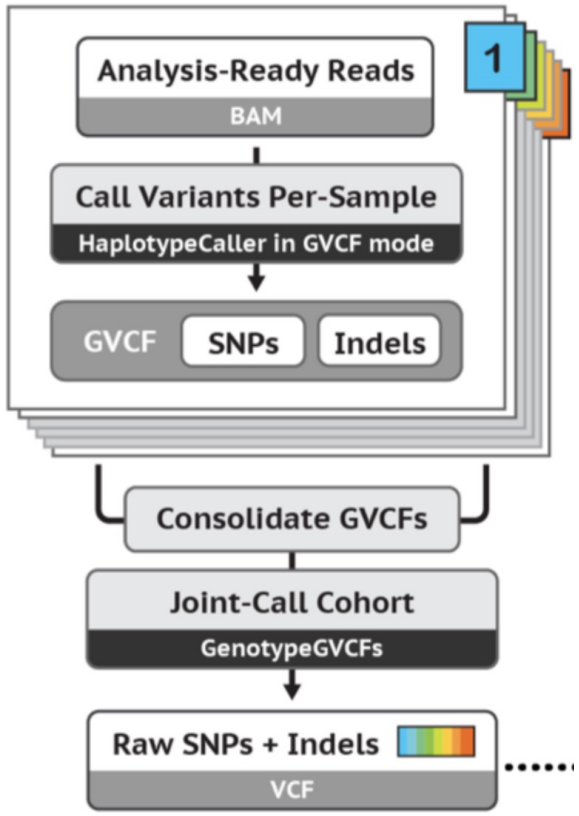


3.1 Pre-processing

3.2 Variant discovery

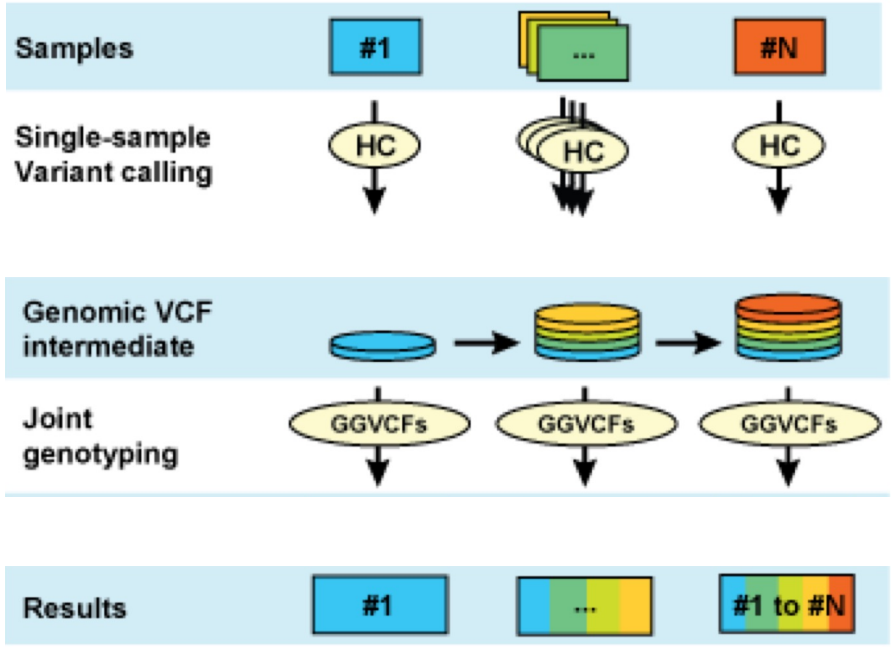
3.3 Refinement

3.2 Variant discovery



Variant discovery

- **Software**

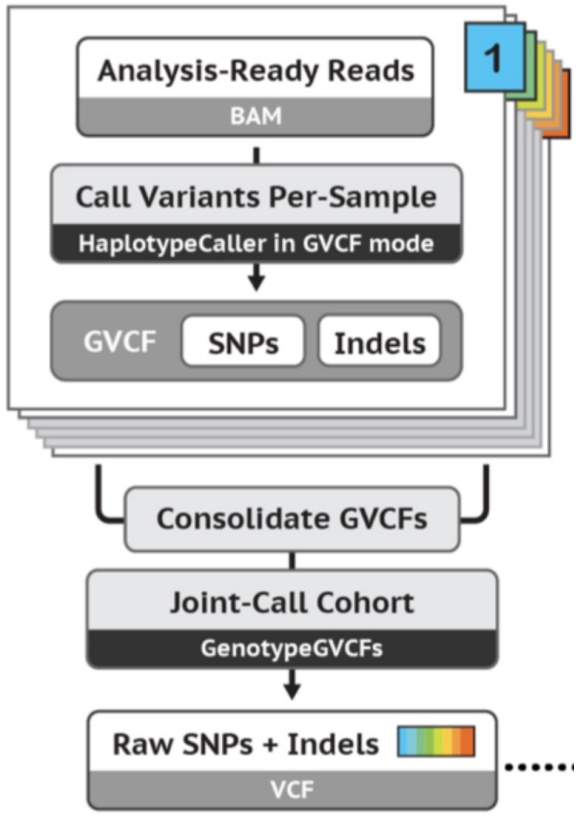


HaplotypeCaller

CombineGVCFs/ GenomicsDBImport

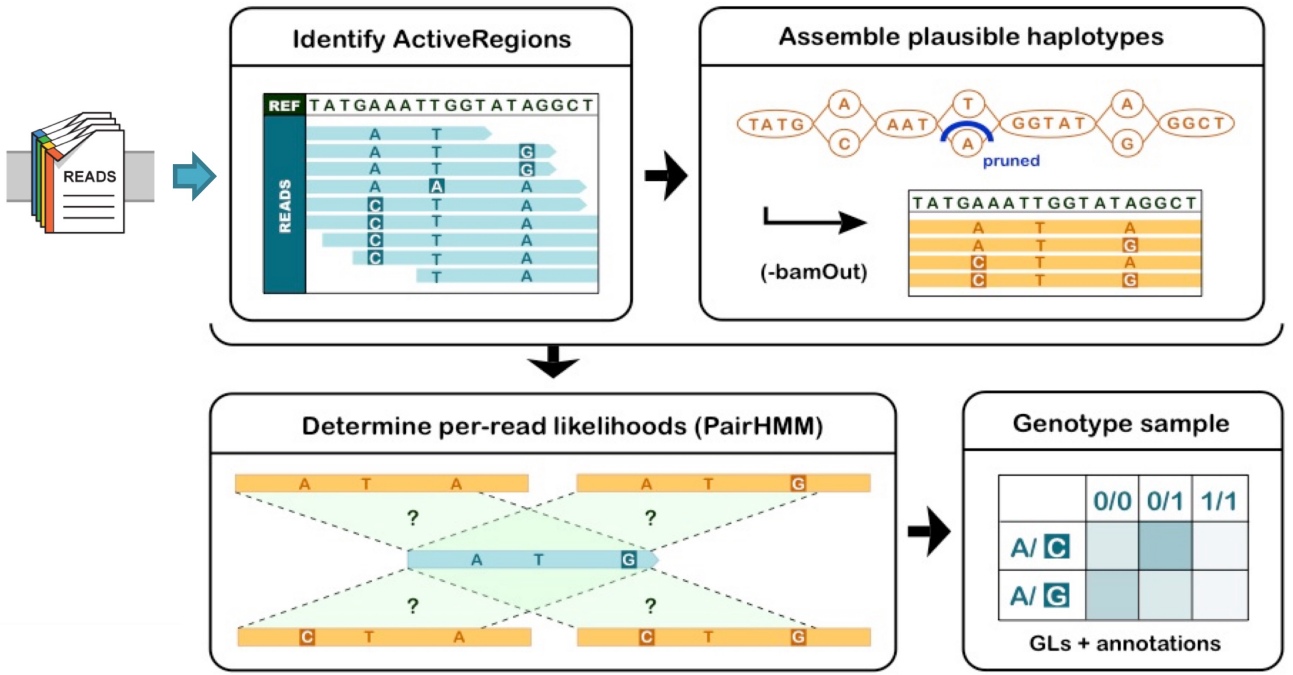
GenotypeGVCFs

3.2 Variant discovery

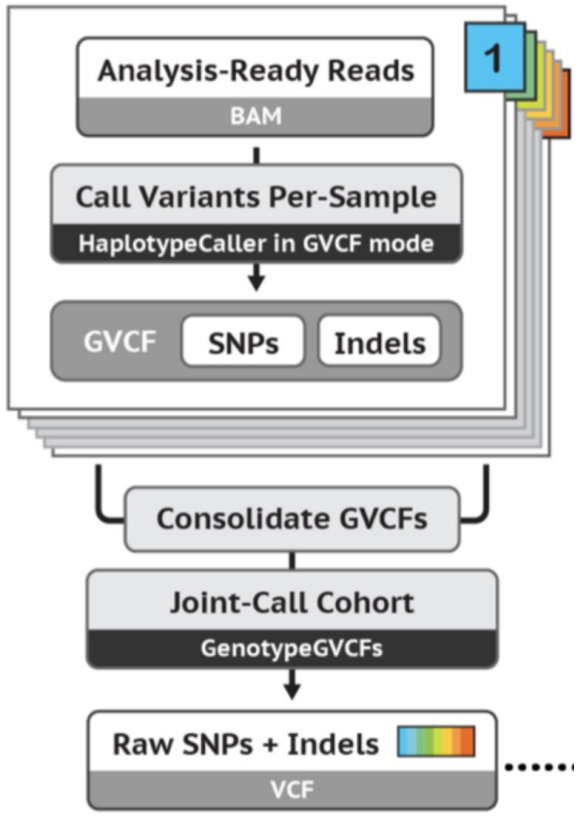


Variant discovery

- **HaplotypeCaller**
- `gatk --java-options "-Xmx4g" HaplotypeCaller -R <reference.fa> -I input.bam -O output.g.vcf.gz -ERC GVCF`



3.2 Variant discovery



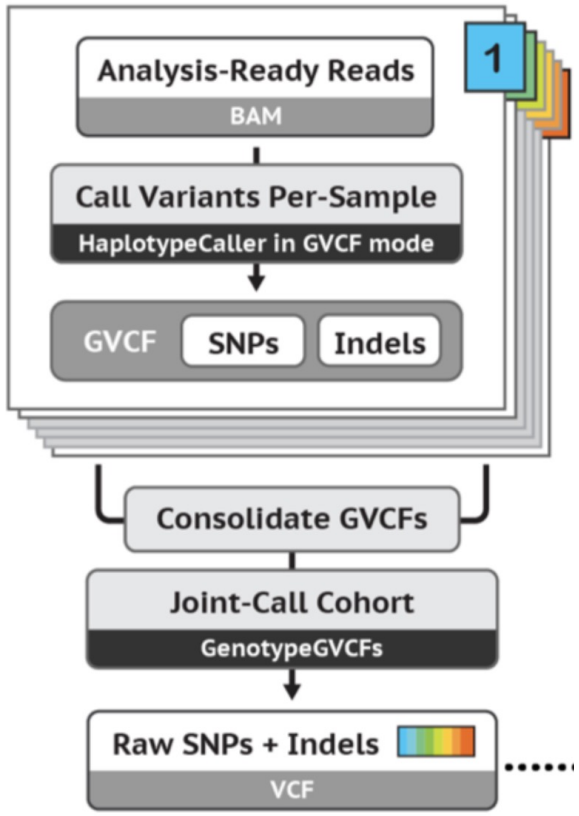
Variant discovery

- **CombineGVCFs**

- `gatk CombineGVCFs R <reference.fa> --variant sample1.g.vcf.gz --variant sample2.g.vcf.gz -O cohort.g.vcf.gz`

- Combine per samples gVCF files (produced by HaplotypeCaller) into a multi-sample gVCF file.

3.2 Variant discovery



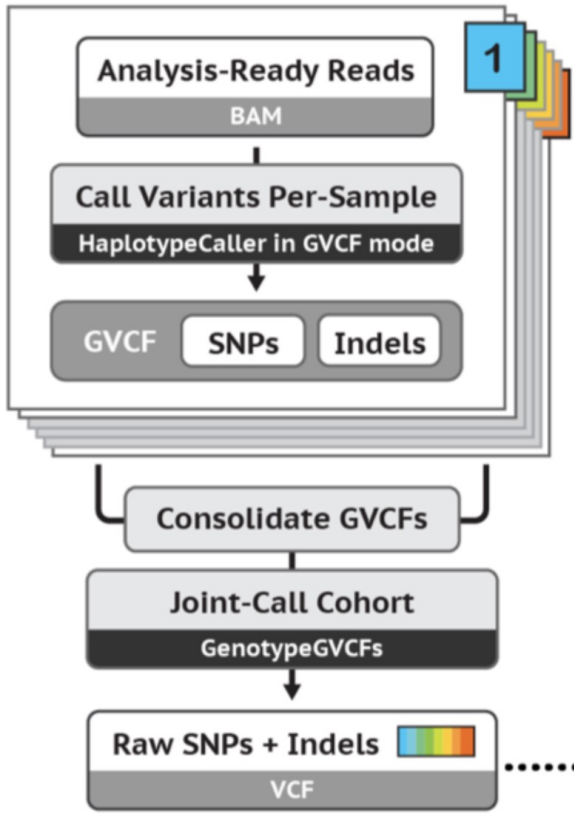
Variant discovery

- **GenotypeGVCFs**

- `gatk --java-options "-Xmx4g" GenotypeGVCFs -R <reference.fa> -V cohort.g.vcf.gz -O output.vcf.gz`

- Combine per samples gVCF files (produced by HaplotypeCaller) into a multi-sample gVCF file.

3.2 Variant discovery



Variant discovery

- Output is a VCF file
- VCF file specifications https://samtools.github.io/hts-specs/VCFv4.2.pdf

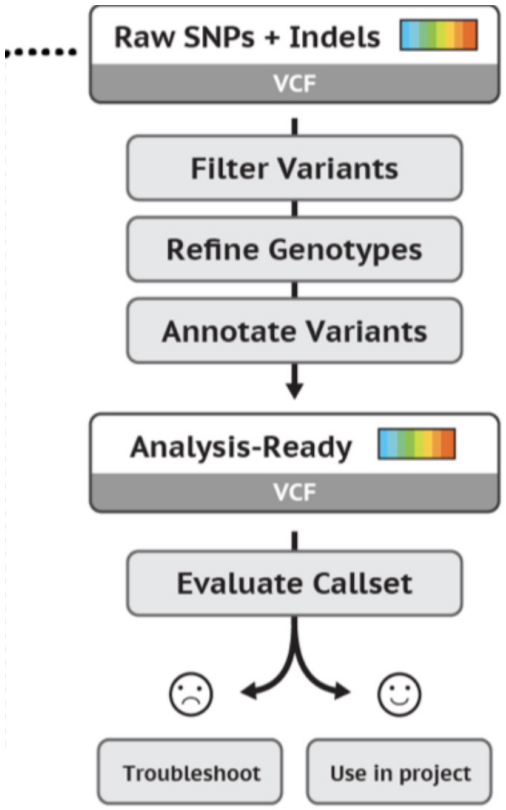
Header

```
#fileformat=VCFv4.2
##FILTER=<ID=PASS,Description="All filters passed">
##contig=<ID=1,length=249250621>
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
```

Variant record

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Sample1
1	567376	.	G	A	146.3	PASS	AC=1;DP=55	GT:AD:DP	0/1:30,25:55

3.3 Variant Refinement



Refinement

- Variant callers are sensitive
- The aim here is to identify potential false positives and apply filters to remove those less likely to be real variants. Strategies include:

1. Variant quality score recalibration (using known sites)
2. Hard filtering on quality criteria
3. Annotation features

Variant record

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Sample
1	567376	.	G	A	146.3	PASS	AC=1;DP=55	GT:AD:DP	0/1:30,25.55

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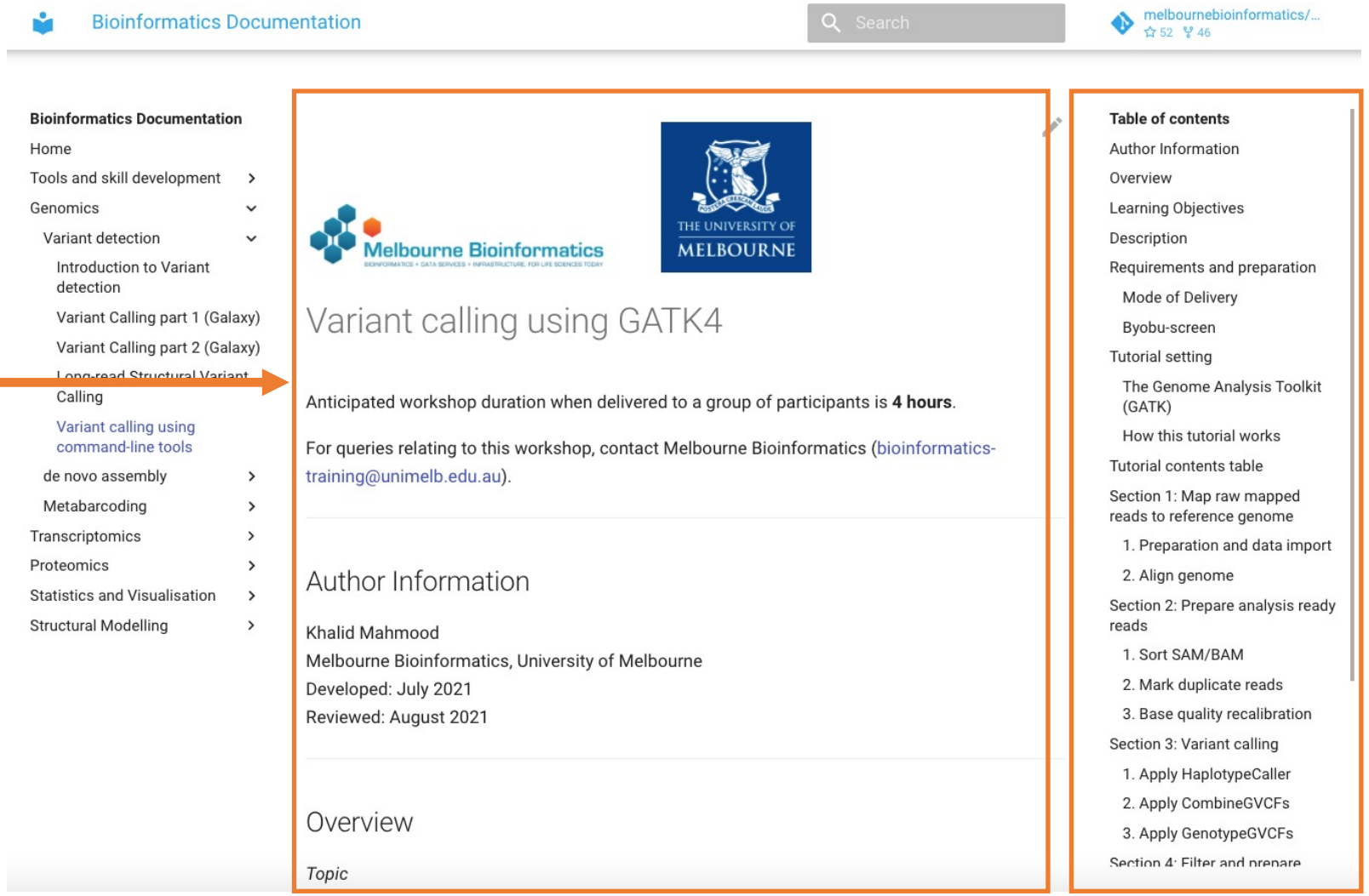
Workshop structure

Workshop structure

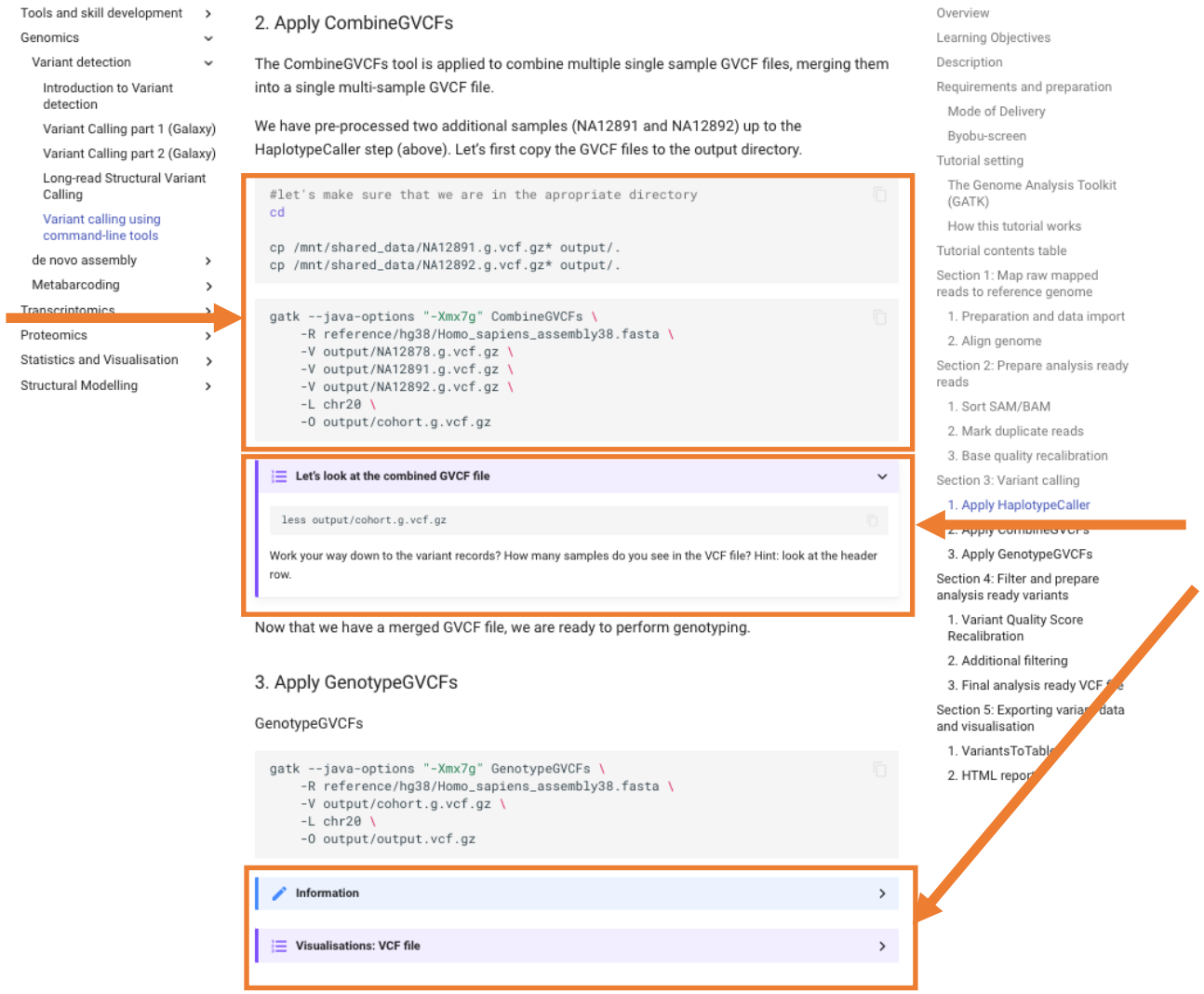
Tutorial content



Tutorial navigation

Workshop structure

Command and output blocks
‘#’ comments - do not run



Interactive sections
Notes, hints, exercises

Workshop structure

Table of contents

Introductory material
Tutorial delivery and some instructions

Workshop content:

- 5 sections
- Each section covers a stage in the variant calling pipeline
- Each section has a text explain the process and links to relevant material
- Sections have multiple steps. Mostly have an input and an expected output file.
- This is a pipeline: input to a step is the output from a previous step

Workshop computers

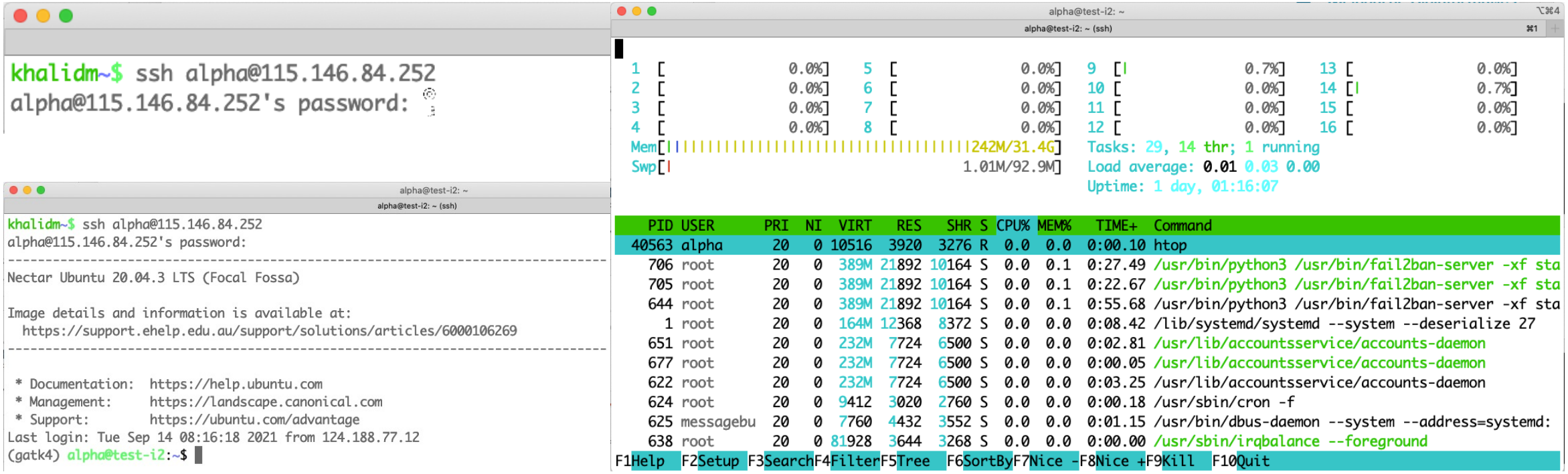
- We will be conducting the workshop on virtual machines
- Hosted on the University of Melbourne Research Cloud service and the ARDC Nectar Research Cloud infrastructure.
- Infrastructure for development and setup of the workshops machines by Simon Gladman

Workshop computers

- Each participant should have a username and a password
- Each participant will be assigned a log in to one of the VM machines:
  - Follow the google sheet link for more details
- Configuration:

Log on to the VMs

- Open a terminal window and on the command prompt type and enter:



Useful Linux commands

- Autofill on command line: **Tab key**
- Abort command: **Ctrl-c**
- List contents of a directory: **ls -l**
- What's the path to my current directory: **pwd**
- Change directory: **cd <path/to/destination>**
- Create a directory: **mkdir <directory name>**
- Copy a file: **cp <source file> <destination path/name>**
- Remove a directory: **rmdir <directory name>**
- Remove a file: **rm <file name>**
- Rename/move a file (this is not copying a file): **mv <source file> <destination file>**
- Open a text editor: **nano**
- Print file content (small files): **cat <file name>**
- Print file content (quick view): **less <file name>**
- Print file content (quick view/first 10 lines of a file): **head <file name>**
- Print file content (quick view/last 10 lines of a file): **tail <file name>**
- curl or wget: download a file from a URL (you will see this in other QIIME2 tutorials)
- Documentation for a command line tool: try **man <tool name>** OR **<command_name> --help**

Workshop data

- Primary data: paired-end sequencing reads from the chr20
  - chr20:2677705-6631126
- Whole genome sequencing data
  - Female
  - Utah resident (European ancestry)
  - 1000 genomes project (NA12878)
- Other data from
  - A male and female
  - Utah resident (European ancestry)
  - 1000 genomes project (NA12891 and NA12892)

Byrska-Bishop, Marta et al. "High coverage whole genome sequencing of the expanded 1000 Genomes Project cohort including 602 trios". *bioRxiv*. (2021).

Byobu-screen

- A terminal multiplexer or a tool to to create multiple ‘windows’ in a single screen
- Improves stability of terminal sessions when connected to a remove computer

- List screen sessions: byobu-screen -ls
- Start new session: byobu-screen -S workshop
- Detach from screen to original window: Ctrl-a-d

- More details:
- https://www.melbournebioinformatics.org.au/tutorials/tutorials/variant_calling_gatk1/variant_calling_gatk1/#byobu-screen

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https://www.melbournebioinformatics.org.au/tutorials/tutorials/variant_calling_gatk1/variant_calling_gatk1/

Break…


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gatk

4. Resources and tools

- GATK resources bundle: collection of files for GATK based analysis working with human sequencing data.

- ftp://gsapubftp-anonymous@ftp.broadinstitute.org/bundle/hg38

1000G_omni2.5.hg38.vcf.gz
1000G_phase1.snps.high_confidence.hg38.vcf.gz
Axiom_Exome_Plus.genotypes.all_populations.poly.hg38.vcf.gz
dbsnp_146.hg38.vcf.gz
hapmap_3.3_grch38_pop_stratified_af.vcf.gz
hapmap_3.3.hg38.vcf.gz
Homo_sapiens_assembly38.dict
Homo_sapiens_assembly38.fasta
Homo_sapiens_assembly38.fasta.gz
Mills_and_1000G_gold_standard.indels.hg38.vcf.gz

4. Resources and tools

- BWA-MEM index
- bwa index Homo_sapiens_assembly38.fasta

Homo_sapiens_assembly38.fasta
Homo_sapiens_assembly38.fasta.amb
Homo_sapiens_assembly38.fasta.ann
Homo_sapiens_assembly38.fasta.bwt
Homo_sapiens_assembly38.fasta.pac
Homo_sapiens_assembly38.fasta.sa

4. Resources and tools

Tools name	function
FastQC	QC tools for raw sequencing reads
MultiQC	QC report aggregator (generates an HTML report)
GATK	Set of tools for variant calling
Picard	A command line tool to analysis and manipulate sequencing files
Samtools	Suite of tools for interacting with mapped sequencing reads (SAM/BAM/CRAM format)
BCFtools	Suite of tools for interacting with variant data (VCF/BCF formats)

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4. Genetic variant resources

- dbSNP
  - An archive of genetic variations – contains ~700 million variants
  - ~90% have a recorded population frequency
- gnomAD
  - Aggregation of variants derived from re-analysis of >125k WES and WGS
- ClinVar
  - Aggregates genetic variations and its relationships with phenotypes
- UCSC genome browser

- UniProt

4. Help

- Tool documentation
- GATK forum
- Online resources (e.g. Biostar)
- GitHub for technical issues/discussions


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