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NATIONAL BIOINFORMATICS
INFRASTRUCTURE SWEDEN

SciLifeLab

Variant-calling Workflow

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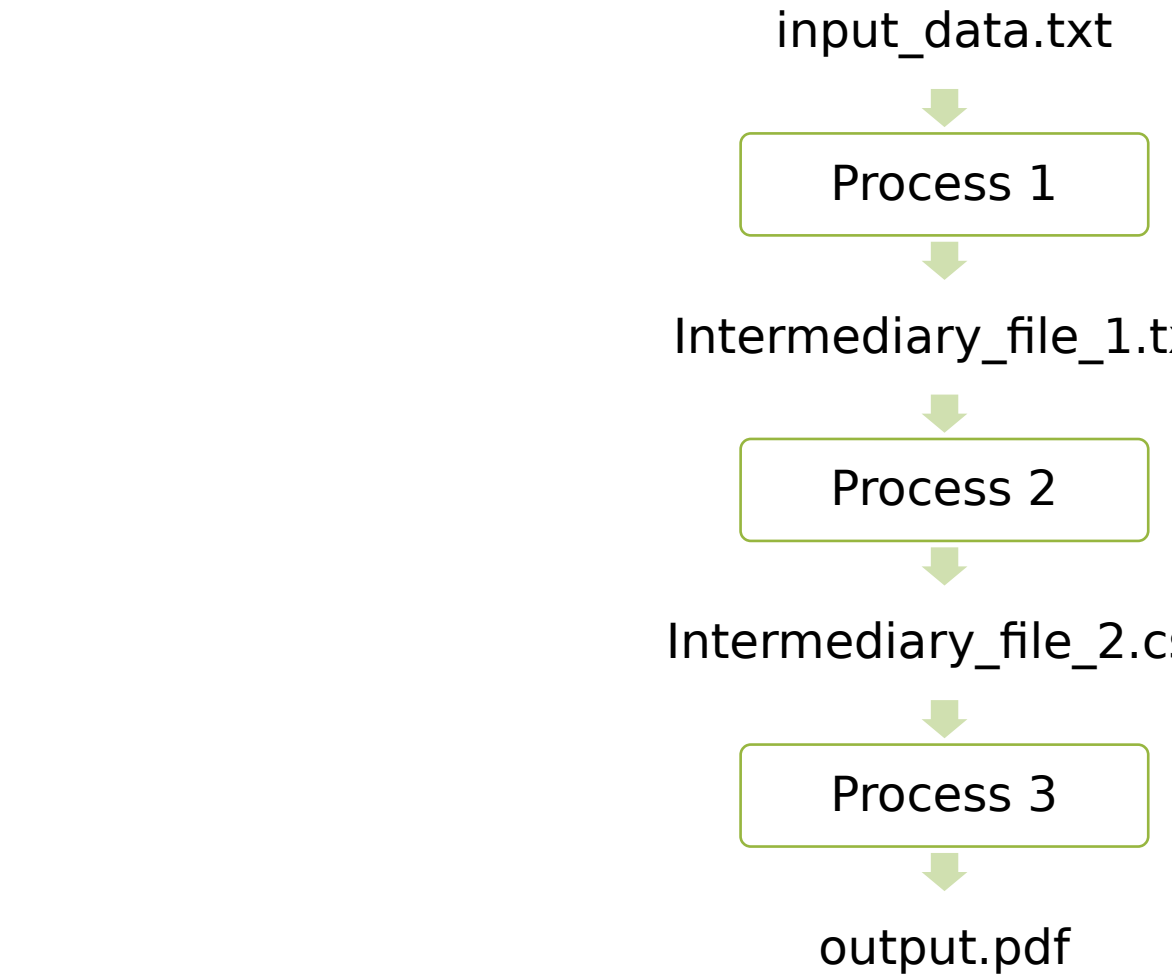
Overview

- Workflows
- Basic variant calling in one sample
- Basic variant calling in cohort
- Introduction to exercise

In separate talk Thursday at 9:

- GATK's Best practices

What is a workflow



Overview

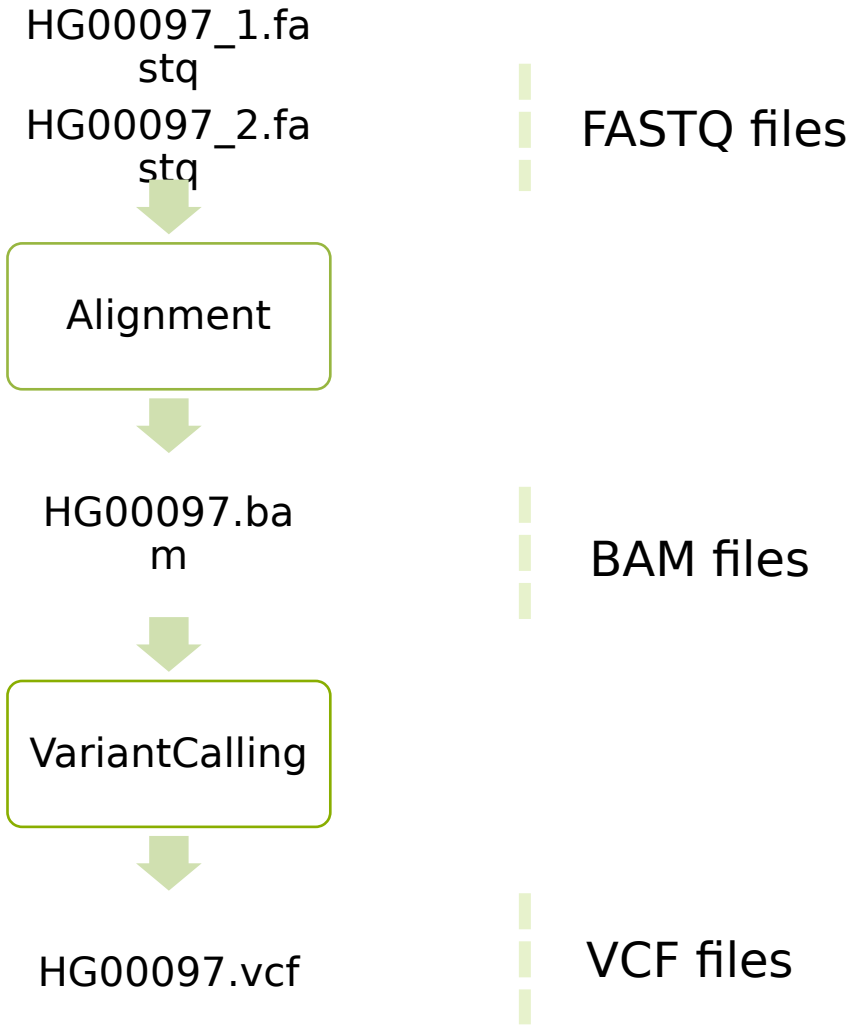
Today:

- Basic variant calling workflow for one sample
- Extend to multiple samples

Tomorrow:

- GATK's Best practices

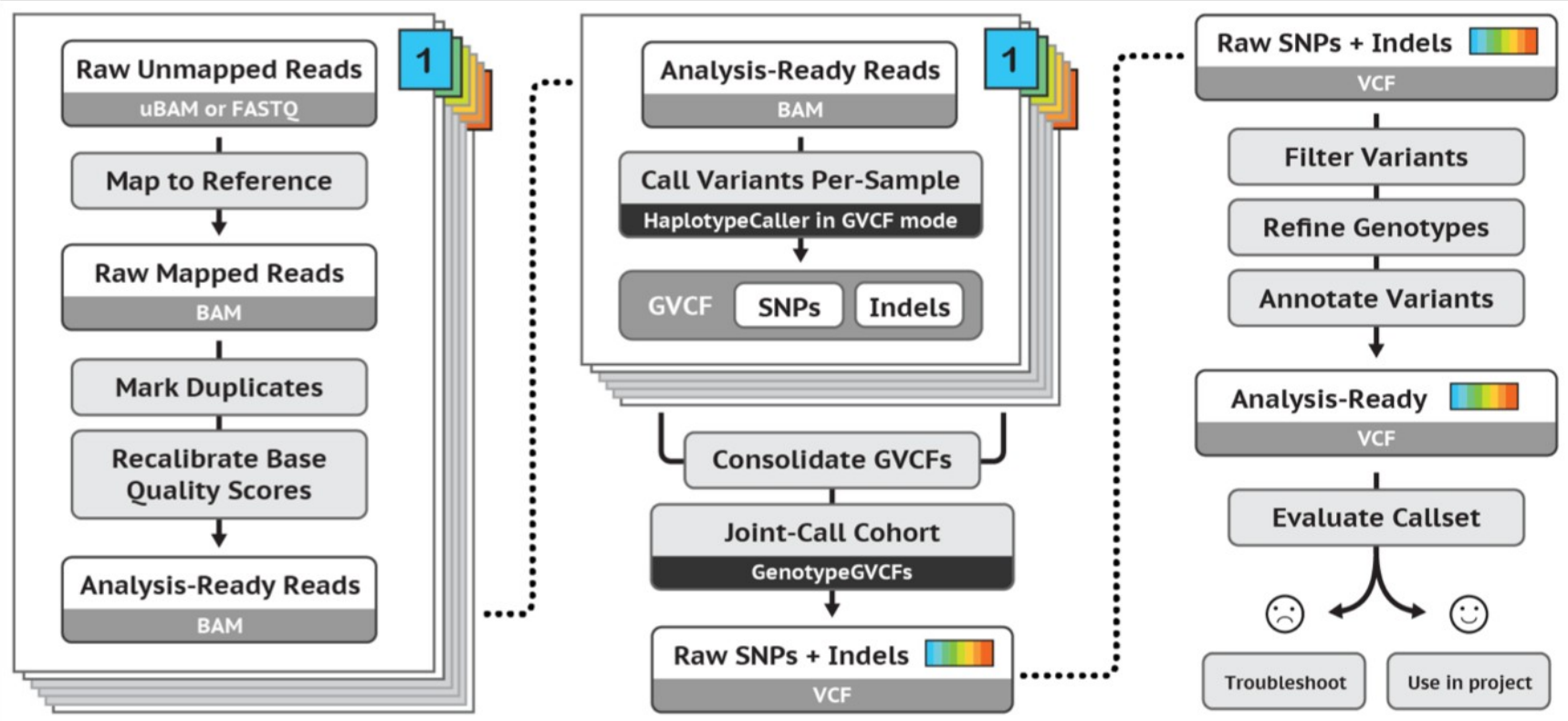
Example: Basic workflow, one sample



Workflow conventions

1. Create a new output file in each process
2. Don’t overwrite the input file
3. Use informative file names
4. Include information of the process + sample
5. Correct name extension e.g. .bam, .vcf, ...

GATK's best practices workflow for germline short variant discovery



https://software.broadinstitute.org/gatk/best-practices/

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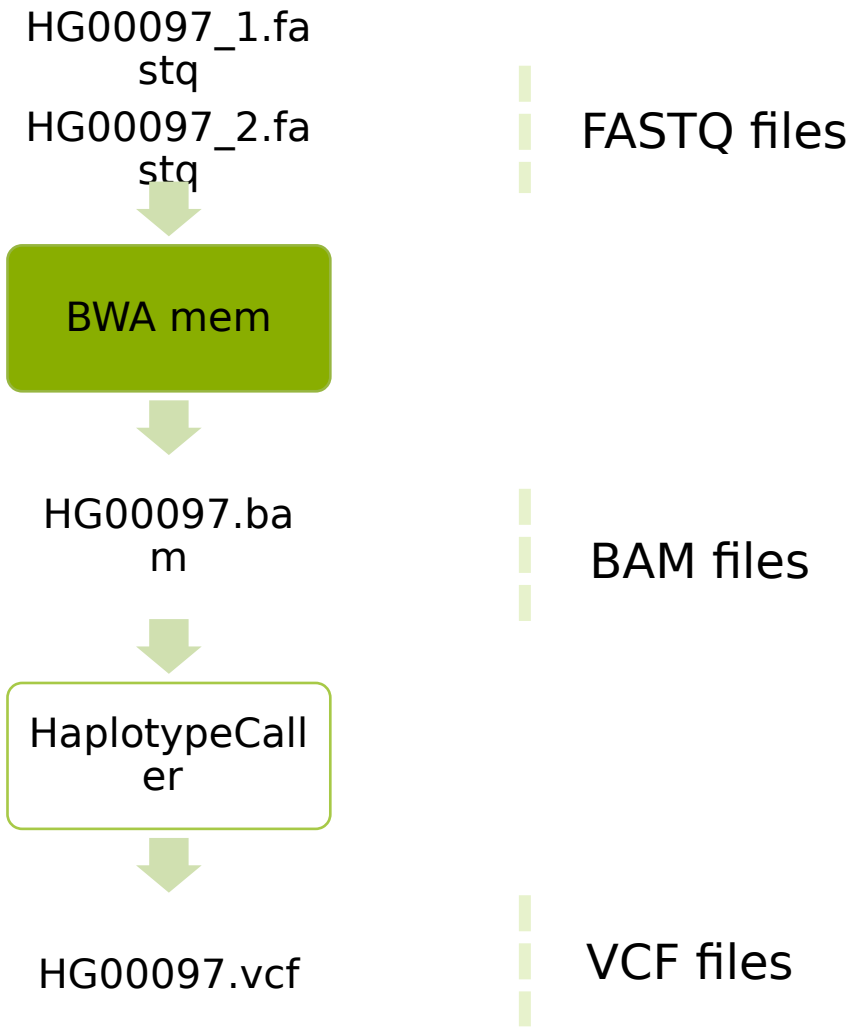
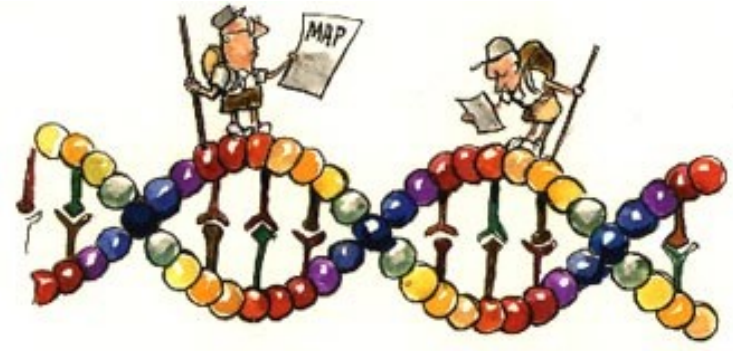
Basic Variant Calling in one sample

Alignment


HG00097_1.fastq
HG00097_2.fastq
FASTQ files
BWA mem
HG00097.bam
BAM files
HaplotypeCaller
HG00097.vcf
VCF files

The reference genome



A reference genome is a haploid nucleic acid sequence which represents a species genome.

The first draft of the human genome contained 150,000 gaps.

GRCh37: 250 gaps

We will work with GRCh37 in the lab.

Keep track of the reference version!

The reference genome sequence is used as input in many bioinformatics applications for NGS data:

- mapping
- variant calling
- annotation

You must keep track of which version of the reference genome your data was mapped to.

The same version must be used in all downstream analyses.

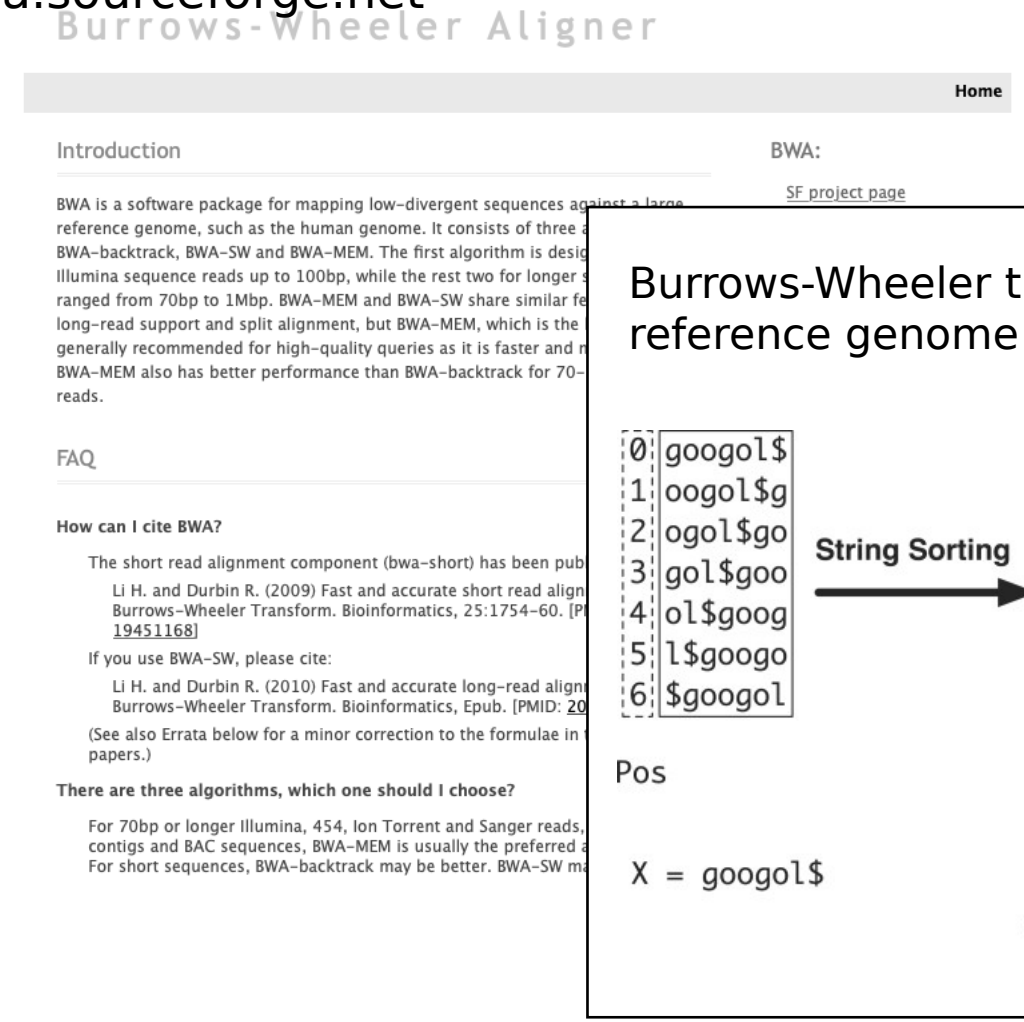
Alignment

AACAGGTATATCTTCCCCGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTACCCTCTTCCTTAGGGACTGTAC

GCTAGCTAGCTAGCTACCCT

Burrows-Wheeler Aligner

http://bio-bwa.sourceforge.net



Output from mapping - Sam format

HEADER SECTION

```
@HD     VN:1.6      SO:coordinate
@SQ     SN:2        LN:243199373
@PG     ID:bwa      PN:bwa      VN:0.7.17-r1188     CL:bwa mem -t 1 human_g1k_v37_chr2.fasta
HG00097_1.fq HG00097_2.fq
@PG     ID:samtools PN:samtools PP:bwa      VN:1.10     CL:samtools sort
@PG     ID:samtools.1PN:samtools PP:samtools VN:1.10    CL:samtools view -H HG00097.bam
```

ALIGNMENT SECTION

```
Read_001    99      2       3843448     0       101M    =       3843625     278
            TTTGGTTCCATATGAACTTT                0F<BFB<FFFBFBFFFBFBB
Read_001    147     2       3843625     0       101M    =       3843448     -278
            TTATTTCATTGAGCAGTGGT                FBBI7IIFIB<BBBB<BBFF
Read_002    163     2       4210055     0       101M    =       4210377     423
            TGGTACCAAAACAGAGATAT                0IIFBFFFIIIFFIFFFBBF
Read_003    99      2       4210066     0       101M    =       4210317     352
            CAGAGATATAGATCAATGGA    0IIFFFIFFFIFIFIIIIIF
```

Start position

Reference sequence name

Read name (usually more complicated)

Sequence

Quality

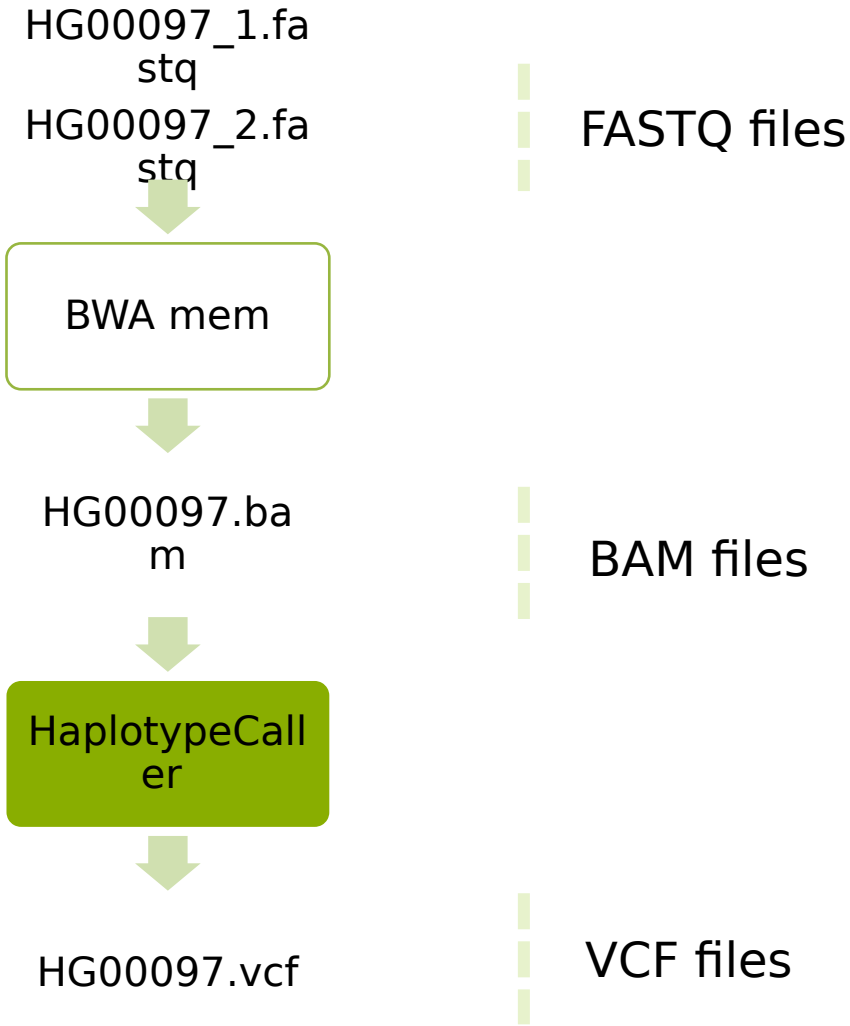
Convert to Bam

Bam file is a binary representation of the Sam file

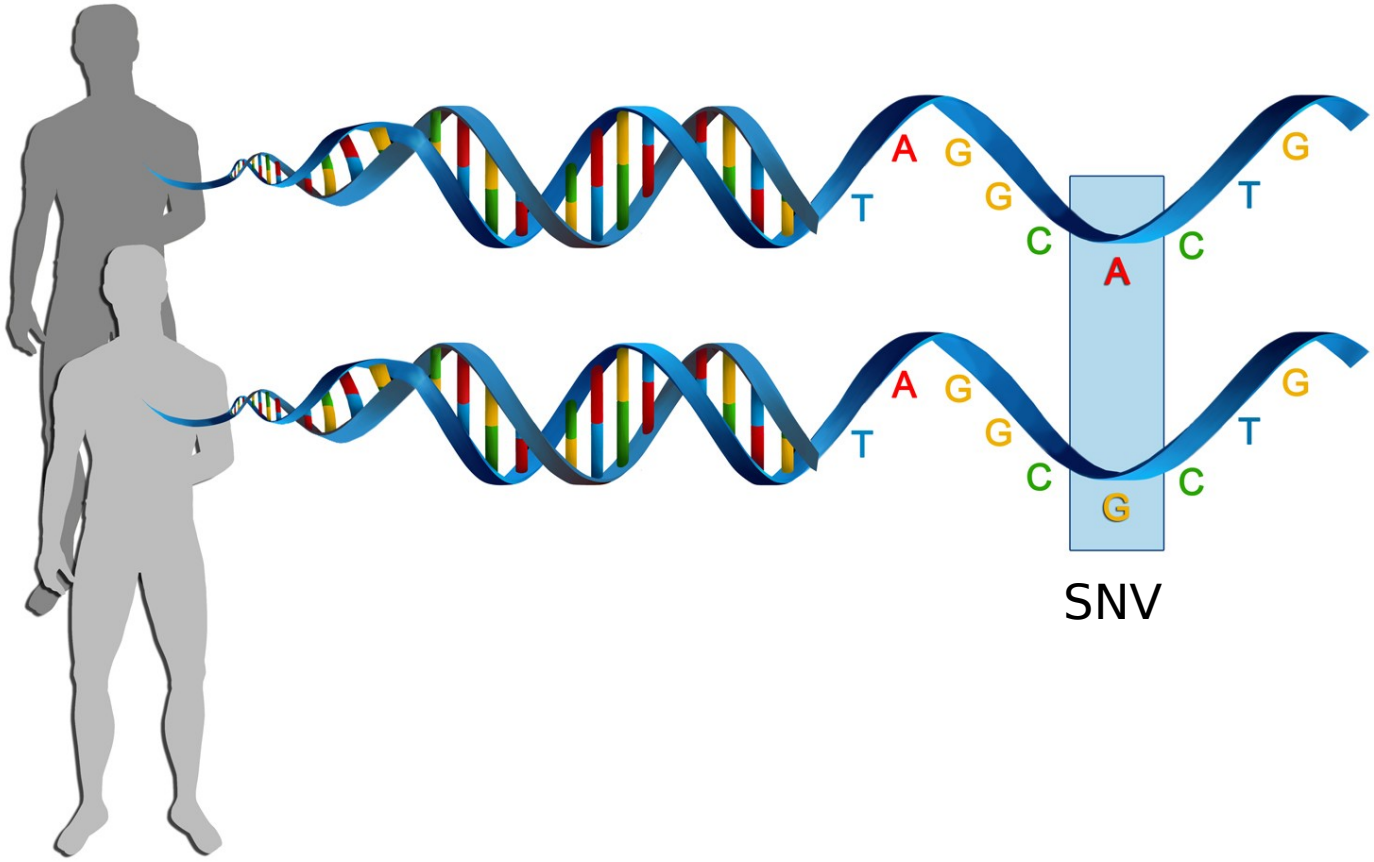
File indices

- Most large files we work with, such as the reference genome (.**fasta**) and the aligned reads (.**bam**) need an index
- The index is a small file
- Allows efficient access to the large file
- Different indices for different file types
- BWA index = Burrows-Wheeler transform of reference genome (**several files**)

Variant calling

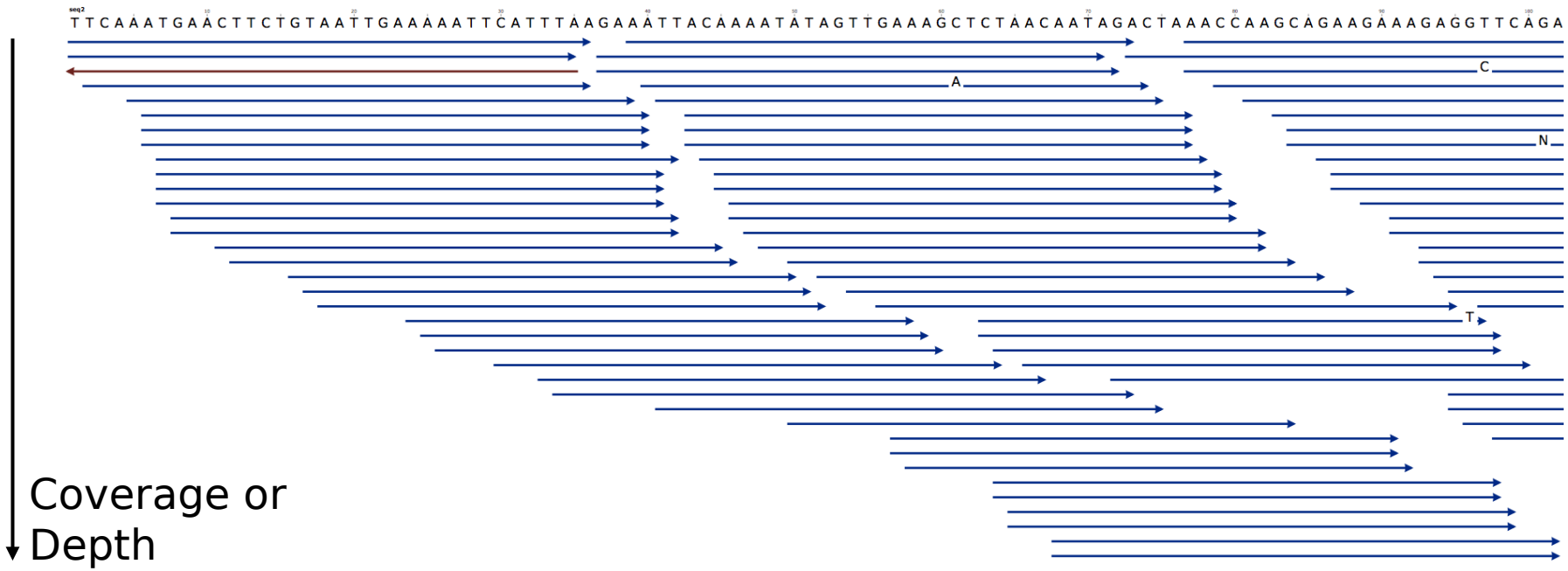


Genetic variation



Genetic variation = differences in DNA among individuals of the same species

Alignment


seq2
TTCAAATGAACTTCTGTAATTGAAAAATTCATTTAAGAAATTACAAAATATAGTTGAAAGCTCTAACAATAGACTAAACCAAGCAGAAGAAAGAGGTTCAGA
A
C
N
T
Coverage or
Depth

Detecting variants in reads

```
Reference:      ...GTGCGTAGACTGCTAGATCGAAGA...
   Sample:   ...GTGCGTAGACTGATAGATCGAAGA...
              ...GTGCGTAGACTGATAGATCGAAGA...
            ...GTGCGTAGACTGCTAGATCGAAGA...
            ...GTGCGTAGACTGCTAGATCGAAGA...
            ...GTGCGTAGACTGATAGATCGAAGA...
            ...GTGCGTAGACTGATAGATCGAAGA...
            ...GTGCGTAGACTGCTAGATCGAAGA...
            ...GTGCGTAGACTGATAGATCGAAGA...
            ...GTGCGTAGACTGCTAGATCGAAGA...
            ...GTGCGTAGACTGATAGATCGAAGA...
```

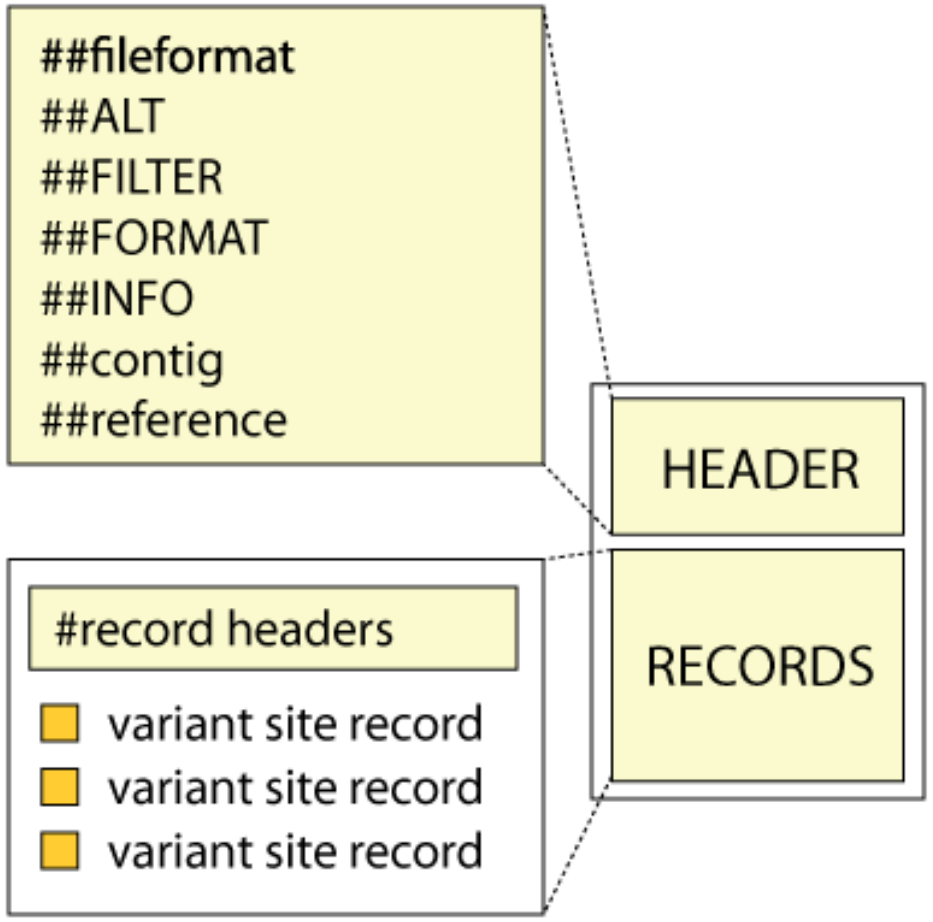
Reference- and alternatve alleles

TGGGCTTTTCCAACAGGTATATCTTCCCCGCTAGCTCGCTAGCTACTTCAAATTCCT

Reference allele	AGCTCGCTA
Alternative allele	AGCTAGCTA

Reference allele = the allele in the refence genome
Alternative allele = the allele NOT in the refence genome

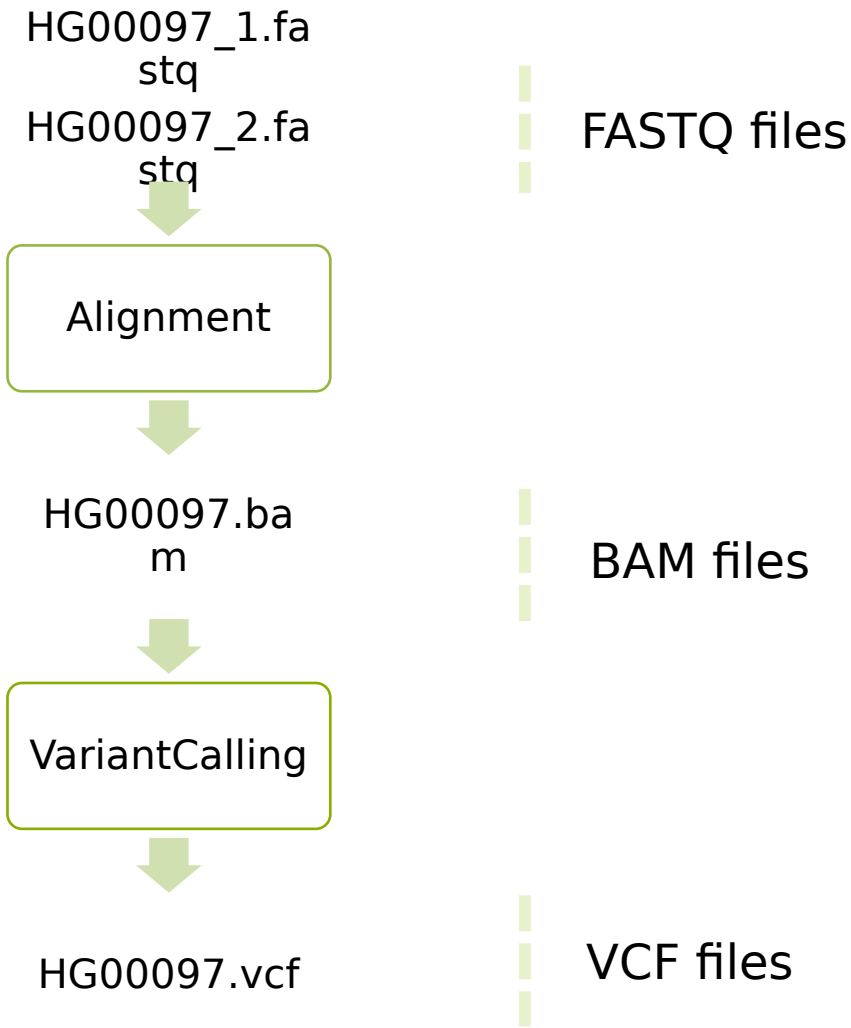
Variant Call Format (VCF)



Variant Call Format (VCF)

```
##fileformat=VCFv4.2
##FILTER=<ID=LowQual,Description="Low quality">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or with bad mates are filtered)">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele, in the same order as listed">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the same order as listed">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##contig=<ID=2,length=243199373>
##source=HaplotypeCaller
#CHROM  POS             ID      REF     ALT     QUAL    FILTER          INFO        FORMAT      HG00097
2       136220992       .       G       GT      30.64   .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:3,2:5
2       136226814       .       GAC     G       44.60   .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:4,2:6
2       136234279       .       C       T       102.60  .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:3,4:7
2       136234284       .       C       T       102.60  .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:3,4:7
2       136263277       .       T       A       148.60  .       AC=1;AF=0.500;AN=2     GT:AD:DP    0/1:8,5:13
…
…
```

Basic workflow, one sample



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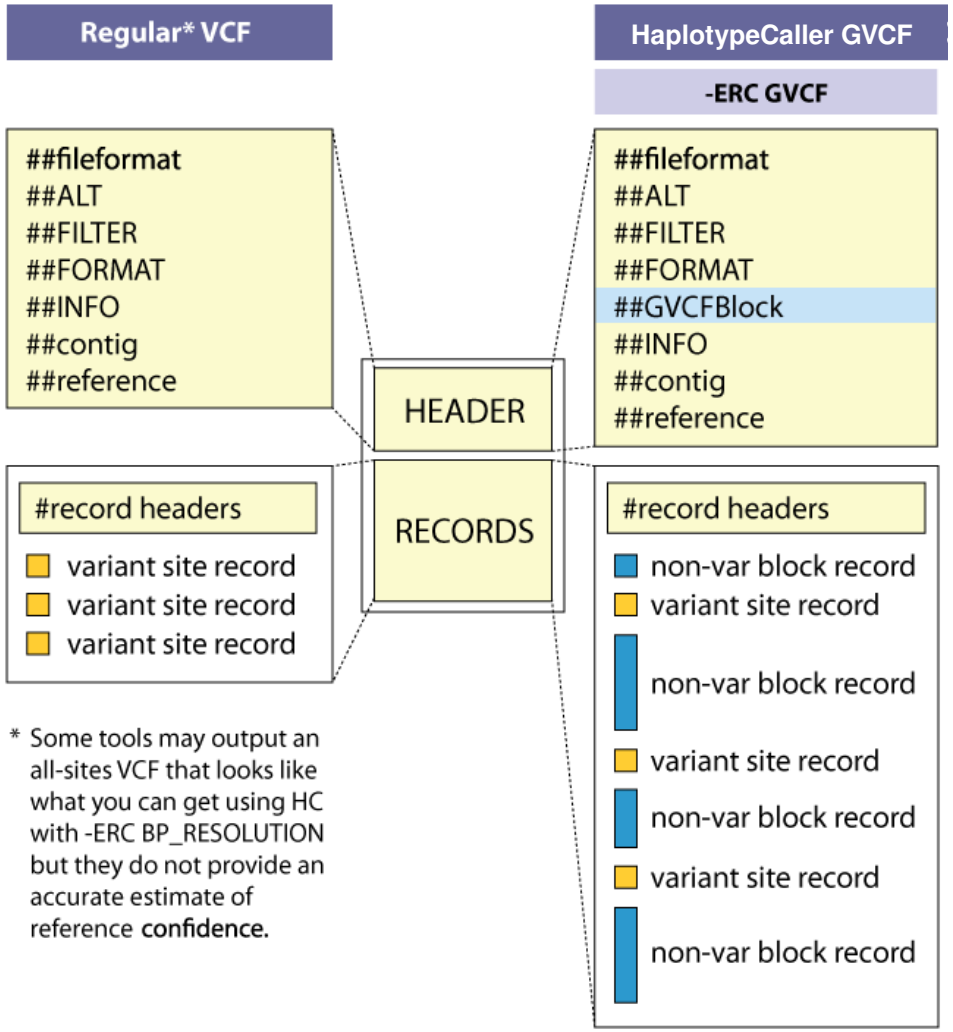
2       136226814       .       GAC     G       44.60   .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:4,2:6
2       136234279       .       C       T       102.60  .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:3,4:7
2       136234284       .       C       T       102.60  .       AC=1;AF=0.500;AN=2  GT:AD:DP    0/1:3,4:7
2       136263277       .       T       A       148.60  .       AC=1;AF=0.500;AN=2     GT:AD:DP    0/1:8,5:13
…
…
```

Basic variant calling in cohort

Basic variant calling in cohort

GVCF Files are valid VCFs with extra information



- GVCF has records for all sites, whether there is a variant call there or not.
- The records include an accurate estimation of how confident we are in the determination that the sites are homozygous-reference or not.
- Adjacent non-variant sites merged into blocks

Basic variant calling in cohort

Variant Call Format (VCF)

```
##fileformat=VCFv4.2
##ALT=<ID=NON_REF,Description="Represents any possible alternative allele at this location">
##FILTER=<ID=LowQual,Description="Low quality">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or with bad mates are filtered)">
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##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##contig=<ID=2,length=243199373>
##source=CombineGVCFs
##source=GenotypeGVCFs
##source=HaplotypeCaller
#CHROM  POS        ID  REF  ALT  QUAL    FILTER  INFO              FORMAT    HG00097     HG00100     HG00101
2       136045826  .   G    A    167.26  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:8,0:8   0/0:13,0:13 0/1:1,5:6
2       136046443  .   CGT  C    129.27  .       AC=3;AF=0.500;AN=6  GT:AD:DP  0/0:8,0:8   0/1:3,1:4   1/1:0,4:4
2       136047387  .   T    C    186.27  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:6,0:6   0/0:16,0:16 0/1:4,6:10
2       136048649  .   C    G    127.26  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:13,0:13 0/0:9,0:9   0/1:1,4:5
2       136052318  .   C    T    107.26  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:7,0:7   0/0:13,0:13 0/1:3,3:6
```

Today's lab

1000 Genomes data

- Low coverage WGS data
- 3 samples
- Small region on chromosome 2

About the samples:
https://www.internationalgenome.org/data-portal/sample

The Lactase enzyme

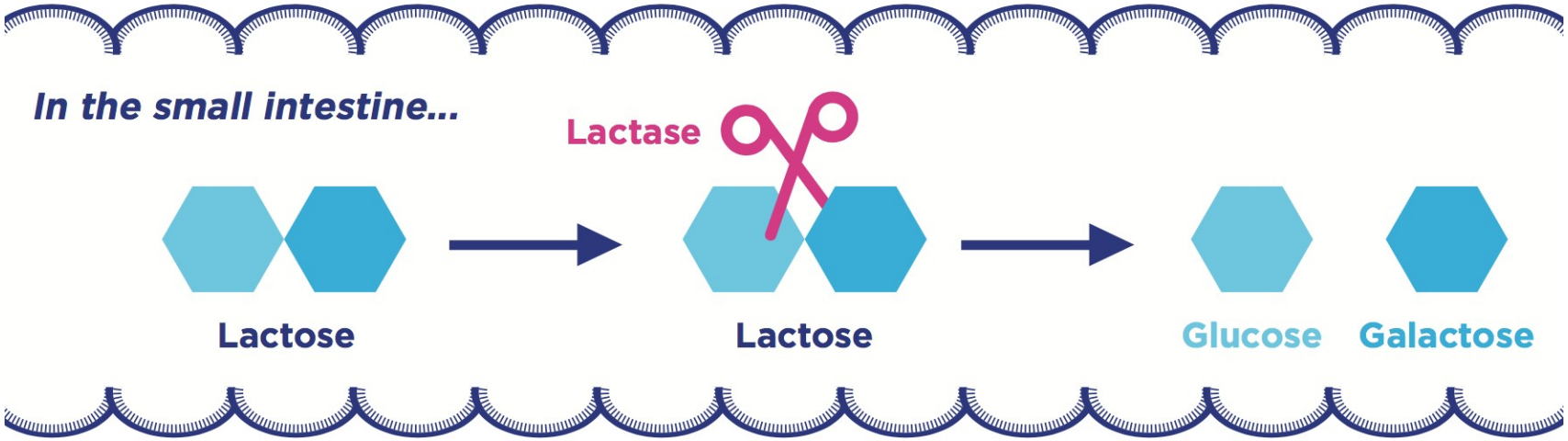
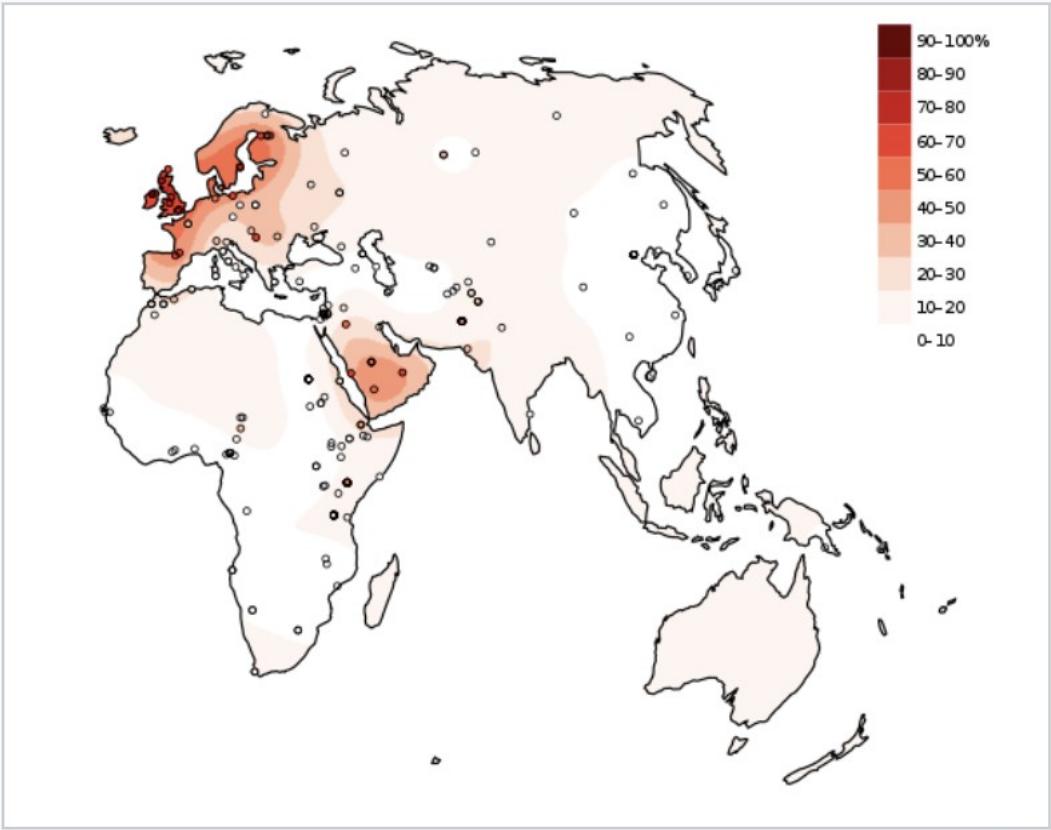


Figure 2. Lactose digestion in the intestine.

- All mammals produce lactase as infants
- Some human produce lactase in adulthood
- Genetic variation upstream of the *LCT* gene cause the lactase persistent phenotype (lactose tolerance)

The Lactase enzyme



Percentage of adults with a known lactase persistence genotype in the indigenous population of the Old World

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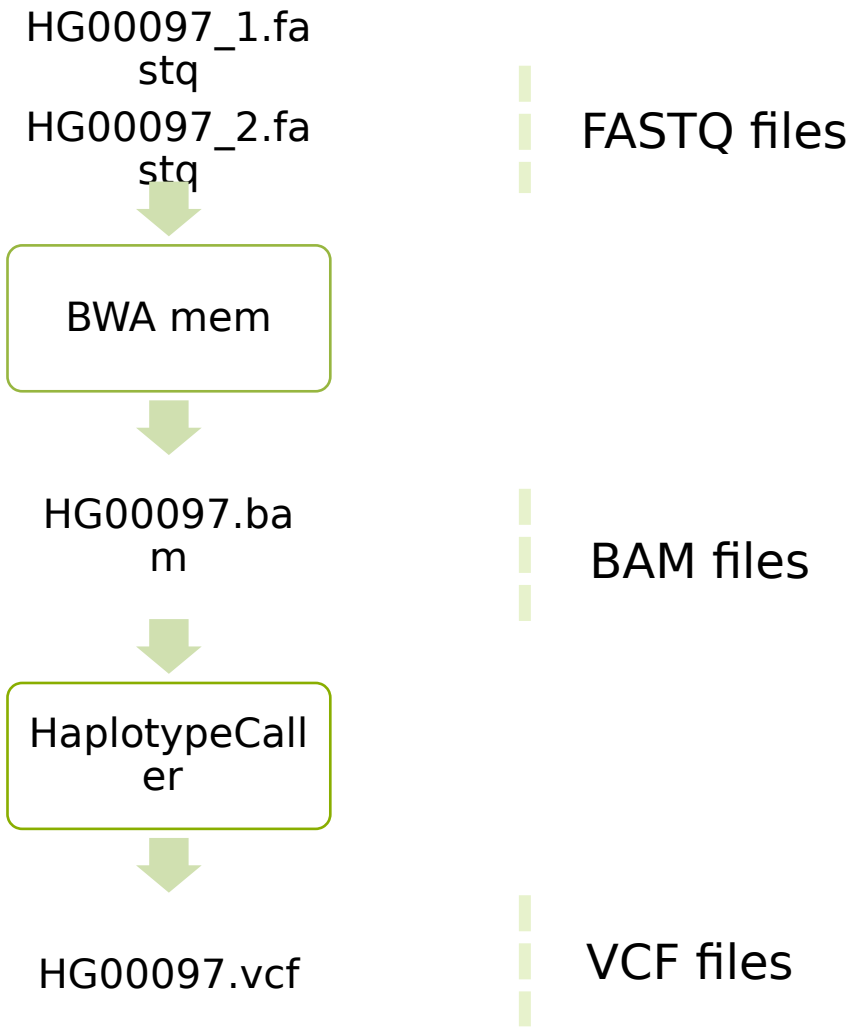
Part 1:

Variant calling in one sample

Basic variant calling in one sample



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Part 2:

Variant calling in cohort

Joint variant calling workflow

Workflow conventions

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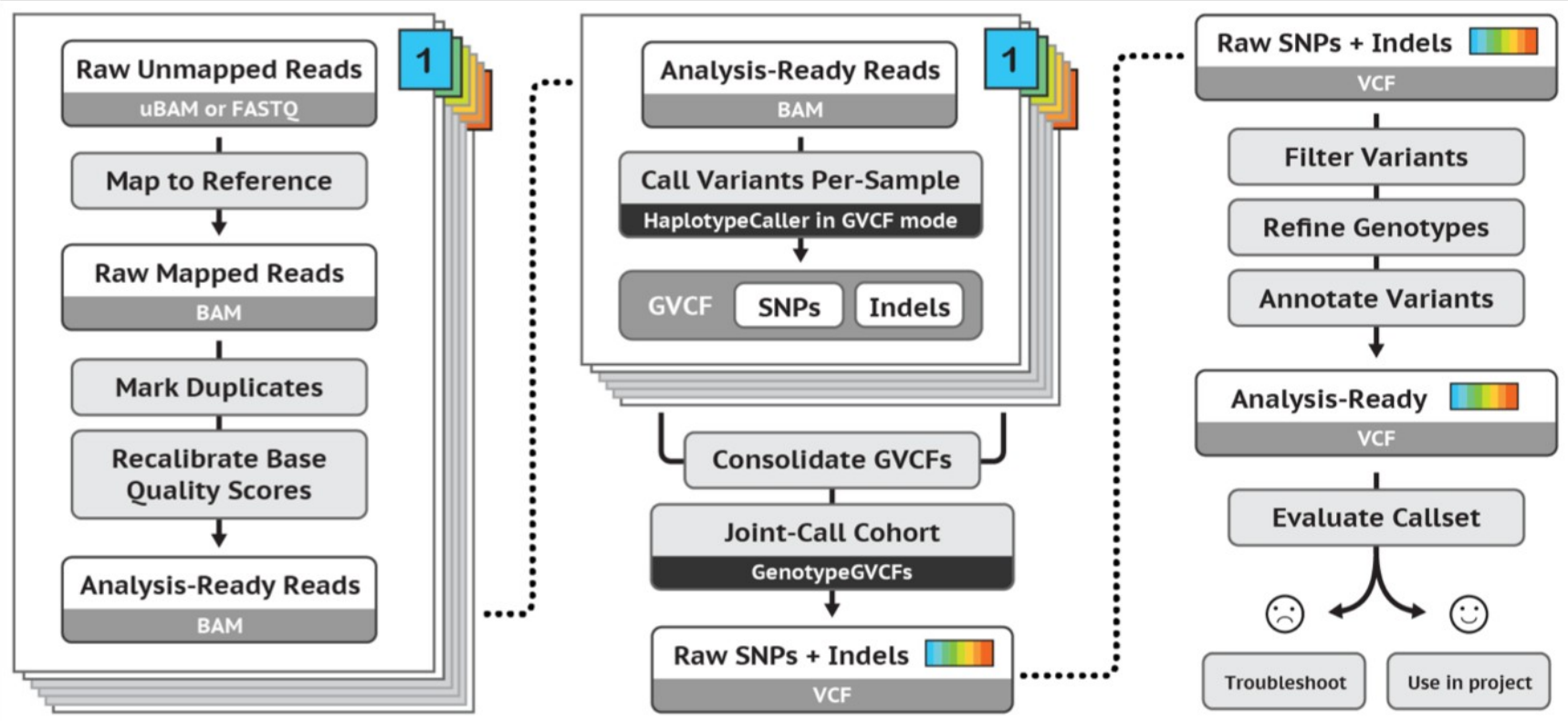
Part 3:

Follow GATK best practices for short variant discovery

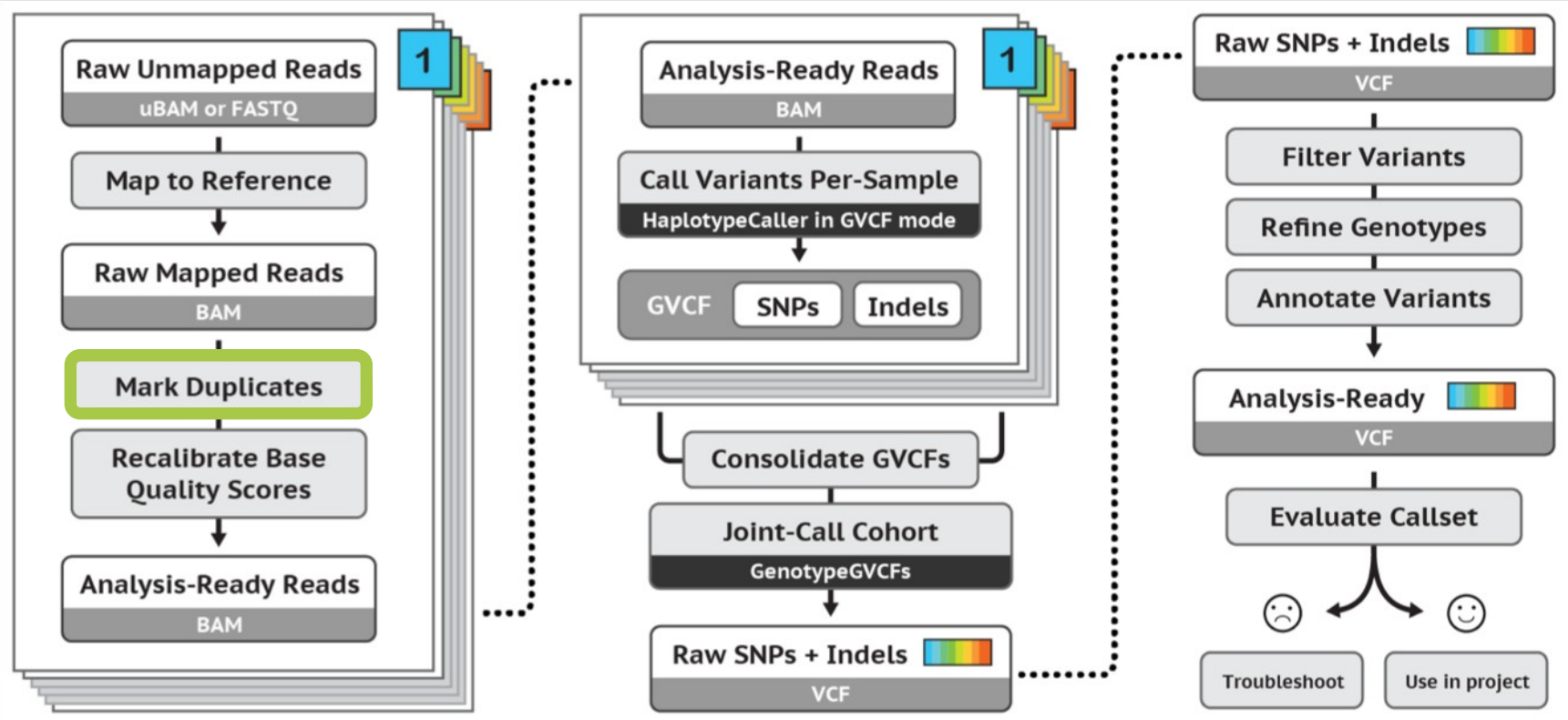
https://gatk.broadinstitute.org

GATK's best practices workflow for germline short variant discovery



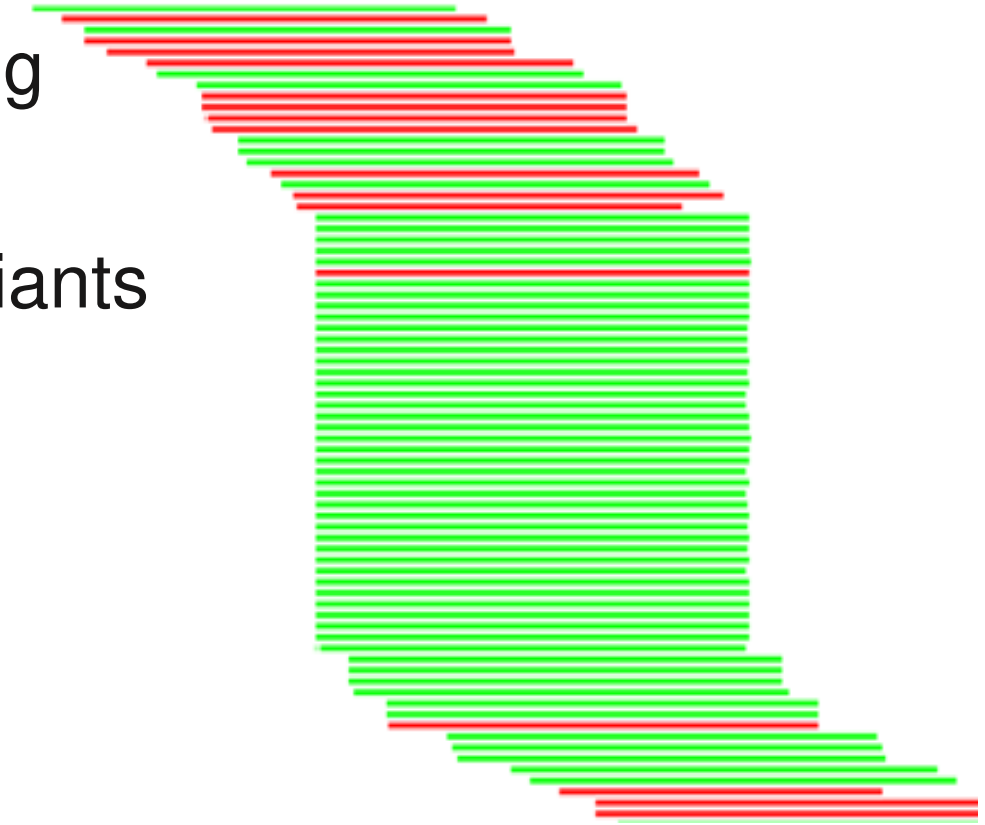
https://software.broadinstitute.org/gatk/best-practices/

Mark Duplicates



Duplicate reads

- PCR duplicates - library preparation
- Optical duplicates - sequencing
- Don’t add unique information
- Gives false allelic ratios of variants
- Should be removed/marked



Need Help?

Search our documentation

MarkDuplicates

MarkDuplicates (Picard) Follow

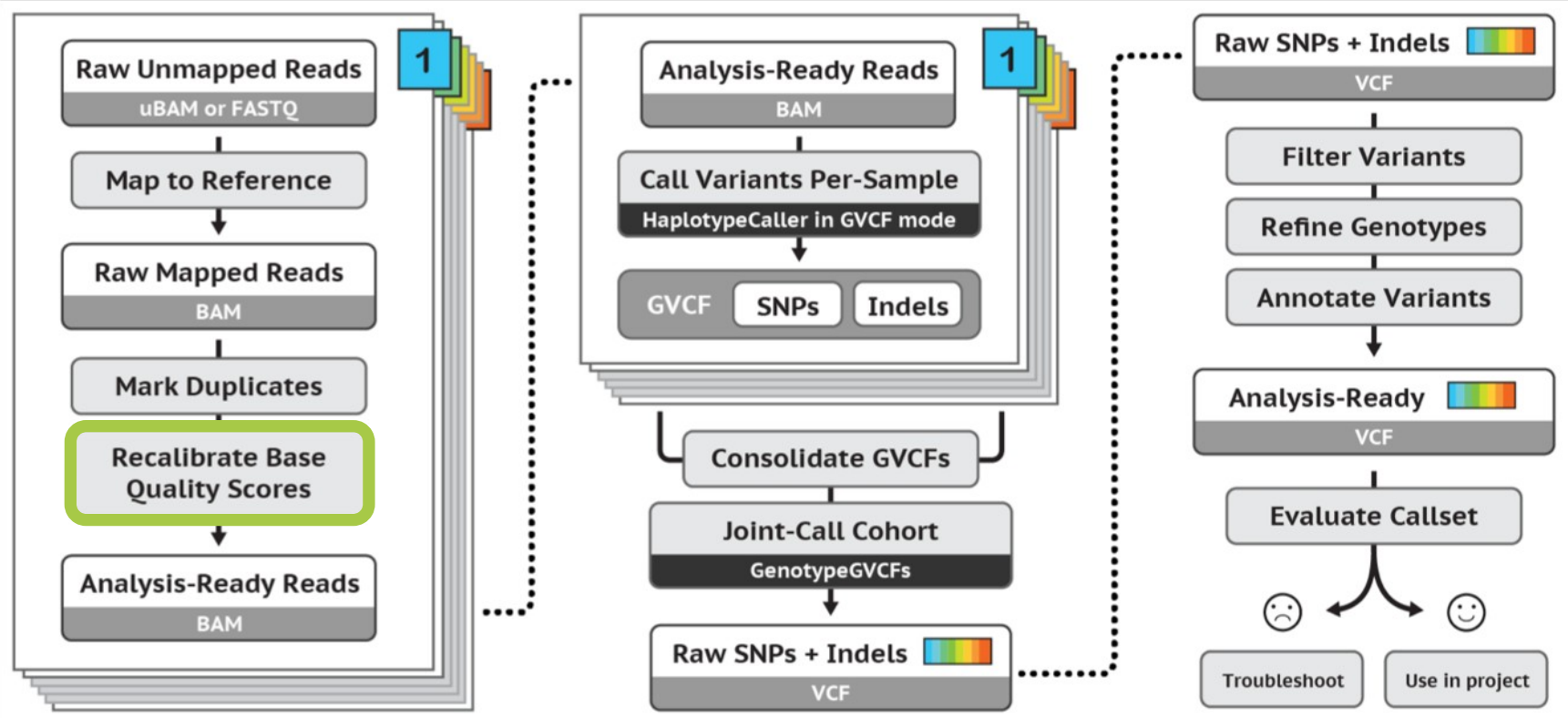
GATK Team
10 months ago · Updated

Identifies duplicate reads.

This tool locates and tags duplicate reads in a BAM or SAM file, where duplicate reads are defined as originating from a single fragment of DNA. Duplicates can arise during sample preparation e.g. library construction using PCR. See also EstimateLibraryComplexity for additional notes on PCR duplication artifacts. Duplicate reads can also result from a single amplification cluster, incorrectly detected as multiple clusters by the optical sensor of the sequencing instrument. These duplication artifacts are referred to as optical duplicates.

```
gatk --java-options -Xmx7g MarkDuplicates \
      -I input.bam \
      -O marked_duplicates.bam \
      -M marked_dup_metrics.txt
```

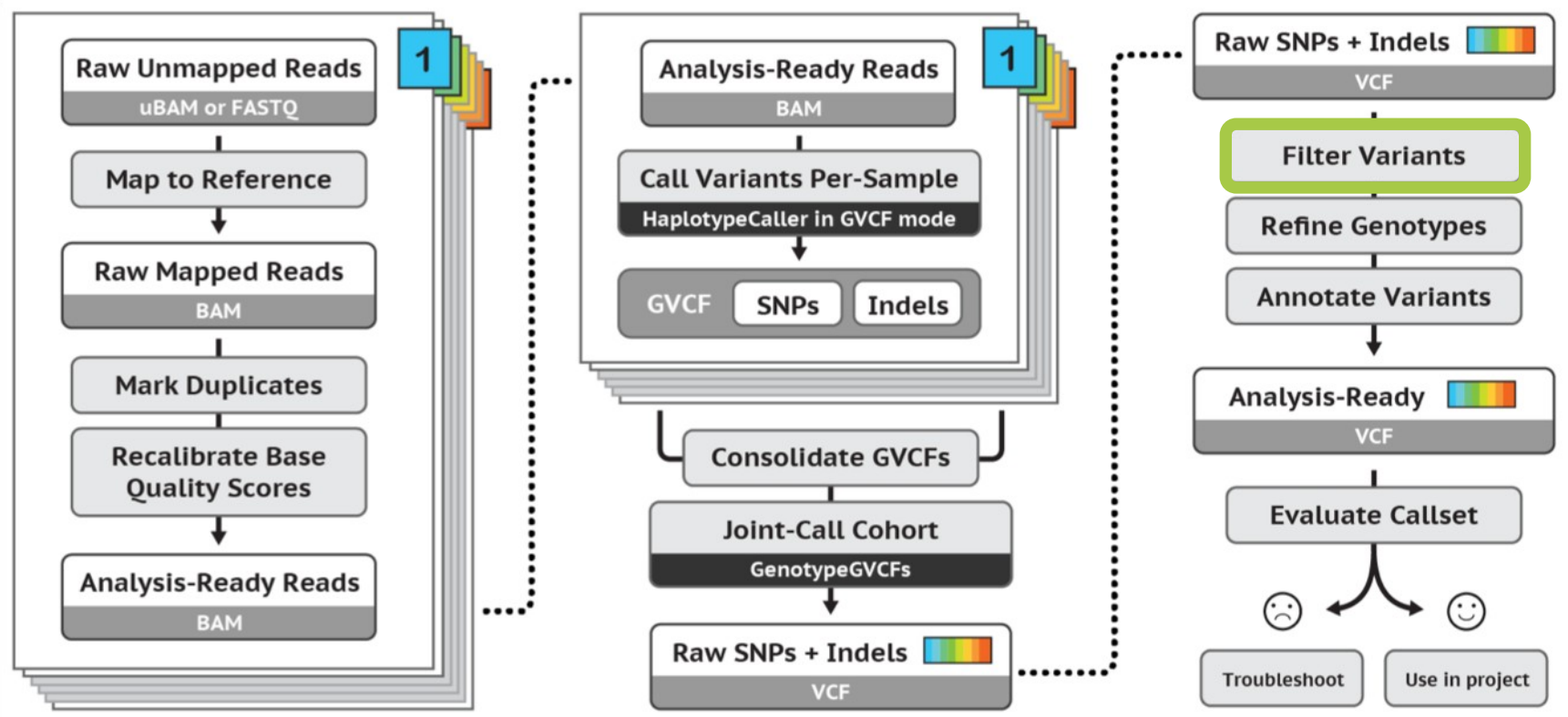
Base Quality Score Recalibration (BQSR)



Base Quality Score Recalibration (BQSR)

1. During base calling, the sequencer estimates a quality score for each base. This is the quality scores present in the fastq files.
2. Systematic (non-random) errors in the base quality score estimation can occur.
   - due to the physics or chemistry of the sequencing reaction
   - manufacturing flaws in the equipment
   - etc
3. Can cause bias in variant calling
4. **Base Qualtiy Score Recalibration** helps to calibrate the scores so that they correspond to the real per-base sequencing error rate (phred scores)

Filter variants



https://software.broadinstitute.org/gatk/best-practices/

Germline short variant discovery (SNPs + Indels)

Variant Call Format (VCF)

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##source=HaplotypeCaller
#CHROM  POS        ID  REF  ALT  QUAL    FILTER  INFO              FORMAT    HG00097     HG00100     HG00101
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2       136046443  .   CGT  C    129.27  .       AC=3;AF=0.500;AN=6  GT:AD:DP  0/0:8,0:8   0/1:3,1:4   1/1:0,4:4
2       136047387  .   T    C    186.27  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:6,0:6   0/0:16,0:16 0/1:4,6:10
2       136048649  .   C    G    127.26  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:13,0:13 0/0:9,0:9   0/1:1,4:5
2       136052318  .   C    T    107.26  .       AC=1;AF=0.167;AN=6  GT:AD:DP  0/0:7,0:7   0/0:13,0:13 0/1:3,3:6
```

Filtering

Variant quality score recalibration (VQSR):

- For large data sets (>1 WGS or >30WES samples)
- GATK has a machine learning algorithm that can be trained to recognise ”likely false” variants
- **We do recommend to use VQSR when possible!**

Hard filters:

- For smaller data sets
- Hard filters on information in the VCF file
- For example: Flag variants with ”Q < 40.0”

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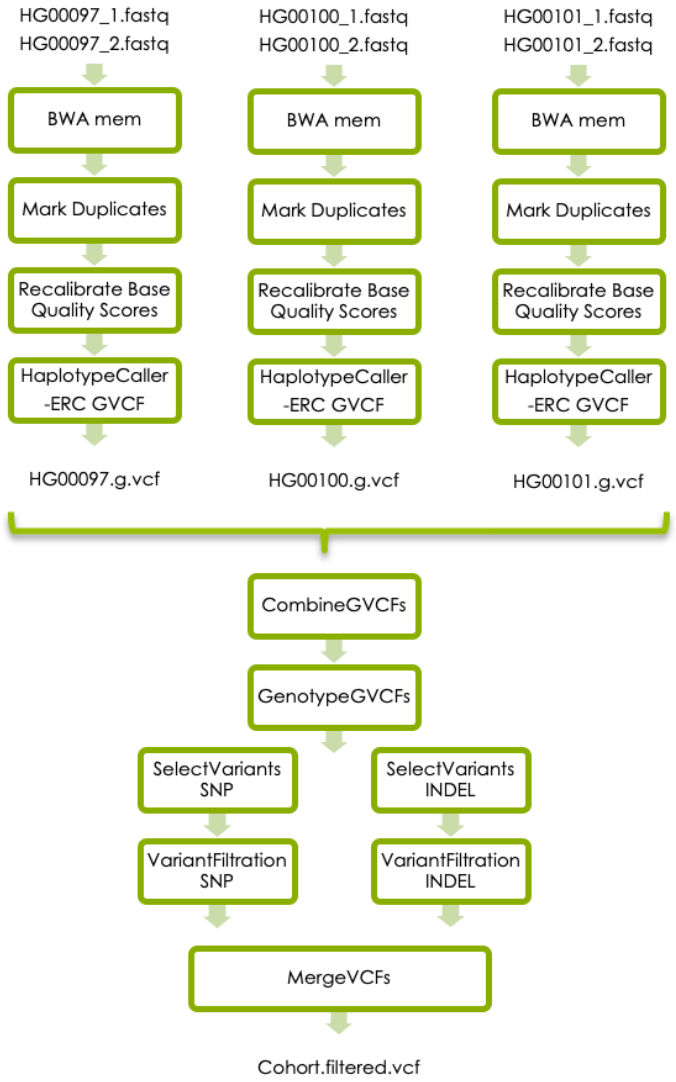
GATK's best practises



https://gatk.broadinstitute.org

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