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NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

SciLifeLab

Variant Calling Workflows

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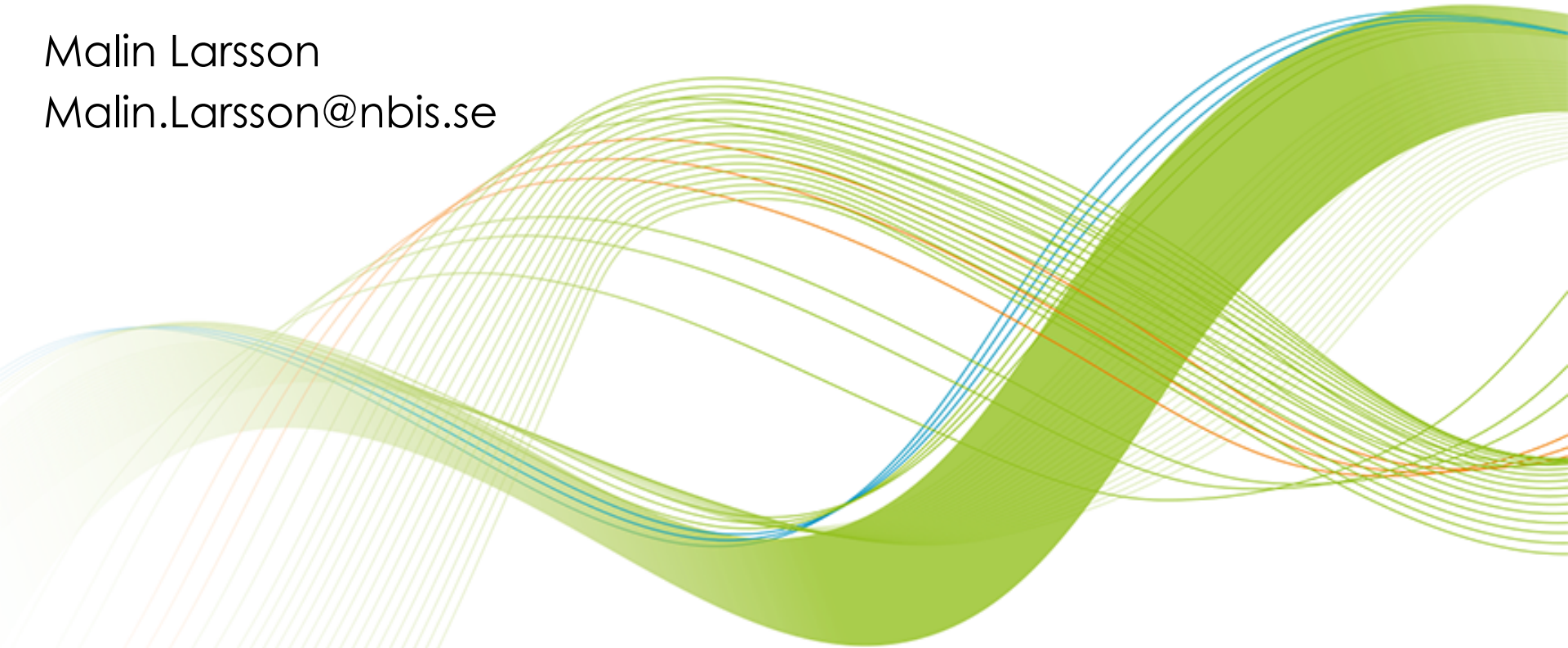


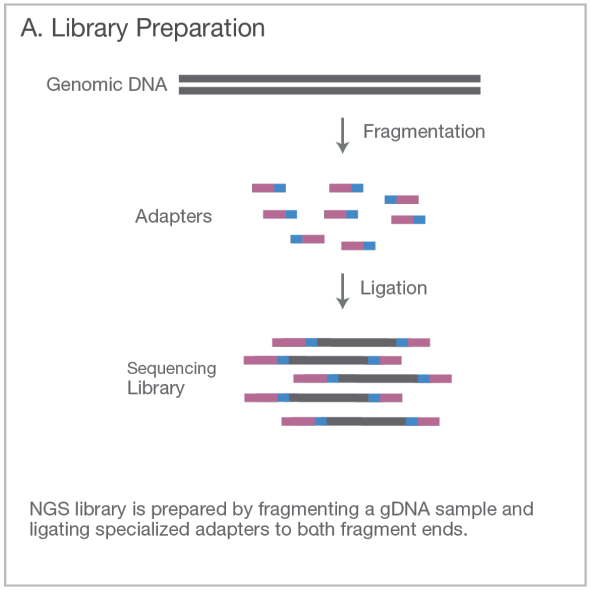
Overview

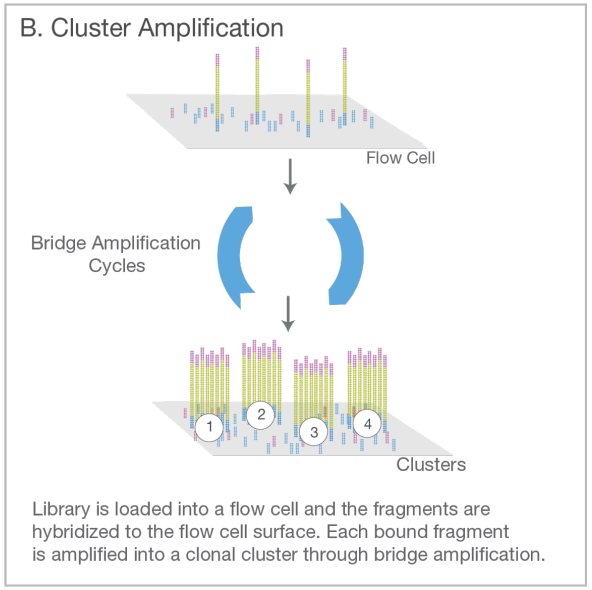
- Workflows
- Basic variant calling in one sample
- Basic variant calling in cohort
- Introduction to exercise

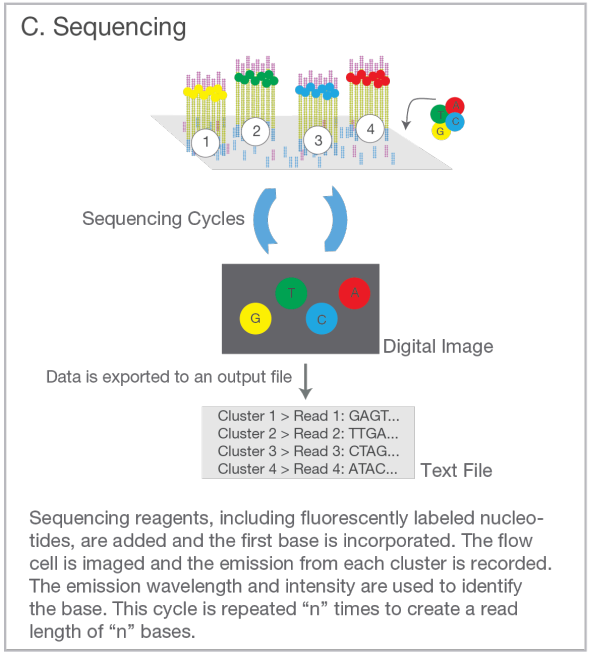
In separate talk Thursday at 9:

- GATK's Best practices

Illumina Sequencing







https://www.youtube.com/watch?v=fCd6B5HRaZ8

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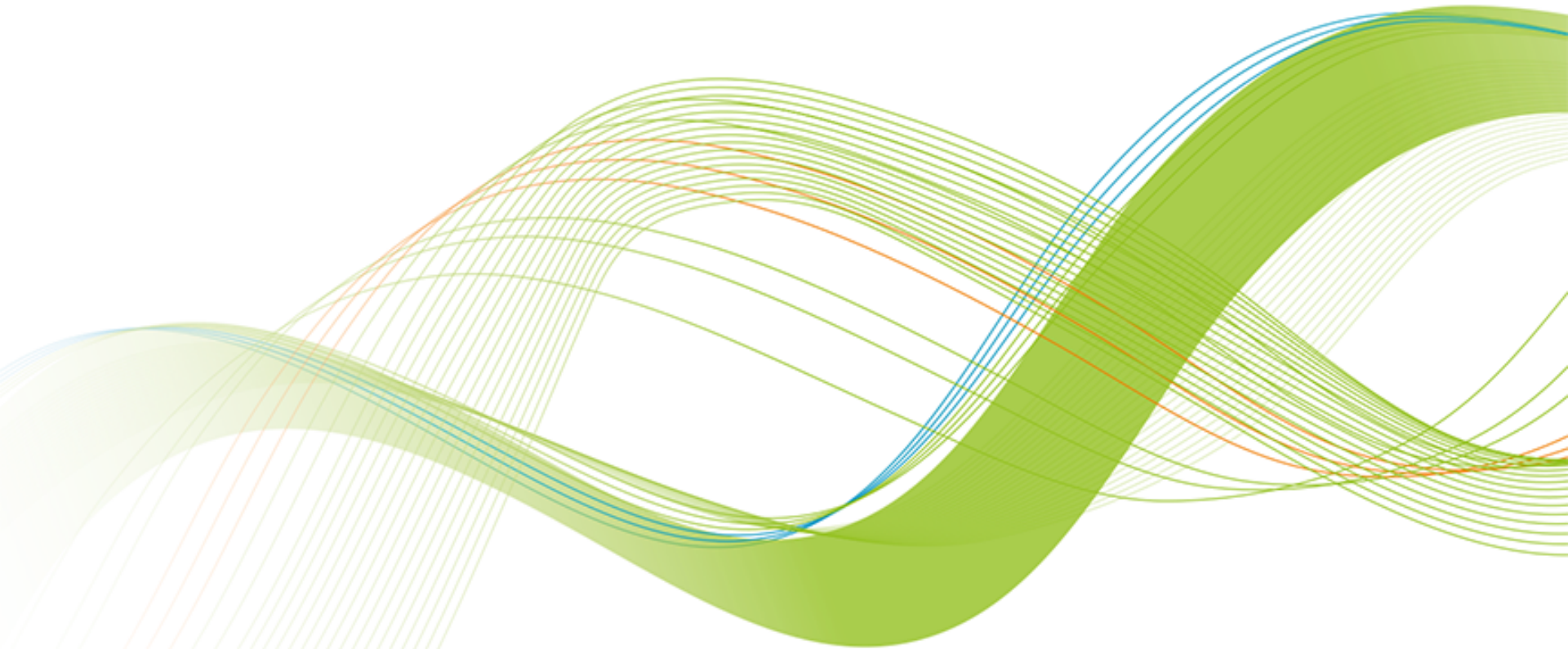
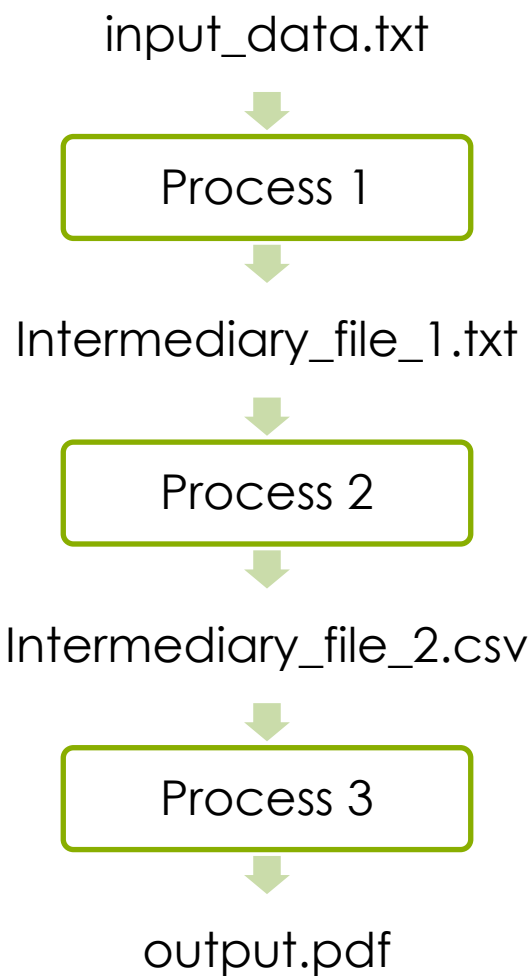
Workflows



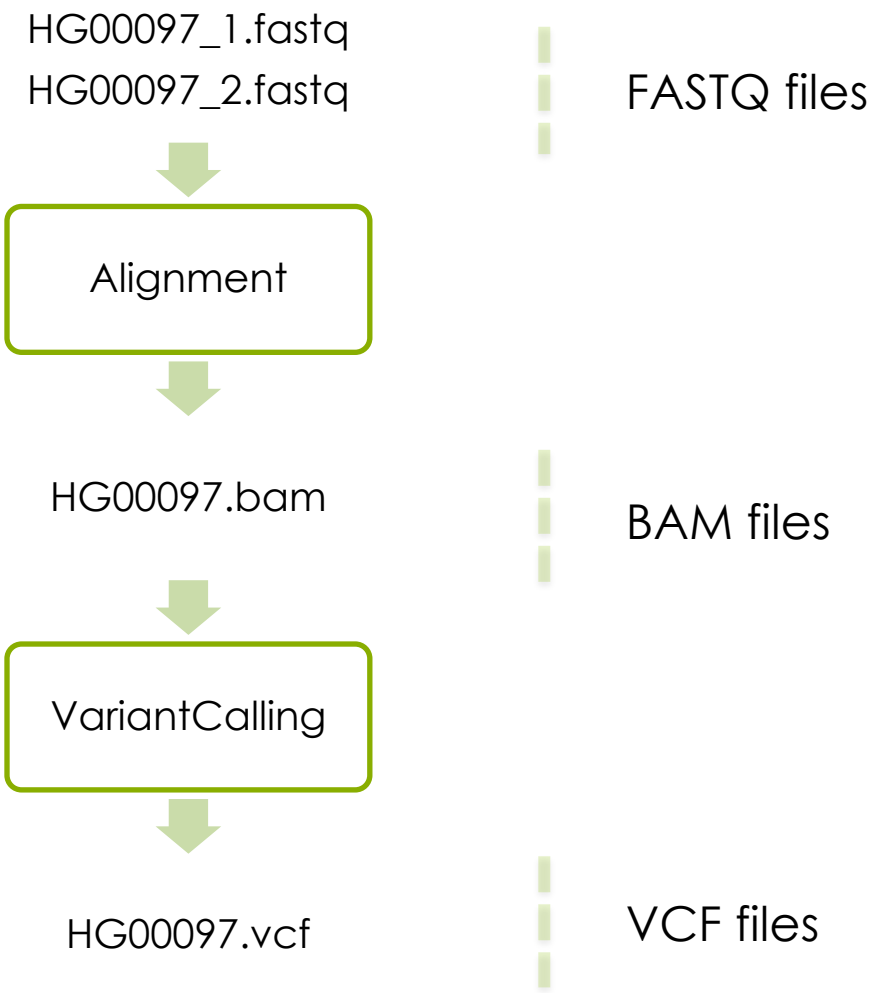
What is a workflow



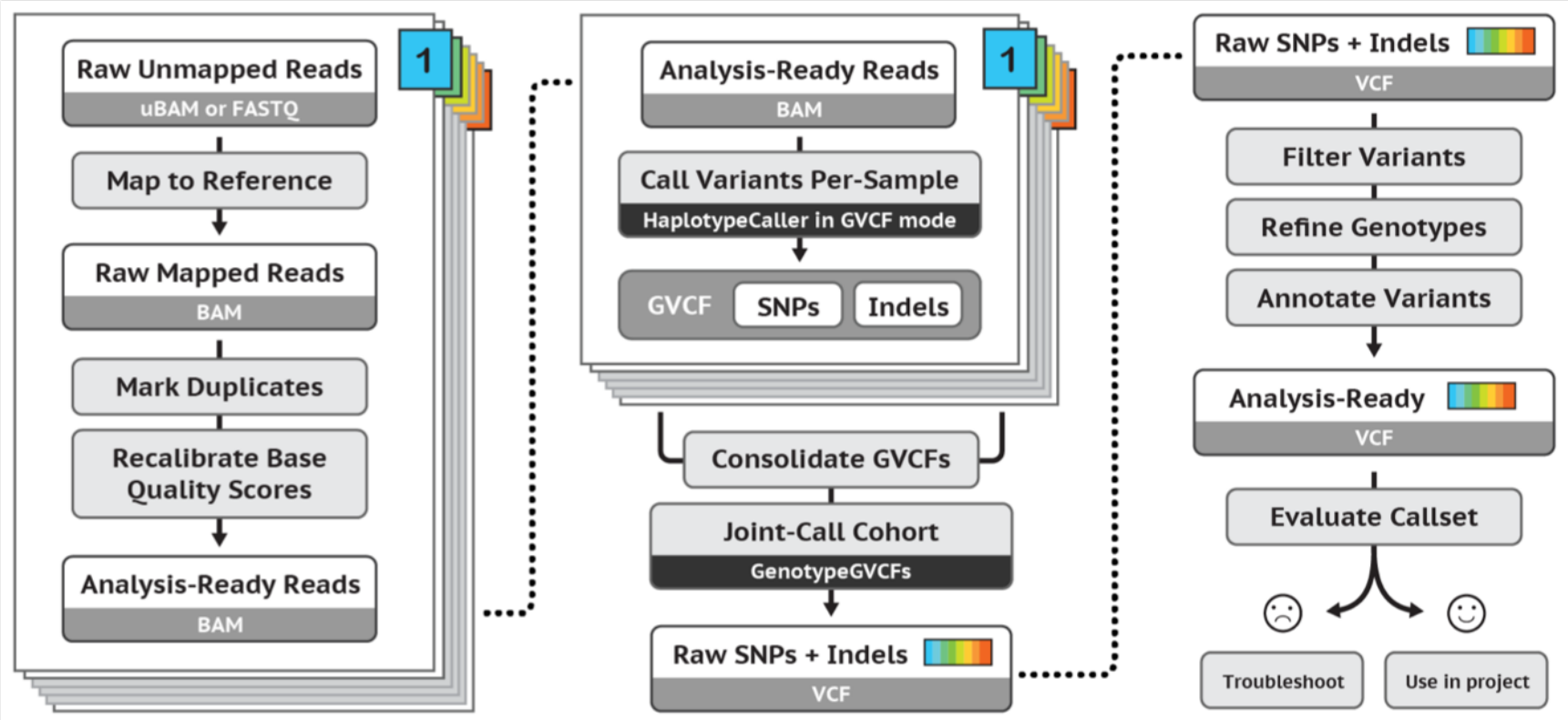
Workflow conventions

- Create a new output file in each process – don't owerwrite the input file
- Use informative file names
- Include information of the process in output file name

Example: Basic variant calling in one sample



GATK's best practices workflow for germline short variant discovery



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

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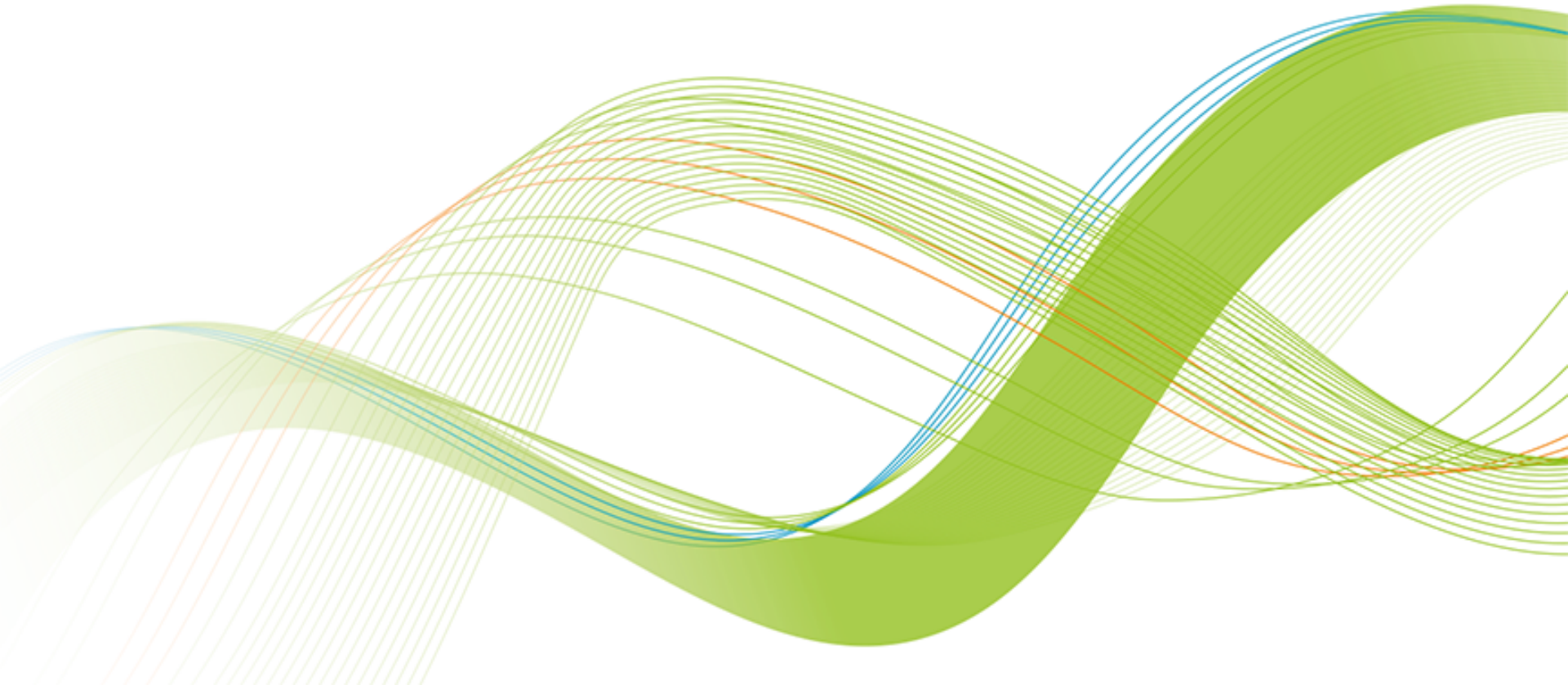
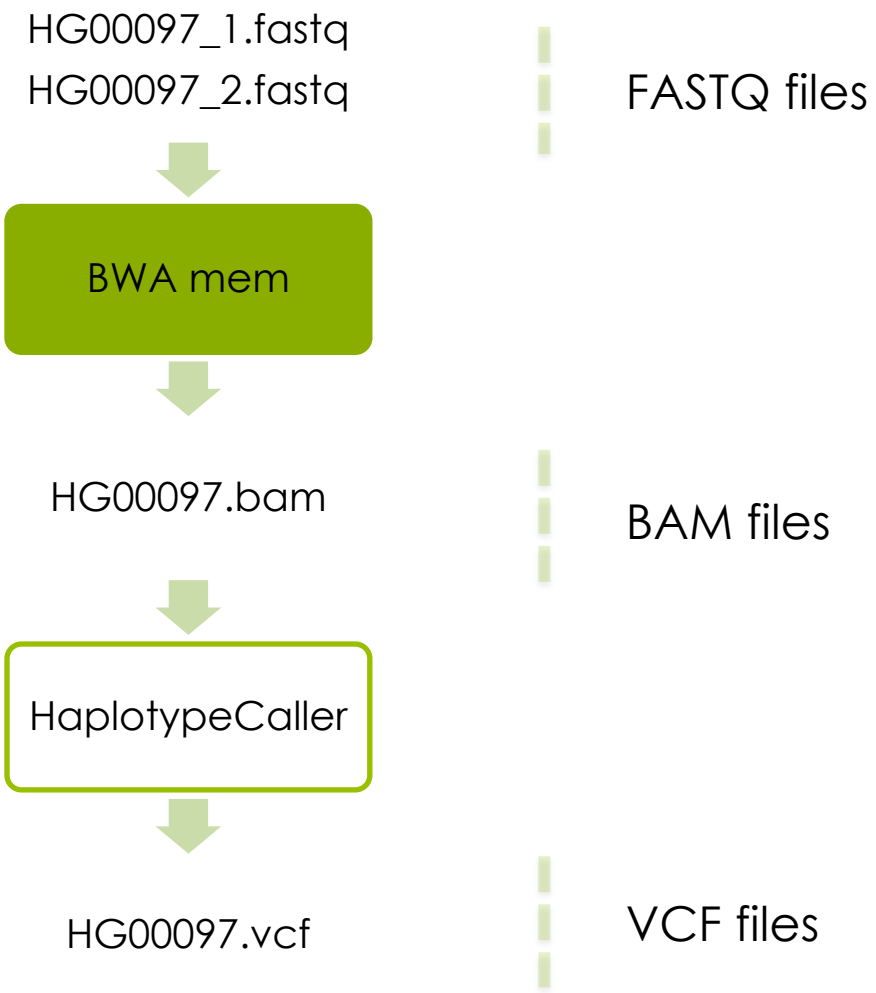
Basic Variant Calling in one sample



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.

HG19: 250 gaps

HG38 is the latest version of the human reference genome, but we will work with HG19.

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.

File Indices

- Most large files we work with, such as the reference genome, need an index
- Allows efficient random access
- Different indices for different file-types
- Bwa index = Burrows-Wheeler transform of reference genome (several files)
- Needs index: fasta, bam vcf files

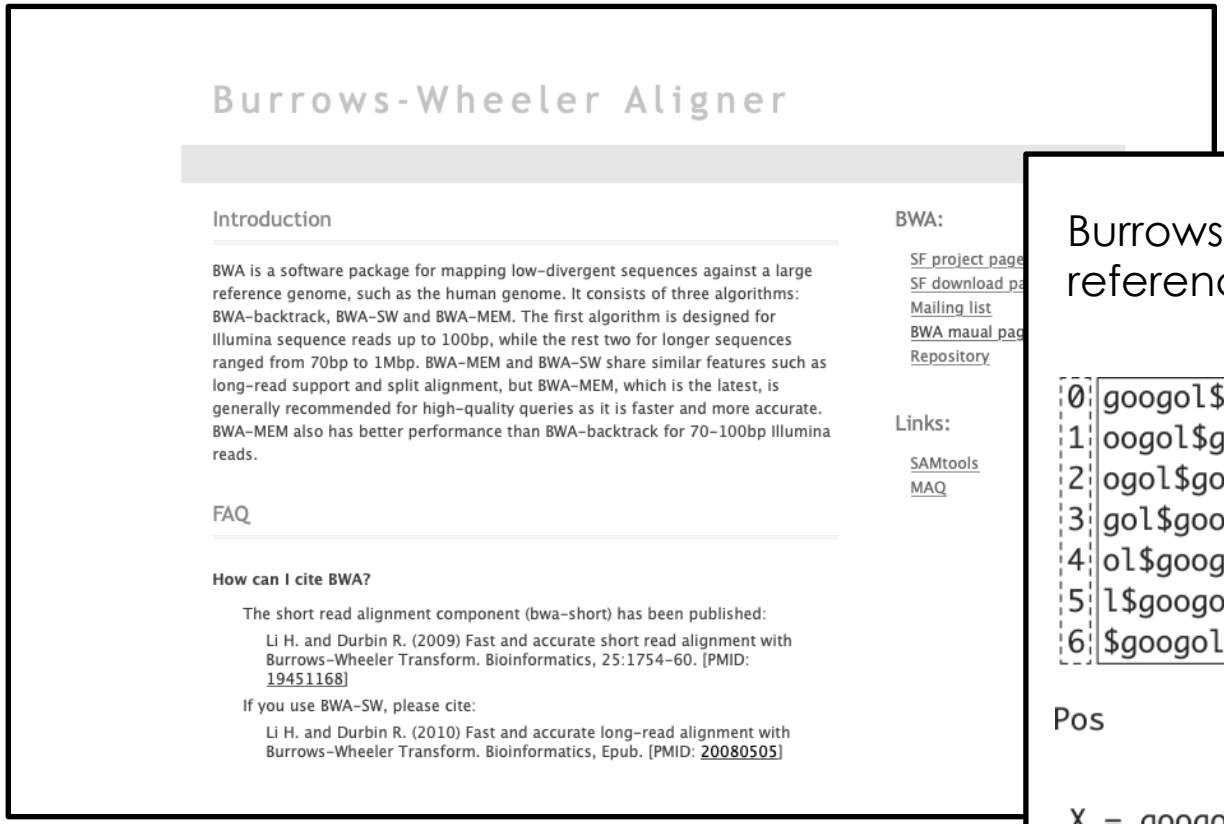
Alignment

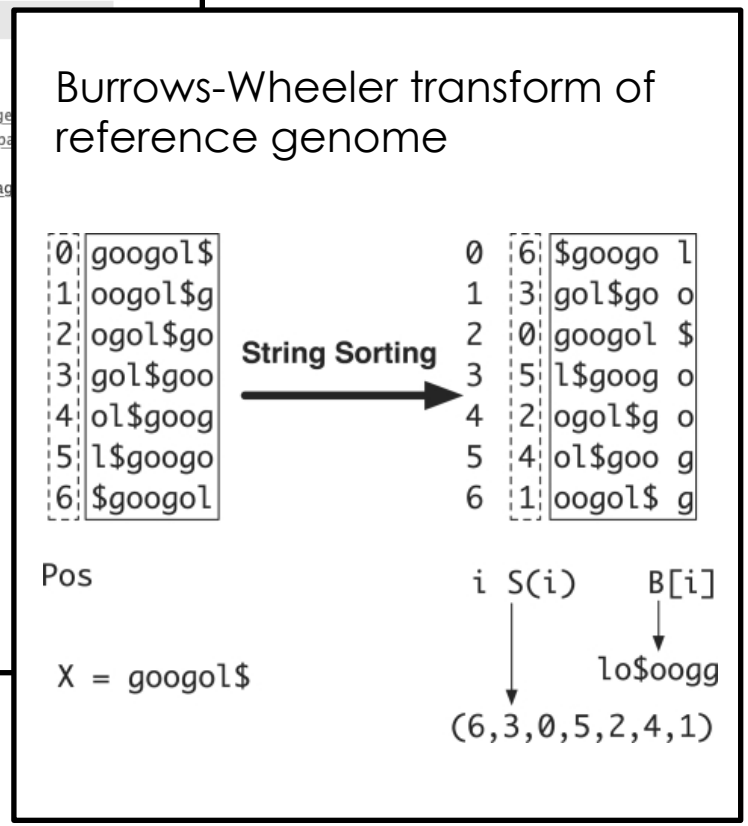
```
module load bwa
```

```
AACAGGTATATCTTCCCCGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTACCCTCTTCCTTAGGGACT
                                                GCTAGCTAGCTAGCTACCCT
```

Burrows-Wheeler Aligner

http://bio-bwa.sourceforge.net





Alignment

```
module load bwa
```

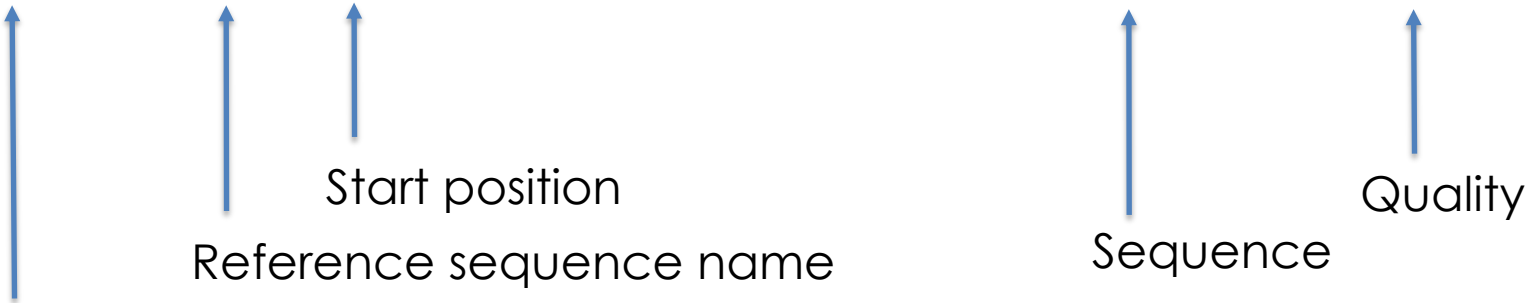
Output from mapping - Sam format

HEADER SECTION

```
@HD    VN:1.6SO:coordinate
@SQ    SN:2  LN:243199373
@PG    ID:bwaPN:bwaVN: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:bwaVN:1.10      CL:samtools sort
@PG    ID:samtools.1      PN: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
```



Convert to Bam

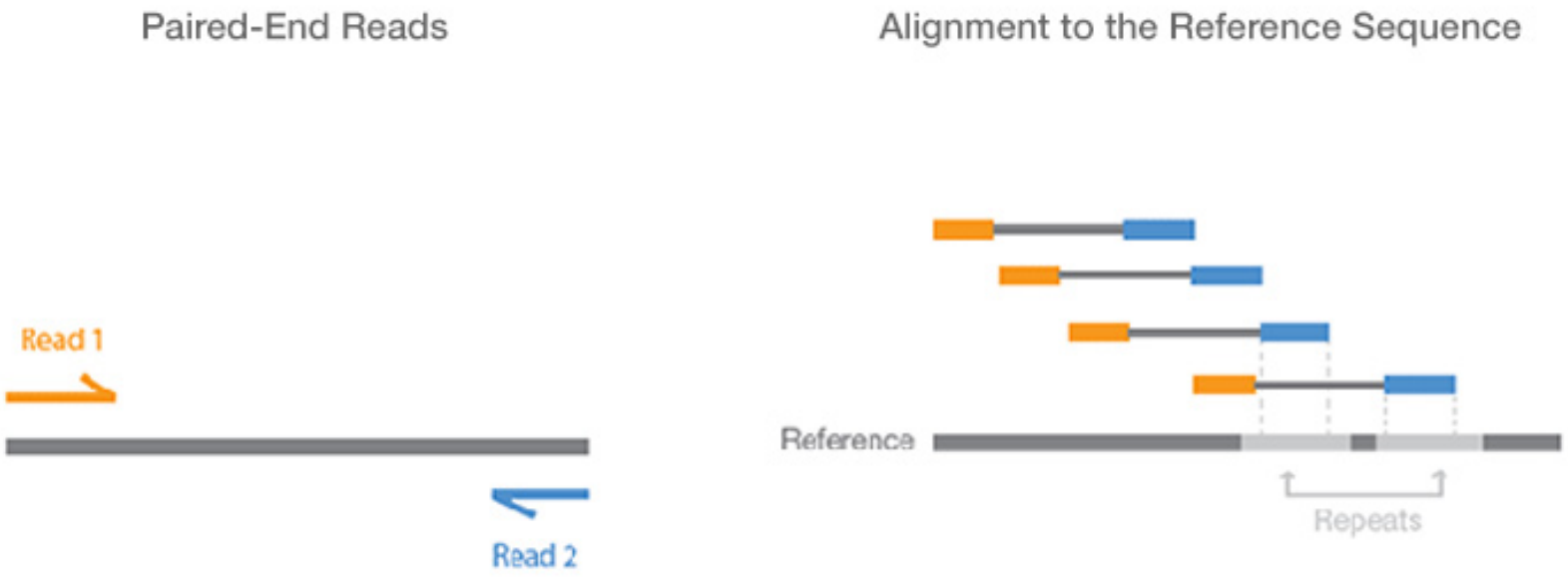
Bam file is a binary representation of the Sam file

Read groups

- Link *sample id*, *library prep*, *flowcell* and *sequencing run* to the reads.
- Good for error tracking!
- Often needed for variant calling
- Detailed description in tutorial or https://gatkforums.broadinstitute.org/gatk/discussion/6472/read-groups

RGID = *combination of the sample id and run id*
RGLB = Library prep
RGPL = Platform (for us ILLUMINA)
RGPU = Run identifier *usually barcode of flowcell*
RGSM = Sample name

Paired-End data



Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in much better alignment of the reads, especially across difficult-to-sequence, repetitive regions of the genome.

Paired-end data

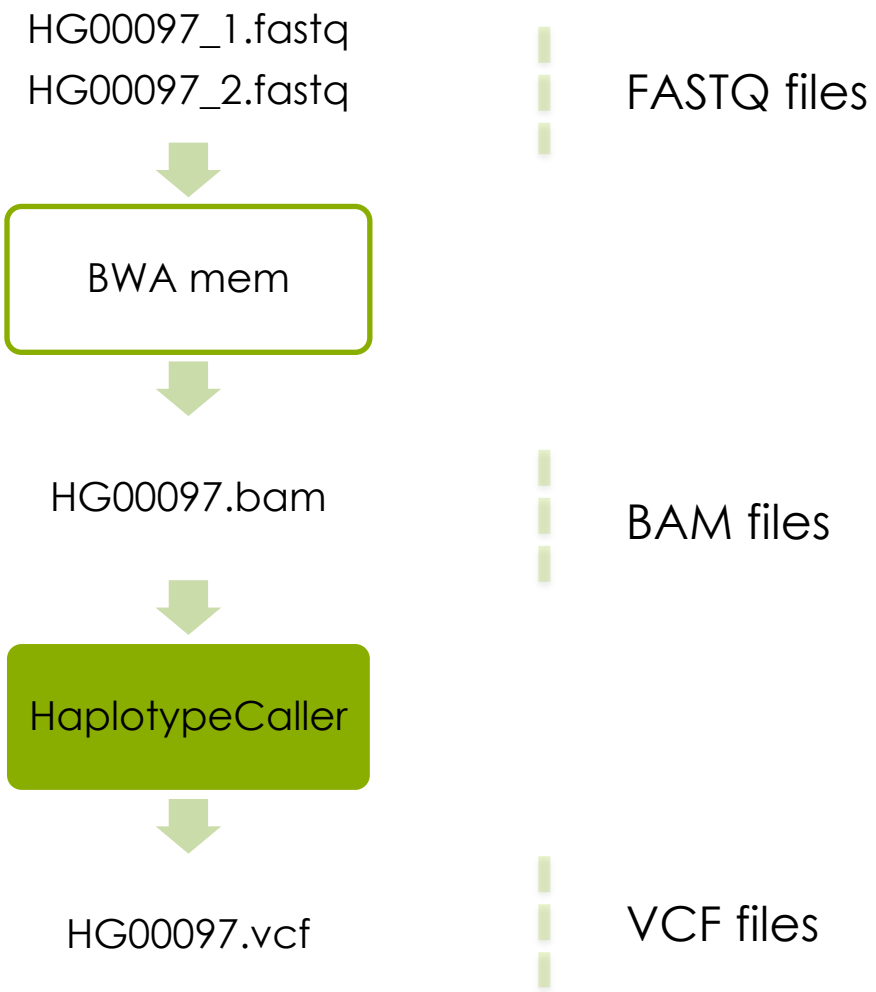
ID_R1_001.fastq

```
@HISEQ:100:C3MG8ACXX:5:1101:1160:2
197 1:N:0:ATCACG
CAGTTGCGATGAGAGCGTTGAGAAGTATAATAGG
AGTTAAACTGAGTAACAGGATAAGAAATAGTGAG
ATATGGAAACGTTGTGGTCTGAAAGAAGATGT
+
B@CFFFFFHHHHHGJJJJJJJJJJFHHIIIIJJ
JIHGIIJJJJIJIJIJJJJIIJJJJJIIEIHHIJ
HGHHHHHDFFFEDDDDDCDDDCDDDDDDDCDC
```

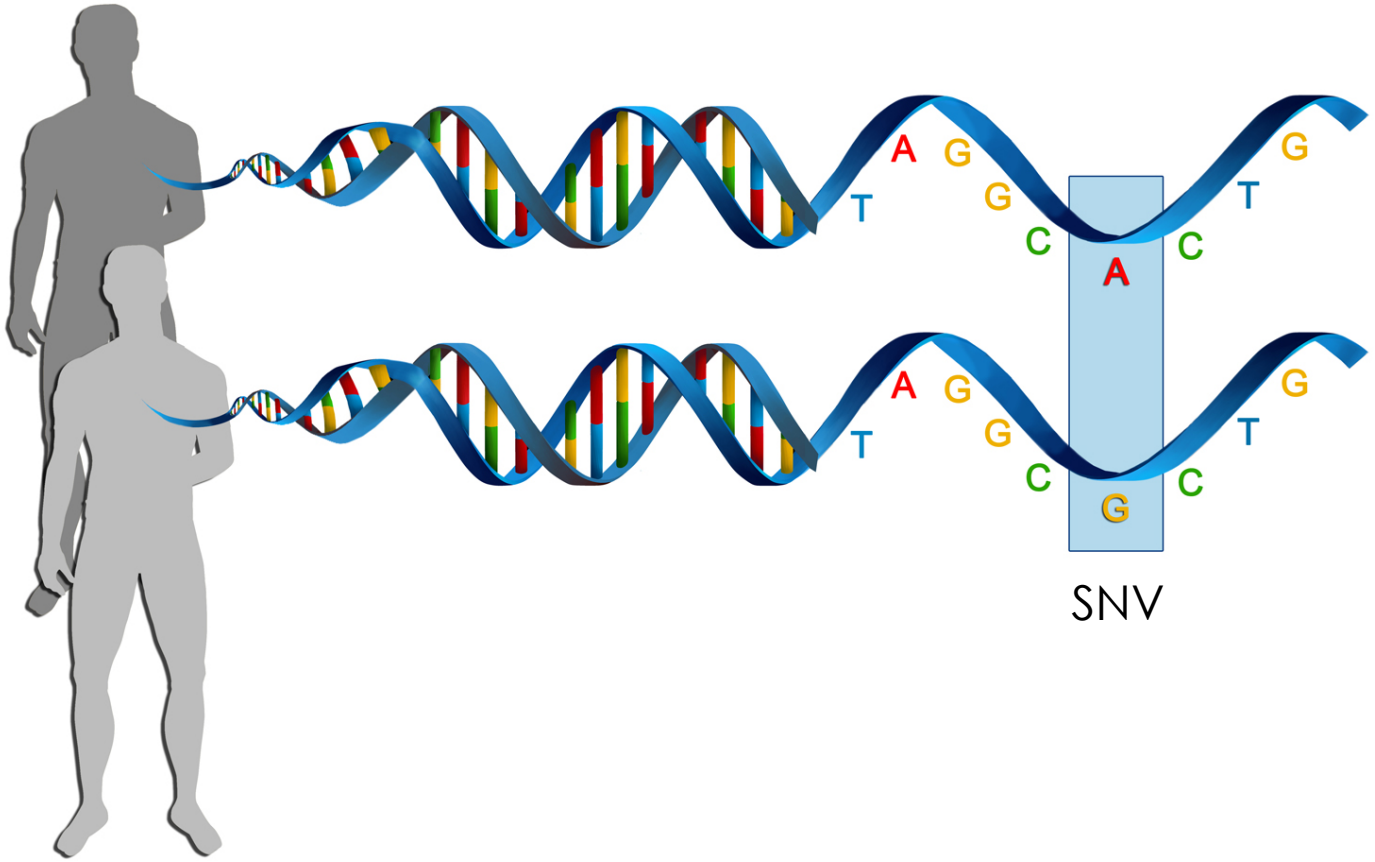
ID_R2_001.fastq

```
@HISEQ:100:C3MG8ACXX:5:1101:1160:
2197 2:N:0:ATCACG
CTTCGTCCACTTTCATTATTCCTTTCATACATG
CTCTCCGGTTTAGGGTACTCTTGACCTGGCCTT
TTTTCAAGACGTCCCTGACTTGATCTTGAAACG
+
CCCFFFFFHHHHHJJJJIJJJJJJJJJJJJJJ
JJJJJJJIJIJGIJHBGHHIIIJIJJJJJJJJI
JJJHFFFFFFDDDDDDDDDDDDDDDDEDCCDDDD
```

Variant calling



Genetic variation



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

Detecting variants in reads

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

Sample:
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...

Reference- and Alternatve Alleles

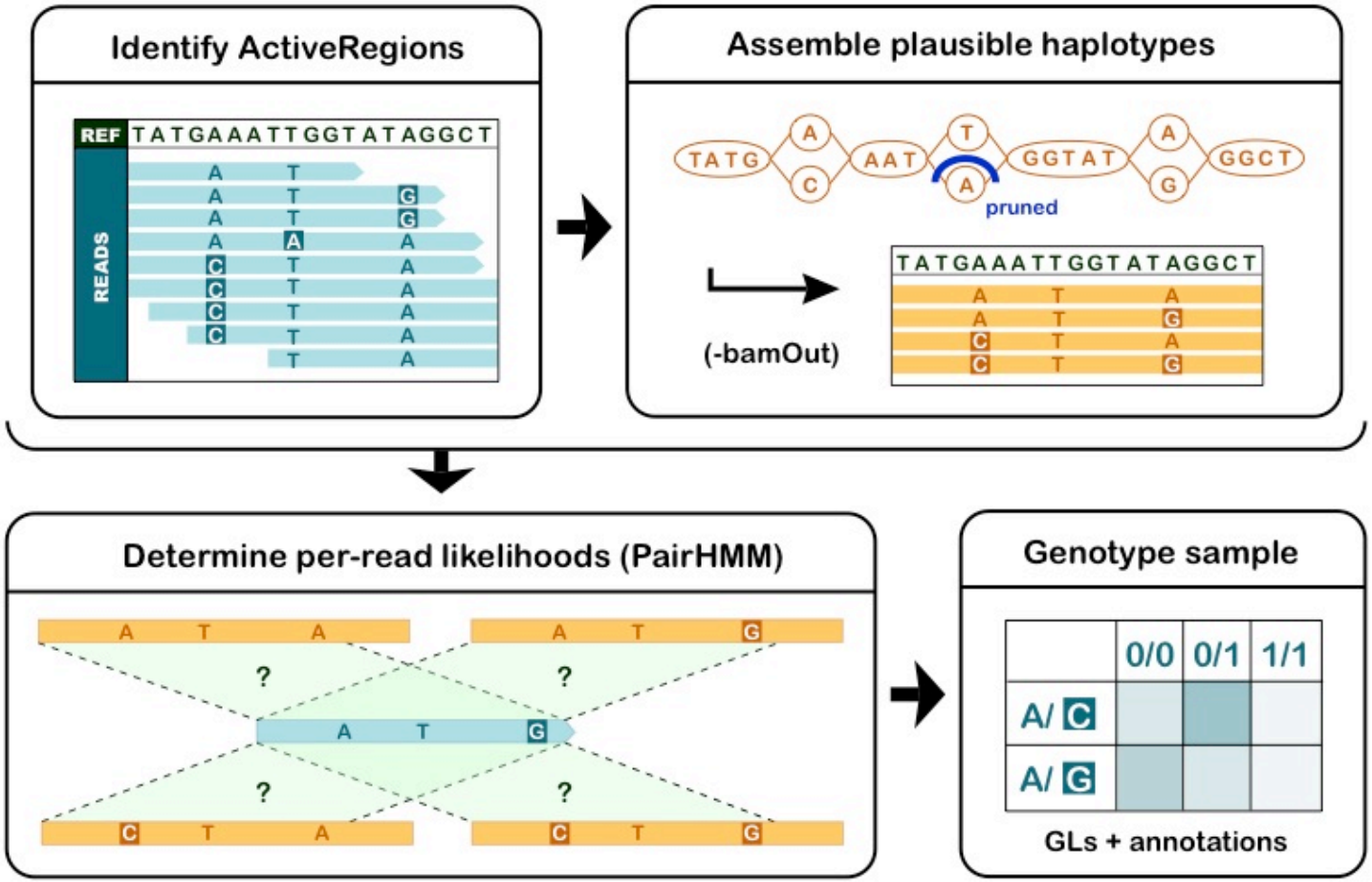
GGCTTTTCCAACAGGTATATCTTCCCCGCTAGCTAGCTAGCTACTTCAAAT

Reference allele AGCTAGCTA

Alternative allele AGCTGGCTA

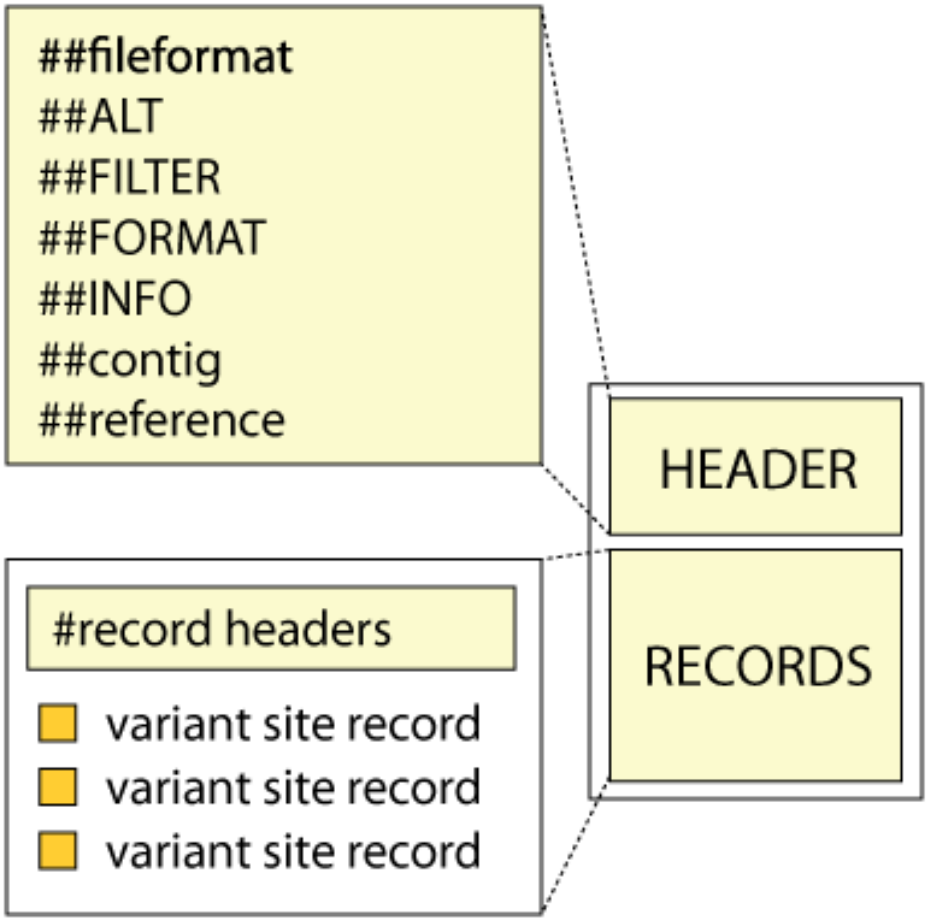
Reference allele = the allele in the refence genome
Alternative allele = the allele NOT in the refence genome

Variant Calling
HaplotypeCaller



For more info: https://www.youtube.com/watch?v=NQHGkVGICpY

Variant Call Format (VCF)



Variant Call Format (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”...
##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">
#CHROM POS     ID        REF ALT    QUAL FILTER INFO                              FORMAT      NA00001
20     14370   rs6054257 G   A      29   PASS   NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP  0|0:48:1
20     17330   .         T   A      3    q10    NS=3;DP=11;AF=0.017     GT:GQ:DP  0|0:49:3
20     1230237 .         T   .      47   PASS   NS=3;DP=13;AA=T         GT:GQ:DP  0|0:54:7
20     1234567 microsat1 GTC G,GTCT 50   PASS   NS=3;DP=9;AA=G          GT:GQ:DP  0|1:35:4
```

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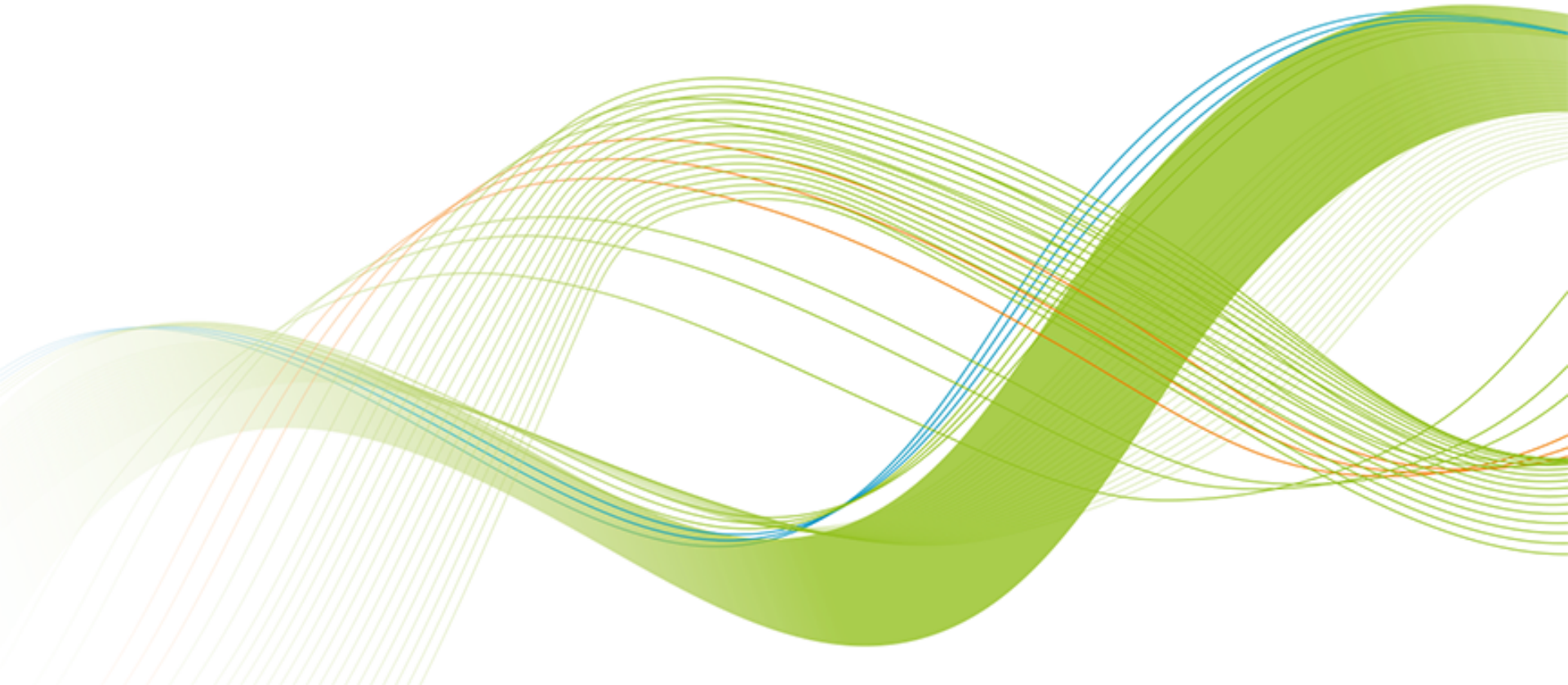
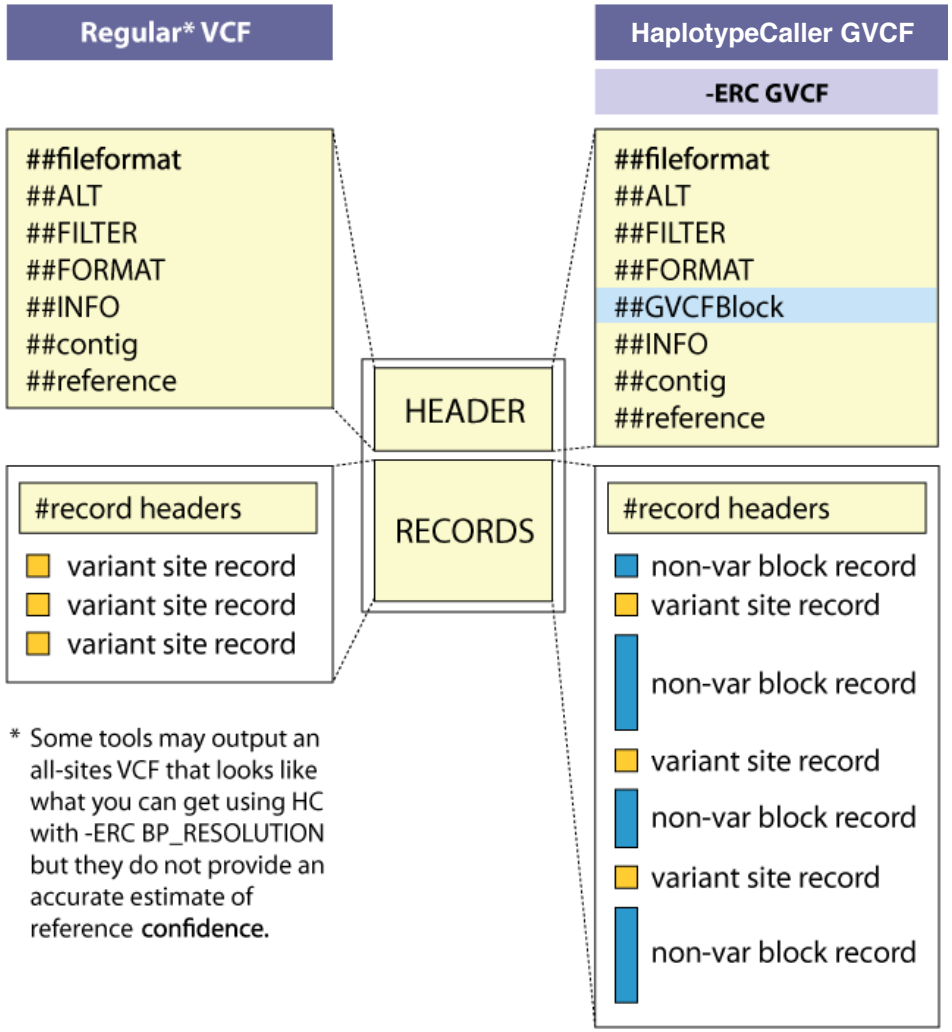
Variant calling in cohort



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=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens”...
##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">
```

#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	0|0:48:1	1|0:48:8	1|1:43:5
20	17330	.	T	A	3	q10	NS=3;DP=11;AF=0.017	GT:GQ:DP	0|0:49:3	0|1:3:5	0|0:41:3
20	1230237	.	T	.	47	PASS	NS=3;DP=13;AA=T	GT:GQ:DP	0|0:54:7	0|0:48:4	0|0:61:2
20	1234567	microsat1	GTC	G,GTCT	50	PASS	NS=3;DP=9;AA=G	GT:GQ:DP	0|1:35:4	0|2:17:2	1|1:40:3

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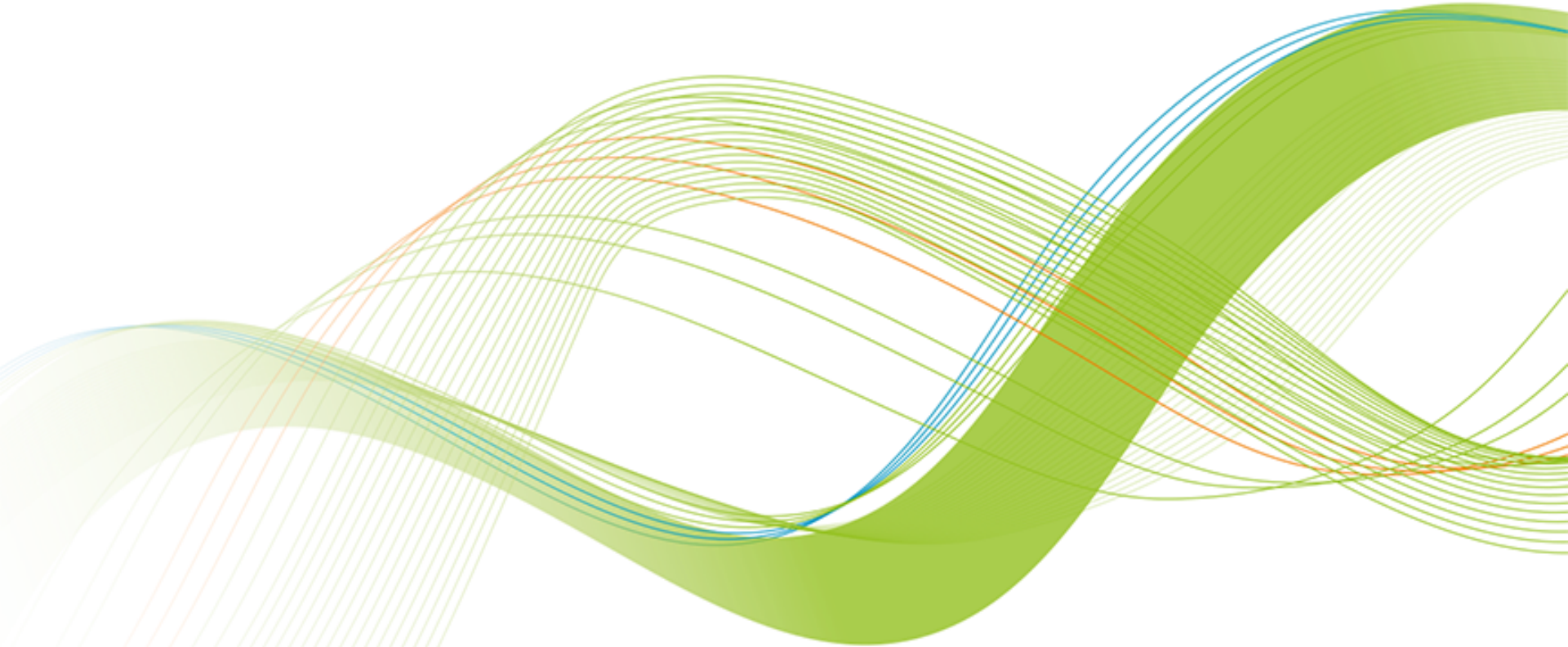
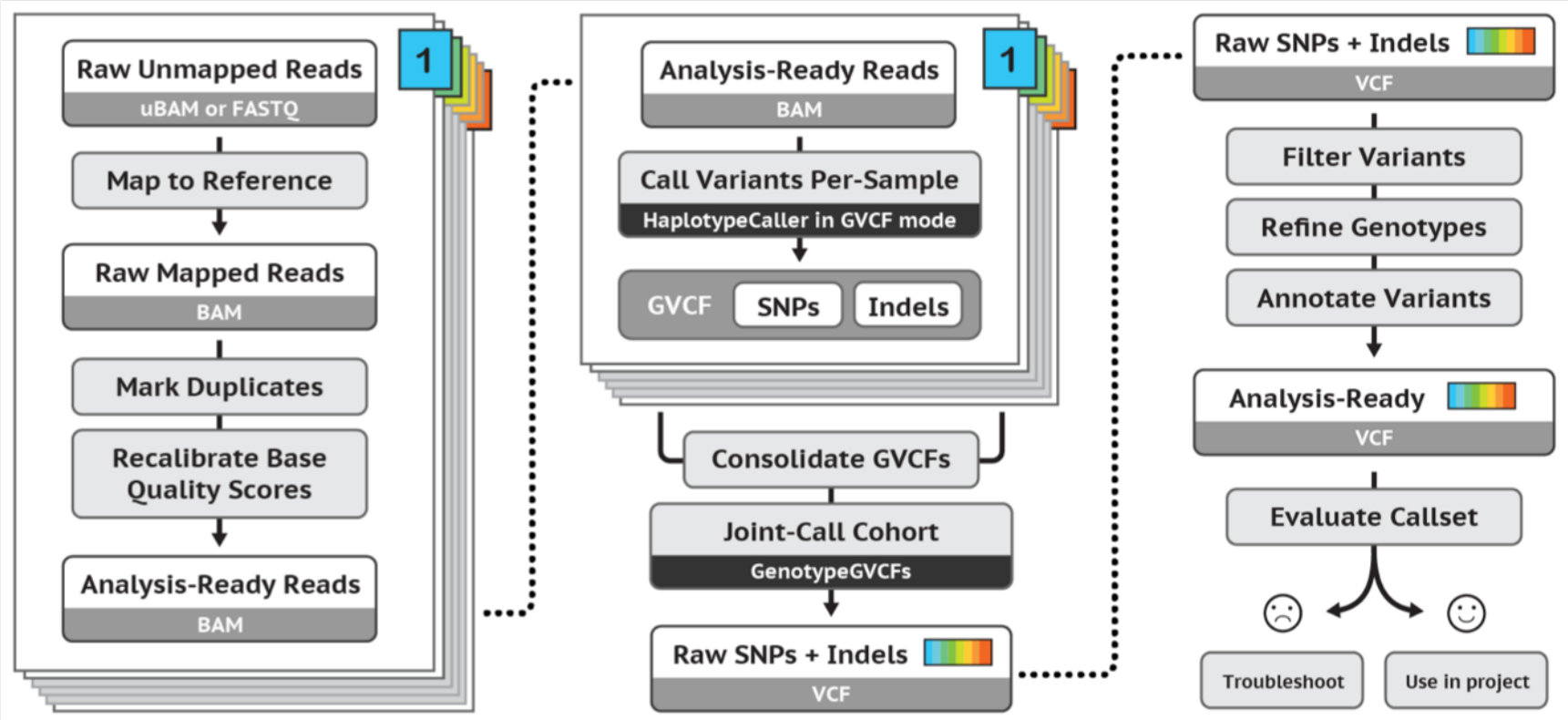
GATK's best practices for germline short variant discovery



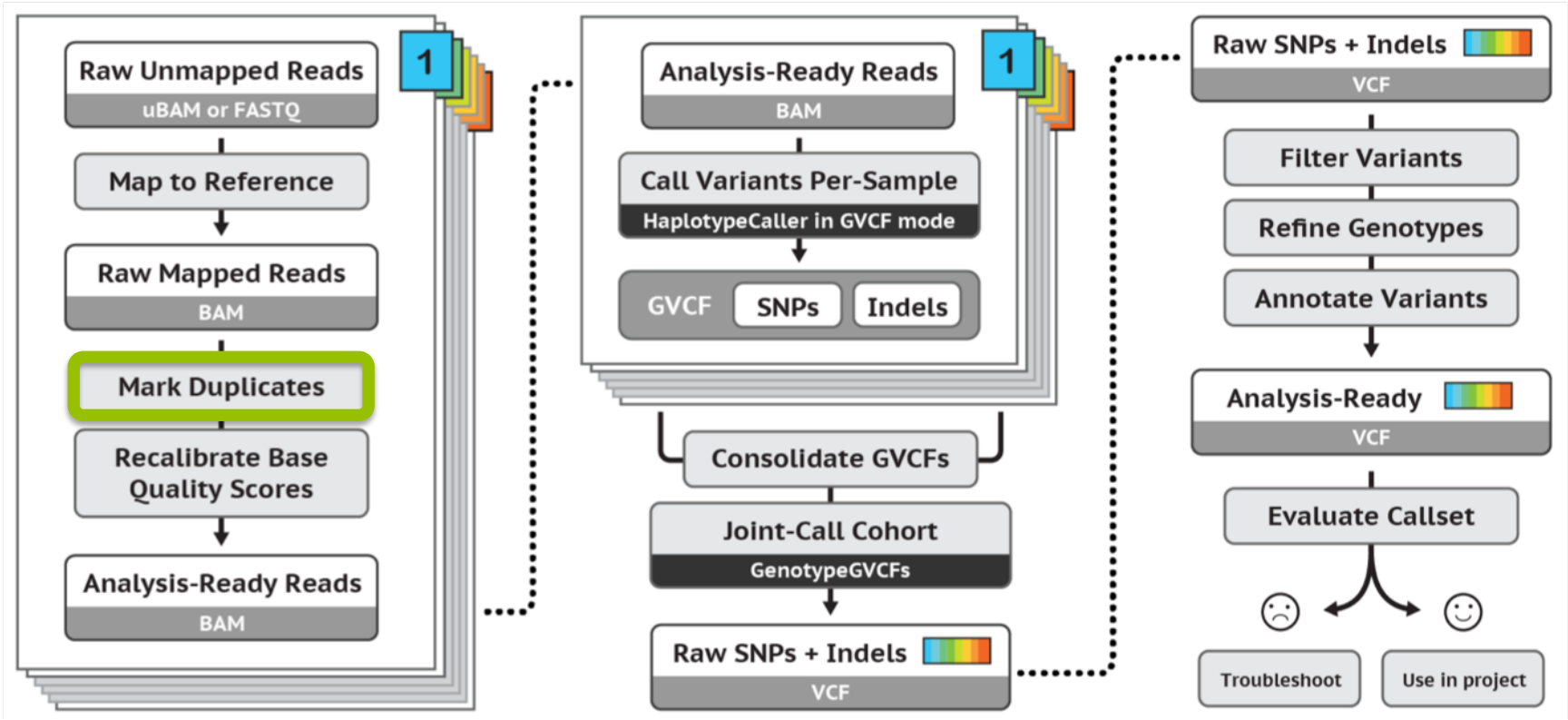
https://gatk.broadinstitute.org

GATK's best practices workflow for germline short variant discovery



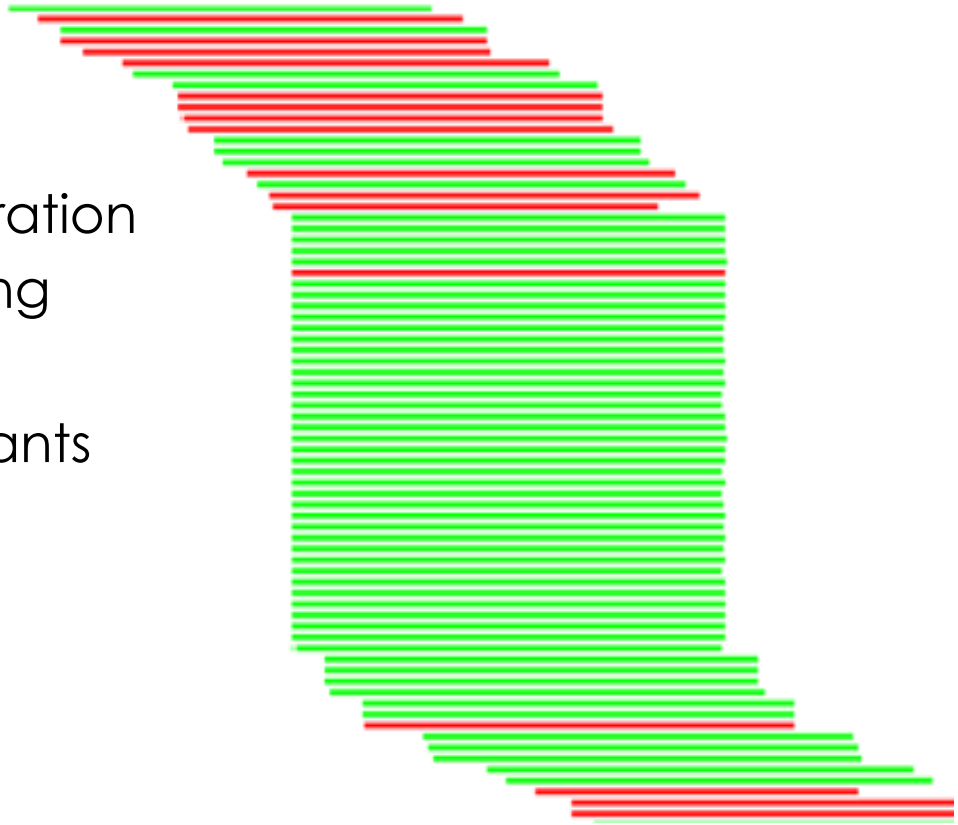
https://software.broadinstitute.org/gatk/best-practices/

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



MarkDuplicates (Picard) Follow

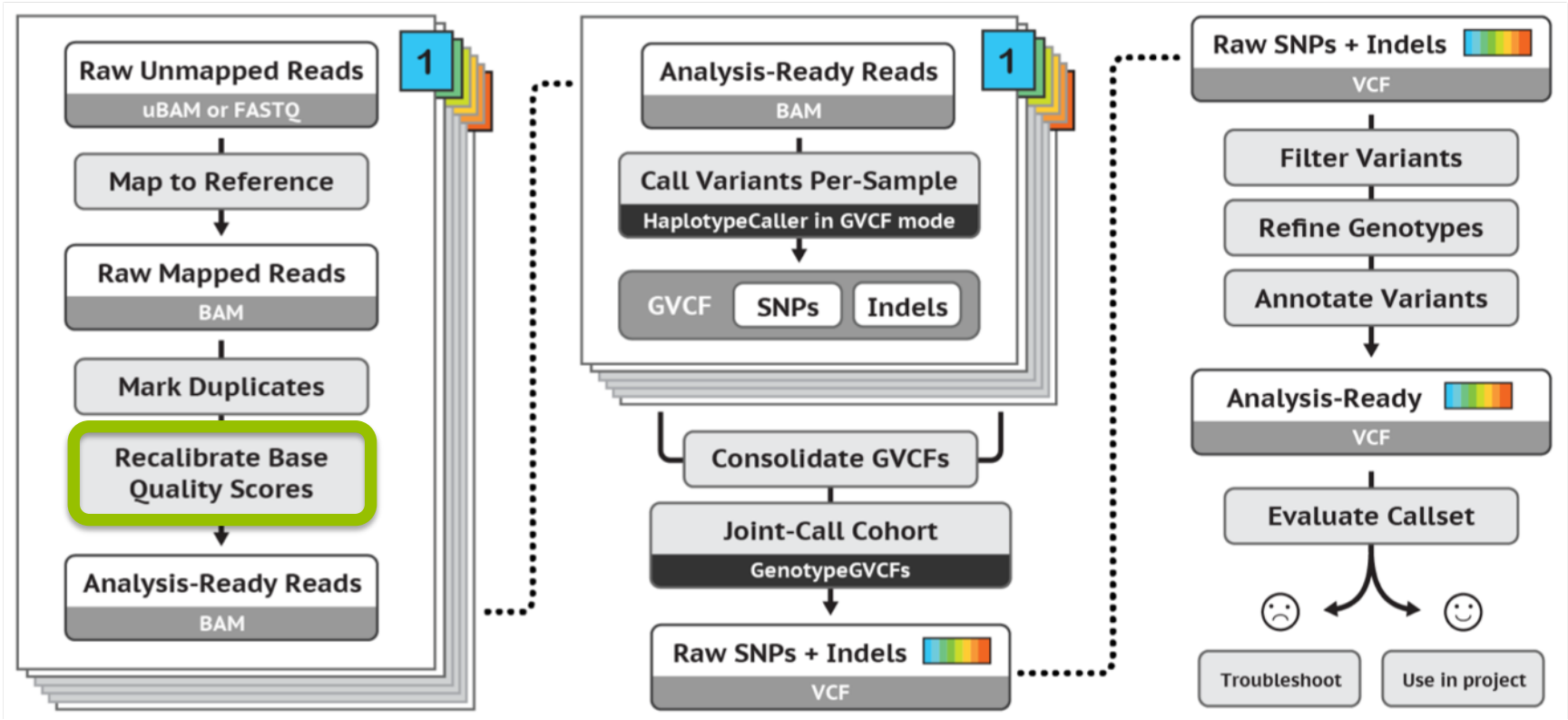
GATK Team
10 months ago · Updated

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)

- During base calling, the sequencer estimates a quality score for each base. This is the quality scores present in the fastq files.

- 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

- Can cause bias in variant calling

- **Base Qualtiy Score Recalibration** helps to calibrate the scores so that they correspond to the real per-base sequencing error rate (phred scores)

Base Quality Score Recalibration (BQSR) Follow

GATK Team

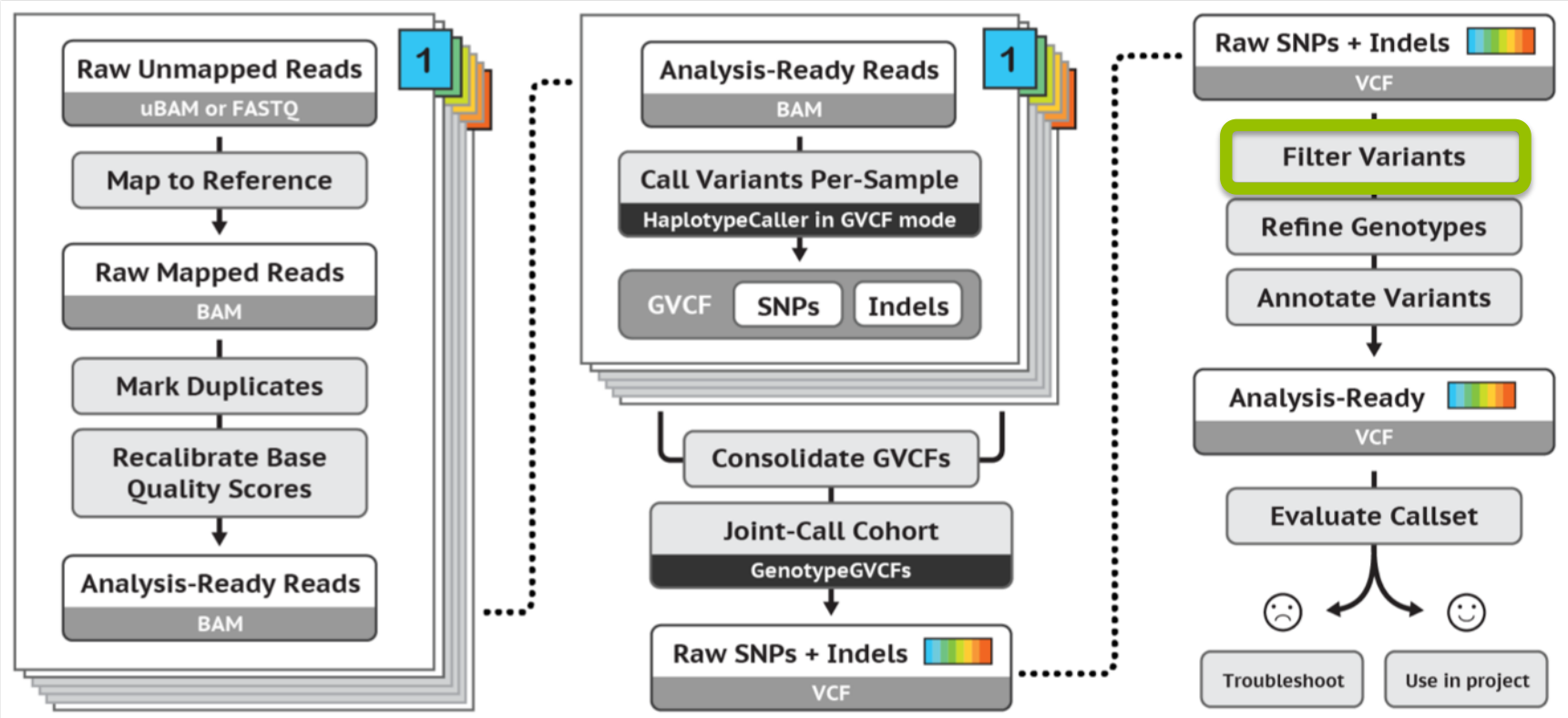
5 days ago · Updated

BQSR stands for Base Quality Score Recalibration. In a nutshell, it is a data pre-processing step that detects systematic errors made by the sequencing machine when it estimates the accuracy of each base call.

*Note that this **base** recalibration process (BQSR) should not be confused with **variant** recalibration (VQSR), which is a sophisticated filtering technique applied on the variant callset produced in a later step. The developers who named these methods wish to apologize sincerely to anyone, especially Spanish-speaking users, who get tripped up by the similarity of these names.*

Contents

Filter variants



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

Germline short variant discovery (SNPs + Indels)

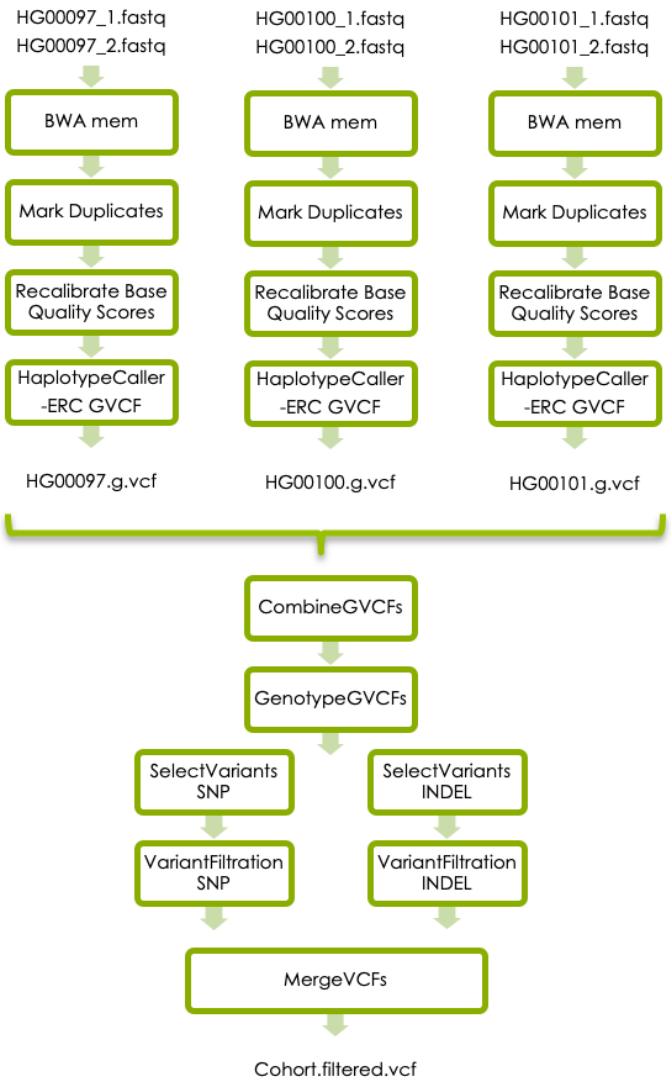
Filtering

- Remove low quality variants

- 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 "QD < 2" and "MQ< 40.0"
  - GATK recommendations on hard filters: https://gatkforums.broadinstitute.org/gatk/discussion/2806/howto-apply-hard-filters-to-a-call-set

GATK's best practices workflow



More details and links to GATK for each step is found in the lab instructions.

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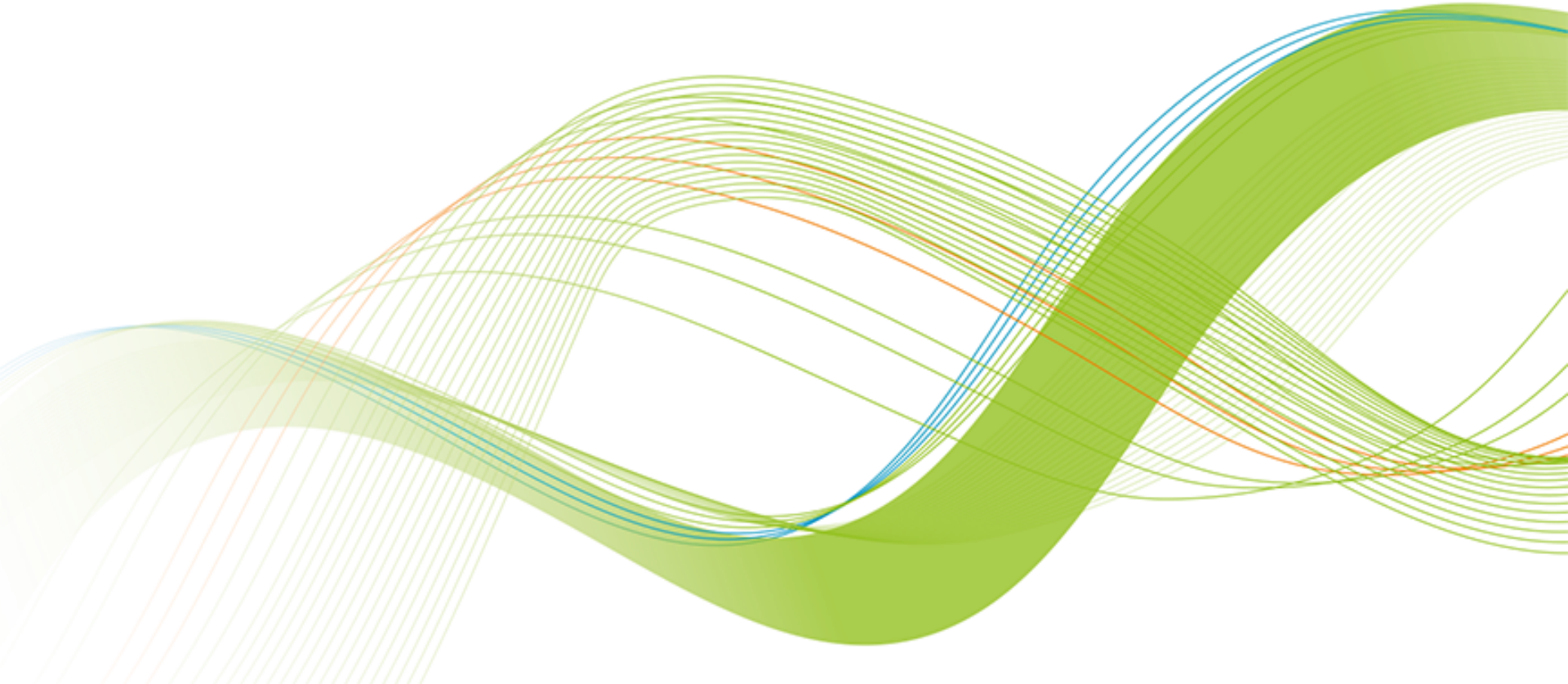
Today's lab



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

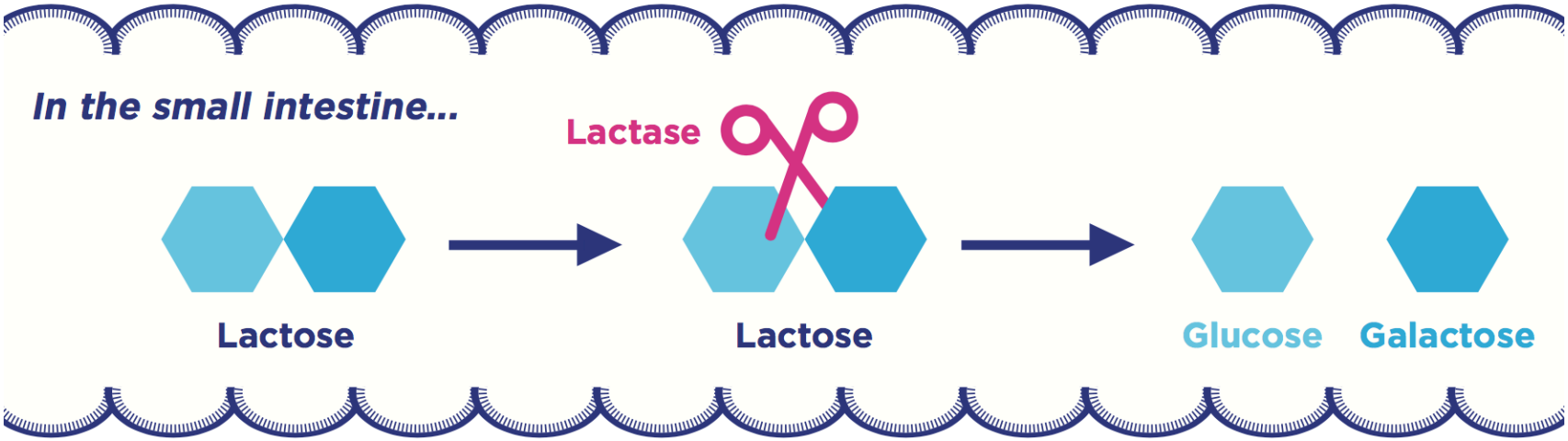


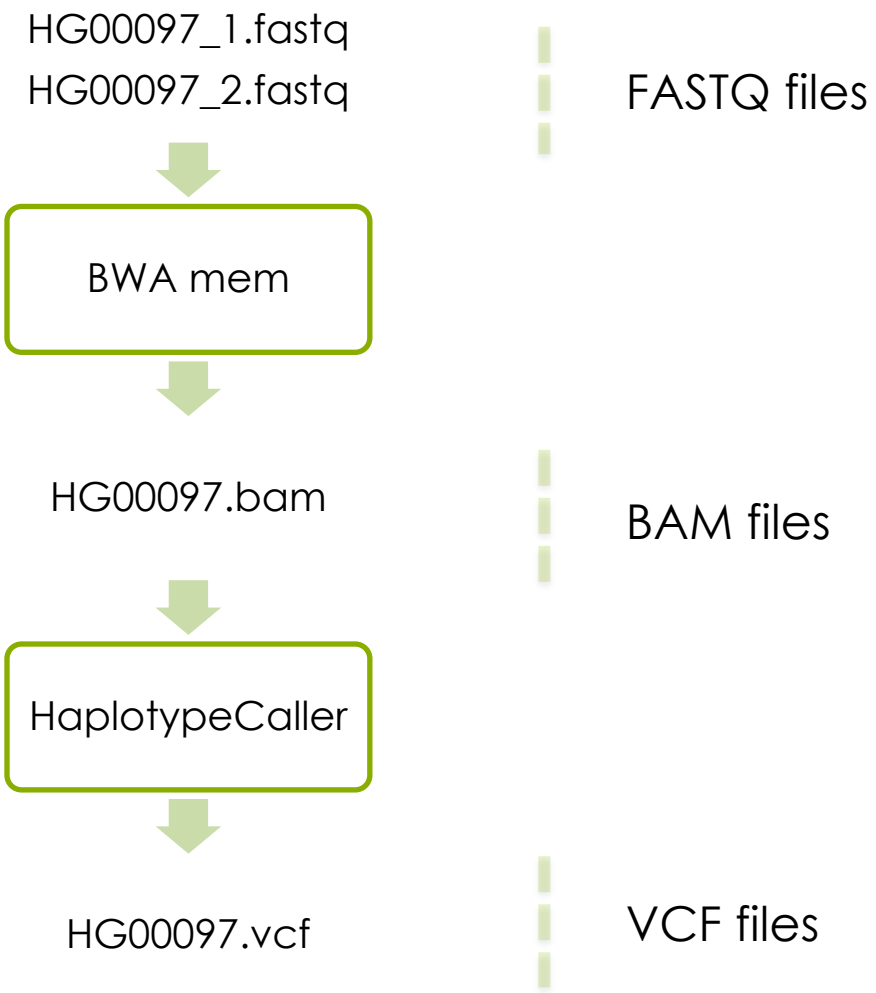
Figure 2. Lactose digestion in the intestine.

- 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)

part one:

variant calling in one sample

Basic variant calling in one sample



Part two (if you have time):

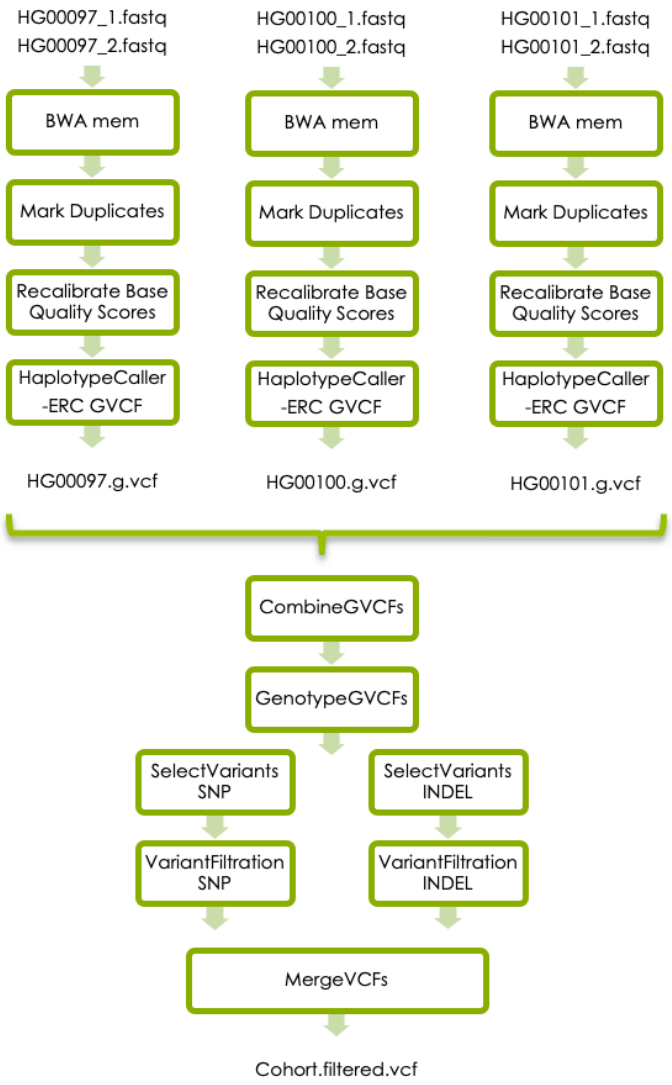
variant calling in cohort

Joint variant calling workflow

Part three (if you have time):

Follow GATK best practices for short variant discovery

GATK's best practises



First look at video about this linked from schedule!

https://gatk.broadinstitute.org

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Genome Analysis Toolkit

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Developed in the Data Sciences Platform at the Broad Institute, the toolkit offers a wide variety of tools with a primary focus on variant discovery and genotyping. Its powerful processing engine and high-performance computing features make it capable of taking on projects of any size. Learn more

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