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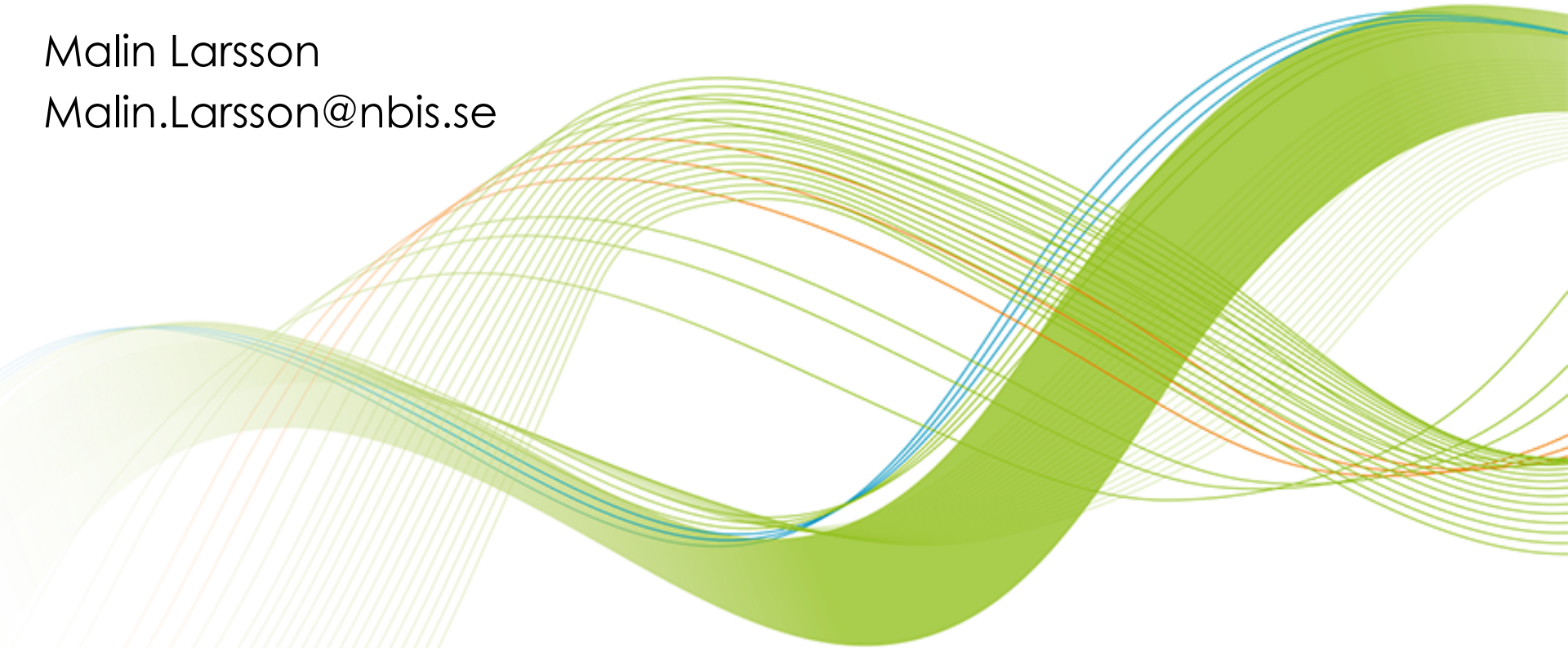
Variant Calling Workflows

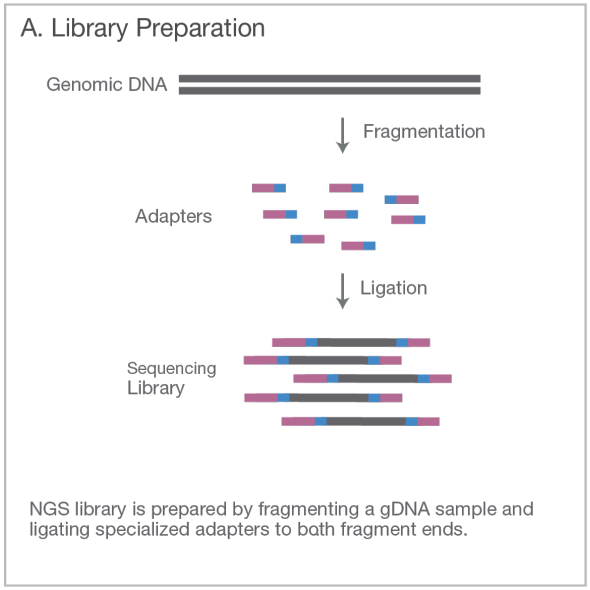
Malin Larsson
Malin.Larsson@nbis.se

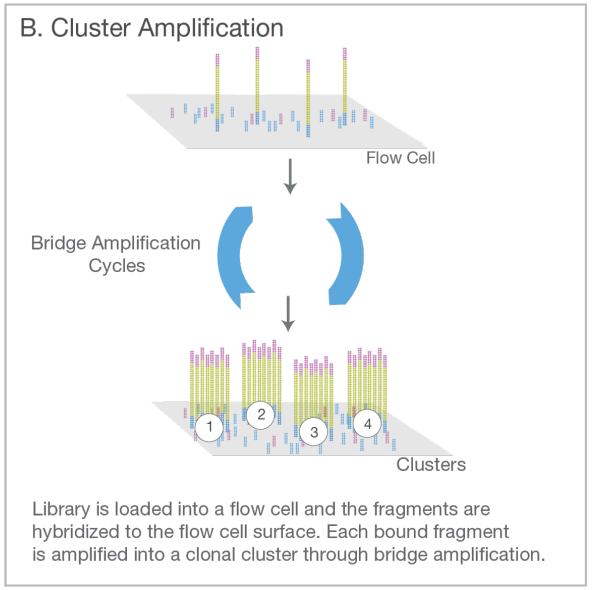


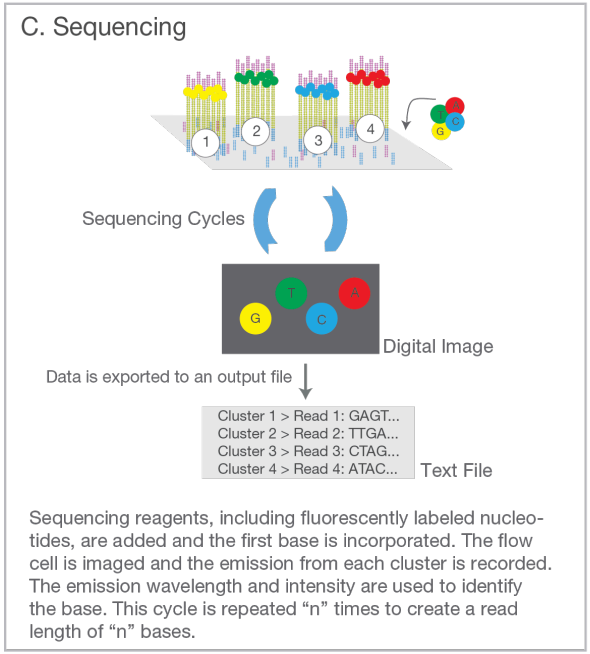
Talk Overview

- The reference genome
- Genetic variation
- Workflows
- Basic variant calling in one sample
- Basic variant calling in cohort
- GATK Best practices
- Introduction to exercise

Illumina Sequencing







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

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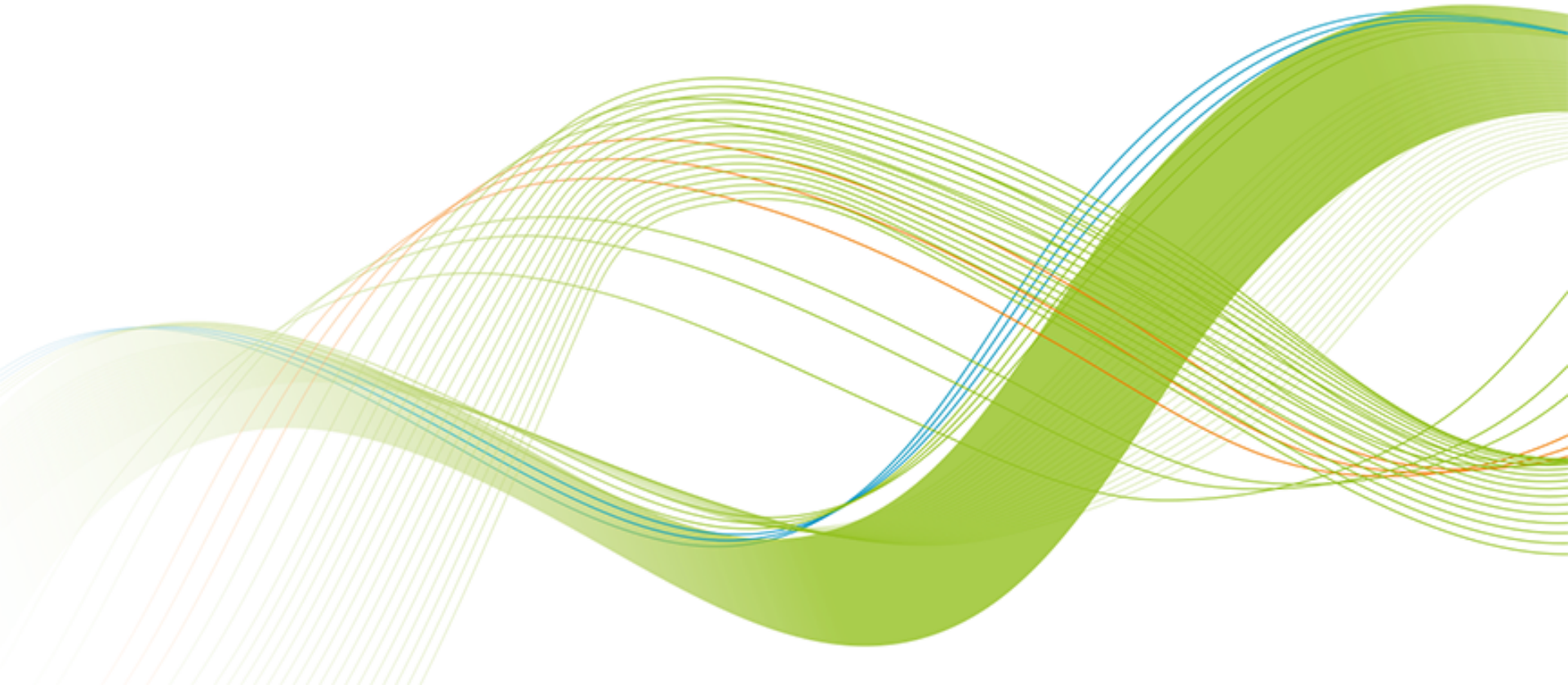
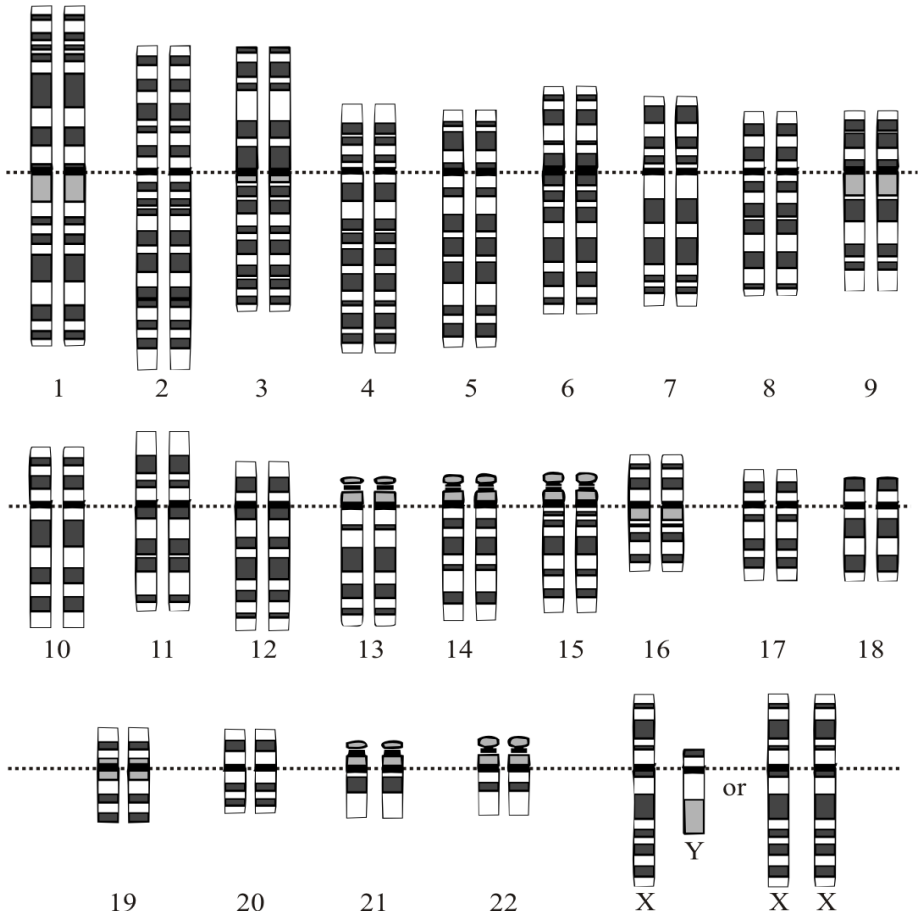
The reference genome sequence



Each chromosome…



…represented by a sequence

```
>chr1
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTCTG
GGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTC
TTTGATTCCTGCCTCATTCTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACCTAC
TAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCGC
TTTCCACACAGACATCATAACAAAAAATTTCCACCAAACCCCCCCCTCCCCCCGCTTCTGGCCACA
GCACTTAAACACATCTCTGCCAAACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTC
AAATTTTATCTTTAGGCGGTATGCACTTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCT
CCCACTCCCATACTACTAATCTCATCAATACAACCCCCGCCCATCCTACCCAGCACACACACACCG
CTGCTAACCCCATACCCCGAACCAACCAAACCCCAAAGACACCCCCCACAGTTTATGTAGCTTACC
TCCTCAAAGCAATACACTGAAAATGTTTAGACGGGCTCACATCACCCCATAAACAAATAGGTTTGG
TCCTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCCGTTCCAGTGAGTTCAC
CCTCTAAATCACCACGATCAAAAGAGGCGGTATGCACTTTTAACAGTCACCCCCAGGCGGTATGCA
```

The reference genome

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

In 2001: The International Human Genome Sequencing Consortium published the first draft of the human genome sequence.
It 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
- visualizing
- variant calling
- annotation
- etc

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

The same reference sequence 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

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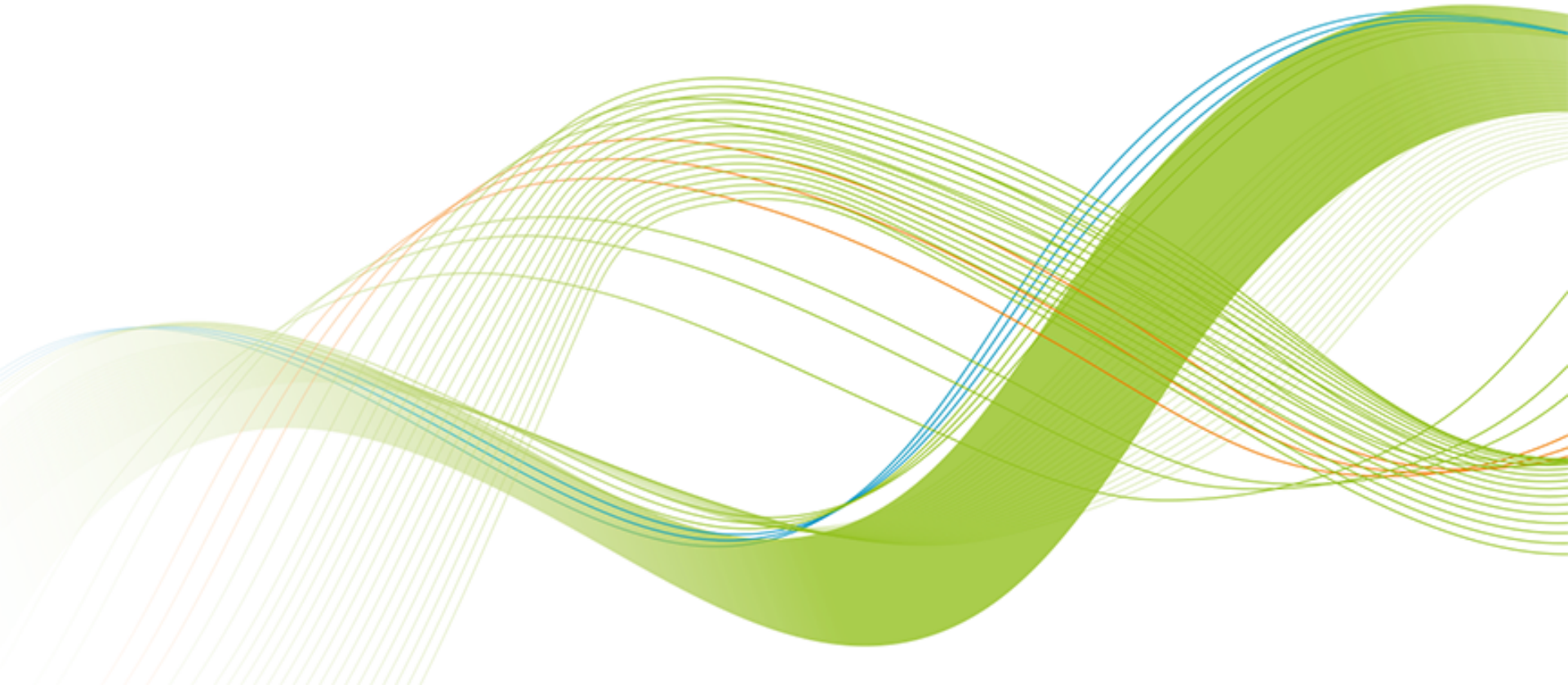
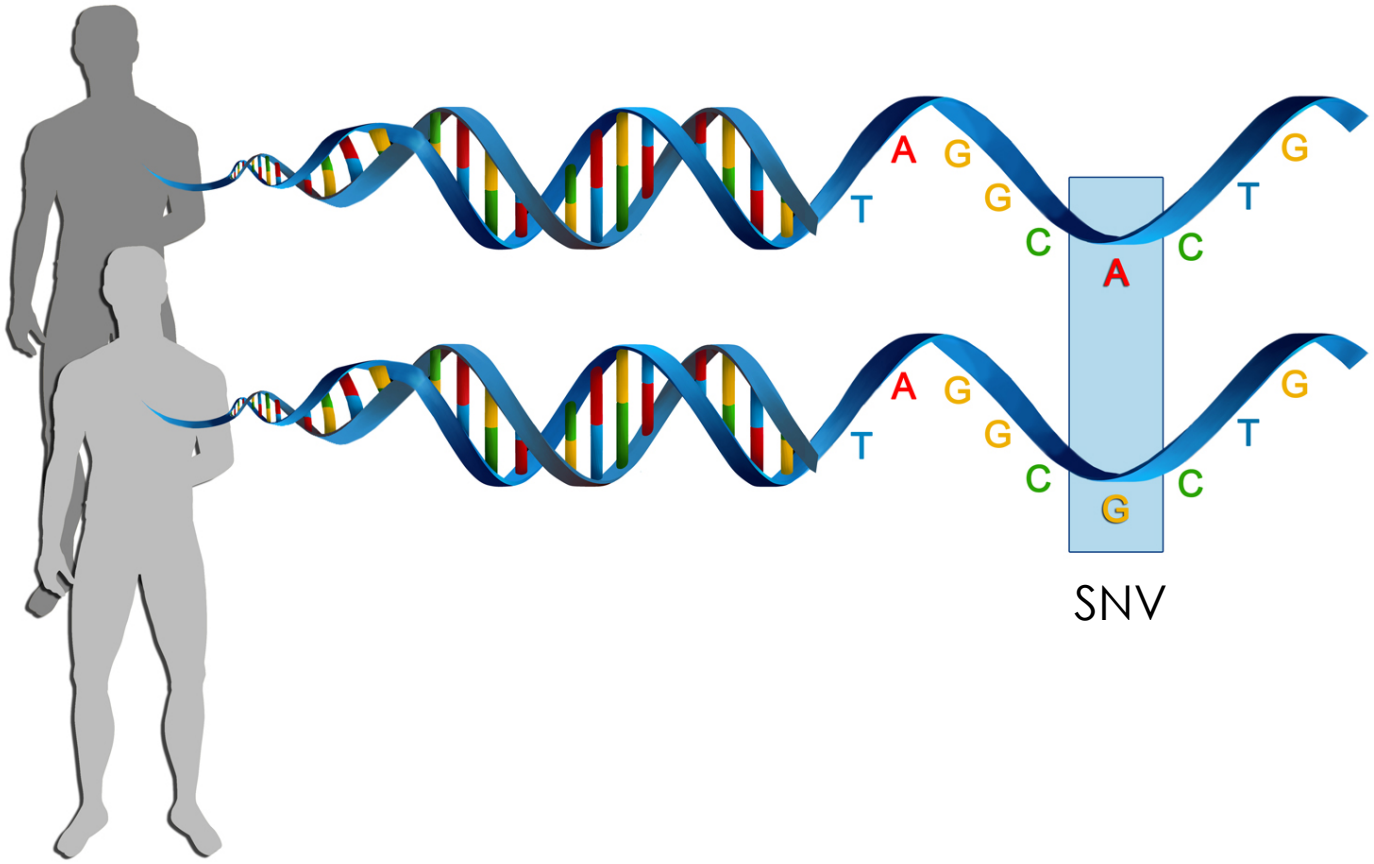
Genetic variation



Genetic Variation

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

Single Nucleotide Variants (SNVs)



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

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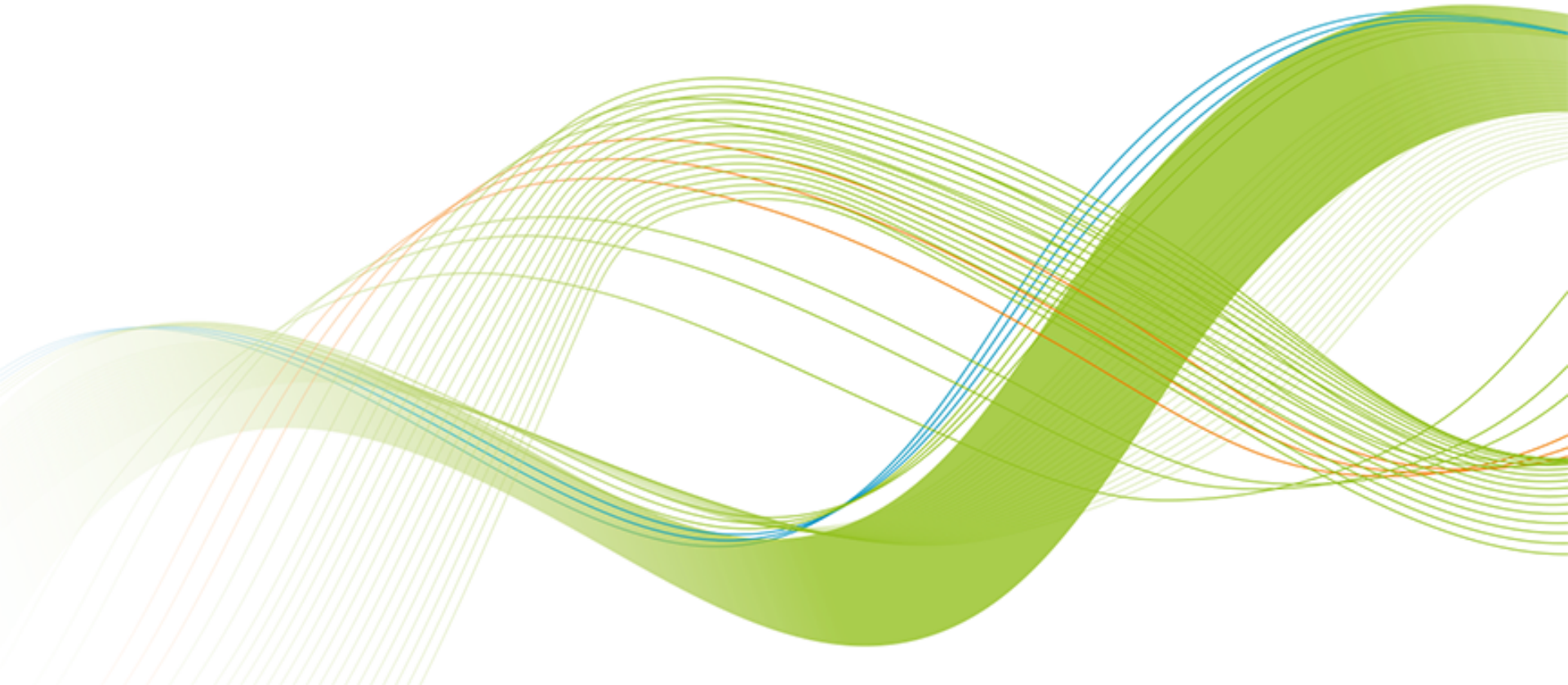
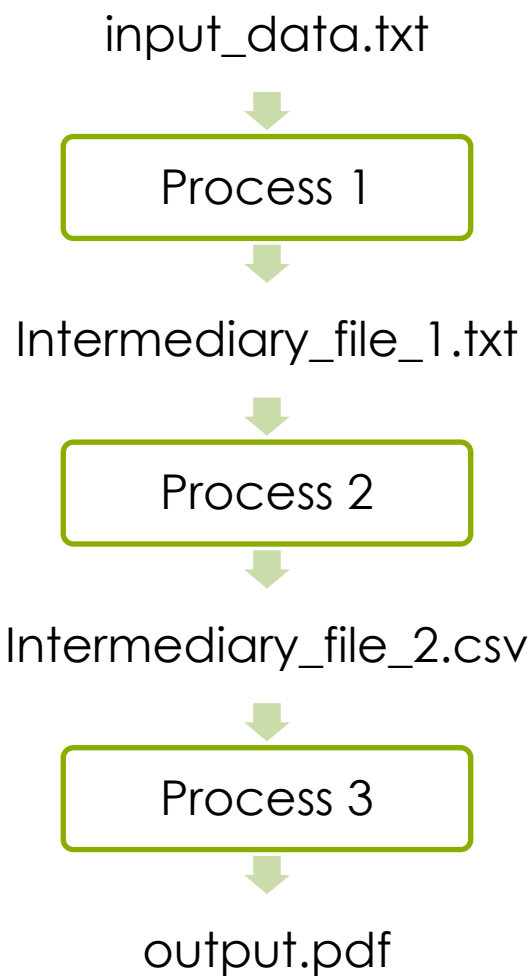
Introduciton to workflows



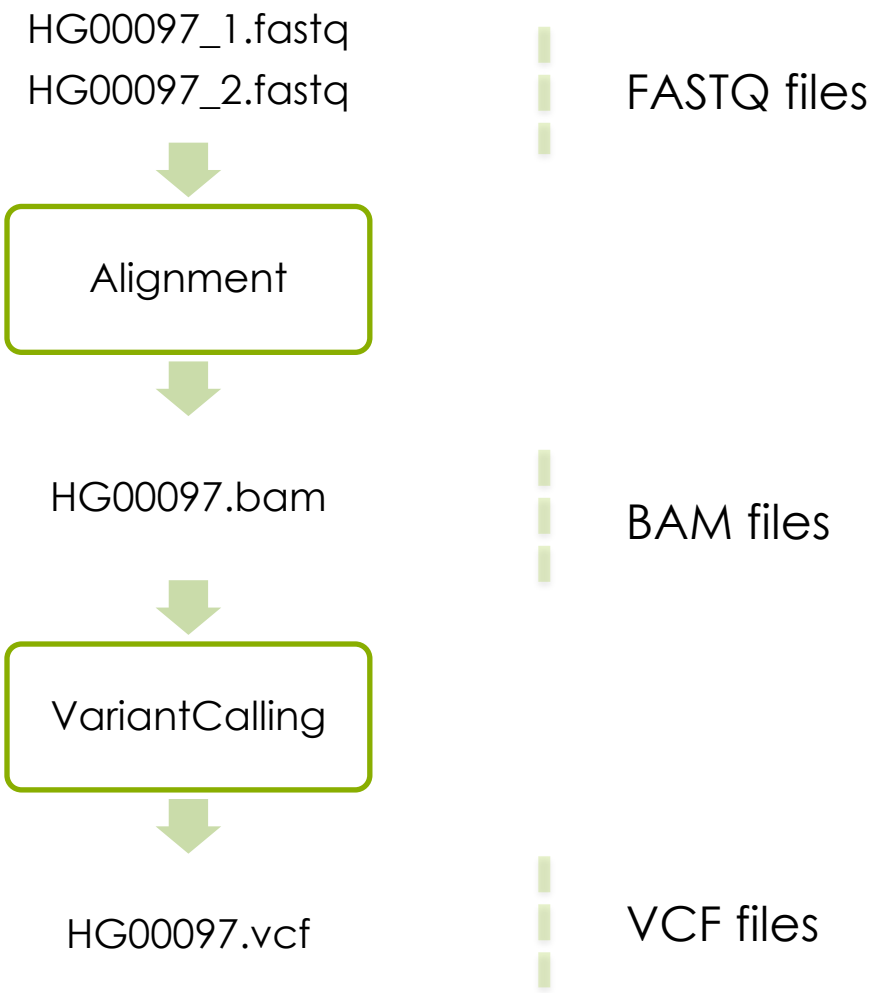
A bioinformatics workflow



Workflow conventions

- Each process has an input and output file(s)
- 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

Basic variant calling in one sample



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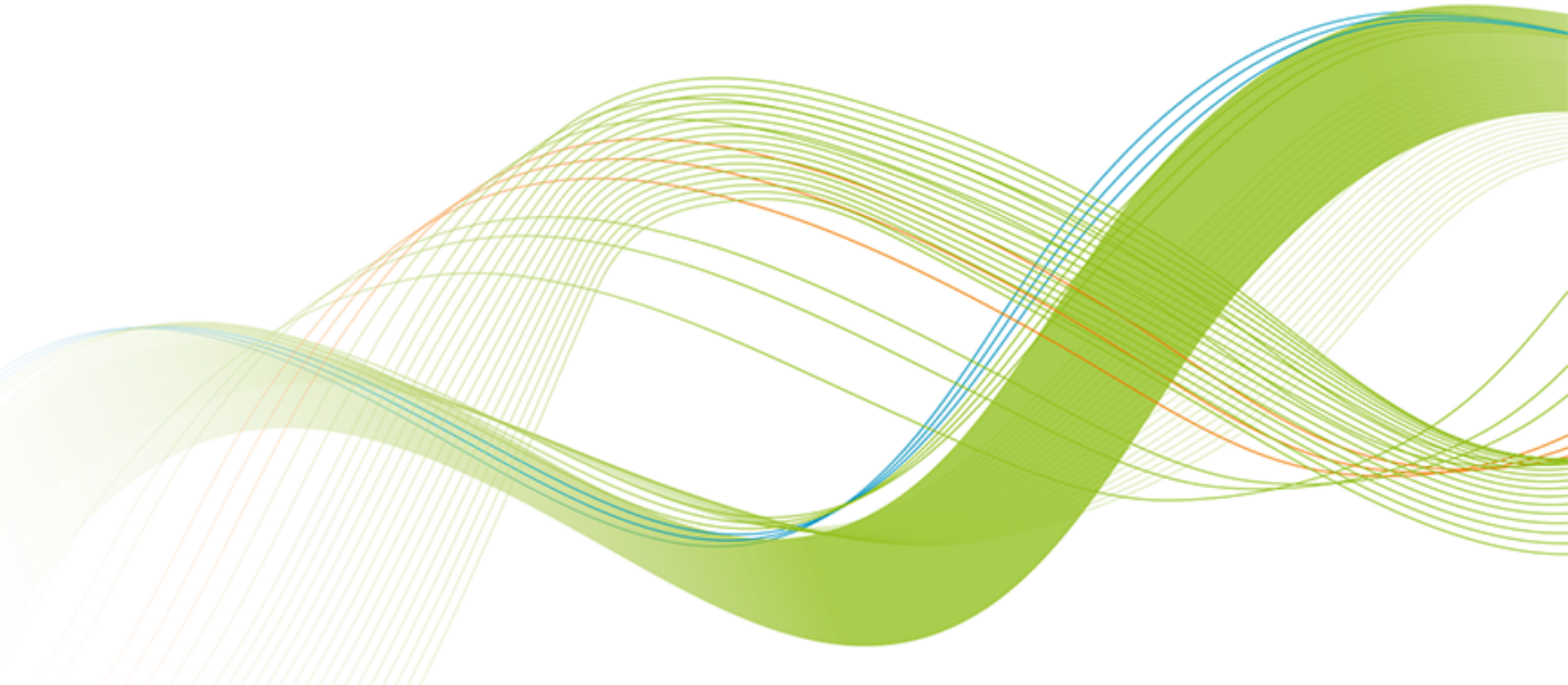
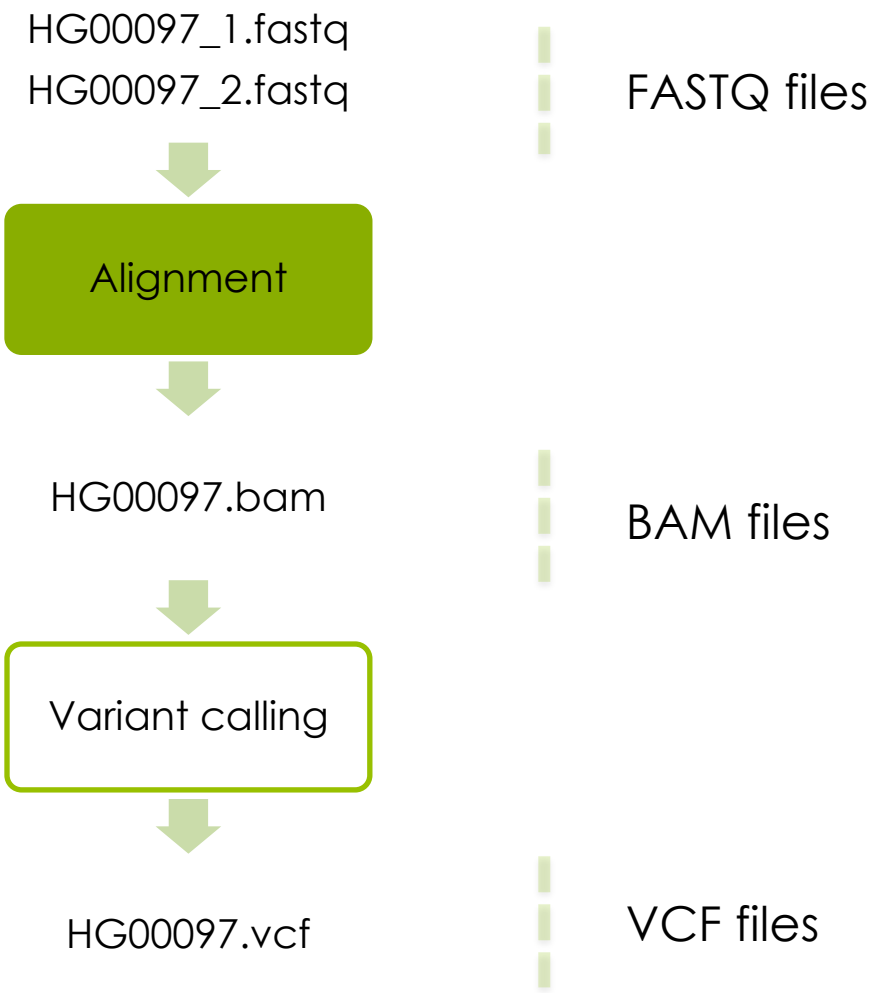
Basic Variant Calling in one sample



Basic variant calling in one sample



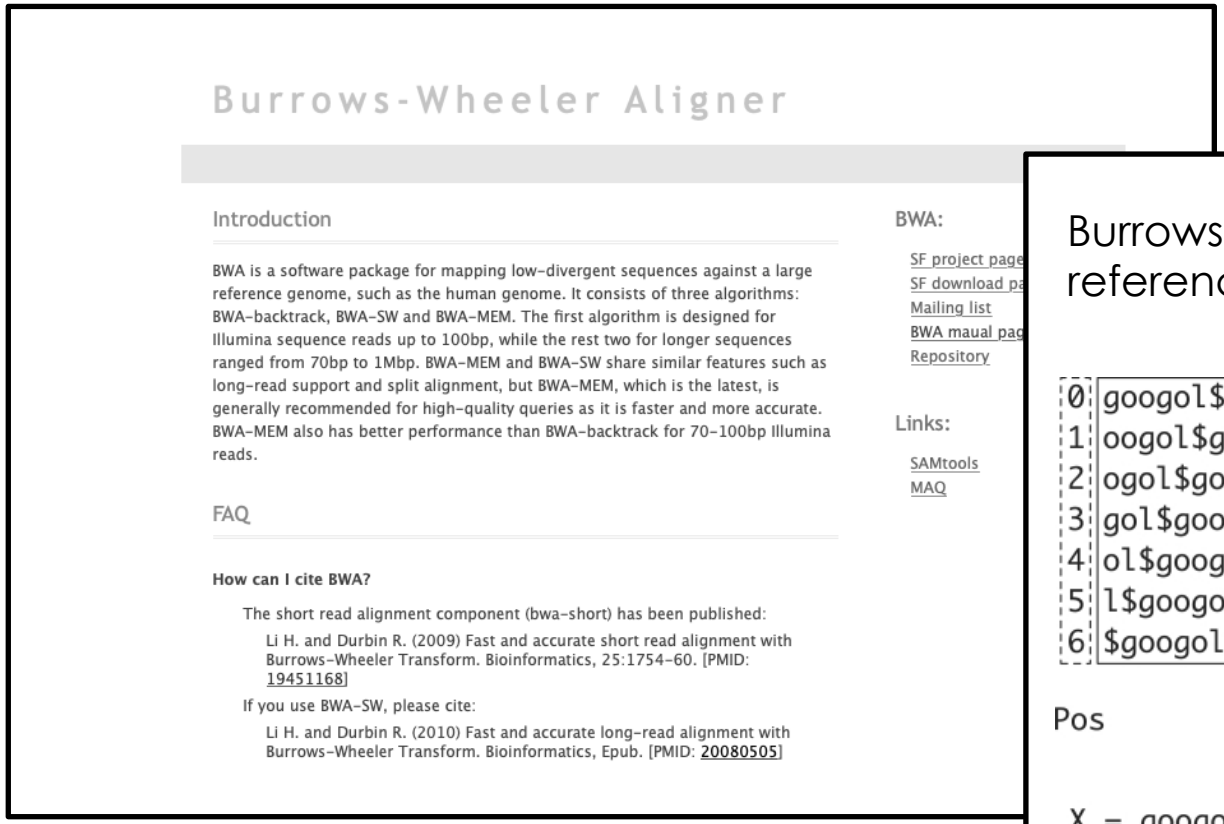
Alignment

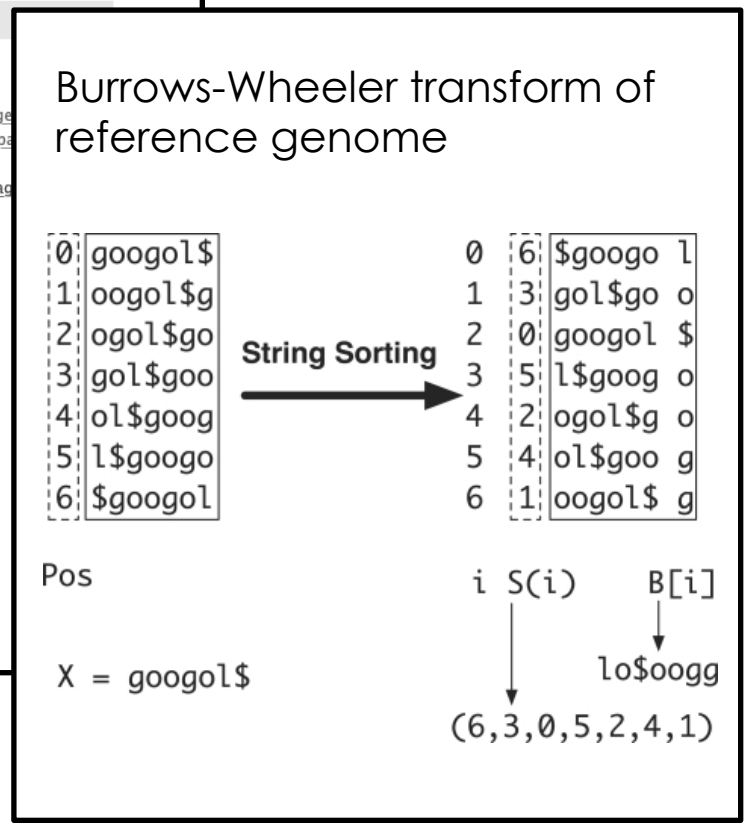
```
module load bwa
```

```
AACAGGTATATCTTCCCCGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTACCCTCTTCCTTAGGGACT
                                                GCTAGCTAGCTAGCTACCCT
```

Burrows-Wheeler Aligner

http://bio-bwa.sourceforge.net





Alignment

```
module load bwa
```

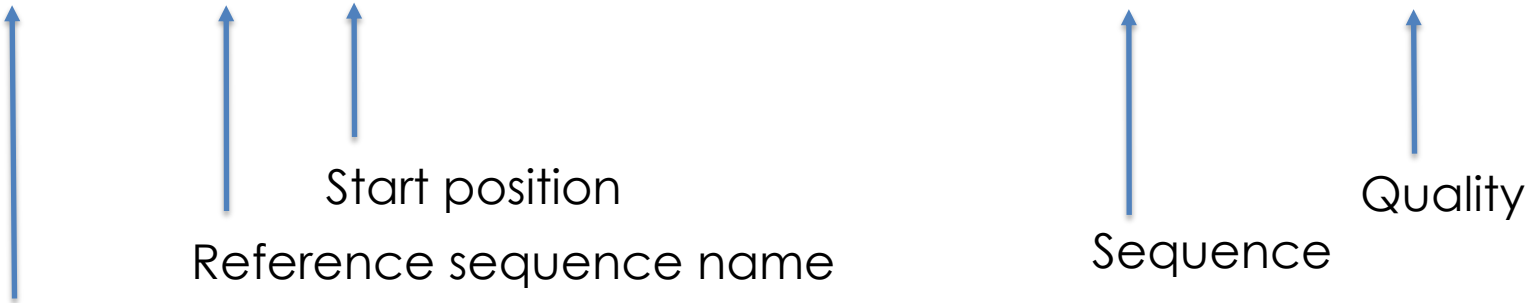
Output from mapping - Sam format

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

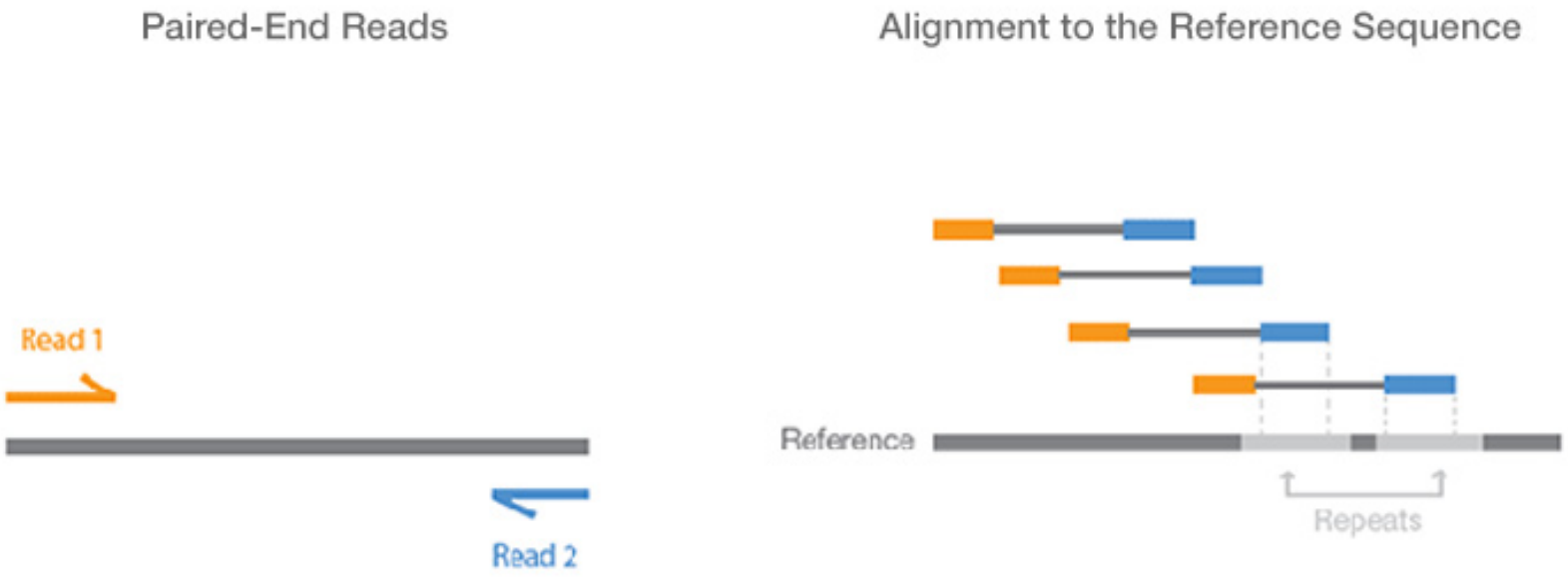
Bam file is a binary representation of the Sam file

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

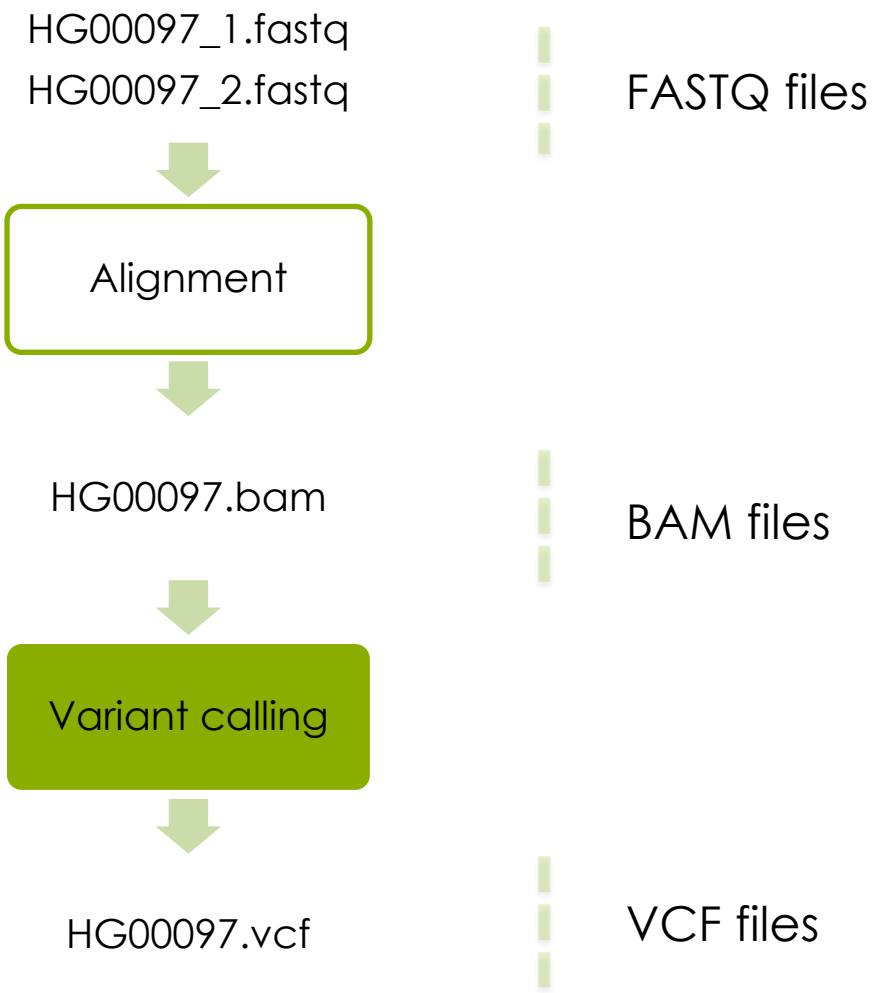
ID_R1_001.fastq

```
@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
```

Basic variant calling in one sample

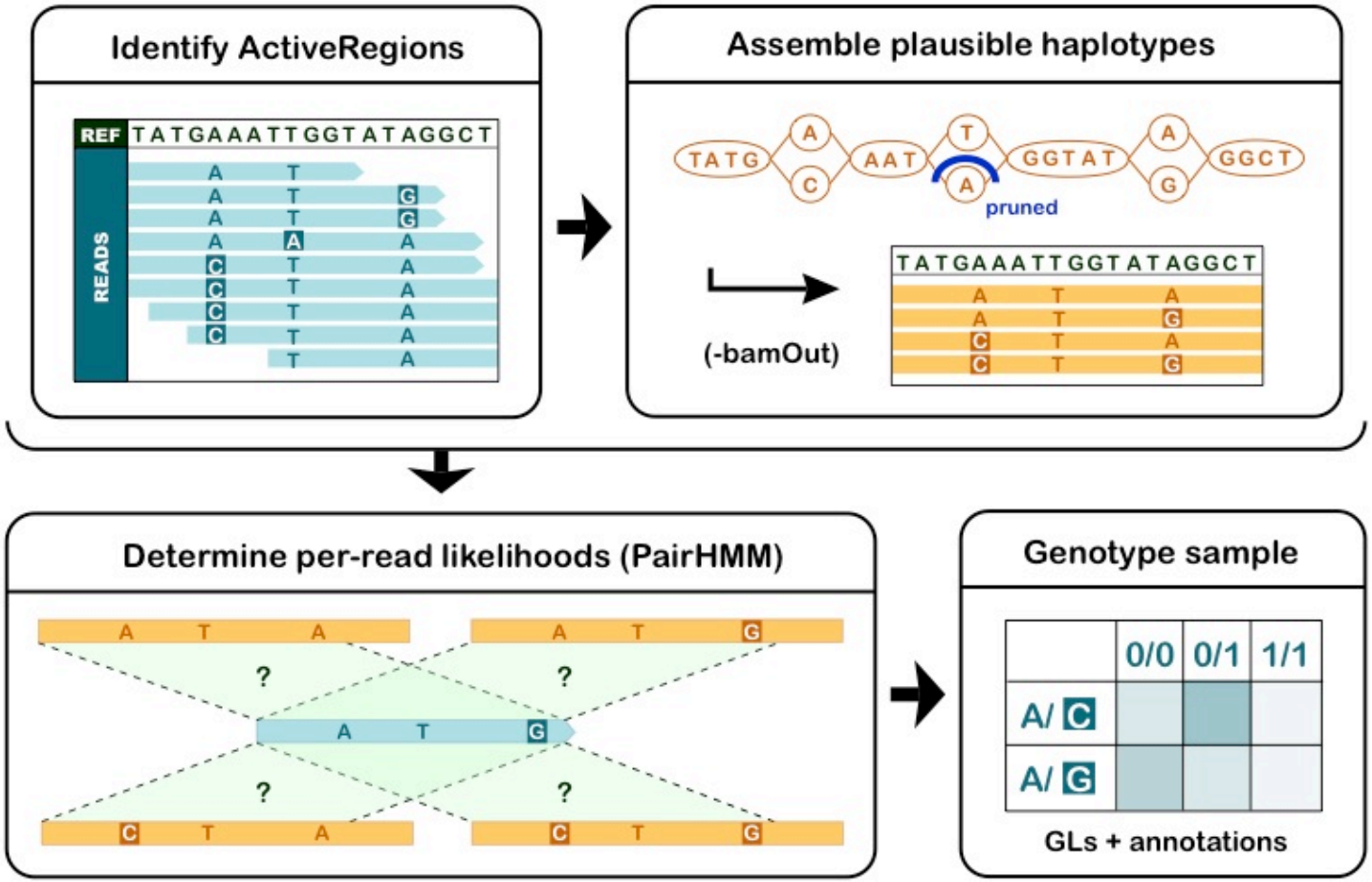


Detecting variants in reads

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

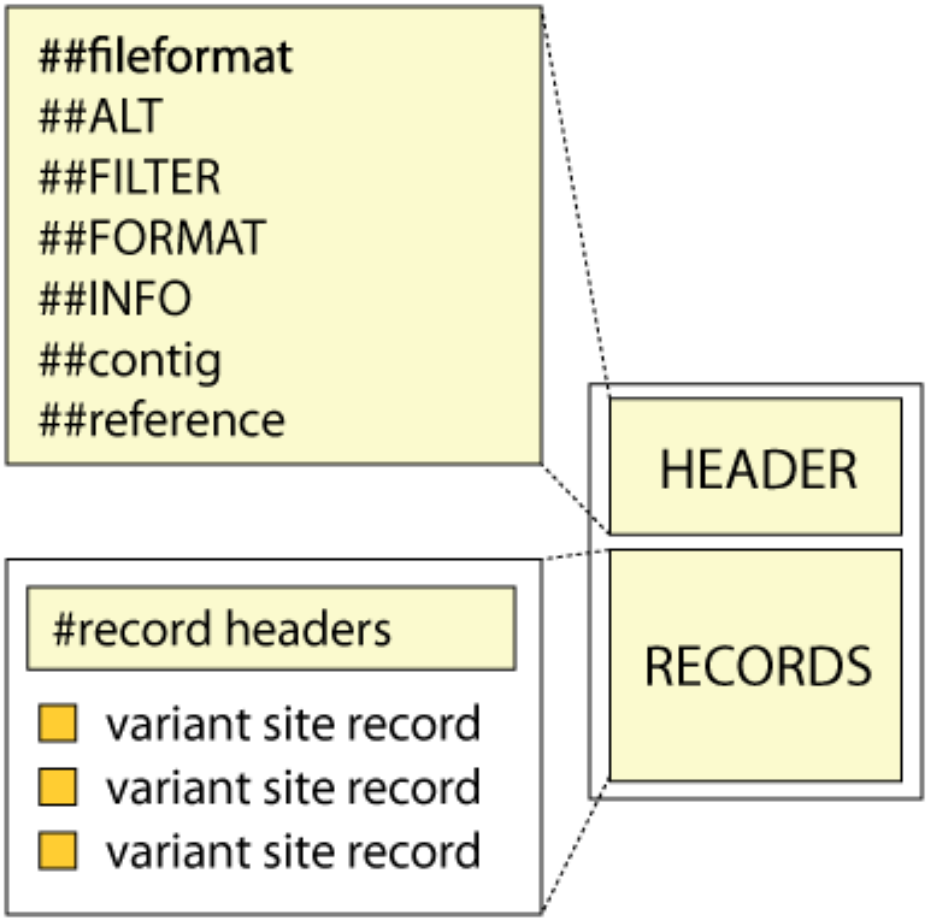
Sample:
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...

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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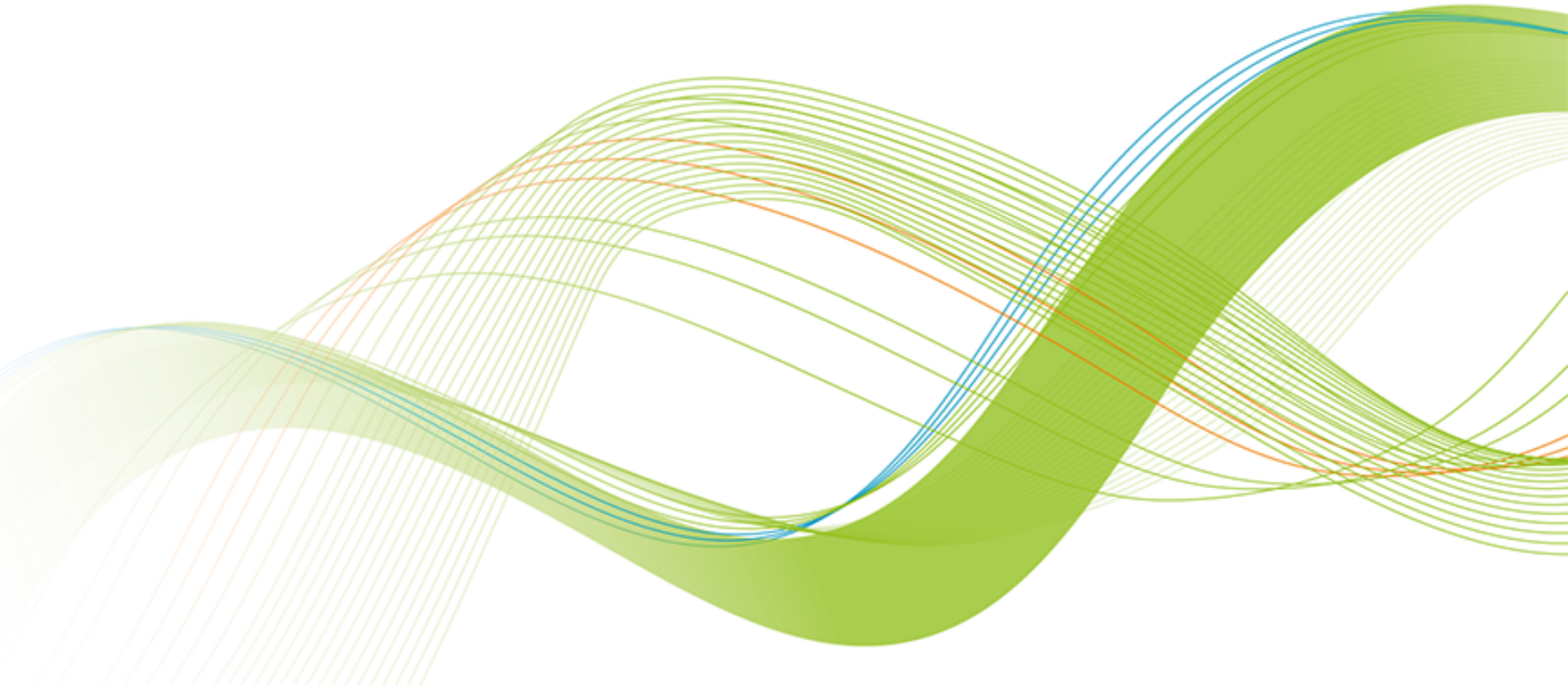
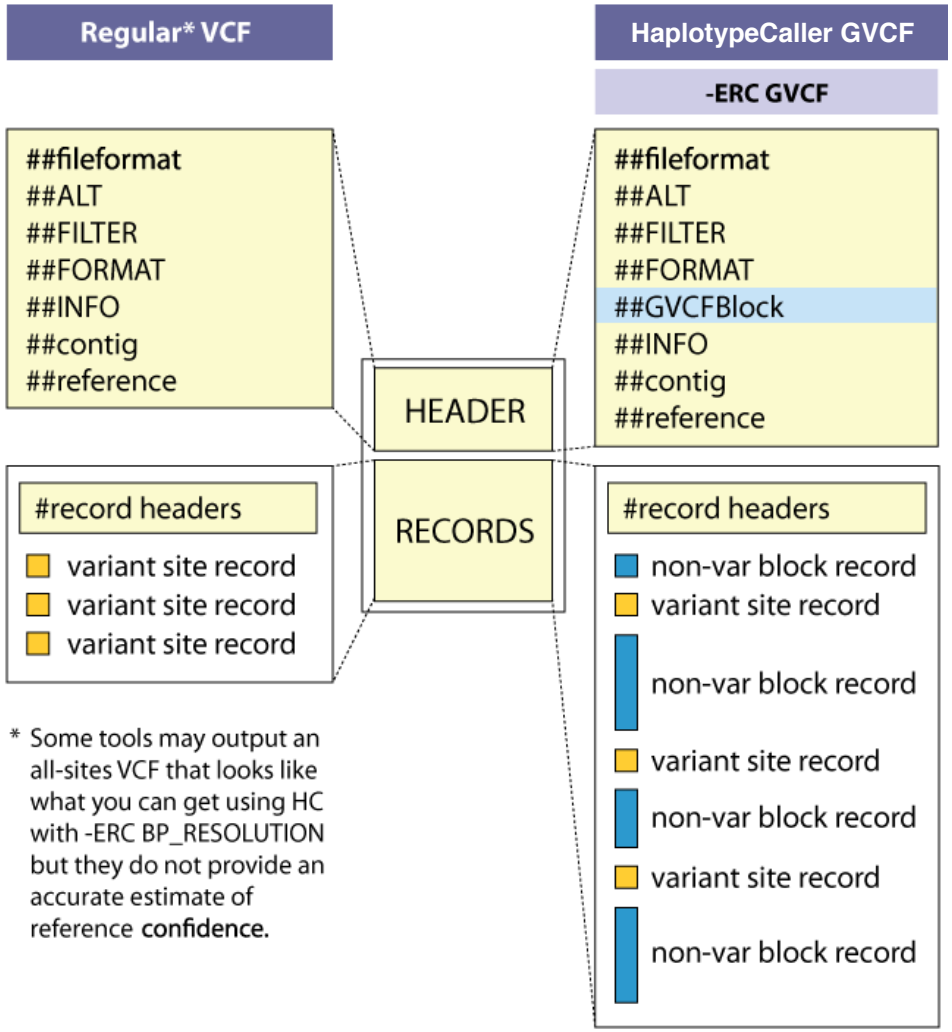
Variant calling in cohort



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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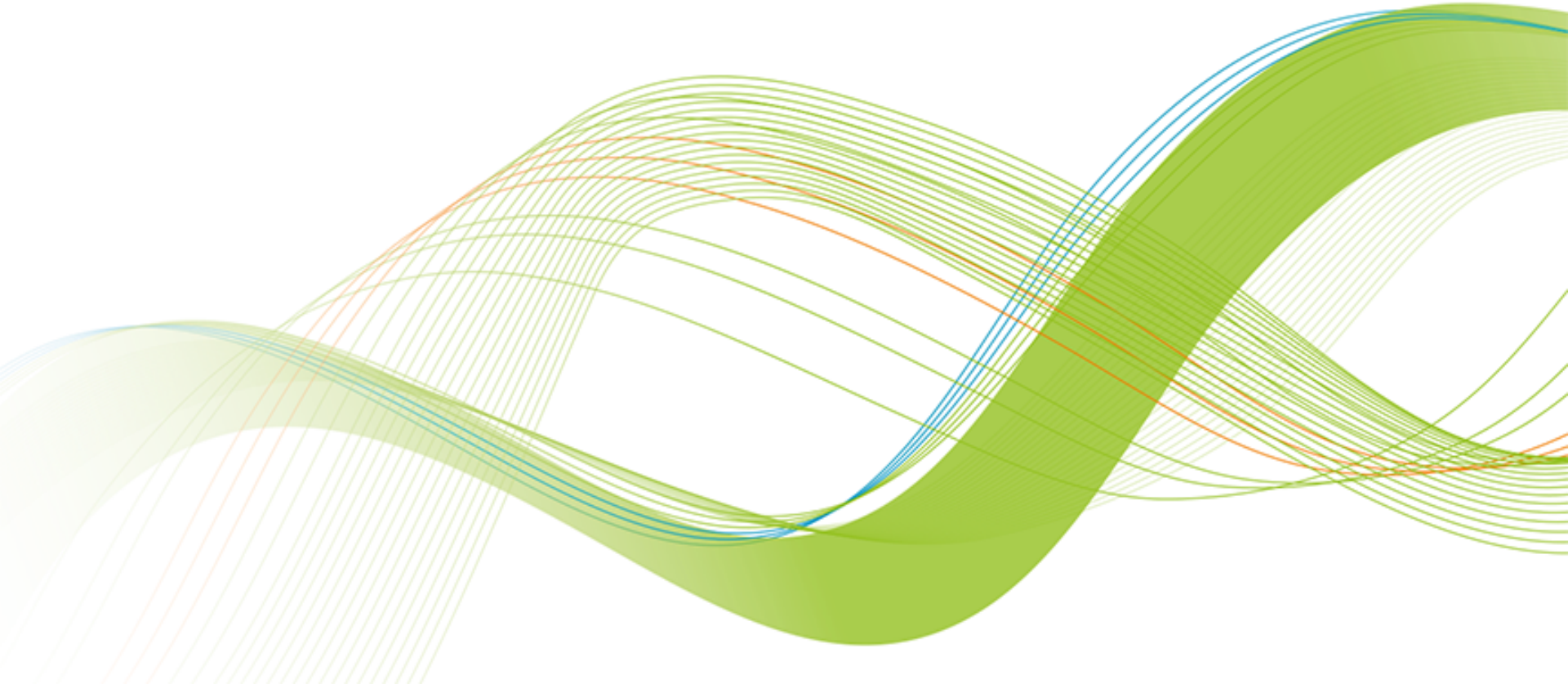
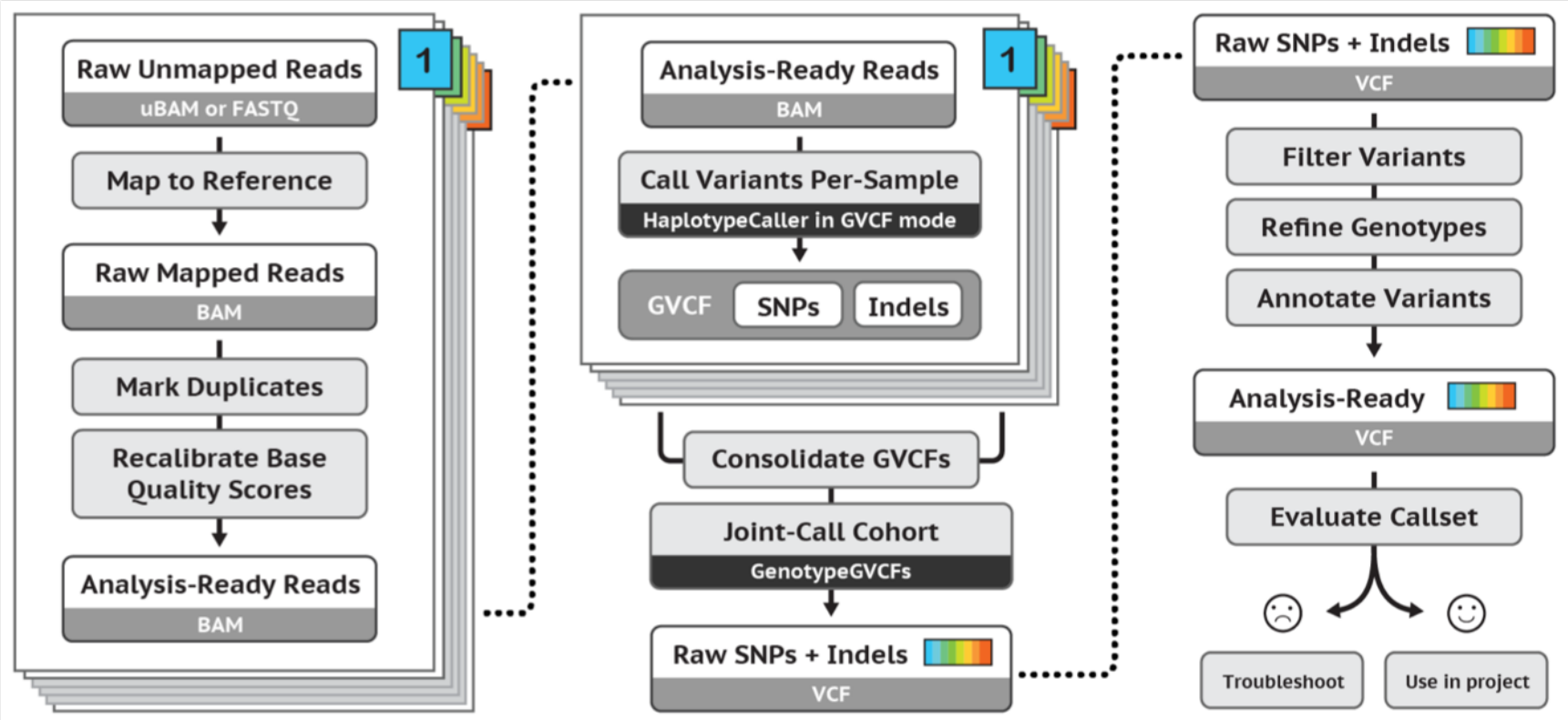
GATK's best practice germline short variant discovery



https://gatk.broadinstitute.org

GATK best practices workflow for variant discovery



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

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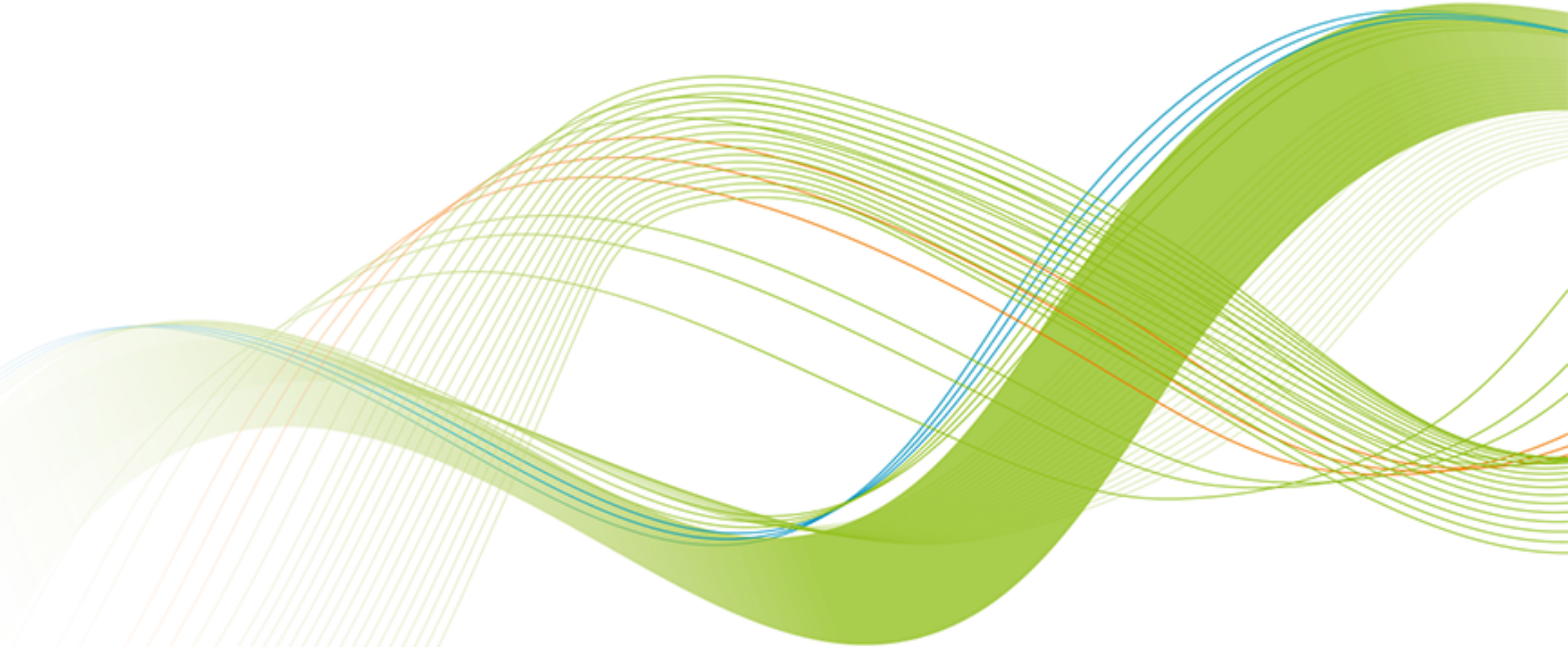
Introduction to computational exercise



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

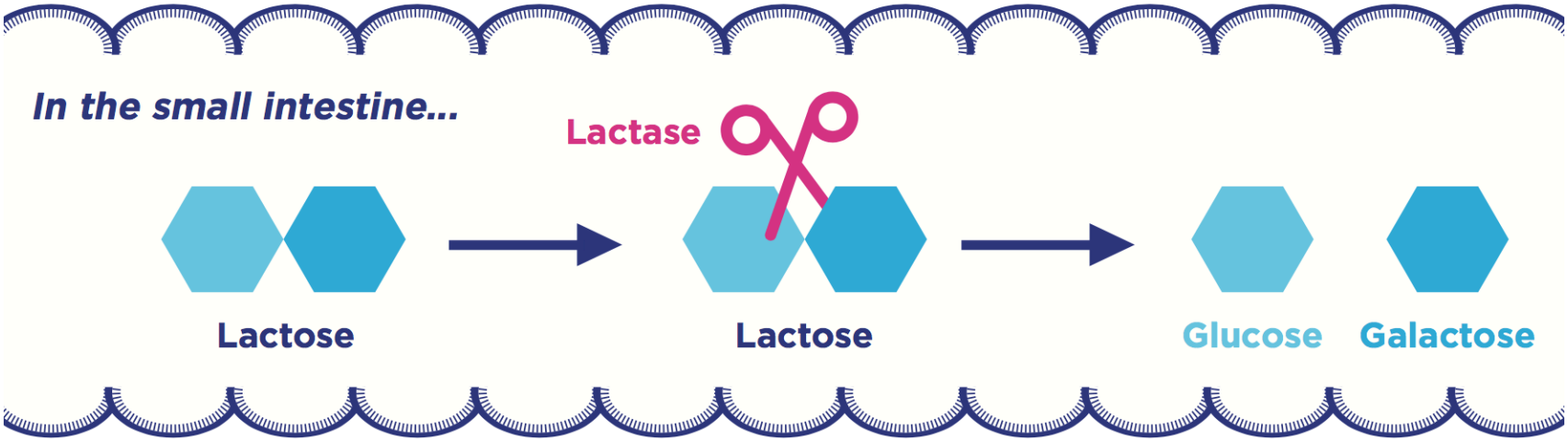


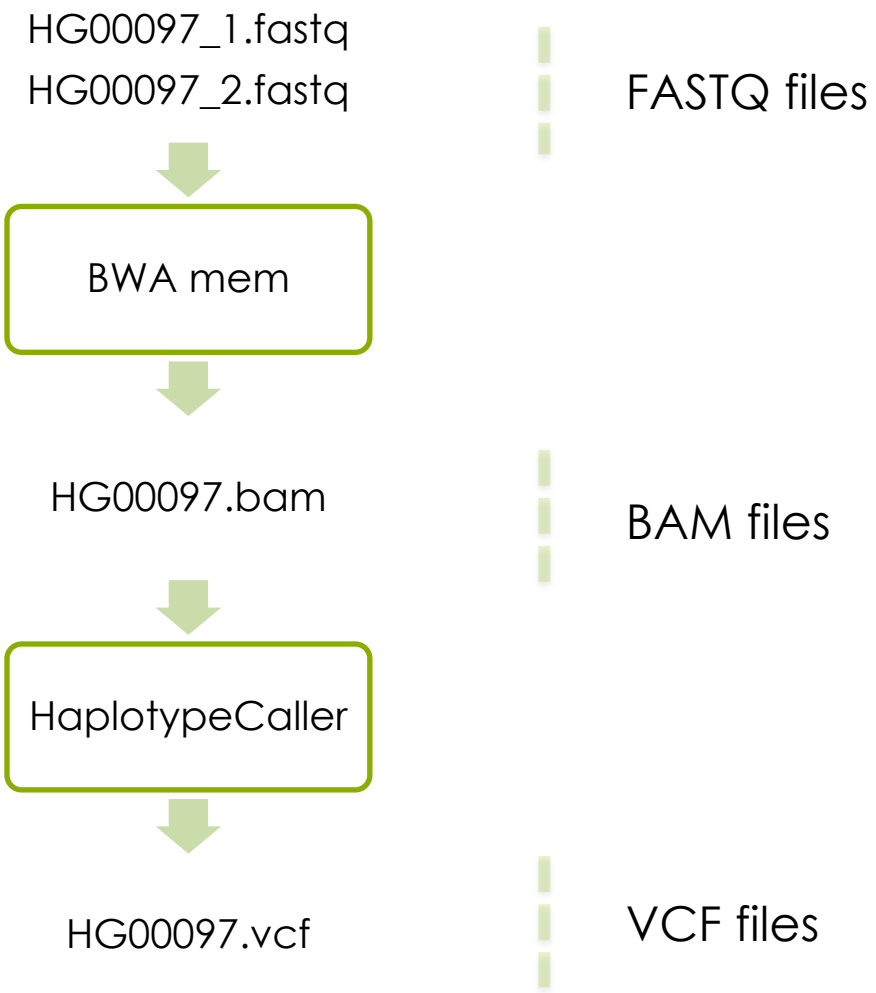
Figure 2. Lactose digestion in the intestine.

- All mammals produce lactase as infants
- Some human produce lactase in adulthood
- The *LCT* gene on chromosome 2 encodes lactase
- Genetic variation upstream of the LCT gene cause the lactase persistent phenotype

part one:

variant calling in one sample

Basic variant calling in one sample



Part two (if you have time):

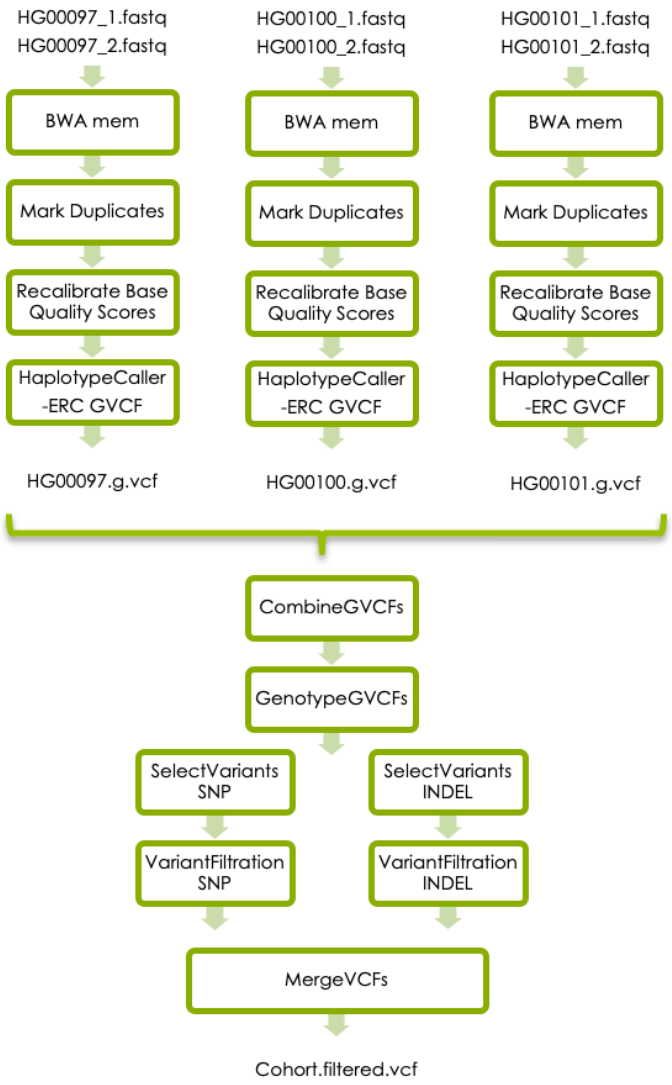
variant calling in cohort

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!

Questions?