From raw reads to variants

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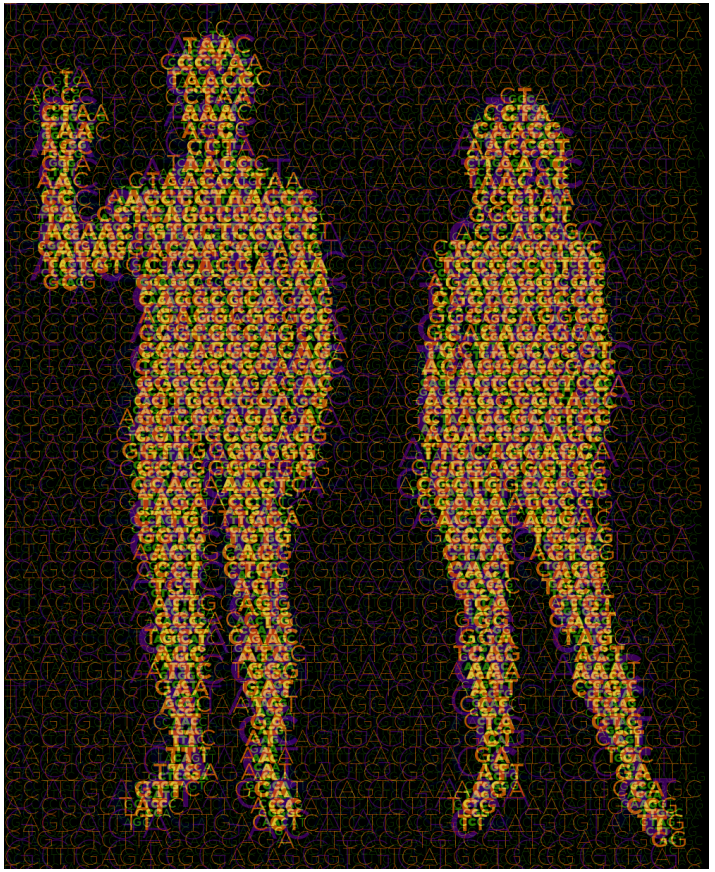
Talk Overview

- Concepts
  - Reference genome
  - Variants
  - Paired-end data
- NGS Workflow
  - Quality control & Trimming
  - Alignment
  - Local realignment
  - PCR duplicates & removal
  - Base Quality Score Recalibration
  - Variant calling
- VCF files
- Joint genotyping & gVCF files
- Annotation & Filtering

Reference genome

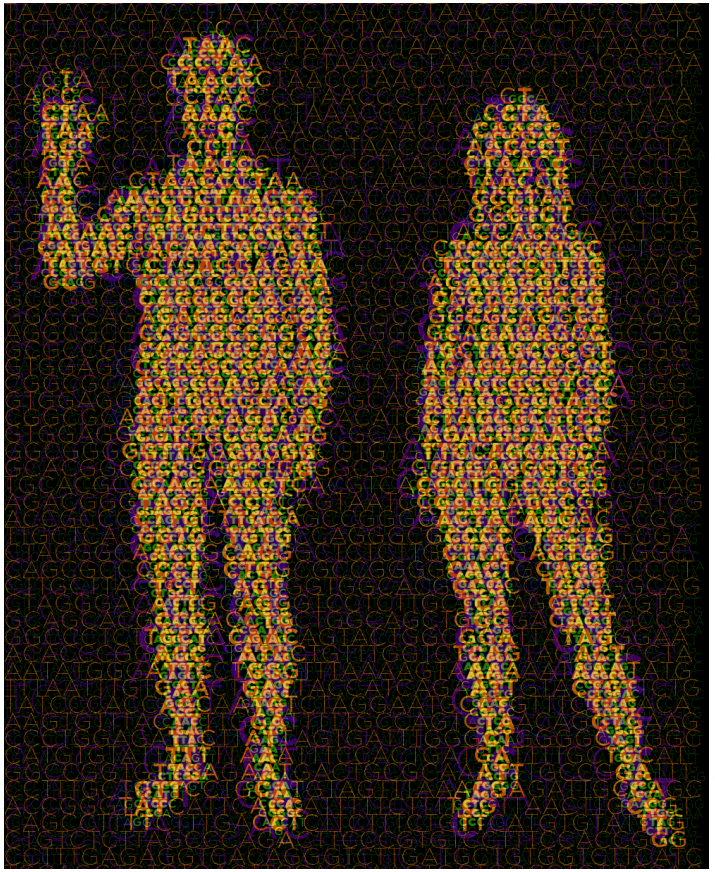
- Genome Reference Consortium
- A mosaic nucleid acid sequence
  - ...GTGCGTAGACTGCTAGATCGAAGA...



Reference genome

- Genome Reference Consortium
- A mosaic nucleid acid sequence
  - ...GTGCGTAGACTGCTAGATCGAAGA...

- What changes between versions?
  - First version: 150,000 gaps
  - HG19: 250 gaps



Variants

A position where sample sequence does not agree with reference genome sequence

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

Variants

A position where sample sequence does not agree with reference genome sequence

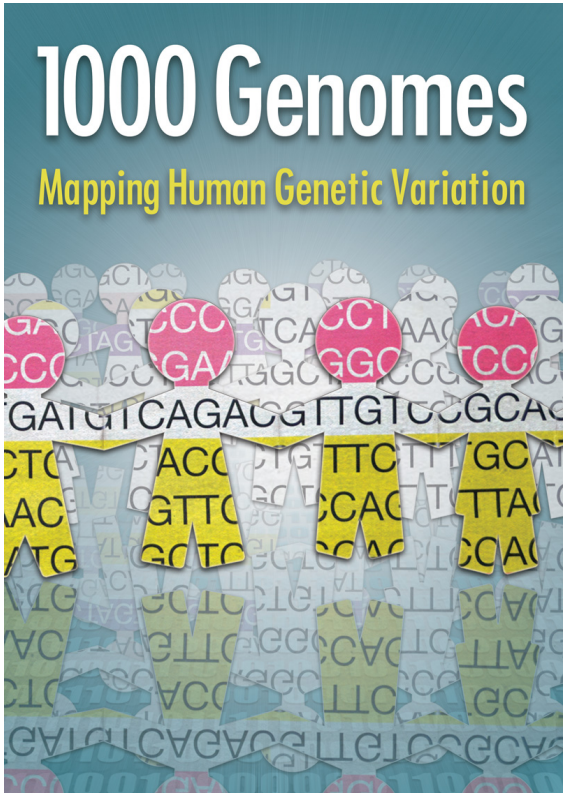
Reference: ...GTGCGTAGACTGCTAGATCGAAGA...
Sample: ...GTGCGTAGACTGATAGATCGAAGA...

Variants

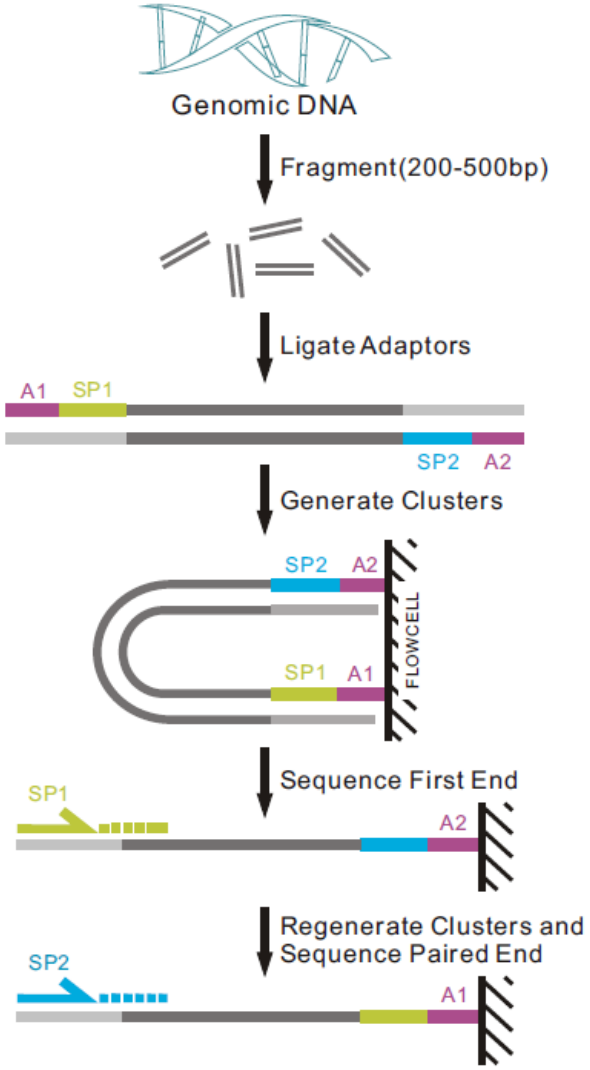
Population based variant projects

UK 10K

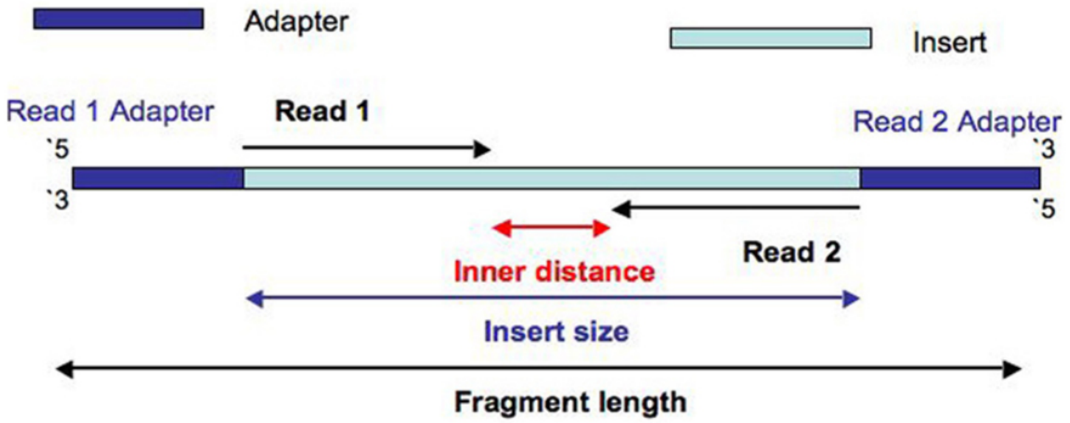
RARE GENETIC VARIANTS IN HEALTH AND DISEASE



Paired-end sequencing



Paired-end data



Paired-end data

The forward and reverse reads are stored in two fastq files.

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
```

Paired-end data

The forward and reverse reads are stored in two fastq files.

The order of pairs and naming is identical, except the designation of forward and reverse.

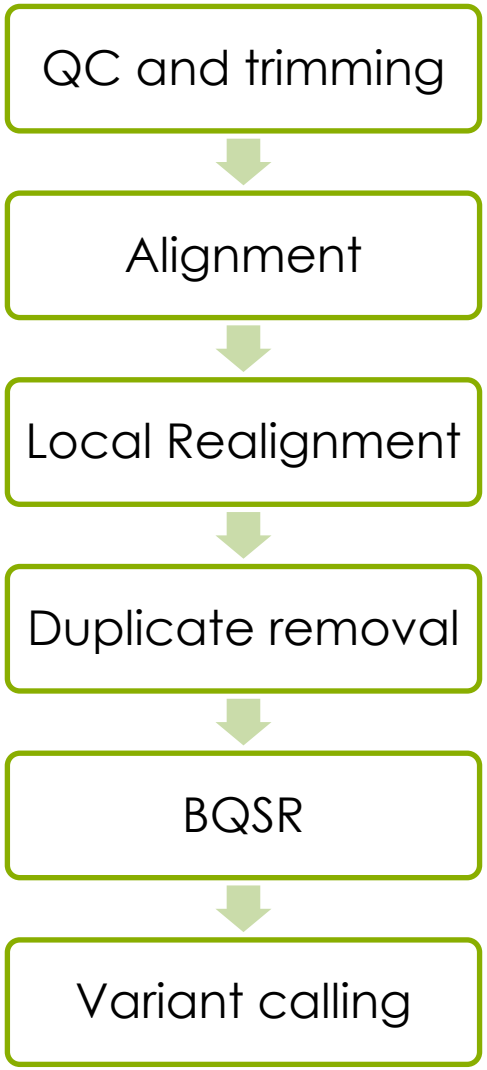
ID_R1_001.fastq

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

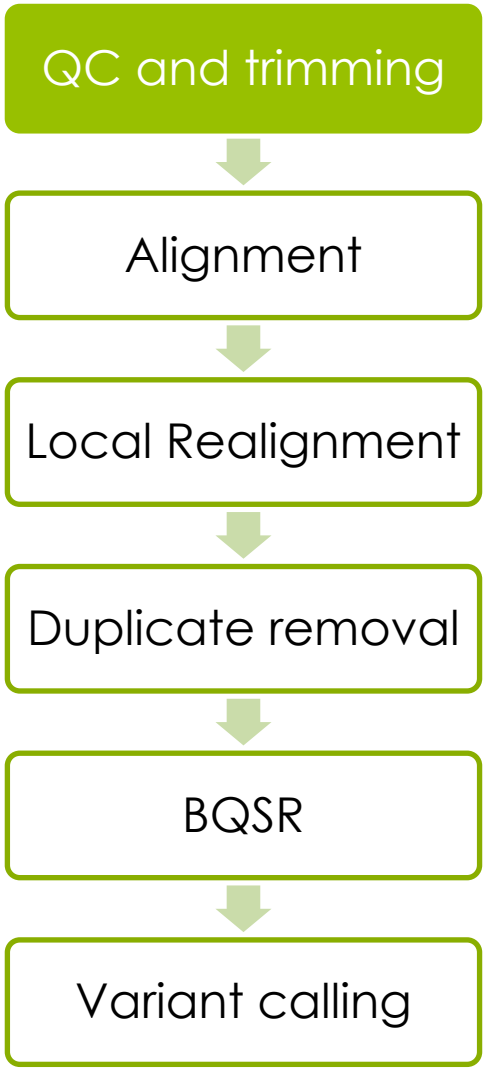
ID_R2_001.fastq

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

NGS workflow



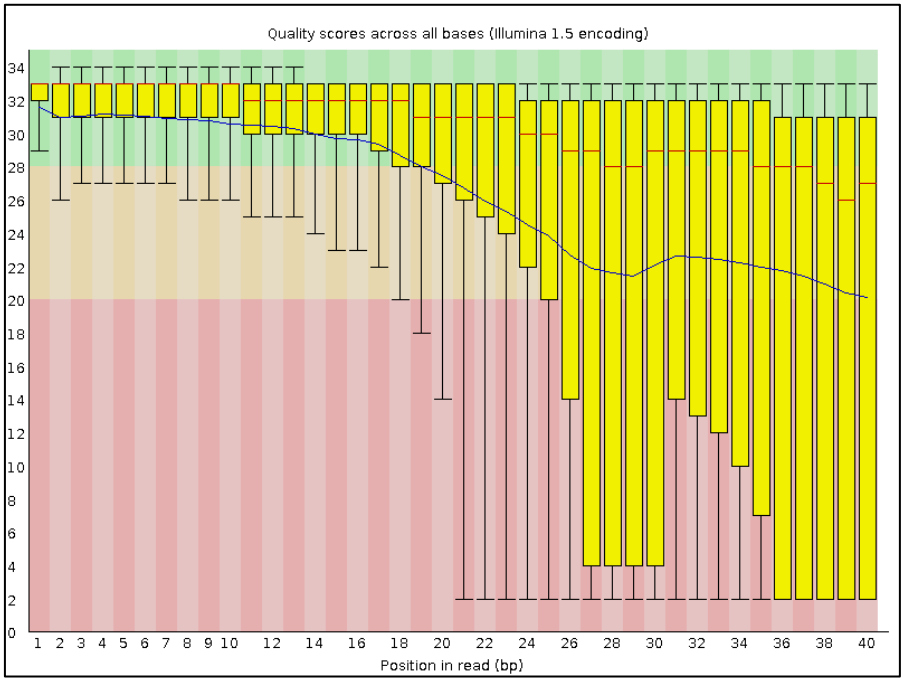
NGS workflow



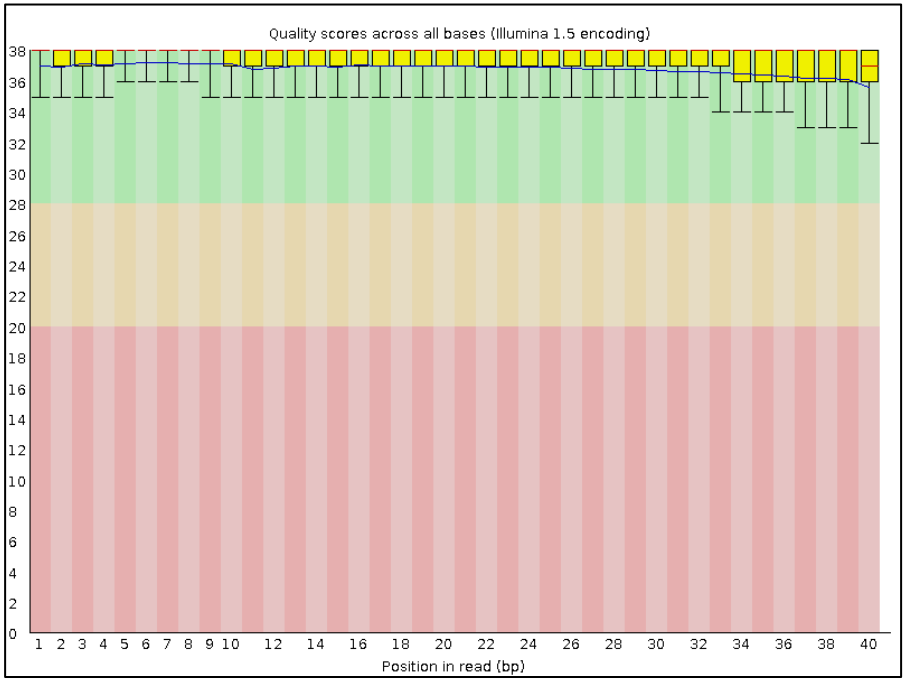
Quality control

```
module load FastQC
```

Bad qualities:



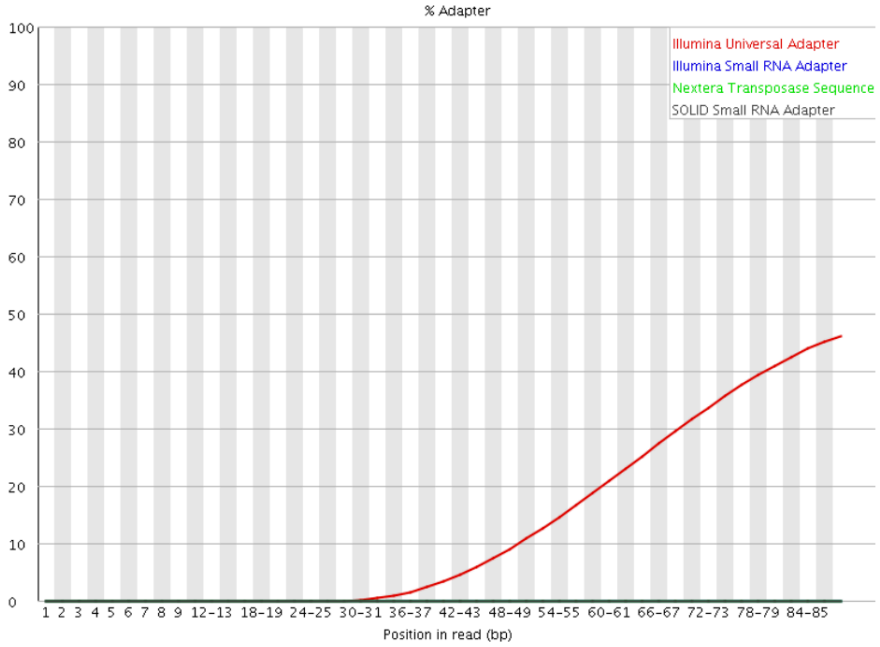
Good qualities:



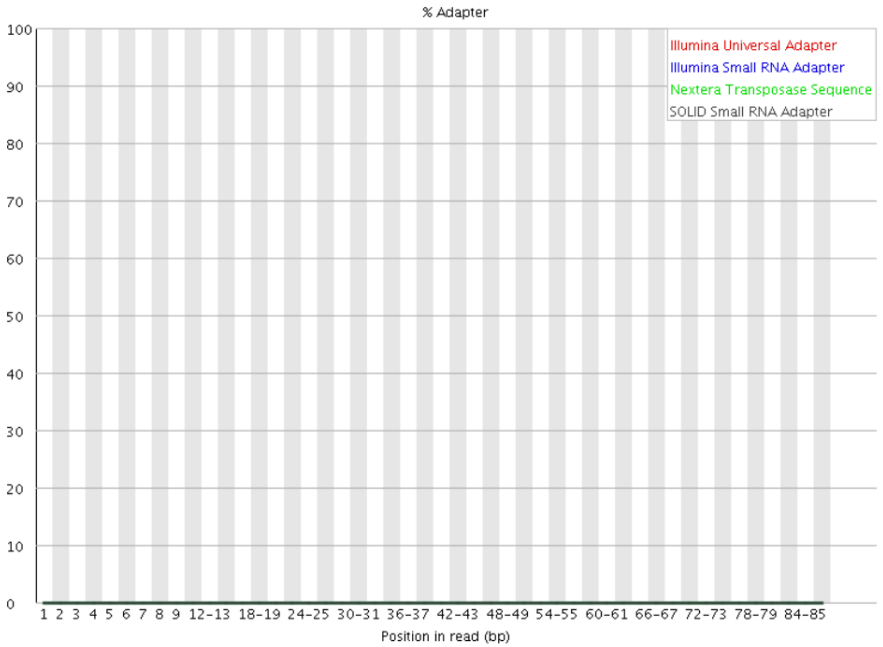
Quality control

```
module load FastQC
```

Adapters present:

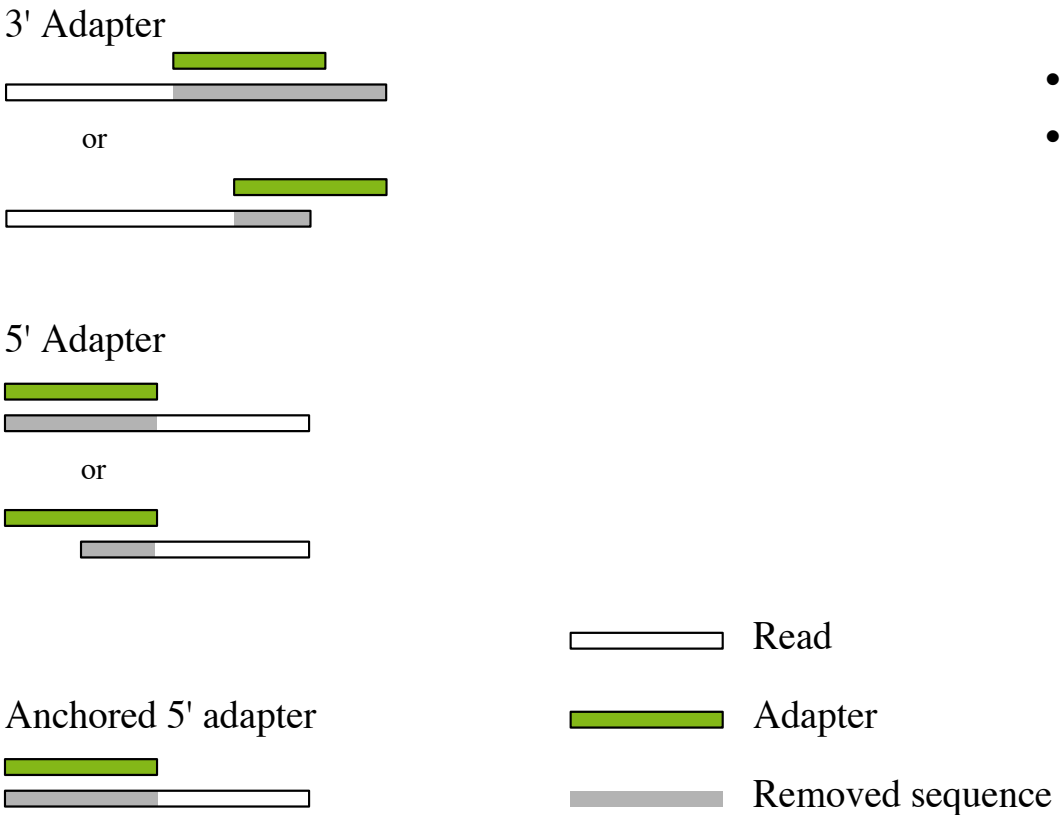


Adapters Absent:



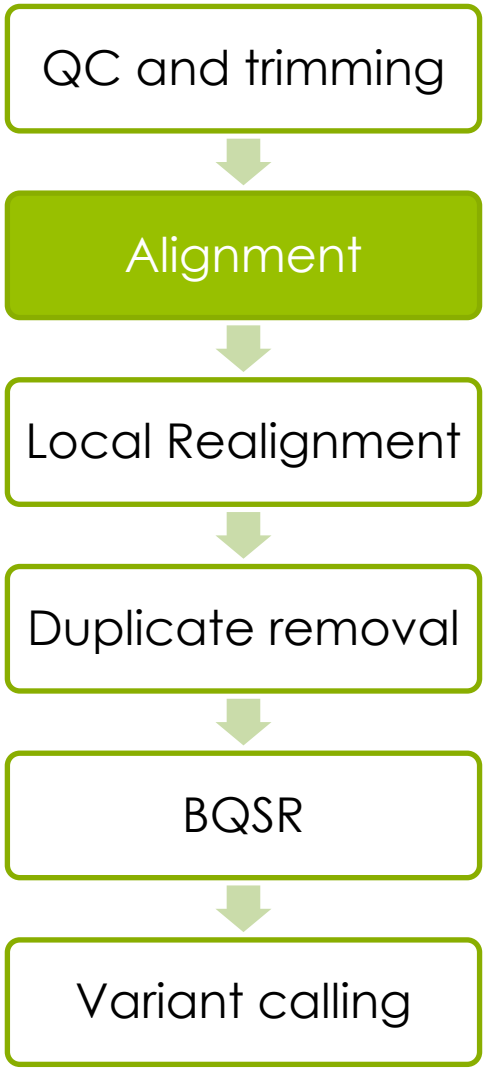
Trimming

```
module load cutadapt / TrimGalore / trimmomatic
```

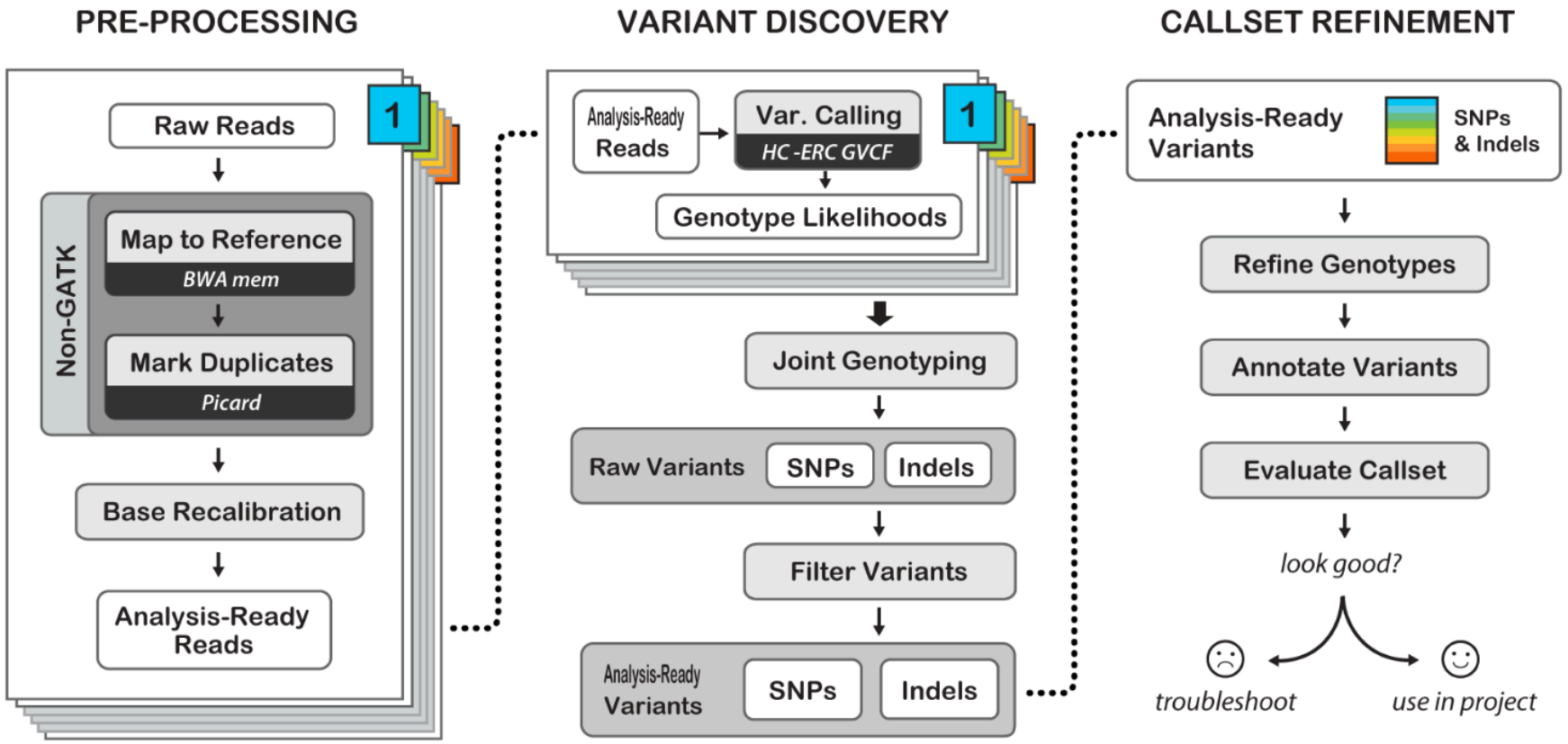


- Remove bad quality reads
- Remove adapters

NGS workflow



GATK Best Practices



Best Practices for Germline SNPs and Indels in Whole Genomes and Exomes - June 2016

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

Alignment

```
module load bwa
```

Read	TCGATCC
Reference	GACCTCATCGATCCCACTG

Alignment

```
module load bwa
```

Read TCGATCC
Reference GACCTCATCGATCCCACTG

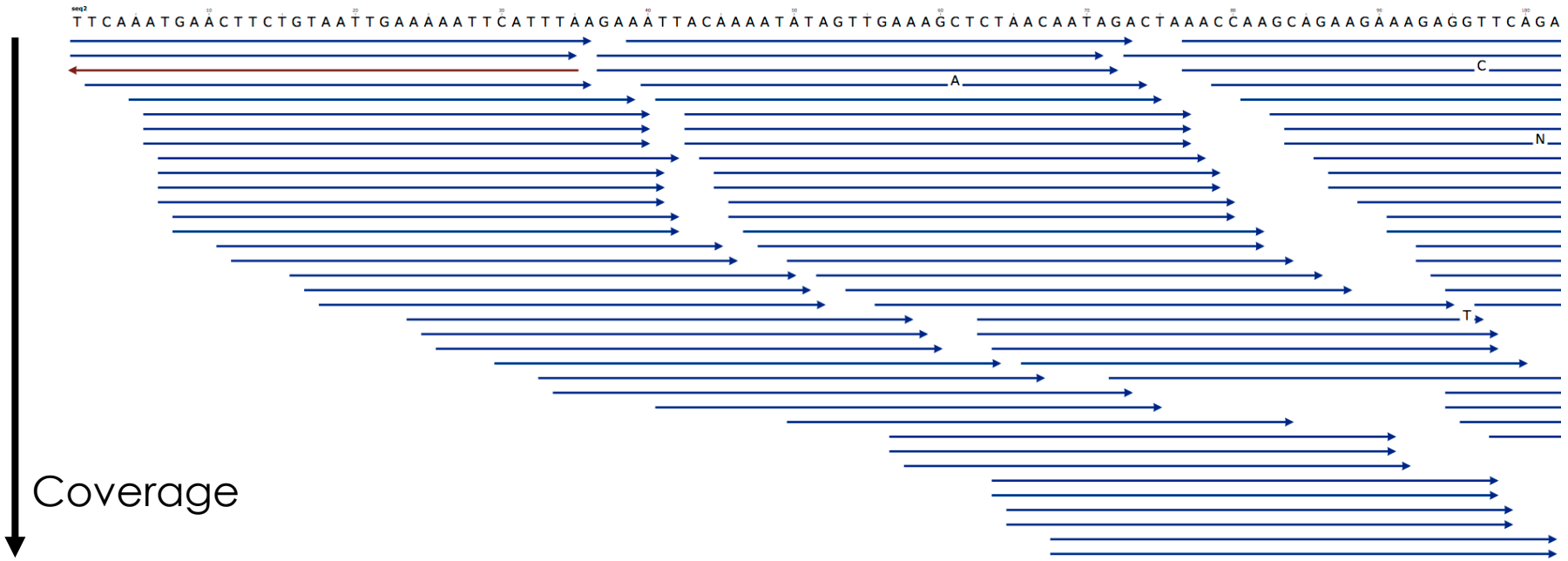
Read TCGATCC
Reference GACCTCATCGATCCCACTG

Alignment

```
module load bwa
```

Alignment

```
module load bwa
```



Paired-end data

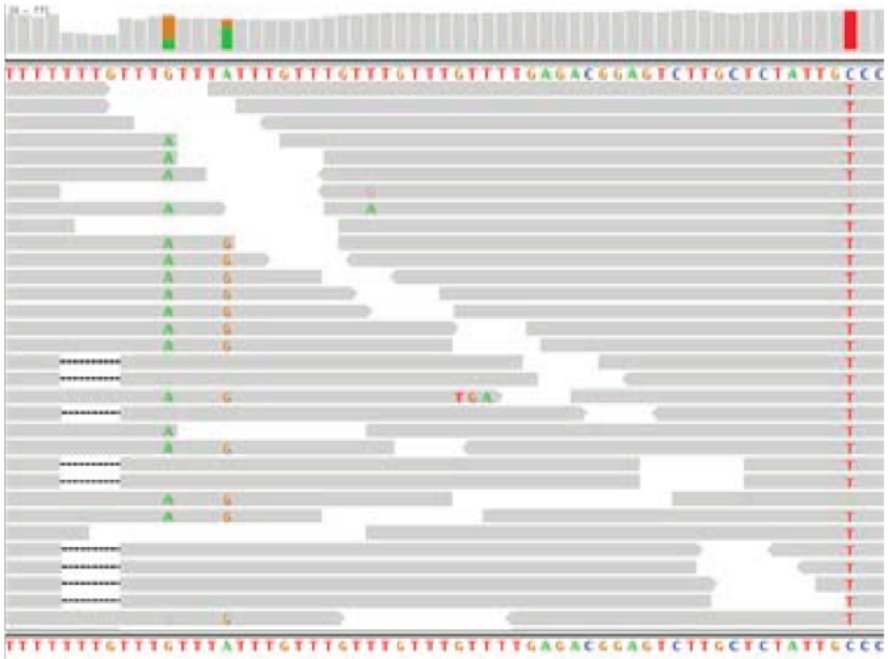
The known distance between paired reads allows improved mapping over repeat regions

NGS workflow

Local realignment

Problem: Reads are mapped **one** read at a time, this sometimes leads to single variants being split into multiple variants

Solution: Realign such a region taking **all** reads into account



HiSeq data, raw BWA alignments

HiSeq data, after MSA

Local realignment

```
module load GATK
```

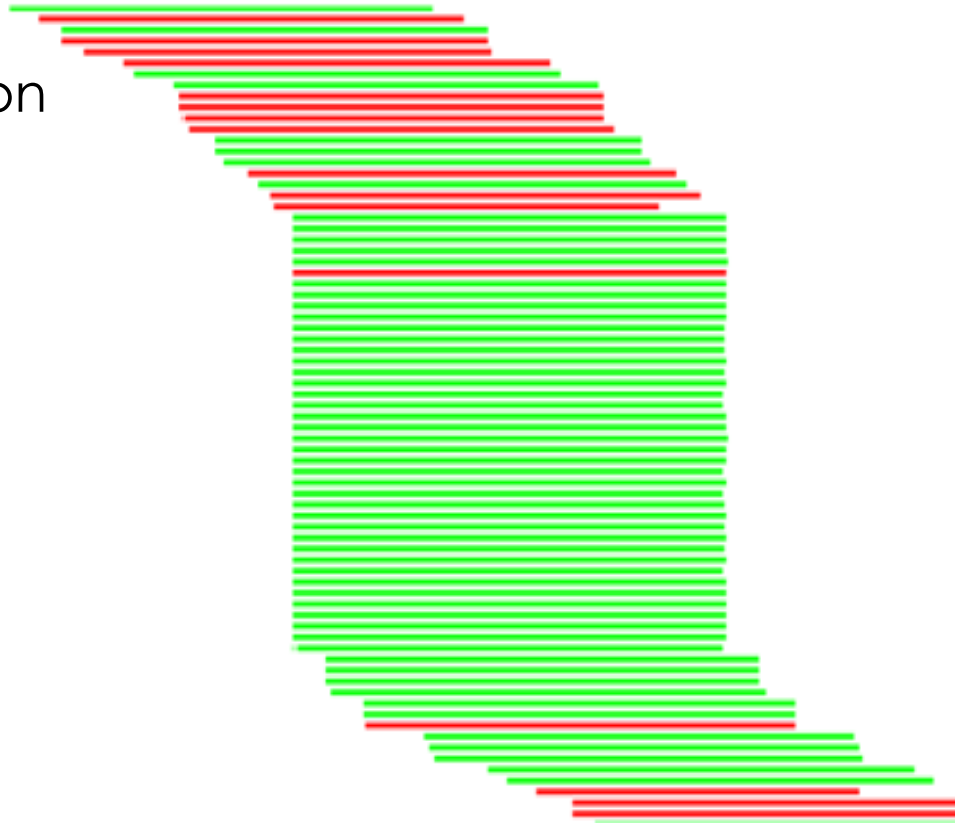
- `Genome Analysis ToolKit`
  - RealignerTargetCreator
  - IndelRealigner
- Local realignment, still needed?
  - HaplotypeCaller (HC)
  - Mutect2

NGS workflow

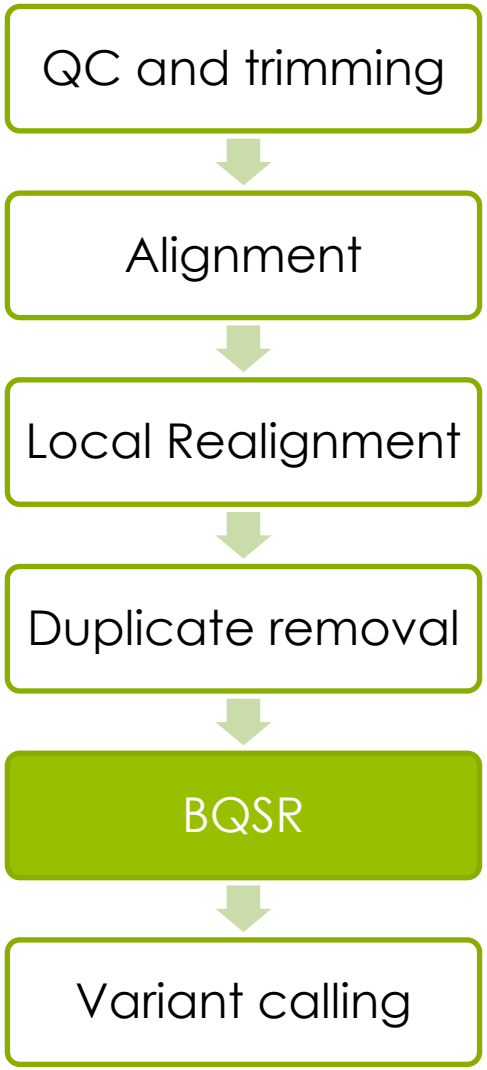
PCR duplicates & removal

```
module load picard
```

- Occur during library preparation
- Optical duplicates
- Don't add unique information



NGS workflow



Base Quality Score Recalibration

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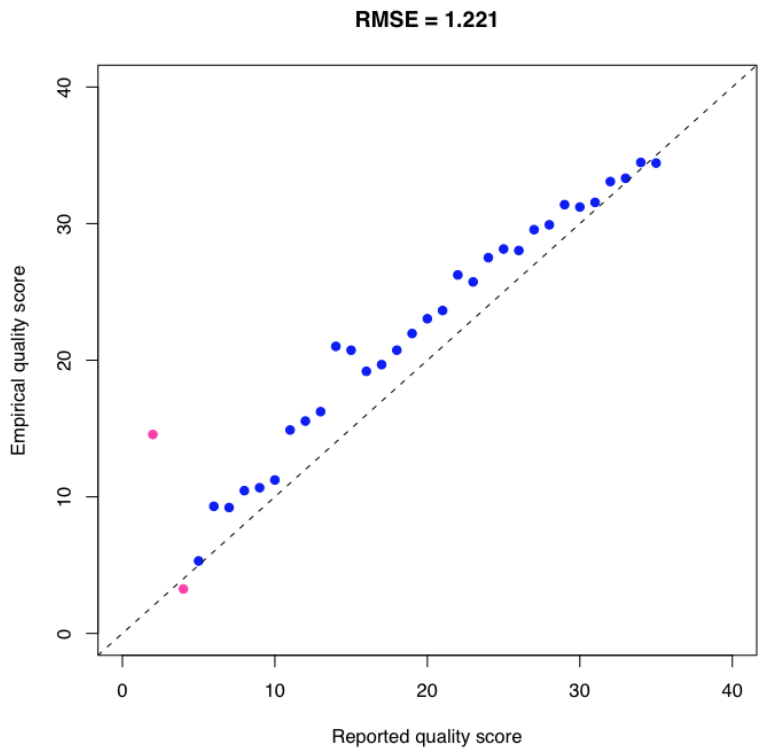
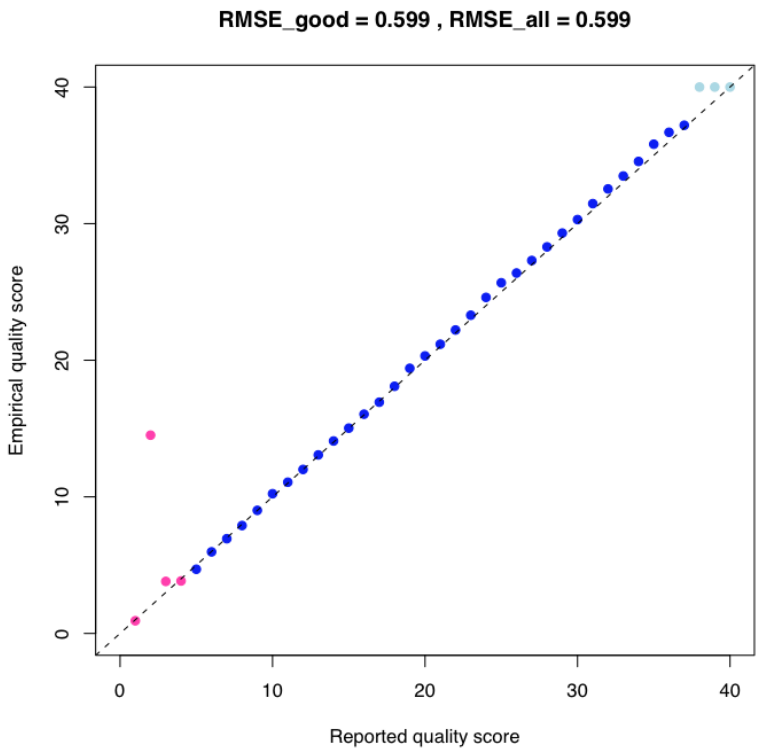
Reported Quality vs. Empirical Quality



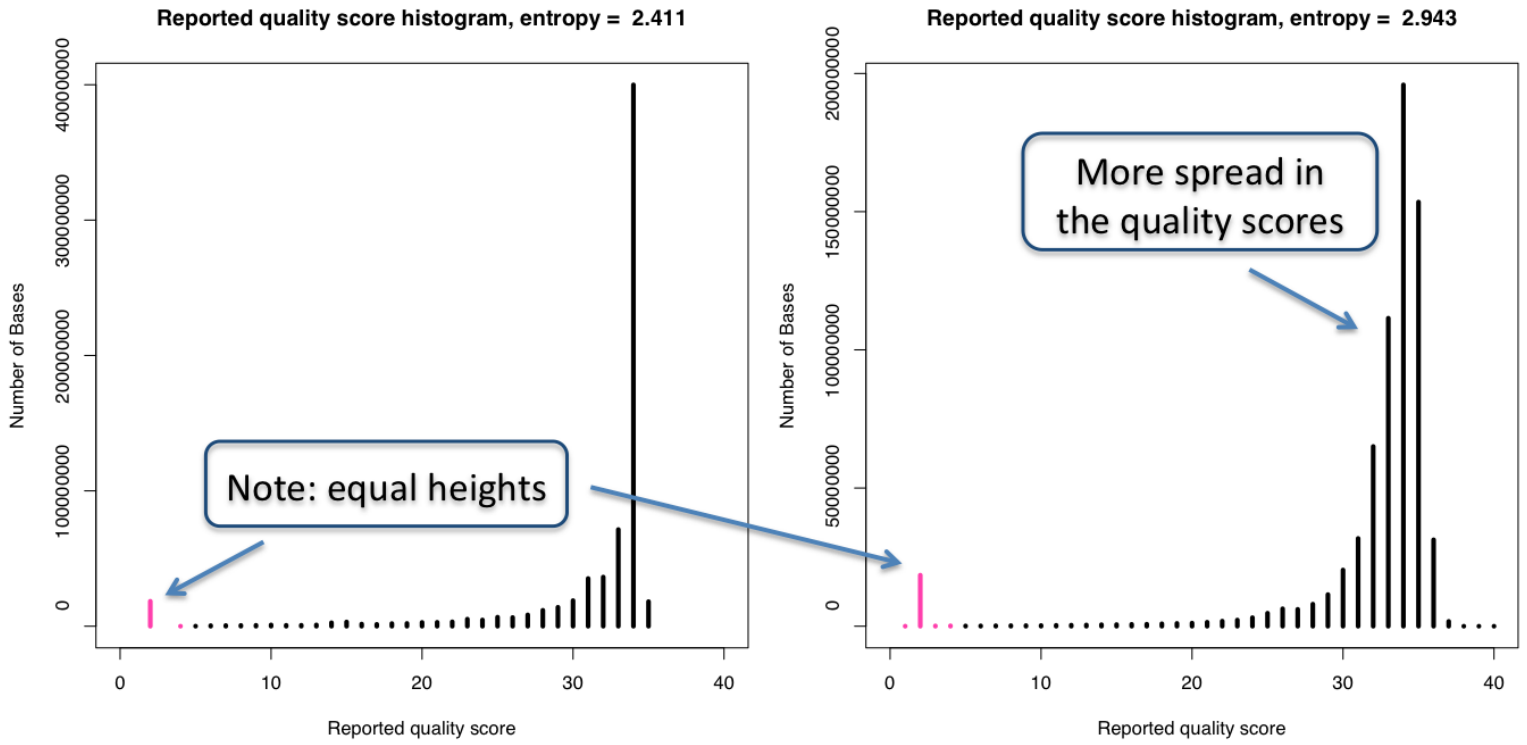
Original Data



After GATK Recalibration

Base Quality Score Recalibration

Distribution of Quality Scores

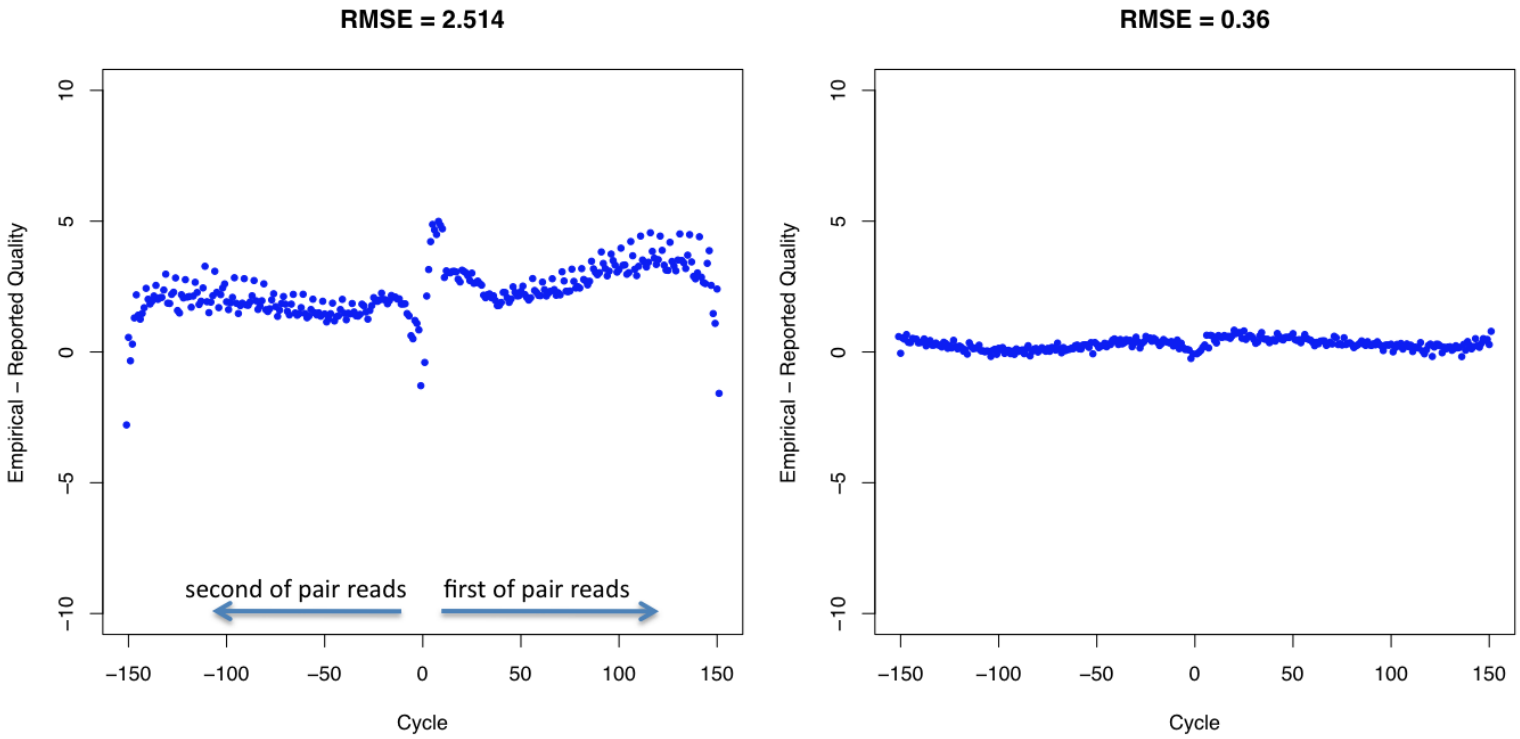


Original Data

After GATK Recalibration

Base Quality Score Recalibration

Residual Error by Machine Cycle



Original Data

After GATK Recalibration

Base Quality Score Recalibration

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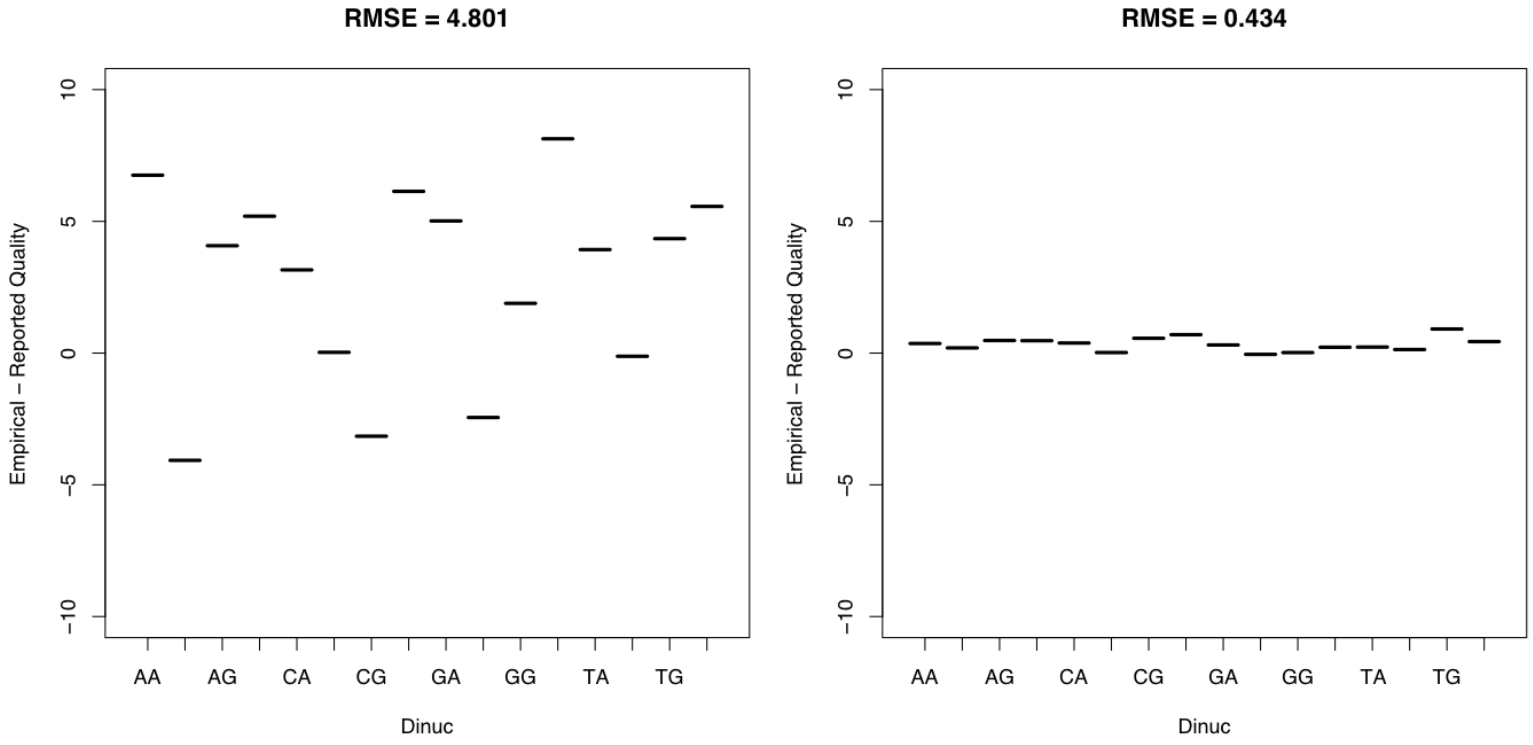
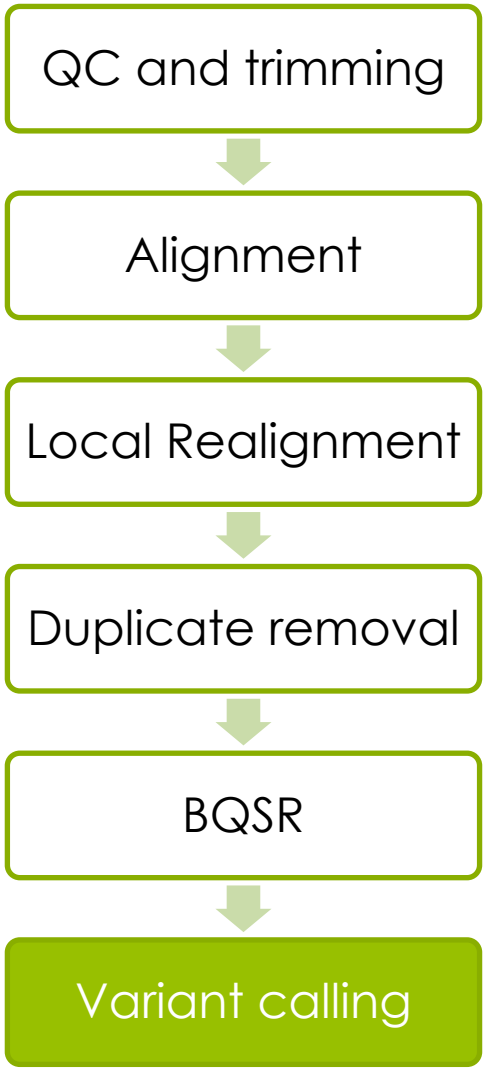
Residual Error by Dinucleotide



Original Data

After GATK Recalibration

NGS workflow



Variant calling

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

Sample: ...GTGCGTAGACTGATAGATCGAAGA...

Variant calling

NBIS SciLifeLab

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

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

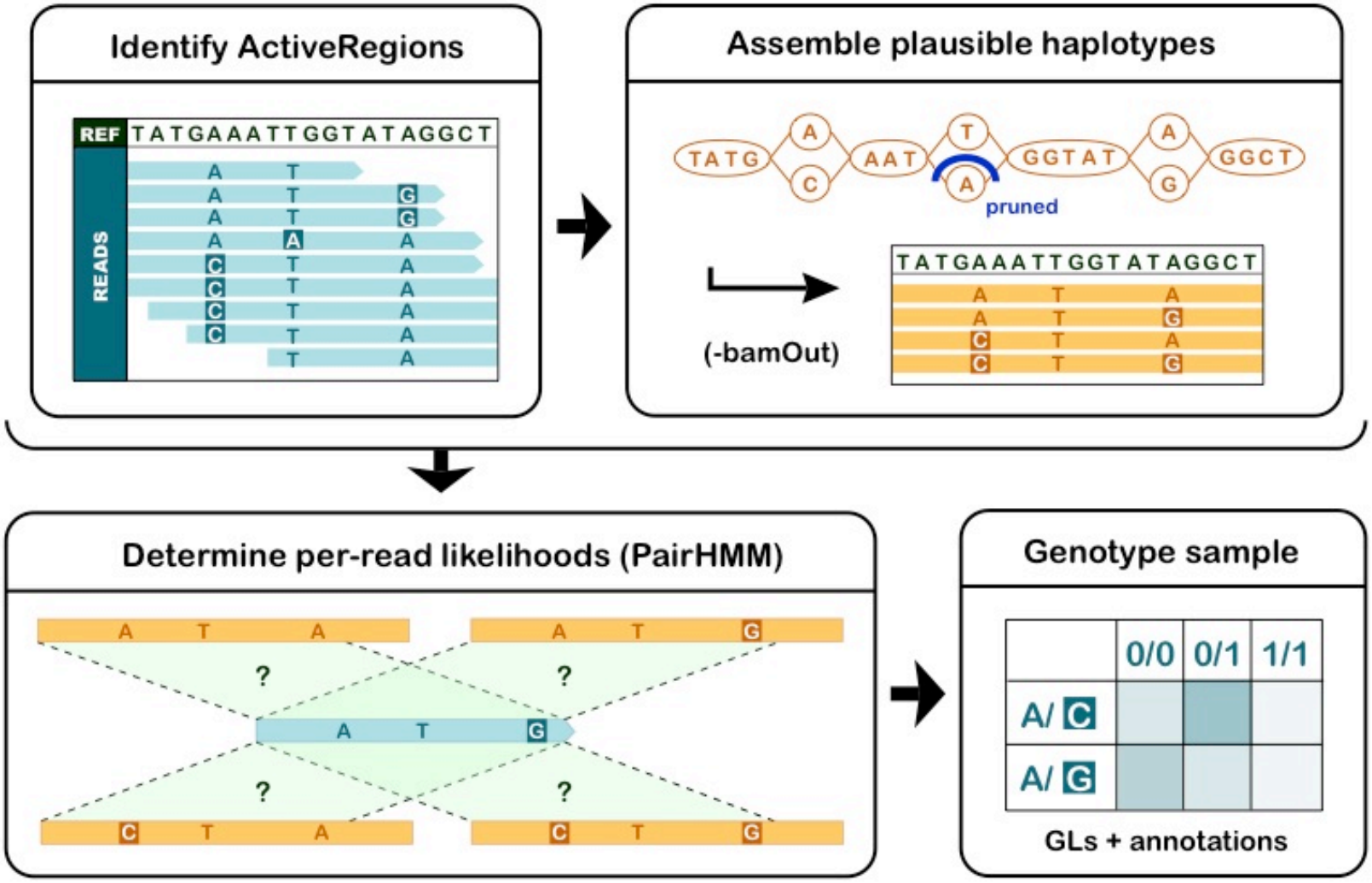
Variant calling

Reference: ...GTGCGTAGACTGCTAGATCGAAGA...

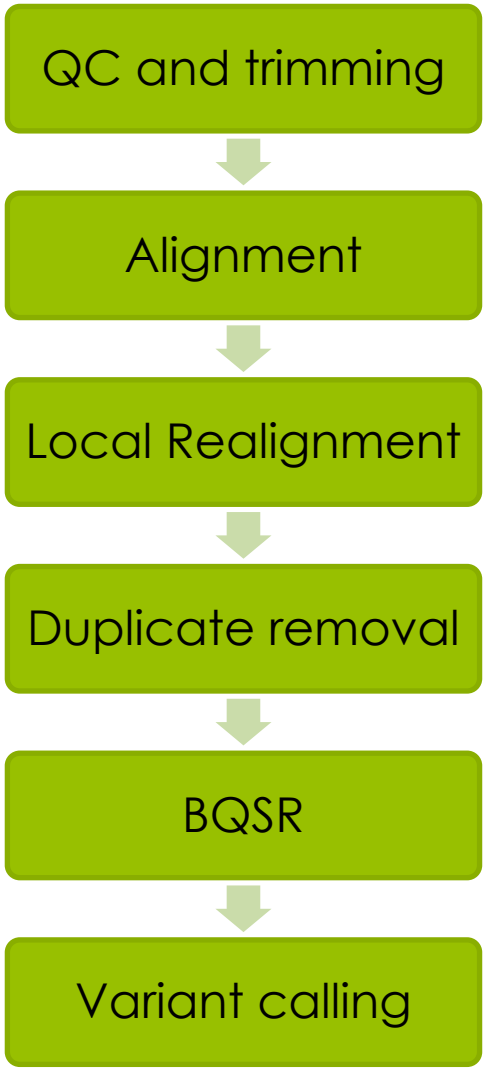
Sample:
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...
...GTGCGTAGACTGCTAGATCGAAGA...
...GTGCGTAGACTGATAGATCGAAGA...

$$\frac{\#Variants\ in\ a\ position}{\#Reads\ in\ a\ position} = A\ variants\ allele\ frequency$$

Variant Calling



NGS workflow



VCF Files

```
##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 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
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
```

VCF Files

NBIS SciLifeLab

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##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
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
```

VCF Files

```
##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 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
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
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##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
```

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
20	14370	rs6054257	G	A	29	PASS	NS=3;DP=14;AF=0.5;DB;H2
20	17330	.	T	A	3	q10	NS=3;DP=11;AF=0.017
20	1110696	rs6040355	A	G,T	67	PASS	NS=2;DP=10;AF=0.333,0.667;AA=T;DB

VCF Files

```
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##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">
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##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
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
```

VCF Files

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##source=myImputationProgramV3.1
##reference=1000GenomesPilot-NCBI36
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##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">
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##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
```
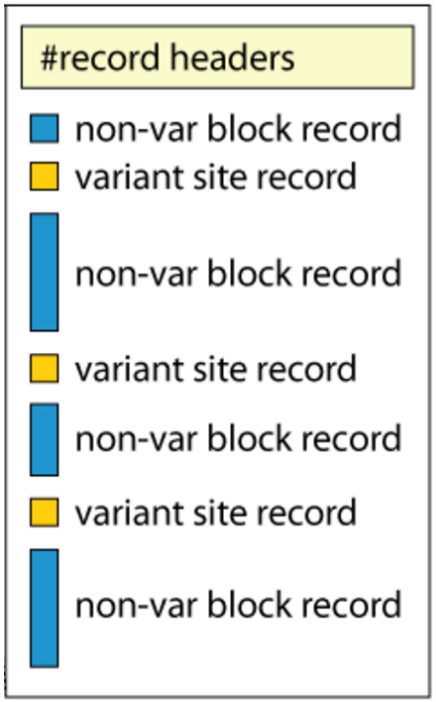
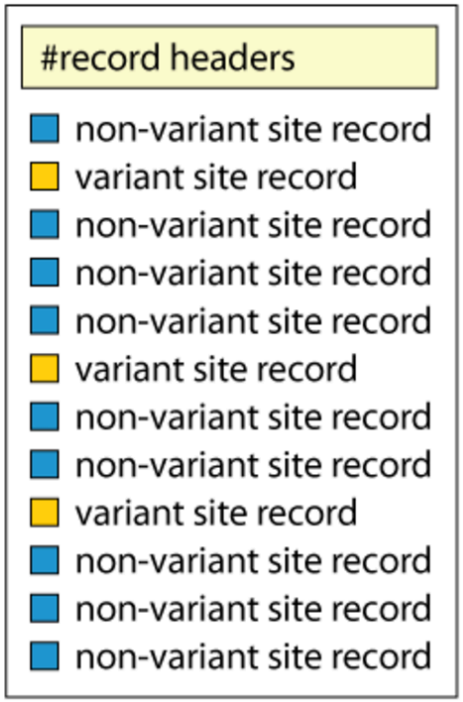

#FORMAT	NA00001	NA00002	NA00003
GT:GQ:DP:HQ	0|0:48:1:51,51	1|0:48:8:51,51	1/1:43:5:.,.
GT:GQ:DP:HQ	0|0:49:3:58,50	0|1:3:5:65,3	0/0:41:3
GT:GQ:DP:HQ	1|2:21:6:23,27	2|1:2:0:18,2	2/2:35:4

gVCF Files

New gVCF

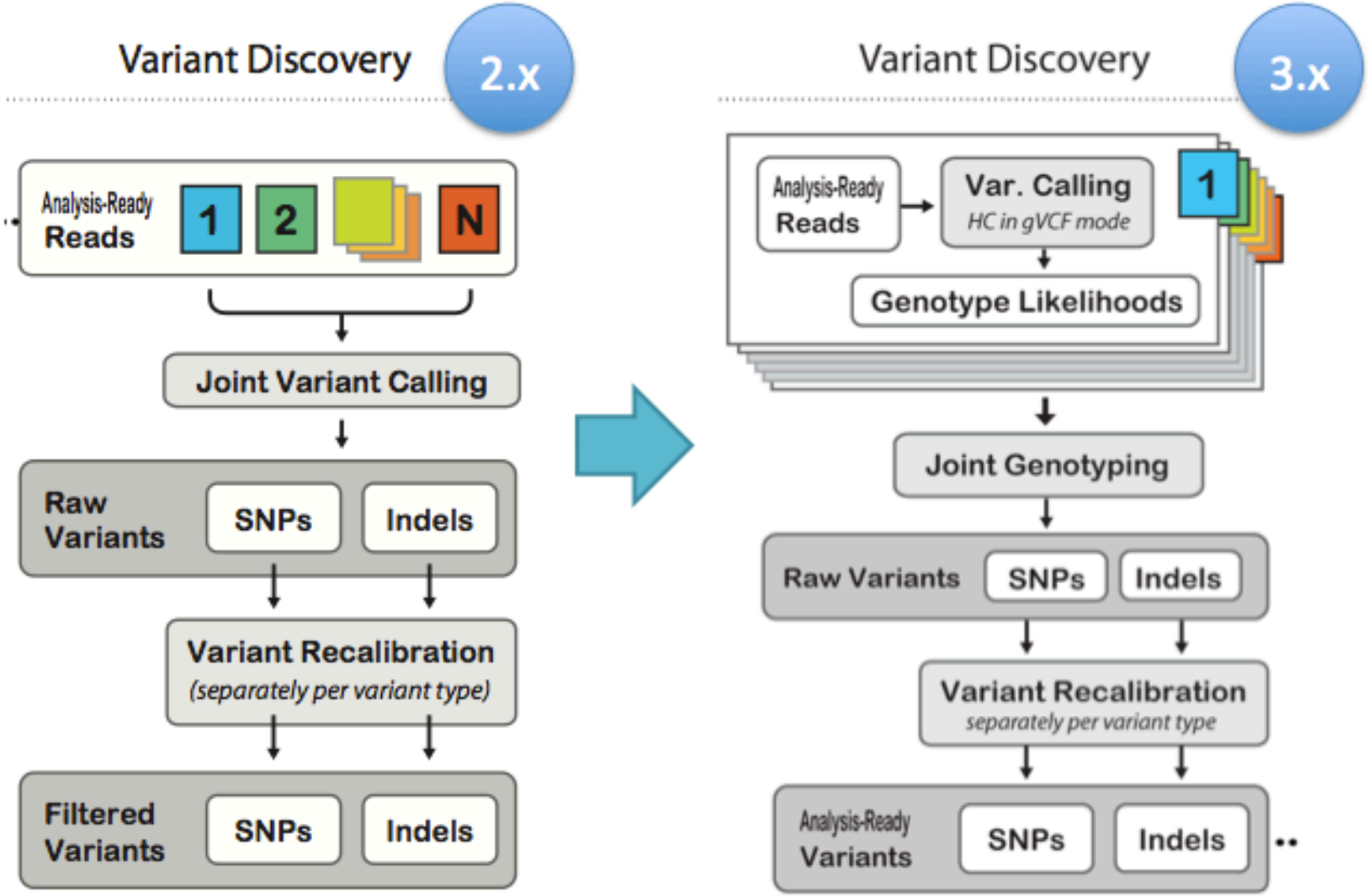


Old gVCF




```
##GVCFBlock=minGQ=0(inclusive),maxGQ=5(exclusive)
##GVCFBlock=minGQ=20(inclusive),maxGQ=60(exclusive)
##GVCFBlock=minGQ=5(inclusive),maxGQ=20(exclusive)
```

Joint genotyping



Annotation & Filtering

```
module load annovar /snpEff / vep

#CHROM POS ID REF ALT QUAL
20 14370 rs6054257 G A 29
```

- Gene-based
  - Non-synonymous/synonymous
- Region-based
  - CpG-islands
  - Conserved regions
  - Predicted transcription factor binding sites
- Filter-based
  - dbSNP
  - 1000G
  - COSMIC

Annotation & Filtering

```
module load annovar /snpEff / vep
```

```
#CHROM POS ID REF ALT QUAL
20 14370 rs6054257 G A 29
```

- Gene-based
  - Non-synonymous/synonymous
- Region-based
  - CpG-islands
  - Conserved regions
  - Predicted transcription factor binding sites
- Filter-based
  - dbSNP
  - 1000G
  - COSMIC

USE THE SAME REFERENCE!

Annotation & Filtering

```
module load GATK
```

```
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT
20 14370 rs6054257 G A 29 PASS NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP:HQ
```

VariantFiltration

--filterExpression "QUAL > 30"

--filterName QUAL_filter

--filterExpression "QUAL / DP < 10.0"

--filterName QUALDP_filter

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Questions?

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Questions?

Work like a professional bioinformatician – Google errors!