Technical notes

Using Kooplex-edu for sequence analysis

a short tutorial

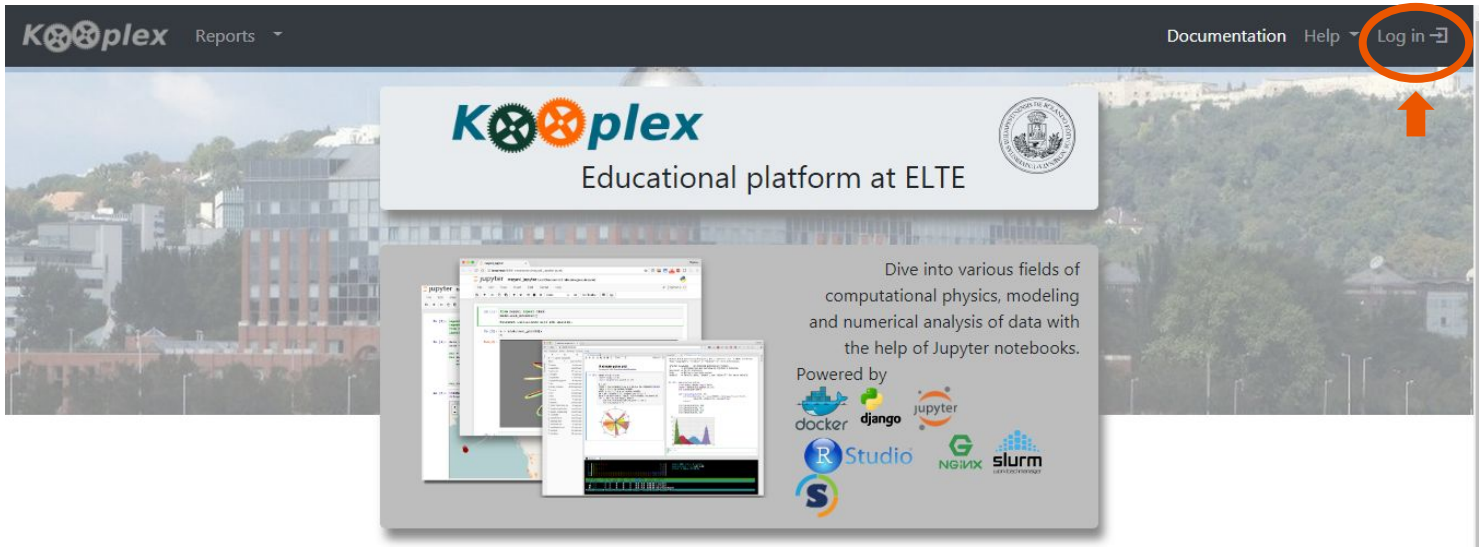
Overview

- Kooplex-edu provides a separate “virtual computer” for each student for each of his/her courses
- Data and tools can be uploaded to Kooplex-edu by teachers to keep necessary resources in the same place
- Assignments can be created, submitted and corrected on Kooplex-edu (with automatic collection at deadline)
- You can use Kooplex-edu for any of your courses if you need a place for programming or documenting pipelines/laboratory exercises

Find the website

https://kooplex-edu.elte.hu

Log in


Kooplex
Reports
Documentation
Help
Log in
Kooplex
Educational platform at ELTE
Dive into various fields of computational physics, modeling and numerical analysis of data with the help of Jupyter notebooks.
Powered by
docker
django
jupyter
Studio
NGINX
slurm

Log in

Sign in with your **caesar**

Log in

Sign in with your **caesar** or **neptun account**

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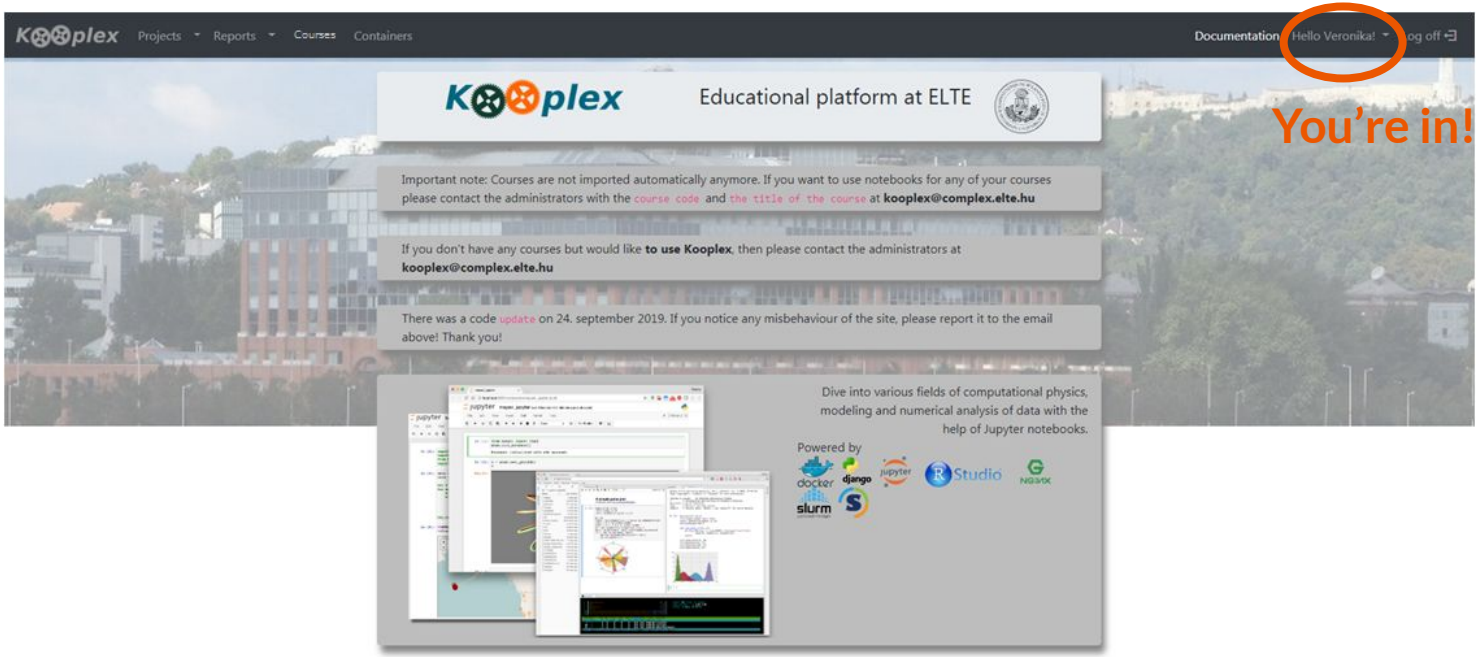
Find the course



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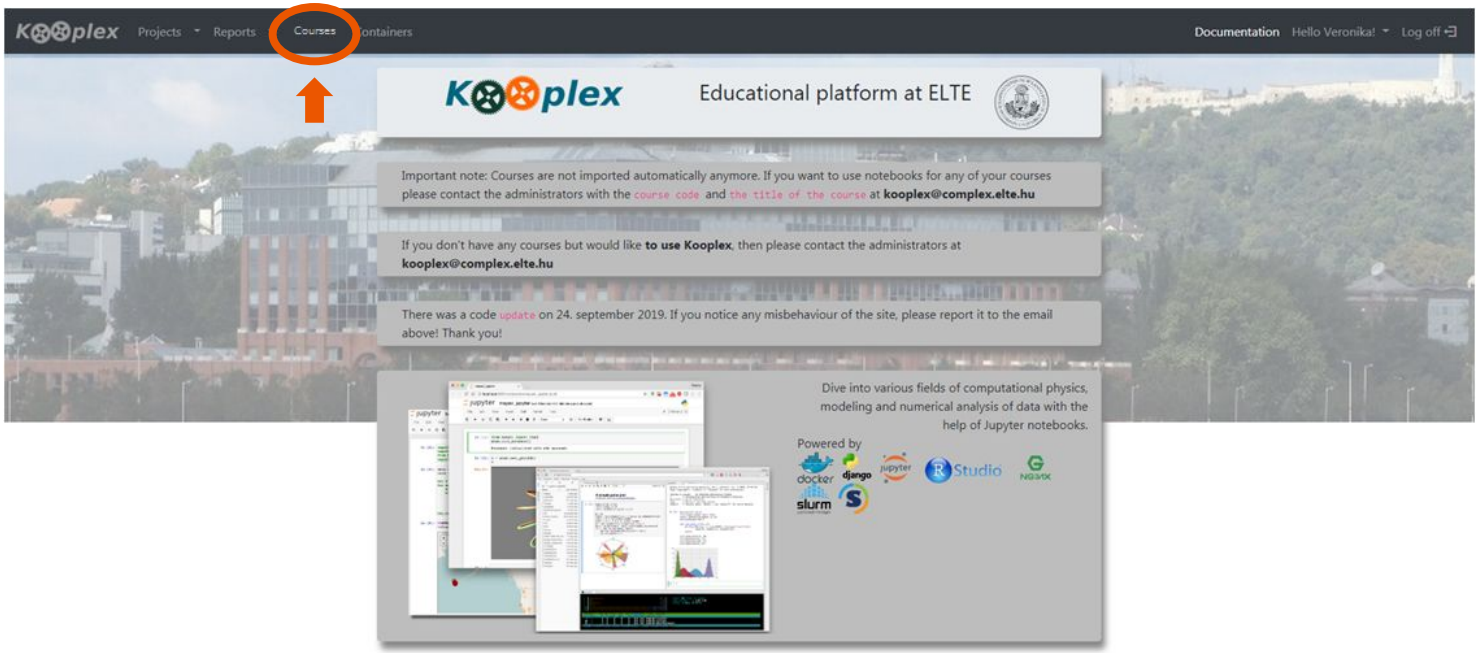
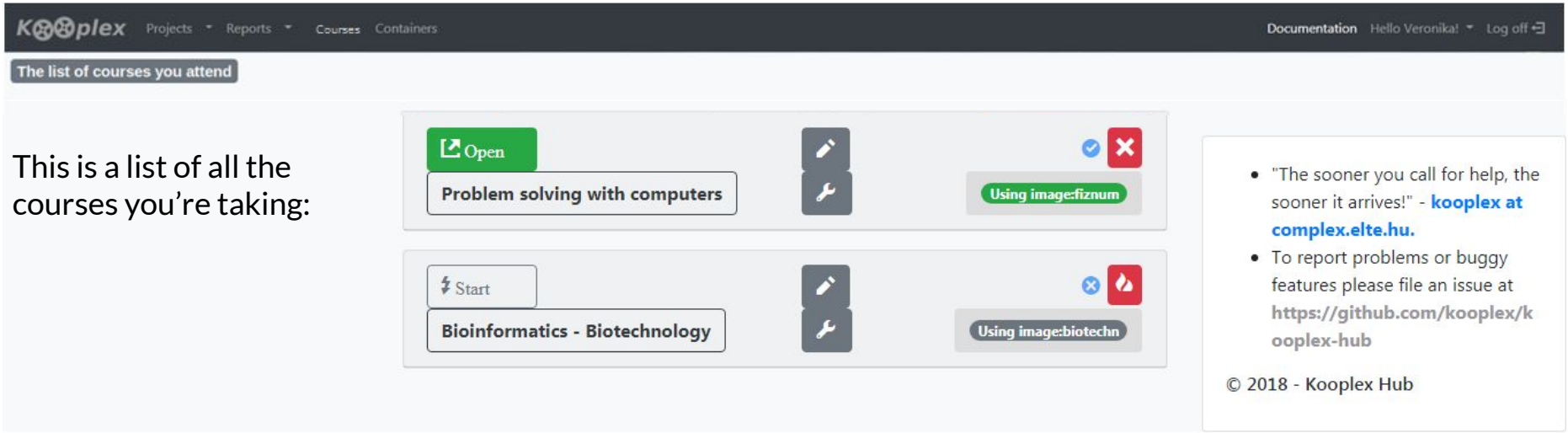
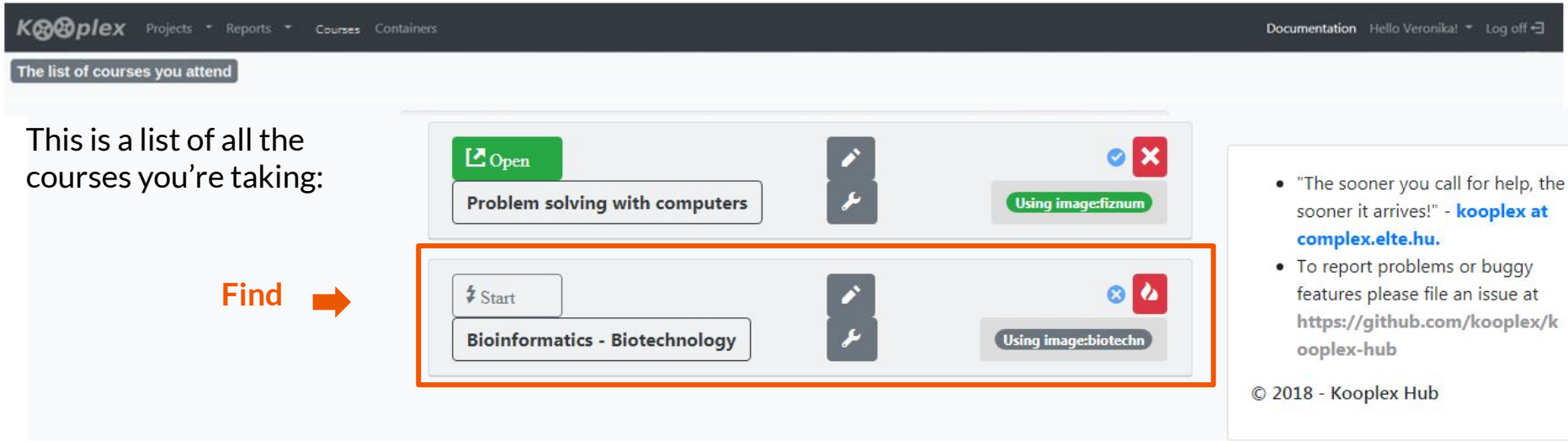


Find the course

This is a list of all the courses you're taking:



Find the course



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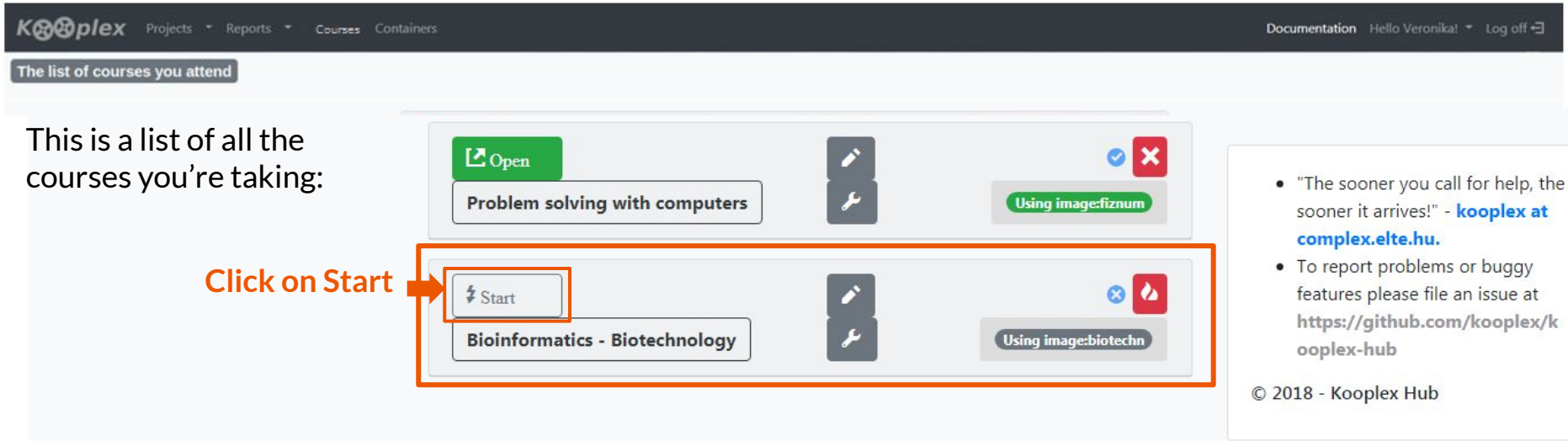
Find

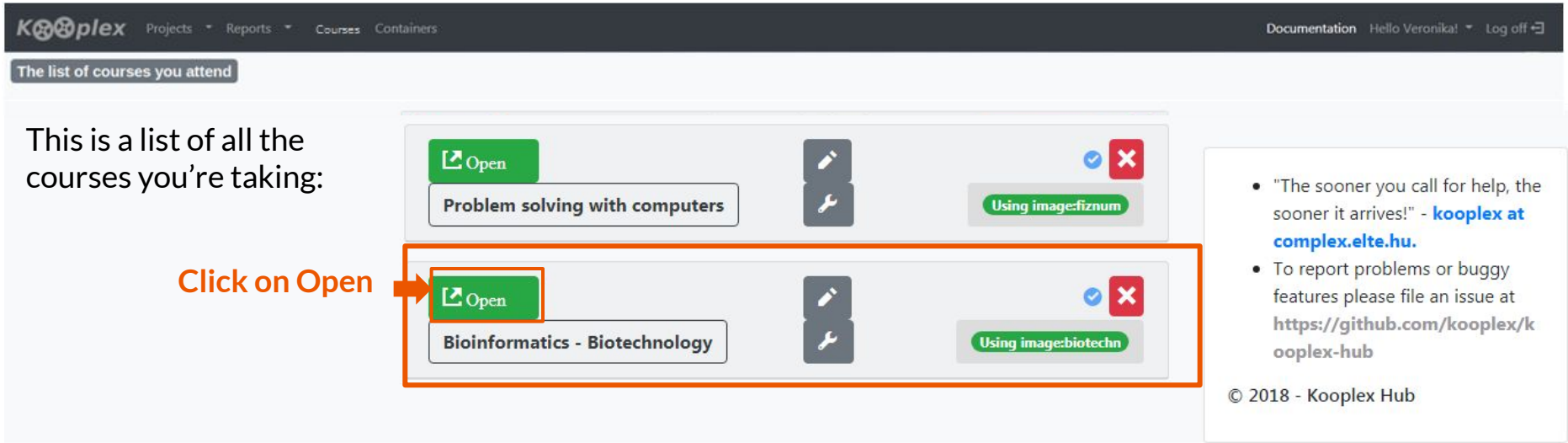
Find the course



This is a list of all the courses you're taking:

Click on Start

Find the course



This is a list of all the courses you're taking:

Click on Open

Assignment directory

Assignment directory

Assignment notebook

Shared files and tools

Not writable!

Shared files and tools

Files Running Clusters

Select items to perform actions on them. Upload New

0 / share / genomeSequencing

Name	Last Modified	File size
..	seconds ago	
ANNOVARresults	an hour ago	
BAM	an hour ago	
FASTQ	an hour ago	
HC_GVCFs	an hour ago	
PoN_VCFs	an hour ago	
PoNs	an hour ago	
PUP	an hour ago	
tools	an hour ago	
VarCallResults	17 minutes ago	

About the notebook

- The notebook uses a python3 kernel and bash magics (`!`) to run external tools in linux bash
- In order to make the most out of jupyter notebooks, invest some time into reading tutorials (beginners guide, tips&tricks, detailed course in Hungarian)

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- Notebook cells are either Markdown

pretty text with formatting, LaTeX formulas, tables, etc.

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actual code that can be run and does things

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pretty text with formatting, LaTeX formulas, tables, etc.

actual code that can be run and does things

looks like code (not formatted), but cannot be run (does nothing)

About the notebook

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- Notebook cells are either Markdown, Code or Raw NBConvert
- All cell types can be run with: Shift+Enter

Things you don't need to know at this point

- Kooplex-edu allows you to submit your solutions to assignments and also collects them automatically at the predefined deadline
- You can install different python packages on Kooplex-edu that you might need for your courses
- There is a python package available for every task, learn to love python
- The Jupyter Notebook is an extremely powerful tool to create whole analysis pipelines that are documented in detail and are thus reproducible
- Reproducibility should be one of the key objectives of science

Technical inquiries

kooplex@complex.elte.hu

Technical problems:

- I can't sign in.
- The website is unavailable.
- I can't open the notebook.
- I can't find the course.
- etc.

Non-technical problems:

- I don't understand what the code does.
- My code doesn't work.
- I require more hints for the task.
- etc.

Genome sequencing

Bioinformatical analysis of
Next Generation Sequencing results

with a focus on human genome sequencing and cancer

Overview

- NGS technology, a (very) brief introduction

  PCR, short reads, base calling from image files

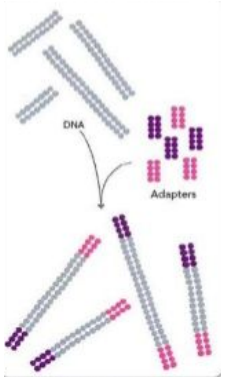
- Data analysis pipeline
  - Alignment
  - Preprocessing alignment files
  - Variant calling (SNVs, indels)
  - Interpreting variant files

NGS technology

Preparing DNA for sequencing

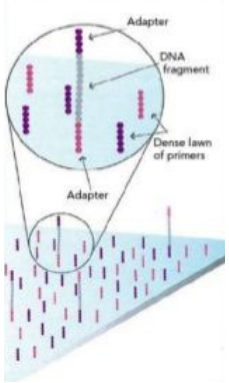
PCR + Short reads

Randomly fragment DNA after PCR and ligate adapters to both ends.



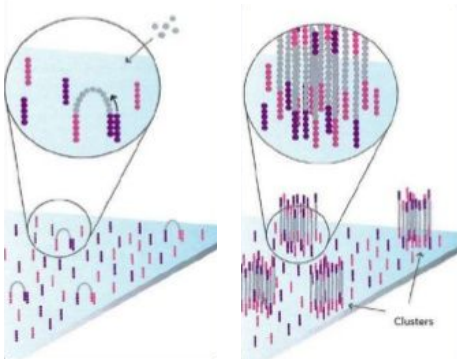
Attach to surface

Bind **single-stranded** fragments randomly to the flow cell.



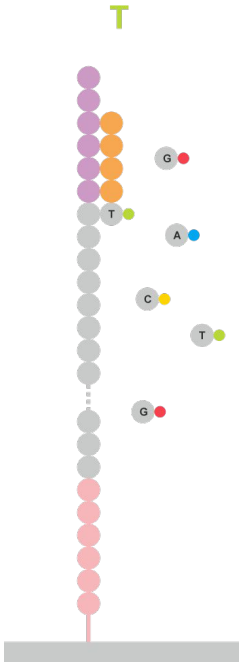
Amplification

Dense clusters of **identical** DNA are generated on the flow cell.

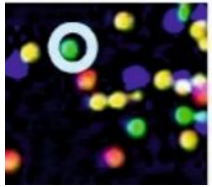


Sequencing by synthesis

1. Flow cell is washed with a mixture of **DNA polymerase enzyme and fluorescently tagged ddNTPs**. (four-colour chemistry: different emission for each base)



Sequencing by synthesis



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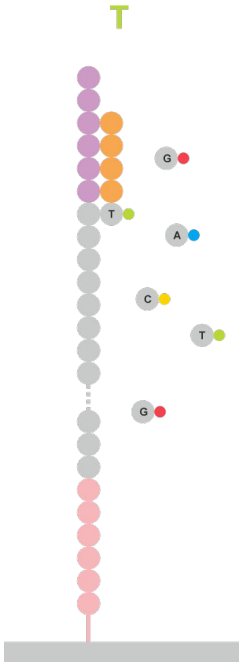
2. **Bright colored patches** (one for each cluster) are recorded as a **photo**.



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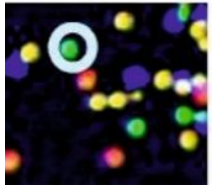

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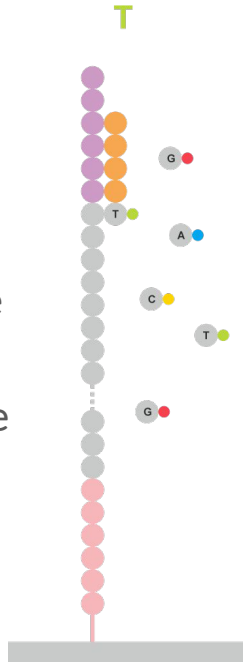

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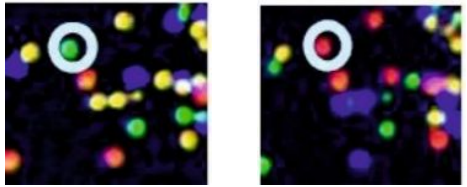

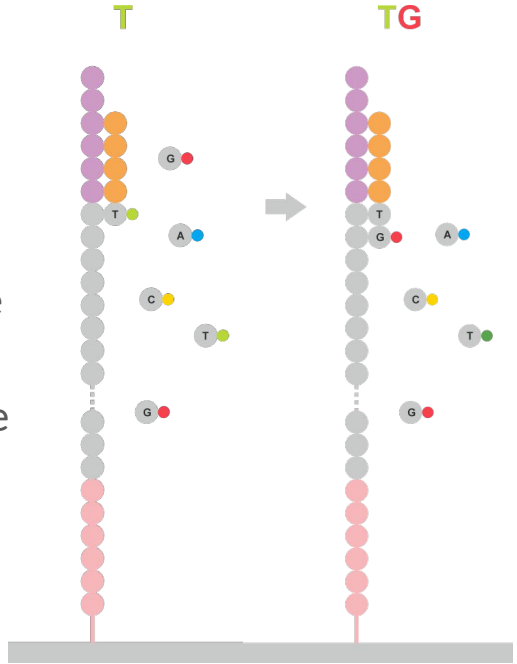

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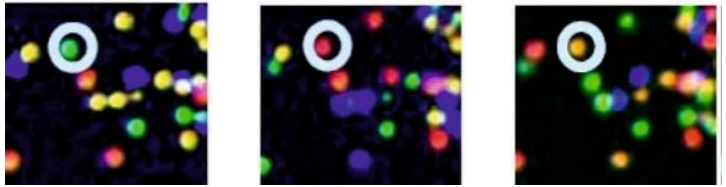
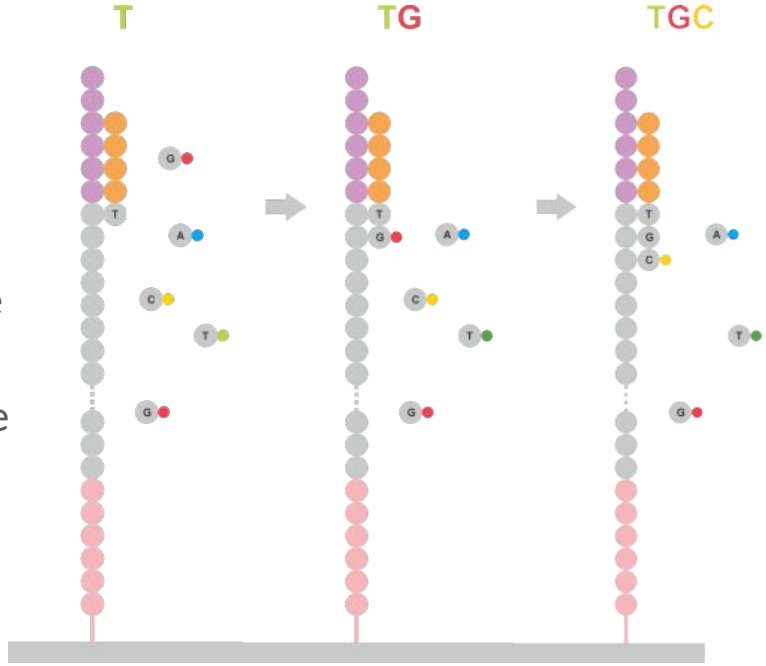
Sequencing by synthesis





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2. **Bright colored patches** (one for each cluster) are recorded as a **photo**.
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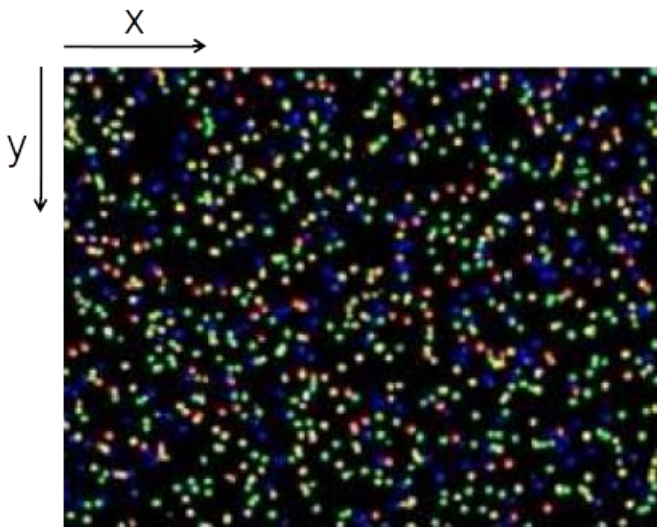
4. The process is repeated again…
5. And again...

From image to text



coordinates color intensity

x	y	A	C	G	T
...	...	...	...	...	...
17	20	4	13	76	3
17	25	2	45	41	10
...	...	...	...	...	...
1001	1253	8	1	2	97
...	...	...	...	...	...

→ G

→ G? C?

→ T

3D: (x,y) and time

Short read: fixed (x,y), changing time →

A A A C G T A C A C A bases

$Q_A Q_A Q_A Q_C Q_G Q_T Q_A Q_C Q_A Q_C Q_A$ base qualities

Base calling error, base quality

The **probability of calling a given base incorrectly**: P

(~ high, when we have trouble deciding between the colors)

Base quality (Phred-score): $Q = -10 \log_{10} P$

(The higher Q is, the more reliable the base call.)

Convert to ASCII:

1. Round to integer value
2. Add 33

$$Q_{ASCII} = round(Q) + 33$$

Output of NGS: FASTQ format

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

Data analysis

A general idea

De-novo assembly

1. Reconstruct the whole genome from the short reads

De-novo assembly

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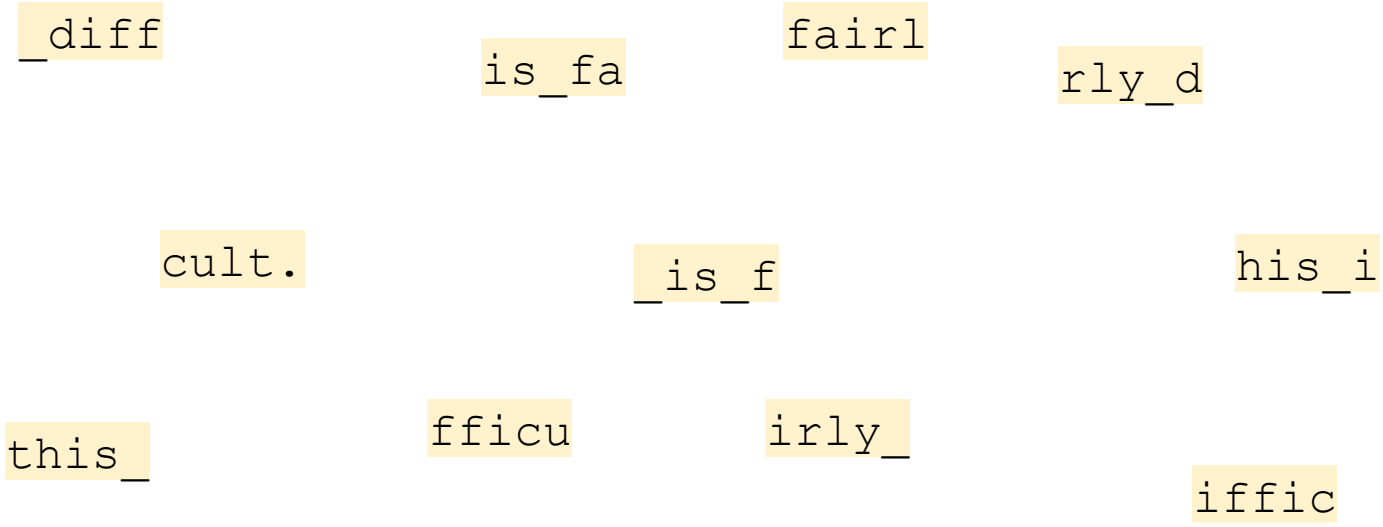


De-novo assembly

1. Reconstruct the whole genome from the short reads

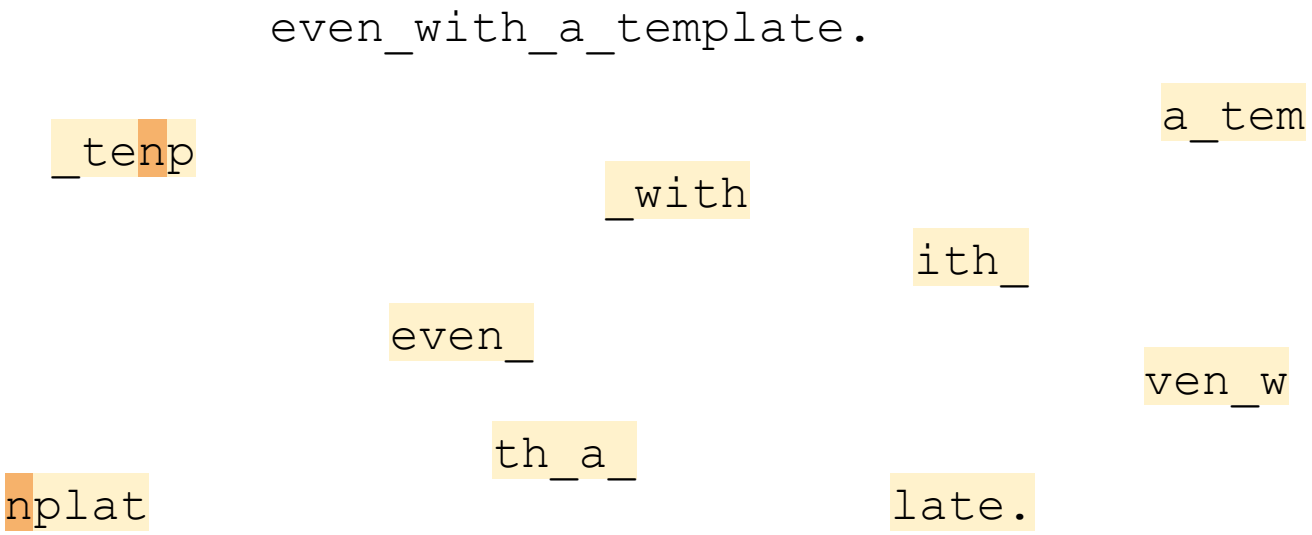
```
this_ is_fa rly_diffic
 his_i  fairl _diff cult.
    _is_f irly_  fficu

this_is_fairly_difficult.
```

(de-novo assembly)

Alignment to a reference genome

1. Reconstruct the whole genome from the short reads



Alignment to a reference genome

1. Reconstruct the whole genome from the short reads

```
even_with_a_template.

even_  ith_a_tem late.
 ven_w  th_a_   nplat
    _with   _tenp
```

(alignment to reference genome)

Variant calling

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even_with_a_template.
even_  ith_a_tem late.
 ven_w  th_a_   nplat
    _with   _tenp
```

(alignment to reference genome)

m > n

2. Compare the reconstructed genome(s) to a reference genome or to each other and find differences (variants/mutations)

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```

m > n

(alignment to reference genome)

Not at all trivial either!

2. Compare the reconstructed genome(s) to a reference genome or to each other and find differences (variants/mutations)

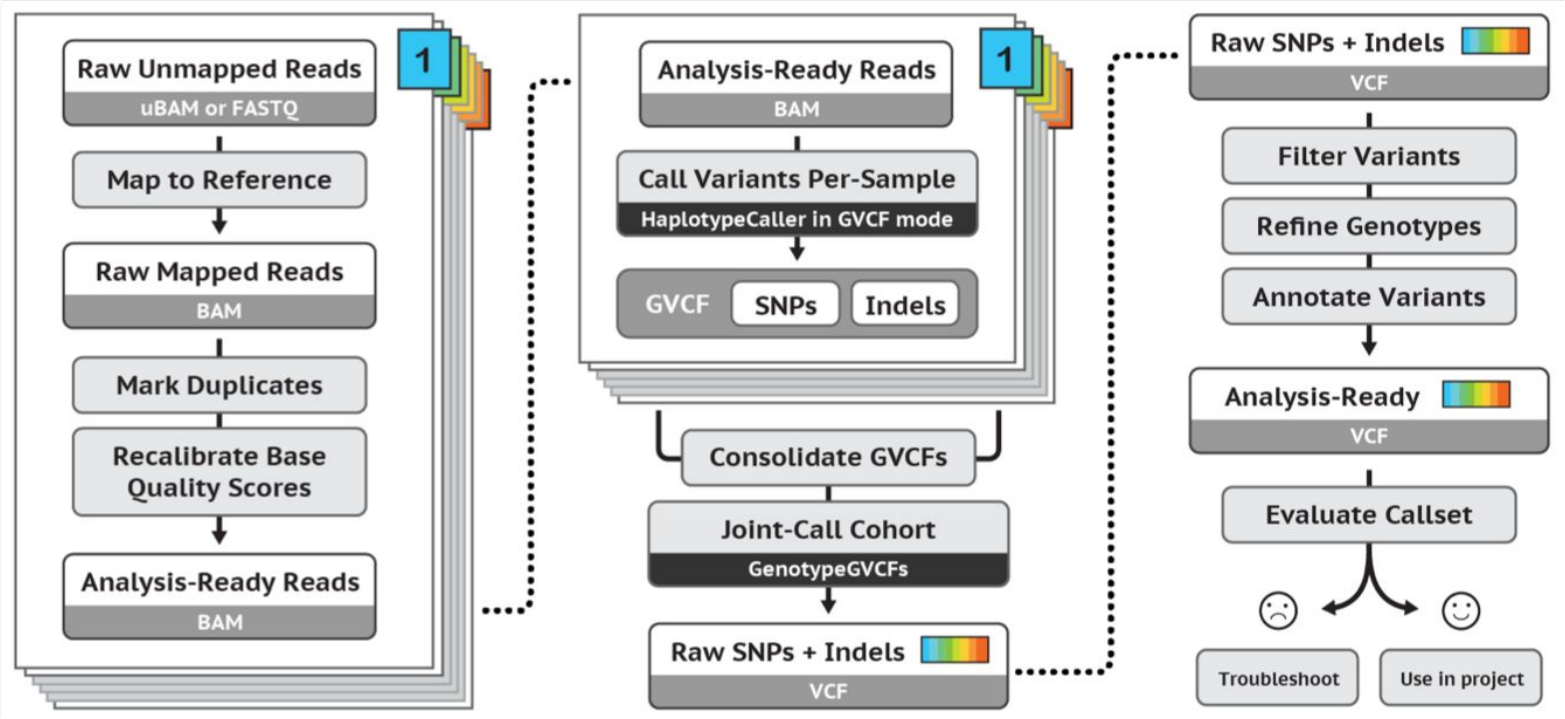
Data analysis

Details

Analysis pipeline: outputs and file formats

NGS	Alignment	Preprocessing	Variant calling	Interpretation
Sequences and base qualities of short reads in **random order**.	Sequences and base qualities of short reads with the **genomic position of where they fit on the reference genome**.	**Sorted** (easy to search) alignment files with sequences labelled with **read groups** and sometimes with **duplicates removed**.	**List of somatic and germline variants** with additional information.	List of **annotated, filtered variants, figures**, etc.
FASTQ files	SAM/BAM files Pileup files	BAM files	VCF files	Image files Filtered VCF files

GATK Best Practices - Main steps for Germline Cohort Data



Preparations for alignment

Not always easy!

1. Find and download the appropriate reference genome (FASTA format)
   (i.e. do not align sequencing data from a chicken to the human reference genome)

   e.g: http://hgdownload.cse.ucsc.edu/goldenPath/hg38/chromosomes/

2. Create an index file for the reference genome

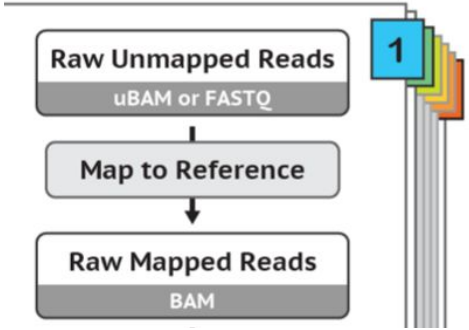
Tools: BWA, samtools

```
samtools faidx refgenome.fa
bwa index refgenome.fa
```

Alignment to a reference genome

Input: short read sequences (and base qualities) in random order (FASTQ files or uBAM files (convert first to FASTQ files))

Goal: determining the order of the short reads by fitting them to a template (reference genome)



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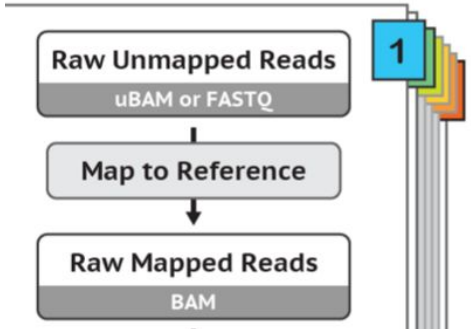
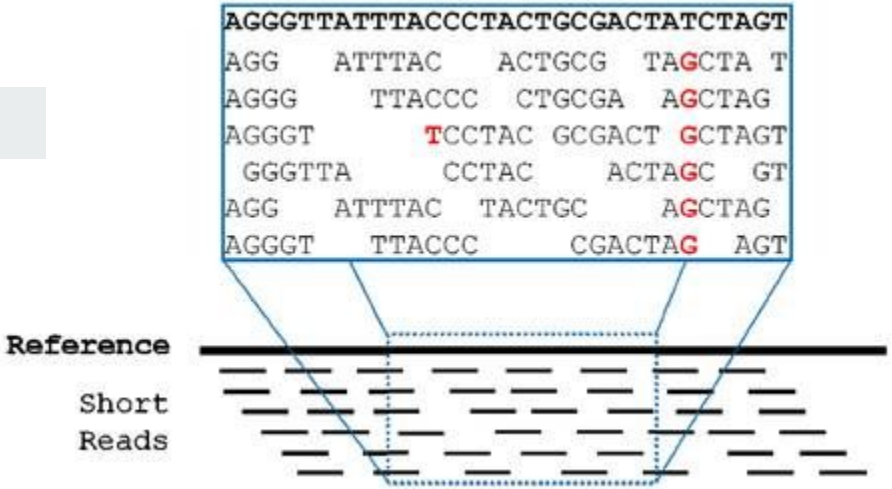
Tool: BWA

```
bwa mem refgenome.fa s1.fq.gz > s1.sam
```





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Tool: BWA

Output: short reads with **position** and **mapping quality** (SAM/BAM files)

```
samtools view -bS s1.sam > s1.bam
```

Conversion tool: samtools

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M     =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662    0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37      AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
      >>>>>>>>>>>>>>>>>>>>>><>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M     =      17644  -314
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X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)

```
1:497:R:-272+13M17D24M     113     1       497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG    0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99      1       17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147     1       17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT    ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
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XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M     =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

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```
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CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37      AM:i:0
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19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
      >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M     =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

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- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position
- MAPQ: mapping quality
- CIGAR string: indicating alignment information

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662    0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37      AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

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- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662    0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

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- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
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- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read
- PNEXT: mapping position for next read

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position
- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read
- PNEXT: mapping position for next read
- TLEN: length of read group

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG   0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT   ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position
- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read
- PNEXT: mapping position for next read
- TLEN: length of read group
- SEQ: short read sequence

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG    0;==-==9;>>>>>=>>>>>>>>>>>>=>>>>>>>>>>   XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
       >>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9   RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT    ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<   XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position
- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read
- PNEXT: mapping position for next read
- TLEN: length of read group
- SEQ: short read sequence
- QUAL: short read base qualities

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662    0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG    0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>    XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
        >>>>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9    RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT    ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<    XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

- QNAME: name of the short read present in FASTQ file
- FLAG: a number (“code”) describing the alignment
- RNAME: name of the reference sequence (often contains chromosome)
- POS: mapping position
- MAPQ: mapping quality
- CIGAR string: indicating alignment information
- RNEXT: reference sequence name for the next read
- PNEXT: mapping position for next read
- TLEN: length of read group
- SEQ: short read sequence
- QUAL: short read base qualities
- TAGS: additional optional information

```
1:497:R:-272+13M17D24M     113    1      497    37     37M    15     100338662     0
CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG    0;==-==9;>>>>>>=>>>>>>>>>>>>=>>>>>>>>>>    XT:A:U NM:i:0 SM:i:37       AM:i:0
X0:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    99     1      17644  0      37M    =      17919  314    TATGACTGCTAATAATACCTACACATGTTAGAACCAT
        >>>>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9    RG:Z:UM0098:1 XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:0 XO:i:0
XG:i:0 MD:Z:37
19:20389:F:275+18M2D19M    147    1      17919  0      18M2D19M      =      17644  -314
GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT    ;44999;499<8<8<<<8<<><<<<><7<;<<<>><<    XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:4
X1:i:0 XM:i:0 XO:i:1 XG:i:2 MD:Z:18^CA19
```

Alignment to a reference genome

Pileup files: `samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup`

Tool: samtools

```
seq1 272 T 24  ,.$.....,,.,,.,,,,,,,..^+. <<<+;<<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,,.,,.,,,,,,,,.A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$....,,.,,.,,,,,,,,...    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$....,,.,,.,,,,,,,,...^l.  <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)

Alignment to a reference genome

Pileup files:

```
samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup
```

Tool: samtools

```
seq1 272 T 24  ,.$....,,.,.,...,,,,,..^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,.,.,...,,,,,..A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$....,,.,.,...,,,,,...    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$....,,.,.,...,,,,,...^l.  <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)
- genomic position on the chromosome (contig)

Alignment to a reference genome

Pileup files:

```
samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup
```

Tool: samtools

```
seq1 272 T 24  ,.$....,,.,.,...,,,,.,..^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,.,.,...,,,,.,..A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$....,,.,.,...,,,,.,...    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$....,,.,.,...,,,,.,...^l.  <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)
- genomic position on the chromosome (contig)
- the reference base at the genomic position

Alignment to a reference genome

Pileup files:

```
samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup
```

Tool: samtools

```
seq1 272 T 24  ,.$....,,.,.,...,,,,.,..^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,,.,.,...,,,,.,..A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$....,,.,.,...,,,,.,...    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$....,,.,.,...,,,,.,...^l.  <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)
- genomic position on the chromosome (contig)
- the reference base at the genomic position
- coverage: the number of short reads aligned to the genomic position

Alignment to a reference genome

Pileup files:

```
samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup
```

Tool: samtools

```
seq1 272 T 24   ,.$.....,,,,,,,,,,,,,,,,..^+.  <<<+;<<<<<<<<<<<=<;<;7<&
seq1 273 T 23   ,.....,,,,,,,,,,,,,,,,,,..A  <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23   ,.$.....,,,,,,,,,,,,,,,,...     7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23   ,$.....,,,,,,,,,,,,,,,,...^l.   <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)
- genomic position on the chromosome (contig)
- the reference base at the genomic position
- coverage: the number of short reads aligned to the genomic position
- the bases aligned to the genomic position (they originate from *different* short reads) (ref: .,)

Alignment to a reference genome

Pileup files:

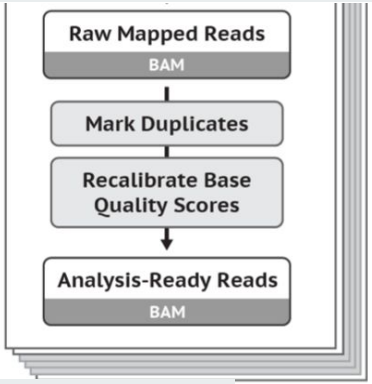
```
samtools mpileup -f refgenome.fa [options] s1.bam > s1.pup
```

Tool: samtools

```
seq1 272 T 24  ,.$.....,,,,,,,,,,,,,,,.^+.  <<<+;<<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,,,,,,,,,,,,,,,.A  <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$....,,,,,,,,,,,,,,,...    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$....,,,,,,,,,,,,,,,...^l.  <+;9*<<<<<<<<<=<<:
```

- name of the reference sequence (usually the chromosome)
- genomic position on the chromosome (contig)
- the reference base at the genomic position
- coverage: the number of short reads aligned to the genomic position
- the bases aligned to the genomic position (they originate from *different* short reads) (ref: .,)
- the base qualities of the aligned bases

Preprocessing alignment files



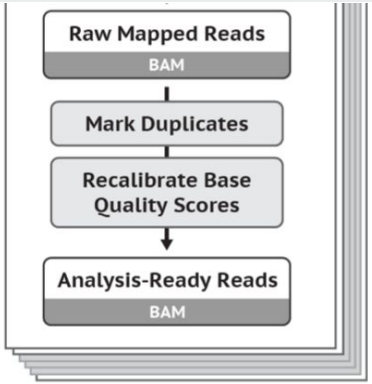
Input: alignment files (BAM files)

Goals:

- Sorting BAM files **Tool: samtools**

```
samtools sort -o s1_sorted.bam s1.bam
```

Preprocessing alignment files



Input: alignment files (BAM files)

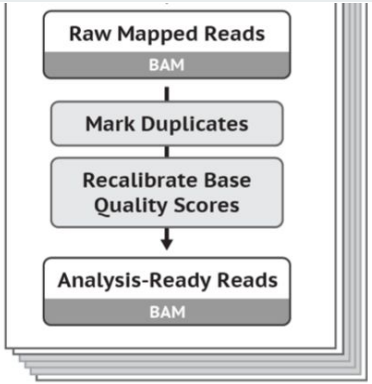
Goals:

- Sorting BAM files
- Marking duplicate reads

Tool: Picard Tools | MarkDuplicates

```
java -jar picard.jar MarkDuplicates \
     I=input.bam \
     O=marked_duplicates.bam \
     M=marked_dup_metrics.txt
```

Preprocessing alignment files



Input: alignment files (BAM files)

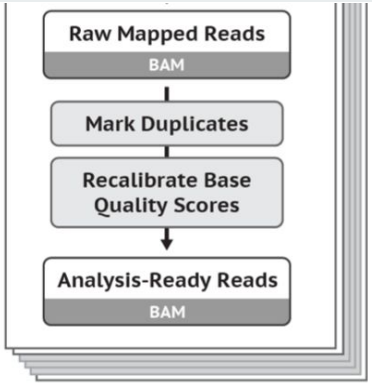
Goals:

- Sorting BAM files
- Marking duplicate reads
- Adding read groups if necessary

Tool: Picard Tools | AddOrReplaceReadGroups

```
java -jar picard.jar AddOrReplaceReadGroups \
      INPUT=s1_RMdup.bam OUTPUT=s1_RG.bam \
      RGLB=lib1 RGPL=illumina RGPU=unit1 \
      RGSM=1
```

Preprocessing alignment files



Input: alignment files (BAM files)

Goals:

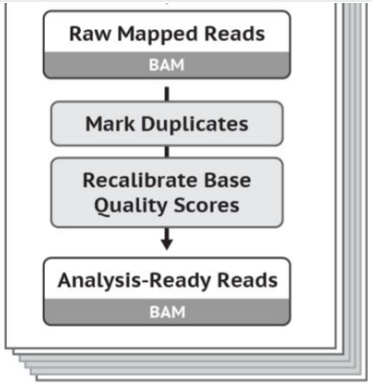
- Sorting BAM files
- Marking duplicate reads
- Adding read groups if necessary
- Recalibrate base quality scores

Tools: GATK Base Recalibrator, ApplyBQSR

```
gatk BaseRecalibrator \
   -I my_reads.bam \
   -R reference.fasta \
   --known-sites sites_of_variation.vcf \
   --known-sites another/optional/setOfSitesToMask.vcf \
    -O recal_data.table
```

```
gatk ApplyBQSR \
   -R reference.fasta \
   -I input.bam \
   --bqsr-recal-file
recalibration.table \
    -O output.bam
```

Preprocessing alignment files



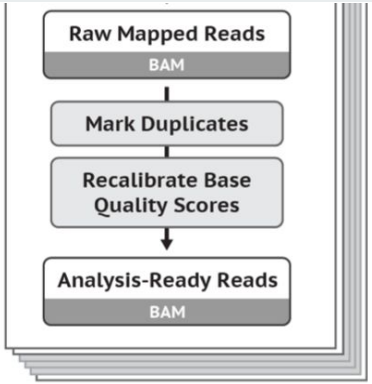
Input: alignment files (BAM files)

Goals:

- Sorting BAM files
- Marking duplicate reads
- Adding read groups if necessary
- Recalibrate base quality scores
- Indexing BAM files **Tool: samtools**

```
samtools index s1_RG.bam
```

Preprocessing alignment files



Input: alignment files (BAM files)

Goals:

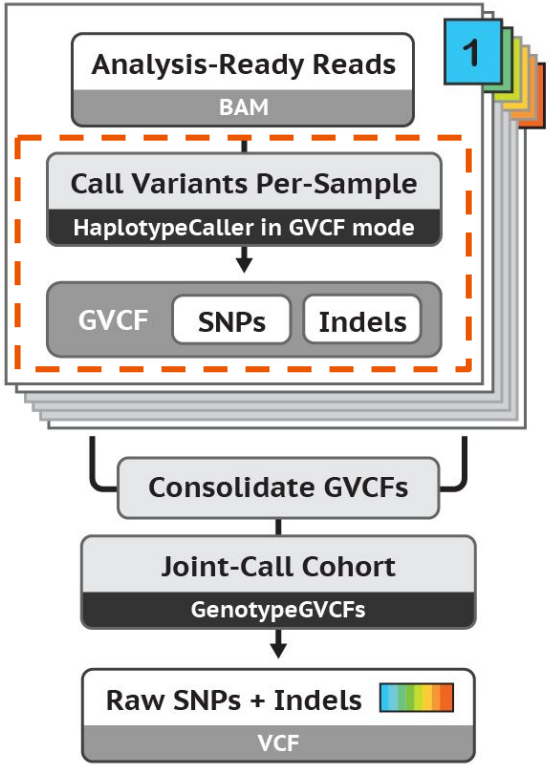
- Sorting BAM files
- Marking duplicate reads
- Adding read groups if necessary
- Recalibrate base quality scores
- Indexing BAM files **Tool: samtools**

```
samtools index s1_RG.bam
```

Output: modified alignment files (BAM files)

Variant calling: germline variants

Tool: GATK

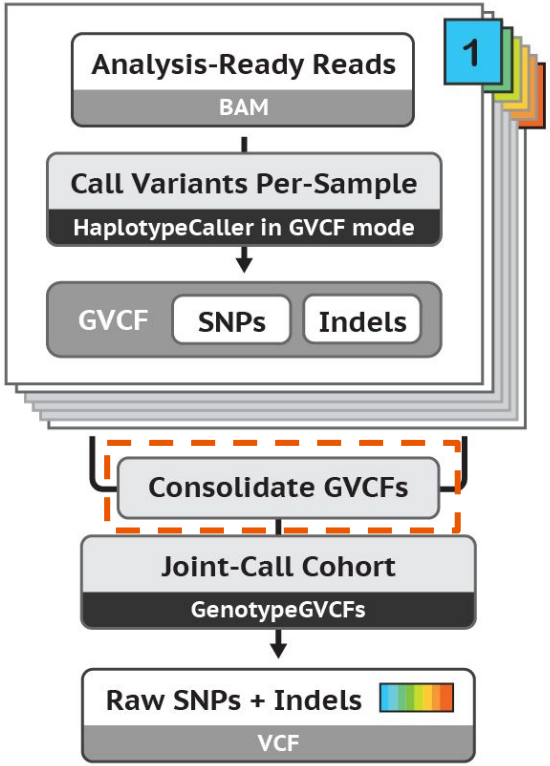


1. Analyse each sample and determine if the given genomic position has any variation (compared to reference) (one GVCF file per sample)

```
gatk HaplotypeCaller \
      -R refgenome.fa \
      -I s1_RG.bam \
      -O s1.raw.g.vcf.gz \
      -ERC GVCF
```

Variant calling: germline variants

Tool: GATK

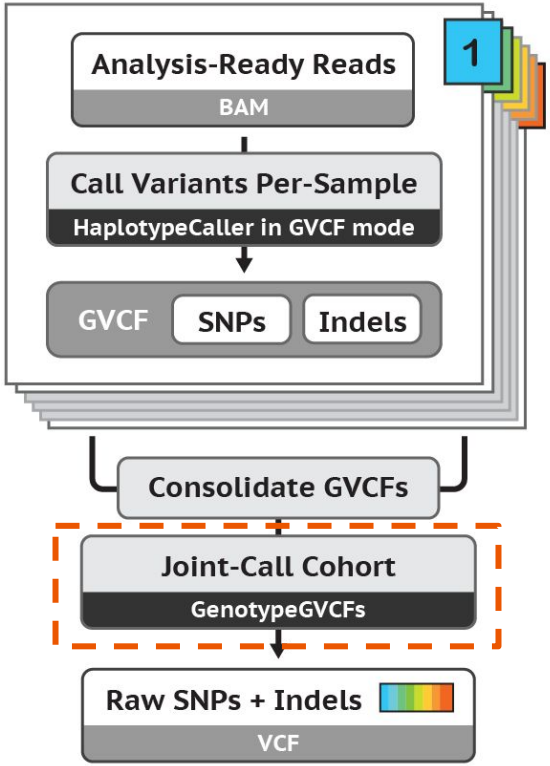


1. Analyse each sample and determine if the given genomic position has any variation (compared to reference) (one GVCF file per sample)
2. Combine GVCF files to a database (one common database for all samples)

```
gatk GenomicsDBImport \
      -V s1.raw.g.vcf.gz \
      -V s2.raw.g.vcf.gz \
      -V s3.raw.g.vcf.gz \
      [-V ...] \
      - V sn.raw.g.vcf.gz \
      --genomicsdb-workspace-path my_database \
      -L chr19
```

Variant calling: germline variants

Tool: GATK

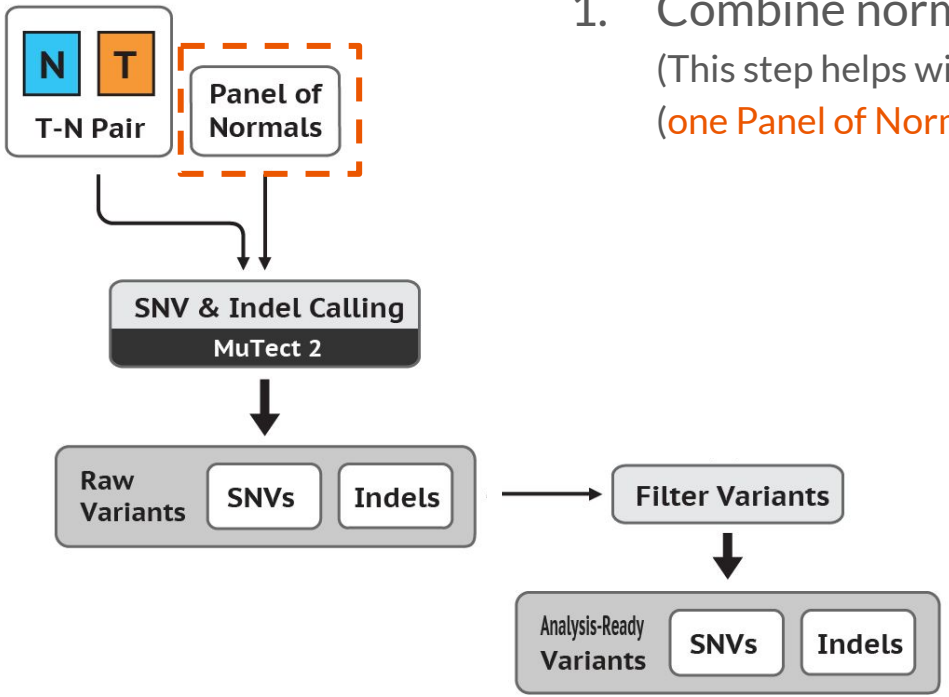


1. Analyse each sample and determine if the given genomic position has any variation (compared to reference) (one GVCF file per sample)
2. Combine GVCF files to a database (one common database for all samples)
3. Check each genomic position and determine the genotype of each sample:
   - homozygous reference (same as reference)
   - heterozygous (about half of the aligned bases are non-reference)
   - homozygous non-reference (none of the aligned bases is reference)

```
gatk GenotypeGVCFs \
   -R refgenome.fa \
   -V gendb://my_database \
   -O jointgenotypingresults.vcf.gz
```

Variant calling: somatic variants

Tool: GATK



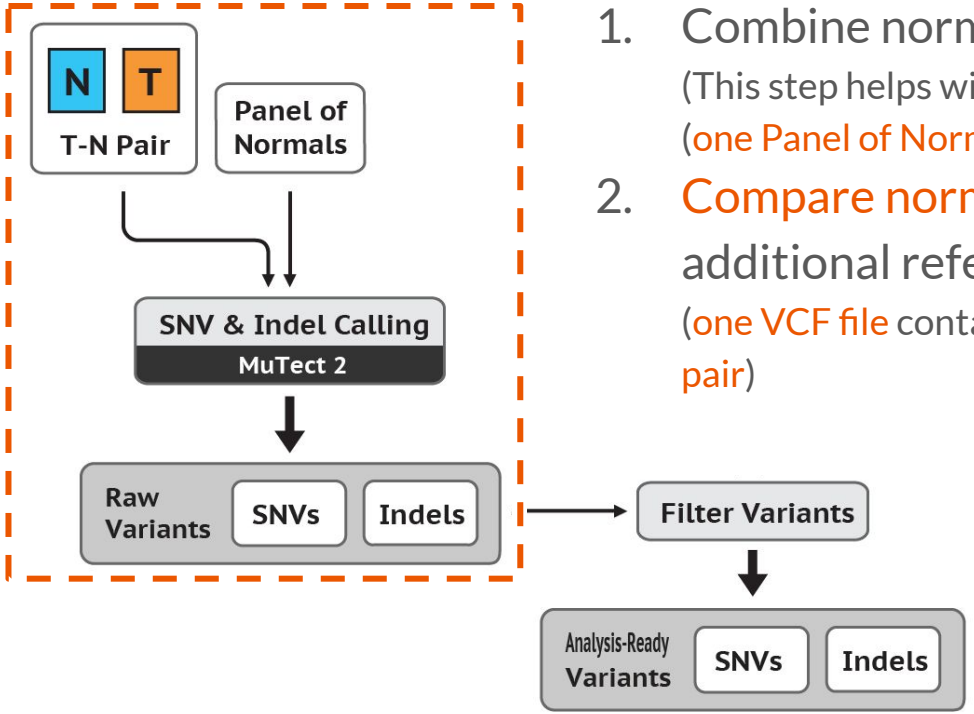
1. Combine normal samples to Panel of Normals (PoN)
   (This step helps with the filtering of common germline variations)
   (one Panel of Normals VCF file from many normal samples)

```
gatk Mutect2 \
      -R refgenome.fa \
      -I s1_RG.bam \
      -tumor s1_sample_name \
      -O s1_pon.vcf.gz
```

```
gatk CreateSomaticPanelOfNormals \
      -vcfs s1_pon.vcf.gz \
      -vcfs s2_pon.vcf.gz \
      [-vcfs ...]
      -vcfs sn_pon.vcf.gz \
      -O combined_pon.vcf.gz
```

Variant calling: somatic variants

Tool: GATK

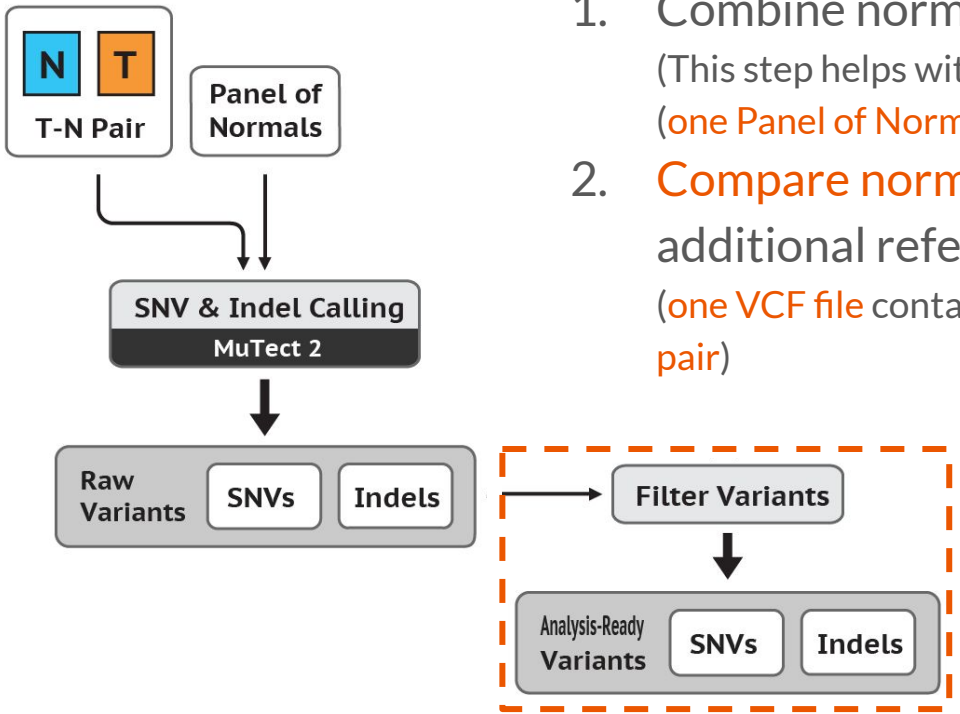


1. Combine normal samples to Panel of Normals (PoN)
   (This step helps with the filtering of common germline variations)
   (one Panel of Normals VCF file from many normal samples)
2. Compare normal-tumor sample pairs and use PoN as an additional reference
   (one VCF file containing raw variant calls for each normal-tumor sample pair)

```
gatk Mutect2 \
      -R refgenome.fa \
      -I s1_RG.bam \
      -I s2_RG.bam \
      -tumor s1_sample_name \
      -normal s2_sample_name \
      -pon combined_pon.vcf.gz \
      -L chr19 \
      -O s1_somatic_m.vcf.gz
```

Variant calling: somatic variants

Tool: GATK

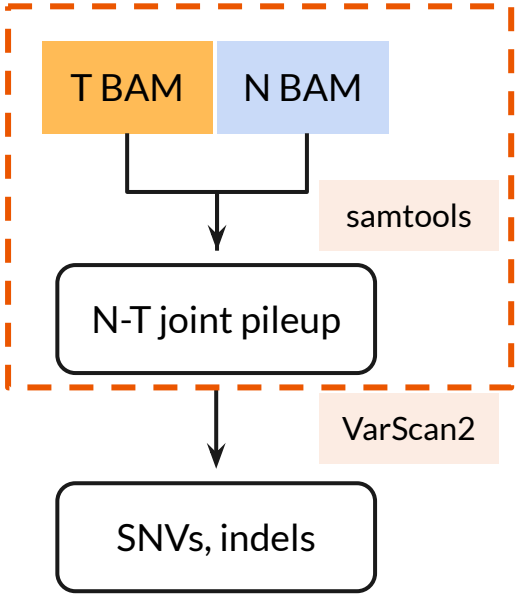


1. Combine normal samples to Panel of Normals (PoN)
   (This step helps with the filtering of common germline variations)
   (one Panel of Normals VCF file from many normal samples)
2. Compare normal-tumor sample pairs and use PoN as an additional reference
   (one VCF file containing raw variant calls for each normal-tumor sample pair)
3. Further filter variants
   (one VCF file containing final variant calls for each normal-tumor sample pair)

```
gatk FilterMutectCalls \
      -V s1_somatic_m.vcf.gz \
      -O s1_somatic_filtered.vcf.gz
```

Variant calling: another method

Tool: VarScan2

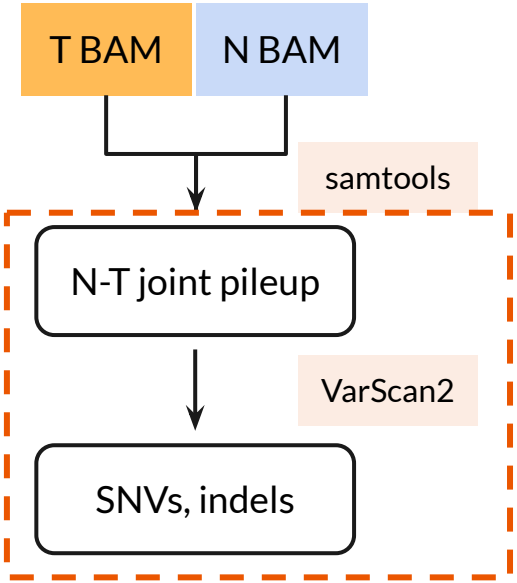


1. Generate a joint pileup file for each tumor normal pair

```
samtools mpileup \
      -f refgenome.fa \
      [options] \
      s2_RG.bam s1_RG.bam > s2_s1.pup
```

Variant calling: another method

Tool: VarScan2



1. Generate a joint pileup file for each tumor normal pair
2. Call short indels and SNVs with VarScan2
   (categorized as “somatic”, “germline” or “LOH”)

```
java -jar VarScan.v2.4.3.jar somatic \
      s2_s1.pup \
      1_somatic_varscan \
      --mpileup 1
```

Variant calling: VCF files

HEADER: starts with “##”, contains information about the structure of the file

```
##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",taxonomy=x>
##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">
##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
20    1230237 .         T      .       47   PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Variant calling: VCF files

Field names: starts with “#”, name of columns

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Variant calling: VCF files

Variants: actual data lines, with columns defined above

```
##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",taxonomy=x>
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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">
##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
20     1230237 .         T      .      47    PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Variant calling: VCF files

Examples: how many samples were analysed?

```
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##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
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#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
20     1230237  .          T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51   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
```

Variant calling: VCF files

Examples: how many samples were analysed?

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##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
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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=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
20     1230237  .          T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51  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
```

1

Variant calling: VCF files

Examples: how many samples were analysed?

```
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##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
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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">
##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">
```

									1	2	
#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
20	1230237	.	T	.	47	PASS	NS=3;DP=13;AA=T	GT:GQ:DP:HQ	0|0:54:7:56,60	0|0:48:4:51,51	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

Variant calling: VCF files

Examples: how many samples were analysed?

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

									1	2	3
#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
20	1230237	.	T	.	47	PASS	NS=3;DP=13;AA=T	GT:GQ:DP:HQ	0|0:54:7:56,60	0|0:48:4:51,51	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

Variant calling: VCF files

Examples: was the 20:17330 variant filtered? Why?

```
##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",taxonomy=x>
##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">
##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
20     1230237  .          T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51   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
```

Variant calling: VCF files

Examples: was the 20:17330 variant filtered? Why?

```
##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",taxonomy=x>
##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">
##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
20    1230237  .          T    .      47   PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Variant calling: VCF files

Examples: was the 20:17330 variant filtered? Why?

```
##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",taxonomy=x>
##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">
##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
20    1230237  .          T    .      47   PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Yes

Variant calling: VCF files

Examples: was the 20:17330 variant filtered? Why?

```
##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",taxonomy=x>
##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">
##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
20    1230237  .          T    .      47   PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Yes, because the quality was below 10.

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20     1230237  .          T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51   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
```

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20     1230237  .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Genotye ID: **GT**

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20     1230237  .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51  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
```

Genotye ID: **GT**

GT: **first field** in FORMAT

Variant calling: VCF files

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Genotye ID: **GT**

GT: **first field** in FORMAT

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

0/0: homozygous reference

Genotye ID: **GT**

GT: **first field** in FORMAT

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

0/0: homozygous reference

1/0: heterozygous

Genotye ID: **GT**

GT: **first field** in FORMAT

Variant calling: VCF files

Examples: what were the genotypes of the 3 samples in position 20:14370?

0/0: homozygous reference

1/0: heterozygous

1/1: homozygous non-reference

Genotye ID: **GT**

GT: **first field** in FORMAT

```
##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",taxonomy=x>
##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">
##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
20    1230237   .         T     .      47    PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 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
```

Interpreting results: general questions

- **How many mutations** were detected in each sample?
  - **Note:** different variant callers result in *extremely different* variant lists
- Are these mutations **novel or already listed in available databases**? (e.g. dbSNP)
- Are the mutations located in **specific genes**? Which ones? What are the **roles of these genes**?
- What are the **consequences** of these mutations?
- Is there a **specific pattern** in mutations? (e.g. most of them are C>T)
- etc.

Interpreting results: annotation

Input: list of variants (final VCF files)

Goal: search for each mutation (chrom, pos, ref, alt) in available databases

Output: annotated variant list (tables, csv files)

Tool: ANNOVAR

Chr	Start	End	Ref	Alt	Func.refGe	Gene.refGene	GeneDetail.refGene	ExonicFunc.refGene	AAChange.refGene	Xref.refGene	ExAC_Freq
1	948921	948921	T	C	UTR5	ISG15	NM_005101:c.-33T>C	.	.	Immunodeficienc	0.941
1	1404001	1404001	G	T	UTR3	ATAD3C	NM_001039211:c.*91G>T	.	.	.	0.054
1	5935162	5935162	A	T	splicing	NPHP4	NM_001291594:exon17:c.1282-2T>A	.	.	Nephronophthisi	0.825
1	162736463	162736463	C	T	intronic	DDR2	.	.	.	Spondylometaepi	.
1	84875173	84875173	C	T	intronic	DNASE2B	.	.	.	.	.
1	13211293	13211294	TC	-	intergenic	PRAMEF36P;F	dist=11566;dist=116902	.	.	.	.
1	11403596	11403596	-	AT	intergenic	UBIAD1;PTCH	dist=55105;dist=135699	.	.	.	.
1	105492231	105492231	A	ATAAA	intergenic	LOC1001291	dist=872538;dist=640085	.	.	.	.
1	67705958	67705958	G	A	exonic	IL23R	.	nonsynonymous SNV	IL23R:NM_144701:exon9:c.G1142A:p.R381Q	.	0.041
2	234183368	234183368	A	G	exonic	ATG16L1	.	nonsynonymous SNV	ATG16L1:NM_198890:exon5:c.A409G:p.T137A;ATG16L1:NM_0	.	0.457
16	50745926	50745926	C	T	exonic	NOD2	.	nonsynonymous SNV	NOD2:NM_001293557:exon3:c.C2023T:p.R675W;NOD2:NM_0	Blau syndrome, A	0.023
16	50756540	50756540	G	C	exonic	NOD2	.	nonsynonymous SNV	NOD2:NM_001293557:exon7:c.G2641C:p.G881R;NOD2:NM_0	Blau syndrome, A	0.009917
16	50763778	50763778	-	C	exonic	NOD2	.	frameshift insertion	NOD2:NM_001293557:exon10:c.2936dupC:p.L980Pfs*2;NOD2	Blau syndrome, A	0.013
13	20763686	20763686	G	-	exonic	GJB2	.	frameshift deletion	GJB2:NM_004004:exon2:c.35delG:p.G12Vfs*2	Bart-Pumphrey sy	0.006038
13	20797176	21105944	0	-	exonic	CRYL1;GJB6	.	frameshift deletion	GJB6:NM_001110220:wholegene;GJB6:NM_001110221:whole	.	.
8	8887543	8887543	A	T	exonic	ERI1	.	stoploss	ERI1:NM_153332:exon7:c.A1049T:p.X350L	.	.
8	8887539	8887539	A	T	exonic	ERI1	.	stopgain	ERI1:NM_153332:exon7:c.A1045T:p.K349X	.	.
8	8887536	8887537	AG	GATT	exonic	ERI1	.	frameshift substitutic	ERI1:NM_153332:exon7:c.1042_1043GATT:p.R348Dfs*2	.	.
8	8887540	8887540	G	GGAA	exonic	ERI1	.	nonframeshift substit	ERI1:NM_153332:exon7:c.1046delinsGGAA:p.R348_K349insR	.	.
5	1295288	1295288	G	A	upstream	TERT	dist=126	.	.	.	.

Problems

- Different tools result in *very* different results. (Sometimes there is no overlap between the list of detected variants.)
  - Which one is better? Which results are true?
  - *Why* do they detect different variants? What filtering steps do they use?
  - How can we compare results?
- Different tools have *very* different runtimes.
  - Is it realistic to use tools that run for weeks?
  - How many samples do we have?
  - What kind of computer do we have?

General guidelines

- Be very patient both with the computer and yourself.
- Many commands take a lot of time to run. Think before you start running a faulty pipeline on many samples.
- You *will* come across error messages. Don't panic, Google.
- Practice makes perfect, don't give up.
- Always make sure your results make sense. (E.g. Try interchanging tumor and normal samples, do you get only a few somatic mutations in normals?)
- If you find anything strange, don't sweep it under the rug.

Good luck!

Thank you for your attention!