Bioinformatics 101

Analysing WGS data

SciLifeLab

Karolinska Institutet

GANATIONALCTAC
ATCAGENOMICSGT
INFRASTRUCTURE

NBIS

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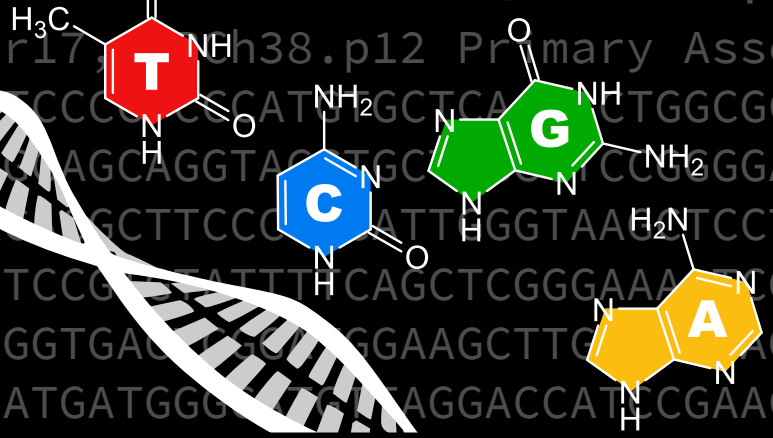
maxulysse.github.io

@MaxUlysse

@gau

Barntumörbanken

```
bwa mem -R \"${readGroup}\" ${extra} -t ${task.cpus
${genomeFile} ${fastqFile1} ${fastqFile2} | \
samtools sort --threads ${task.cpus} -m 2G - > ${id
>Homo sapiens chr17, GRCh38.p12 Primary Assembly
```



Plan

- DNA: From the sequencing to files

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- Preprocessing: What to do with these files?

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- Preprocessing: What to do with these files?
- Variant Calling and Annotation: Finally getting some results

DNA: From the sequencing to files

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- Sequencing
- Formats

How to store nucleotide sequence?

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```
AGCATCATACGGGGCTTTGG
CTGTACTGTACAGTTACTGT
AGGGGCAGTGACGCCGC
```

FASTA

FASTA: text-based format for storing either nucleotide or peptide sequences.

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```

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```
> My sequence|P3X-974|Homo Sapiens|GRCh38
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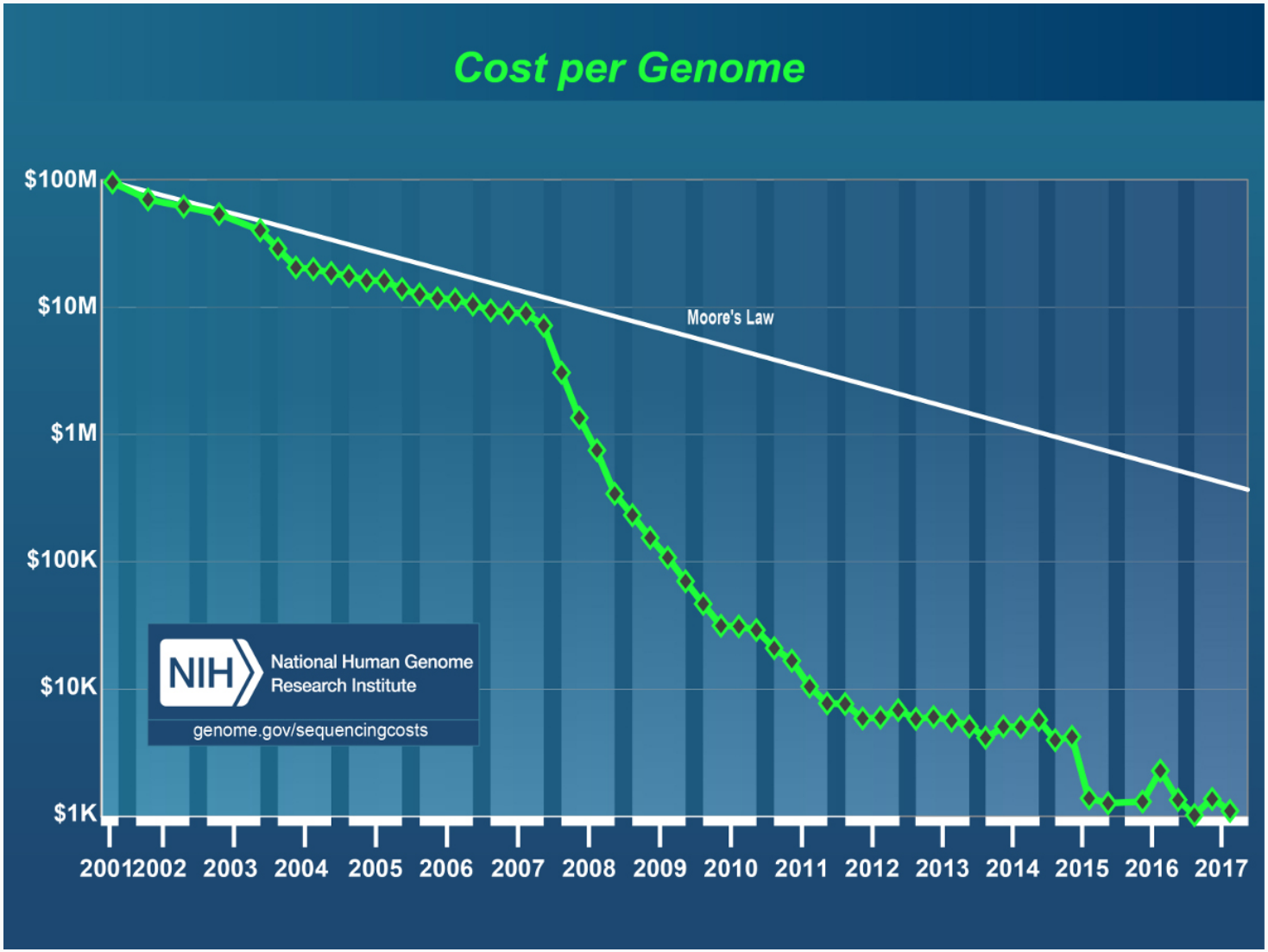
CTGTACTGTACAGTTACTGT
AGGGGCAGTGACGCCGC
```

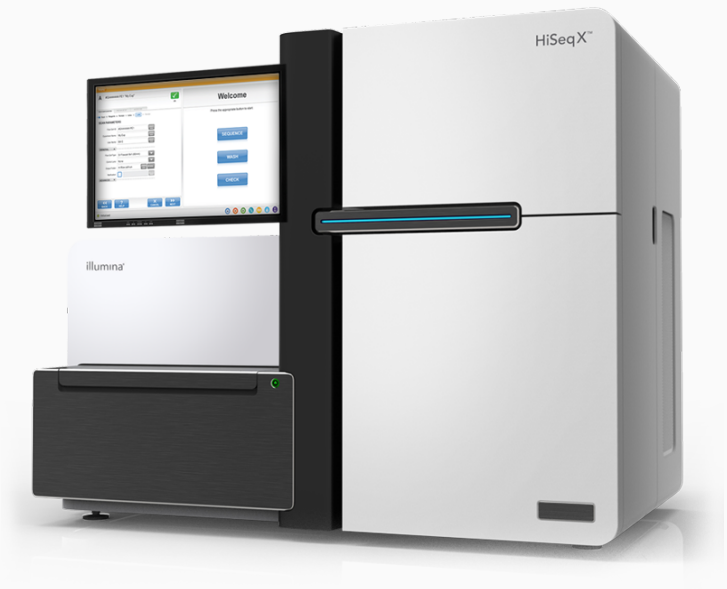
Moore's law in Bioinformatics



Sequencing with Illumina

Illumina's HiSeq X

Sequencing with Illumina



Illumina's HiSeq X

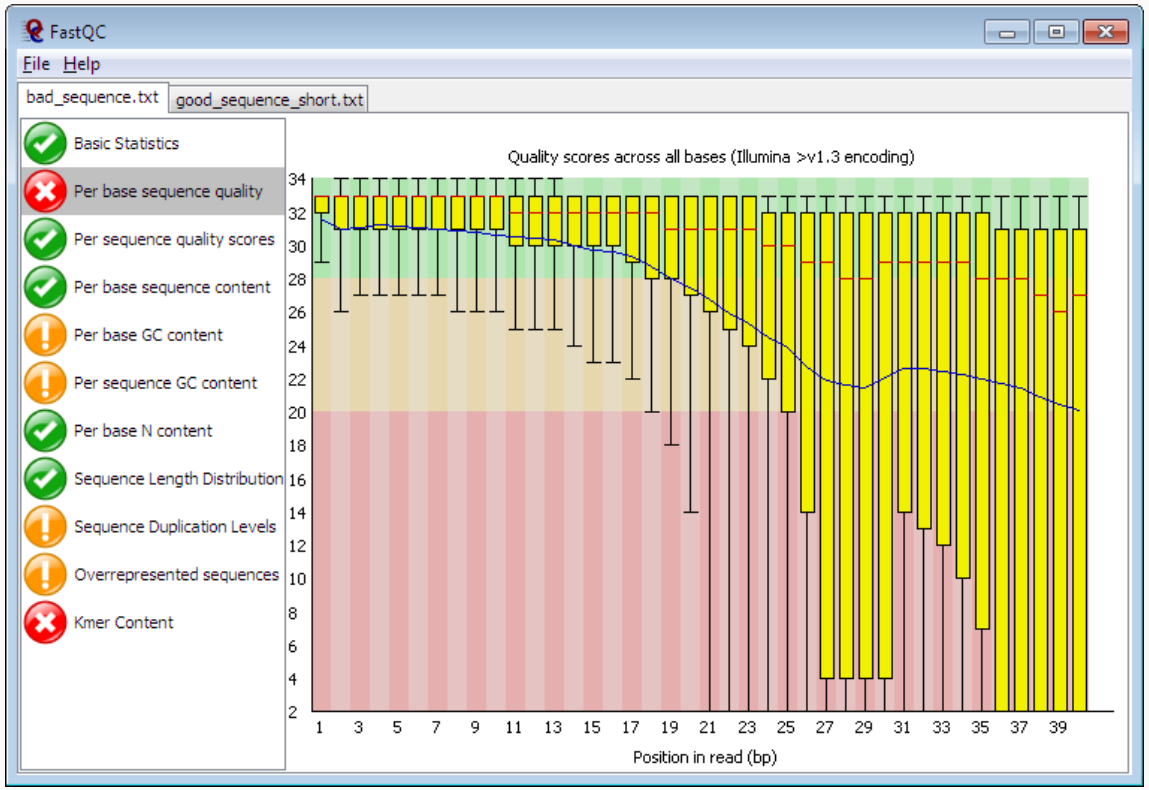
- Short reads ($\sim$ 120 -> 150 bp)

Sequencing with Illumina

Illumina's NovaSeq

- Short reads ($\sim$ 120 -> 150 bp)

Back to sequencing



Each base in a read is assigned a quality score probability of error

FASTQ Files

FASTQ: text-based format for storing both nucleotide sequence and corresponding quality scores.

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

FASTQ Files

FASTQ: text-based format for storing both nucleotide sequence and corresponding quality scores.

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

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
```

First conclusion

- FASTA: Used to store sequences
  - You might use or even open such file

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- FASTA: Used to store sequences
  - You might use or even open such file
- FASTQ: Used to store sequences and quality
  - You will see that
  - You won't use that directly
  - You will never open such file
  - You will transform it

Preprocessing: What to do with these files?

- Assembly

Preprocessing: What to do with these files?

- Assembly
- Cleanup

Assembly - the reads

Difficult question is coming

- Human Genome: $\sim$ 3,234.83 Mb

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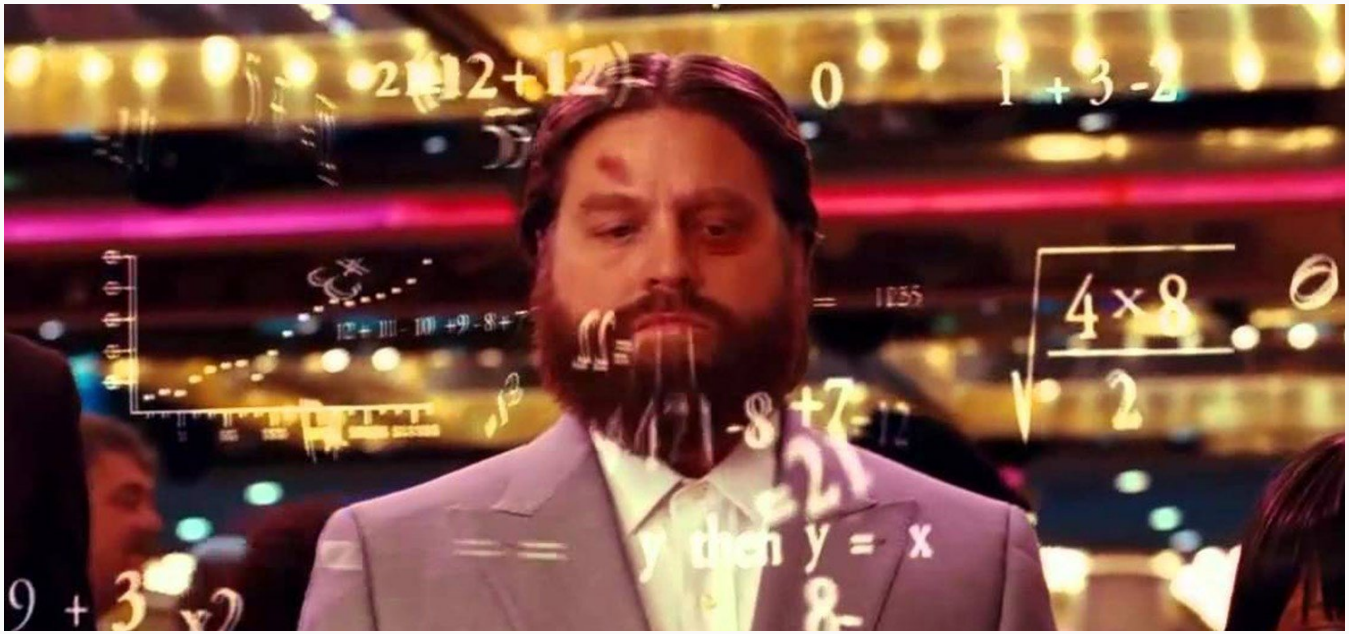

How many reads in the end?

- Human Genome: $\sim$ 3,234.83 Mb
- Depth of sequencing: 30X
- Reads: $\sim$ 120 -> 150 bp

Assembly - the reads

600 M reads

- Human Genome: $\sim$ 3,234.83 Mb
- Depth of sequencing: 30X
- Reads: $\sim$ 120 -> 150 bp

Assembly with short reads - I

```
                TACGGGG                                GGCTTTG
                            AGCATCA
ATCATAC                                     ACGCCGC
```

Some short reads

Assembly with short reads - II

```
            GGCTTTG
      TACGGGG
   ATCATAC
AGCATCA
```

Some can be assembled

Assembly with short reads - III

```
            GGCTTTG
        TACGGGG
   ATCATAC
AGCATCA                                                  ACGCCGC
AGCATCATACGGGGCTTTGGCTGTACTGTACAGTTACTGTAGGGGCAGTGACGCCGC
```

Easier with a Reference

Quality guidelines for processing data

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

From the Broad Institute: GATK Best Practices (GATK 4.0)

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- Reads mapped to reference genome with `bwa`

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- Reads mapped to reference genome with `bwa`
- Duplicates marked with `picard MarkDuplicates`

Quality guidelines for processing data

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

From the Broad Institute: GATK Best Practices (GATK 4.0)

- Reads mapped to reference genome with `bwa`
- Duplicates marked with `picard MarkDuplicates`
- Recalibrate with `GATK BaseRecalibrator`

Tools

Burrows-Wheeler Aligner

http://bio-bwa.sourceforge.net/

Software package for mapping low-divergent sequences against a large reference genome.

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GATK/Picard

https://software.broadinstitute.org/gatk/
https://broadinstitute.github.io/picard/

Sets of bioinformatic tools for analyzing/manipulating high-throughput sequencing (HTS) data.

Command lines

```
bwa mem -R \"@RG\tID:group1\tSM:file1\tPL:illumina\tLB:lib1\tPU:unit1\" -M \
Reference.fasta file1.fastq file2.fastq | \
samtools sort - > file.bam

gatk MarkDuplicates \
--INPUT file.bam \
--METRICS_FILE file.bam.metrics \
--ASSUME_SORT_ORDER coordinate \
--CREATE_INDEX true \
--OUTPUT file.md.bam

gatk BaseRecalibrator \
--input file.md.bam \
--output file.recal.table \
-R Reference.fasta
```

BAM files

Binary Alignment Map (BAM): compressed binary representation for storing biological sequences aligned to a reference sequence.

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```
@HD VN:1.6 SO:coordinate
@SQ SN:ref LN:45
r001 99 ref 7 30 8M2I4M1D3M = 37 39 AGCATCATACGGGGCTTTG *
```

BAM files

Binary Alignment Map (BAM): compressed binary representation for storing biological sequences aligned to a reference sequence.

```
@HD VN:1.6 SO:coordinate
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r001 99 ref 7 30 8M2I4M1D3M = 37 39 AGCATCATACGGGGCTTTG *
```

1	QNAME	String	Query template NAME
2	FLAG	Int	bitwise FLAG
3	RNAME	String	References sequence NAME
4	POS	Int	1-based leftmost mapping POSition
5	MAPQ	Int	MAPping Quality
6	CIGAR	String	CIGAR String
7	RNEXT	String	Ref. name of the mate/next read
8	PNEXT	Int	Position of the mate/next read
9	TLEN	Int	observed Template LENgth
10	SEQ	String	segment SEQuence
11	QUAL	String	ASCII of Phred-scaled base QUALity+33

CRAM files

Basically even more compressed BAM.

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Basically even more compressed BAM.

Not yet widely adopted, but good to know about.

Second conclusion

- Follow Best Practices, unless you know what you're doing

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- Follow Best Practices, unless you know what you're doing
- Try to save space
  - Keep only your latest BAMs and your FASTQs
  - Look at BAM files with visualization tools (IGV…)

Variant Calling

- Differences to Reference genome

How many variants?

- According to 1 000 Genomes Project:

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- According to 1 000 Genomes Project:
  - $\sim$ 1 000 deletions

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  - $\sim$ 160 copy number variation

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- According to 1 000 Genomes Project:
  - $\sim$ 1 000 deletions
  - $\sim$ 1 000 insertions
  - $\sim$ 160 copy number variation
  - $\sim$ 10 inversions

Some Variant Calling tools

- SNVs[1] and small indels[2]

[1] Single Nucleotide Variant
[2] insertion or deletion

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  - HaplotypeCaller (GATK)
  - Strelka2 (Illumina)

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- SNVs[1] and small indels[2]
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Some Variant Calling tools

- SNVs[1] and small indels[2]
  - HaplotypeCaller (GATK)
  - Strelka2 (Illumina)
- Structural variants:
  - Manta (Illumina)

[1] Single Nucleotide Variant
[2] insertion or deletion

Annotation

- VEP, SnpEff, ANNOVAR...
- 🗄 ClinVar, COSMIC, dbSNP, GENCODE, gnomAD, polyphen, sift, etc.

VCF files

The Variant Call Format (VCF): text-based format for storing gene sequence variations.

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```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##phasing=partial
#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:H
  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
```

Third conclusion

- Lots of different variant callers
  - Lots of variants found

Third conclusion

- Lots of different variant callers
  - Lots of variants found
  - Need to filter
  - Need to annotate
  - Can even improve that with prioritisation

What do we want?

Do analysis!

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Do analysis!

- Easy to use
- Easy to install

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Do analysis!

- Easy to use
- Easy to install
- Reproducible

What do we need?

- Tools

What do we need?

- Tools
  - Installed
  - Specific version

What do we need?

- Tools
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  - Specific version
- Reference files

What do we need?

- Tools
  - Installed
  - Specific version
- Reference files
  - Dowloaded
  - Specific version

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  - Specific version
- Annotation files / databases

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  - Installed
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- Annotation files / databases
  - Dowloaded
  - Specific version
- Works with cluster executor

What is Sarek?

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http://sarek.scilifelab.se/

- Analysis germline and somatic workflow

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- Whole genome or targeted sequencing

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- Analysis germline and somatic workflow
- Whole genome or targeted sequencing
- Developed with NGI and NBIS

NATIONAL GENOMICS INFRASTRUCTURE

NBIS

What is Sarek?

http://sarek.scilifelab.se/

- Analysis germline and somatic workflow
- Whole genome or targeted sequencing
- Developed with NGI and NBIS
- Support from The Swedish Childhood Tumor Biobank

Build with

nextflow

https://www.nextflow.io/

- Data-driven workflow language
- Portable (executable on multiple platforms)
- Shareable and reproducible

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https://www.nextflow.io/

- Data-driven workflow language
- Portable (executable on multiple platforms)
- Shareable and reproducible

https://www.sylabs.io/singularity/

- Docker-like container engine
  - Specific for HPC environnment

Sarek exists in multiple flavors

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Sarek

Sarek Germline

Sarek exists in multiple flavors

Sarek

Sarek Germline

Sarek Somatic

Sarek exists in multiple flavors

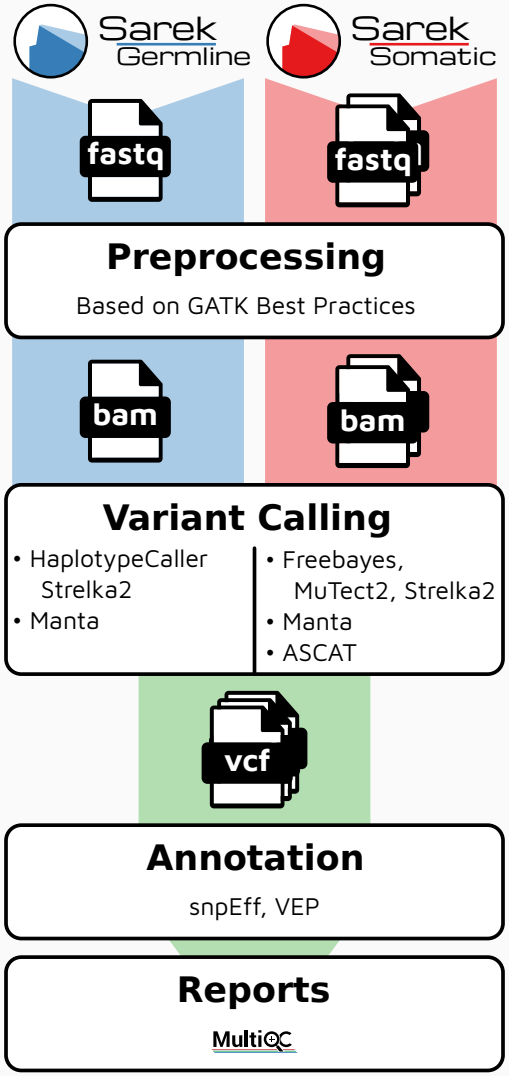
Sarek

Sarek Germline

Sarek Somatic

Sarek Exome

Data and files workflow



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Any questions?

- http://sarek.scilifelab.se/
- https://github.com/SciLifeLab/Sarek
- https://maxulysse.github.io/2018-Analysing-WGS-data/
- http://rsg-sweden.iscbsc.org/
- https://www.biostars.org/