Sarek

SciLifeLab

Karolinska Institutet

GANATIONALCTAC ATCAGENOMICSGT INFRASTRUCTURE

NBIS

An introduction

Maxime U. Garcia

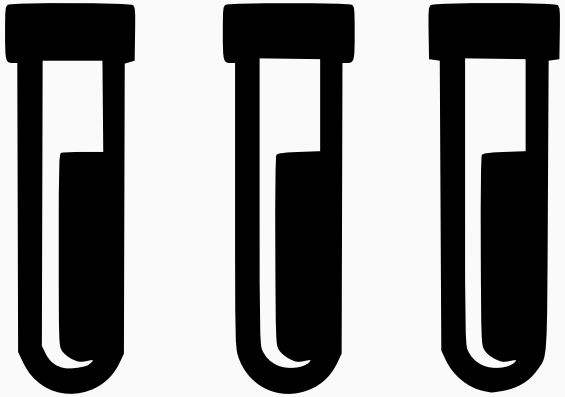
maxulysse.github.io

@MaxUlysse

@gau

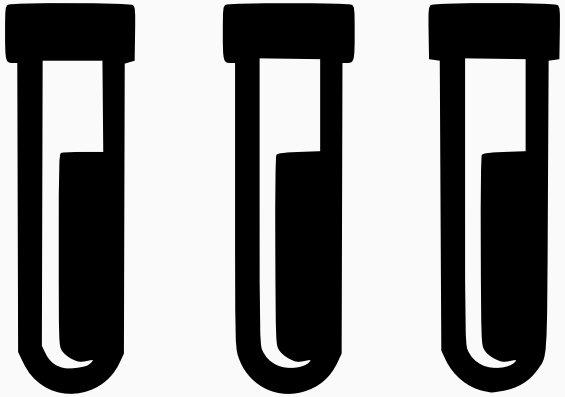
Barntumörbanken

Samples



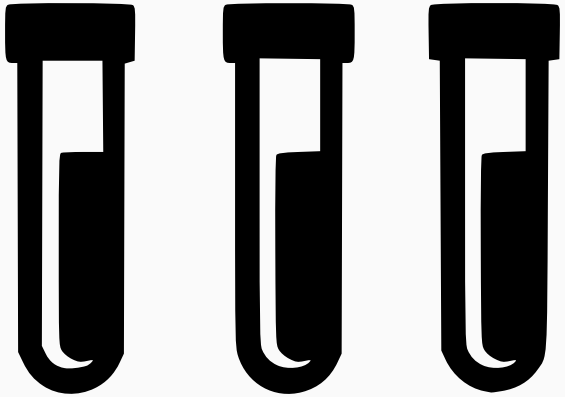
- Normal sample -> blood

Samples



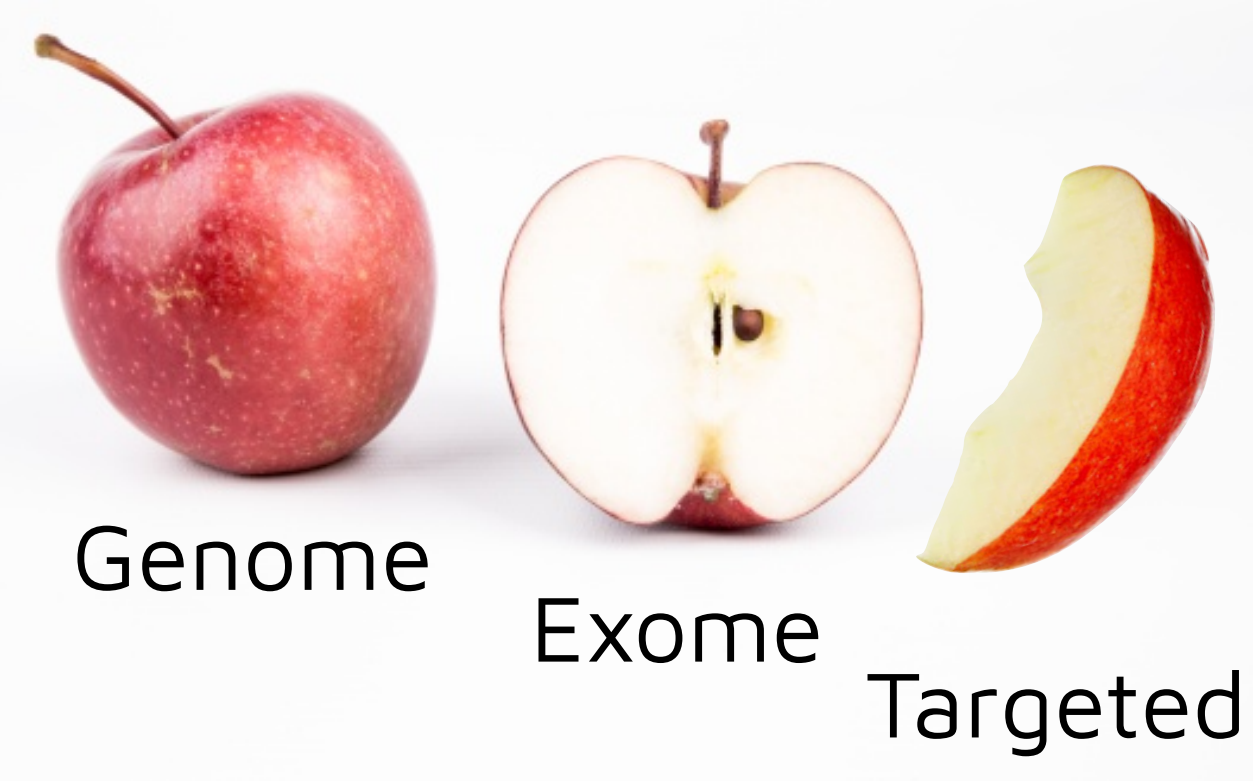
- Normal sample -> blood
- Tumor sample

Samples



- Normal sample -> blood
- Tumor sample
- Eventual relapse or metastasis

WGS - WES - Targeted sequencing



Sequencing

Illumina's HiSeq X

- Short reads

FASTQ Files

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

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

Preprocessing

- Map short reads to reference genome

Preprocessing

- Map short reads to reference genome
- Cleanup

SAM/BAM files

Sequence Alignment Map (SAM): text-based format 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 TTAGATAAAGGATACTG *
r002 0 ref 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *
r003 0 ref 9 30 5S6M * 0 0 GCCTAAGCTAA * SA:Z:ref,29,-,6H5M,17,0;
r004 0 ref 16 30 6M14N5M * 0 0 ATAGCTTCAGC *
r003 2064 ref 29 17 6H5M * 0 0 TAGGC * SA:Z:ref,9,+,5S6M,30,1;
r001 147 ref 37 30 9M = 7 -39 CAGCGGCAT * NM:i:1
```

Variant Calling

- Germline

Variant Calling

- Germline
  - Differences to Reference genome

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  - Differences to Germline genome

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
  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
```

What do we want?

Do analysis!

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- Tools

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- Tools
  - Installation
  - Version

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  - Version
- Works with cluster executor

What is Sarek?

http://sarek.scilifelab.se/

- Analysis germline and somatic workflow

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

Nextflow

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- Data-driven workflow language

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- Shareable and reproducible (with containers)

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- Docker-like container engine
  - Specific for HPC environnment

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- Without the root user security problem
- Supported by Nextflow
- Can pull containers from Docker-hub

Sarek exists in multiple flavors

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Sarek Germline

Sarek exists in multiple flavors

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Sarek Germline

Sarek Somatic

Sarek exists in multiple flavors

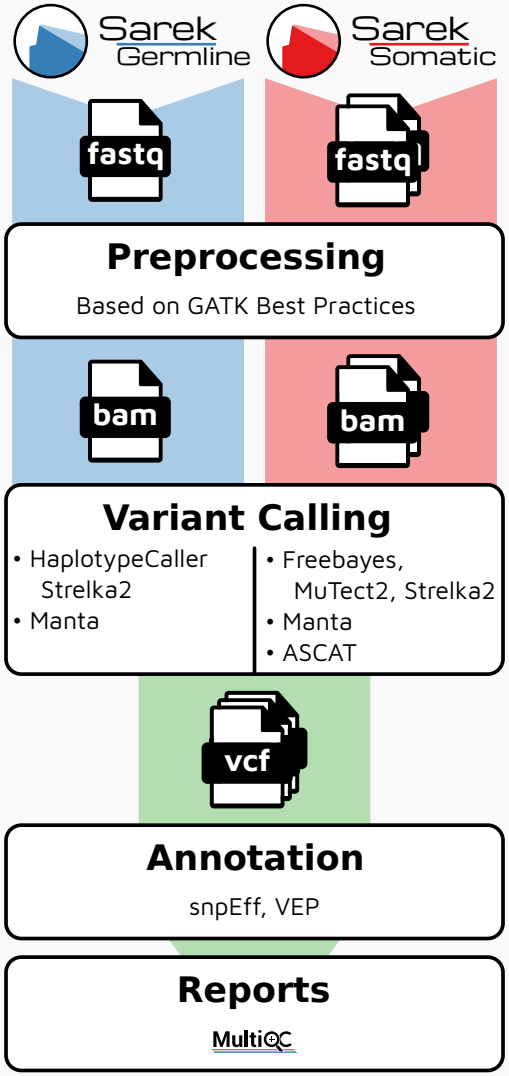
Sarek

Sarek Germline

Sarek Somatic

Sarek Exome

Data and files workflow



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gatk ☑ Best Practices

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

Based on GATK Best Practices (GATK 4.0)

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- Reads mapped to reference genome with `bwa`

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gatk ☑ Best Practices

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Based on GATK Best Practices (GATK 4.0)

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

Preprocessing

gatk ☑ Best Practices

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

Based on GATK Best Practices (GATK 4.0)

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

Germline Variant Calling

- SNVs and small indels:

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  - HaplotypeCaller
  - Strelka2

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- SNVs and small indels:
  - MuTect2
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  - Manta
- Sample heterogeneity, ploidy and CNVs:
  - ASCAT
  - Control-FREEC (🔧 adding)

Annotation

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

Prioritization

- First step towards clinical use

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- Rank scores are computed for all variants
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- Rank scores are computed for all variants
  - COSMIC, ClinVar, SweFreq and MSK-IMPACT (cancerhotspots.org)
- Findings are ranked in three tiers
  - 1st tier: well known, high-impact variants
  - 2nd tier: variants in known cancer-related genes
  - 3rd tier: the remaining variants

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Any questions?

http://sarek.scilifelab.se/

https://github.com/SciLifeLab/Sarek