RUNNING THE PIPELINE (Part I)

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INSTITUTO GULBENKIAN DE CIÊNCIA

Practical: VARIANT DETECTION DAY1 CASE STUDY

- Variant calling:
  - Detection of somatic SNV and indels
  - Detection of gene copy-number variants

- Quality Control on:
  - Sequencing data.
  - Capture and Library construction.

Overview of the case study: Exome analysis (OVCA)

Patient suffering ovarian cancer.

Whole-exome sequencing data from two samples from the patient:

- Tumour sample.
- Matched normal sample (healthy tissue) from epithelium.

Library protocol: Agilent SureSelect V5 Human All Exons.

Sequencing platform: HiSeq 2000 (Illumina)

Expected outcome:

- ~docens germ-line variants.
- A few somatic cancer mutations (SNV, indel or CNA).

NOTE: This data was simulated and reduced in order to perform the computational analysis in 30 minutes.

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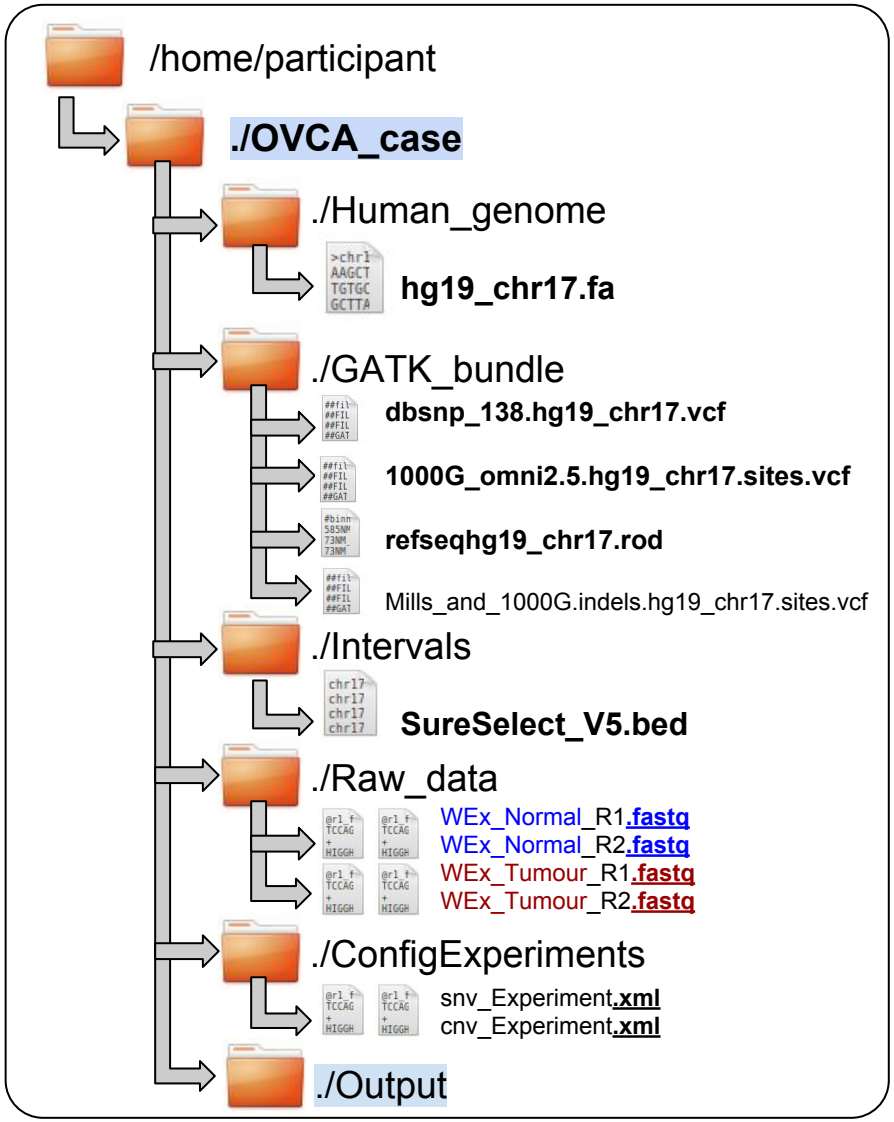
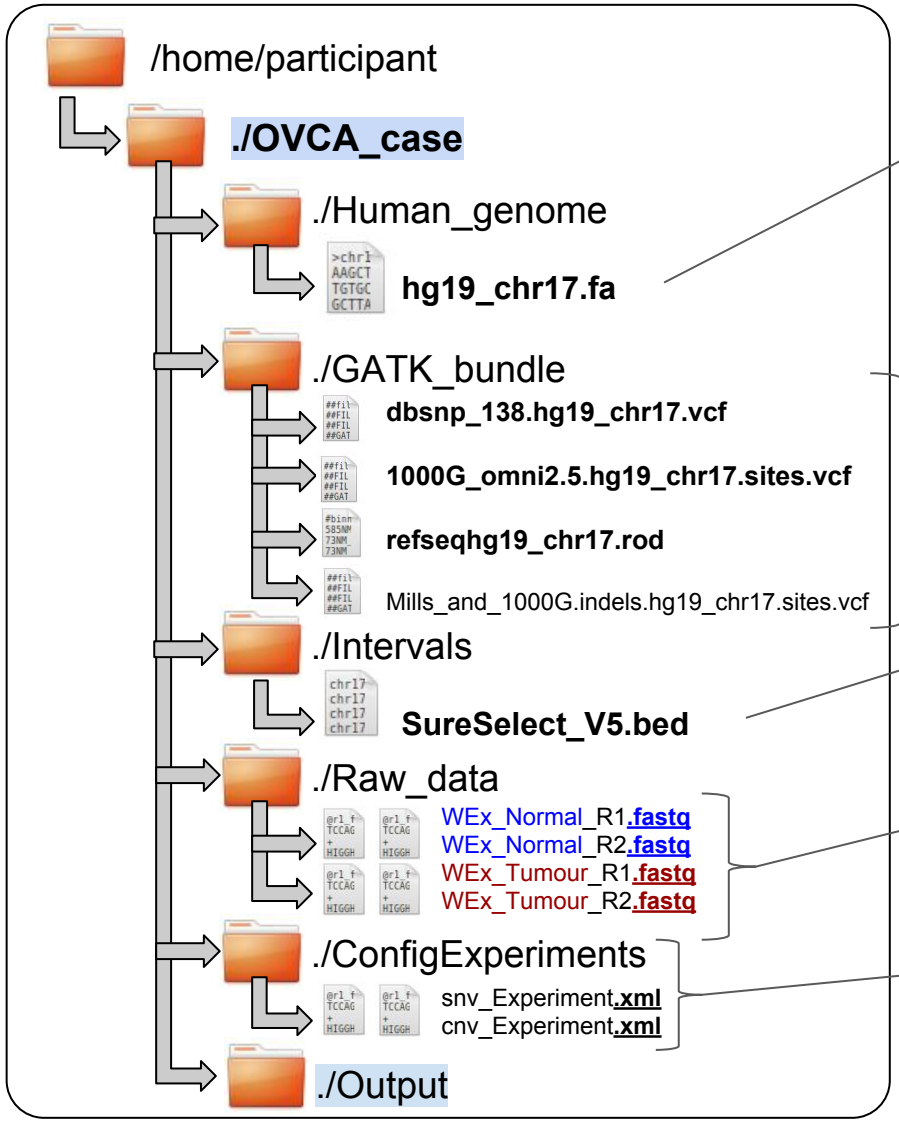


Exome analysis (OVCA) :: Input files



Human reference genome.
Alignment on a consensus genome reference.
Source: UCSC / 1000G project

`dbsnp_138.hg19_chr17.vcf` dbSNP release before 1000G phase I are set of known SNP to be used in *Base Recalibration* and annotation of VCF files (ID field in output files).

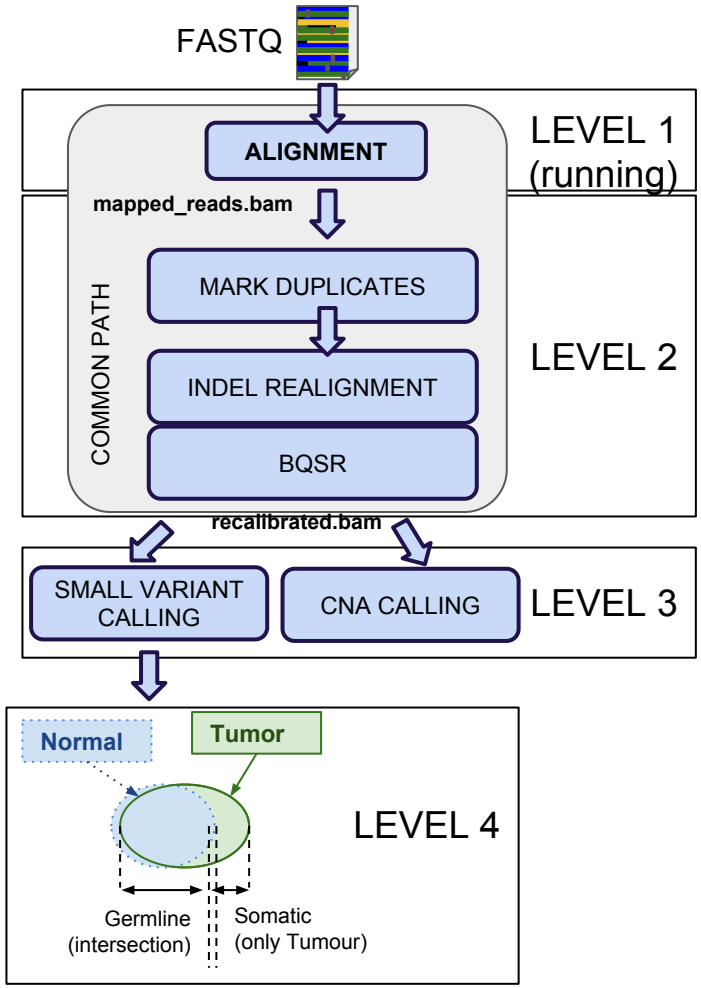
`1000G_omni2.5.hg19_chr17.sites.vcf` (1000G phase I) and **`Mills_and_1000G.indels.hg19_chr17.sites.vcf`** are set of known indels to be used for *local realignment*.

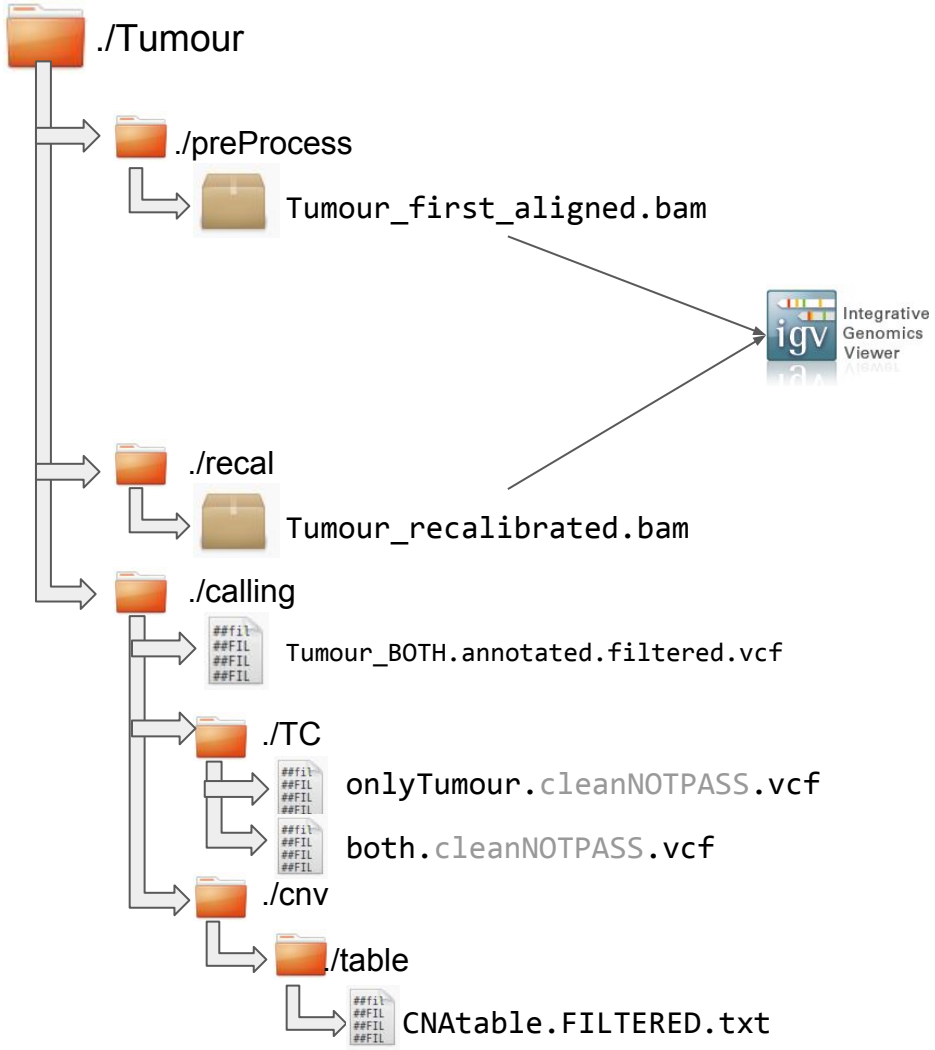
WEx Library design.
Predefined genomic regions of interest.
Source: manufacturer

Raw exome sequencing data.
Patient's sample data, generated by the sequencing machine.
Source: Collaborator / Consumer provider

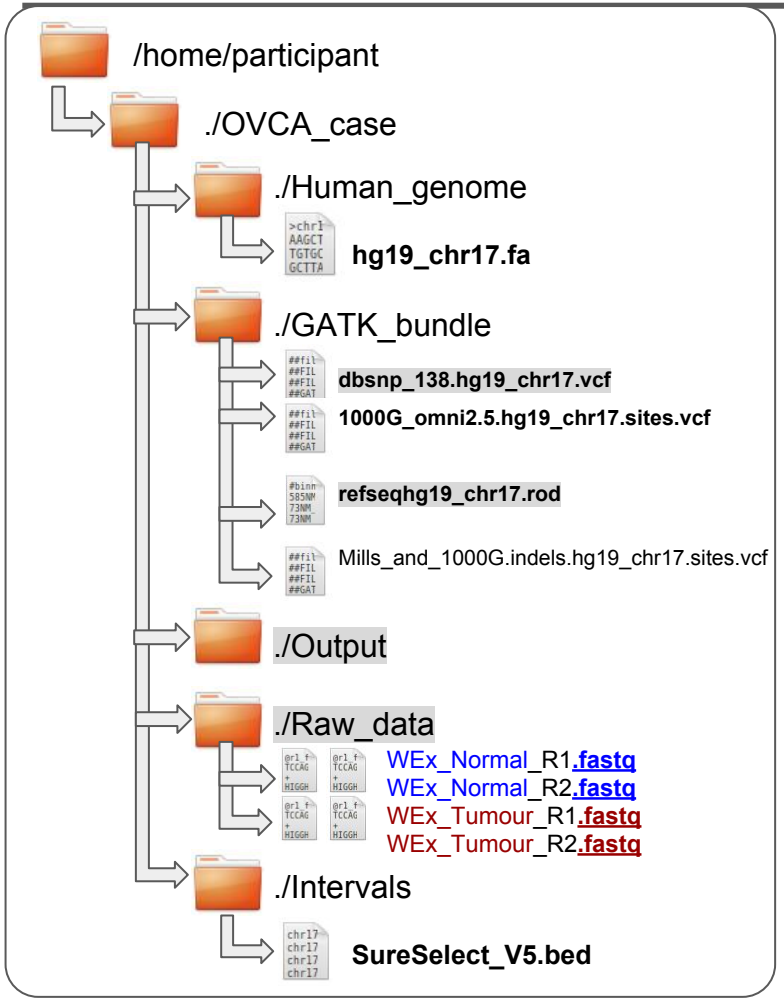
Experiment files.
Source: Templates from RUbioSeq (see Manual).

Exome analysis (OVCA) :: output files



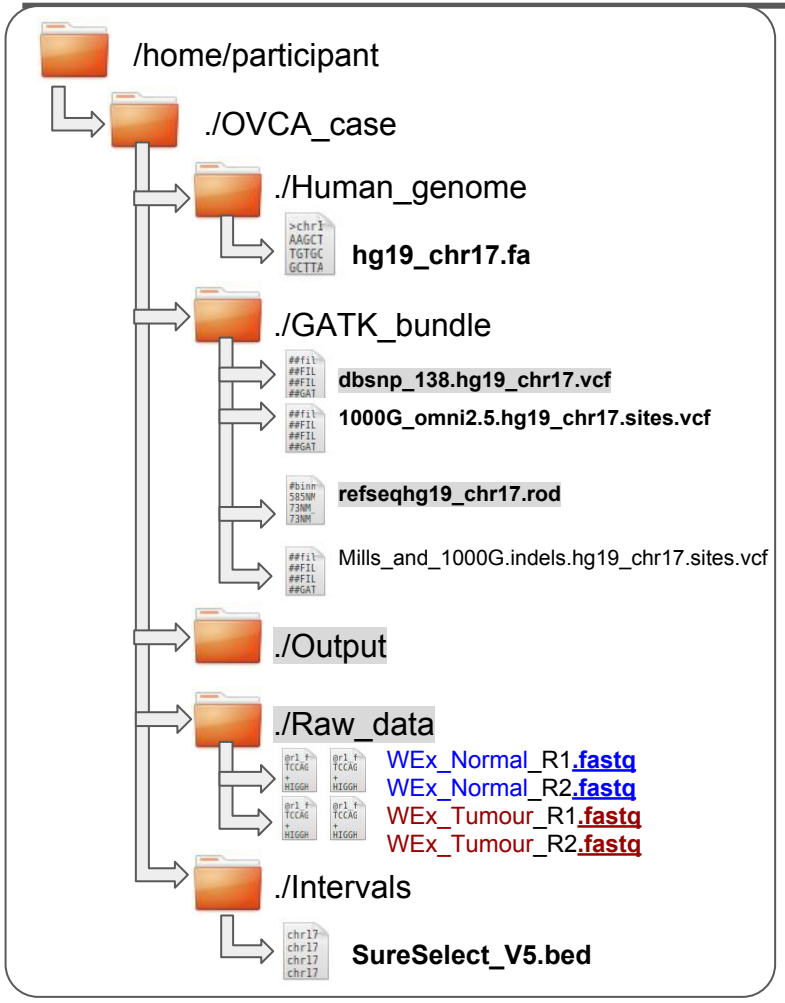


Create the experiment file (SNV) :: ~ 30 minutes




```
<?xml version="1.0" encoding="UTF-8"?>
<!-- EXAMPLE RUBIOSEQ EXPERIMENT CONFIG FILE -->
<configData branch="SNV">
        <!-- GENOME REFERENCE PATH :: MANDATORY -->
        <GenRef>___path_to_hg19.fa___</GenRef>
        <!-- DBSNP ANNOTATION PATH :: MANDATORY -->
        <DbSnpAnnot>___path_to_dbsnp_version.vcf___</DbSnpAnnot>
        <!-- 1000 Genomes ANNOTATION PATH :: MANDATORY -->
        <Genomes1000Annot>___path_to_1000G_omni2.5.hg19.sites.vcf___</Genomes1000Annot>
        <!-- REFSEQ ANNOTATION PATH :: MANDATORY -->
        <IndelAnnot>___path_to_refseqhg19_chr17.rod___</IndelAnnot>
        <!-- INTERVALS PATH :: OPTIONAL -->
        <Intervals>___path_to_WExLibrary.bed___</Intervals>
        <!-- KNOWN INDELS FOR REALIGNING :: OPTIONAL -->
        <KnownIndels>___path_to_Mills_and_1000G.indels.hg19.sites.vcf___</KnownIndels>
        <!-- PLATFORM :: MANDATORY -->
        <Platform>illumina</Platform>
        <!-- checkCasava :: OPTIONAL. -->
        <checkCasava>0</checkCasava>
        <!-- OUTPUT DIRECTORY :: DEFAULT: Home directory -->
        <dirOutBase>/home/participant/OVCA_case/</dirOutBase>
        <!-- PROJECT NAME :: MANDATORY -->
        <ProjectId>Output</ProjectId>
        <!-- USER NAME :: OPTIONAL(default Undefined) -->
        <UserName>participant</UserName>
        <!-- RAW DATA PATH :: MANDATORY -->
        <InDirPreProcess>/home/participant/OVCA_case/Raw_data/</InDirPreProcess>
        <Sample>
                <!-- SAMPLE NAME :: MANDATORY -->
                <SampleName>Tumor</SampleName>
                <SampleFiles>WEx_Tumour</SampleFiles>
                <!-- SUFFIX :: MANDATORY -->
                <SampleSuffix>.fastq</SampleSuffix>
                <!-- READ TYPE - 1: single-end 2:paired-end :: MANDATORY -->
                <SampleType>2</SampleType>
        </Sample>
        <Sample>
                <!-- SAMPLE NAME :: MANDATORY -->
                <SampleName>Normal</SampleName>
                <SampleFiles>WEx_Normal</SampleFiles>
                <!-- SUFFIX :: MANDATORY -->
                <SampleSuffix>.fastq</SampleSuffix>
                <!-- READ TYPE - 1: single-end 2:paired-end :: MANDATORY -->
                <SampleType>2</SampleType>
        </Sample>
```

Create the experiment file (SNV) :: ~ 30 minutes

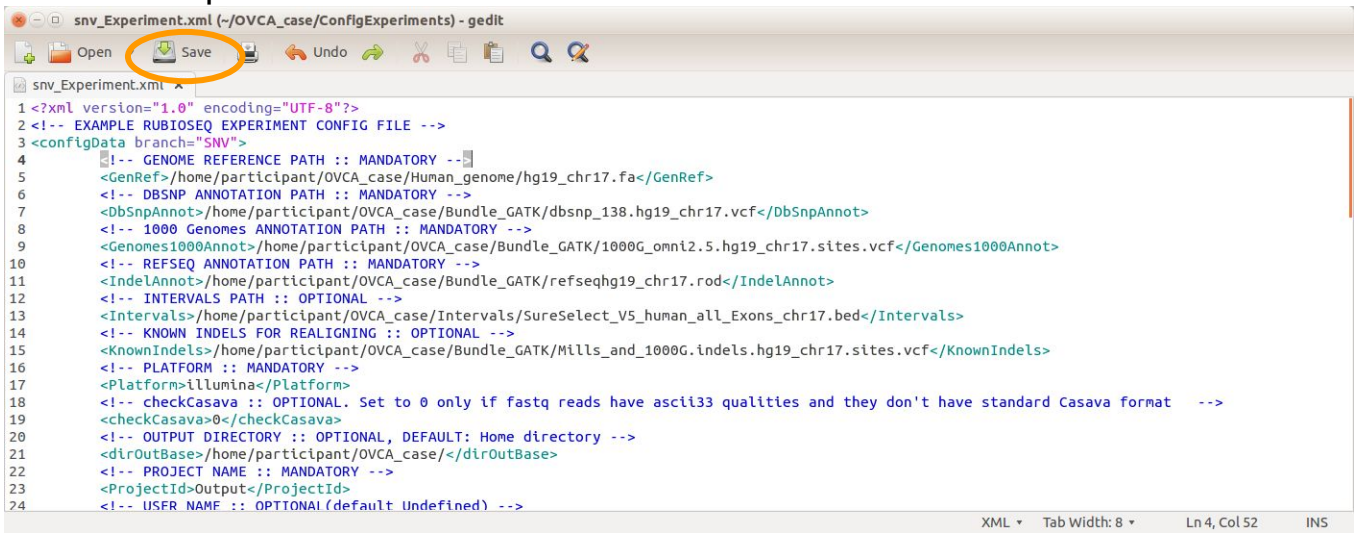



```
        [ . . . ]

        <!-- CALL TYPE :: OPTIONAL (default BOTH) -->
        <CallingType>BOTH</CallingType>
        <!-- GATKoutputMode - variants:EMIT_VARIANTS_ONLY, others:EMIT_ALL_SITES,
EMIT_ALL_CONFIDENT_SITES ::default (EMIT_VARIANTS_ONLY) -->
        <GATKoutputMode>EMIT_VARIANTS_ONLY</GATKoutputMode>
        <!-- Clean dbSNP output entries ::  1, clean dbSNPs (default 0) :: OPTIONAL -->
        <rsFilter>0</rsFilter>
        <!-- RUbioSeq_Mode Values: 0: standalone multisample, 1: joint multisample
execution (default 0) :: OPTIONAL-->
        <RUbioSeq_Mode>0</RUbioSeq_Mode>
        <!-- Run fastqc analysis  ::  1, run analysis (default 0) :: OPTIONAL -->
        <fastqc>0</fastqc>
        <!-- Run TEQC analysis ::  1, convert file (default 0) :: OPTIONAL -->
        <!--<bedTEQCFlag>0</bedTEQCFlag>-->
        <!-- VEP analysis ::  1,execute analysis (default 0) :: OPTIONAL -->
        <VEPFlag>0</VEPFlag>
        <!-- Tumor/Control flag :: OnlyTumor and Germline analyses:: 1 (default 0) ::
OPTIONAL -->
        <TCFlag>1</TCFlag>
        <!-- Markduplicates flag (WARNING:: ONLY FOR ADVANCED USERS) Enable
markduplicates step :: 1, Disable markduplicates step :: 0 (default 1) :: OPTIONAL -->
        <MDFlag>1</MDFlag>
        <!-- Min phred-scaled confidence threshold for calling (default 30.0) -->
        <standCallConf>30.0</standCallConf>
        <!-- Min phred-scaled confidence threshold for emitting (default 30.0)-->
        <standEmitConf>30.0</standEmitConf>
        <!-- Queue project :: OPTIONAL (default none) -->
        <queueSGEProject>none</queueSGEProject>
        <!-- Whole exome and target sequencing analyses filtering -->
        <HardFilters>
                <!-- VCF Depth filter :: OPTIONAL -->
                <DPmin>15</DPmin>
                <!-- VCF min quality filter :: OPTIONAL -->
                <minQual>100</minQual>
                <!-- Optional. ONLY FOR ADVANCED USERS. Hard filter custom name -->
                <!--<HfilterNameSNP>QDFilter</HfilterNameSNP>-->
                <!-- Optional. ONLY FOR ADVANCED USERS. Hard filter custom rule -->
                <!--<HfilterRuleSNP>QD&lt;2.0</HfilterRuleSNP>-->
        </HardFilters>
</configData>
```

Check that everything is ready for running the pipeline

- Save the experiment file



- Getting help to run the pipeline

```
$ perl /home/participant/Sotware/RUbioSeq+/RUbioSeq3.7/RUbioSeq.pl -h
    RUbioSeq.pl --analysis analysisType --config config_file  [--level level_number]

          Getting help:
                [--help]
          Analysis Types:
                variantCalling : Variant Calling Workflow.(default)
                cnvCalling: CNV Calling Workflow.
                ChIPseq: ChIPseq workflow.
                methylationCalling : Methylation Calling Workflow.
          Example:
                ./RUbioSeq.pl --analysis variantCalling --config /dir/config.xml  --level 3
```

Launch the SNV and Indel calling

open a terminal, and execute the cmd:

```
$ perl /home/participant/Sotware/RUbioSeq+/RUbioSeq3.7/RUbioSeq.pl --analysis variantCalling
    --config /home/participant/OVCA_case/configExperiments/snv_Experiment.xml
```

VARIANT CALLING ANALYSIS
bwaPath: /local/participant/Soft/NGS/bwa-0.7.10/
javaRam: -Xmx16G
samtoolsPath: /local/participant/Soft/NGS/samtools-0.1.19/
BFASTPath: /opt/NGS/bfast+bwa/0.7.0b/bin/
gatkpath: /local/participant/Soft/NGS/GenomeAnalysisTK-3.1-1/
picardPath: /local/participant/Soft/NGS/picard-tools-1.107/picard-tools-1.107
[. . .]
TCFlag: 1
CallingType: BOTH
RUbioSeq_Mode: 0
IndelAnnot: /home/participant/OVCA_case/Bundle_GATK/refseqhg19_chr17.rod
MDFlag: 1
checkCasava: 0
Genomes1000Annot: /home/participant/OVCA_case/Bundle_GATK/1000G_omni2.5.hg19_chr17.sites.vcf
InDirPreProcess: /home/participant/OVCA_case/Raw_data/
Intervals: /home/participant/OVCA_case/Intervals/SureSelect_V5_human_all_Exons_chr17.bed
fastqc: 1
minQual: 100

The analysis is using these parameters (you just input them)

EMIT ALL SITES for TC analysis activated
Directory /home/jperales/OVCA_case//Output/ exists
Executed command perl /home/jperales/Soft/RUbioSeq+/RUbioSeq3.7/variantCalling/../common/indexReference.pl /home/jperales/Soft/samtools-0.1.19/ /home/jperales/Soft/picard-tools-1.107/ /home/jperales/OVCA_case/Human_genome/hg19_chr17.fa -Xmx4G > /home/jperales/OVCA_case//Output//log_S0.txt 2>&1

Level 0

Executed command perl /home/jperales/Soft/RUbioSeq+/RUbioSeq3.7/variantCalling/../common/sampleAlign.pl /home/jperales/OVCA_case//Output/Tumor /home/jperales/OVCA_case/Raw_data/ /home/jperales/Soft/bwa-0.7.10/ /home/jperales/Soft/samtools-0.1.19/ /home/jperales/Soft/picard-tools-1.107/ /home/jperales/Soft/bfast-bwa-ed42c18ea7f48af862935be52f1c072b1d5609cc/bin/ /home/jperales/OVCA_case/Human_genome/hg19_chr17.fa WEx_Tumour .fastq Tumor jperales illumina Tumor Output 2 4 -Xmx4G 1 /home/jperales/Soft/FastQC/ 0 0 > /home/jperales/OVCA_case//Output/Tumor/log_S1_WEx_Tumour.txt 2>&1

Level 1: Tumor

[Level 2, Level 3 on Tumor sample]

Executed command perl /home/jperales/Soft/RUbioSeq+/RUbioSeq3.7/variantCalling/../common/sampleAlign.pl /home/jperales/OVCA_case//Output/Normal /home/jperales/OVCA_case/Raw_data/ /home/jperales/Soft/bwa-0.7.10/ /home/jperales/Soft/samtools-0.1.19/ /home/jperales/Soft/picard-tools-1.107/ /home/jperales/Soft/bfast-bwa-ed42c18ea7f48af862935be52f1c072b1d5609cc/bin/ /home/jperales/OVCA_case/Human_genome/hg19_chr17.fa WEx_Normal .fastq Normal jperales illumina Normal Output 2 4 -Xmx4G 1 /home/jperales/Soft/FastQC/ 0 0 > /home/jperales/OVCA_case//Output/Normal/log_S1_WEx_Normal.txt 2>&1

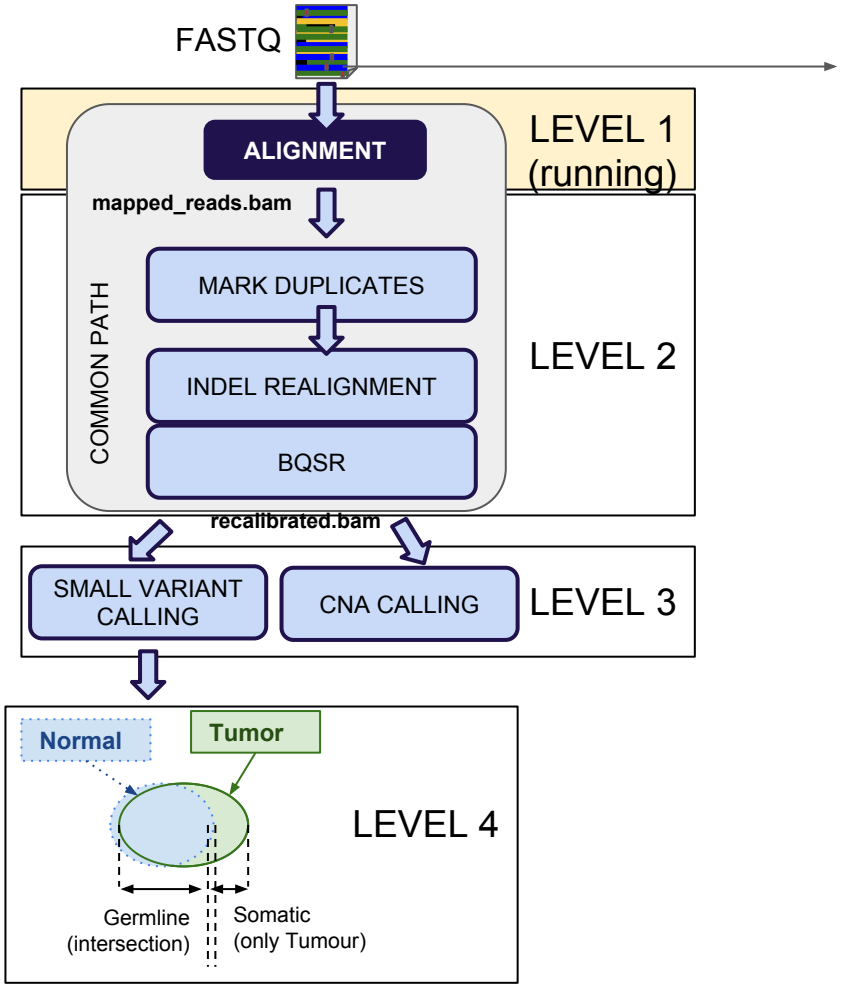
Level 1: Normal

[Level 2, Level 3 on Normal sample]

Executed command perl /home/jperales/Soft/RUbioSeq+/RUbioSeq3.7/variantCalling/postProcess.pl /home/jperales/OVCA_case//Output/Tumor/calling/TC /home/jperales/OVCA_case//Output/Tumor/calling/TC/onlyControl.vcf /home/jperales/OVCA_case/Human_genome/hg19_chr17.fa 0 1 > /home/jperales/OVCA_case//Output/Tumor/log_S4_OC.txt 2>&1
Executed command perl /home/jperales/Soft/RUbioSeq+/RUbioSeq3.7/variantCalling/postProcess.pl /home/jperales/OVCA_case//Output/Tumor/calling/TC /home/jperales/OVCA_case//Output/Tumor/calling/TC/bothTC.vcf /home/jperales/OVCA_case/Human_genome/hg19_chr17.fa 0 1 > /home/jperales/OVCA_case//Output/Tumor/log_S4_B.txt 2>&1

Level 4: Tumor-Matched Normal

Hands-on Quality Control



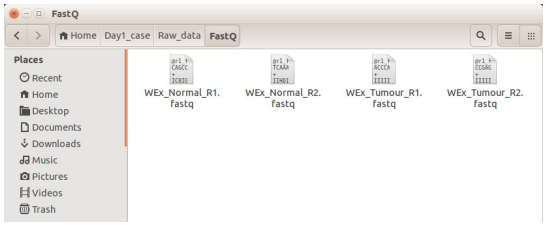
We will perform the Quality Control assessment in the raw data in the meantime the alignment (Level 1 from the pipeline) is running in background.

Hands-on Quality Control

We will carry out a QC on the Case study raw data.

Remember the data:

- Whole-exome sequencing (Illumina platform)
- paired-end sequencing (2 samples, 2 files each)

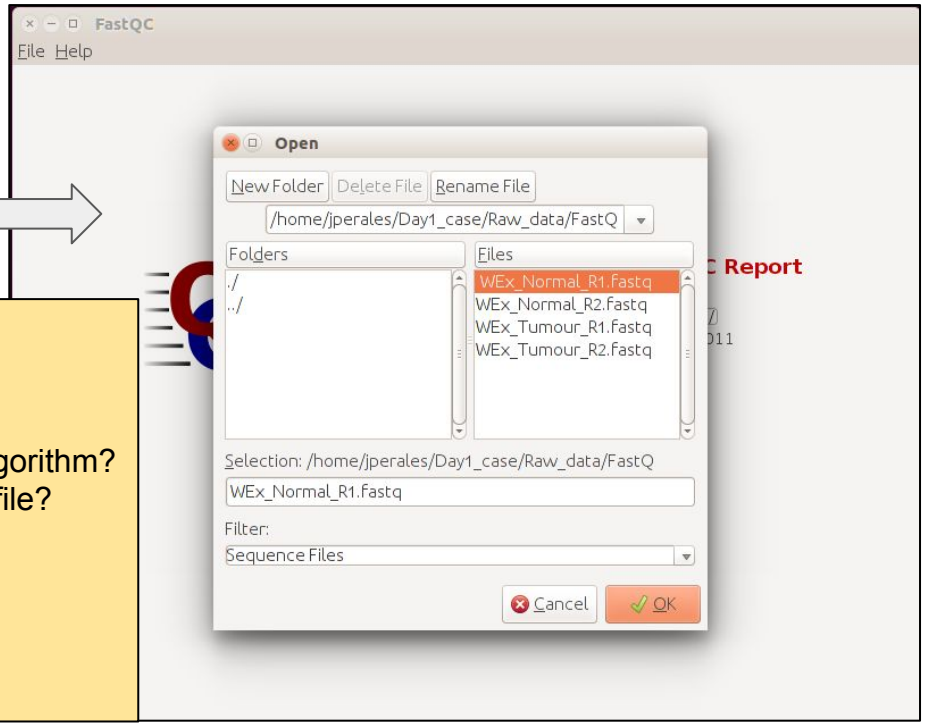


We must open the QC software: FastQC

So open a terminal, and execute the cmd:

```
perl ~/Software/FastQC/fastqc
```

File > Open: each file `.fastq` (4x)



Try to answer to the following questions:

1. What seq depth was run in the experiment? (No. sequenced reads)
2. What Phred Score encoding is detected by the algorithm?
3. How is the general QC state of each sequencing file?
4. Is there any plot with an error or a warning? why?

~ 10 minutes!

Manual: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/

Webpage: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Hands-on Quality Control

Try to answer to the following questions:

Q: What seq depth was run in the experiment? (No. sequenced reads)

A: _____.

Q: What Phred Score encoding is detected by the algorithm?

A: _____.

Q: How is the general QC state of each sequencing file?

A: _____.

Q: Is there any plot with an error or a warning? Why?

A: _____.

Coffee Break time

16:00 - 16:30

RUNNING THE PIPELINE (Part II)

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Practical: VARIANT DETECTION
DAY1 CASE STUDY
(Part II)

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- Matched normal sample (healthy tissue) from epithelium.

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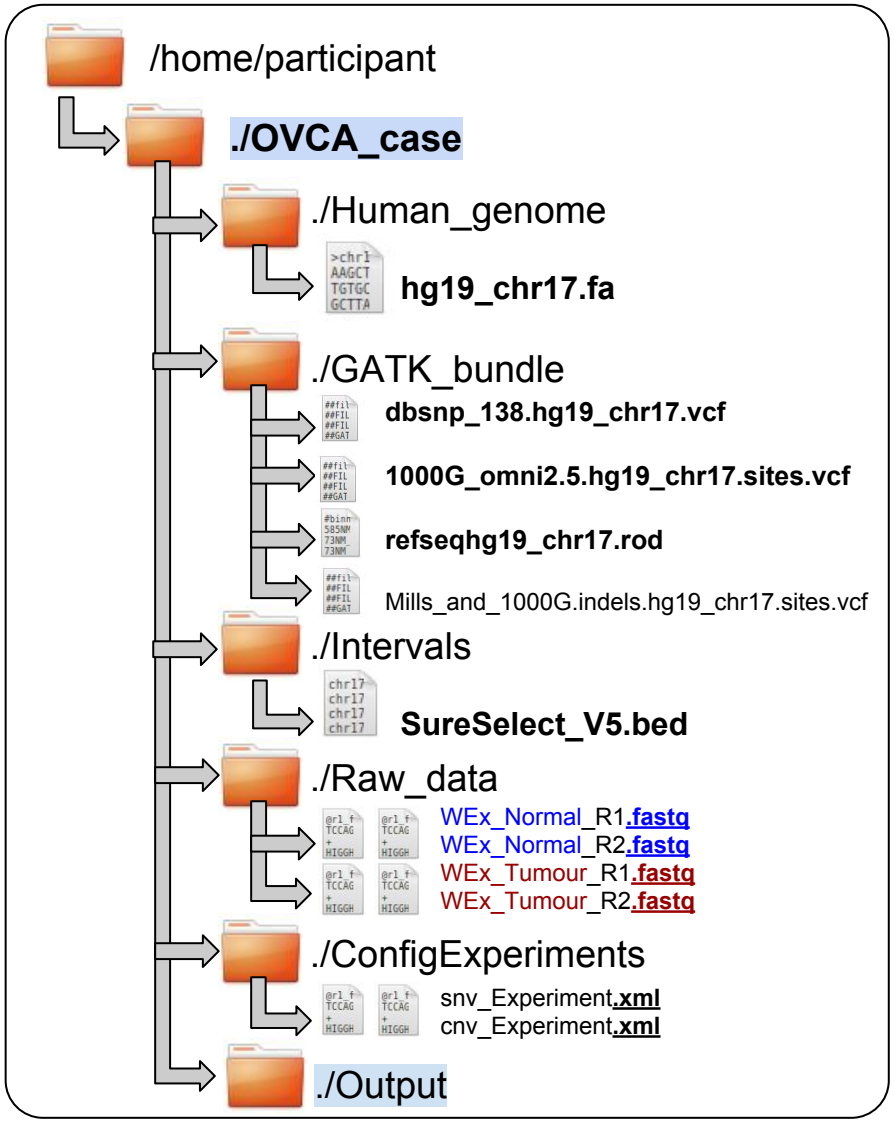
Sequencing platform: HiSeq 2000 (Illumina)

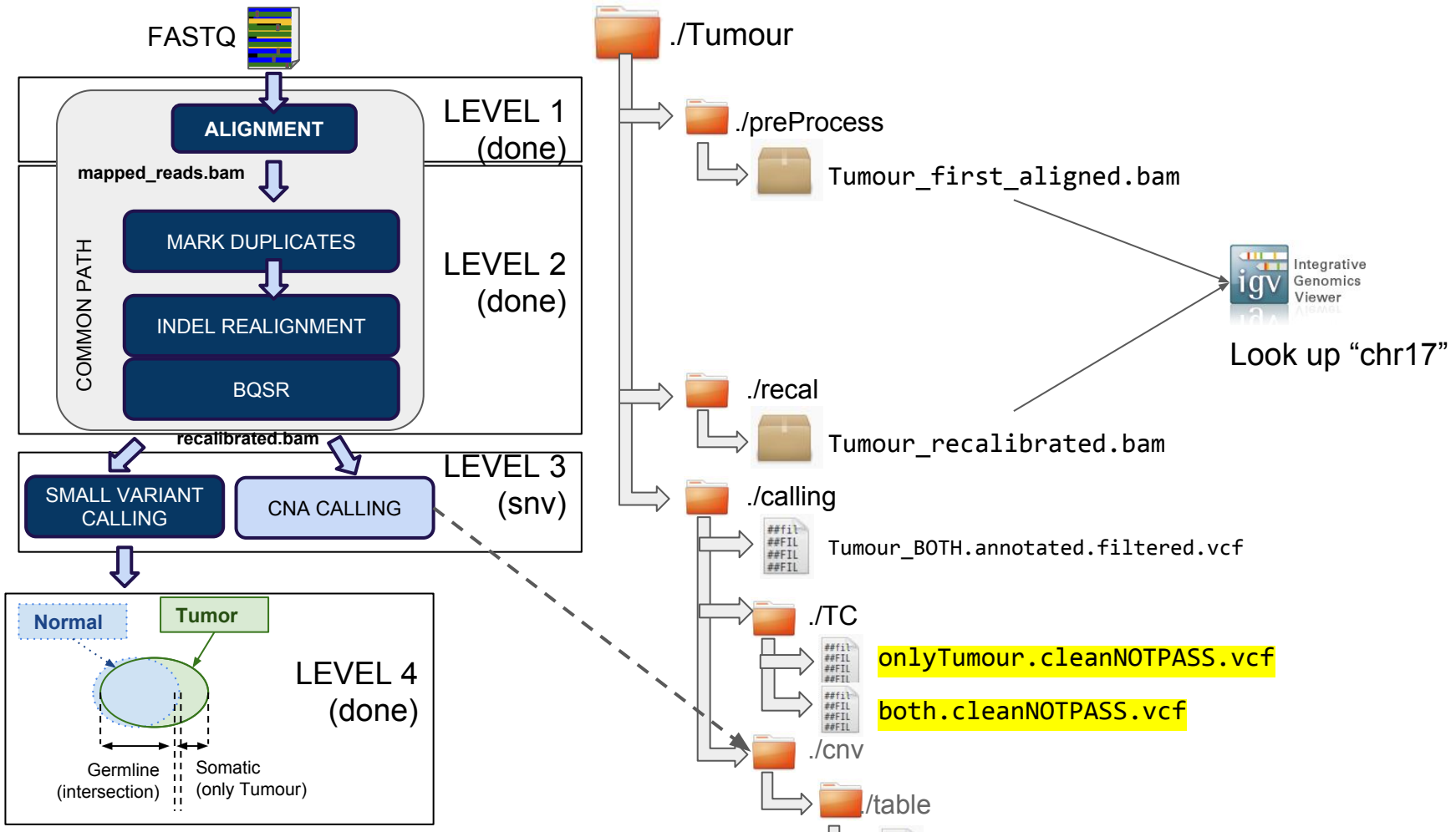
Expected outcome:

- ~docens germ-line variants.
- A few somatic cancer mutations (SNV, indel or CNA).

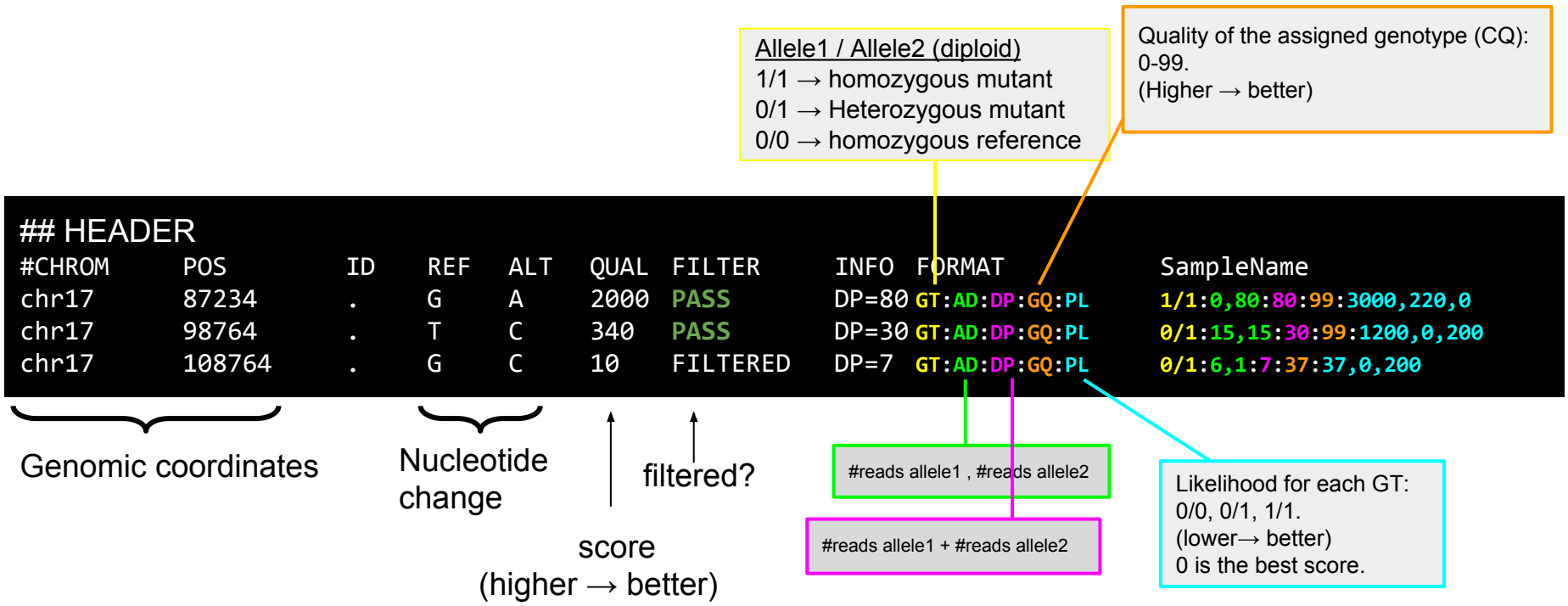
NOTE: This data was simulated and reduced in order to perform the computational analysis in 30 minutes.



Exome analysis (OVCA) :: output files



Variant Call Format (vcf) from GATK callers

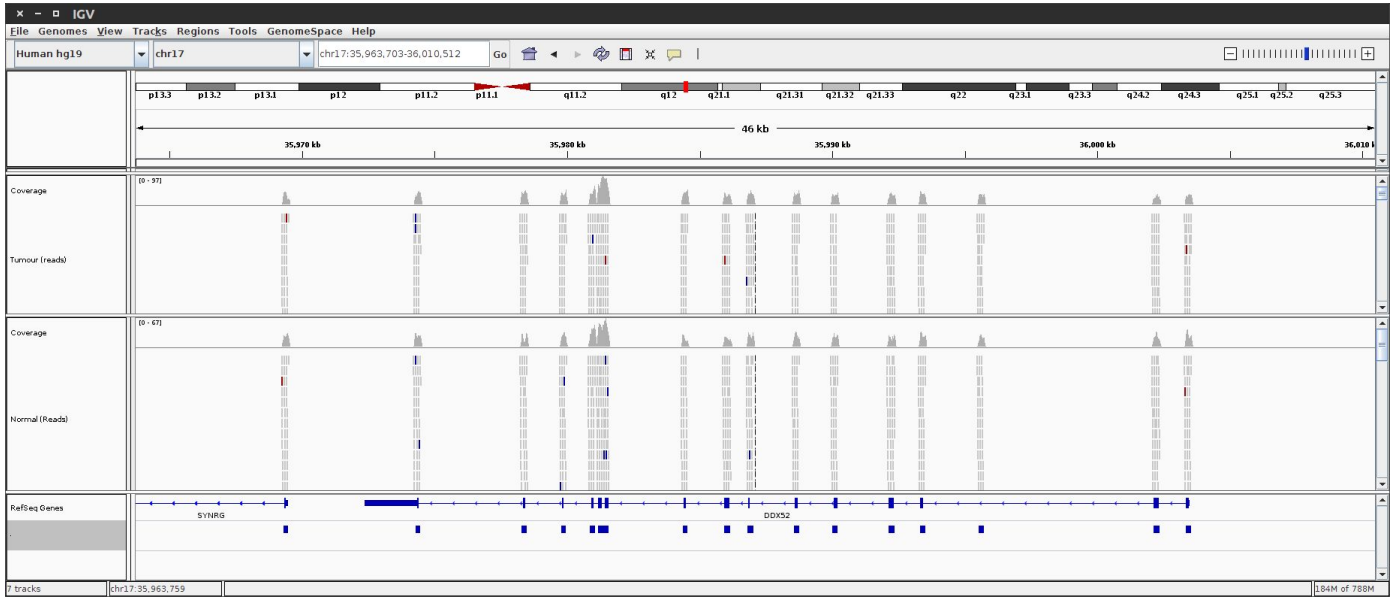


More info.:
https://www.broadinstitute.org/gatk/guide/article?id=1268

IGV : Genome Browser

open a terminal, and execute the cmd:

```
$ java -jar /home/participant/Software/IGV_2.3.66/igv.jar
```



Open the BAM files from each sample:

`recalibrated.bam`

*Case study :: **Point mutations results***

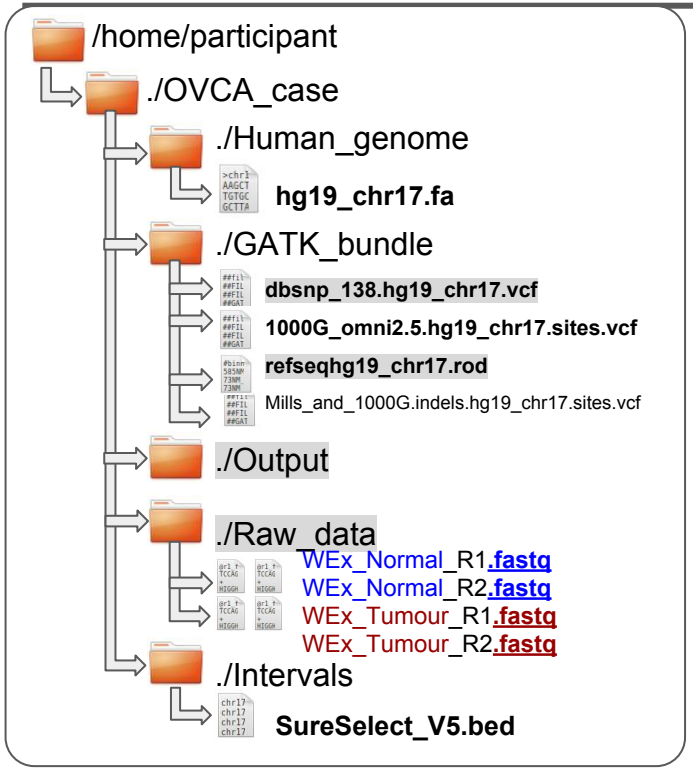
- Germline variants

There were detected __ germline variants in total:
- __ Single Nucleotide Variants.
- __ Indels.

- Somatic variants

There were detected __ somatic SNVs.
Genes affected and type of mutations
(see alignment using **IGV on chr17**):
- _____.
- _____.

Create the experiment file (**CNV**) :: ~ 15 minutes

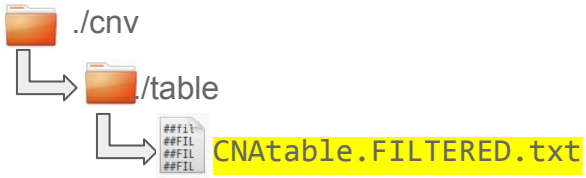



```
<?xml version="1.0" encoding="UTF-8"?>
<!-- EXAMPLE RUBIOSEQ EXPERIMENT CONFIG FILE -->
<configData branch="CNV">
        <!-- GENOME REFERENCE PATH :: MANDATORY -->
        <GenRef>___path_to_hg19.fa___</GenRef>
        <!-- DBSNP ANNOTATION PATH :: MANDATORY -->
        <DbSnpAnnot>___path_to_dbsnp_version.vcf___</DbSnpAnnot>
        <!-- REFSEQ ANNOTATION PATH :: MANDATORY -->
        <IndelAnnot>___path_to_refseqhg19_chr17.rod___</IndelAnnot>
        <!-- INTERVALS PATH :: OPTIONAL -->
        <Intervals>___path_to_WExLibrary.bed___</Intervals>
        <!-- KNOWN INDELS FOR REALIGNING :: OPTIONAL -->
        <KnownIndels>___path_to_Mills_and_1000G.indels.hg19.sites.vcf___</KnownIndels>
        <!-- PLATFORM :: MANDATORY -->
        <Platform>illumina</Platform>
        <!-- checkCasava :: OPTIONAL. -->
        <checkCasava>0</checkCasava>
        <!-- OUTPUT DIRECTORY :: DEFAULT: Home directory -->
        <dirOutBase>/home/participant/OVCA_case/</dirOutBase>
        <!-- PROJECT NAME :: MANDATORY -->
        <ProjectId>Output</ProjectId>
        <!-- USER NAME :: OPTIONAL(default Undefined) -->
        <UserName>participant</UserName>
        <!-- RAW DATA PATH :: MANDATORY -->
        <InDirPreProcess>/home/participant/OVCA_case/Raw_data/</InDirPreProcess>
        <Sample>
                <!-- SAMPLE NAME :: MANDATORY -->
                <SampleName>Tumor</SampleName>
                <SampleFiles>WEx_Tumour</SampleFiles>
                <!-- SUFFIX :: MANDATORY -->
                <SampleSuffix>.fastq</SampleSuffix>
                <!-- READ TYPE - 1: single-end 2:paired-end :: MANDATORY -->
                <SampleType>2</SampleType>
        </Sample>
        <Sample>
                <!-- SAMPLE NAME :: MANDATORY -->
                <SampleName>Normal</SampleName>
                <SampleFiles>WEx_Normal</SampleFiles>
                <!-- SUFFIX :: MANDATORY -->
                <SampleSuffix>.fastq</SampleSuffix>
                <!-- READ TYPE - 1: single-end 2:paired-end :: MANDATORY -->
                <SampleType>2</SampleType>
        </Sample>
```

WARNING!! Use '`--level 3`' in the command because the 'Common Path' has been already done during the `snvCalling`.

```
$ perl /home/participant/Sotware/RUbioSeq+/RUbioSeq3.7/RUbioSeq.pl --analysis cnvCalling
    --config /home/participant/OVCA_case/configExperiments/cnv_Experiment.xml --level 3
```

*Case study :: **Copy-number variants results***



There were detected __ somatic CNVs (see table).
Genes affected and type of mutations
(see alignment using **IGV on chr17**):

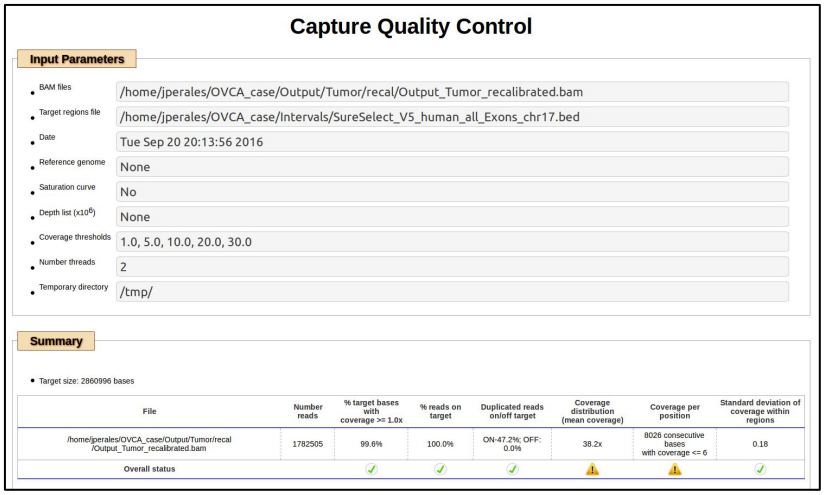
- _____.
- _____.

ngsCAT

Evaluation of the performance of the capture step in targeted high-throughput sequencing experiments in terms of:

- **Sensitivity** : quality of the coverage on target regions.
- **Specificity** : on-target / off-target reads.
- **Uniformity** : sequencing biases.

```
$ python /home/participant/Software/ngscat.v0.1/ngscat.py \
    --bam /home/participant/OVCA_case/Output/Tumor/recal/Output_Tumor_recalibrated.bam \
    --bed /home/participant/OVCA_case/Intervals/SureSelect_V5_human_all_Exons_chr17.bed \
    --out /home/participant/OVCA_case/Output/Tumor/Quality
```



Capture Quality Control

Input Parameters

BAM files	/home/jperales/OVCA_case/Output/Tumor/recal/Output_Tumor_recalibrated.bam
Target regions file	/home/jperales/OVCA_case/Intervals/SureSelect_V5_human_all_Exons_chr17.bed
Date	Tue Sep 20 20:13:56 2016
Reference genome	None
Saturation curve	No
Depth list ($x10^6$)	None
Coverage thresholds	1.0, 5.0, 10.0, 20.0, 30.0
Number threads	2
Temporary directory	/tmp/

Summary

- Target size: 2860996 bases

File	Number reads	% target bases with coverage >= 1.0x	% reads on target	Duplicated reads on/off target	Coverage distribution (mean coverage)	Coverage per position	Standard deviation of coverage within regions
/home/jperales/OVCA_case/Output/Tumor/recal/Output_Tumor_recalibrated.bam	1782505	99.6%	100.0%	ON-47.2%; OFF: 0.0%	38.2x	8026 consecutive bases with coverage <= 6	0.18
Overall status		✓	✓	✓	⚠	⚠	✓

WEB : http://bioinfo.cipf.es/ngscat/ngscat/download/start
Paper: Lopez-Domingo FJ et al. (2014) Bioinformatics.

Target Region Coverage

Representation of the Fraction of Capture targeted bases in a data set (several samples).

```
$ Rscript /home/participant/Software/targetedRegionCoverage.R \
--bams
/home/participant/OVCA_case/Output/Tumor/recal/Output_Tumor_recalibrated.bam,/home/participant/OVCA_case/Output/N
ormal/recal/Output_Normal_recalibrated.bam   \
--bed /home/participant/OVCA_case/Intervals/SureSelect_V5_human_all_Exons_chr17.bed   \
--out /home/participant/OVCA_case/Output/coverage.png
```