DATA FORMATS & METHODS IN VARIANT CALLING

Javier Perales-Patón
jperales@cnio.es

Fátima Al-Shahrour
[falshahrour@cnio.es]
Elena Piñeiro-Yáñez
[epineiro@cnio.es]
Pedro Fernandes
[pfern@igc.gulbenkian.pt]

Centro Nacional de Investigaciones Oncológicas

Translational Bioinformatics Unit
CNIO. Madrid, Spain.

INSTITUTO GULBENKIAN DE CIÊNCIA

Data formats cheat sheet

Format	Uses	Example	File type	Software Management	File Extension
Fasta	**Human genome** Define biological sequences (DNA, RNA, cDNA, proteins).	`human_genome.fa`	Plain text	samtools, picard-tools	.fa; .fasta
FastQ	**Raw sequencing data** Single-end sequencing → 1 file Paired-end sequencing → 2 files (R1 and R2 for each end, respectively)	`DNAseq_raw_data.fastq` (`DNAseq_R1.fastq` and `DNAseq_R2.fastq`)	Plain text	samtools, picard-tools **Aligners**	.fq; .fastq
SAM	Define read alignments. Store alignment meta-info (reference, methods, one- or multi-sample).	`mapped_reads.sam`	Plain text	samtools, picard-tools	.sam
BAM	**VISUALIZE ALIGNMENTS (IGV)** The same as SAM, but compressed and indexed. Also to store UNMAPPED reads (compressed).	`mapped_reads.bam` `unmapped_reads.bam`	Binary	samtools, picard-tools, **IGV** (Integrative Genome Viewer)	.bam
VCF ★	**SNV & Indels calls** Indicate genomic variations. Store Variant calling meta-info (reference, methods,one- or multi-sample).	`detected_pointvariants.vcf`	Plain text	bcftools, vcftools, Unix	.vcf
BED	**Intervals** Delimit genomic regions (i.e. intervals) w or w/o annotations.	`targeted_regions.bed` `intervals.bed`	Plain text	bedtools, Unix **GATK, picard-tools**	.bed
TSV or CSV	Create data matrix (rows X Columns)	`CONTRA_output.tsv` `annotated_variants.tsv`	Plain text	Unix, Microsoft Excel, OpenOffice	.tsv; .csv; .txt
XML	RUbioSeq configuration file (internal) Define software internal configuration.	`config.xml`	Plain	Unix, Firefox	.xml

FASTA & FASTQ formats

- FASTA format: simple sequences

```
>DNA_SEQUENCE_1
NNNNNNCTCTGGGGGACAGAACCCTATGGTGGCCCCGGCTCCTCCCCAGTATCCAGTCCT
CCGTGAAGATGGAGCCATATTCC
```

60 chars

Each sequence is composed by
at least two consecutive lines.
- "`>`" Sequence name
- Multiple lines with the whole sequence.

Possible chars: DNA → ATCGatcgNRY...,
Protein→ 1 letter amino acid code

https://en.wikipedia.org/wiki/FASTA_format

- FASTQ format: raw sequencing data.

_R1

```
@SN1083:379:H8VA1ADXX:2:1101:1248:2144 1:N:0:12
CCTAAATGGTGCCATGCTAGGAGGCCGTGCCCTTCTTGAAAAGTTGTATGTGAA
+
BBBFFFFFFBFFFIIIIFI<FFIIIIIFIIIIFBFIIIIIIIIFFFIIIIFIII
```

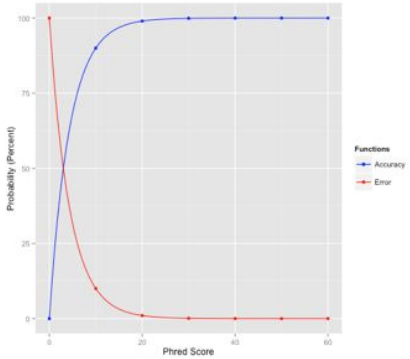
_R2

```
@SN1083:379:H8VA1ADXX:2:1101:1248:2144 2:N:0:12
CATTTTCGACGTTGTTAATAAGCTCTGCGTACTTGCAAGCTATCTGCGCGAACG
+
BBBFFFFFFFFFFIIIIIIIIIIIIIIIIIFIIIIIIIIIIIIIIIIIIIIFFF
```

Each sequence read is composed by 4 lines:
- "`@`"read name
- Sequence
- "`+`" (optionally: repeat the read name)
- Base Quality Score: Phred scale (0,40)
  transformed in ASCII chars.

1 unique sample could have:
If Single-end Seq → 1 file (suffix "`.fastq`")
If Paired-end Seq → 2 files (suffixes "`_R1.fastq`" "`_R2.fastq`")

Phred Score (0,40)



https://en.wikipedia.org/wiki/FASTQ_format

FASTQ format in detail

Just to show how one single read is: 4 consecutive lines.

readname
sequence
comment
Quality

```
@HWI-EAS209_0006_FC706VJ:5:58:5894:21141      ATCACG
TTAATTGGTAAATAAATCTCCTAATAGCTTAGATNTTACCTTNNNNNNNNNNTAGTTTCTTGAGATTTGTTGGGGGAGACATTTTTGTGATTGCCTTGAT
+
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}JJJJJJJ
```

Encoding:

```
SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
.........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX......................
...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII......................
................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ......................
LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
|                         |    |        |                              |                     |
33                        59   64       73                            104                   126
0........................26...31.......40
                          -5....0........9.............................40
                                0........9.............................40
                                   3.....9.............................40
0.2......................26...31........41
```

Different platforms have been using different encodings.

```
S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

WARNING: There are many codifications for the Phred Score, which overlap each other. Therefore, you must know the actual codification.

https://en.wikipedia.org/wiki/FASTQ_format

FASTA & FASTQ formats

```
@9VMIV:01561:02299
TGGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCTGCAGCAAGGGCTGGGCGATATCCCCTTGCTCGCAGC
+
@C?DA?B@ABCCB?ACAC>A@?B?==>C@C@@A?AA@B@?>BC@?9A@EC@C@>B@D@?E@AB@BAAB@?@CCACC>?<AB>D@?DB@>?>DD???=AC?A?>><CCC>A@8;>=;
@9VMIV:00358:01397
GCTGCGAGCAAGGGGATATCGCCCAGCCCTTGCTGCAGCCCAACAACTATCAGTTCTGCTGAGGAGTTGACGACAGGGAGTACCACTCTCCCCTCCTCCAAACTTCAACTCCTCCAGACCAGTGAGGGAAGTGAGGACGTACACT
+
A>BB?:A@>CB@>>>CA@A?:><;B@>>>BBC>AA=D@>?>DB?EC@CAC@B?@B@BC>BBA@?C@@CBC@9B?C?=?@@@@?;B>?@A@>><A?>C@>DCA?A??B====9;;:A@A@;B@@AA@>?CB?@47821/14:0403
@9VMIV:01056:02786
GCTGCGAGCAAGGGGATATCGCCCAGCCCTTGCTGCAGCCCAACAACTATCAGTTCTGCTGAGGAGTTGACGACAGGGAGTACCACTCTCCCCTCCTCCAAACTTCAACTCCTCCC
+
A>DD?<B@?B==<<<@A@A@:>>=B@>>>BBC>BC>E@>?>DB@DB@CAC@E@AC@BC?CCC@?B@@CBA?<A?B@=?B@?@@>B?<@<<==7==:=?:?@:<===B===@;;;<8
@9VMIV:03590:01718
GCTGCGAGCAAGGGGATATCGCCCAGCCCTTGCTGCAGCCCAACAACTATCAGTTCTGCTGAGGAGTTGACGACAGGGAGTACCACTCTCCCCTCCTCCAAACTTCAACTCCTCCAAAGC
+
A>BB?=A@>B?@??<@A@A?8<?<A@?><A=<;:;:B?>>5;;?B>@A::9A:?8::9>?AC@?B@ABCA@<@>A?=>B?:=87;9:9:@>><:=;==:?B?<===B==4323914:753
@9VMIV:00229:02357
GGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCTGCAGCAAGGGCTGGGCGATATCCCCTTGCTCGCAGC
+
A?DA@A@ABCCB@ACCC?B@?B@>>?C@C@@A>AA@B@?=BBA?9A@EB@C@>A@B@>D@A@>CCCB@?@BCABC>??AB>D@>DC@??>DC>?=<?@=8;<<8;<<>A?8;><;
@9VMIV:00282:01752
GGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCTGCAGCAAGGGCTGGGCGATATCCCCTTGCTCGCAGC
+
@>DA@C@ABC@B?@ABC?C@?B?>=?B@B@AC?AA@B@?>BC@?9A@BB@B@>C@D@?D@BC?BBBB@@@@A@AA>>?=@=E@>CC?>?>BC>?=:AA?=;==:;=<>?<<<>@;
@9VMIV:00290:02125
GGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCTGCAGCAAGGGCTGGGCGATATCCCCTTGCTCGCAGC
+
A?DA?C@ACCAB@@BAB?B@?C@>>?A@A@AC?@@@B@=>CCA?:A@DB@B@>B@E@=D@BB?BBBBA?@BA@AA>?<BB>D@>CA?>?>BB??=9BC?<:>><ABC?A<37654
@9VMIV:00398:00013
GGAGGAGTTGAAGTTTGGAGGAGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGCTGCAG
+
A>DA@C@@?@CC@ACBB?B@?A??=A@A@AC?@@?B@?>BCA@:A@B@>C@>B@D@>D??=<BA@B@:79578558<?>?<
@9VMIV:00571:02383
GGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCGTGCAGCAAGGGCCTGGGCGATATCCCCTTGCTCGCAGC
+
@>AA?AAACCAB@ACBC?C@?B@?>>C@C@AC?@@???<=?CA?<A@DB@A@>B@B@=B@AA@CAAA@@@?>@<5<584645B=>EB@>;<;?=>597::8:9<<198<7@?054<<
@9VMIV:00585:00910
GGAGGAGTTGAAGTTTGGAGGAGGGGAGAGTGGTACTCCCTGTCGTCAACTCCTCAGCAGAACTGATAGTTGTTGGGCTGCAGCAAGGGCTGGGCGATATCCCCTTGCTCGCCAGC
+
A?BA@B@ABCCB@AACA?C@?C@???C@B@@C?AA@B?=>CB@@:A@DB@B@;A@D@?E@BB?AAAA<>?CCABC?=<=?=E@>EB@?==4B;;;233322::1453;10.244<;
```

A single sample could have Millions of reads. My experience is that...

Panel of 50 genes→ 70,000 reads

Whole-exome seq → 30,000,000 to 70,000,000 reads

Quality assessment of raw sequencing data

Performed by **FastQC software**: easy-to-use software.
Input : FastQ files. **Output report**: html (web-like) with plots

Basic Statistics

Measure	Value
Filename	good_sequence_short.txt
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	250000
Sequences flagged as poor quality	0
Sequence length	40
%GC	45

Additional tests: over-represented contaminants (adaptor/vector), technical biases,etc.

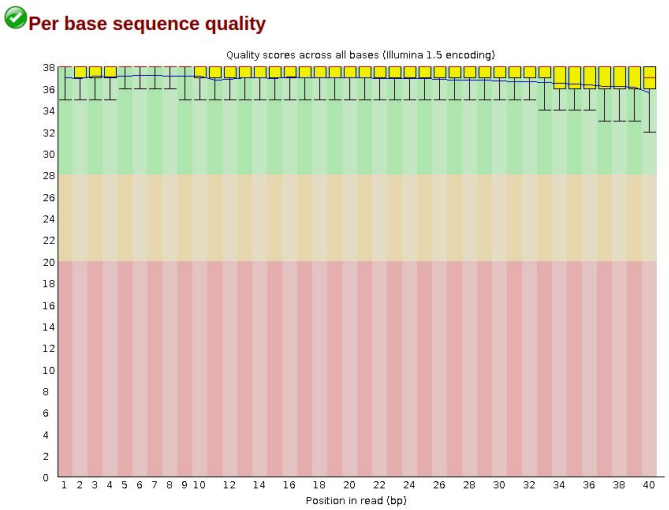
Integrated in RUbioSeq! Easy-to-use.

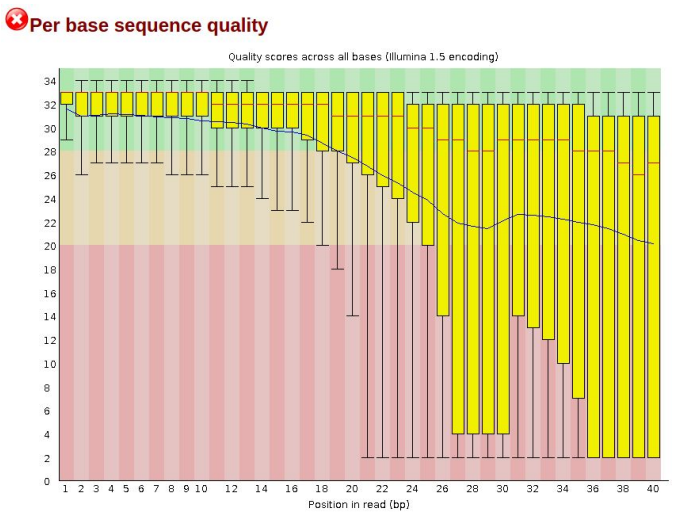
Manual: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/
Webpage: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Quality assessment of raw sequencing data

Performed by **FastQC software**: easy-to-use software.

Input : FastQ files. **Output report**: html (web-like) with plots





GOOD SAMPLE **BAD SAMPLE**

Additional tests: over-represented contaminants (adaptor/vector), technical biases,etc.

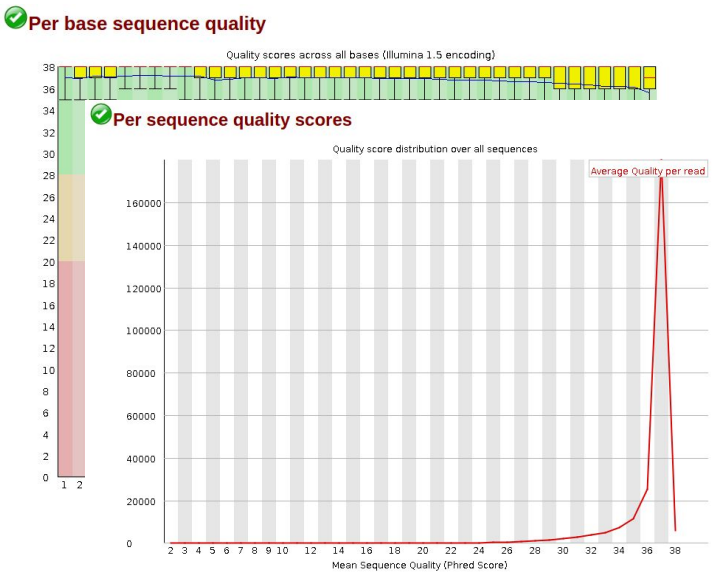
Integrated in RUbioSeq! Easy-to-use.

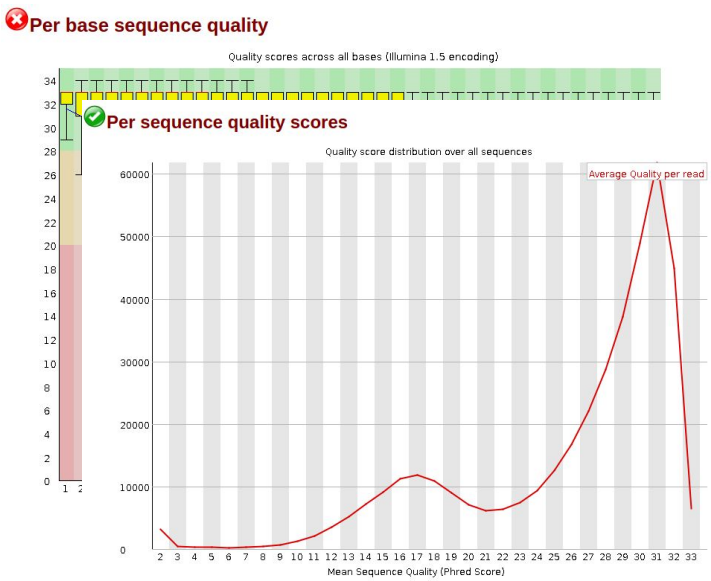
Manual: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/
Webpage: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Quality assessment of raw sequencing data

Performed by **FastQC software**: easy-to-use software.

Input : FastQ files. **Output report**: html (web-like) with plots





GOOD SAMPLE **BAD SAMPLE**

Additional tests: over-represented contaminants (adaptor/vector), technical biases,etc.

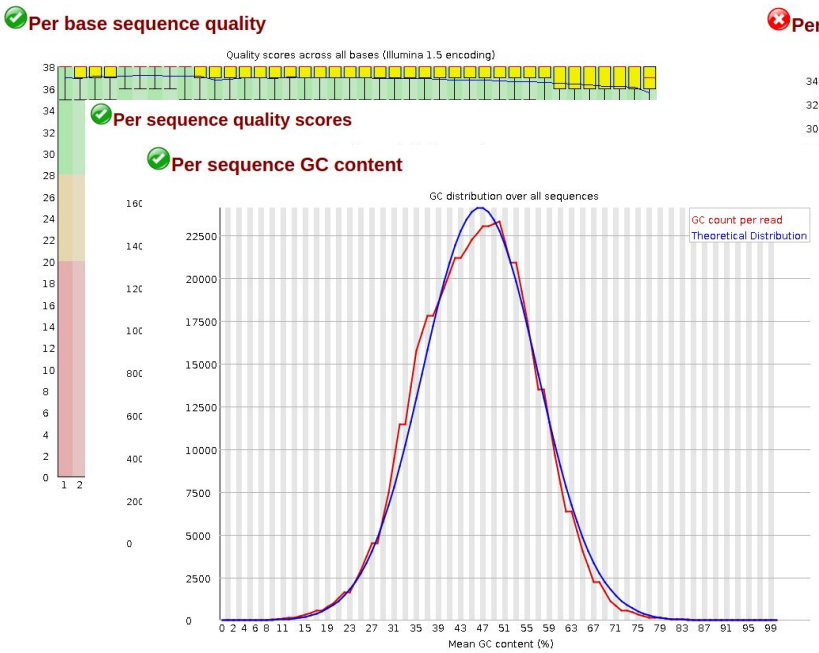
Integrated in RUbioSeq! Easy-to-use.

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

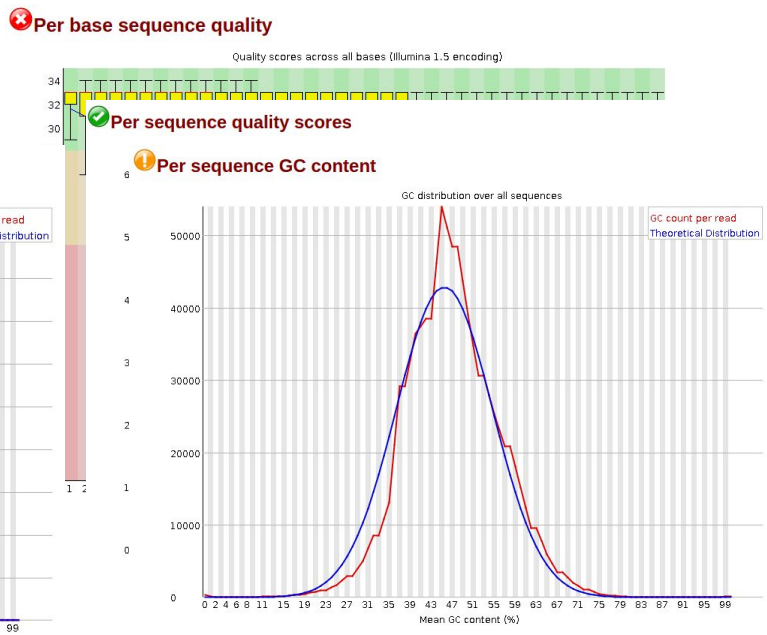
Quality assessment of raw sequencing data

Performed by **FastQC software**: easy-to-use software.

Input : FastQ files. **Output report**: html (web-like) with plots



GOOD SAMPLE



BAD SAMPLE

Additional tests: over-represented contaminants (adaptor/vector), technical biases,etc.

Integrated in RUbioSeq! Easy-to-use.

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

SAM & BAM FORMAT :: Part 1 - header

Terminal

```
jperales@machine:recal $ (samtools view -H experiment-output_tumour_recalibrated.bam; samtools view experiment-output_tumour_recalibrated.bam | head -n 1)
@HD     VN:1.4  GO:none SO:coordinate
@SQ     SN:chr1 LN:249250621
@SQ     SN:chr2 LN:243199373
@SQ     SN:chr3 LN:198022430
@SQ     SN:chr4 LN:191154276
@SQ     SN:chr5 LN:180915260
@SQ     SN:chr6 LN:171115067
@SQ     SN:chr7 LN:159138663
@SQ     SN:chr8 LN:146364022
@SQ     SN:chr9 LN:141213431
@SQ     SN:chr10        LN:135534747
@SQ     SN:chr11        LN:135006516
@SQ     SN:chr12        LN:133851895
@SQ     SN:chr13        LN:115169878
@SQ     SN:chr14        LN:107349540
@SQ     SN:chr15        LN:102531392
@SQ     SN:chr16        LN:90354753
@SQ     SN:chr17        LN:81195210
@SQ     SN:chr18        LN:78077248
@SQ     SN:chr19        LN:59128983
@SQ     SN:chr20        LN:63025520
@SQ     SN:chr21        LN:48129895
@SQ     SN:chr22        LN:51304566
@SQ     SN:chrX LN:155270560
@SQ     SN:chrY LN:59373566
@RG     ID:Undefinedtumour      PL:illumina     PU:AA   LB:experiment-output    SM:tumour
@PG     ID:GATK IndelRealigner  VN:3.1-1-g07a4bf8       CL:knownAlleles=[(RodBinding name=knownAlleles source=/local/jperales/REFERENCES/bundle_GATK/2.8/dbsnp_138.hg19.vcf), (R
odBinding name=knownAlleles2 source=/local/jperales/REFERENCES/bundle_GATK/2.8/Mills_and_1000G_gold_standard.indels.hg19.sites.vcf)] targetIntervals=/home/jperales/D1_overnight
/experiment-output/tumour/realign/experiment-output_tumour_forRealigner.intervals LODThresholdForCleaning=5.0 consensusDeterminationModel=USE_READS entropyThreshold=0.15 maxRea
dsInMemory=150000 maxIsizeForMovement=3000 maxPositionalMoveAllowed=200 maxConsensuses=30 maxReadsForConsensuses=120 maxReadsForRealignment=20000 noOriginalAlignmentTags=false
nWayOut=null generate_nWayOut_md5s=false check_early=false noPGTag=false keepPGTags=false indelsFileForDebugging=null statisticsFileForDebugging=null SNPsFileForDebugging=null
@PG     ID:bfast        VN:0.7.0b
@PG     ID:bwa  PN:bwa  VN:0.7.10-r789  CL:/home/jperales/Soft/bwa-0.7.10/bwa samse -f /home/jperales/D1_overnight/experiment-output/tumour/preProcess/experiment-output_tumour_
BWA.sam /local/jperales/REFERENCES/hg19_bwa-0.7.10_bfast-0.7.0b/hg19.fa /home/jperales/D1_overnight/experiment-output/tumour/preProcess/tumour_aln_sa.sai /home/jperales/D1_over
night/Raw_data/patientCHP_tumour.fastq
@PG     ID:GATK PrintReads      VN:3.1-1-g07a4bf8       CL:readGroup=null platform=null number=-1 sample_file=[] sample_name=[] simplify=false no_pg_tag=false
9VMIV:02395:02034       0       chr1    43814873        8       2I5M2I1M1D10M3I5M2I7M1I3M2D5M1I2M3D5M5I3M1D4M3I1M1D6M2I2M5I7M4I2M5D112M1D6M     *       0       0       CAGCAGGT
CAGAGAGCGGGGGGCCACAGTCGCCCGGGATGGCGTAGCTCCCCTTCCCGATGTTCTCAAACAACTTGTAGATGTTGTCCCCTTCGAAGGGGTCTGCATCTAGTGCTGGGCCTCAGCGCCGTCCTGGGCCTGCTGCTGCTGAGGTGGCAGTTTCCTGCACACTACAGGTACCGCCC
CCGCCAGGCAGGAGACTGGCGGTGGACAGGTG        =B==>=<====?=>><8=<=<9==A>@=>?8=<<8<==>@=>8=<==>==<8=:<<;8=?A==========@>>==A>;<>>@=;>43::4:563<9<=<9<>=@=@>=><==?<=@<=;;==@><8<97<<<<>;7
<7<>;=><<<:;<342132110:<2015::=<><;<=<::95;0378811586;:58>=558<;>87,7468.+.6856        XA:i:3  MD:Z:2T3^T3G7T1C11^GC2A4^TGA6T1^G5^T1G1C6T6^TGACC1C110^C6      PG:Z:bfast      R
G:Z:Undefinedtumour     IH:i:1  NH:i:3  HI:i:1  NM:i:54 AS:i:2850
```

Header (start with @) meta-info.

Contigs (chr names) & lengths

Post-alignment processing program
Aligners (versions)

PL : Platform

SM : Sample name

Alignment of a single read

[...] the rest of reads.

- SAM format specs: http://samtools.github.io/hts-specs/SAMv1.pdf
- SAM flags: http://broadinstitute.github.io/picard/explain-flags.html

SAM & BAM FORMAT :: Part 2 - alignment

#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
ReadName	99	chr10	2	30	3MD2M1I1M	=	14	20	CATCTAG	jjjjjjj	z:Aligner

```
RefPos:      1 2 3 4 5 6 7   8 9
Reference:   C C A T A C T - G A
Read:          C A T - C T A G

POS: 2
CIGAR:               3M1D2M1I1M
```

CIGAR

Op	Description
M	alignment match (can be a sequence match or mismatch)
I	insertion to the reference
D	deletion from the reference
N	skipped region from the reference
S	soft clipping (clipped sequences present in SEQ)
H	hard clipping (clipped sequences NOT present in SEQ)
P	padding (silent deletion from padded reference)
=	sequence match
X	sequence mismatch

FIELDS

#Col	Field	Description
1.	QNAME	read name
2.	FLAG	bitwise FLAG* (unmapped, pair unmapped, properly mapped, …)
3.	RNAME	Reference sequence name (e.g. chr1).
4.	POS	1-based leftmost position.
5.	MAPQ	Mapping Quality (Phred-scaled). Scale 0 to 255.
6.	CIGAR	extended CIGAR string.
7.	MRNM	Paired-end: Mate Reference sequence Name (= if same as RNAME).
8.	MPOS	Paired-end: 1-based Mate position.
9.	TLEN	Paired-end: Insert size
10.	SEQ	Read sequence
11.	QUAL	Base Quality Score from the Read sequence.
12.	OPT	Optional Tags.

- SAM format specs: http://samtools.github.io/hts-specs/SAMv1.pdf
- SAM flags: http://broadinstitute.github.io/picard/explain-flags.html

BED FORMAT

```
track name="CHP2_Designed" description="Amplicon_Insert_CHP2" type=bedDetail ionVersion=4.0
chr1   43814968    43815086    CHP2_MPL_1    .   GENE_ID=MPL
chr1   115252185   115252269   CHP2_NRAS_3   .   GENE_ID=NRAS
chr1   115256504   115256584   CHP2_NRAS_2   .   GENE_ID=NRAS
chr1   115258689   115258774   CHP2_NRAS_1   .   GENE_ID=NRAS
chr2   29432572    29432680    CHP2_ALK_2    .   GENE_ID=ALK
chr2   29443607    29443729    CHP2_ALK_1    .   GENE_ID=ALK
chr2   209113103   209113206   CHP2_IDH1_1   .   GENE_ID=IDH1
chr2   212288904   212288990   CHP2_ERBB4_8  .   GENE_ID=ERBB4
chr2   212530051   212530180   CHP2_ERBB4_7  .   GENE_ID=ERBB4
```

The first three are required BED fields, the rest optional.

1. **chrom** - The name of the chromosome (e.g. chr3, chrY, chr2).
2. **chromStart** - The starting position of the feature in the chromosome. The first base in a chromosome is **numbered 0**.
3. **chromEnd** - The ending position of the feature in the chromosome or scaffold.

Adittionally, 9 optional fields:

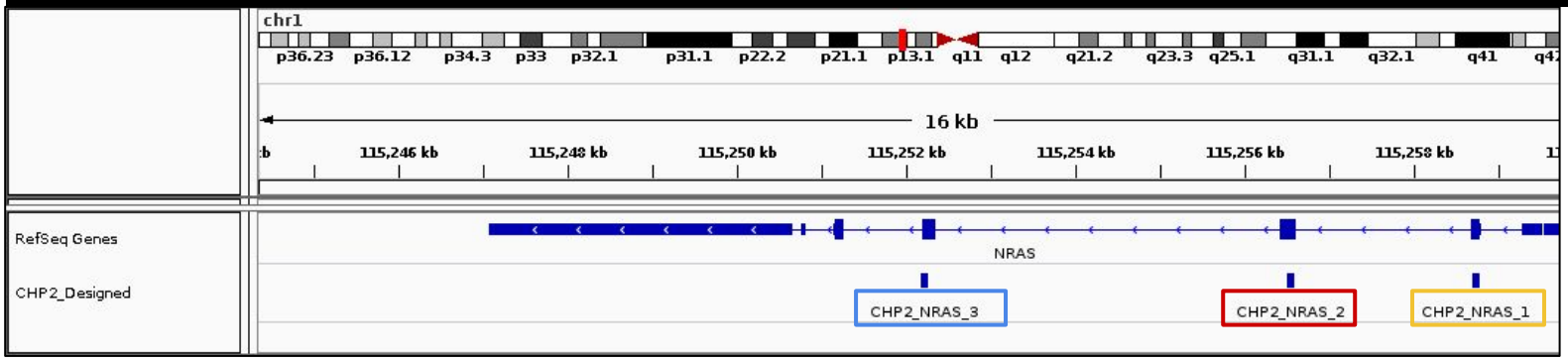
4. **name** - Defines the name of the BED line.
5. Score (. or a number between 0 and 1000).
6. strand (+ forward, - reverse)
7. thickStart
8. thickEnd
9. itemRgb (255,0,0).
10. blockCount; 11. blockSizes; 12. blockStarts

The BED format is 0-based.

Official specs: http://genome.ucsc.edu/FAQ/FAQformat#format1
http://bedtools.readthedocs.org/en/latest/content/general-usage.html

BED FORMAT

```
track name="CHP2_Designed" description="Amplicon_Insert_CHP2" type=bedDetail ionVersion=4.0
chr1    43814968    43815086    CHP2_MPL_1     .    GENE_ID=MPL
chr1    115252185   115252269   CHP2_NRAS_3    .    GENE_ID=NRAS
chr1    115256504   115256584   CHP2_NRAS_2    .    GENE_ID=NRAS
chr1    115258689   115258774   CHP2_NRAS_1    .    GENE_ID=NRAS
chr2    29432572    29432680    CHP2_ALK_2     .    GENE_ID=ALK
chr2    29443607    29443729    CHP2_ALK_1     .    GENE_ID=ALK
chr2    209113103   209113206   CHP2_IDH1_1    .    GENE_ID=IDH1
chr2    212288904   212288990   CHP2_ERBB4_8   .    GENE_ID=ERBB4
chr2    212530051   212530180   CHP2_ERBB4_7   .    GENE_ID=ERBB4
```



WARNING: You must know what library designed was used to sequence your data, each library has particular regions to be sequenced. You will call variants along these regions.

Examples: Agilent SureSelect v5 Human all Exon, IonTorrent comprehensive cancer panel, etc

VCF format

Header

```
##fileformat=VCFv4.2
##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">
```

single Variant calls

#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

Genotype variables ↔ Individual Samples

valid?

Genotype specifications

- VCF records are oriented to provide details of single variant calls.
- Not all records in a VCF are true calls, the **`FILTER`** column specifies those which passed the calling.
- **`QUAL`** is the score assigned to a given call. The greater QUAL is, the more reliable is. It is in log-scale.
- **`ID`** is an identifier. E.g. a dbSNP id.

A PDF with the v4.2 specifications: http://samtools.github.io/hts-specs/VCFv4.2.pdf

What is a VCF and how to interpret it : https://software.broadinstitute.org/gatk/guide/article?id=1268

TSV

Tab-separated Text (tabular structure).

```
Column1    Column2    Column3    Column4    ...    ColumnN
var_r1c1   var_r1c2   var_r1c3   var_r1c4   ...    var_r1cN
var_r2c1   var_r2c2   var_r2c3   var_r2c4   ...    var_r2cN
var_r3c1   var_r3c2   var_r3c3   var_r3c4   ...    var_r3cN
var_r4c1   var_r4c2   var_r4c3   var_r4c4   ...    var_r4cN
...        ...        ...        ...        ...    ...
var_rNc1   var_rNc2   var_rNc3   var_rNc4   ...    var_rNcN
```

Excel

No format specifications.
There are no limits in terms of the matrix dimension (rows X columns).
However, there are good practices in Bioinformatics:

1. Use intuitive column names without spaces or rare chars. Instead of those, use "." or "_".
2. Data dimension: higher number of rows than columns.
3. Rows → individual observations or records. E.g. genomic positions.
4. Columns→ Individual variables for each individual observation. E.g. Chromosome, position, Score, Gene mutated, etc.
5. Do NOT mix data from different observations

Tidy data principles:
http://vita.had.co.nz/papers/tidy-data.pdf