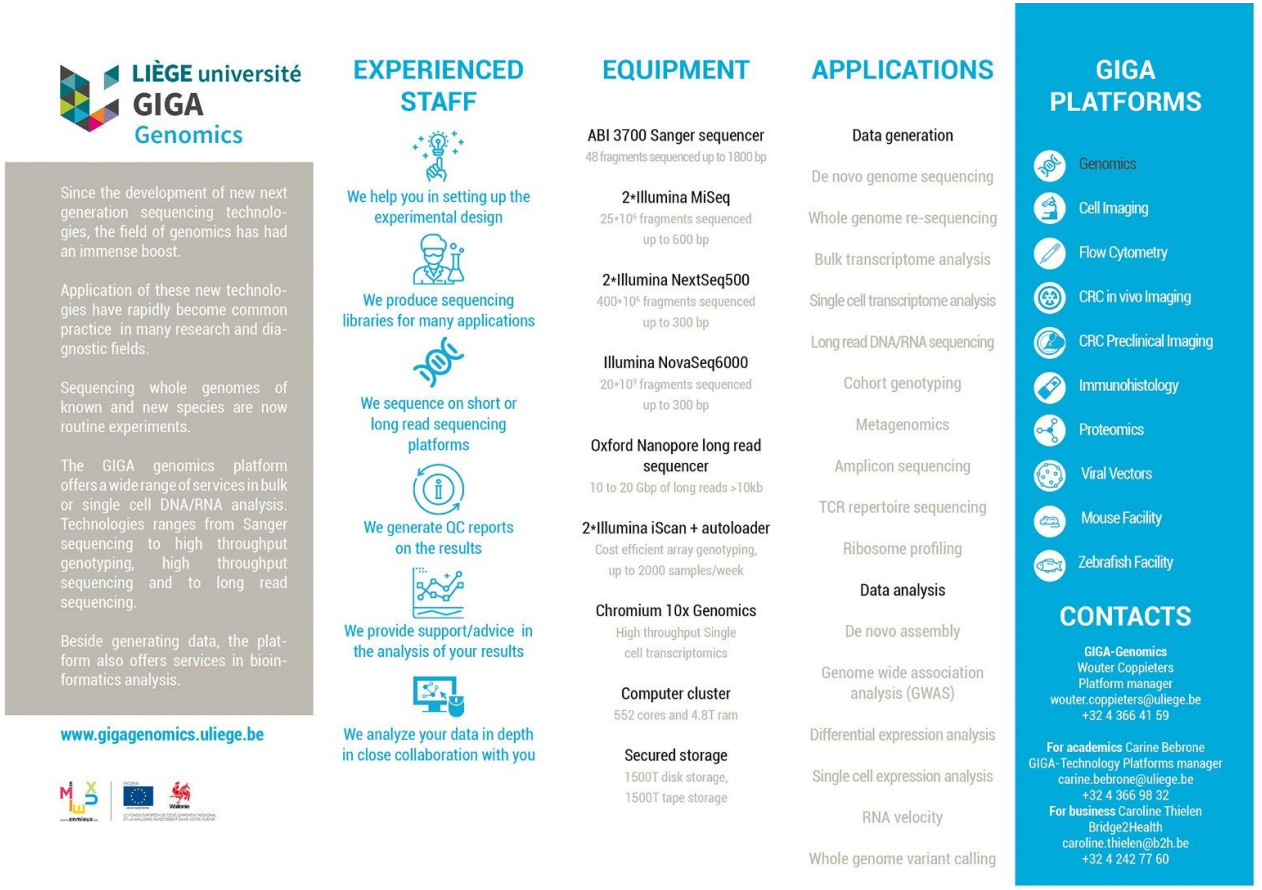
DATA PROCESSING IN GENOMICS: FROM THE SEQUENCERS TO THE RESULTS

GIGA Doctoral School for Health Sciences
Introduction to scientific computing

ARNAUD LAVERGNE
GIGA-Genomics
Bioinformatic team

GIGA - GENOMICS

- Platform
  - Sequencing services
    - Biological materials
    - DNA/RNA
    - Libraries
    - Sequencers
    - Data
  - High Throughput Sequencing (HTS) / Next Generation Sequencing (NGS)
  - Bioinformatic team
    - Data analysis
    - And more ...



SEQUENCERS

MiSeq
540 Mb -15 Gb
4 – 56 hours

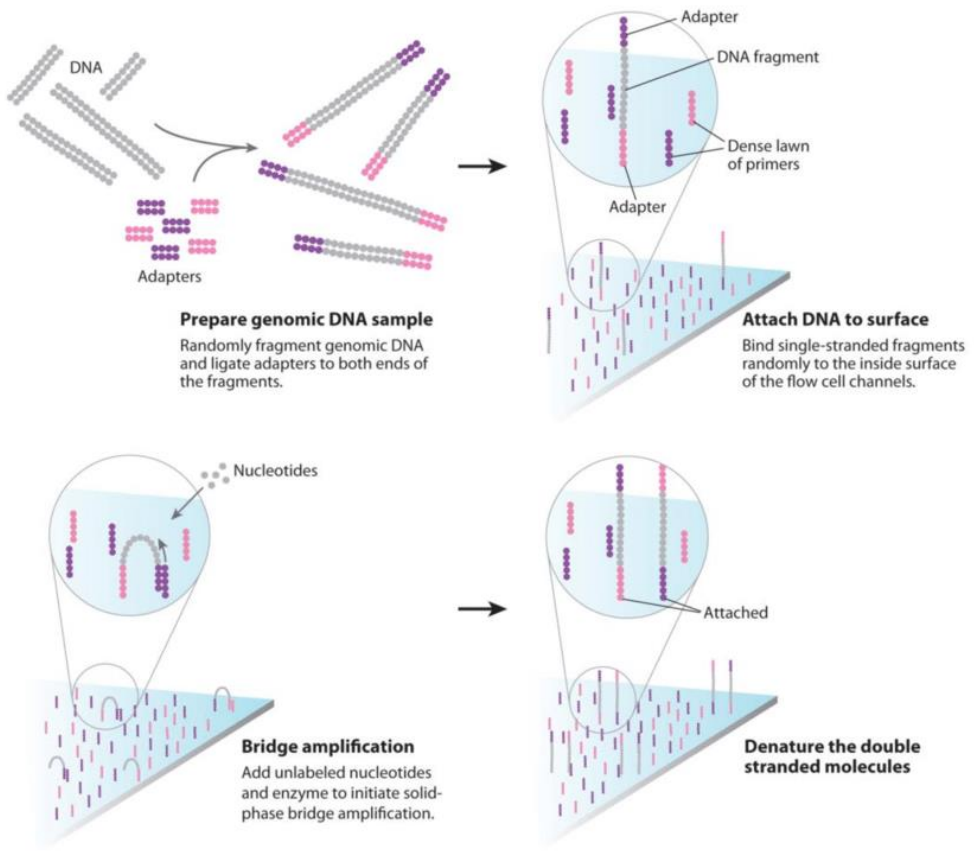
HiSeq
105 Gb - 1,5 Tb
1 – 3,5 days

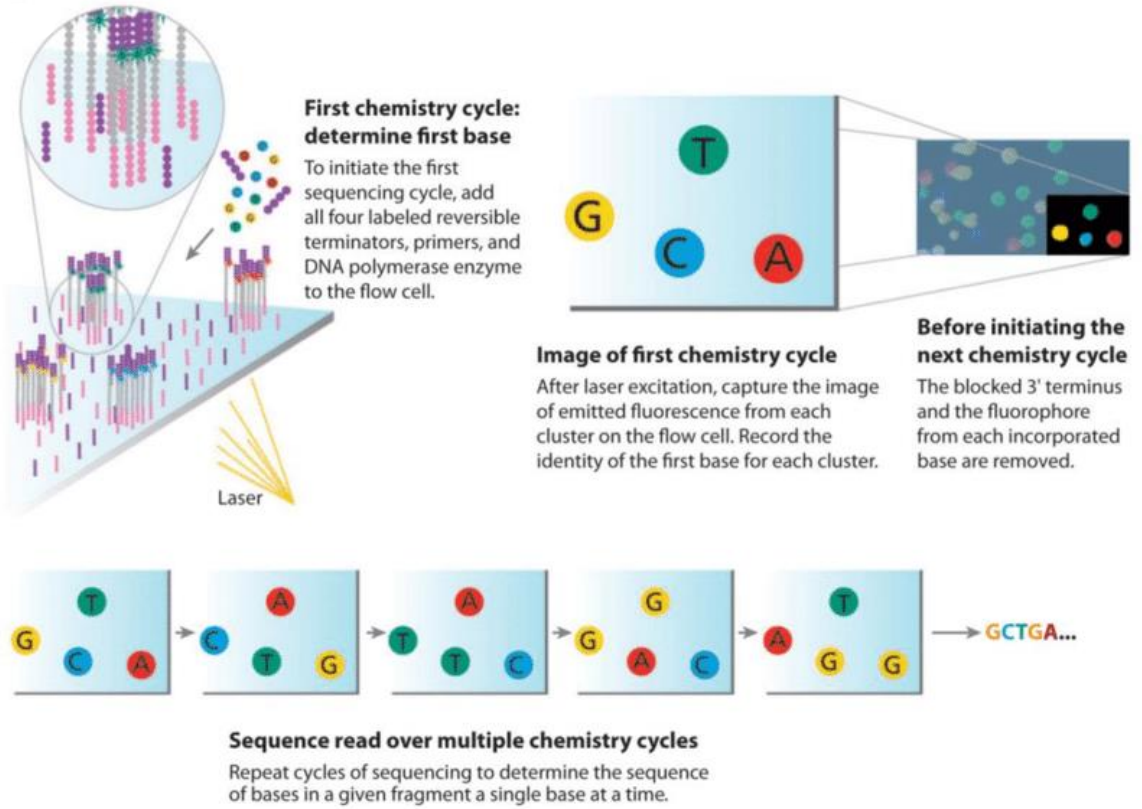
NextSeq
16,25 Gb - 120 Gb
11 – 29 hours

NovaSeq
65 Gb – 3 Tb
13 – 44 hours

Adapted from Illumina

SEQUENCING (HTS/NGS)

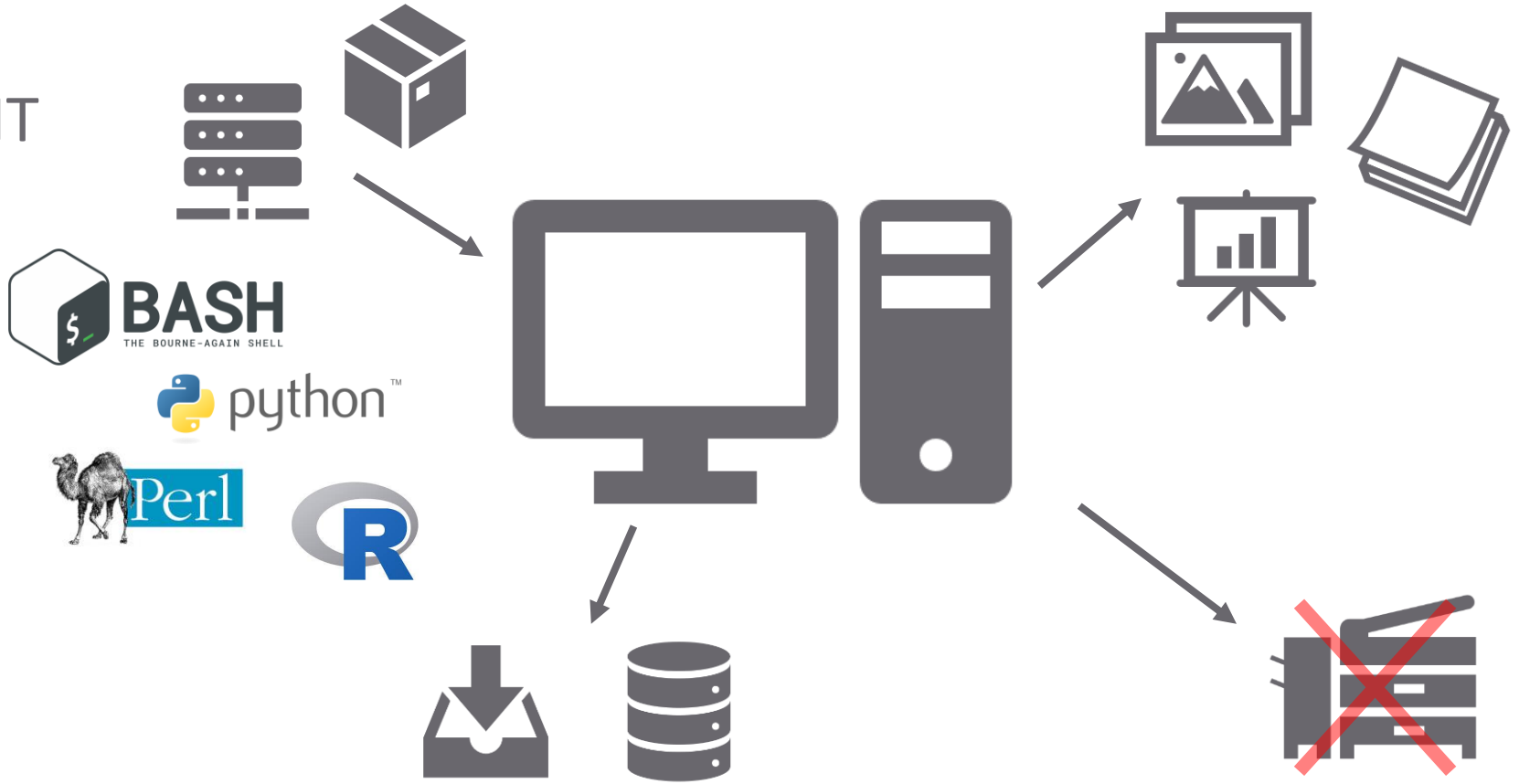




Adapted from Illumina

COMPUTING

- DATA MANAGEMENT
  - DATA PROCESSING
  - STORAGE
  - RESULTS
- REPRODUCTIBILITY
  - PIPELINES
  - CONTAINERS



DATA MANAGEMENT

LAPTOP
250Gb – 1Tb
4-8 Go RAM
4-8 CPUs

LAB COMPUTER
1 - 5 Tb
32 - 128 Go RAM
8-16 CPUs

CLUSTER/STORAGE
1.5 Pb
256 Go RAM
32 CPUs

DATA MANAGEMENT

August 28, 2020

- 1.2 Tb
- 12.8 Billions of reads
- 612 samples
  - Multiple experiments
  - Unique combinations of indexes

- « Run »
- No storage on device

DATA PROCESSING

- DEMULTIPLEXING
  - Reads → Samples
  - ~ 20M / sample
  - « Fastq »
    - Identifier
    - Sequence
    - Separator
    - Quality score
      - Ascii +33
  - + 1.2 Tb (2.4 Tb)

```
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CTTCTGGAGAGGAGTTCCTCTGATATGAATTAAGGTTTTCCCTTCTGTCATGACCAGAAGAAGGTTTTATCTGTGCCACACTACTTTTCATTTTCTGTTGCCAGTTGGTCCAATA
AATCAAAGATGNTTCAAACTGGTCCAAATAACAAGT
+
FFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF,FFFFFFFFFFFFFFF#FFFFFFFFFFFFFFFF
FFFFFFFF
@A00801:49:H2VGKDSXY:2:1101:2022:1016 1:N:0:NGCTTAAG+TCGTGACC
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TCAAATAGTAGNTTTTTAAAAAATTAAAGACATGTC
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TTCAGCCTGGAANGCAGATACTCTCAATAATATTAAT
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFF:FFFFFFF:FFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFF:FFF,FFFFFFFFFFFF:FFF#FFFFF:FFFFFFFFFFFF
FFFFFF
```

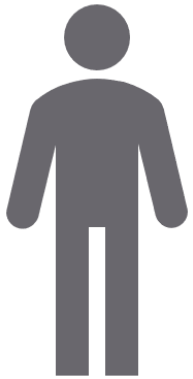
Symbol	ASCII Code	Q-Score	Symbol	ASCII Code	Q-Score
!	33	0	6	54	21
"	34	1	7	55	22
#	35	2	8	56	23
$	36	3	9	57	24
%	37	4	:	58	25
&	38	5	;	59	26
'	39	6	<	60	27
(	40	7	=	61	28
)	41	8	>	62	29
*	42	9	?	63	30
+	43	10	@	64	31
,	44	11	A	65	32
-	45	12	B	66	33
.	46	13	C	67	34
/	47	14	D	68	35
0	48	15	E	69	36
1	49	16	**F**	**70**	**37**
2	50	17	G	71	38
3	51	18	H	72	39
4	52	19	I	73	40
5	53	20			

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90,0000%
20	1 in 100	99,0000%
30	1 in 1000	99,9000%
40	1 in 10,000	99,9900%
50	1 in 100,000	99,9990%
60	1 in 1,000,000	99,9999%

RESEARCHERS

RESEARCHERS

Dr GIGA
52 Gb



- 12 samples
- Fastq
- 52 Gb
- RNA-Seq

Transcriptomics

« Gene Expression »

WORKFLOW

- QC Sequencing
- Mapping
- Quantification
- QC Mapping/Quantification

- Downstream Analysis
  - Clustering
  - Differential Expression

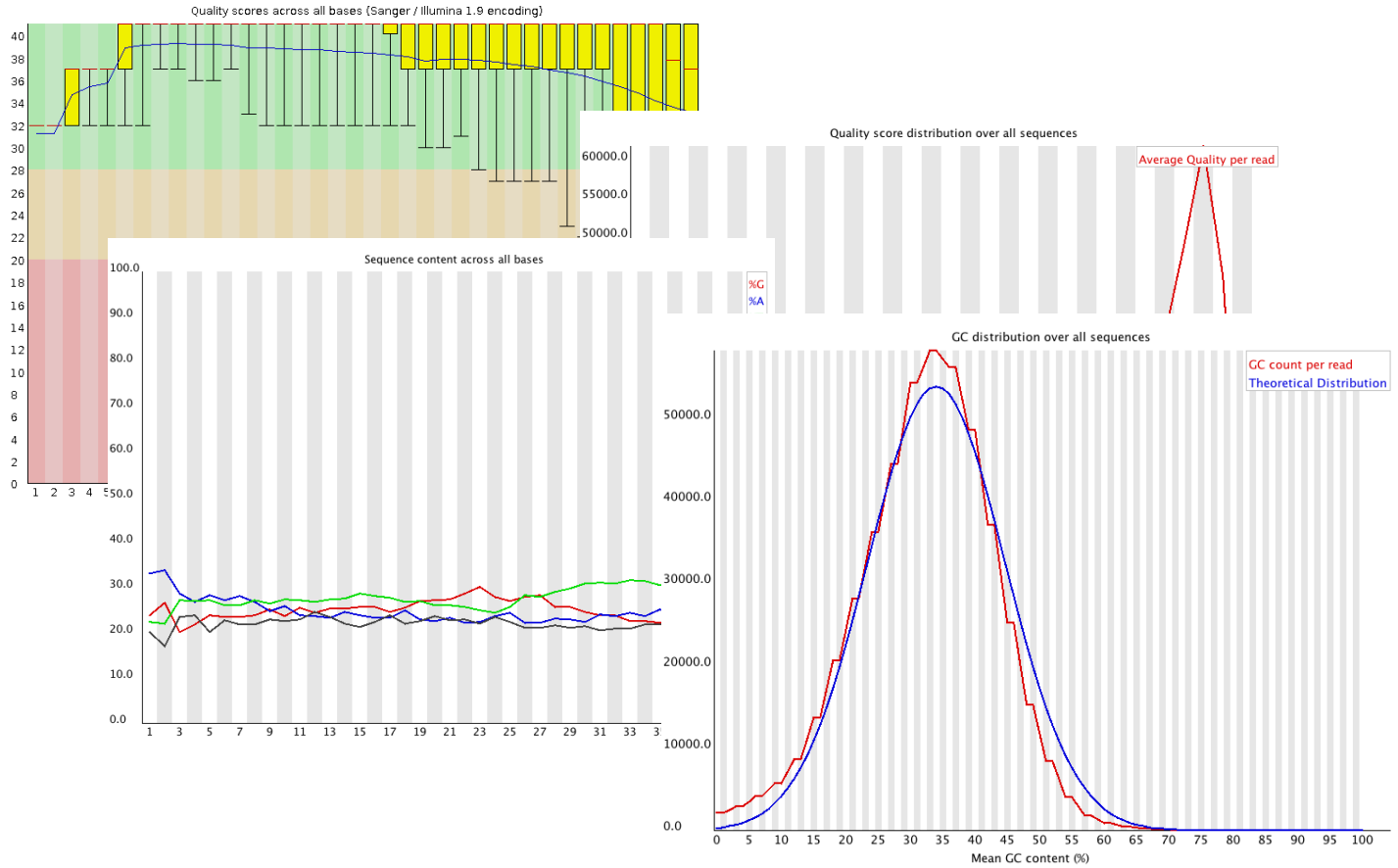
QC SEQUENCING

Dr GIGA
52 Gb

FastQC

- Number of reads
- Base calling quality
- Sequece quality
- GC content
- Sequence length
- Duplication levels
- Adapter content
- Overrepresented sequences
- …

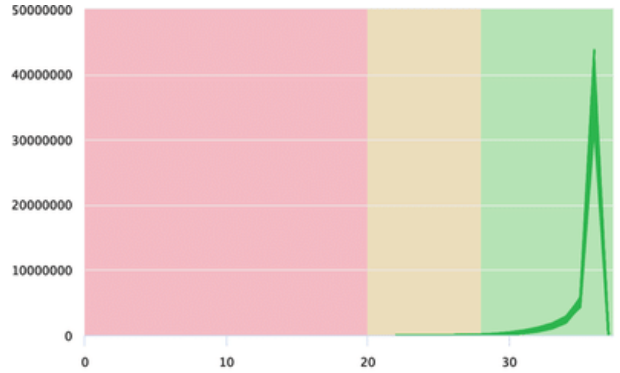
< 1 Mb

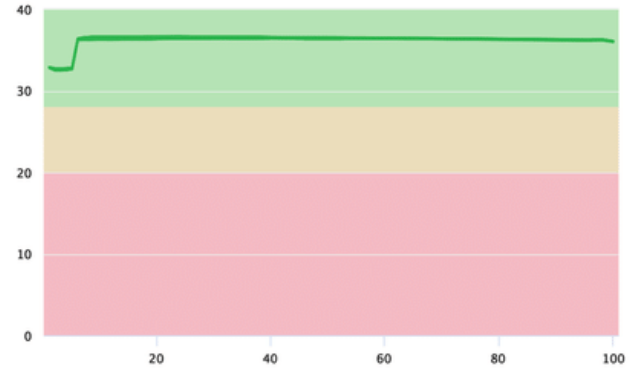


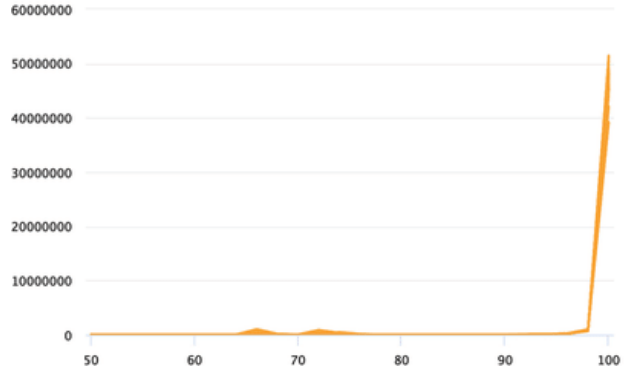
QC SEQUENCING

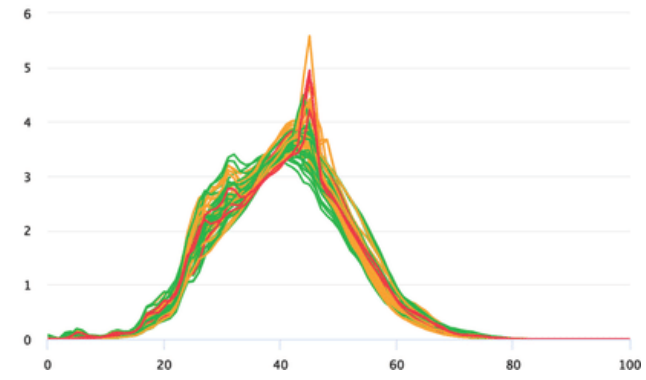
Dr GIGA
52 Gb

MultiQC







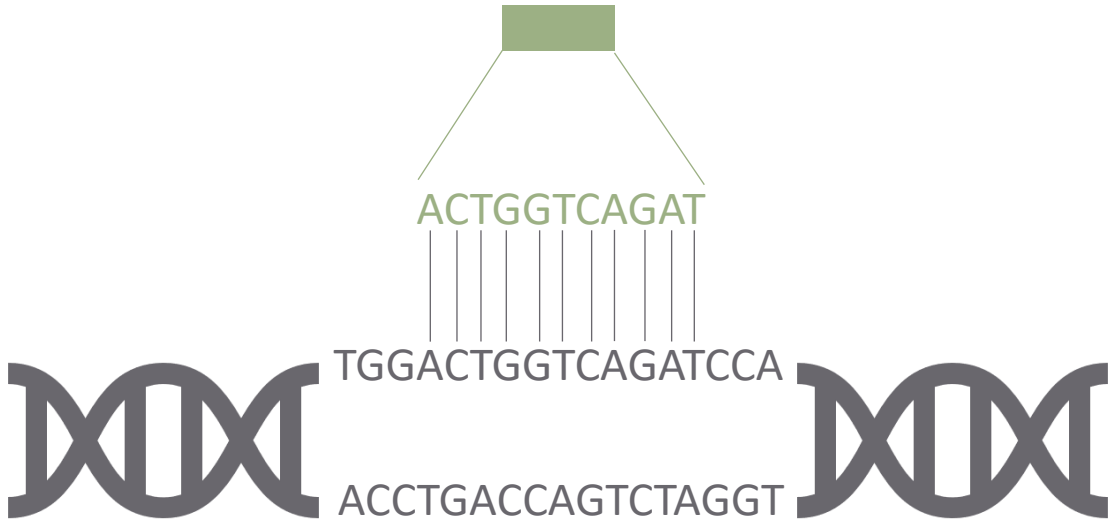


MAPPING

- Alignment
- Origin of reads

- Reference
  - Genome sequence
  - Gene set

- Database
  - Ensembl, UCSC, …



REFERENCE

Genome (FASTA)

>1 dna:chromosome chromosome:GRCh38:1:1:248956422:1 REF
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Gene Set (GTF)

#!genome-build GRCh38.p12
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#!genebuild-last-updated 2019-03
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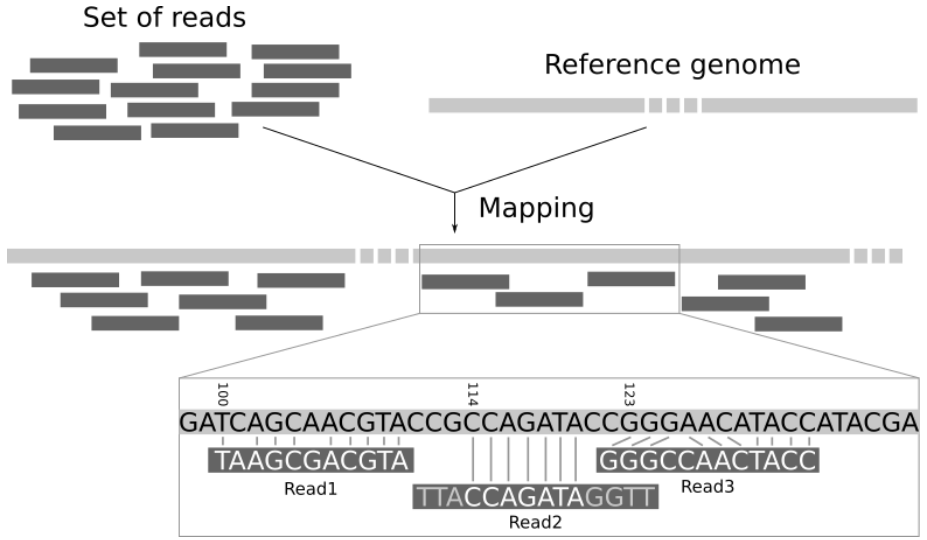
MAPPING

Dr GIGA
56 Gb

- Homo Sapiens
  - Genome (3.2 Gb)
  - Gene Set (1.2 Gb)
- Softwares
  - STAR
  - HISAT
  - …
- High RAM/CPUs



MAPPING

Dr GIGA
86 Gb

- Genome Indexing
  - Quick queries
  - 20M reads

- High RAM/CPUs

- H.Sapiens ~30 Gb

MAPPING

Dr GIGA
118 Gb

- SAM/BAM files (~ 32 Gb)
  - FLAG - Information
  - RNAME - Chromosome
  - POS – Location of 1st base
  - MAPQ – Quality score
  - CIGAR - Operations

Flag	Description
1	read is mapped
2	read is mapped as part of a pair
4	read is unmapped
8	mate is unmapped
16	read reverse strand
32	mate reverse strand
64	first in pair
128	second in pair
256	not primary alignment
512	read fails platform/vendor quality checks
1024	read is PCR or optical duplicate

Paired-End

A00801:76:HGJCYDSXY:4:1544:20401:36699 **99** **1** **3112677** **255** **150M** **=** **3112770** **244**

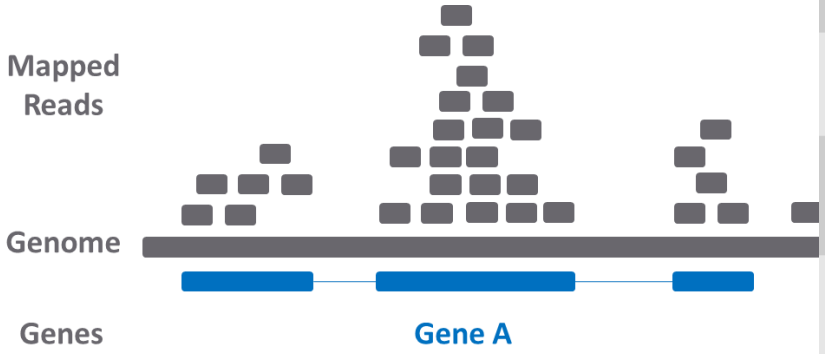
CTAGGAGATAGTAGGGATTGGGAAGCAACTACTGAAAGGTCTGTGTCTTCTTTGTGGATGATAAAATATTCTGGAATTATATTGTATGCTAGGCGCACAATCTTGTGACCATAGTACAGATATTCAACAGATAAATTTTGTGTGCTATGA

F:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFF:FFFFF

NH:i:1 HI:i:1 AS:i:299 nM:i:0 RG:Z:SV2-CTRL2_NGS20-O393_AHGJCYDSXY_S241_L004_R1_001

QUANTIFICATION (RNA)

- Gene Expression



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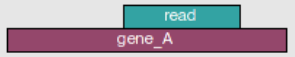
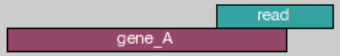
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
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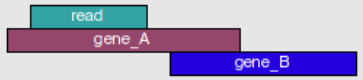
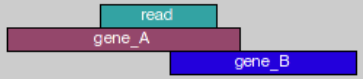
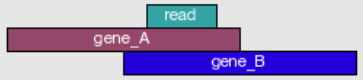
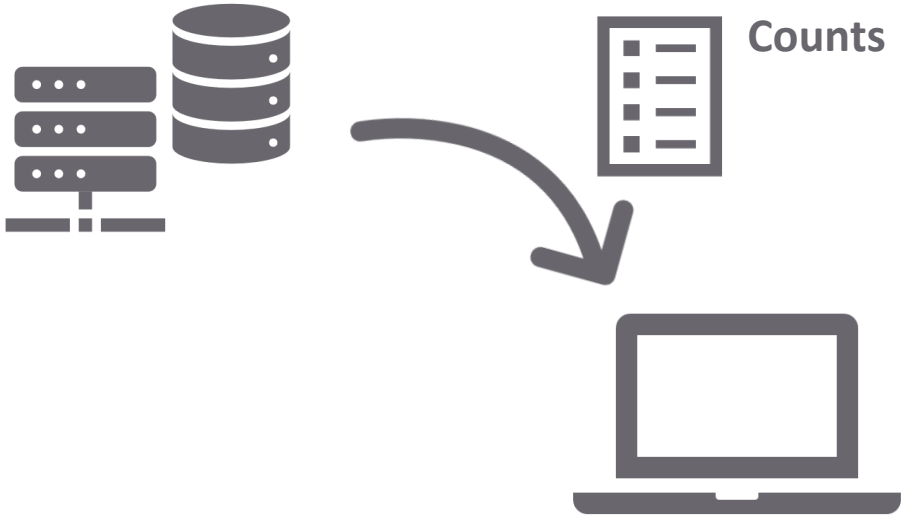
	gene_A	gene_A	gene_A
	ambiguous	gene_A	gene_A
	ambiguous	ambiguous	ambiguous

QUANTIFICATION (RNA)

Dr GIGA
118 Gb

- Gene Expression
- « Count matrix » (3.2 Mb)
- Major output



Each column is a sample

Each row is a gene

GENE ID	KD.2	KD.3	OE.1	OE.2	OE.3	IR.1	IR.2	IR.3
1/2-SBSRNA4	57	41	64	55	38	45	31	39
A1BG	71	40	100	81	41	77	58	40
A1BG-AS1	256	177	220	189	107	213	172	126
A1CF	0	1	1	0	0	0	0	0
A2LD1	146	81	138	125	52	91	80	50
A2M	10	9	2	5	2	9	8	4
A2ML1	3	2	6	5	2	2	1	0
A2MP1	0	0	2	1	3	0	2	1
A4GALT	56	37	107	118	65	49	52	37
A4GNT	0	0	0	0	1	0	0	0
AA06	0	0	0	0	0	0	0	0
AAA1	0	0	1	0	0	0	0	0
AAAS	2288	1363	1753	1727	835	1672	1389	1121
AACS	1586	923	951	967	484	938	771	635
AACSP1	1	1	3	0	1	1	1	3
AADAC	0	0	0	0	0	0	0	0
AADACL2	0	0	0	0	0	0	0	0
AADACL3	0	0	0	0	0	0	0	0
AADACL4	0	0	1	1	0	0	0	0
AADAT	856	539	593	576	359	567	521	416
AAGAB	4648	2550	2648	2356	1481	3265	2790	2118
AAK1	2310	1384	1869	1602	980	1675	1614	1108
AAMP	5198	3081	3179	3137	1721	4061	3304	2623
AANAT	7	7	12	12	4	6	2	7
AARS	5570	3323	4782	4580	2473	3953	3339	2666

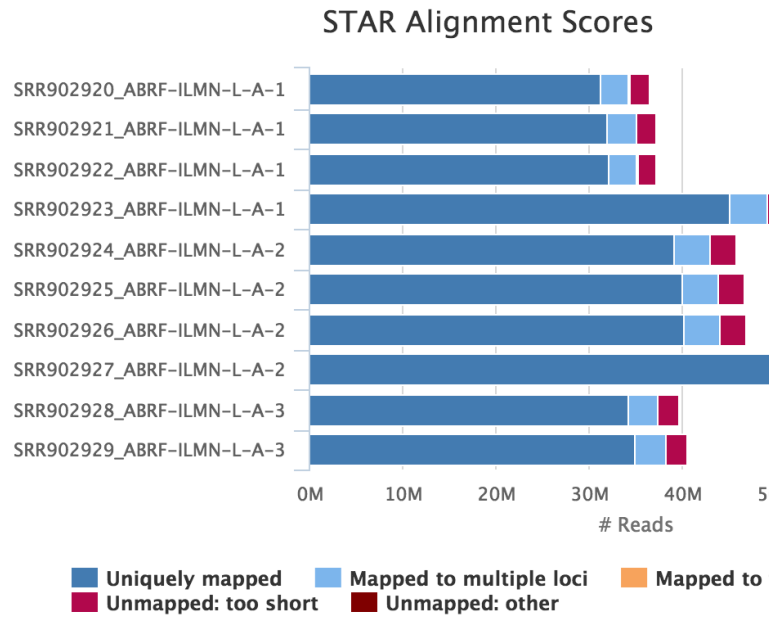
QC MAPPING/QUANTIFICATION

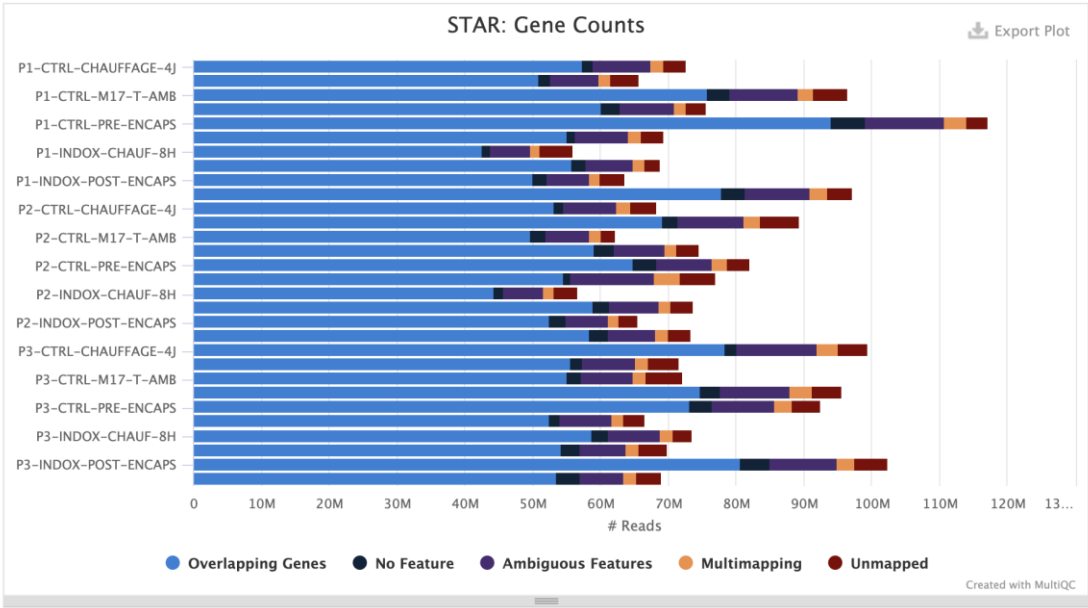
MultiQC

General Statistics

Copy table | Configure Columns | Plot | Showing 8/8 rows and 8/10 columns.

Sample Name	% Assigned	M Assigned	% Aligned	M Aligned	% Trimmed	% Dups	% GC	M Seqs
			%	97.8	4.0%	78.9%	51%	104.4
			%	87.1	3.5%	77.2%	49%	92.0

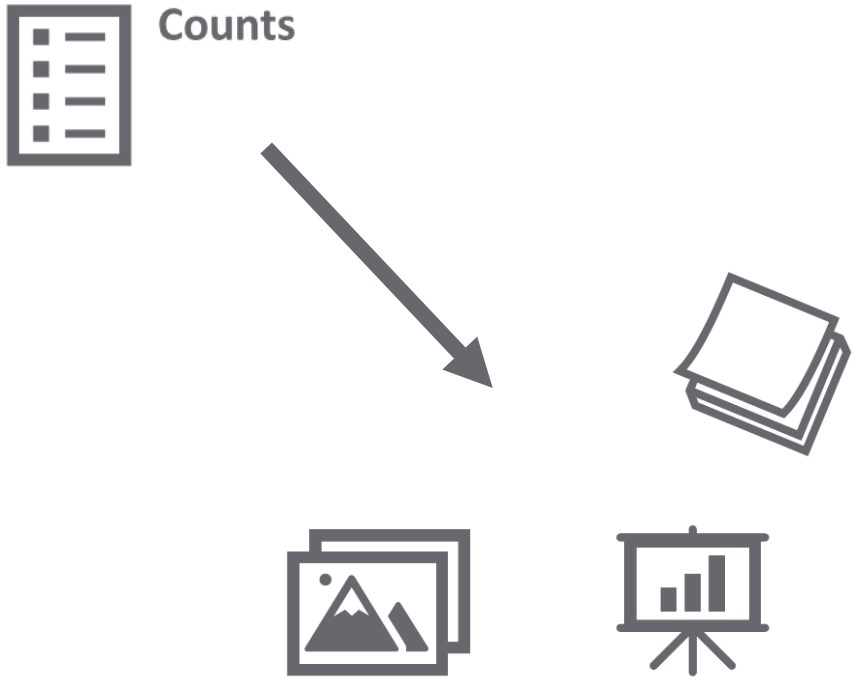




DOWNSTREAM ANALYSIS

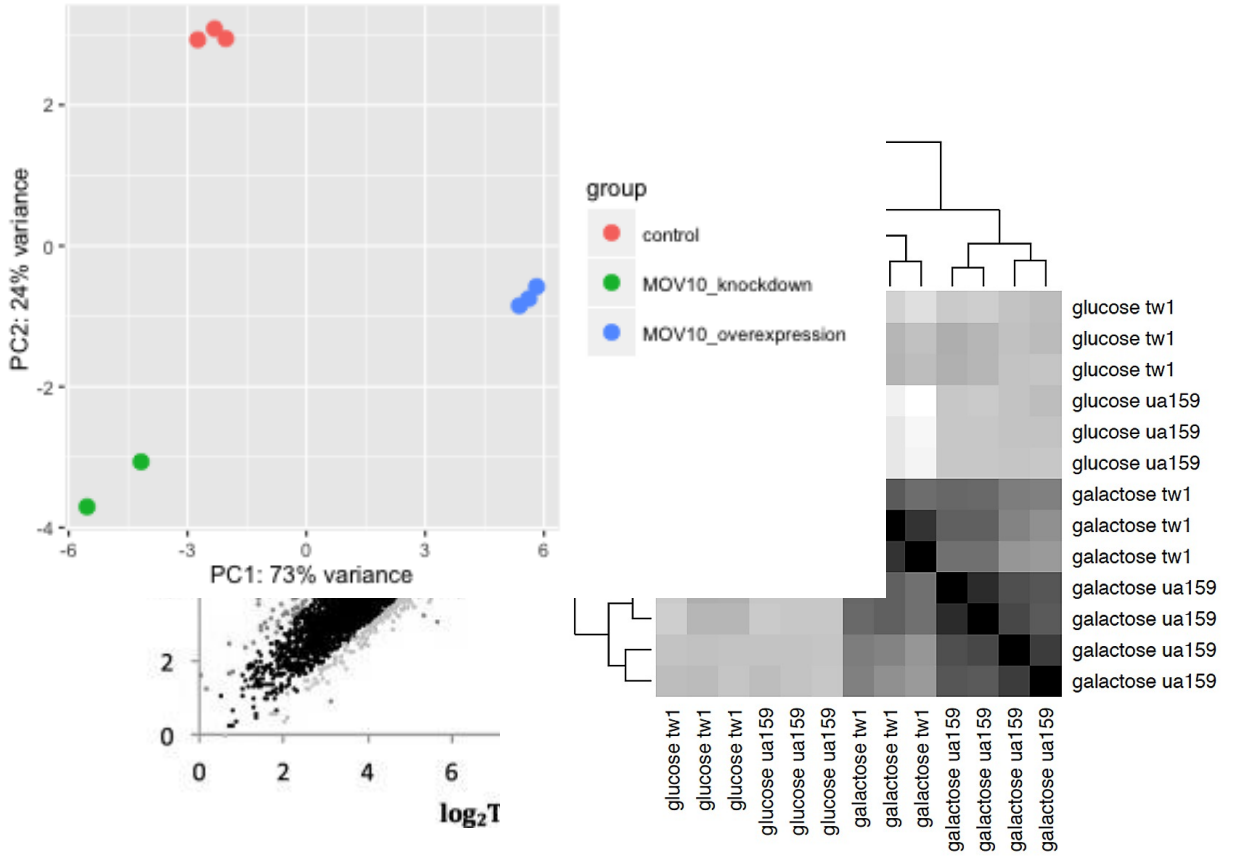
- Experimental Design

- R
  - DESeq2
  - EdgeR
  - Voom
  - ROTS
  - Limma
  - …



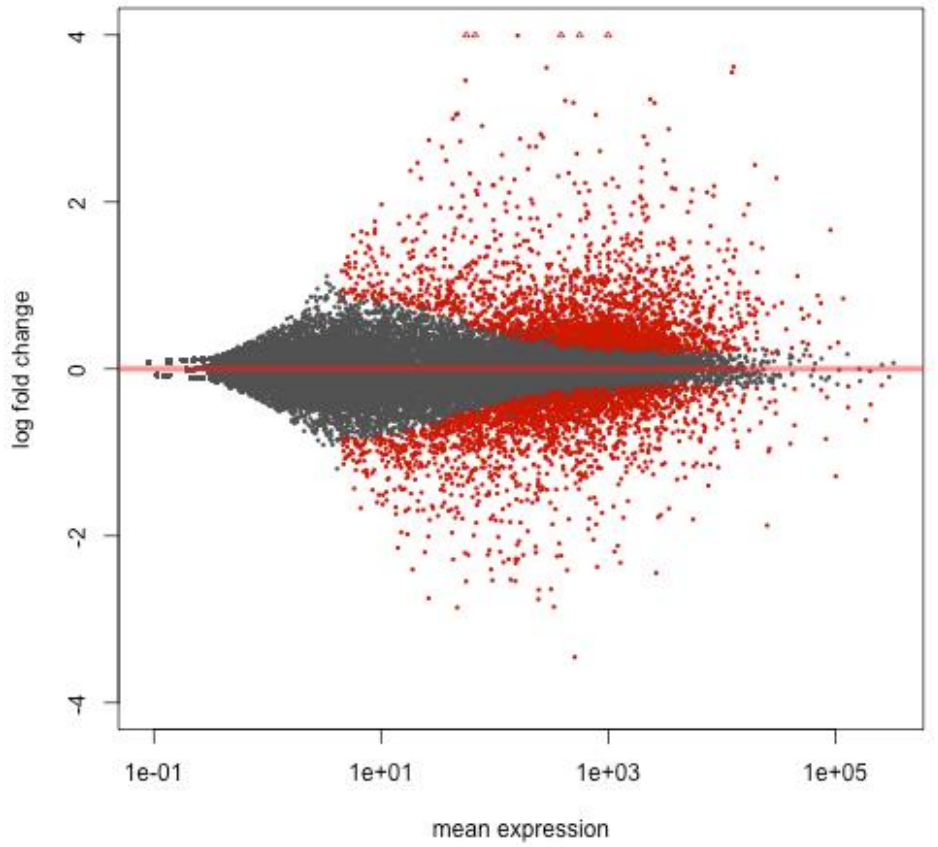
DOWNSTREAM ANALYSIS

- Experimental Design
- Clustering
  - Sample correlation
  - Euclidian distance
  - Principal Component Analysis



DOWNSTREAM ANALYSIS

- Experimental Design
- Clustering
  - Sample correlation
  - Euclidian distance
  - Principal Component Analysis
- Differential Expression Analysis
  - Pairwise comparisons
  - MA plot



DOWNSTREAM ANALYSIS

- Experimental Design
- Clustering
  - Sample correlation
  - Euclidian distance
  - Principal Component Analysis
- Differential Expression Analysis
  - Pairwise comparisons
  - MA plot
  - DE genes

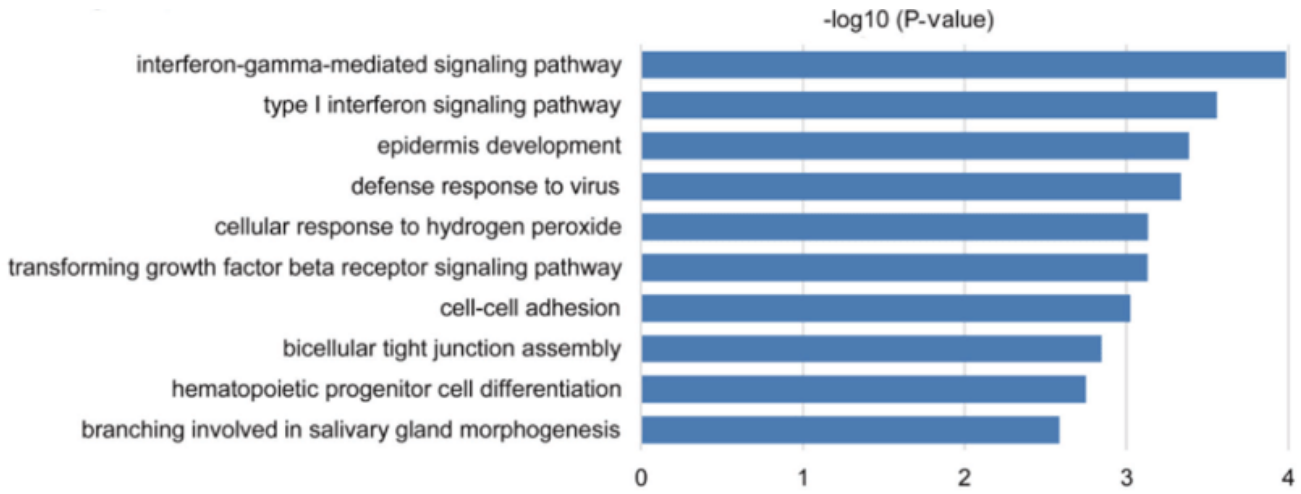
	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
PAX5	1531,91362	6,1280938	0.14505696	28,091789	1,23E-173	1,59E-169
SOX9	348,04912	3,5537120	0.15748166	20,475861	3,53E-93	2,27E-89
PDX1	830,75570	-1,8973788	0.12438094	-15,203018	3,38E-52	1,45E-48
ISL1	655,25202	-1,9729198	0.13344796	-14,715372	5,14E-49	1,65E-45
ARX	526,74210	2,2554297	0.15754888	14,227414	6,19E-46	1,59E-42

Summary

out of 21769 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up) : 985, 4.5%
LFC < 0 (down) : 929, 4.3%
outliers [1] : 0, 0%
low counts [2] : 8914, 41%
(mean count < 8)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results

DOWNSTREAM ANALYSIS

- Biological meaning
- Gene ontology / Gene Set Enrichment Analysis
  - GSEA
  - Enrichr
  - GOrilla
  - PANTHER
  - …



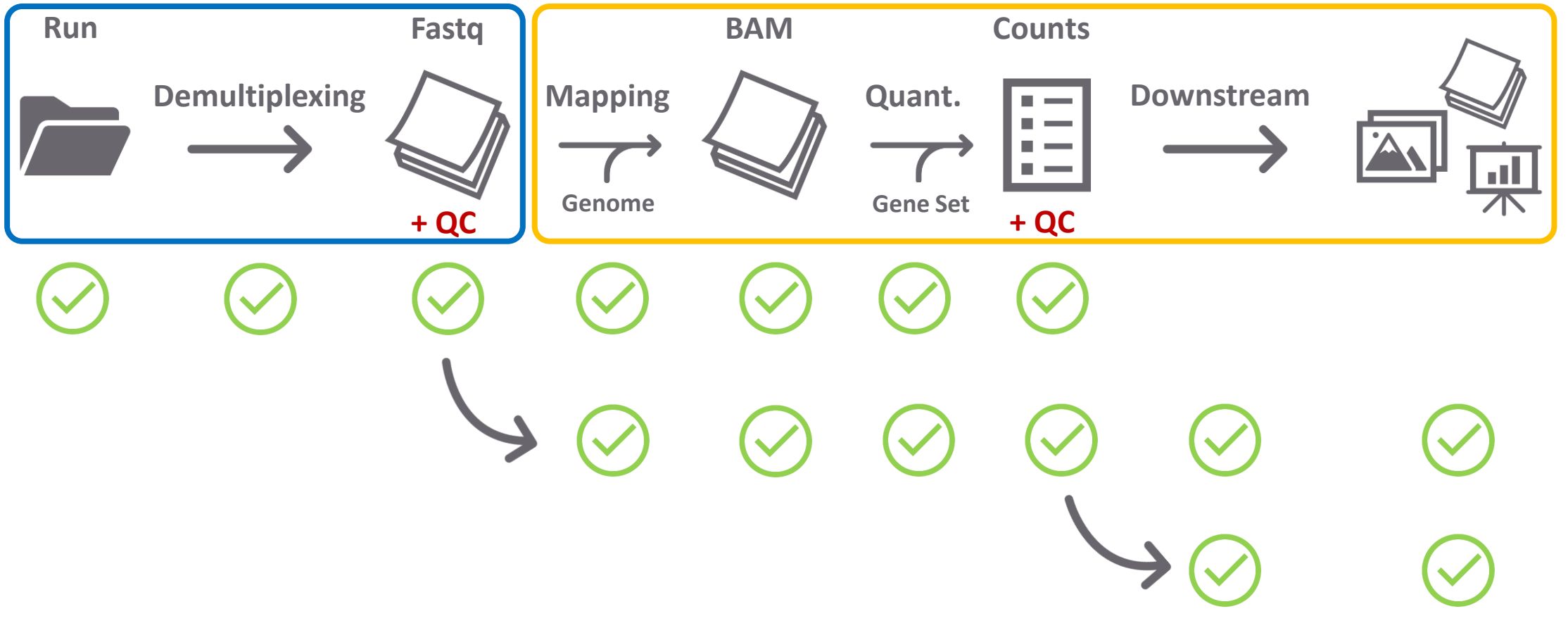
GSEA
Gene Set Enrichment Analysis

Enrichr

GORILLA
Gene Ontology enRIchment anaLysis and visuaLizAtion tool

PANTHER
Classification System

SUMMARY


Run
Demultiplexing
Fastq
+ QC
BAM
Mapping
Genome
Quant.
Gene Set
Counts
+ QC
Downstream

REPRODUCTIBILITY

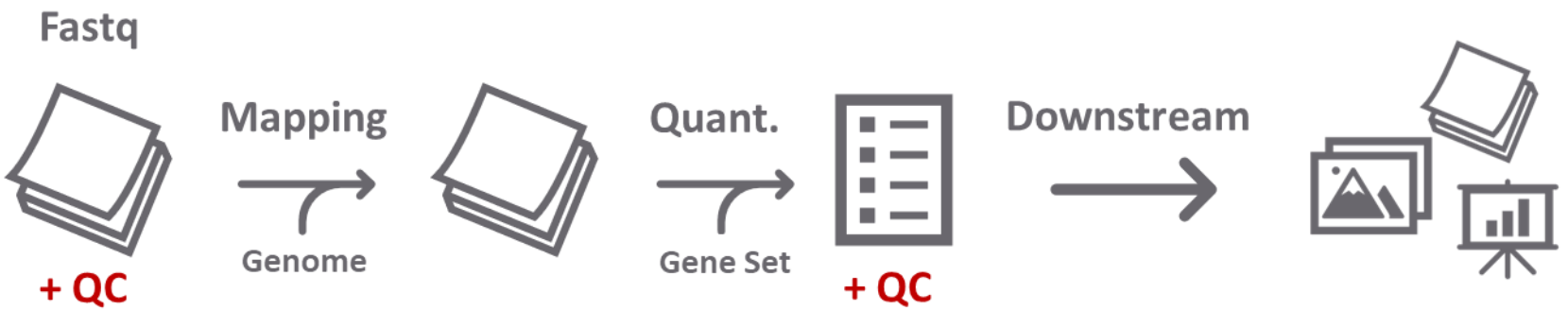
PIPELINES & CONTAINERS

REPRODUCTIBILITY

- Pipelines
  - Set of successive actions
    - Softwares
    - Parameters
    - References

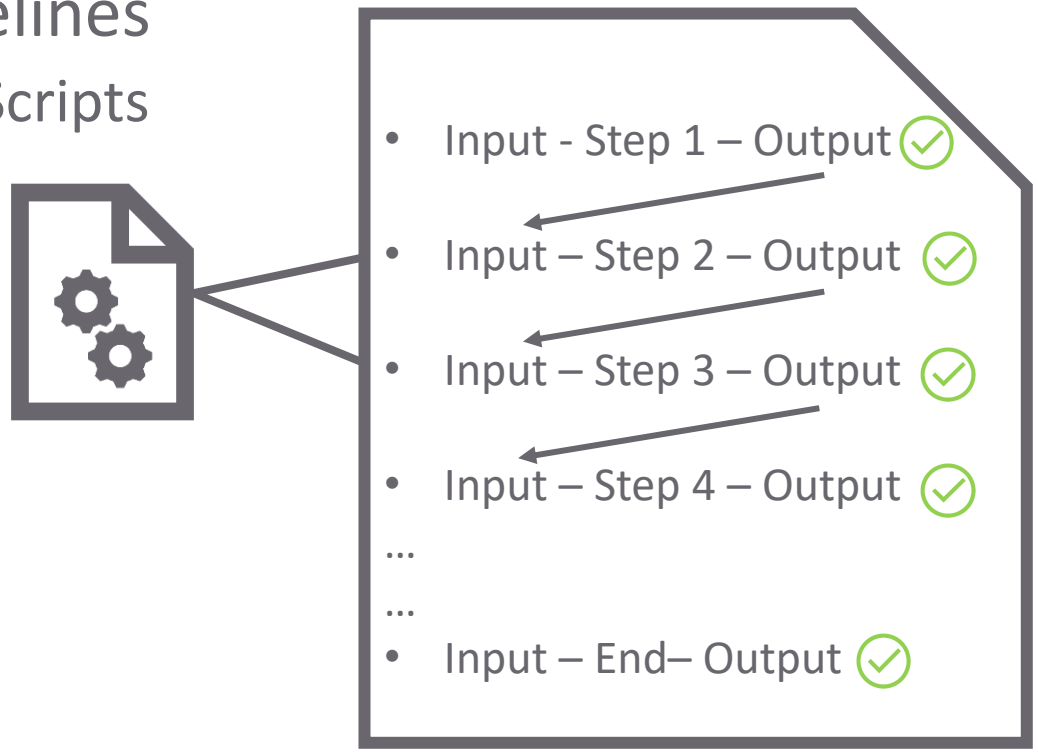
nextflow

Snakemake



REPRODUCTIBILITY

- Pipelines
  - Scripts

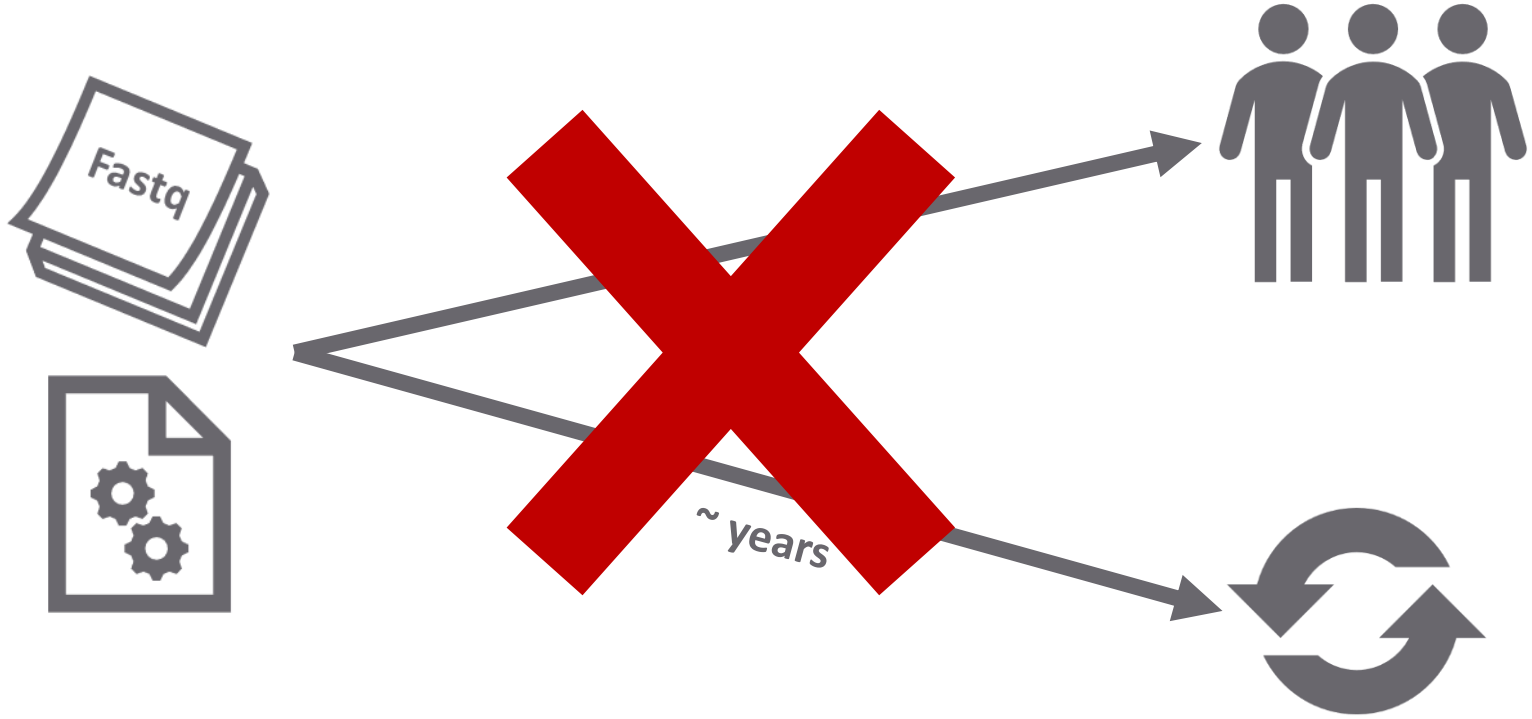


nextflow

Snakemake

nf-core

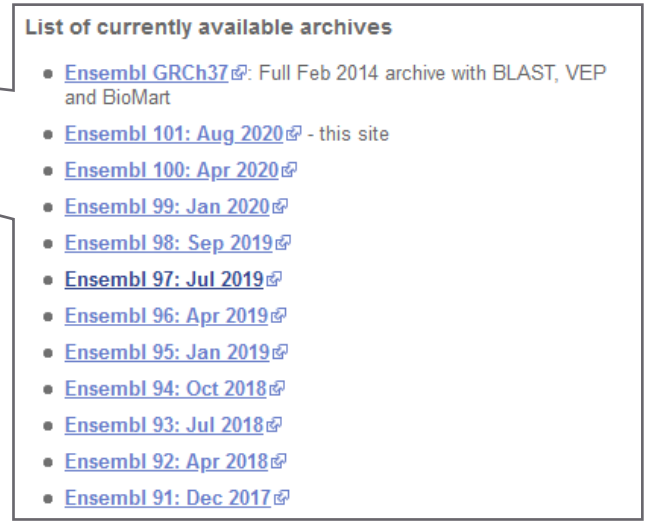
REPRODUCTIBILITY



REPRODUCTIBILITY

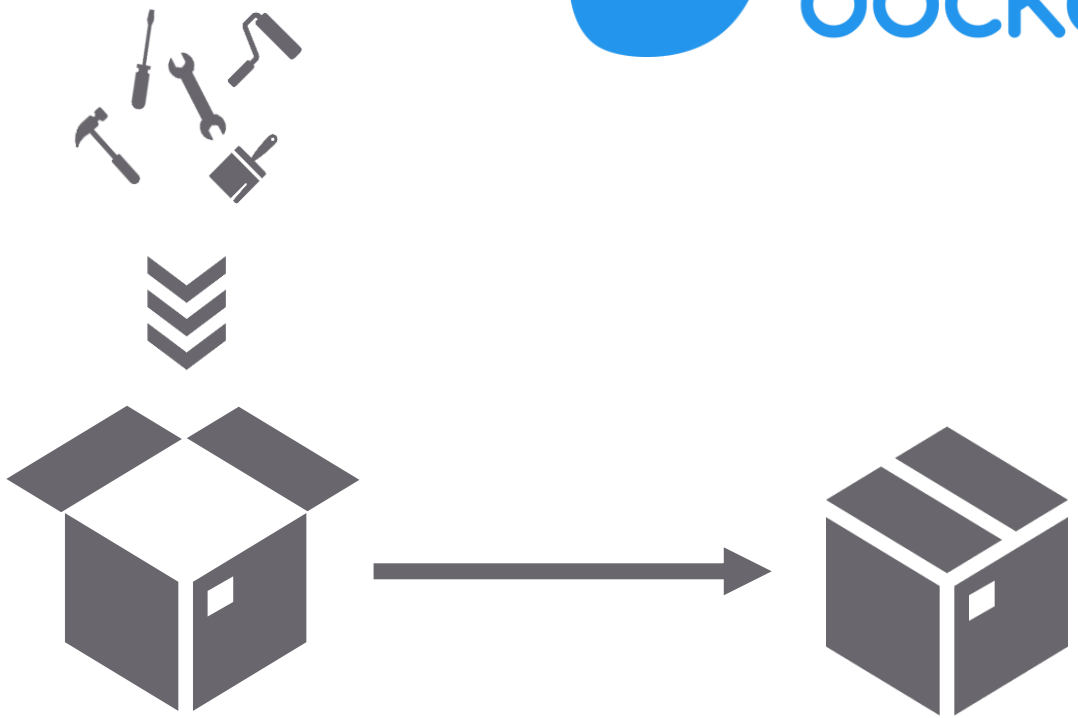
- Variability
  - Updates / Versioning
    - Softwares
    - References
  - Compatibility
  - Format
  - Knowledge

STAR 2.7.5b - 2020/08/01
STAR 2.7.5c - 2020/08/16
STAR 2.7.6a - 2020/09/19

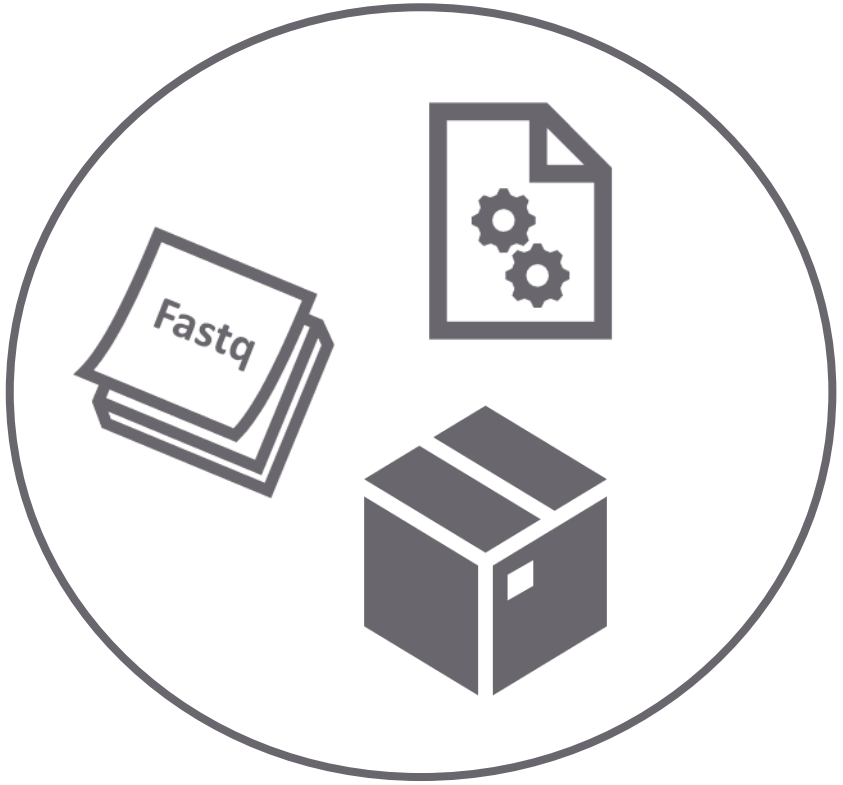


REPRODUCTIBILITY

- CONTAINERS
  - Docker
  - Singularity
- Softwares
  - Versions



REPRODUCTIBILITY



DATA DEPOSITORY

- Gene Expression Omnibus (NCBI)
- ArrayExpress (EMBL-EBI)

THANK YOU FOR YOUR ATTENTION