LIÈGE université
GIGA
Bioinformatics

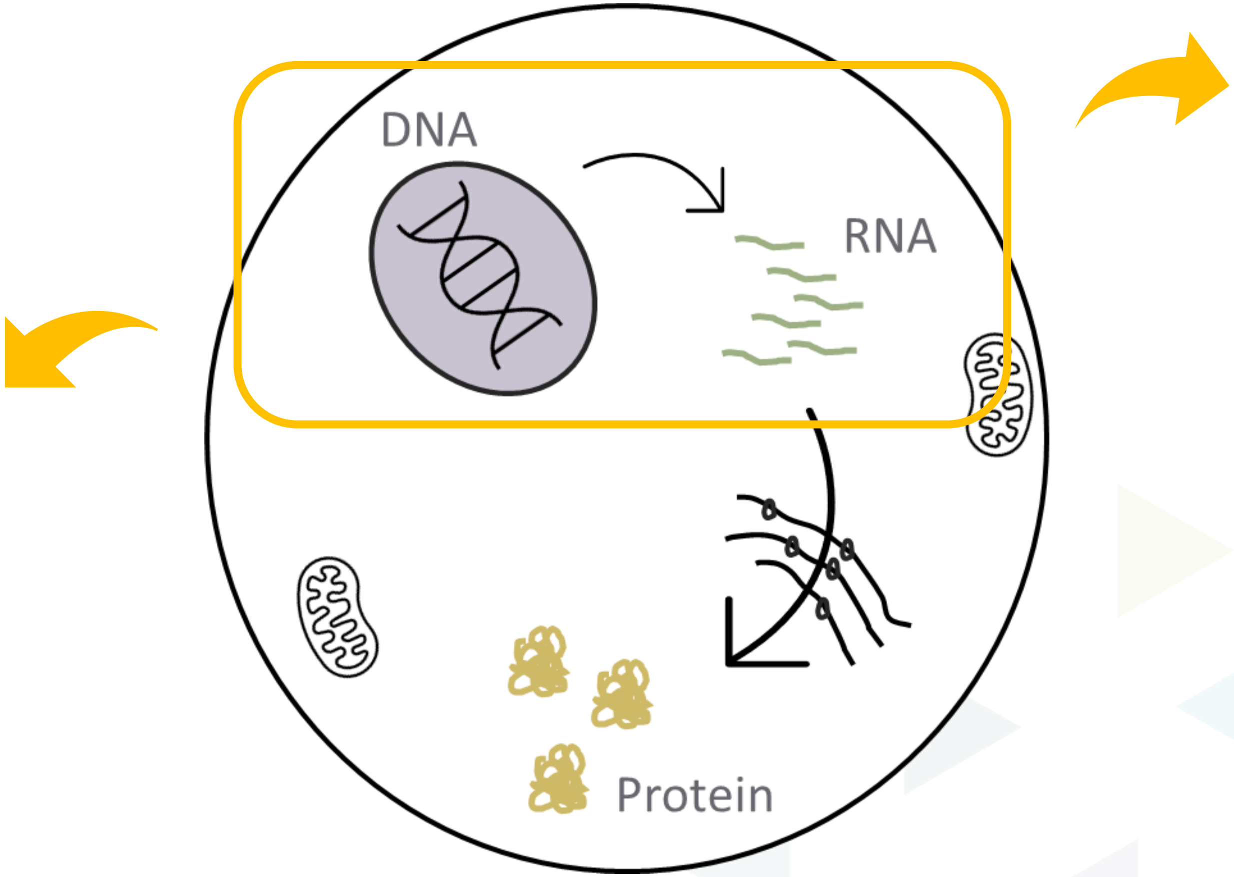
GIGA Doctoral School for Health Sciences
**Introduction to scientific computing**

# DATA PROCESSING IN GENOMICS: FROM THE SEQUENCERS TO THE RESULTS

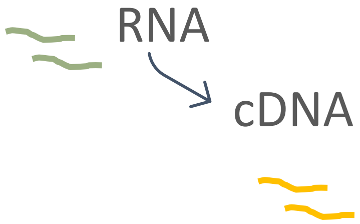
**ARNAUD LAVERGNE, PhD**

GIGA-Bioinformatics

# CLASSIFICATION

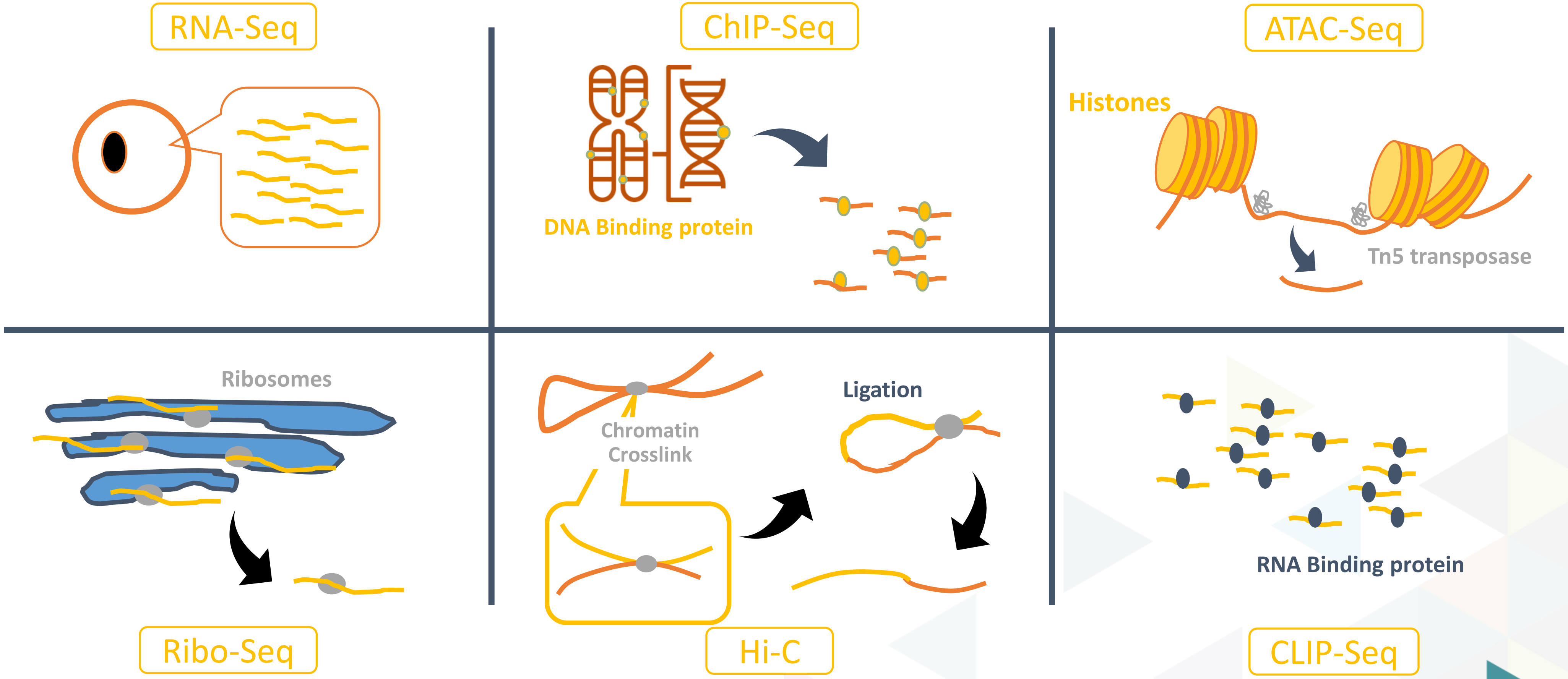


**« Transcriptomics »**



**« Genomics »**

# SAMPLING



RNA-Seq

ChIP-Seq

ATAC-Seq

Ribo-Seq

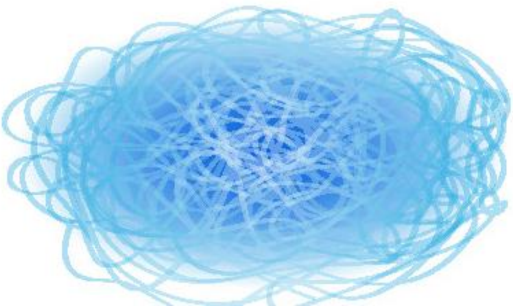
Hi-C

CLIP-Seq

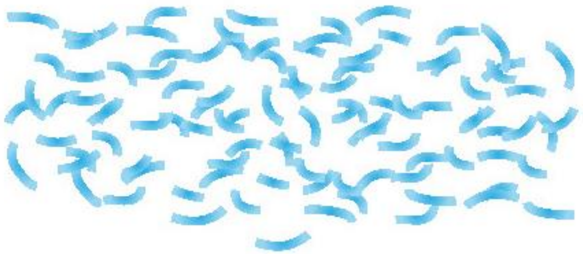
# NEXT-GENERATION SEQUENCING

## = High-Throughput Sequencing (HTS)

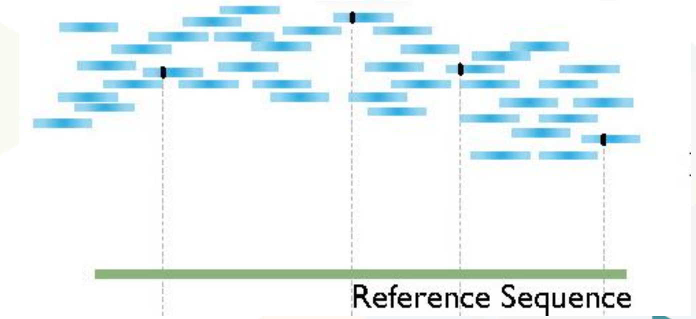
### « Genome-wide »



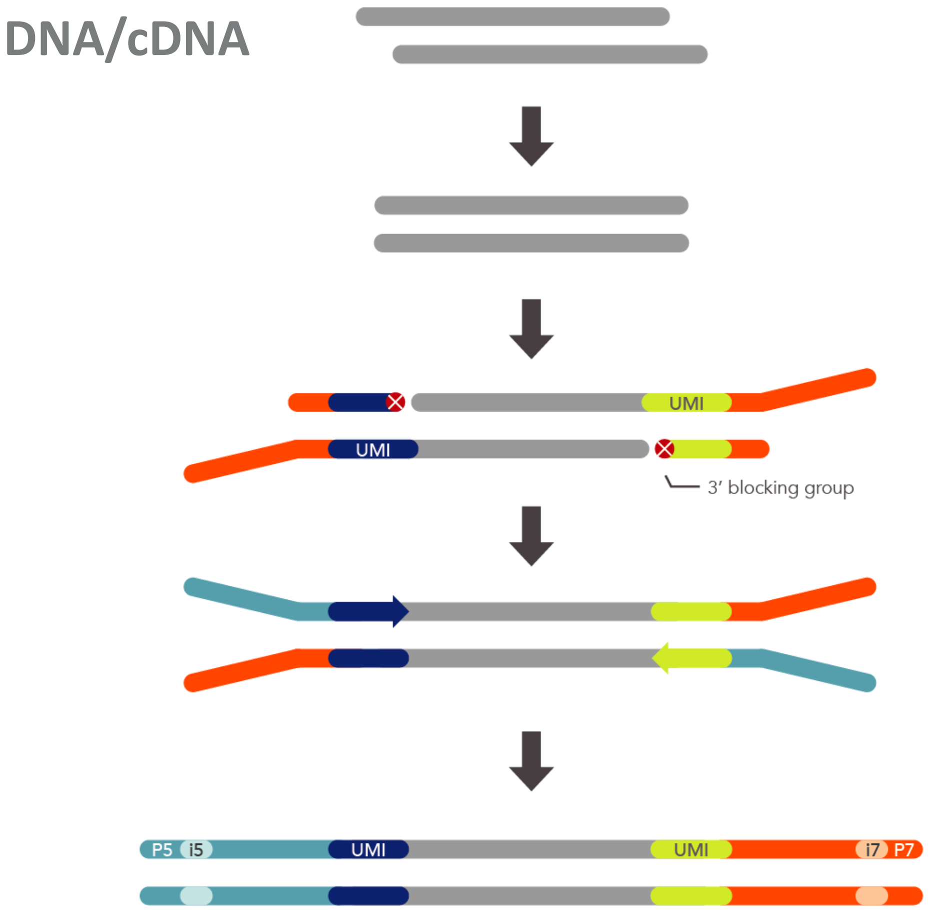
### Massive parallel sequencing



### References



# LIBRARY PREPARATION



- Fragmented input
- End-repair
- Adding adaptors
  - Priming sequences
  - Indexes
  - UMI
  - FlowCell complementary sequences
- Amplification

# SEQUENCERS

**MiSeq**
540 Mb -15 Gb
4 – 56 hours

**HiSeq**
105 Gb - 1,5 Tb
1 – 3,5 days

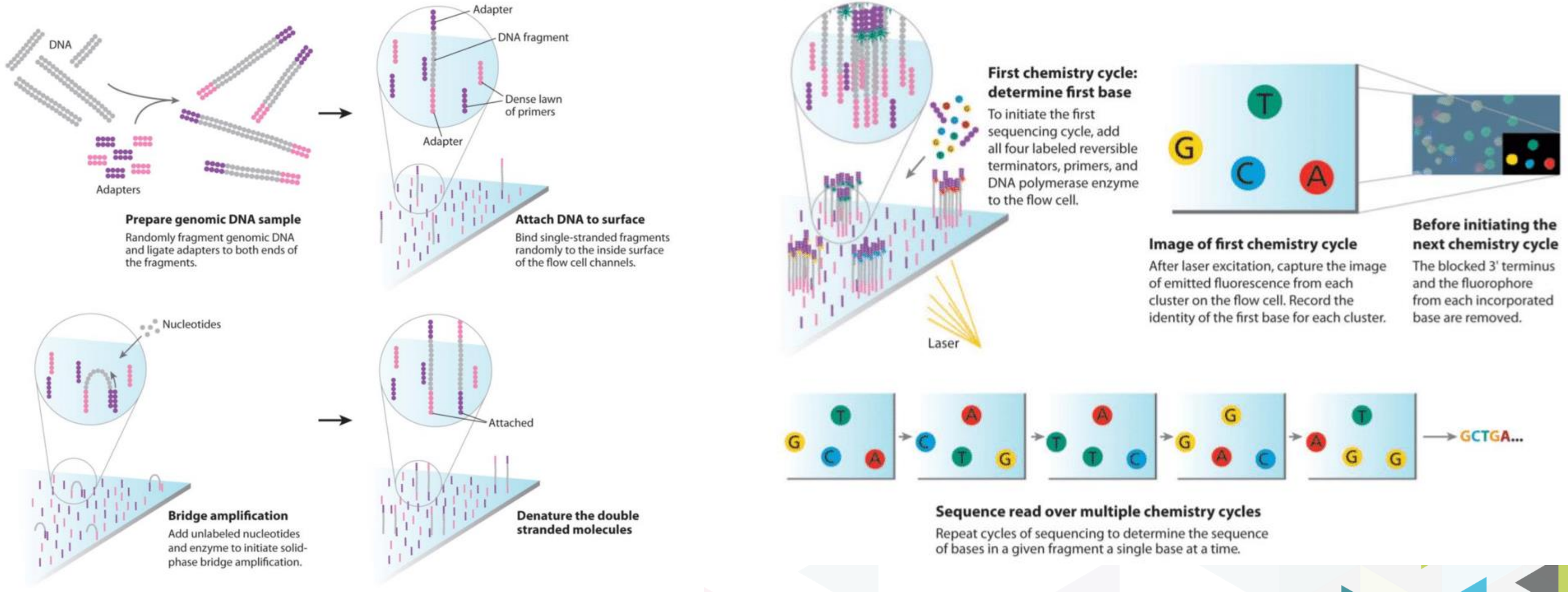
**NextSeq**
16,25 Gb - 120 Gb
11 – 29 hours

**NovaSeq**
65 Gb – 3 Tb
13 – 44 hours

*Adapted from Illumina*

# ILLUMINA SEQUENCING BY SYNTHESIS

# ILLUMINA SEQUENCING BY SYNTHESIS



**Prepare genomic DNA sample**
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

**Attach DNA to surface**
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

**Bridge amplification**
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

**Denature the double stranded molecules**

**First chemistry cycle: determine first base**
To initiate the first sequencing cycle, add all four labeled reversible terminators, primers, and DNA polymerase enzyme to the flow cell.

**Image of first chemistry cycle**
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

**Before initiating the next chemistry cycle**
The blocked 3' terminus and the fluorophore from each incorporated base are removed.

**Sequence read over multiple chemistry cycles**
Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at a time.

*Adapted from Illumina*

# SEQUENCING

# SEQUENCING

# SEQUENCING

## DIGITALIZATION OF THE INFORMATION

# SEQUENCING

# DATA ANALYSIS

## DATA FORMAT & QC SEQUENCING

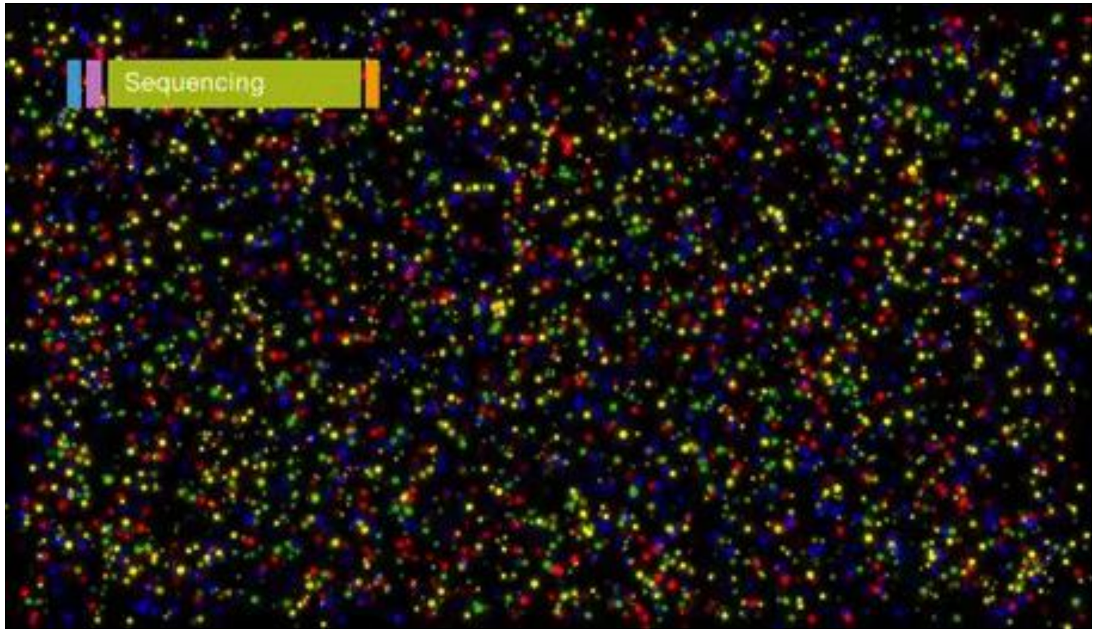
# SEQUENCERS

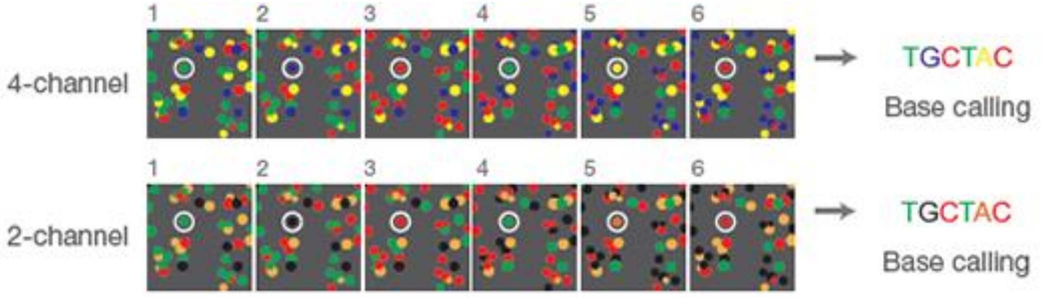
- 1.2 Tb of data
- 12.8 Billions of reads
- Hundreds of samples
  - Multiple experiments
  - Unique combinations of indexes

- « Run »
- No storage on device

# DATA FORMAT

- Bcl files
  - Each position
  - Each cycle
    - Base calls
    - Base call quality scores
  - Raw data files
    - Binary format





.bcl

CONVERSION

.fastq

# DATA FORMAT

- DEMULTIPLEXING
  - Reads → Samples
    - Indexes
  - Fastq format
    - Identifier
    - Sequence
    - Separator
    - Quality score

.fastq

```
@A00801:49:H2VGKDSXY:2:1101:1624:1016 1:N:0:NGCTTAAG+TCGTGACC
CTTCTGGAGAGGAGTTCCTCTGATATGAATTAAGGTTTTCCCTTCTGTCATGACCAGAAGAAGGTTTTATCTGTGCCACACTACTTTTCATTTTCTGTTGCCAGTTGGTCCAATAAATCAAA
GATGNTTCAAACTGGTCCAAATAACAAGT
+
FFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF,FFFFFFFFFFFFFFF#FFFFFFFFFFFFFFFFFFFFFF
FF
@A00801:49:H2VGKDSXY:2:1101:2022:1016 1:N:0:NGCTTAAG+TCGTGACC
TGACAAAAAGATACCTCATTTATGGGGAAATTGAGGAAGATACATATACAAGCACCCCAACCCATATTTAACATATTTGGCAATAACTCCCTTCCCATTCTTCCCCCTCCAATTTTCAAATA
GTAGNTTTTTAAAAAAATTAAAGACATGTC
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFF,FFFFFF:FFFFFFF,#FFFFFFF,FFFFFFFF:F:FF,FF
@A00801:49:H2VGKDSXY:2:1101:4137:1016 1:N:0:NGCTTAAG+TCGTGACC
TTTTTTTGCCTTTTCAAGTGTTTATTTTTATACATTTTTTTGTATTAAAAAGAAAAGCATAATTACCACAAATTACAAAGGACTAAAGCAGGACTAGAATAATGAATGAATCACTTCAGCCT
GGAANGCAGATACTCTCAATAATATTAAT
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFF:FFFFFFF:FFFFF:FFFFFFFFFFFFFFFFFFFFFFFF:FFF,FFFFFFFFFFFF:FFF#FFFFF:FFFFFFFFFFFFFFFFF
```

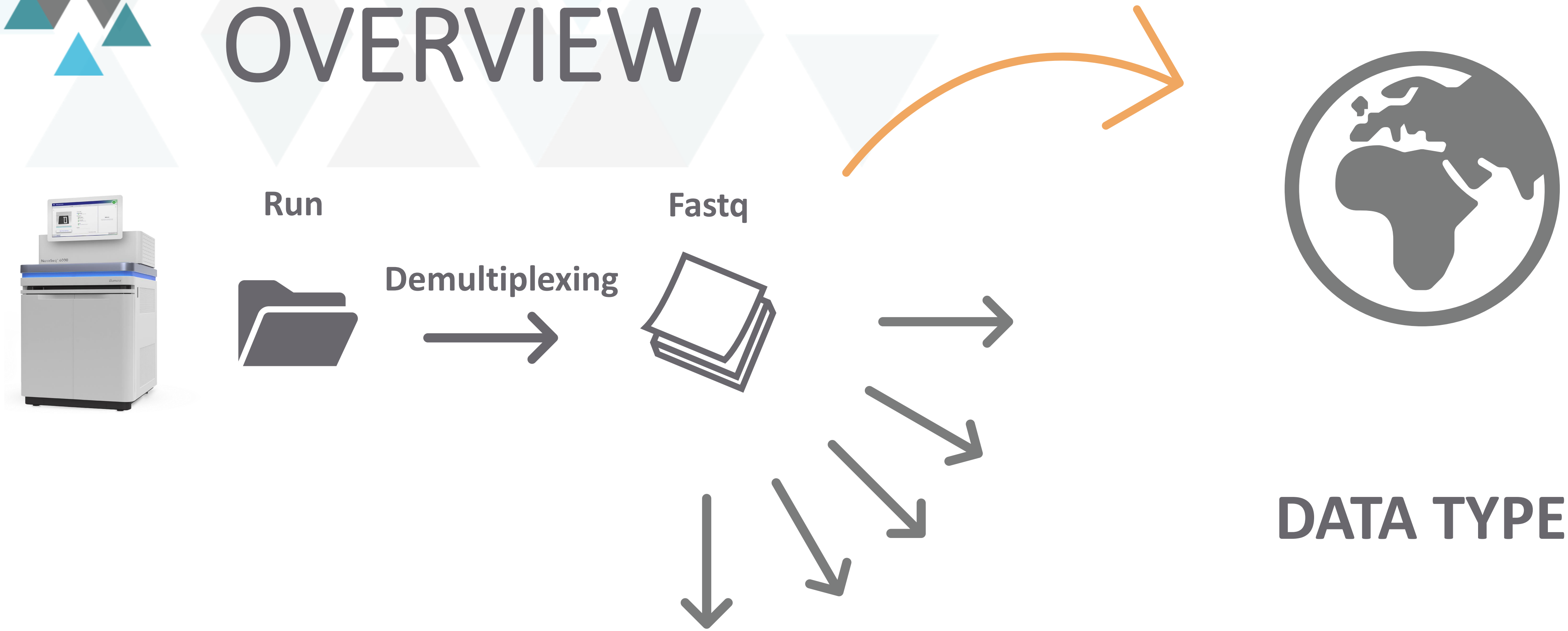
# DATA FORMAT

- DEMULTIPLEXING
  - Reads → Samples
    - Indexes
  - Fastq format
    - Identifier
    - Sequence
    - Separator
    - Quality score
      - Phred +33 encoded using ASCII

| 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% |

| 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 | | | |

# OVERVIEW



Run

Demultiplexing

Fastq

DATA TYPE

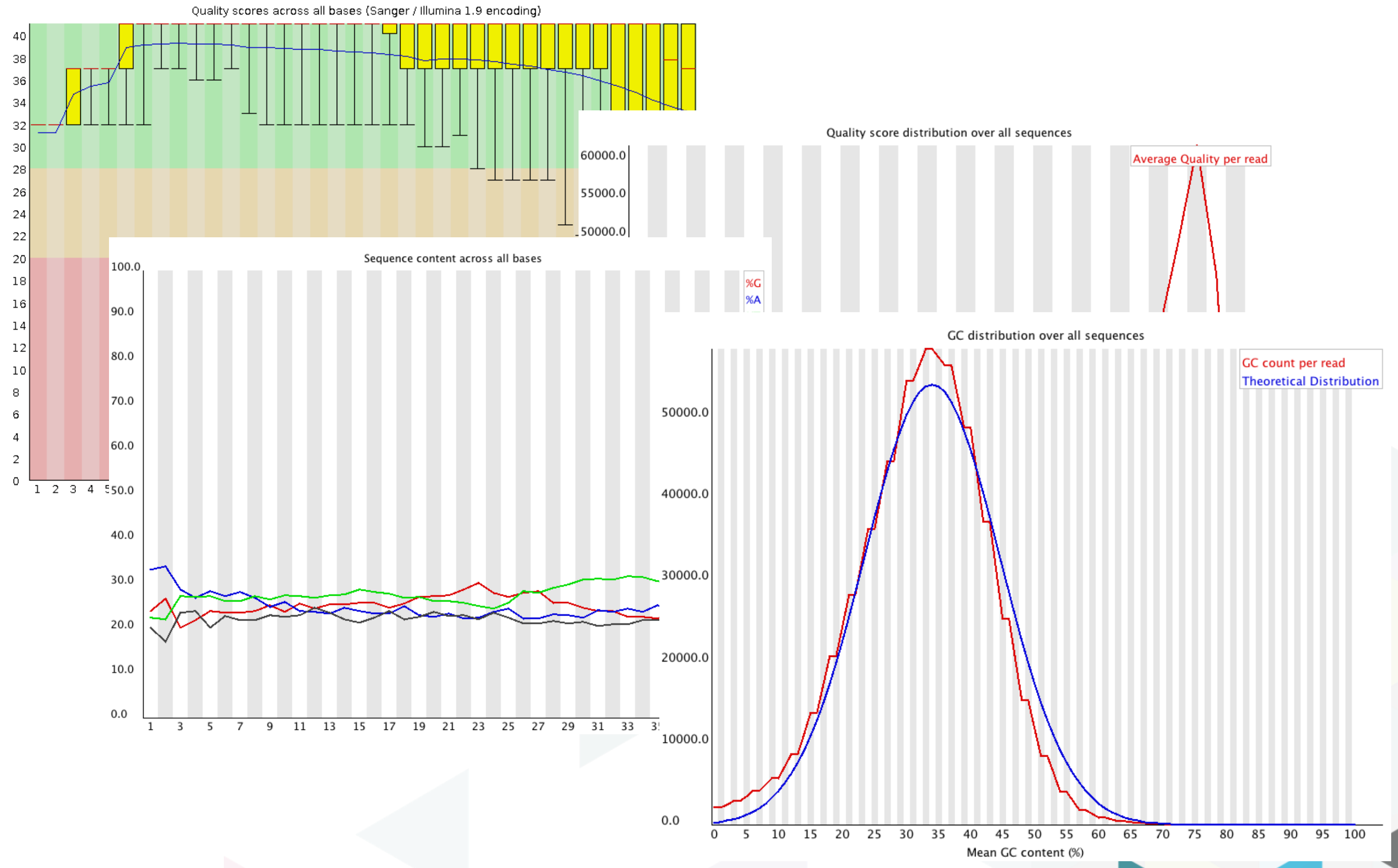
EXPERIMENTAL DESIGN

Fastq.gz

# QC SEQUENCING

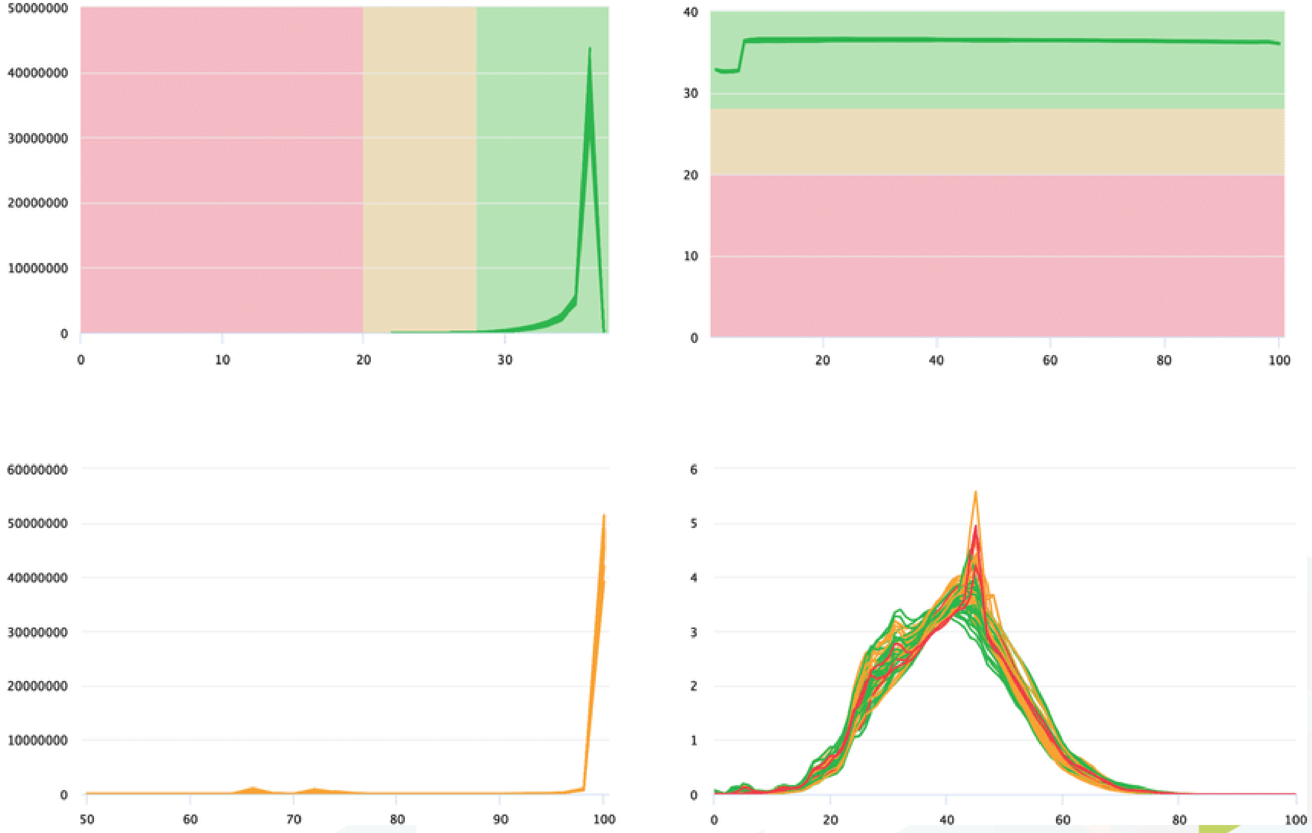
## FastQC

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



# QC SEQUENCING

MultiQC



# DATA ANALYSIS

## MAPPING, BAM FILES & QC MAPPING

# MAPPING

**Reference**

- Genome sequence
- Gene set

ACTGG[..]TCAGAT

TGGACTGG[..]TCAGATCCA

ACCTGACC[..]AGTCTAGGT

**Alignment / mapping**

e!Ensembl

UCSC

# REFERENCE

## Genome (FASTA)

```
>1 dna:chromosome chromosome:GRCh38:1:1:248956422:1 REF
CCCTAACCCCTAACCCTAACCCTAACCCTAACCCTAACCTAACCCTAACCCTAACCCTAAC
CCTAACCCTAACCCTAACCCTAACCCTAACCCCTAACCCTAACCCTAAACCCTAAACCCTA
ACCCTAACCCTAACCCTAACCCTAACCCCAACCCCAACCCCAACCCCAACCCCAACCCCA
ACCCTAACCCCTAACCCTAACCCTAACCCTACCCTAACCCTAACCCTAACCCTAACCCTAA
CCCTAACCCCTAACCCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCT
AACCCTAACCCTAACCCTAACCCTCGCGGTACCCTCAGCCGGCCCGCCCGCCCGGGTCT
GACCTGAGGAGAACTGTGCTCCGCCTTCAGAGTACCACCGAAATCTGTGCAGAGGACA
ACGCAGCTCCGCCCTCGCGGTGCTCTCCGGGTCTGTGCTGAGGAGAACGCAACTCCGC
CGTTGCAAAGGCGCGCCGCGCCGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGC
AGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGG
CGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGCGCAGAGAGGCGCGCCGCGC
CGGCGCAGGCGCAGACACATGCTAGCGCGTCGGGGTGGAGGCGTGGCGCAGGCGCA
GAGAGGCGCGCCGCGCCGGCGCAGGCGCAGAGACACATGCTACCGCGTCCAGGGGT
GGAGGCGTGGCGCAGGCGCAGAGAGGCGCACCGCGCCGGCGCAGGCGCAGAGACA
CATGCTAGCGCGTCCAGGGGTGGAGGCGTGGCGCAGGCGCAGAGACGCAAGCCTACG
GGCGGGGGTTGGGGGGGCGTGTGTTGCAGGAGCAAAGTCGCACGGCGCCGGGCTG
GGGCGGGGGGAGGGTGGCGCCGTGCACGCGCAGAAACTCACGTCACGGTGGCGCGG
CGCAGAGACGGGTAGAACCTCAGTAATCCGAAAAGCCGGGATCGACCGCCCCTTGCTT
GCAGCCGGGCACTACAGGACCCGCTTGCTCACGGTGCTGTGC
```

## Gene Set (GTF)

```
#!genome-build GRCh38.p12
#!genome-version GRCh38
#!genome-date 2013-12
#!genome-build-accession NCBI:GCA_000001405.27
#!genebuild-last-updated 2019-03
1       havana  gene    11869   14409   .       +       .
gene_id "ENSG00000223972"; gene_version "5"; gene_name "DDX11L1"; gene_source "havana"; gene_biotype
"transcribed_unprocessed_pseudogene";
1       havana  transcript 11869 14409  .       +       .       gene_id "ENSG00000223972"; gene_version "5";
transcript_id "ENST00000456328"; transcript_version "2"; gene_name "DDX11L1"; gene_source "havana"; gene_biotype
"transcribed_unprocessed_pseudogene"; transcript_name "DDX11L1-202"; transcript_source "havana"; transcript_biotype "lncRNA"; tag
"basic"; transcript_support_level "1";
1       havana  exon    11869   12227   .       +       .       gene_id "ENSG00000223972"; gene_version "5";
transcript_id "ENST00000456328"; transcript_version "2"; exon_number "1"; gene_name "DDX11L1"; gene_source "havana";
gene_biotype "transcribed_unprocessed_pseudogene"; transcript_name "DDX11L1-202"; transcript_source "havana"; transcript_biotype
"lncRNA"; exon_id "ENSE00002234944"; exon_version "1"; tag "basic"; transcript_support_level "1";
1       havana  exon    12613   12721   .       +       .       gene_id "ENSG00000223972"; gene_version "5";
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gene_biotype "transcribed_unprocessed_pseudogene"; transcript_name "DDX11L1-202"; transcript_source "havana"; transcript_biotype
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"transcribed_unprocessed_pseudogene"; transcript_name "DDX11L1-201"; transcript_source "havana"; transcript_biotype
"transcribed_unprocessed_pseudogene"; tag "basic"; transcript_support_level "NA";
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transcript_id "ENST00000450305"; transcript_version "2"; exon_number "1"; gene_name "DDX11L1"; gene_source "havana";
gene_biotype "transcribed_unprocessed_pseudogene"; transcript_name "DDX11L1-201"; transcript_source "havana"; transcript_biotype
"transcribed_unprocessed_pseudogene"; exon_id "ENSE00001948541"; exon_version "1"; tag "basic"; transcript_support_level "NA";
```

# INDEXING

- Genome Indexing
  - Quick queries
  - « 20M reads »

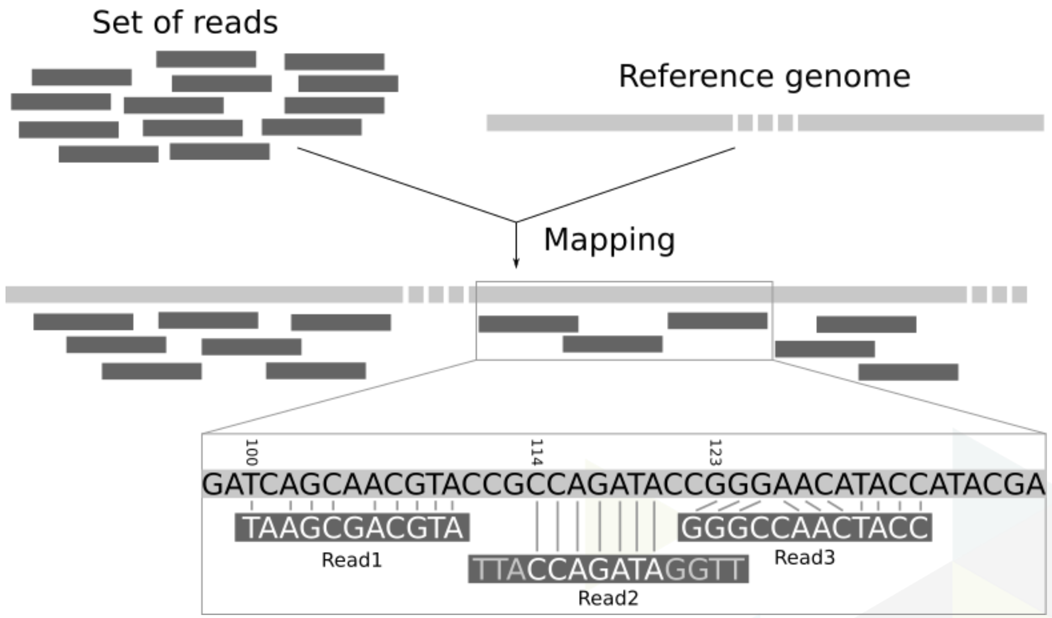
- High RAM/CPUs

- H.Sapiens ~30 Gb

# MAPPING

- Softwares
  - STAR
  - HISAT
  - Bowtie
  - BWA
  - …

- High RAM/CPUs



# BAM FILES

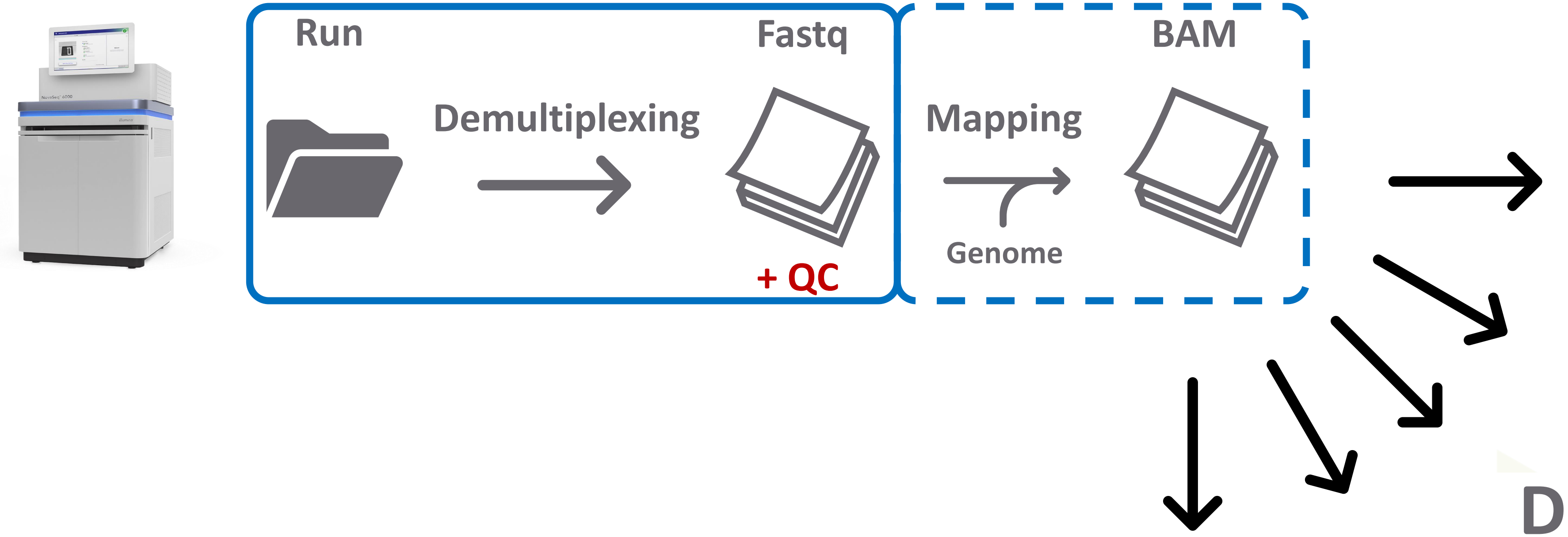
- SAM/BAM files
  - FLAG - Information
  - RNAME - Chromosome
  - POS – Location of 1st base
  - MAPQ – Quality score
  - CIGAR - Operations

| 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:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF: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
```

# OVERVIEW



Run

Demultiplexing

Fastq

+ QC

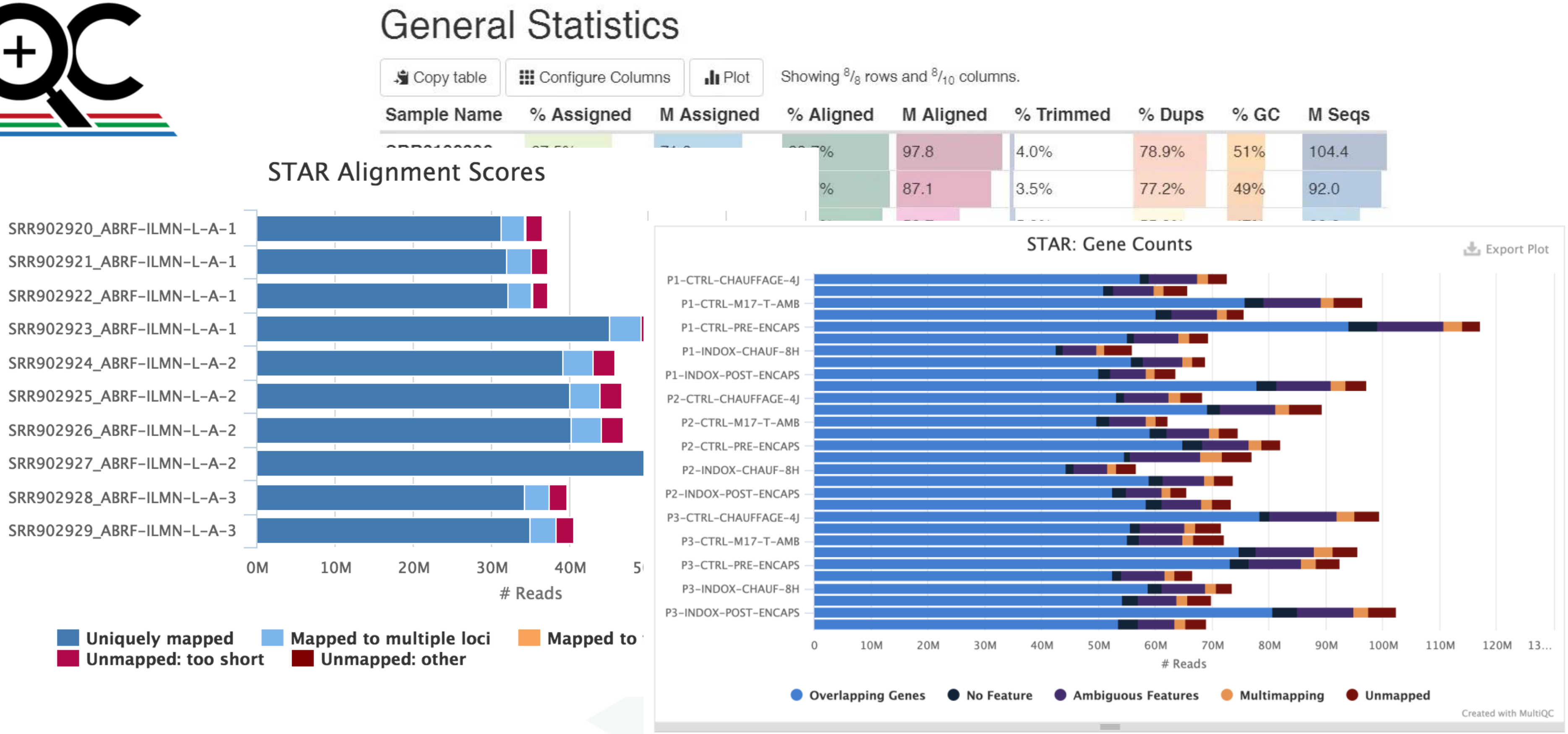
Mapping

Genome

BAM

DOWNSTREAM ANALYSES

# QC MAPPING

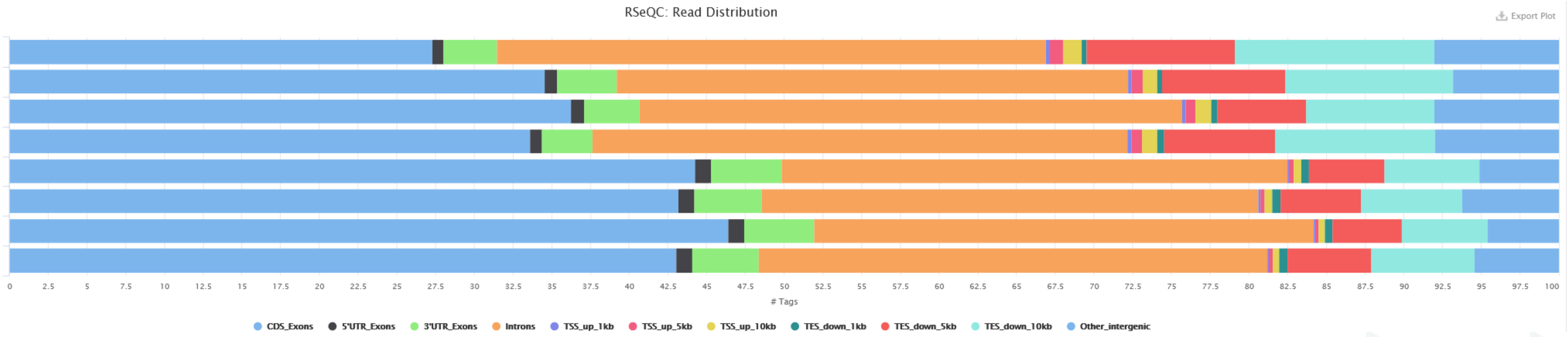


## General Statistics

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 |

# QC MAPPING



Mapping distribution

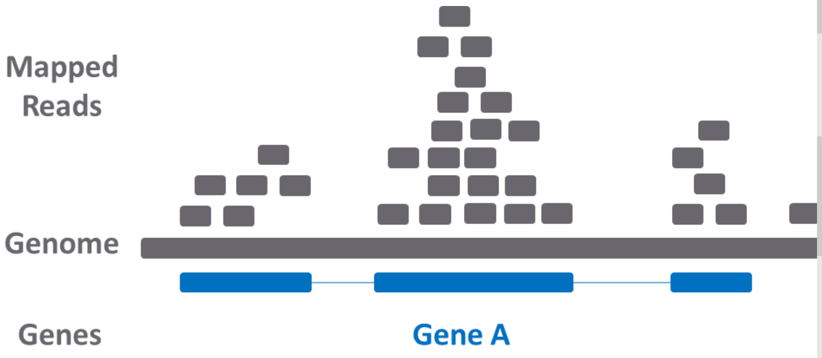
- Proportion of exonic, intronic and intergenic locations
- DNA ≠ total RNA ≠ mRNA
- Experiments

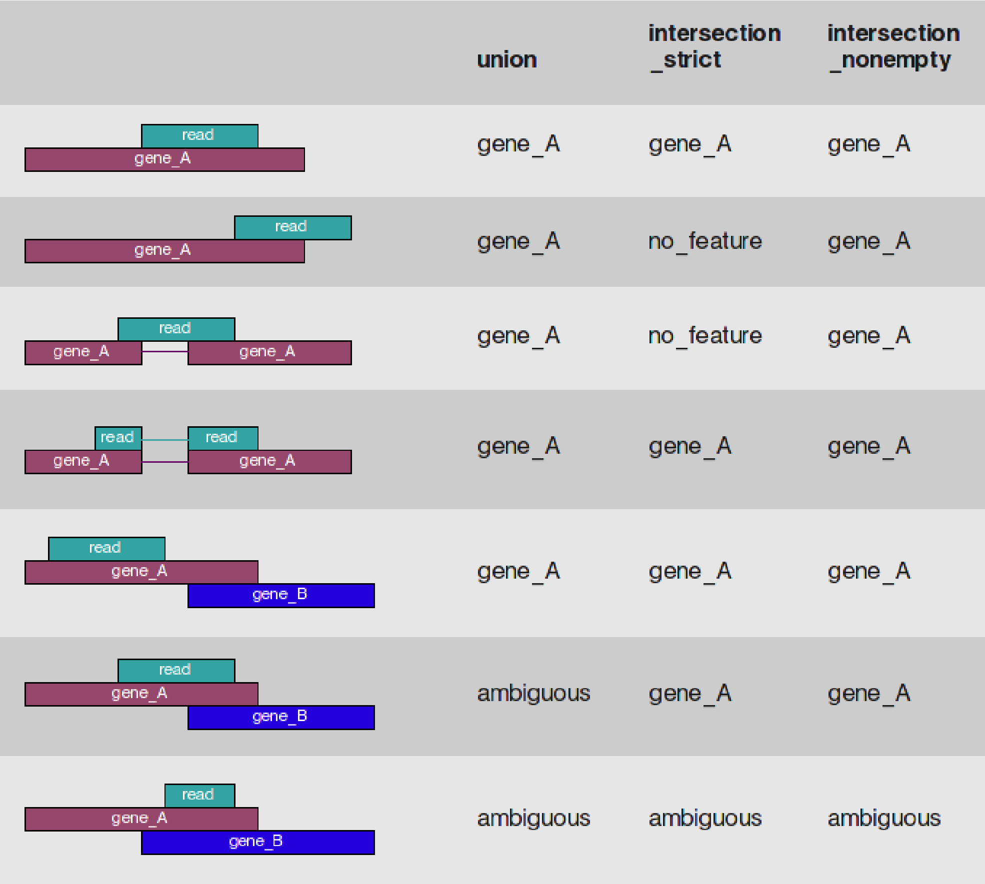
# DATA ANALYSIS

## DOWNSTREAM ANALYSIS

# RNA - QUANTIFICATION

- Gene Expression

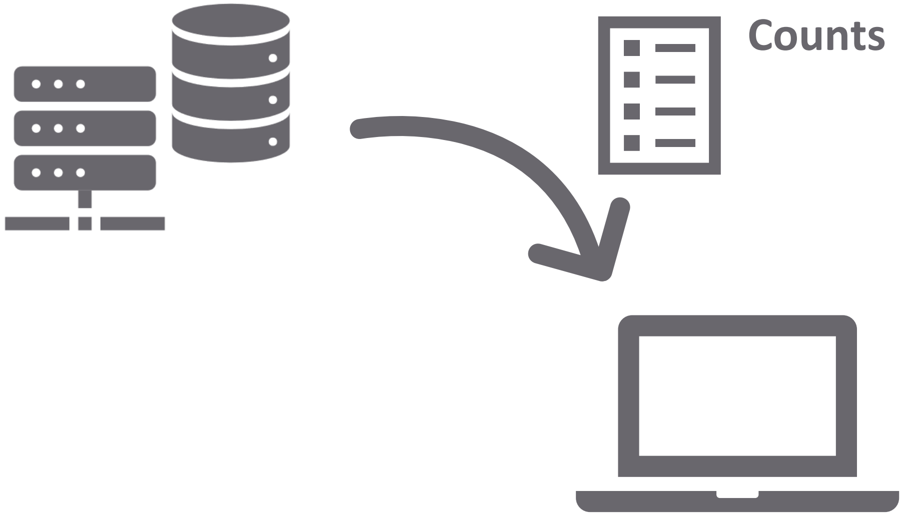




| | union | intersection_strict | intersection_nonempty |
|---|---|---|---|
| read<br>gene_A | gene_A | gene_A | gene_A |
| read<br>gene_A | gene_A | no_feature | gene_A |
| read<br>gene_A gene_A | gene_A | no_feature | gene_A |
| read read<br>gene_A gene_A | gene_A | gene_A | gene_A |
| read<br>gene_A<br>gene_B | gene_A | gene_A | gene_A |
| read<br>gene_A<br>gene_B | ambiguous | gene_A | gene_A |
| read<br>gene_A<br>gene_B | ambiguous | ambiguous | ambiguous |

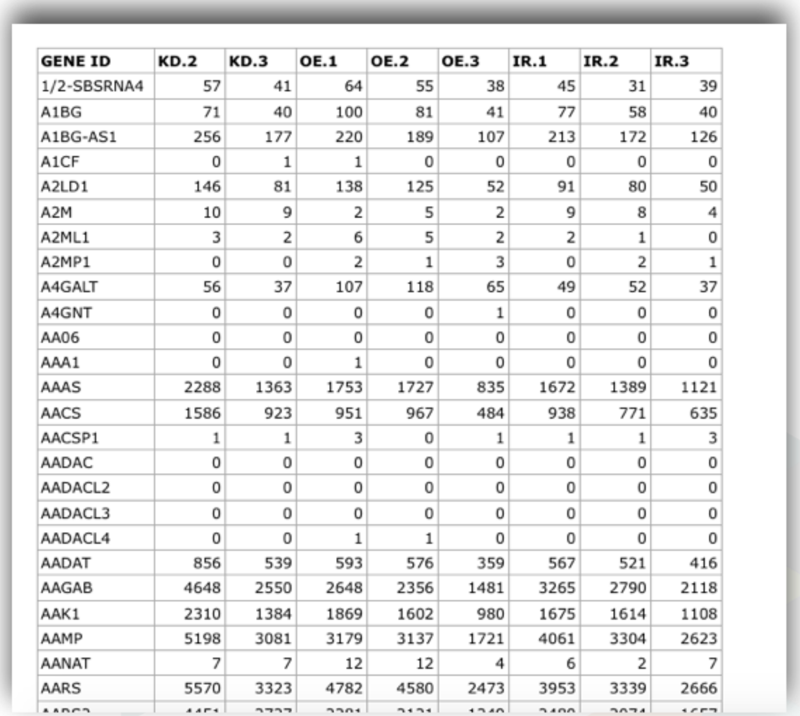
# RNA - QUANTIFICATION

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



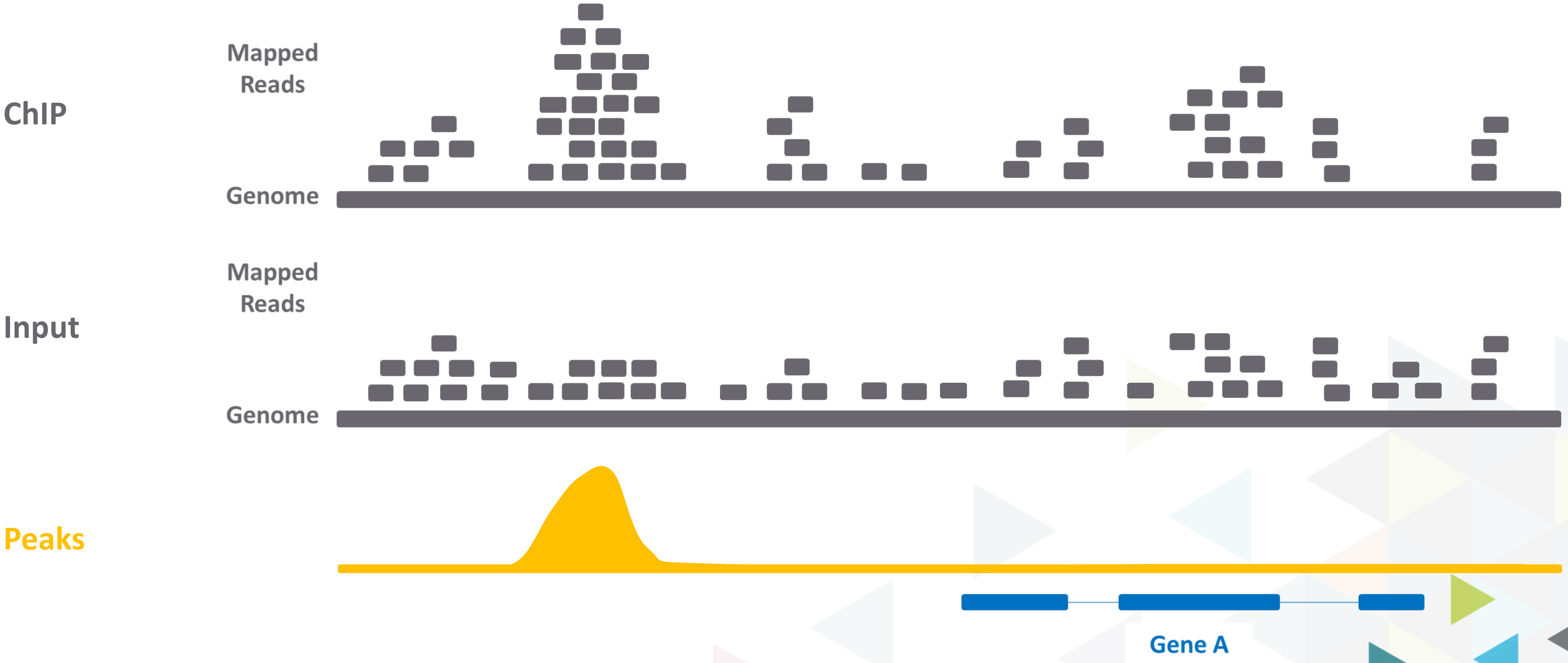
Each column is a sample

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 |

# DNA - PEAK CALLING



# DNA - SNP

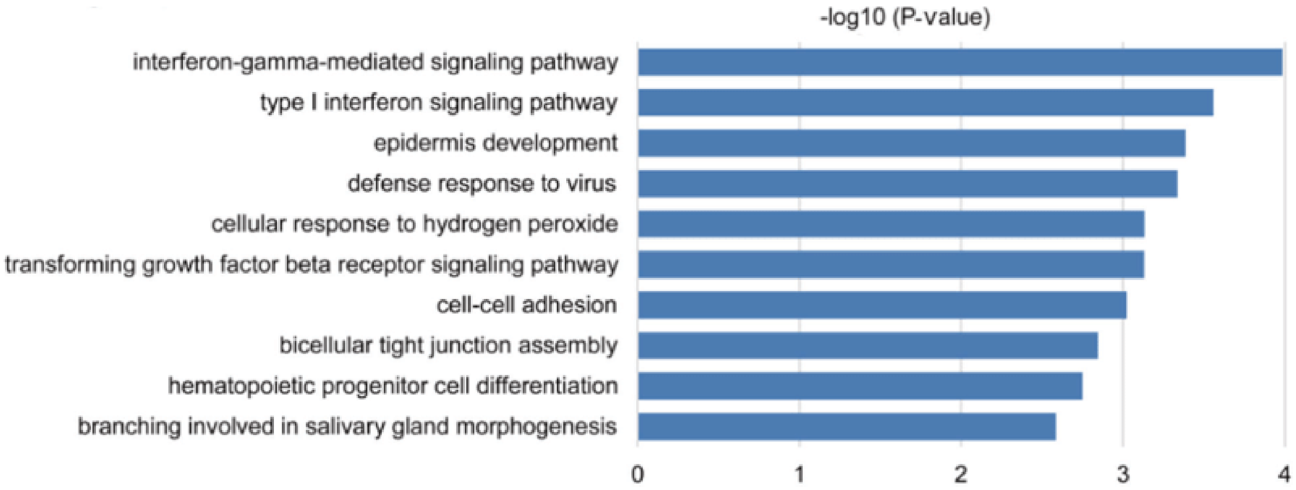
**REFERENCE** …TGGACTGGA..ATCGGCTCGAAGCTTGCATCA..GATCCA…

**DATA** …TGGAATGGA..ATCGGCTCTAAGCTTGCATCA..GATTCA…

**→ VARIANT / GENOTYPE / GWAS**

# DOWNSTREAM ANALYSIS

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



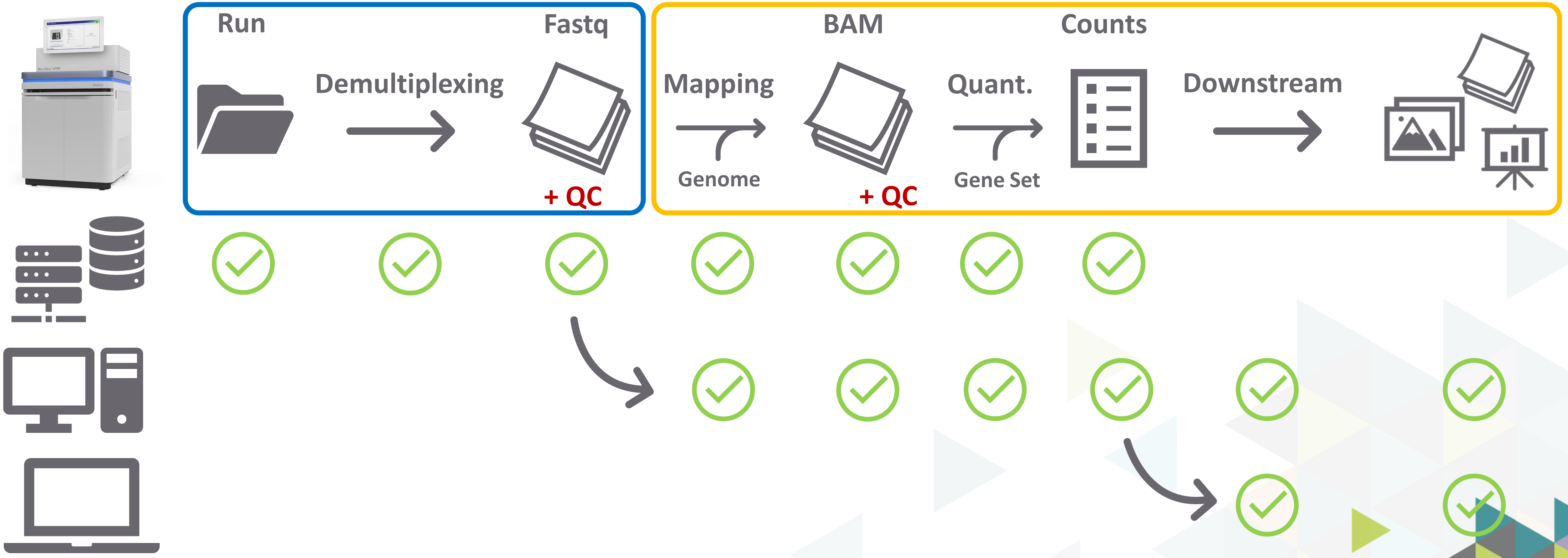
GSEA
Gene Set Enrichment Analysis

Enrichr

GORILLA
Gene Ontology enRIchment anaLysis and visuaLizAtion tool

PANTHER
Classification System

# SUMMARY



Run

Demultiplexing

Fastq

+ QC

BAM

Counts

Mapping

Genome

+ QC

Quant.

Gene Set

Downstream

# REPRODUCTIBILITY

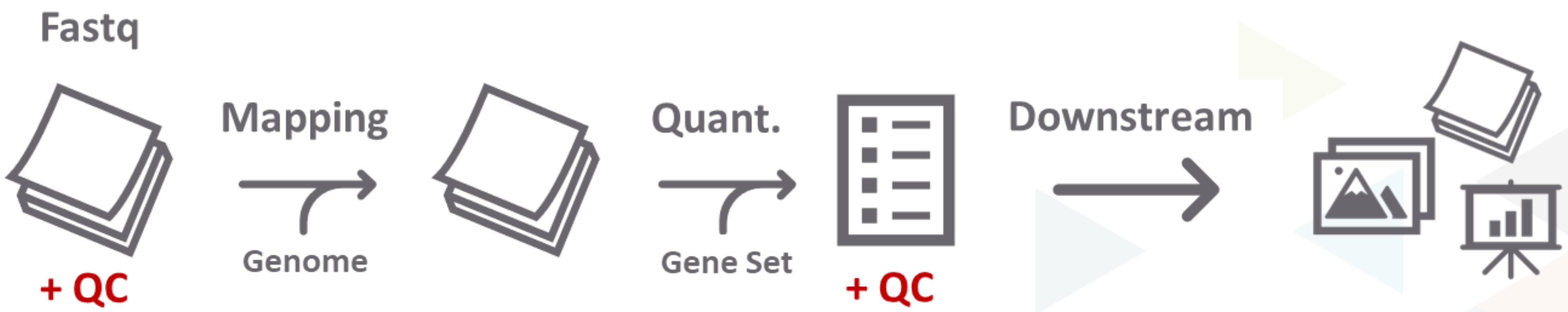
PIPELINES & CONTAINERS

# REPRODUCTIBILITY

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

nextflow

Snakemake



Fastq

+ QC

Mapping

Genome

Quant.

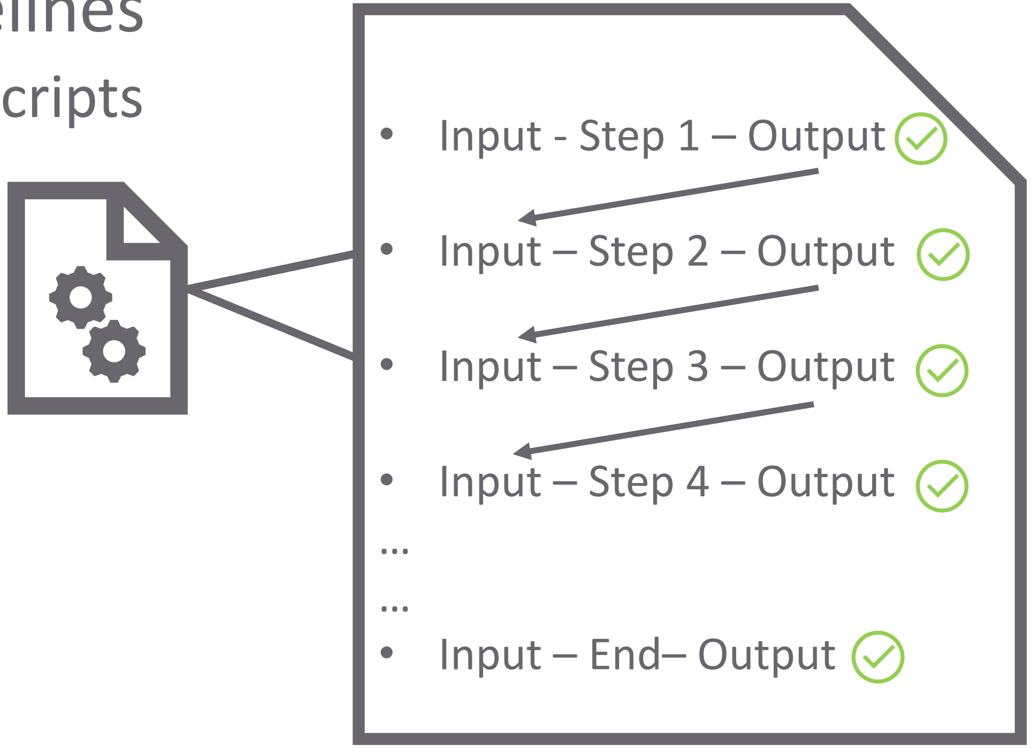
Gene Set

+ QC

Downstream

# REPRODUCTIBILITY

- Pipelines
  - Scripts



- Input - Step 1 – Output ✓
- Input – Step 2 – Output ✓
- Input – Step 3 – Output ✓
- Input – Step 4 – Output ✓

…

…

- Input – End– Output ✓

nextflow

Snakemake

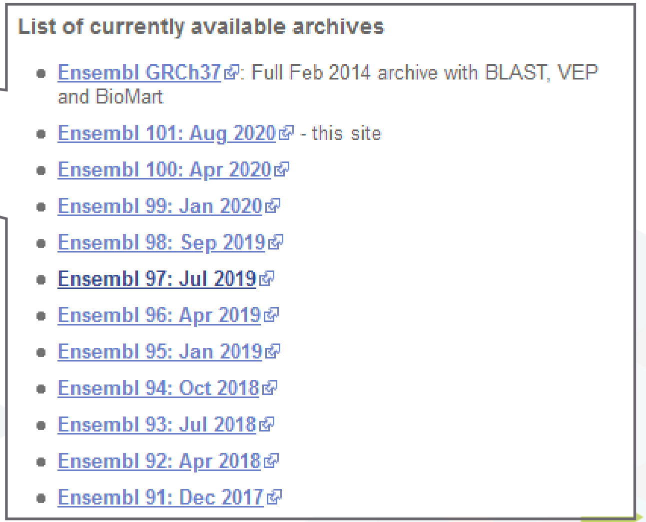
nf-core

# 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



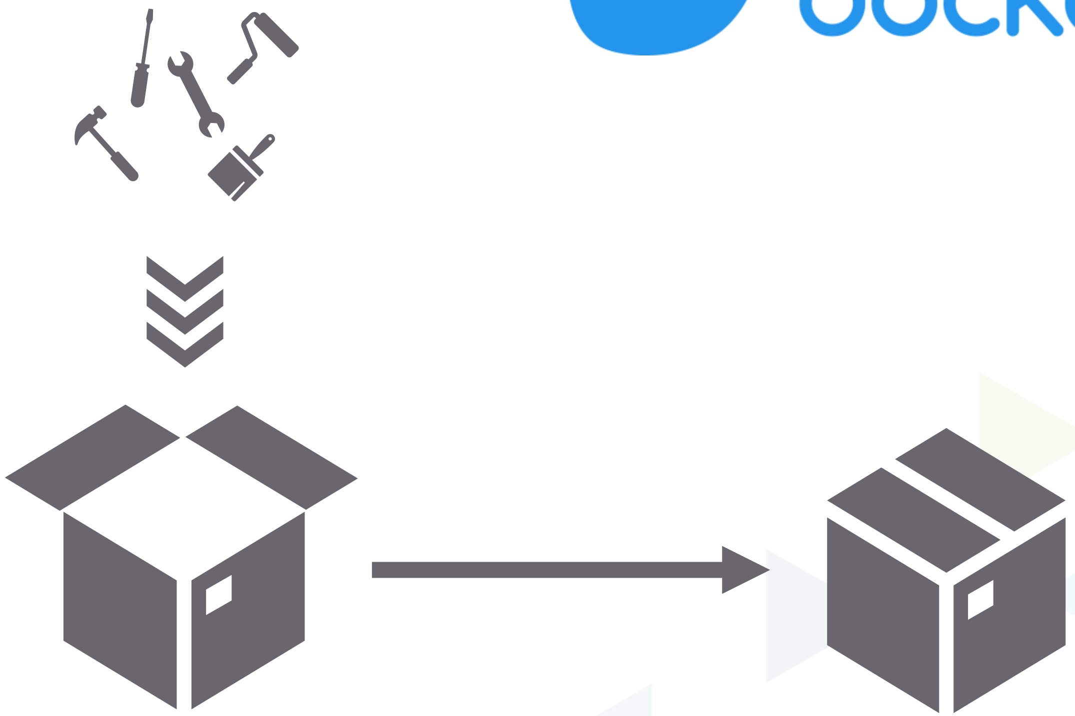
List of currently available archives

- Ensembl GRCh37: Full Feb 2014 archive with BLAST, VEP and BioMart
- Ensembl 101: Aug 2020 - this site
- Ensembl 100: Apr 2020
- Ensembl 99: Jan 2020
- Ensembl 98: Sep 2019
- Ensembl 97: Jul 2019
- Ensembl 96: Apr 2019
- Ensembl 95: Jan 2019
- Ensembl 94: Oct 2018
- Ensembl 93: Jul 2018
- Ensembl 92: Apr 2018
- Ensembl 91: Dec 2017

# REPRODUCTIBILITY

- CONTAINERS
  - Docker
  - Singularity

- Softwares
  - Versions



# REPRODUCTIBILITY

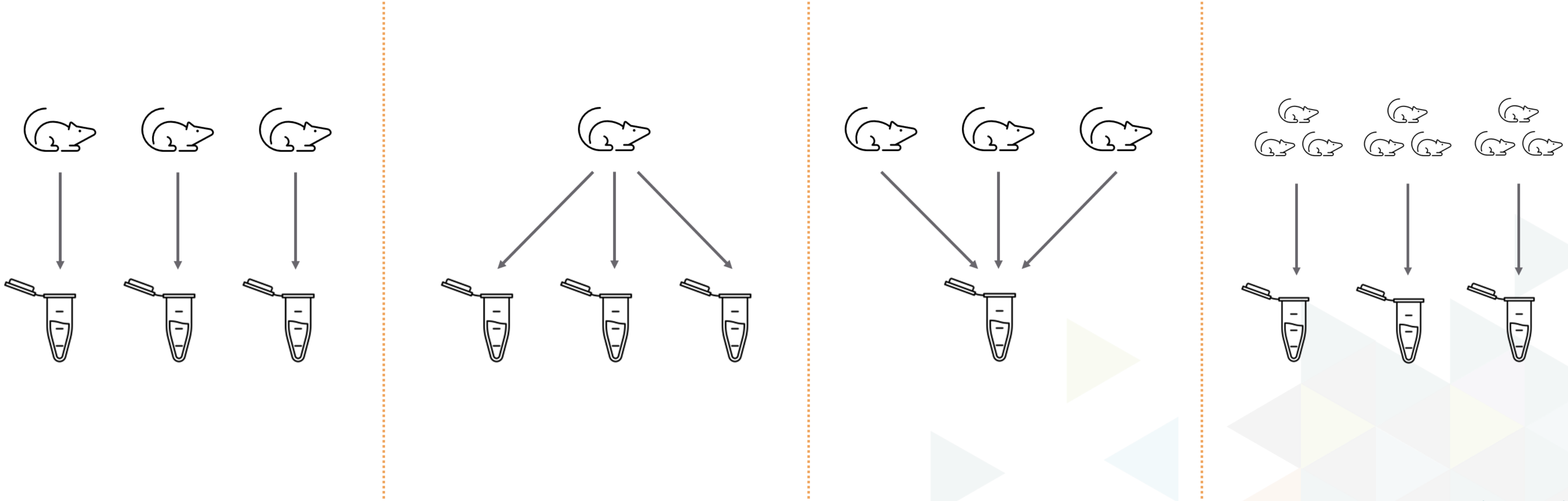
# DATA DEPOSITORY

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

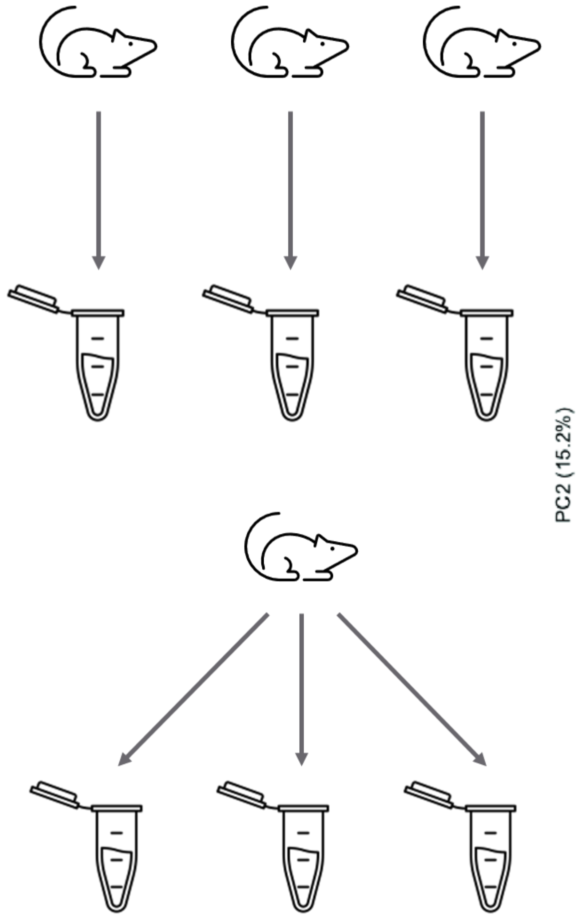
# EXPERIMENTAL DESIGN

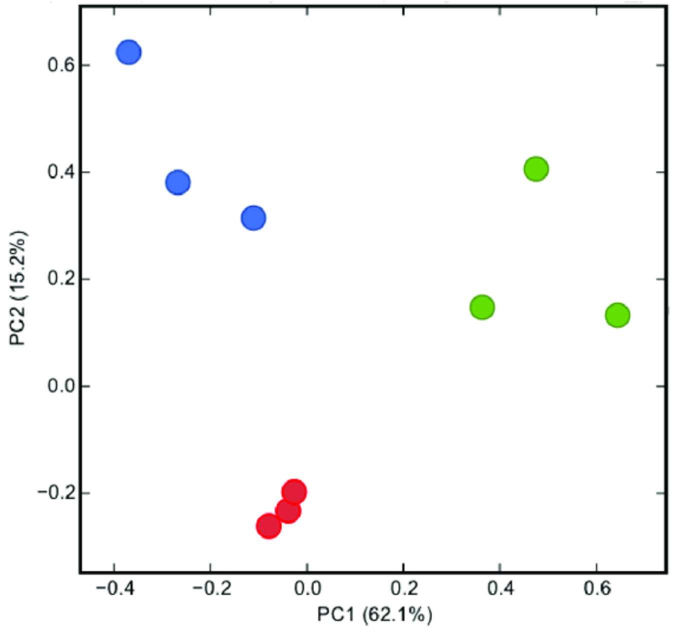
REPLICATES, POOLING & TRACKING

# DESIGN



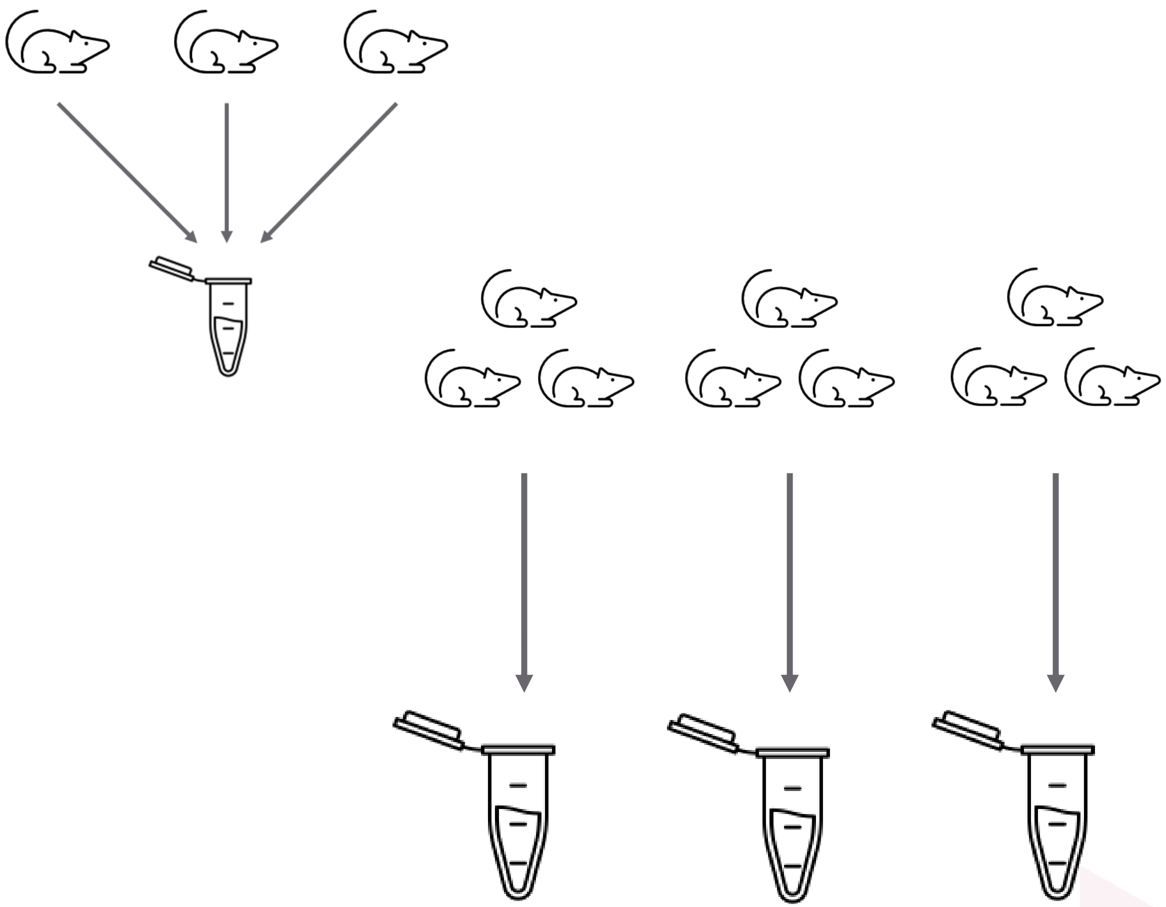
# REPLICATES





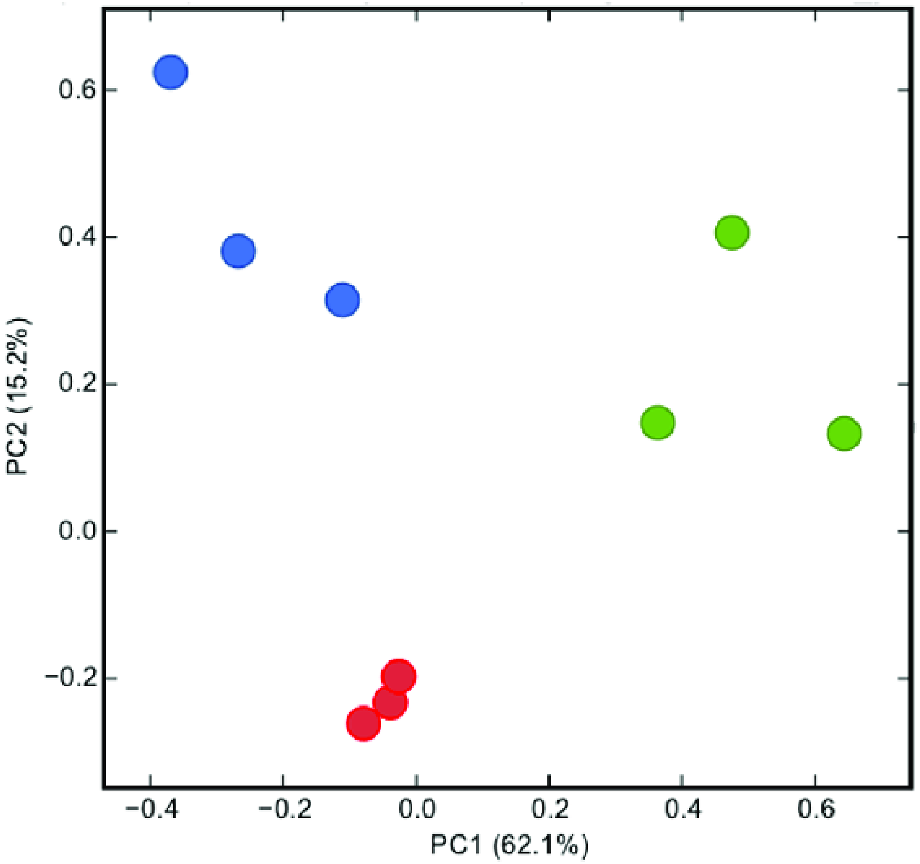
- 3 replicates / condition
  - Intra-variability < Inter-variability
  - Outliers
  - Different sources of variability
- The more, the better
  - Costs ++
- « Statistical test »
  - P-value
  - SE

# POOLING



- Reduces sample variability
  - « More stability »
  - Reduces outlier impact
  - Costs --
- No individual informations
- 1 sample = no statistics

# TRACKING



- « Sample history »
- Very sensitive

- Dissociate biological variability from experiment variability

- Age, Date, Time, Sex, Weigth, …
- Researcher, Kit, Instruments, …
- Observations

LIÈGE université
GIGA
Bioinformatics

# THANK YOU FOR YOUR ATTENTION