MultiQC:
RNA-seq data quality reports

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RNA-seq data analysis

Steps taken so far:

- Generated raw data (FASTQ files)
- Adapter & quality trimming *(fastp)*
- Alignment of reads *(salmon)*
- Generate gene counts *(salmon)*

→ *Seq2science*

seq2science

install with bioconda | Anaconda.org 0.9.5 | downloads 21k | stars 107 | Test Status | docs passing

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Seq2science is the attempt of the *van heeringen lab* to generate a collection of generic pipelines/workflows which can be used by complete beginners to bioinformatics and experienced bioinformaticians alike. Please take a look at our docs for help with installation, how to run it, and best practices.

Our supported workflows:

- Downloading of fastq
- Alignment
- ATAC-seq
- RNA-seq
- ChIP-seq
- scATAC-seq
- scRNA-seq

Performs all of these steps (and many more!) in a reproducible manner

Seq2science

Samples.tsv & config.yaml

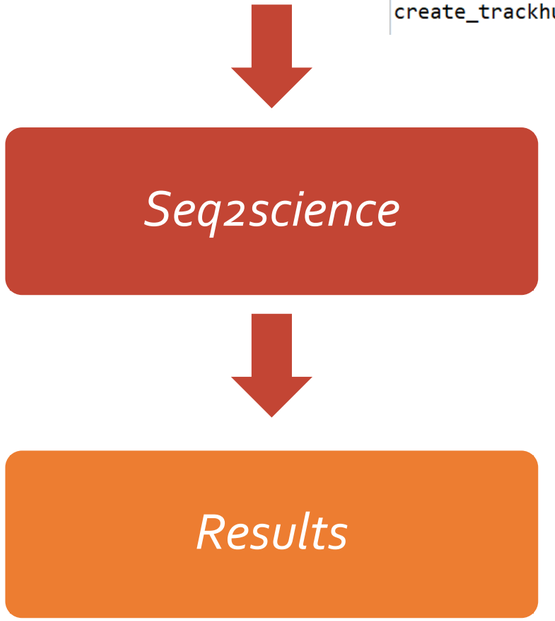
sample	assembly	technical_replicates	descriptive_name
Sample1	GRCh38	Sample1	Heart
Sample1_2	GRCh38	Sample1	Heart
Sample2	GRCh38	Sample2	Lung
Sample2_2	GRCh38	Sample2	Lung
Sample3	GRCh38	Sample3	Brain
Sample3_2	GRCh38	Sample3	Brain

```yaml
# tab-separated file of the samples
samples: samples.tsv

# pipeline file locations
result_dir: ./results  # where to store results
genome_dir: ../genome  # where to look for or download the genomes
fastq_dir: ../fastqs_students  # where to look for or download the fastqs

# contact info for multiqc report and trackhub
email:

# produce a UCSC trackhub?
create_trackhub: true
```



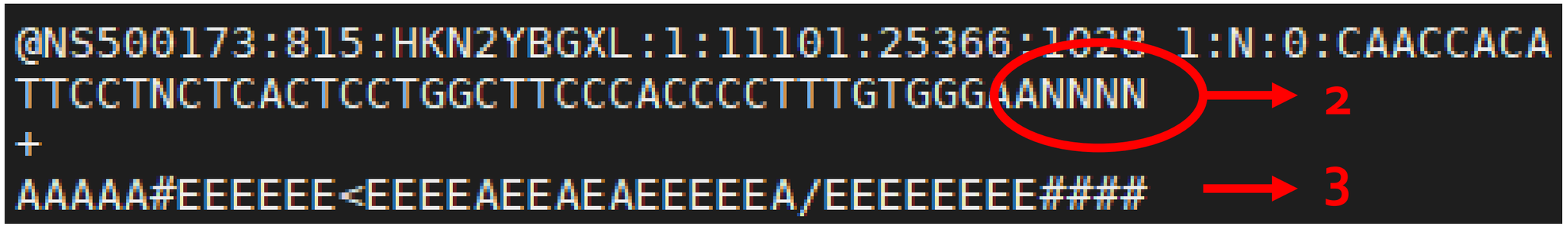
Great! What could possibly go wrong?

Potential problems:

Already taken care of with fastp:

1. Removed adapter reads

2. Removed reads with many low confidence bases (Ns)
3. Removed reads with low quality scores



Other issues to look out for:

Duplicates: reads that maps to the same gene as other reads

- Natural: the same RNA fragment occurs and is sequenced twice
- Artificial: during sequencing a copy of the same read is created and sequenced

Some duplication is expected, but very high rates of duplication may be related to:

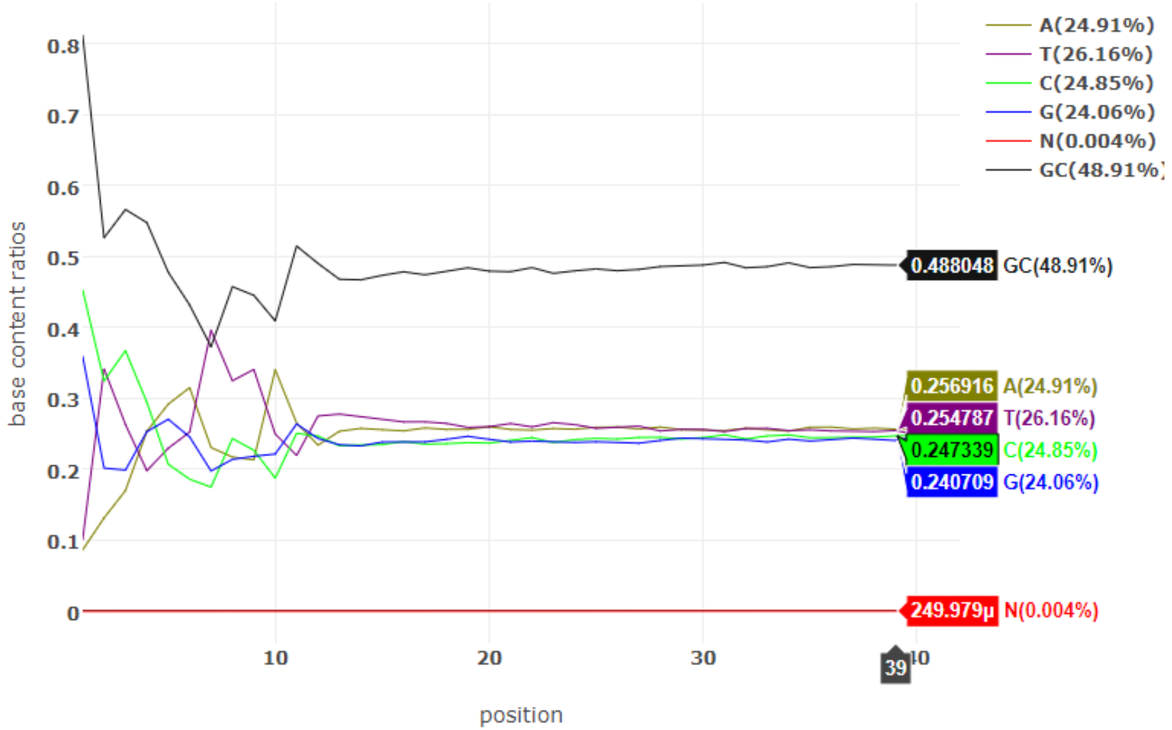
- Low starting material in PCR amplification
- Large variance of fragment size overrepresentation of smaller fragments

→ Duplicates are usually not removed, especially not when there is sufficient library complexity

Other issues to look out for:

Sequence specific bias: random primers used during library prep do not have equal binding efficiencies to each read, regardless of their nucleotide sequence

- For each base across all reads, the percentages of A, C, G, and T should be roughly equal
    - Due to the primers, some bias is expected in the first 10-12 bases



GC content: check whether the GC content matches that expected in your organism

- Generally between 41-50% for humans

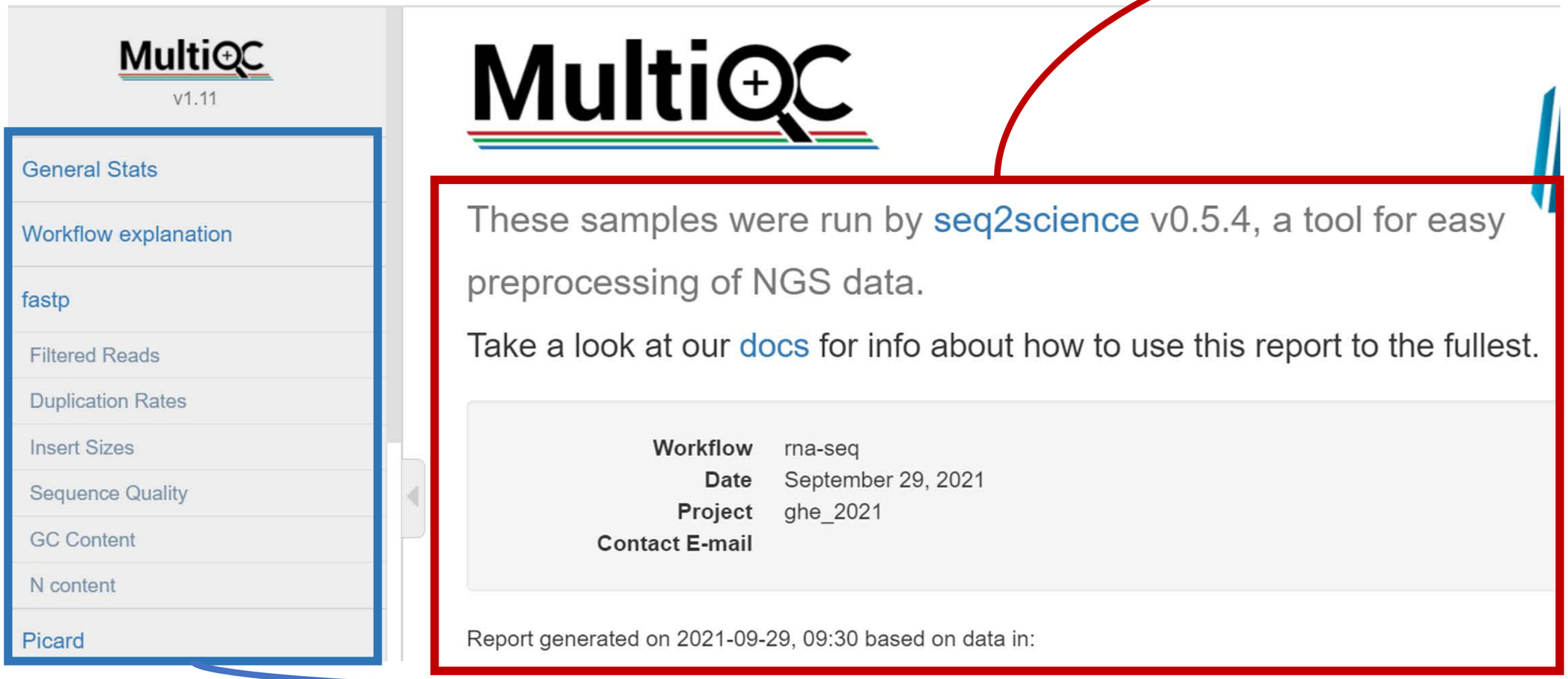
Data Quality Control (QC)

For each processing step that seq2science runs, it generates reports on the quality of your data.

- **MultiQC (also run with seq2science) aggregates results from all these reports and creates a clear overview of all QC metrics**
  - + Interactive
  - + Highly customisable

Example report

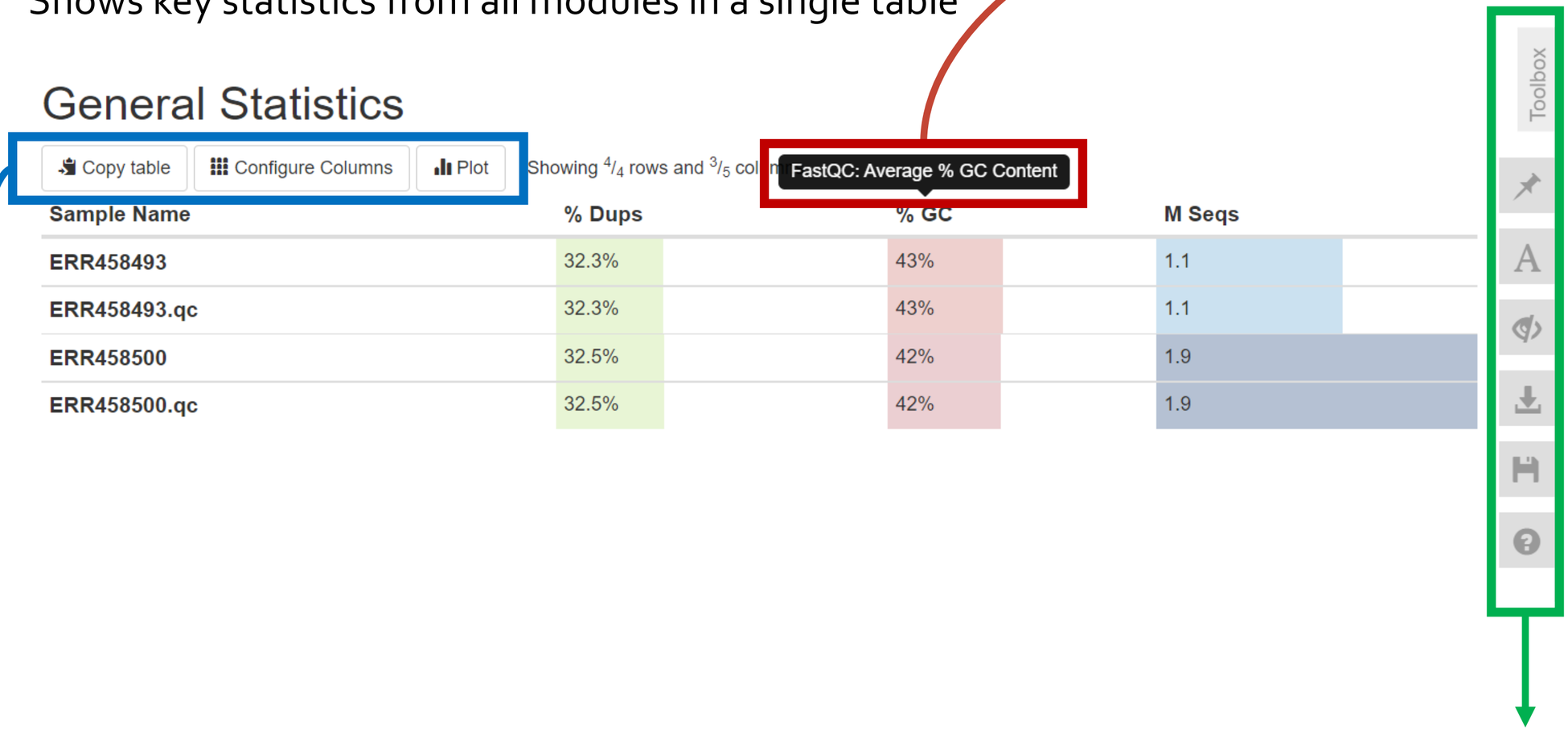
How the report was generated, when, and based on what data



Sections of your report, matching the modules run using seq2science which produce QC metrics.
The 'Workflow explanation' section tells you which modules were used.

General statistics

Shows key statistics from all modules in a single table



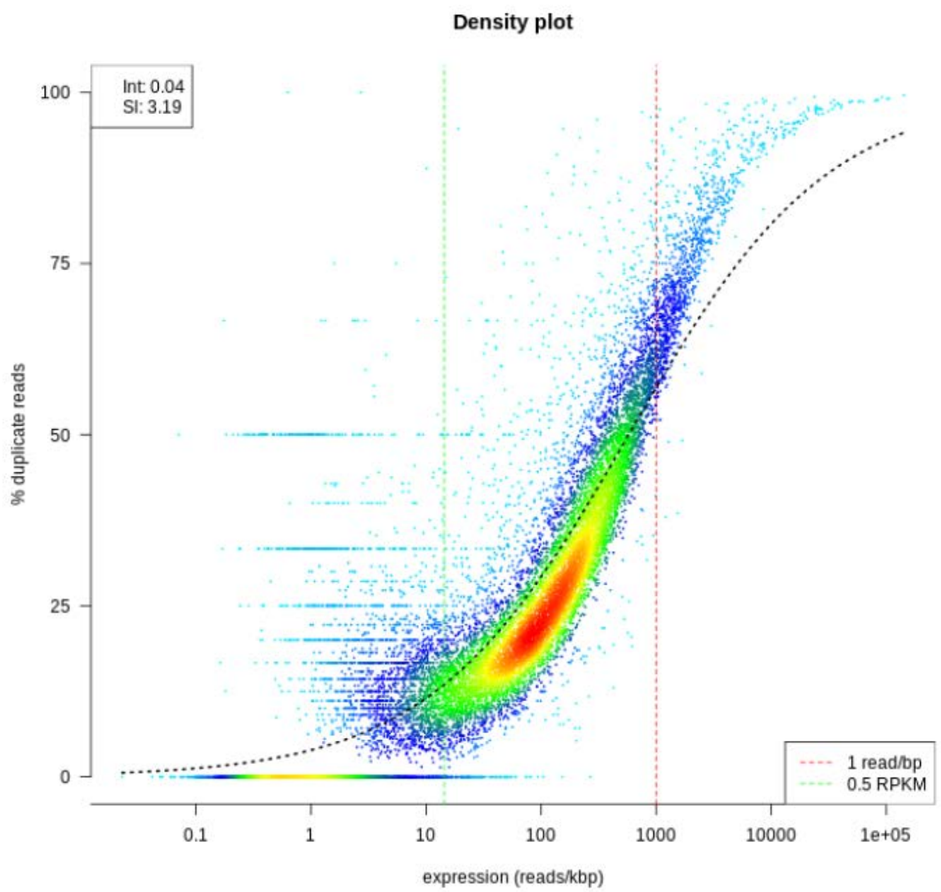
Tells you which module generated this QC metric

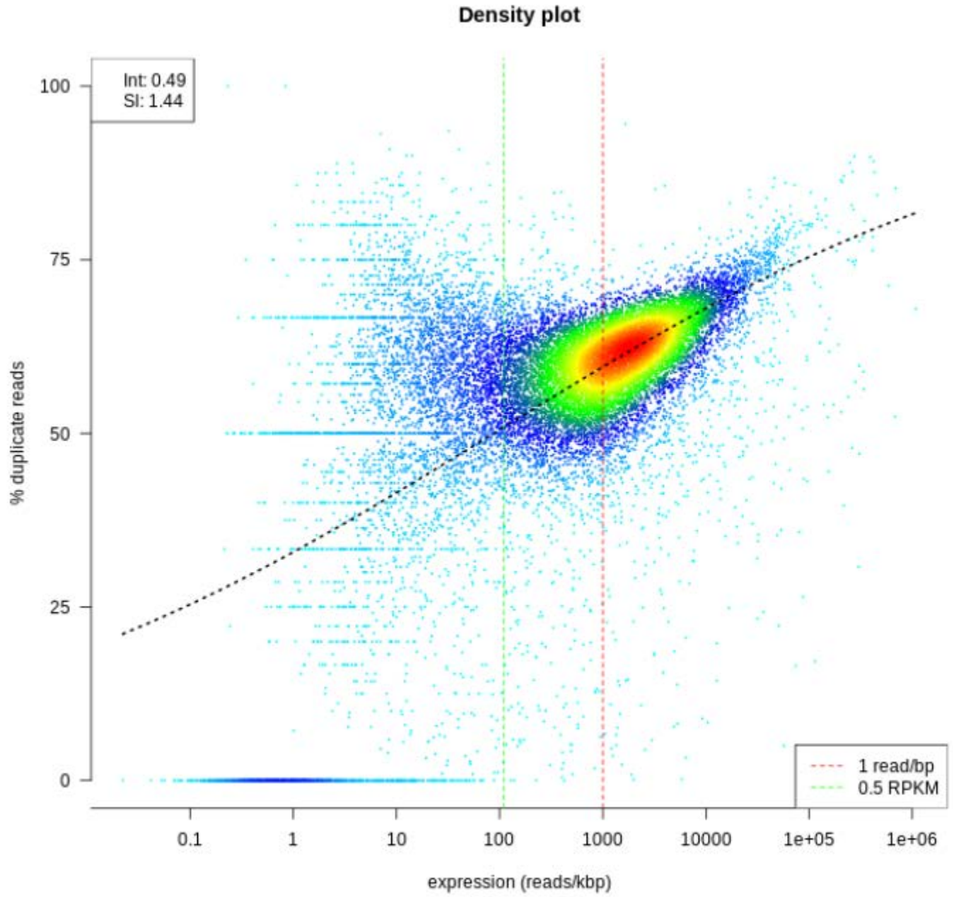
***Interactive**: select which columns to view/compare, and select columns to plot against each other*

Lets you highlight, hide, or rename samples throughout the report

Other QC metrics: dupRadar

- Plots expression of genes versus the number of duplicate reads

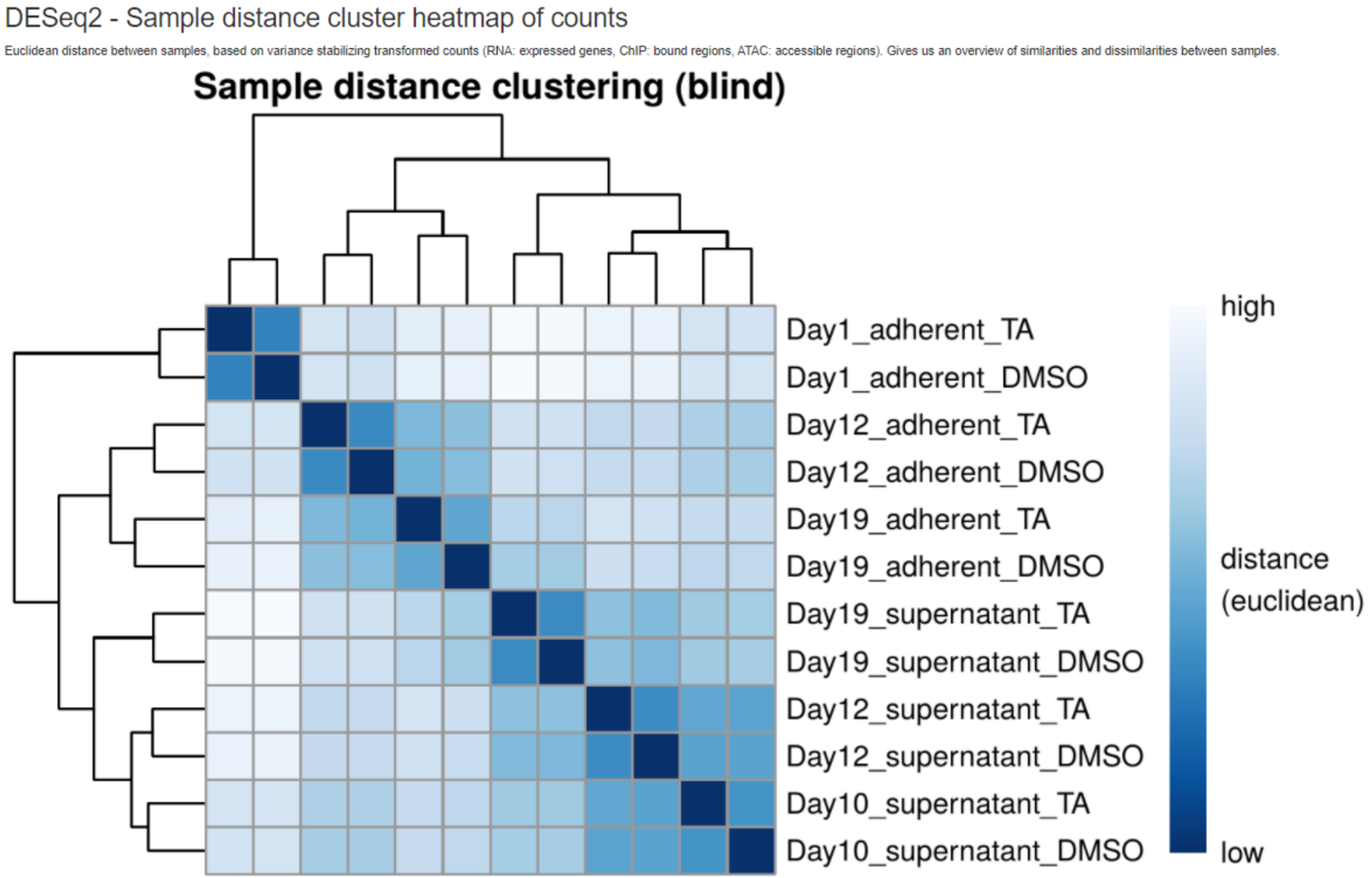




Other QC metrics: Heatmap of counts

Sample clustering

- Another form of quality control
- Shows how different each sample is
  - Samples you expect to be similar should cluster together
- Can reveal
  - Switched samples
  - Contaminations
  - Technical variations



MultiQC reports

- Most reports will show many more QC metrics which you can inspect!
- Some figures and tables have a 

button in the top right corner, which tells you more about what the metric shows and what is expected.

Useful link: https://multiqc.info/docs/

Contains further information on:

- Using and editing multiQC reports (e.g. selecting only samples of interest)
- Modules that you may see metrics from

Your own multiQC report:

https://mbdata.science.ru.nl/ghe_2022/day1/

See whether you can assess the quality of your own data. Are all samples OK for further processing?