After sequencing QC

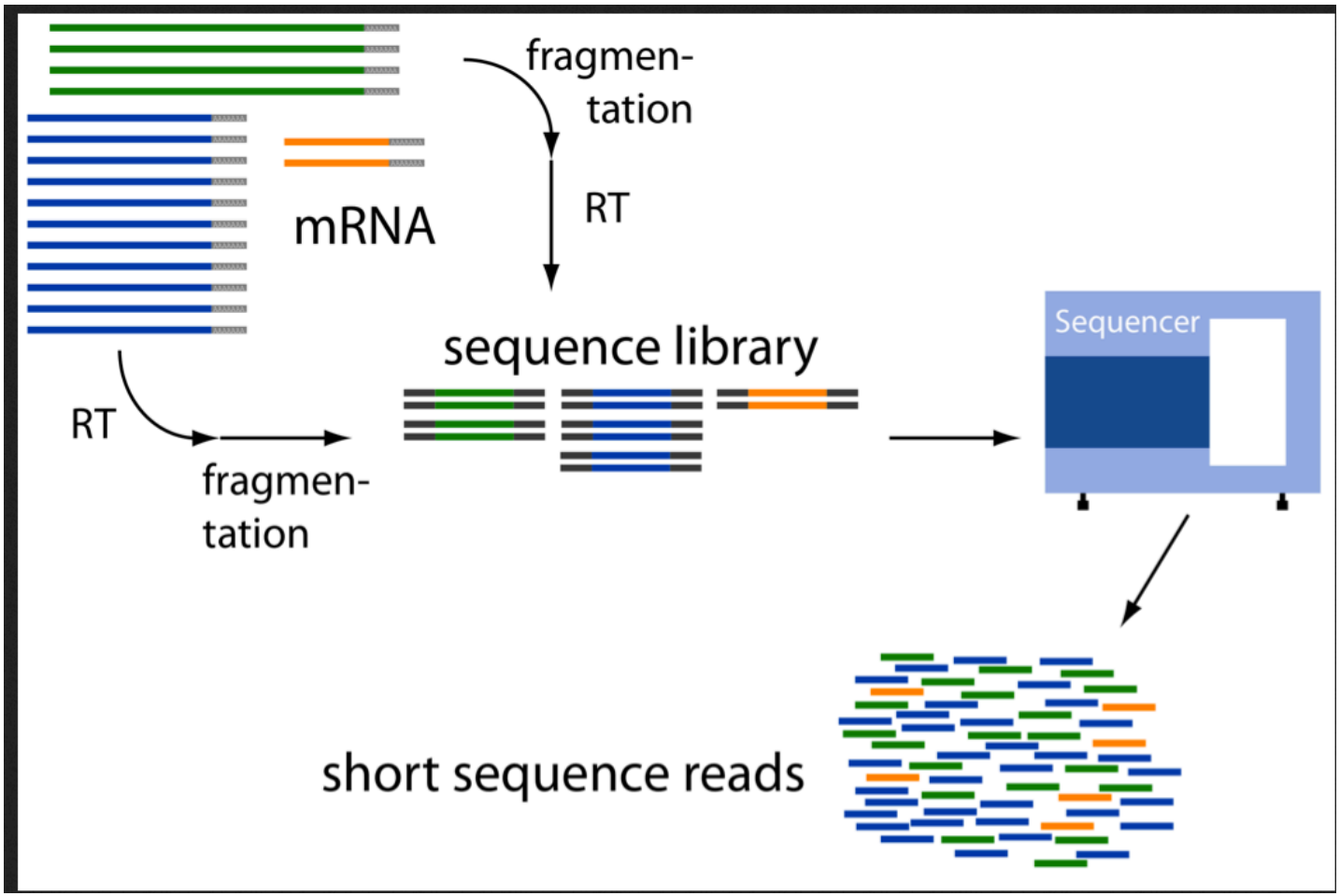
RNA-seq data analysis

Johan Reimegård | 13-May-2019

Overview

- What can affect your data?
- FastQC – read based QC
- RSeQC – mapping based QC
- PCA
- Preventive measurements: spike-in controls, experimental design

RNA-seq libraries



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- RNA quality:

  :es)

- Library prep:

  - Contaminations

- Se

  - Contaminations
  - Sequence complexity

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  - Contaminations (pathogens or other sources)
  - GC-bias
  - Nuclear *vs* organelle reads
- Library prep:
  - Contaminations
- Se
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- Library prep:
  - Failed reactions
  - RNA / Adapter ratios – primer dimers
  - Clonal duplicates
  - Chimeric reads
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- Library prep:
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  - Clonal duplicates
  - Chimeric reads
  - Contaminations
- Sequencing:
  - Base calling errors
  - Uncalled bases
  - Low quality bases (3’ end)
  - Contaminations
  - Sequence complexity

From samples to reads

- may not be what you think they are

- Mixing samples
  - 30 samples with 5 steps from samples to reads has 24 300 000 potential mix ups of samples
  - Error rate 1/ 100 with 5 steps suggest that one of every 20 sample is mislabeled
- Experiments go wrong
  - 30 samples with 5 steps from samples to reads has 150 potential steps for errors
  - Error rate 1/100 with 5 steps suggest that one of every 20 samples the reads does not represent the sample
- Combine the two error sources and approximately one in every 10 samples is wrong

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- How do we understand what went wrong?

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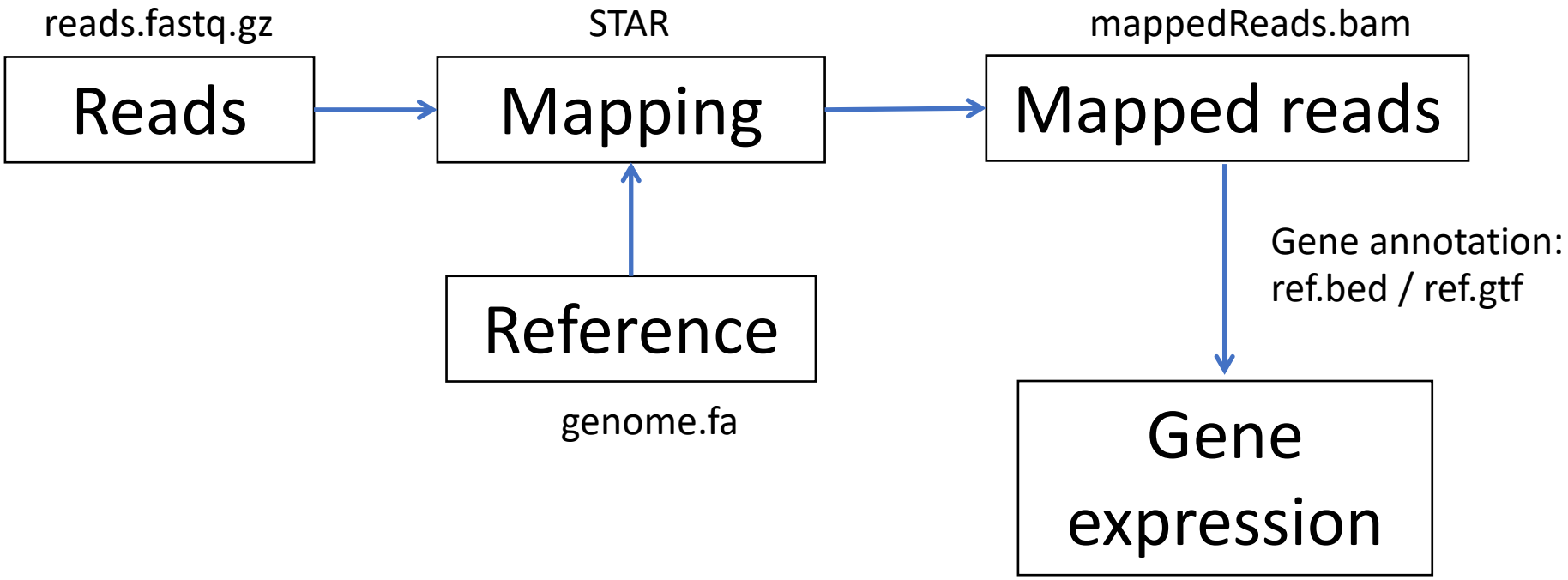
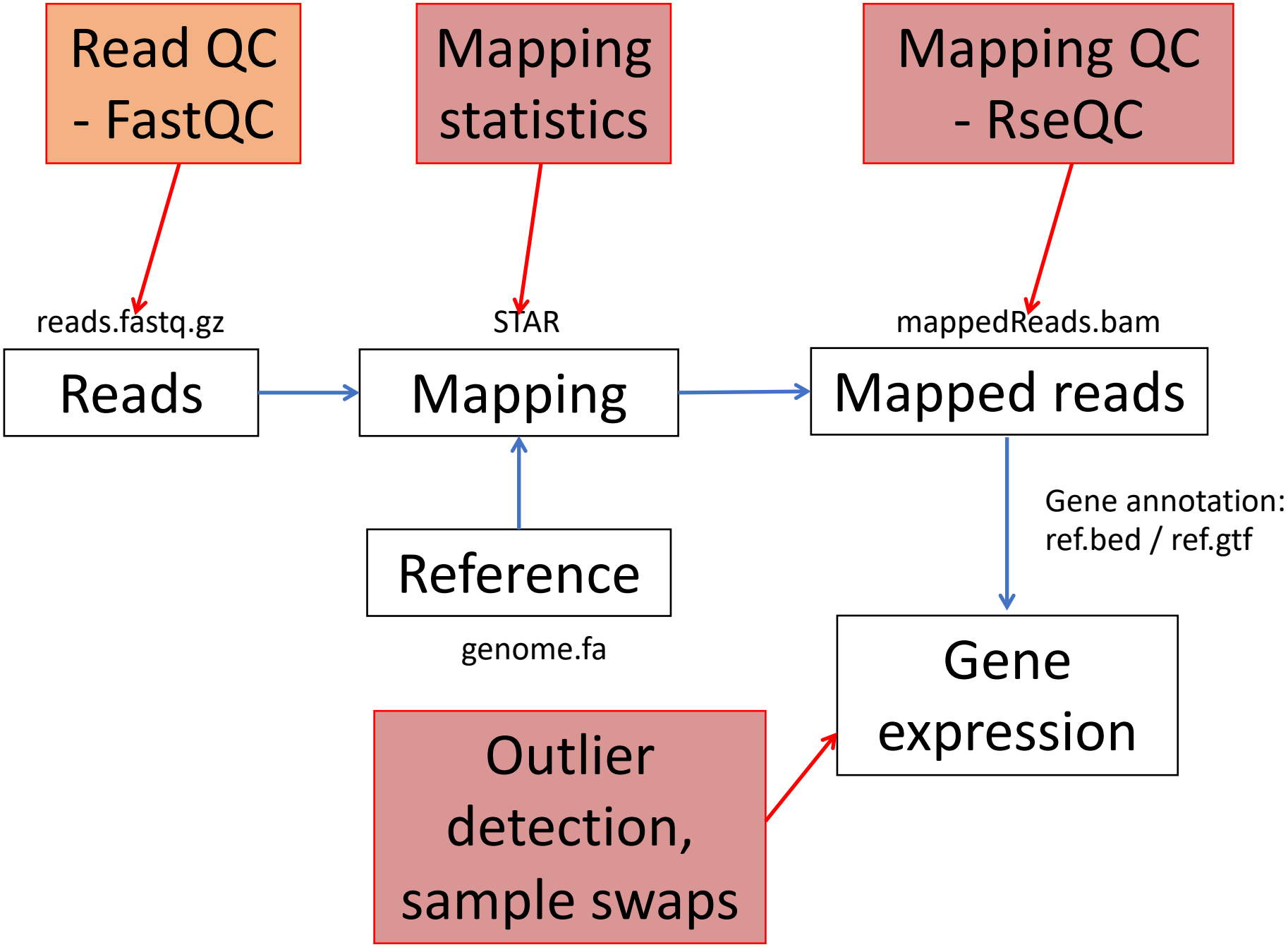
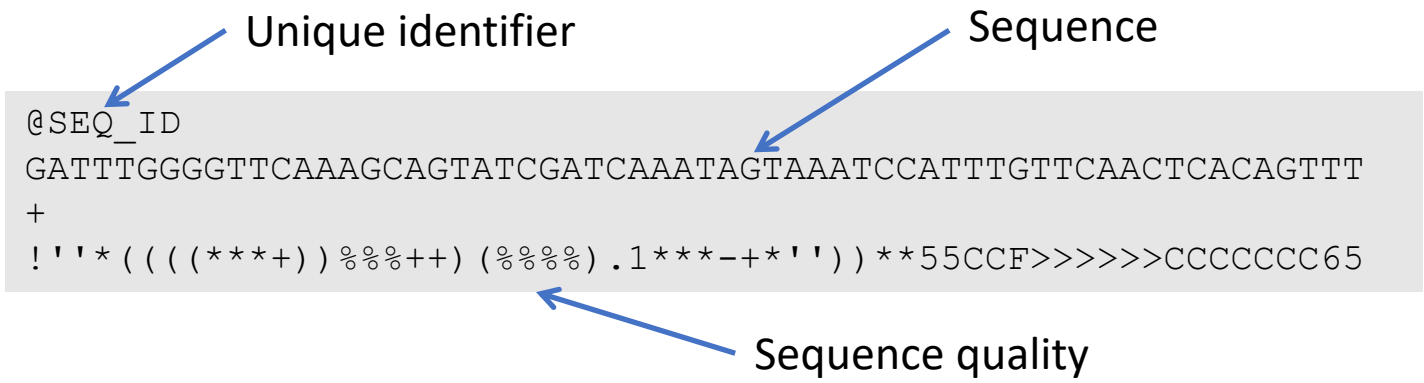
RNA-seq analysis workflow



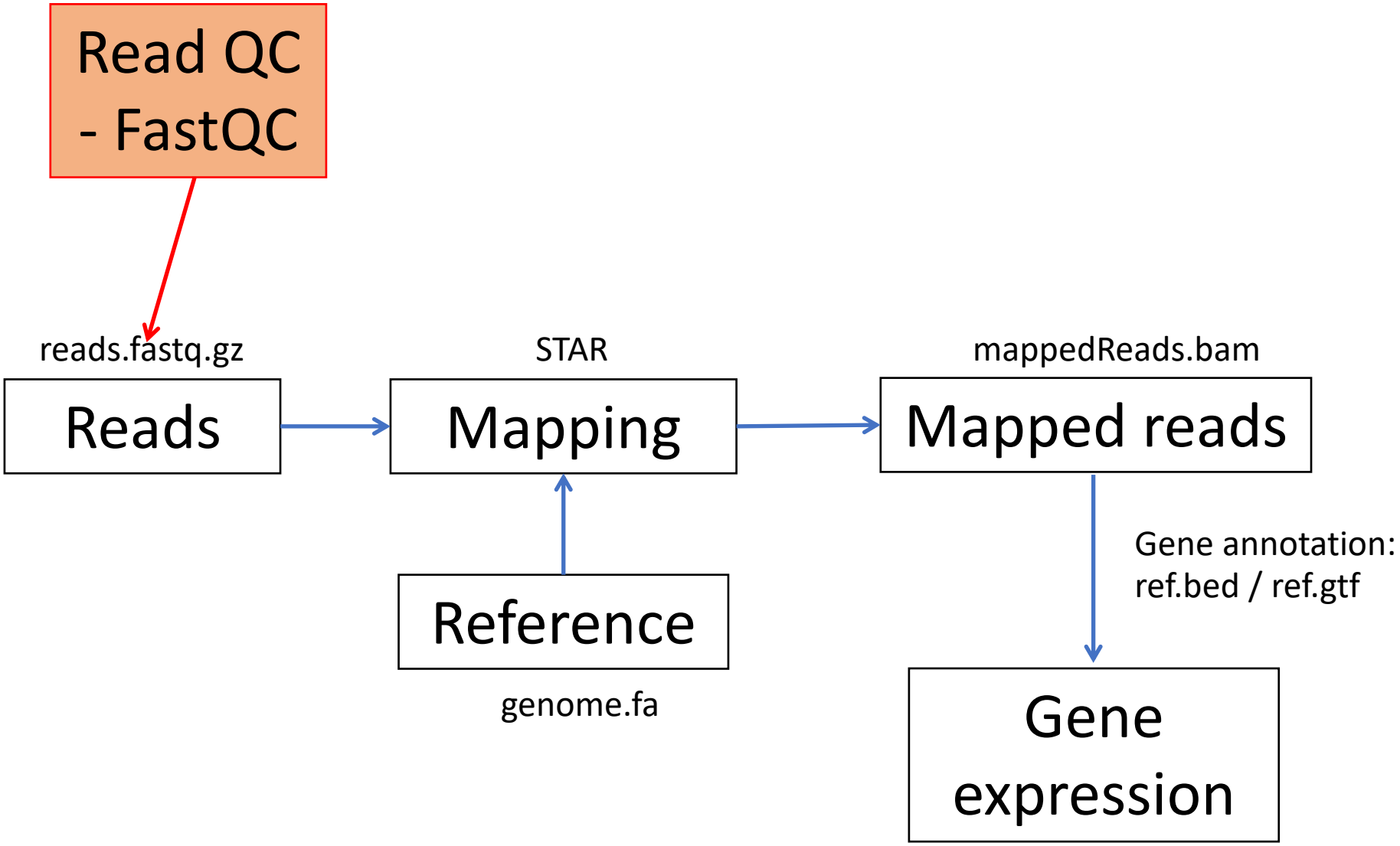

Read QC
- FastQC
Mapping
statistics
Mapping QC
- RseQC
reads.fastq.gz
STAR
mappedReads.bam
Reads
Mapping
Mapped reads
Gene annotation:
ref.bed / ref.gtf
Reference
genome.fa
Gene
expression
Outlier
detection,
sample swaps

Fastq – read file format



Paired end data usually in format sampleX_1.fastq and sampleX_2.fastq with same SEQ_ID for both mate pairs, followed by /1 and /2 (or _f and _r)

Fastq – read file format


Read QC
- FastQC
reads.fastq.gz
Reads
STAR
Mapping
Reference
genome.fa
mappedReads.bam
Mapped reads
Gene annotation:
ref.bed / ref.gtf
Gene
expression

Basic read metrics with FastQC

A program that analyses some of the basic metrics on fastq raw read files.

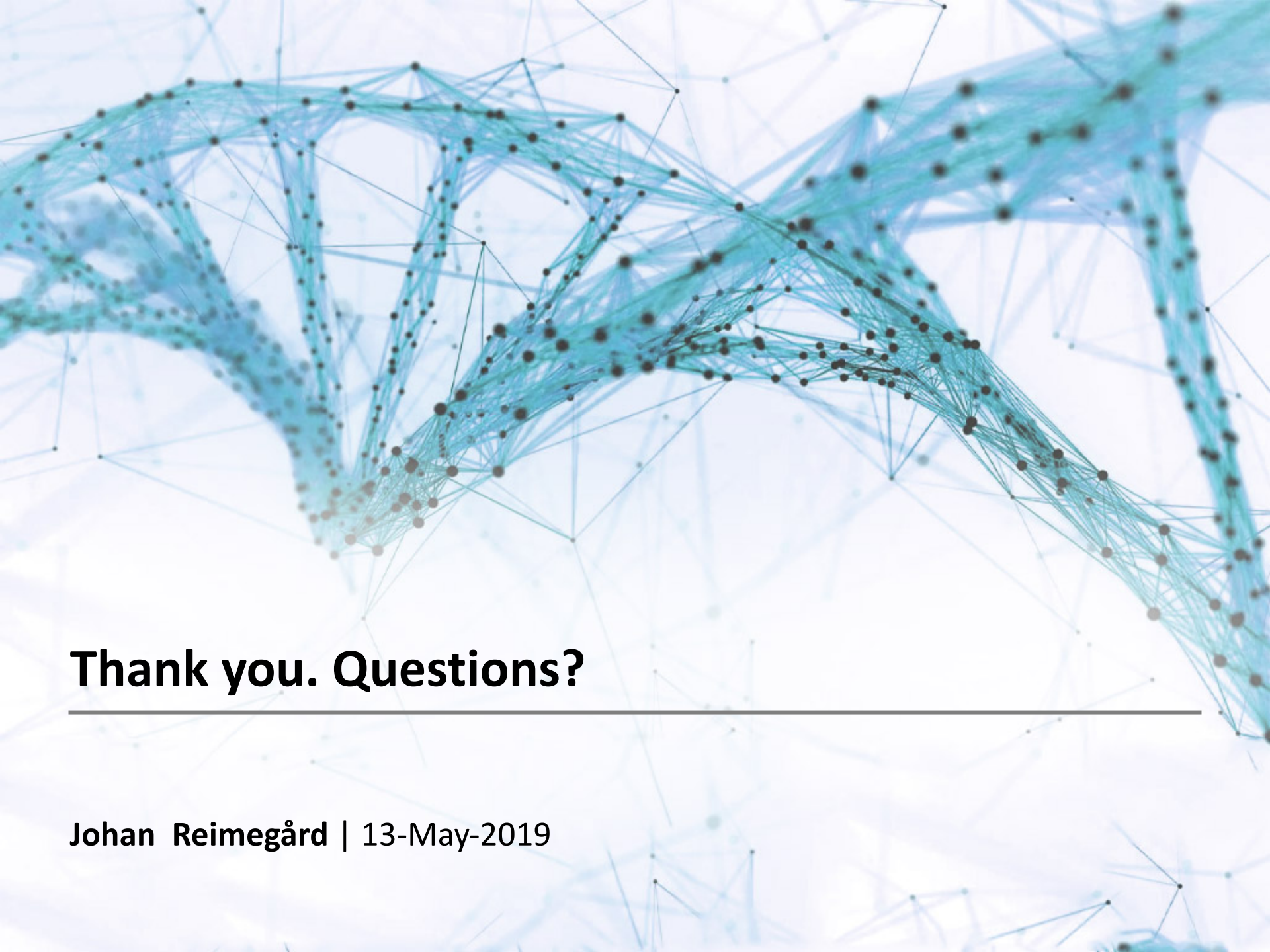
- Quality
- Length
- Sequence bias
- GC content
- Repeated sequences
- Adapter contamination

Code

```
$ module load bioinfo-tools
$ module load FastQC/0.11.2

$ fastqc –o outdir seqfile.fastq
# multiple files:
$ fastqc –o outdir seqfile_*.fastq
```

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



Thank you. Questions?

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