CEITEC

Central European Institute of Technology

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Modern Genomic Technologies (LF:DSMGT01)

Lecture 2 : NGS libraries and basic data quality control

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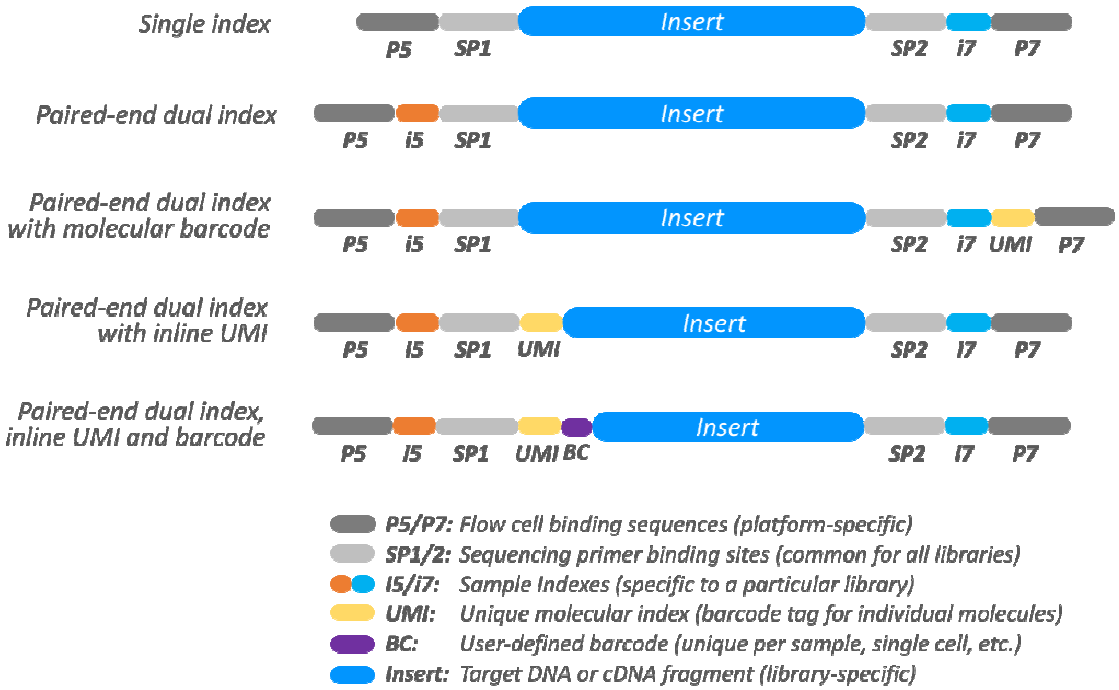
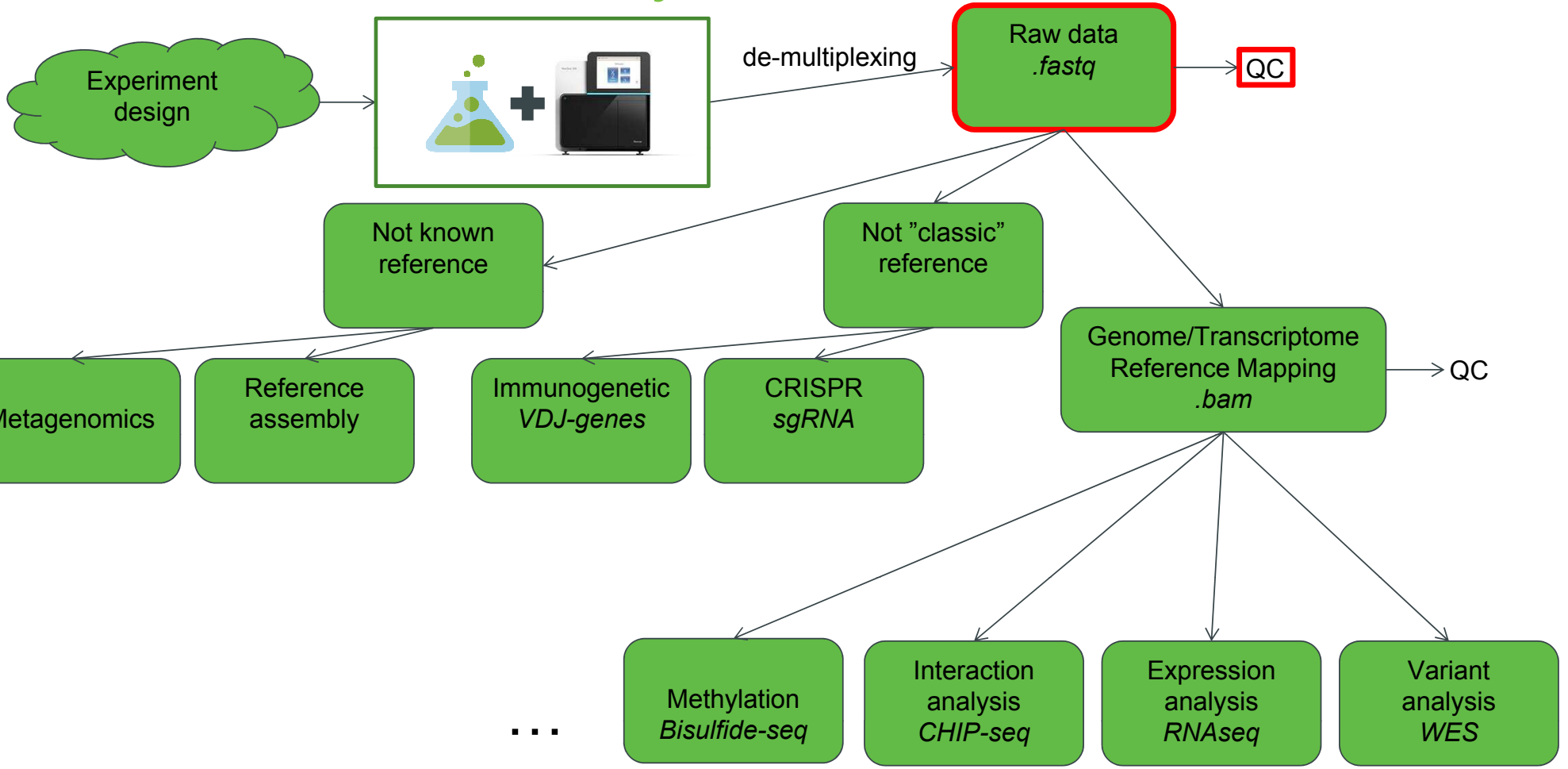
De-multiplexing

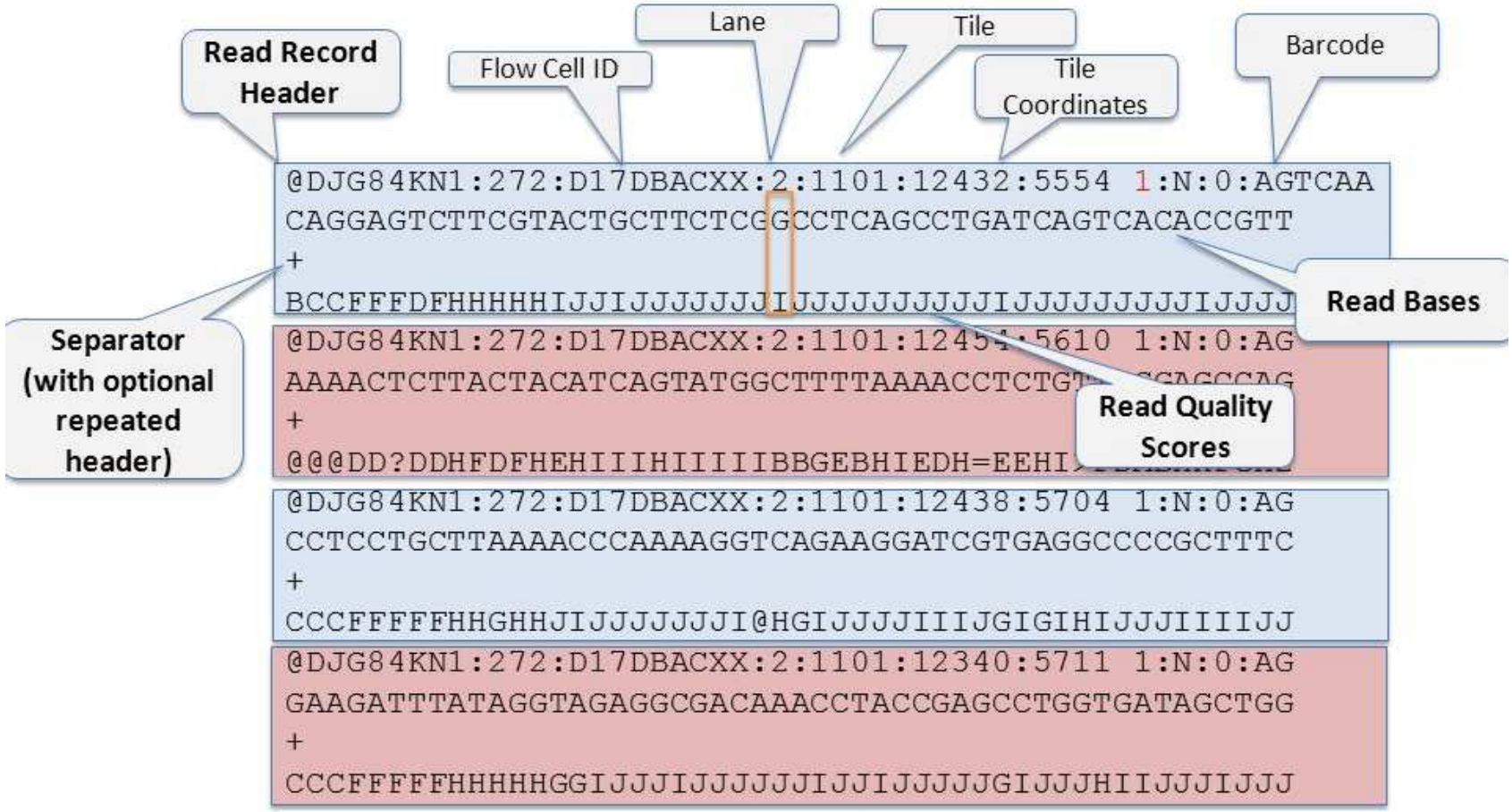
- Bcl2fastq tool
  - *Needs sample sheet with indexes*
  - *Number of barcode mismatches*
    - *Check undetermined*



NGS data analysis



Primary data – fastq file



NOTE: for paired-end runs, there is a second file with one-to-one corresponding headers and reads.

Fastq format - quality

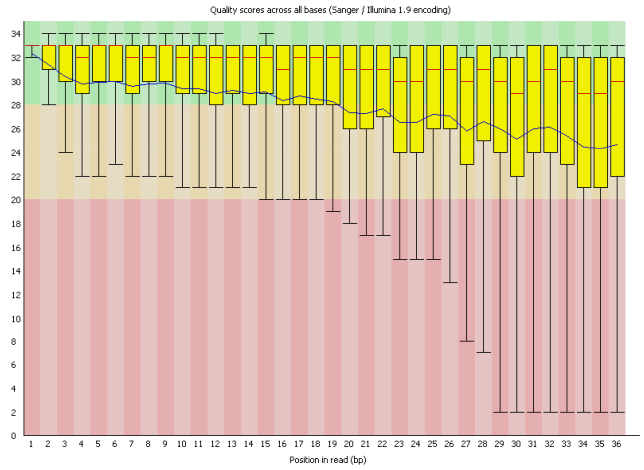
- **Fastq - q stands for quality – coded phred score**

```
CFFFFEFFGCEEGECFGGGGAFF87@E:++6C<++3:,8,33,,:,,,:,,:,,,
```

$$Q = -10 \cdot \log_{10} P$$

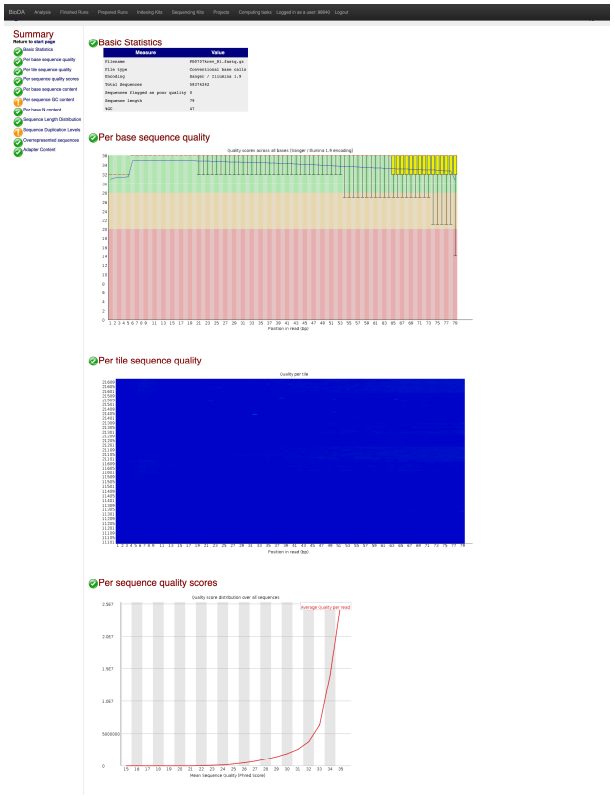
Quality	Error probability
5	31%
10	10%
20	1%
30	0.1%

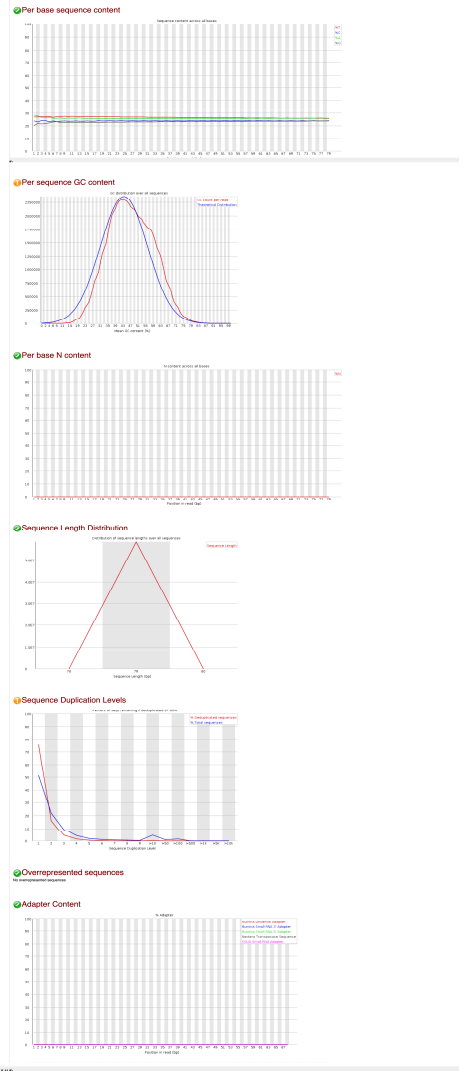
- **Very good for early problem detection**
- **Reasonable for trimming and read filtering**
  - **RNA seq - above phred score 5**



Fastq – quality control

- Fastqc - tool





FastQC Report

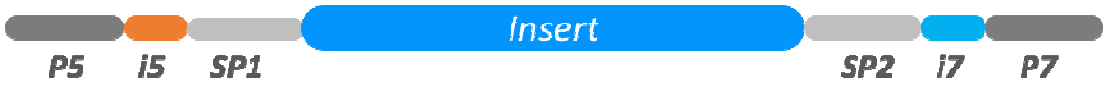
Summary

Return to start page

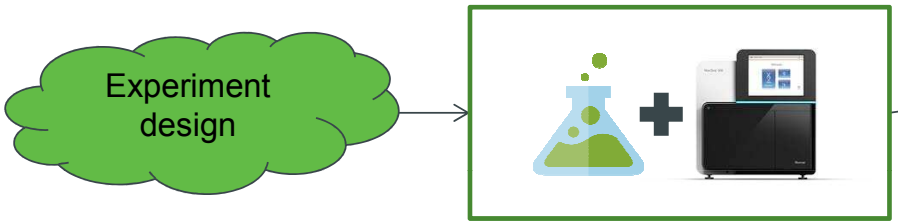
- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

Basic Statistics

Measure	Value
Filename	MU_a_ytHl_R1.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	252819865
Sequences flagged as poor quality	0
Sequence length	161
%GC	40



NGS data analysis



Experiment design

de-multiplexing

Raw data
.fastq

QC

Not known reference

Not "classic" reference

Metagenomics

Reference assembly

Immunogenetic
VDJ-genes

CRISPR
sgRNA

Genome/Transcriptome Reference Mapping
.bam

QC

...

Methylation
Bisulfide-seq

Interaction analysis
CHIP-seq

Expression analysis
RNAseq

Variant analysis
WES

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Thank you for your attention!

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