NGS - quality control, alignment, visualisation

Quality control + database retrieval

Why Quality control?

1. How is the base quality?
2. What is the read length?
3. Are there adapters/barcodes in my sequences?
4. Are there overrepresented sequences?

Dedicated software

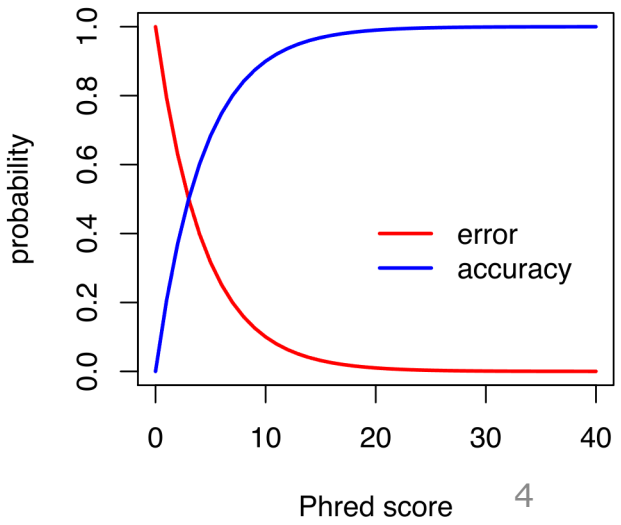
- Manufacturers' software
- Illumina: fastQC
- ONT: pycoQC
- ONT + PacBio: NanoPlot

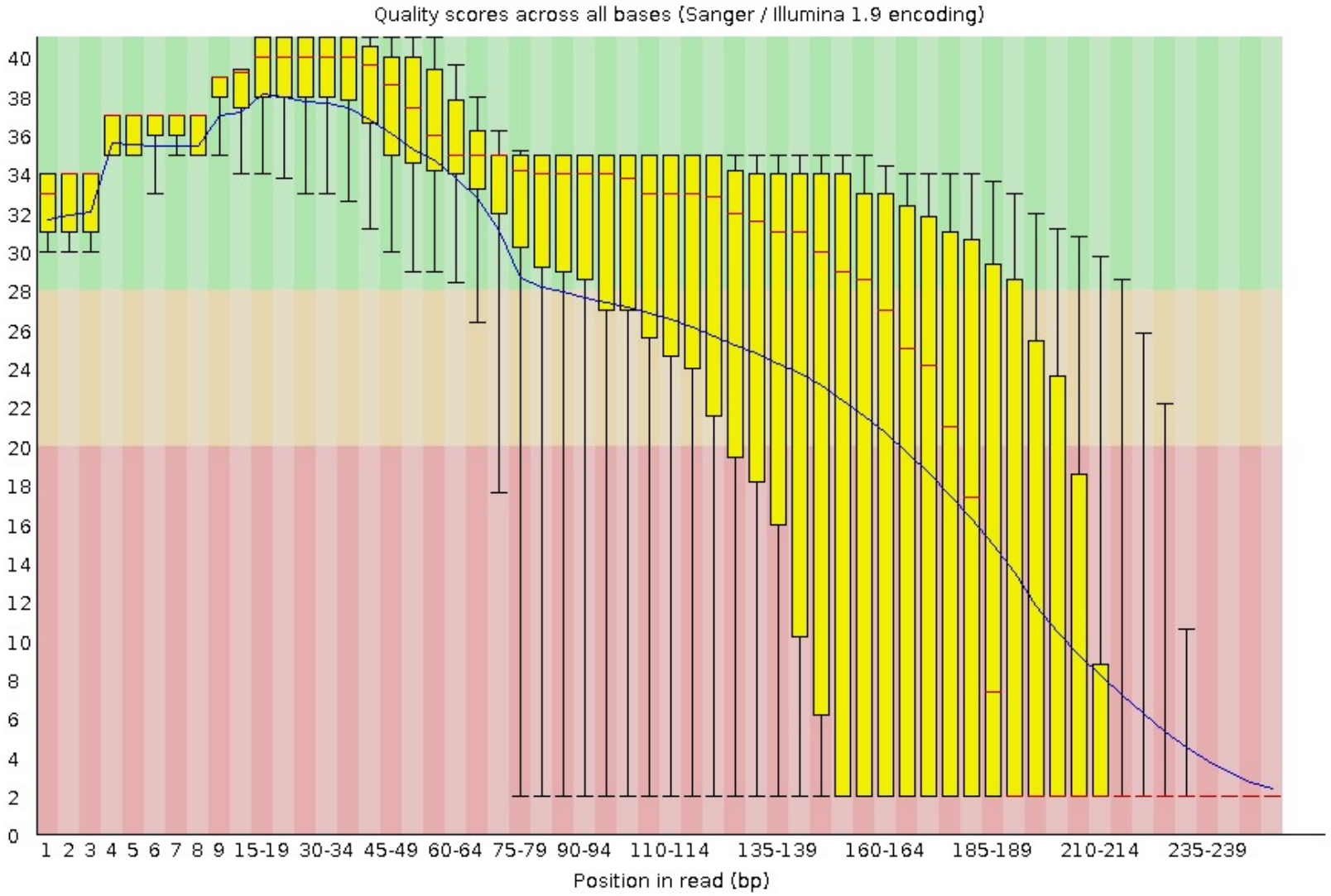
fastq

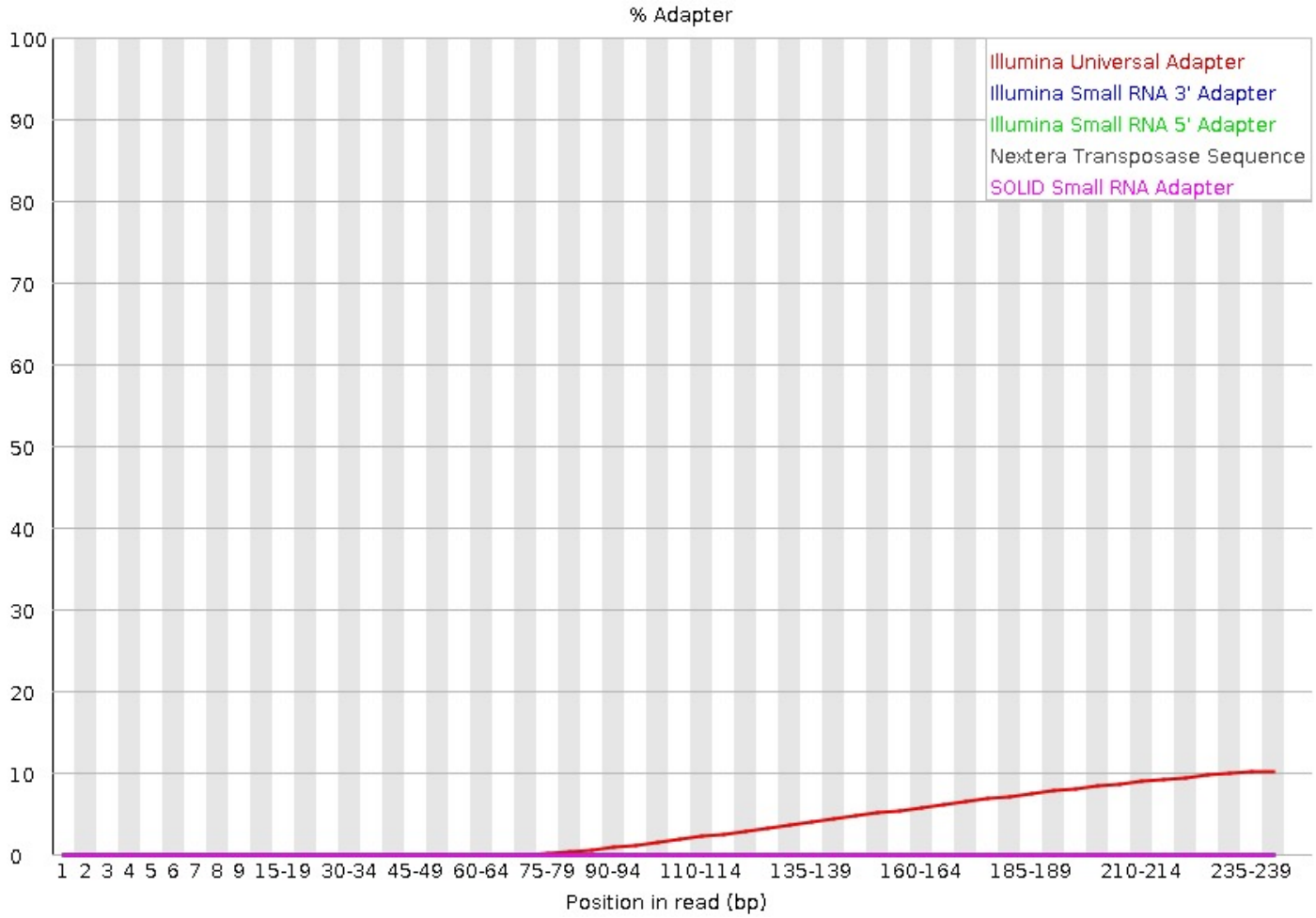
fasta + basequality (fasta + q = fastq)

$$BASEQ = -10log_{10}\Pr\{base\ is\ wrong\}$$

$$-10log_{10}(0.01) = 20$$
$$-10log_{10}(0.1) = 10$$
$$-10log_{10}(0.5) = 3$$

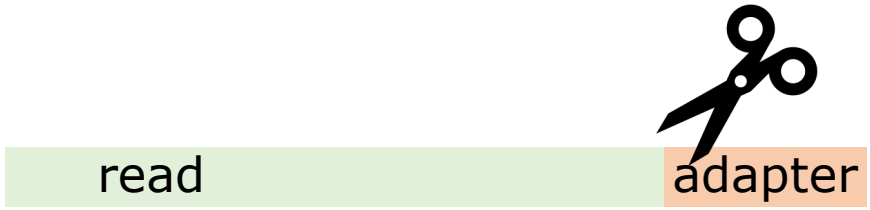



Quality scores across all bases (Sanger / Illumina 1.9 encoding)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 15-19 30-34 45-49 60-64 75-79 90-94 110-114 135-139 160-164 185-189 210-214 235-239
Position in read (bp)


% Adapter
100
90
80
70
60
50
40
30
20
10
0
Illumina Universal Adapter
Illumina Small RNA 3' Adapter
Illumina Small RNA 5' Adapter
Nextera Transposase Sequence
SOLID Small RNA Adapter
1 2 3 4 5 6 7 8 9 15-19 30-34 45-49 60-64 75-79 90-94 110-114 135-139 160-164 185-189 210-214 235-239
Position in read (bp)

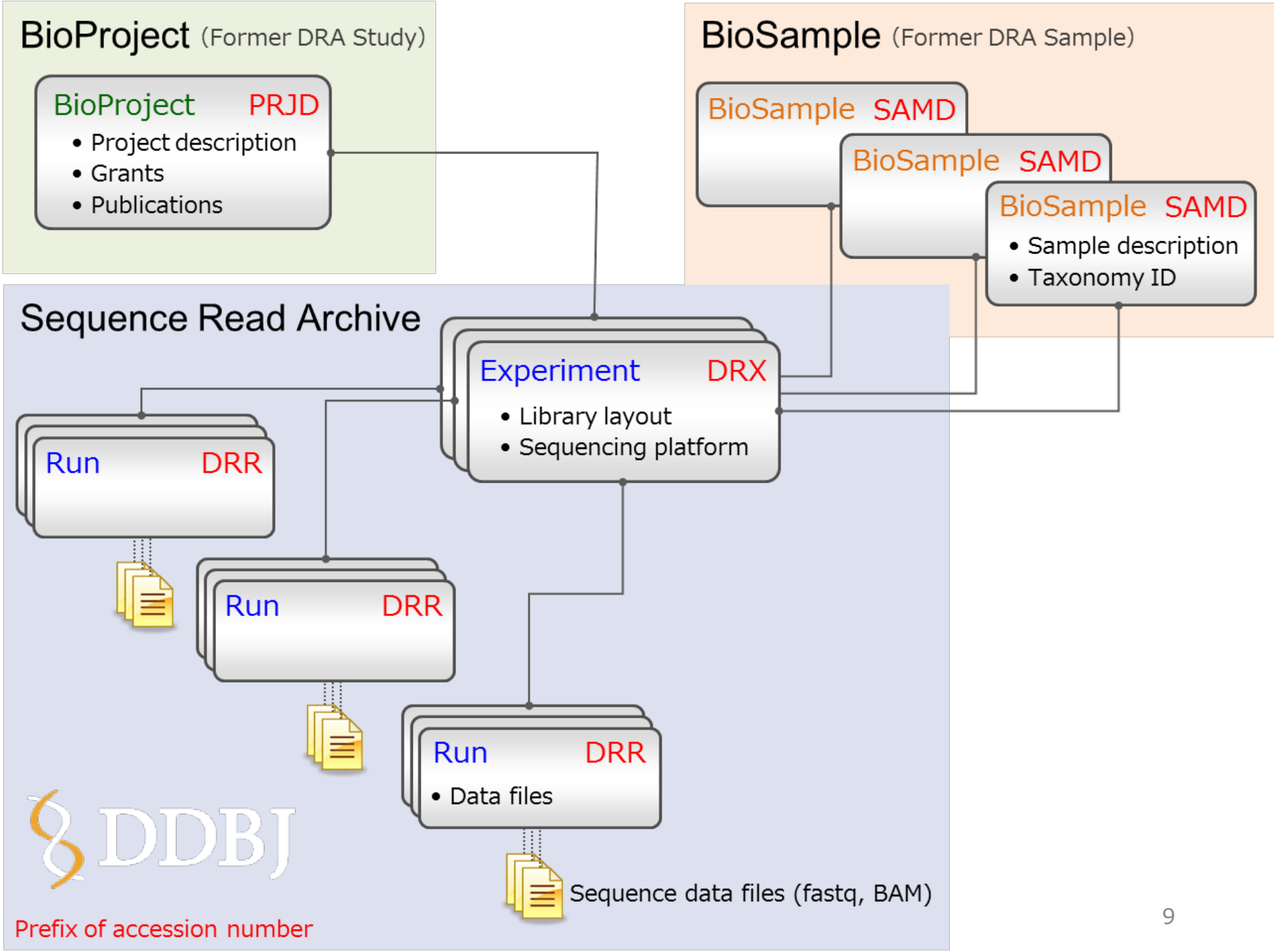
Trimming

- Find and remove:
  - Regions or reads with low base quality
  - Adapter sequences
- Software: `cutadapt` (or `trimmomatic`, `trim_galore`, `bbduk ..`)



Databases

INSDC: **I**nternational **N**ucleotide **S**equence **D**atabase **C**ollaboration


BioProject (Former DRA Study)
BioProject PRJD
• Project description
• Grants
• Publications
BioSample (Former DRA Sample)
BioSample SAMD
BioSample SAMD
BioSample SAMD
• Sample description
• Taxonomy ID
Sequence Read Archive
Experiment DRX
• Library layout
• Sequencing platform
Run DRR
Run DRR
Run DRR
• Data files
DDBJ
Sequence data files (fastq, BAM)
Prefix of accession number

Command line tools

- Retrieve raw data: SRA-tools
  - `prefetch`
  - `fastq-dump`
- Retrieve sequences: Entrez Direct
  - `esearch`
  - `efetch`