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

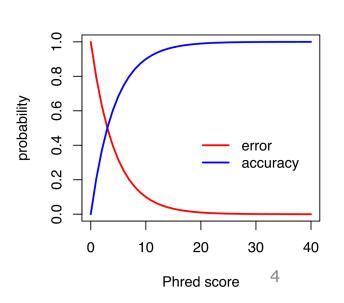
fasta + basequality (fasta + q = fastq)

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

$$-10log_{10} (0.01) = 20$$

$$-10log_{10} (0.1) = 10$$

$$-10log_{10} (0.5) = 3$$



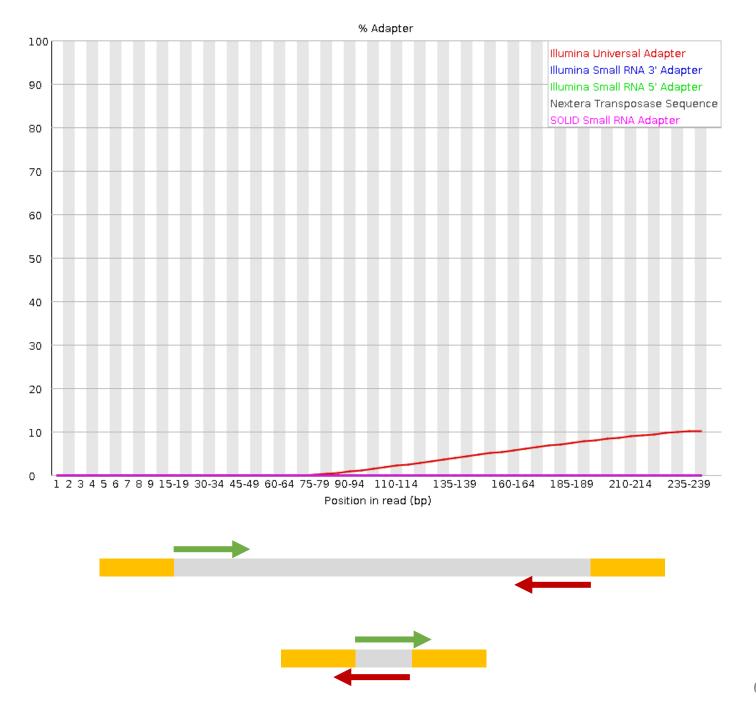
Quality scores across all bases (Sanger / Illumina 1.9 encoding)

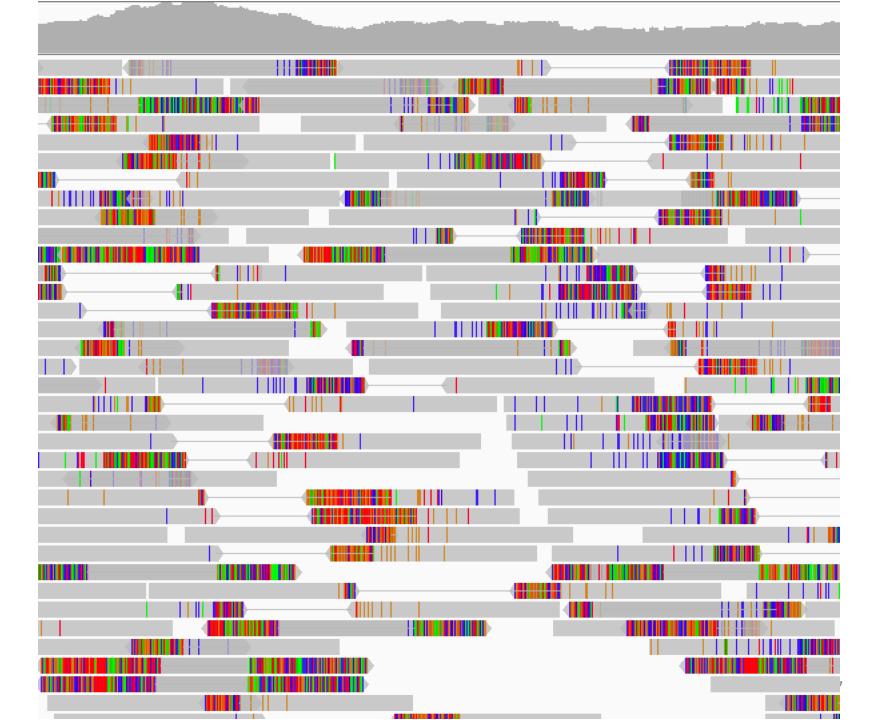
Position in read (bp)

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

235-239

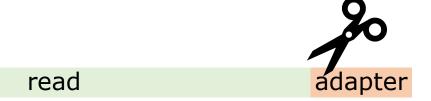
210-214





Trimming

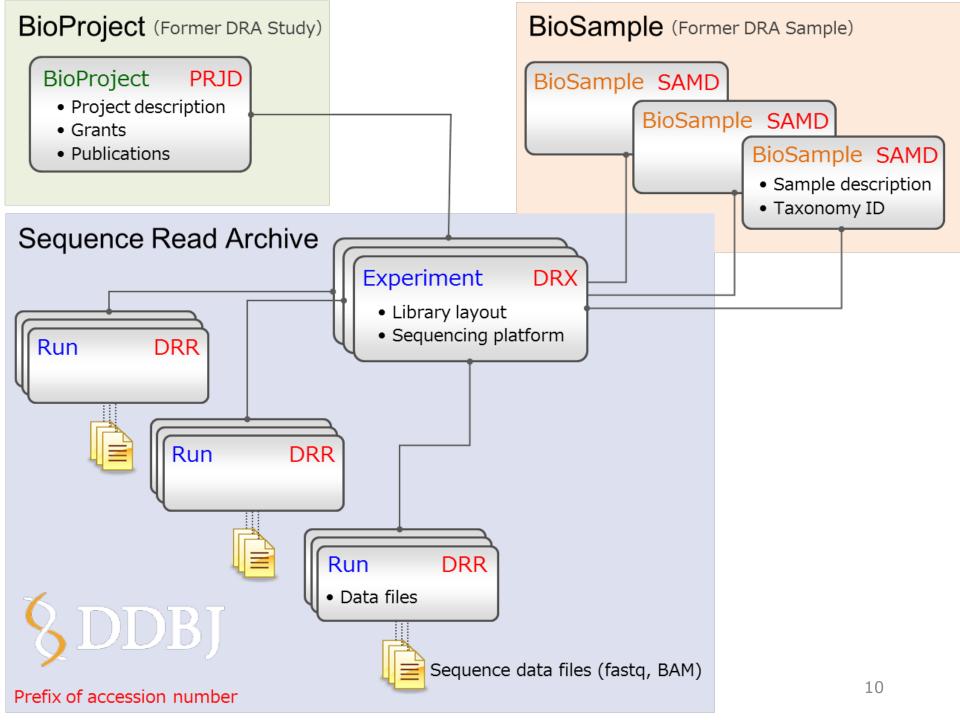
- Find and remove:
 - Regions or reads with low base quality
 - Adapter sequences
- Software: cutadapt (or trimmomatic, trim_galore, bbduk ..)



Databases



INSDC: International Nucleotide Sequence Database Collaboration 9



Command line tools

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