# 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

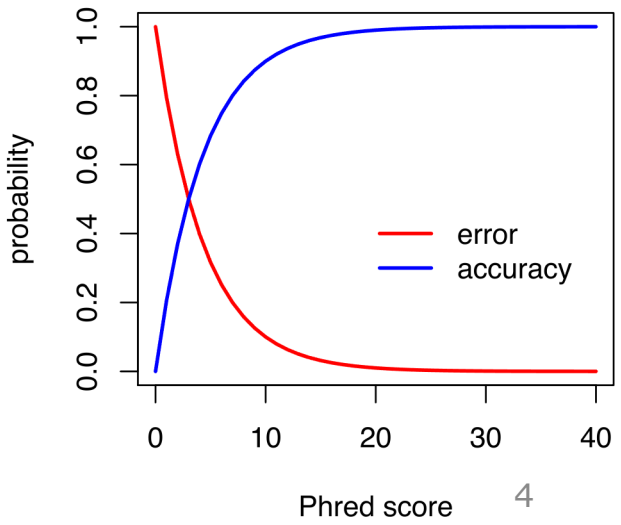
- Manufacturers' software
- Illumina: fastQC
- ONT: pycoQC
- ONT + PacBio: NanoPlot
- ..

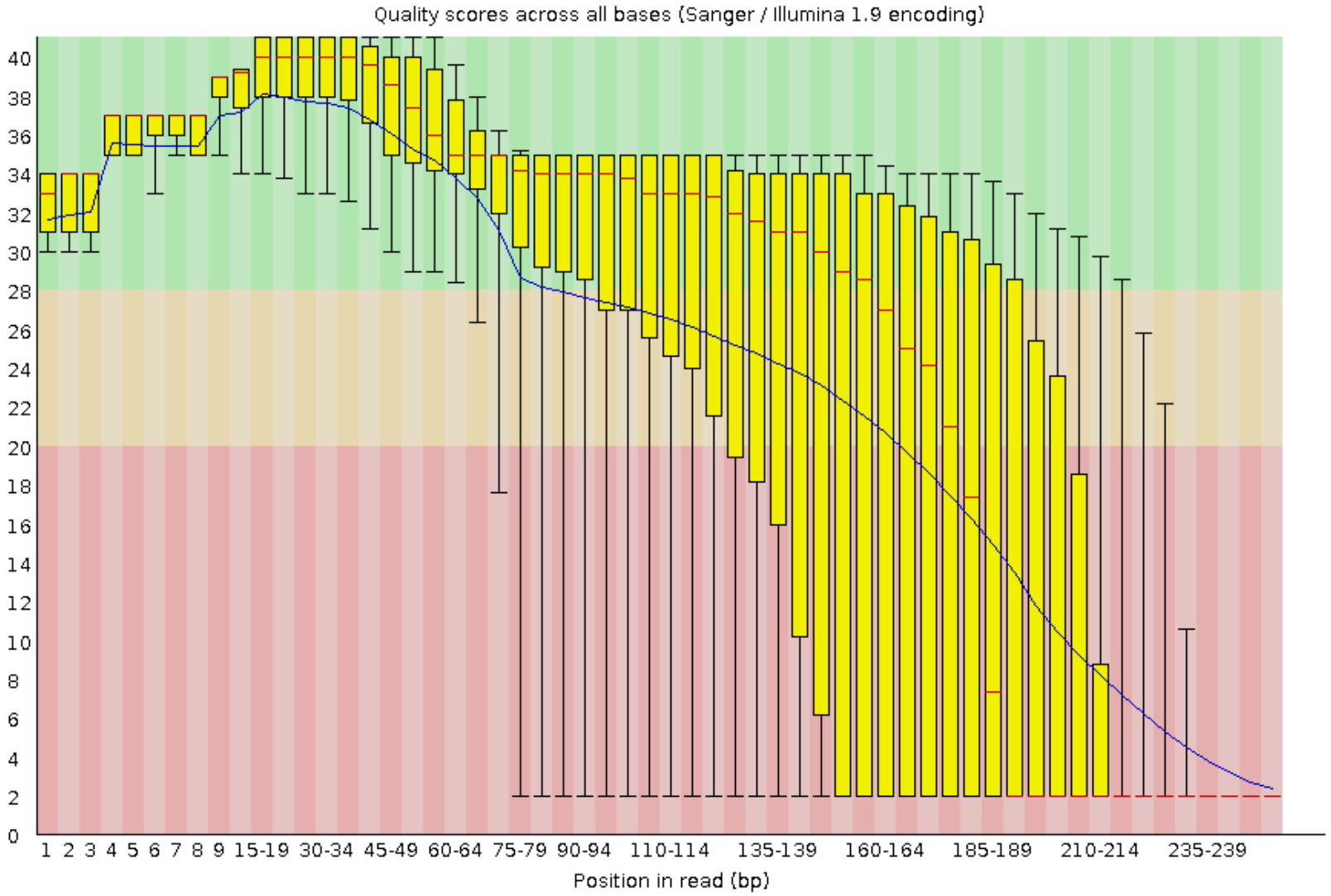
# fastq

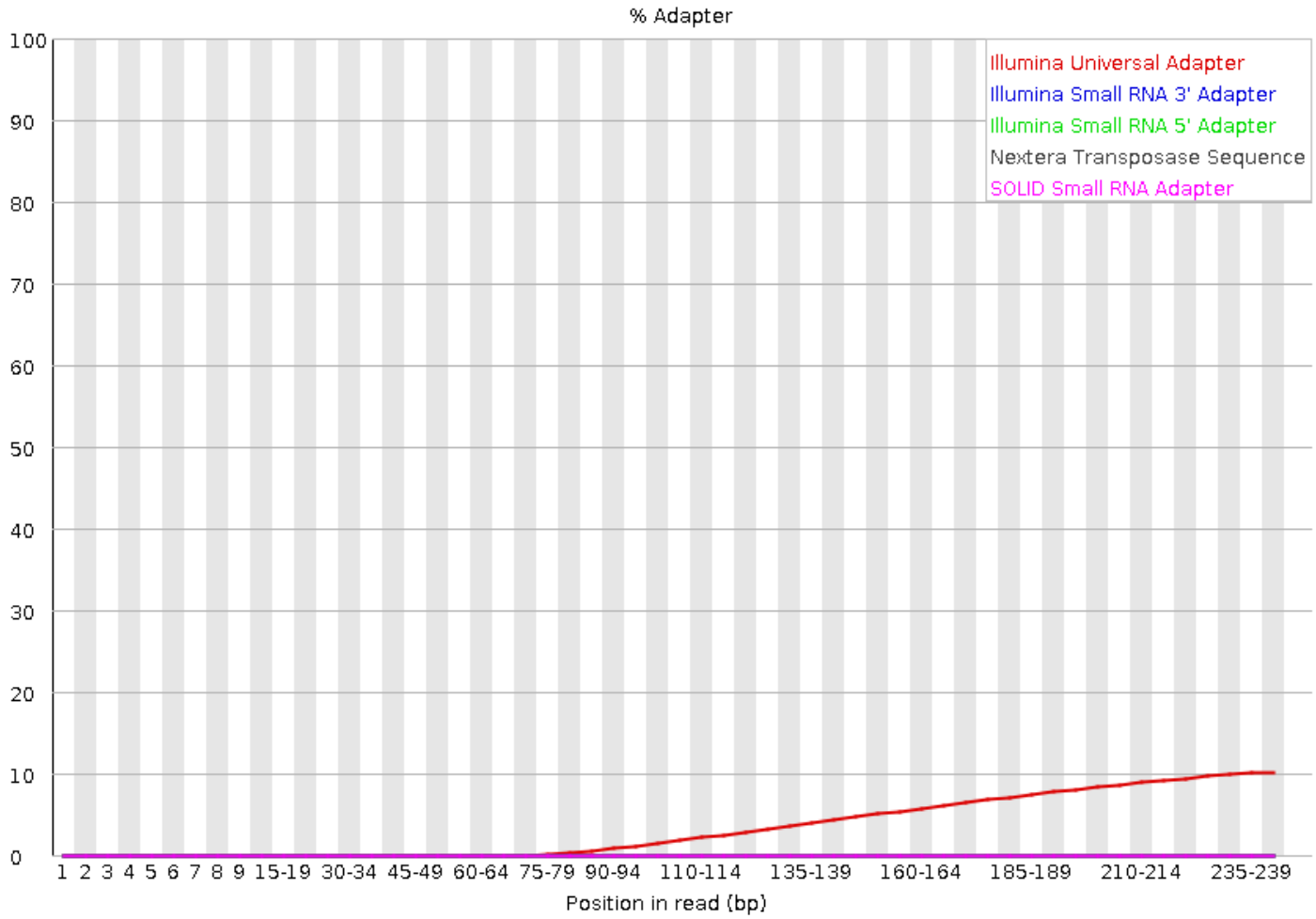
fasta + basequality (fasta + q = fastq)

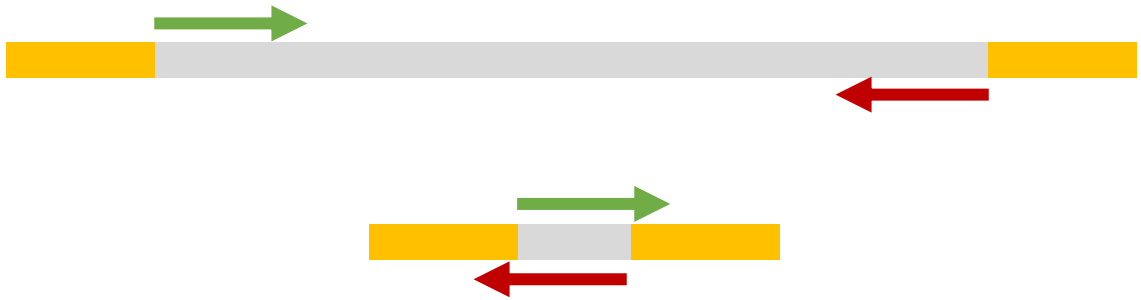
$$BASEQ = -10log_{10}\Pr\{base\ is\ wrong\}$$

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




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
Illumina Universal Adapter
Illumina Small RNA 3' Adapter
Illumina Small RNA 5' Adapter
Nextera Transposase Sequence
SOLID Small RNA Adapter
100
90
80
70
60
50
40
30
20
10
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)



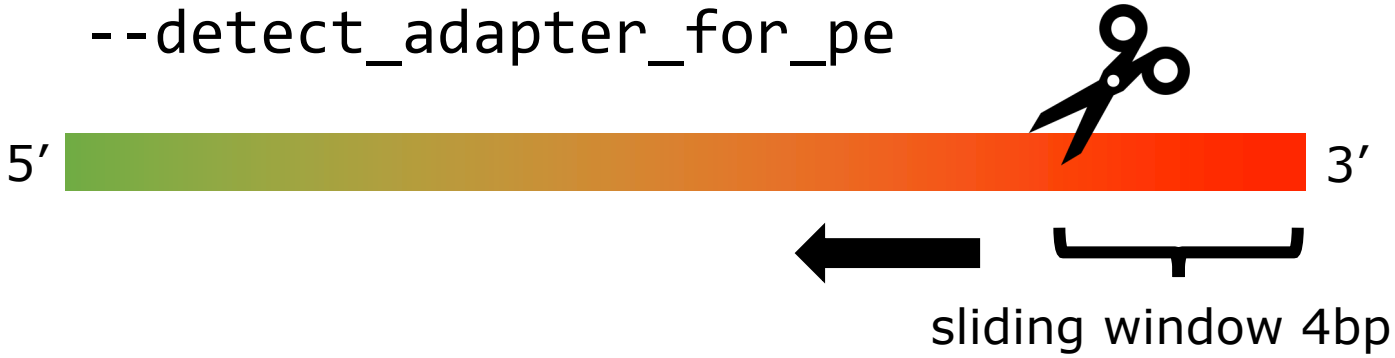
# Trimming

- Find and remove:
  - Regions or reads with low base quality
  - Adapter sequences
  - poly G sequences (e.g. with NovaSeq 6000)
- Software: `fastp` (or `cutadapt`, `trimmomatic`, `trim_galore`, `bbduk ..`)
- Articles on frequently occurring issues: [sequencing.qcfail.com](sequencing.qcfail.com)

read adapter

# Quality trimming with fastp

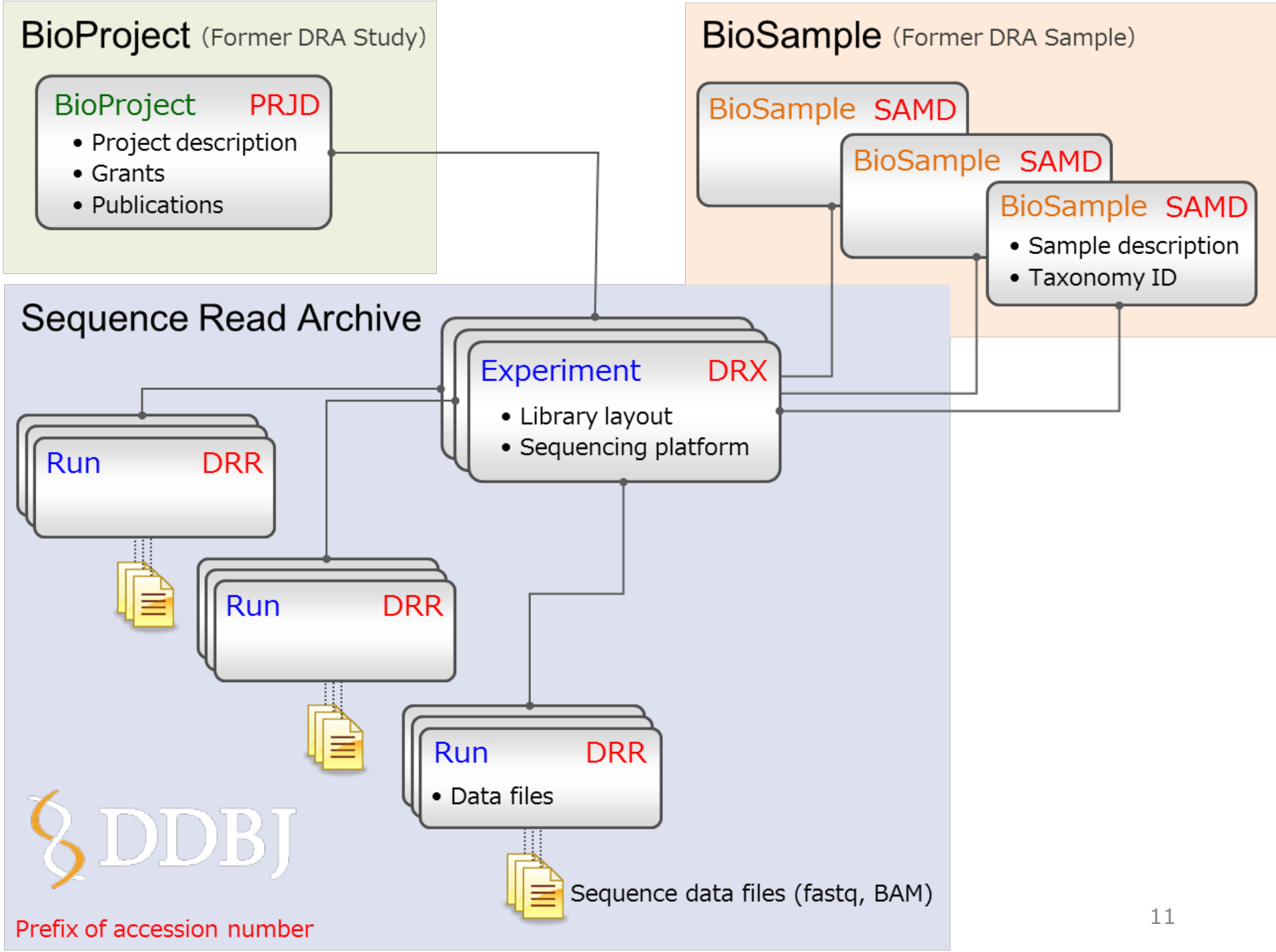
- Default:
  - Remove reads with >40% bases <Q15
  - Trim poly N (and poly G)
  - Autodetect adapters in R1, for both:
    `--detect_adapter_for_pe`




- ‘Classical’ trimming: sliding window
  - options `--cut_front` and `--cut_tail`

# 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`