CEITEC

Středoevropský technologický institut

BRNO | ČESKÁ REPUBLIKA

Sequencing libraries

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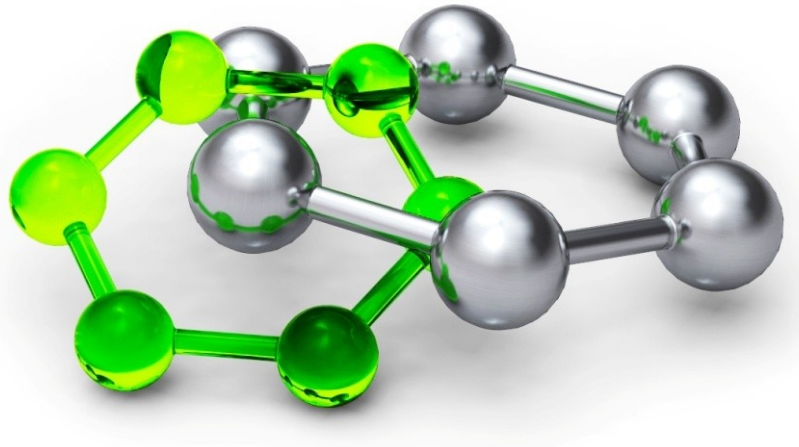
CF Genomics

Brno, 29.3.2021



EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

2007–13
OP Výzkum a vývoj pro inovace

Sequencing library

DNA fragments modified to allow unified access to fragments

Classic cDNA/DNA library

- Cloning of DNA/cDNA into vectors (plasmid etc.)

NGS library

- Adding of seuences that allow amplification (clonal) and sequencing

NGS libraries

Hundreds of types

Input
- DNA, RNA, short RNA, crosslinked DNA/RNA

Adaptor adding strategy
- Ligation, tagmentation, PCR

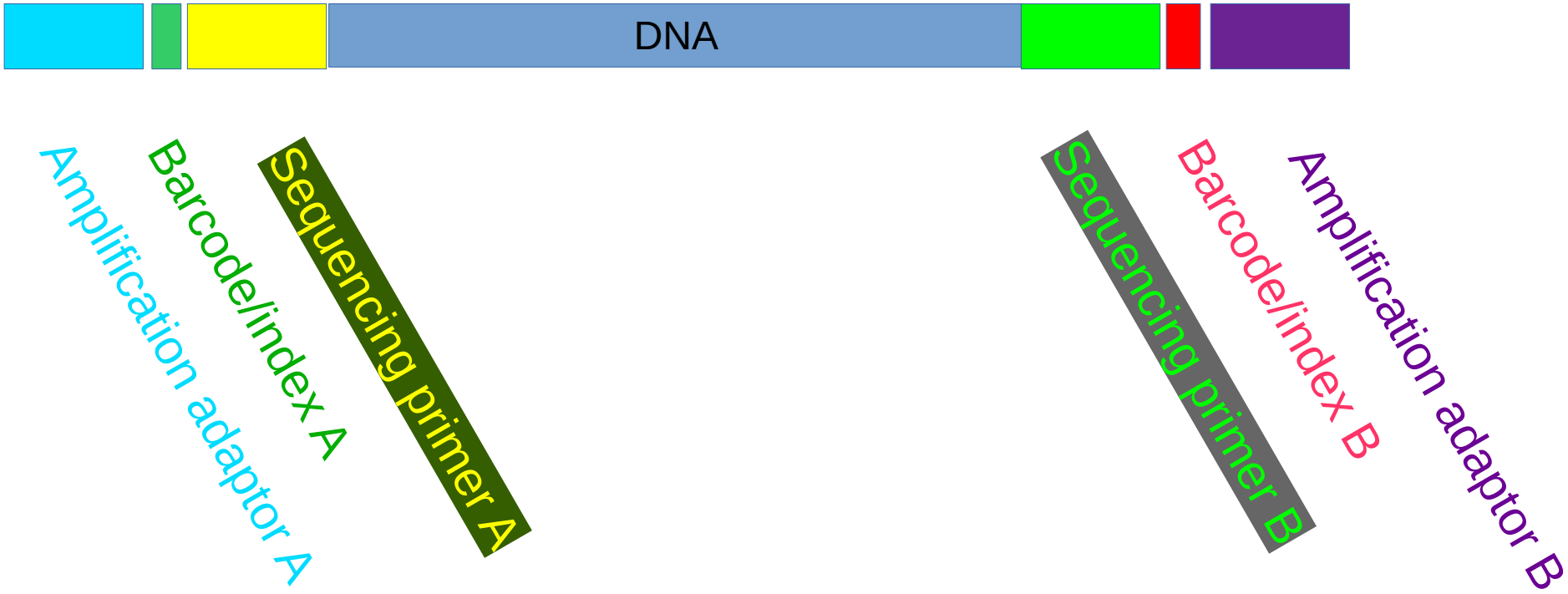
Specific sequence enrichment
- Hybridization, PCR, imunoprecipitation

DNA fragmentation

- Mechanical
  - Ultrasound (Covaris)
  - Hydrodynamic
  - Nebulization
- Enzymatic
  - Restriction endonucleases
  - Fragmentase®
  - Transposase

- Illumina
  - Cluster density depends on fragment length
  - Short fragments cluster better
  - Mix of longer and shorter fragments is problematic
  - Max <1000bp
  - Longer fragments are problematic
    - Broader length distribution =>
      - Uneven cluster generation effectivity
      - Problematic conc. measurment

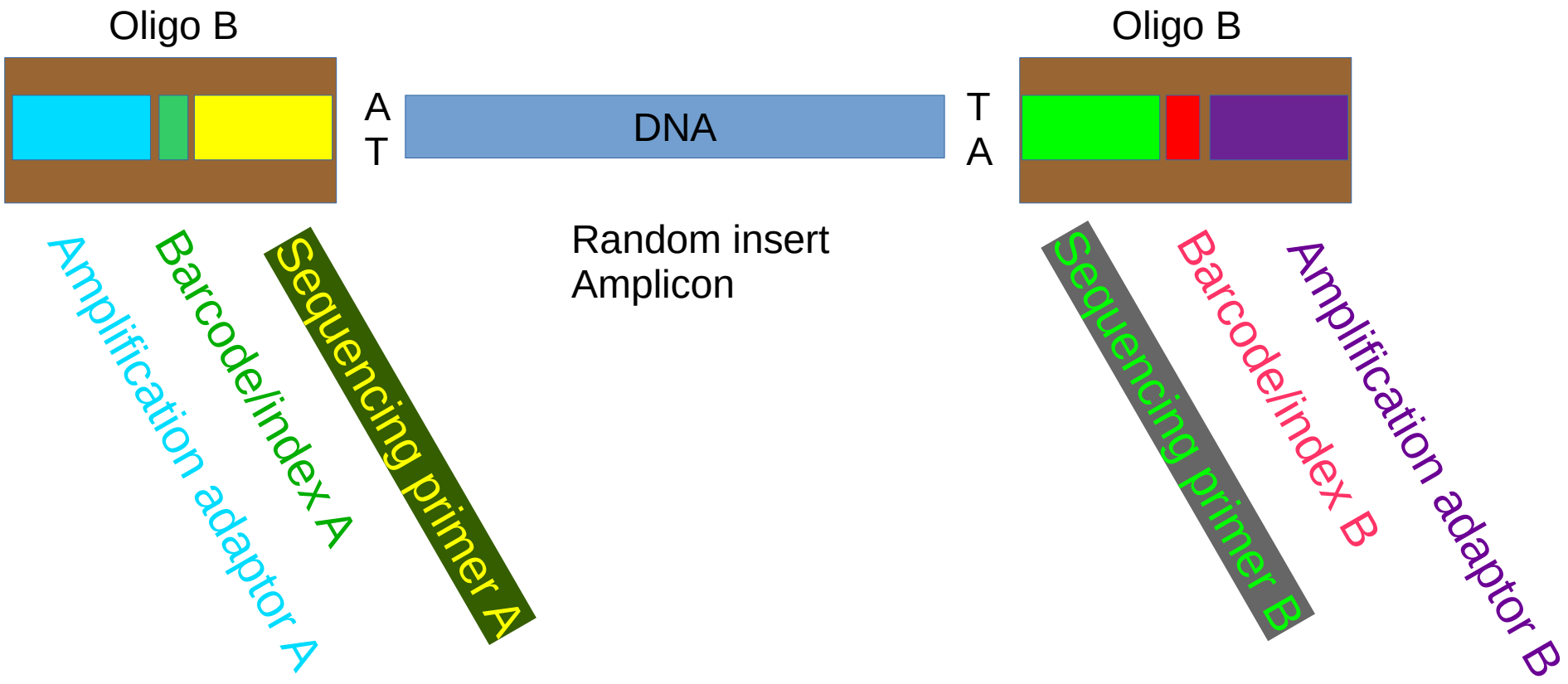
Illumina NGS library



Illumina NGS library - Ligation

Whole genomes
Enrichment libraries - input
Amplicons
ChIP, cDNA

3 steps:
- End repair
- A-tailing
- Ligation (A-T)

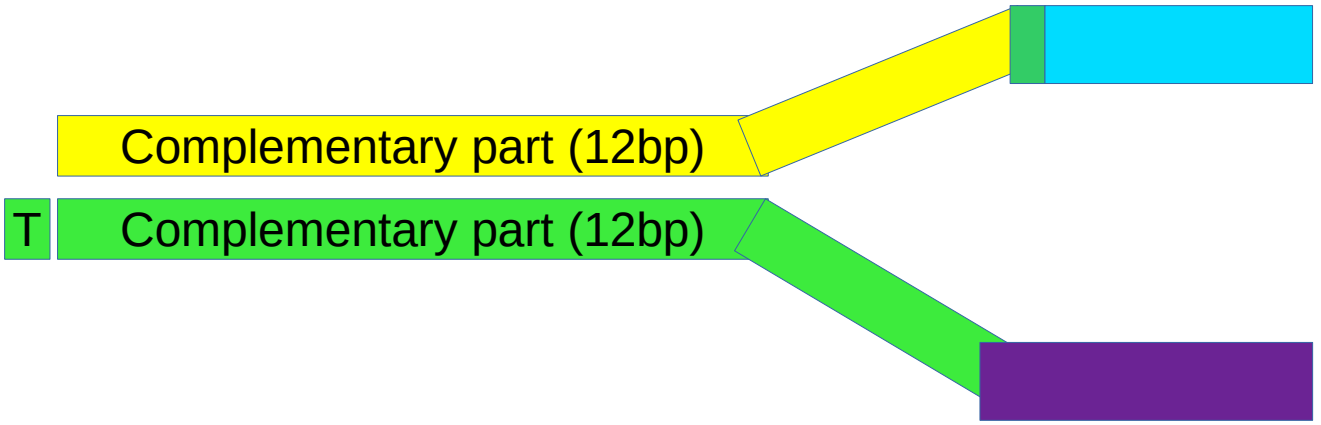


Library preparation

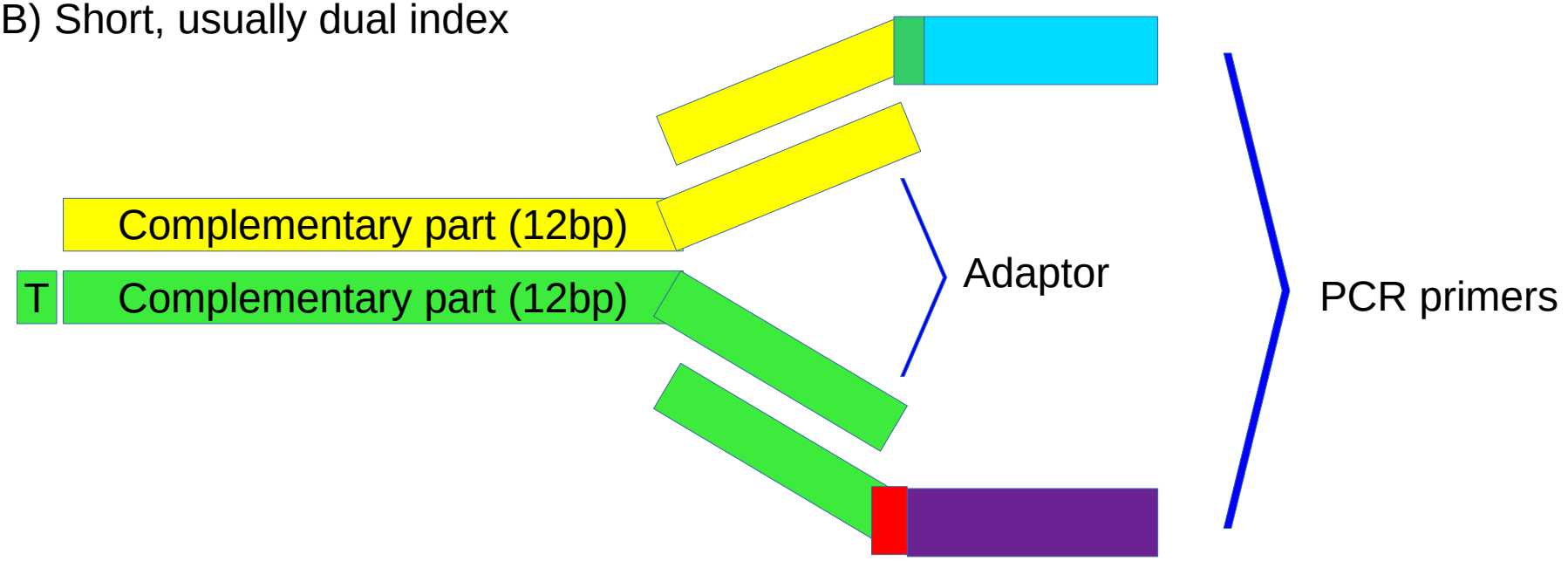
Adaptor structure

Ligation

A) Full-length, usually single index

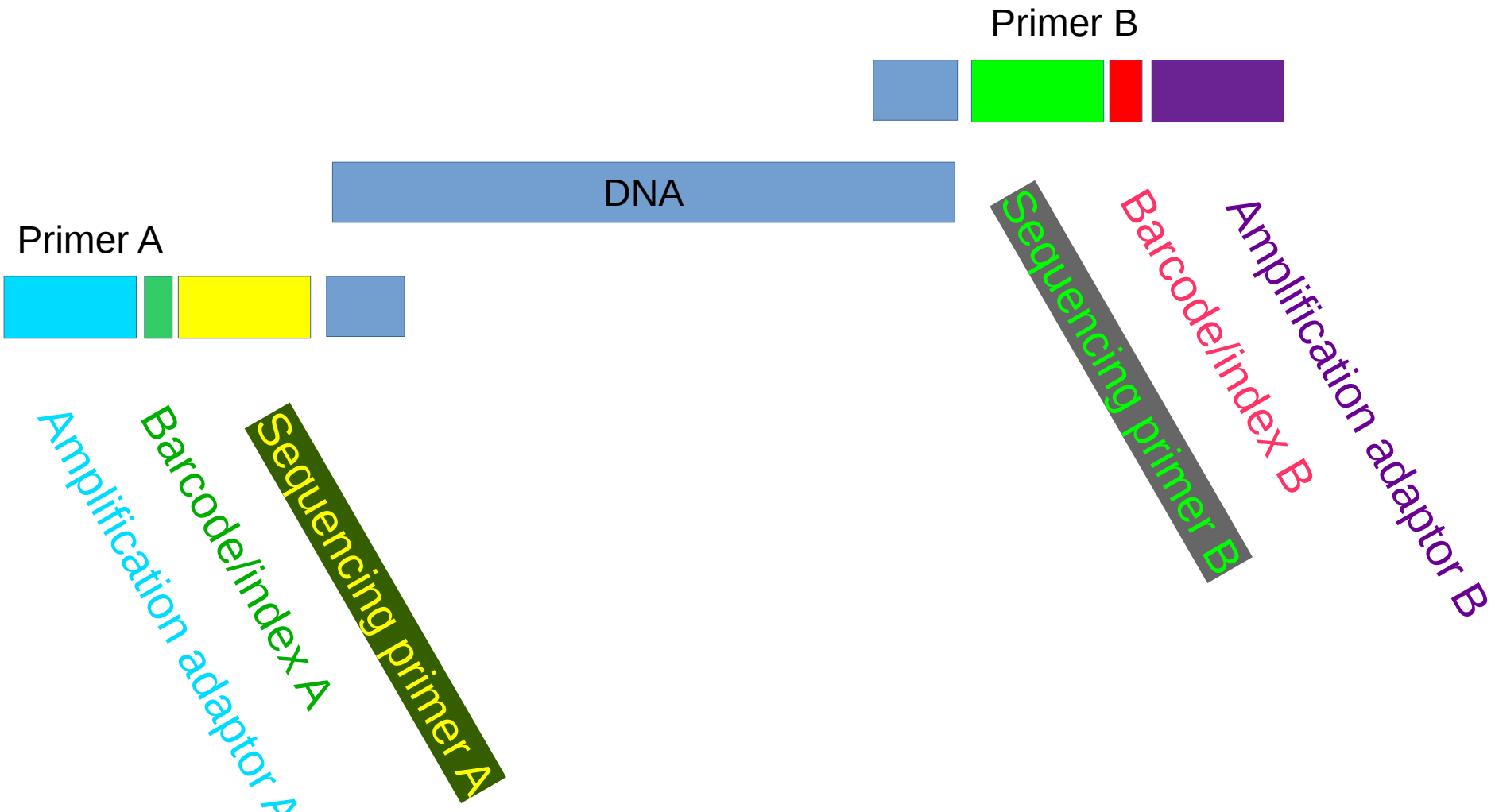


B) Short, usually dual index



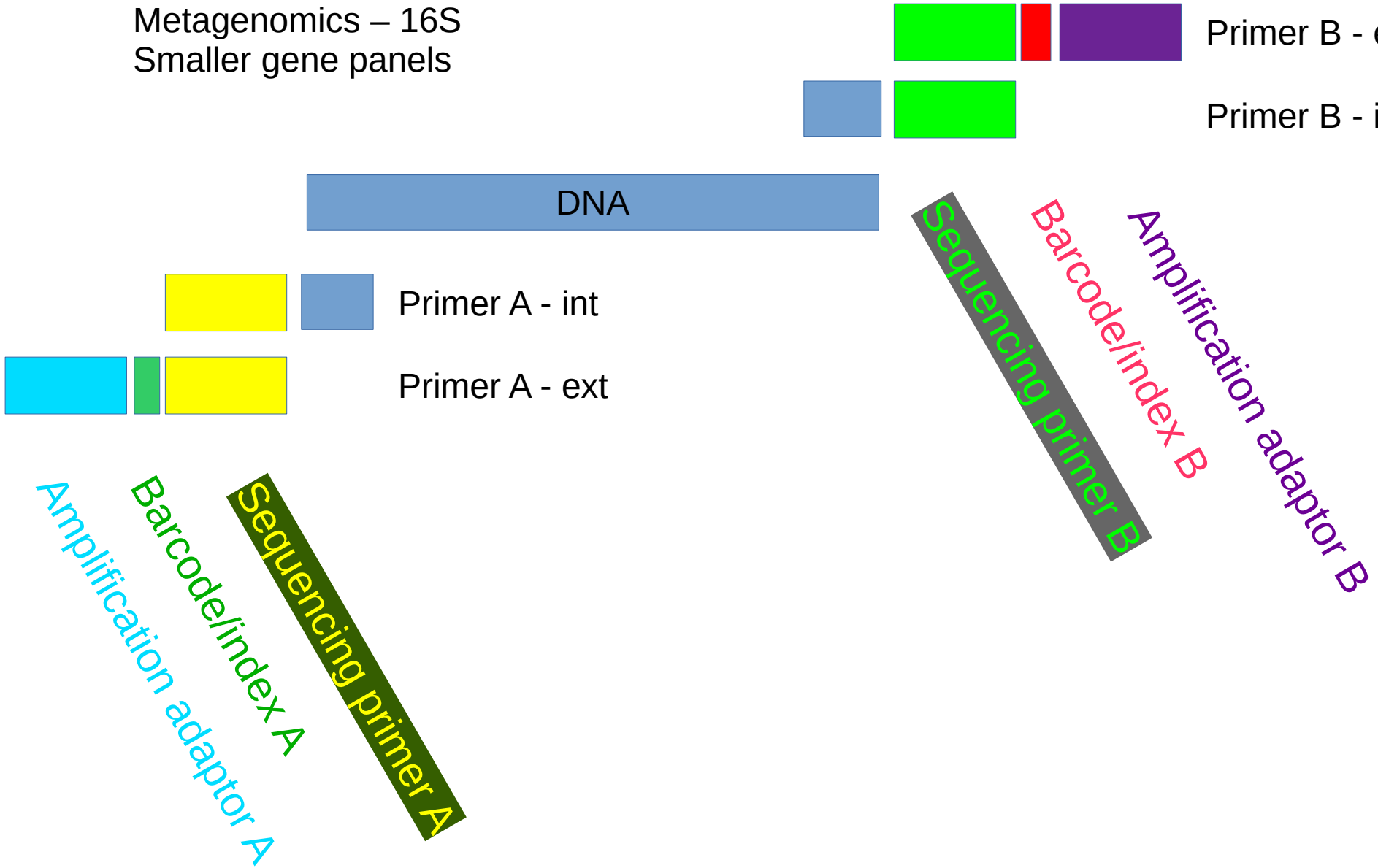
Illumina NGS library - PCR

Single genes/exons
Metagenomics – 16S
Smaller gene panels



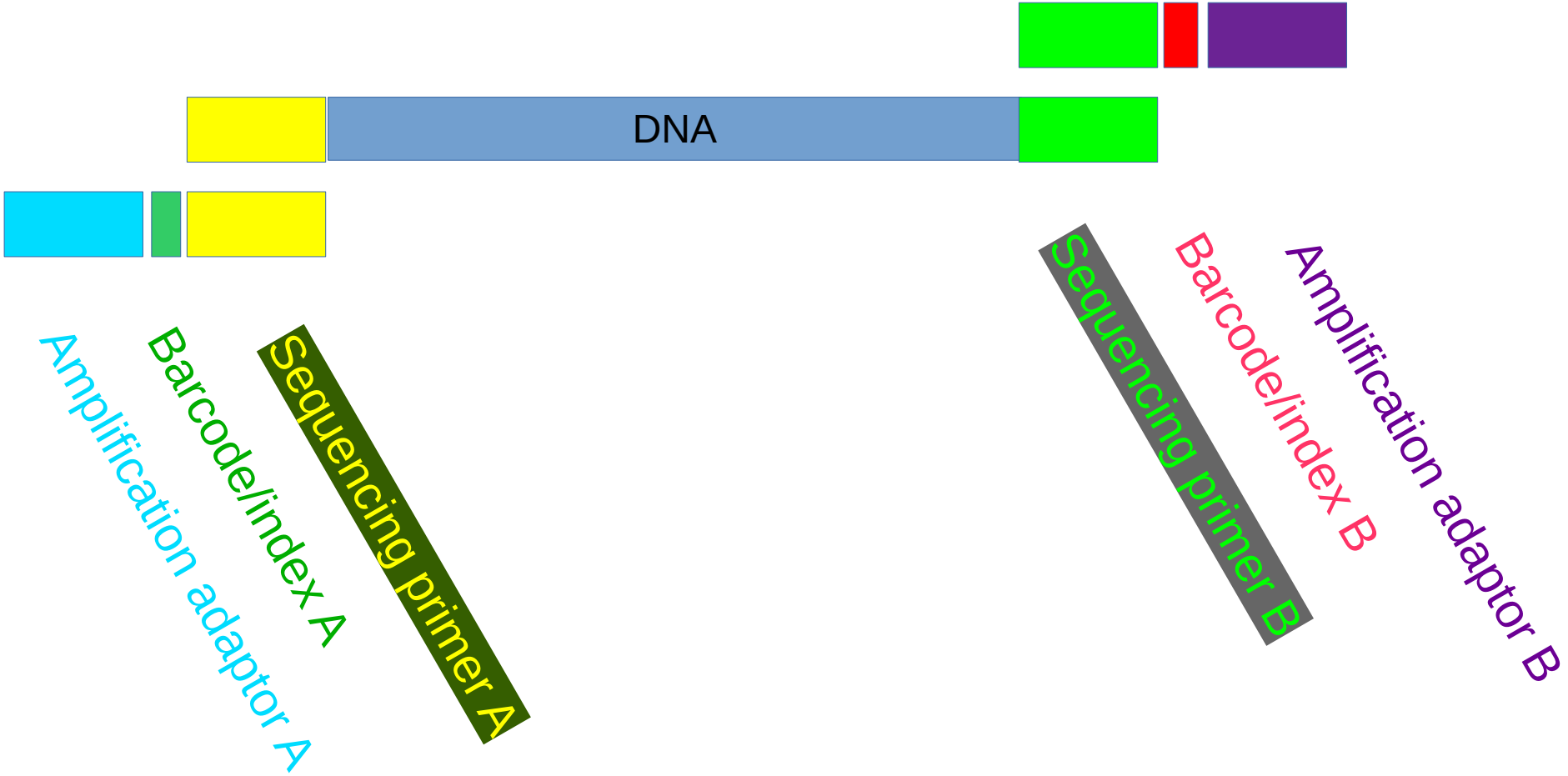
Illumina NGS library – two-round PCR

Single genes/exons
Metagenomics – 16S
Smaller gene panels



Illumina NGS library - tagmentation

Fragmentation and adaptor addition in single step
Transposase
Indexing with PCR

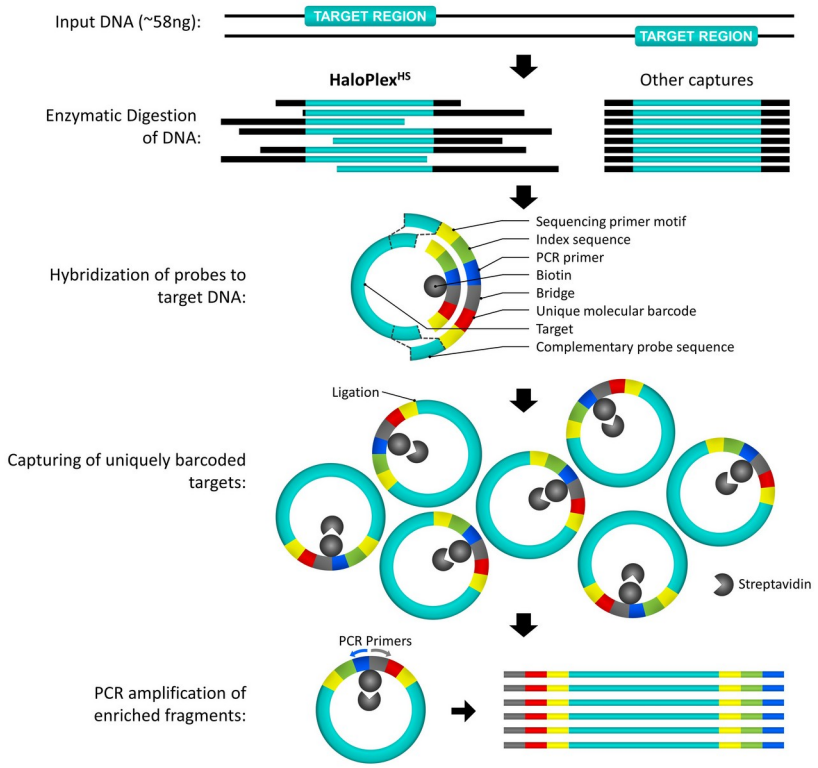


Library preparation

Molecular barcodes

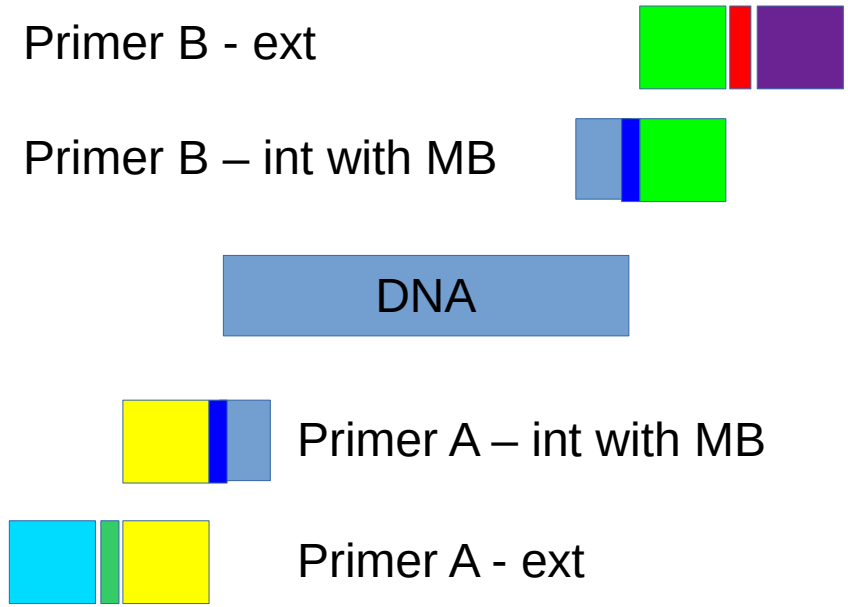
Tag each input molecule with random sequence before PCR amplification =>
- Lower coverage for variant calling
- Better quantification of variants (eg. species in metagenomics)

Haloplex HS



SAFE-Seq

First round – 2 cycles
ExoSAP treatment
Second round

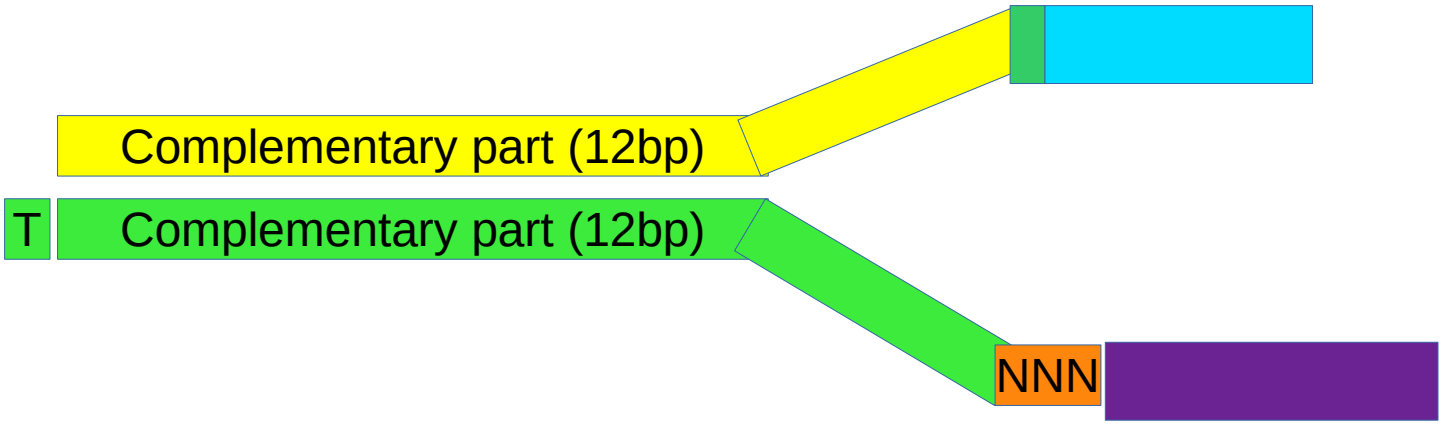


Library preparation

Adaptor structure - UMI

Ligation

A) Full-length, usually single index



B) Short, usually dual index