



Středoevropský technologický institut
BRNO | ČESKÁ REPUBLIKA

Sequencing libraries

Boris Tichý

CF Genomics

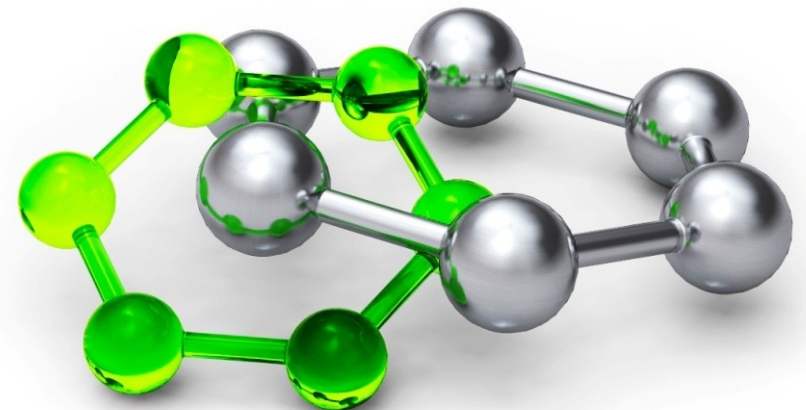
Brno, 29.3.2021



EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI



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 sequences 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, immunoprecipitation

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. measurement

Illumina NGS library



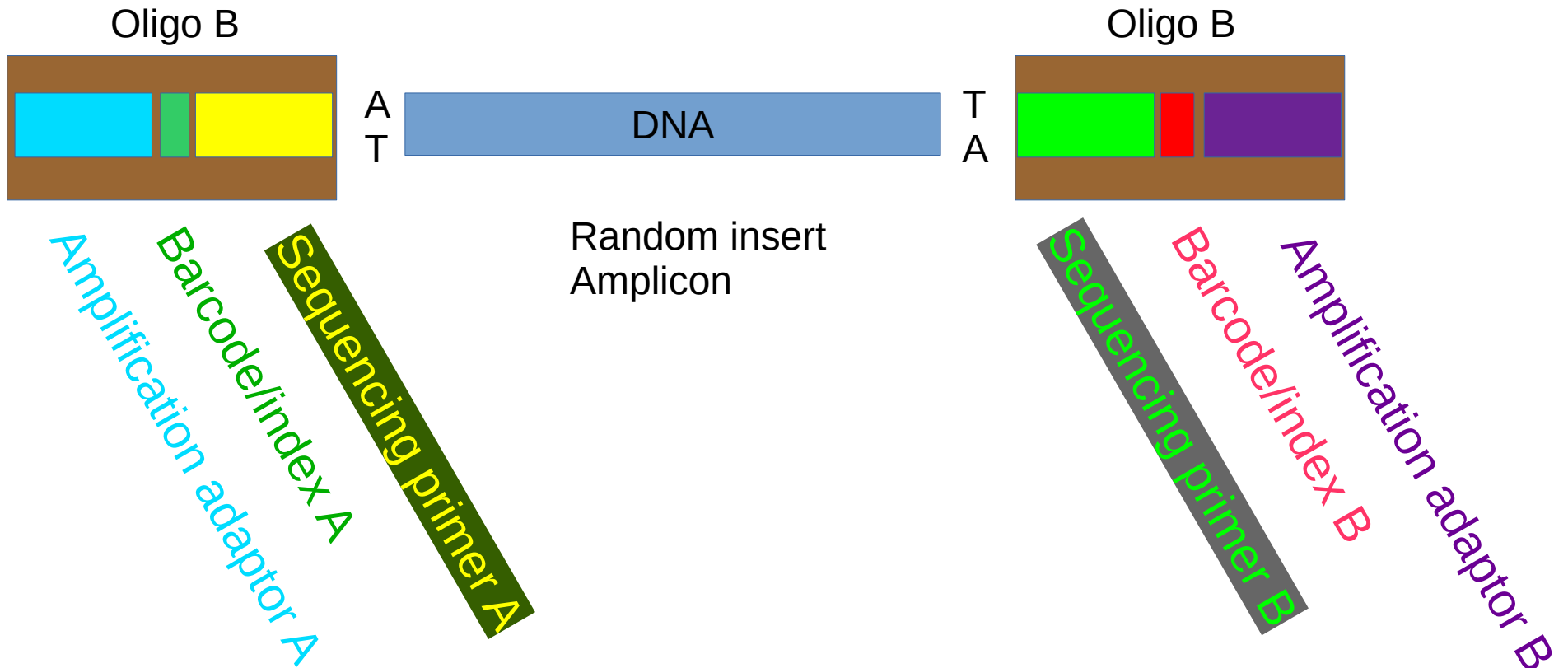
Amplification adaptor A
Barcode/index A
Sequencing primer A

Sequencing primer B
Barcode/index B
Amplification adaptor B

Illumina NGS library - Ligation

Whole genomes
Enrichment libraries - input
Amplicons
ChIP, cDNA

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

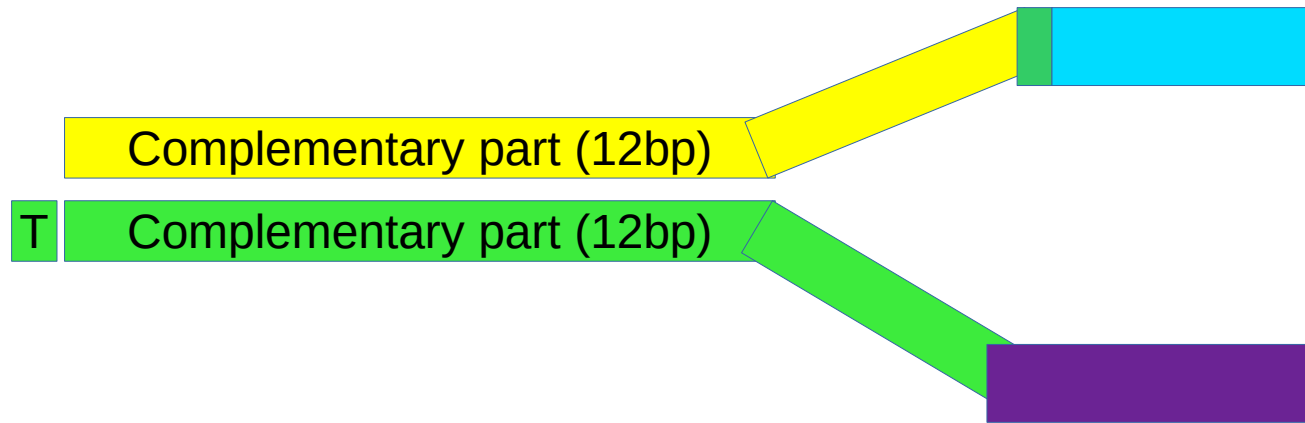


Library preparation

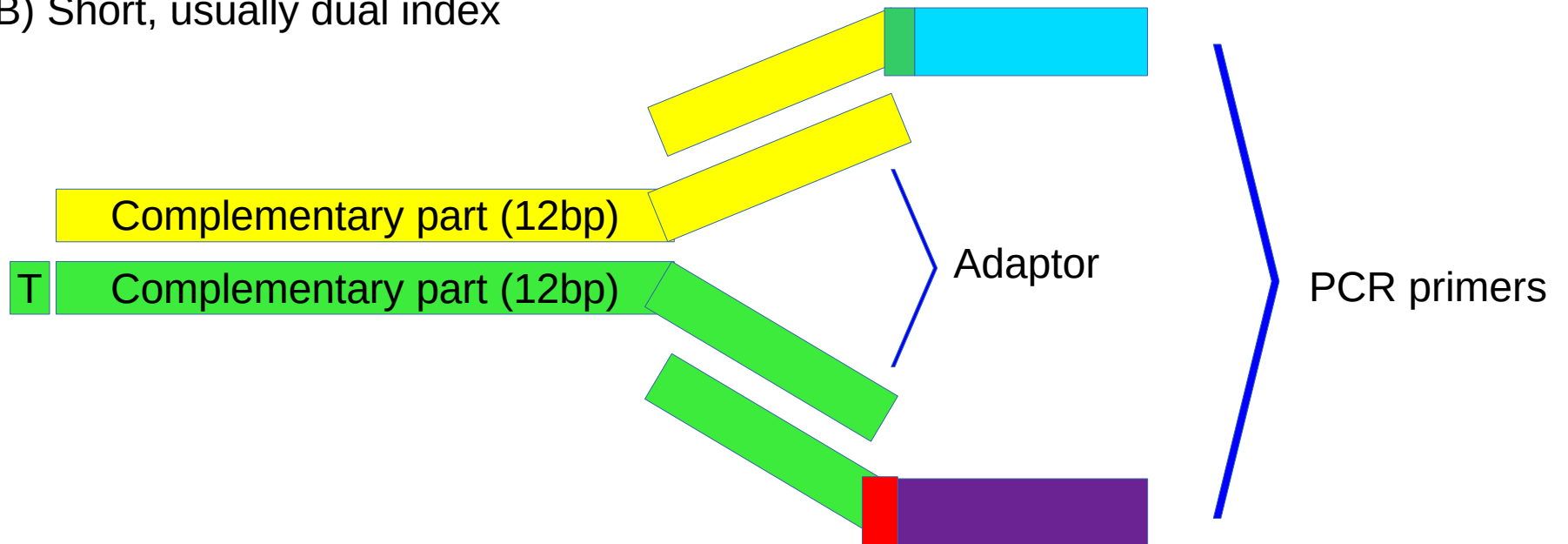
Adaptor structure

Ligation

A) Full-length, usually single index

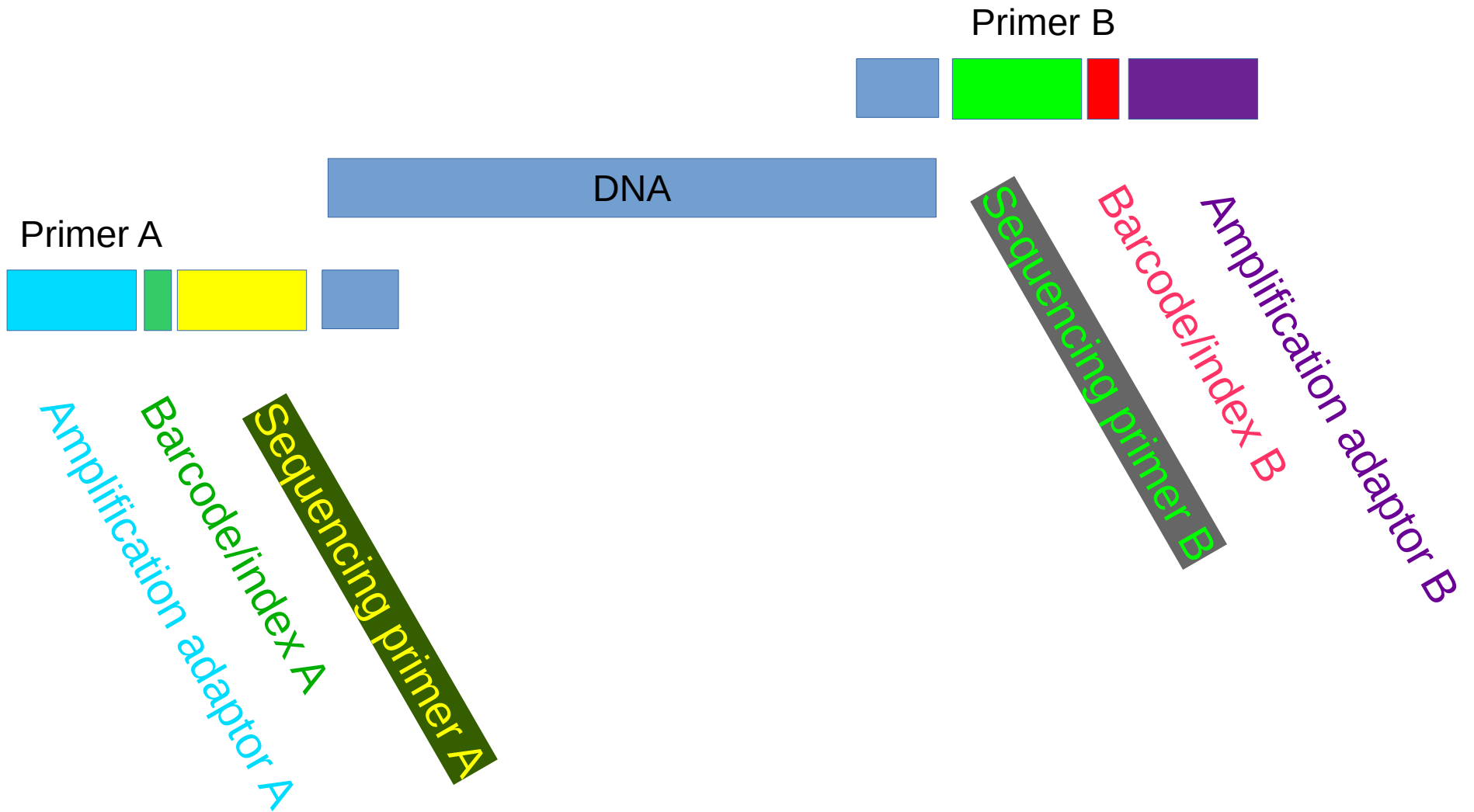


B) Short, usually dual index



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

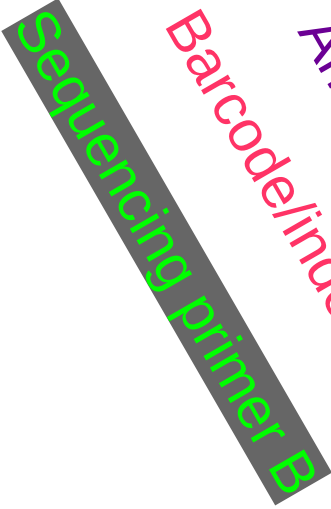


 Primer B - ext

 Primer B - int

 DNA

 Primer A - int

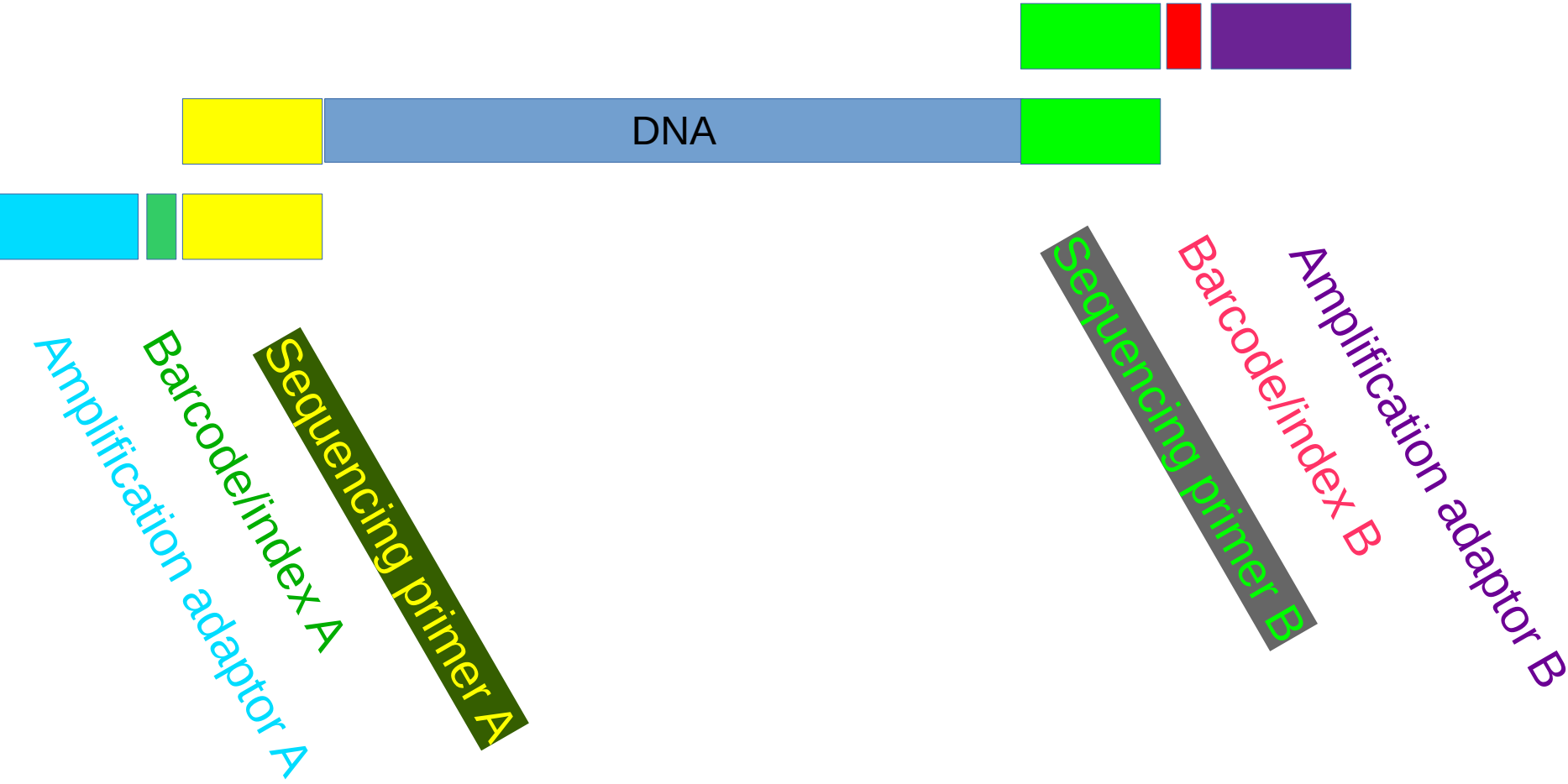
 Primer A - ext

 Sequencing primer B
 Barcode/index B
 Amplification adaptor B

 Amplification adaptor A
 Barcode/index A
 Sequencing primer A

Illumina NGS library - tagmentation

Fragmentation and adaptor addition in single step
Transposase
Indexing with PCR

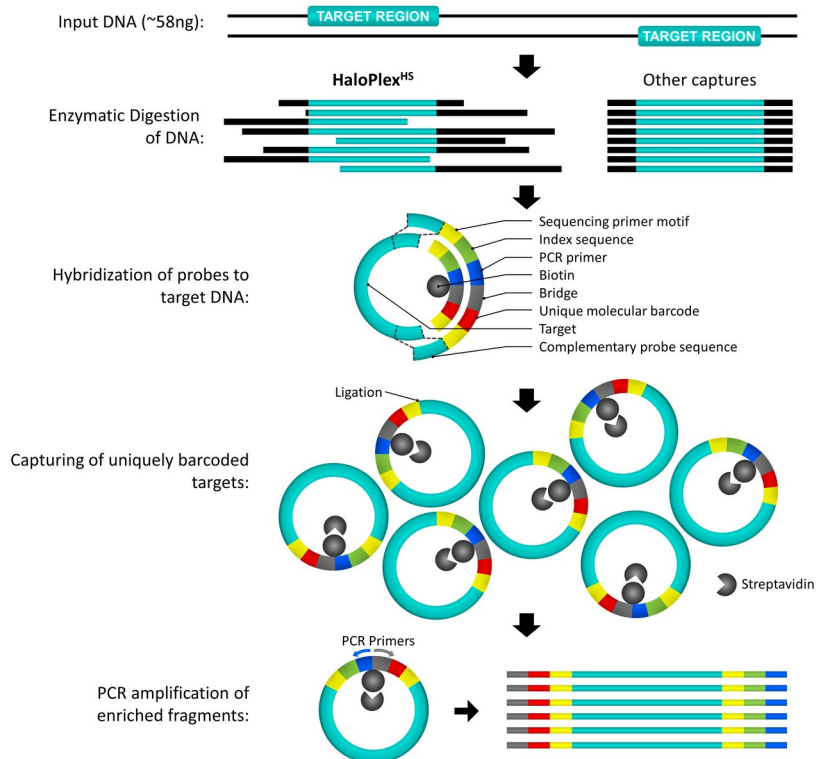


Library preparation

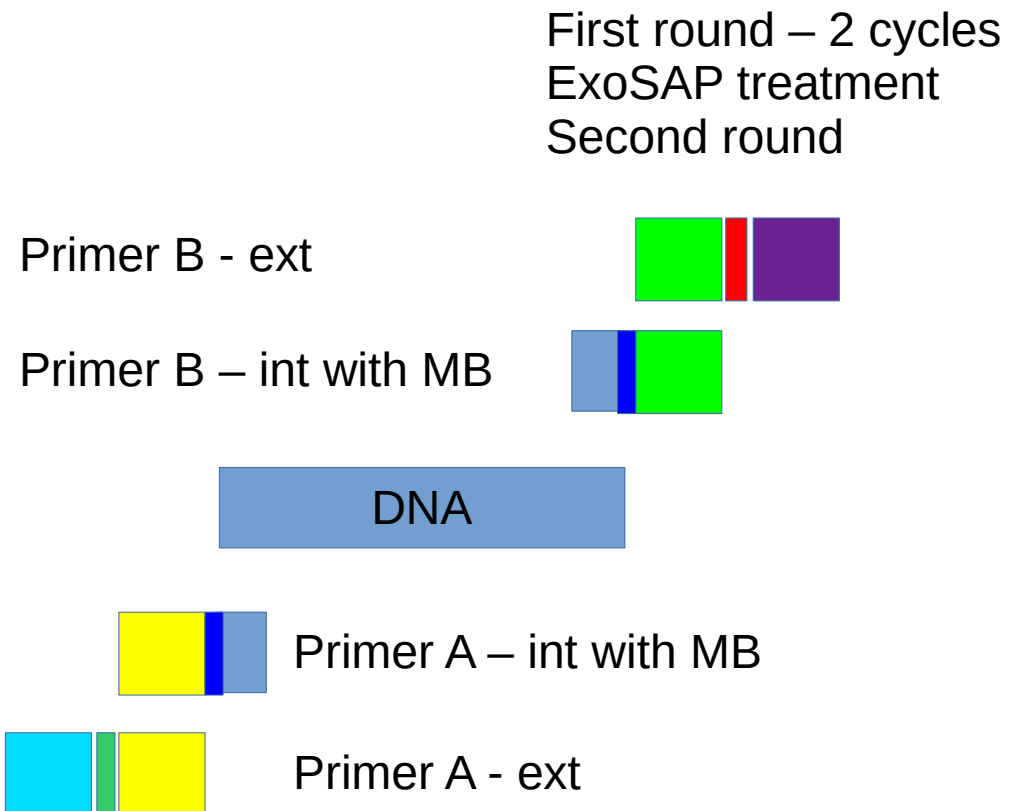
Molecular barcodes

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

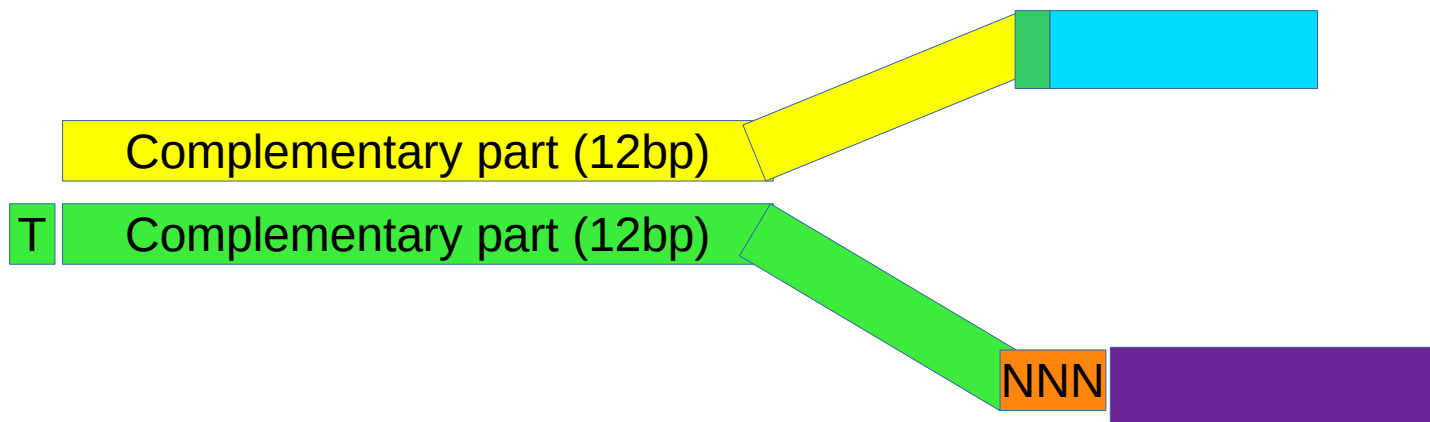


Library preparation

Adaptor structure - UMI

Ligation

A) Full-length, usually single index



B) Short, usually dual index

