

IN-BIOS[9,5]000 2022

HTS

Library prep

Arvind Sundaram

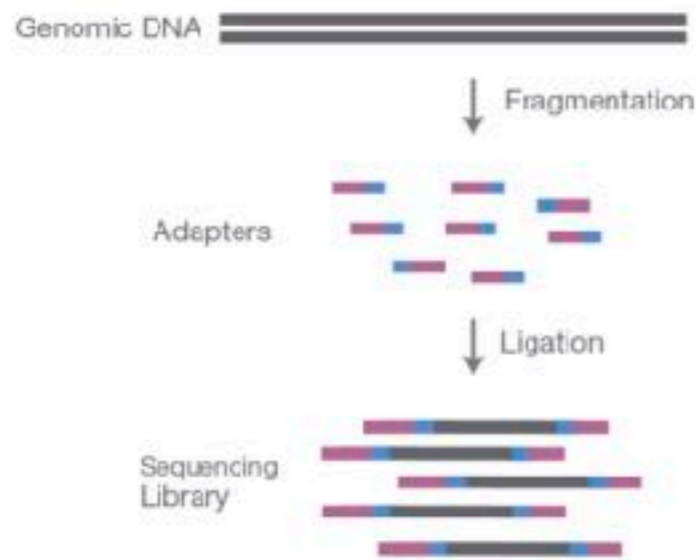
Oct 18, 2022

Norwegian Sequencing Centre
OUS, Ullevål, Oslo



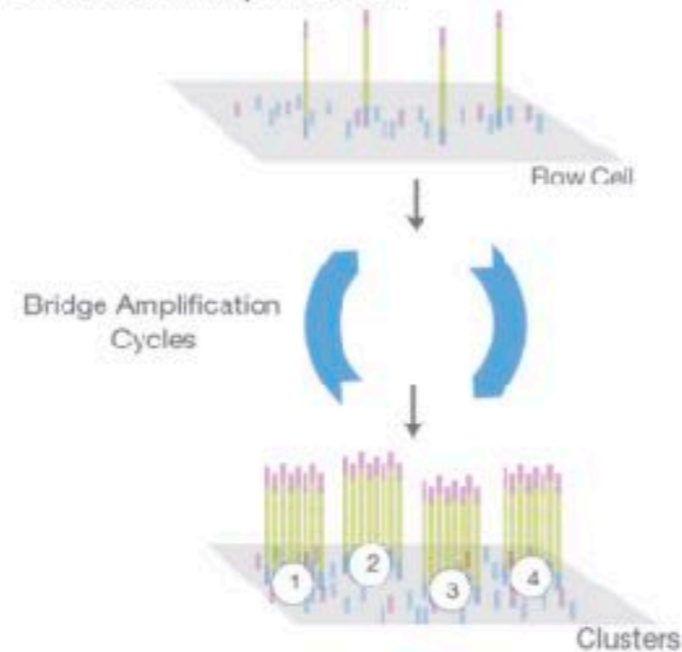
Sample to sequence data

A. Library Preparation



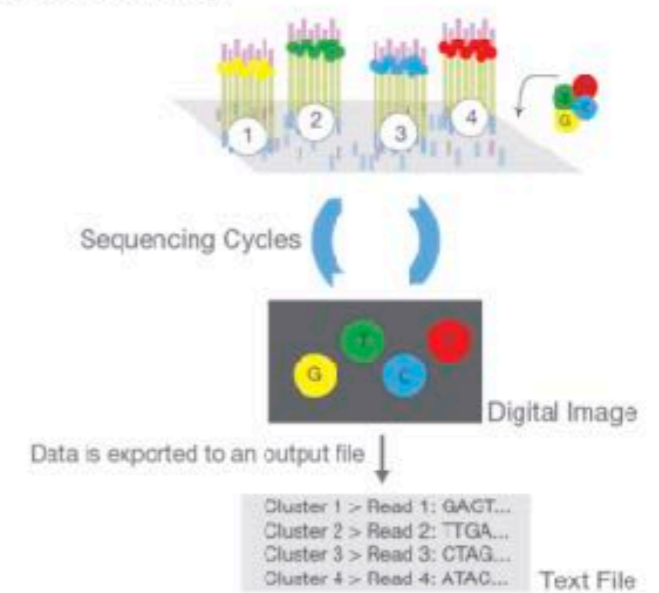
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

B. Cluster Amplification



Library is loaded into a flow cell and the fragments are hybridized to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

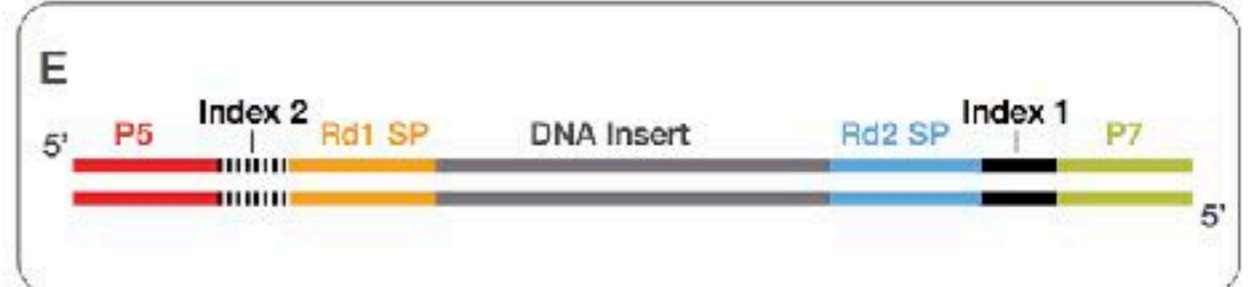
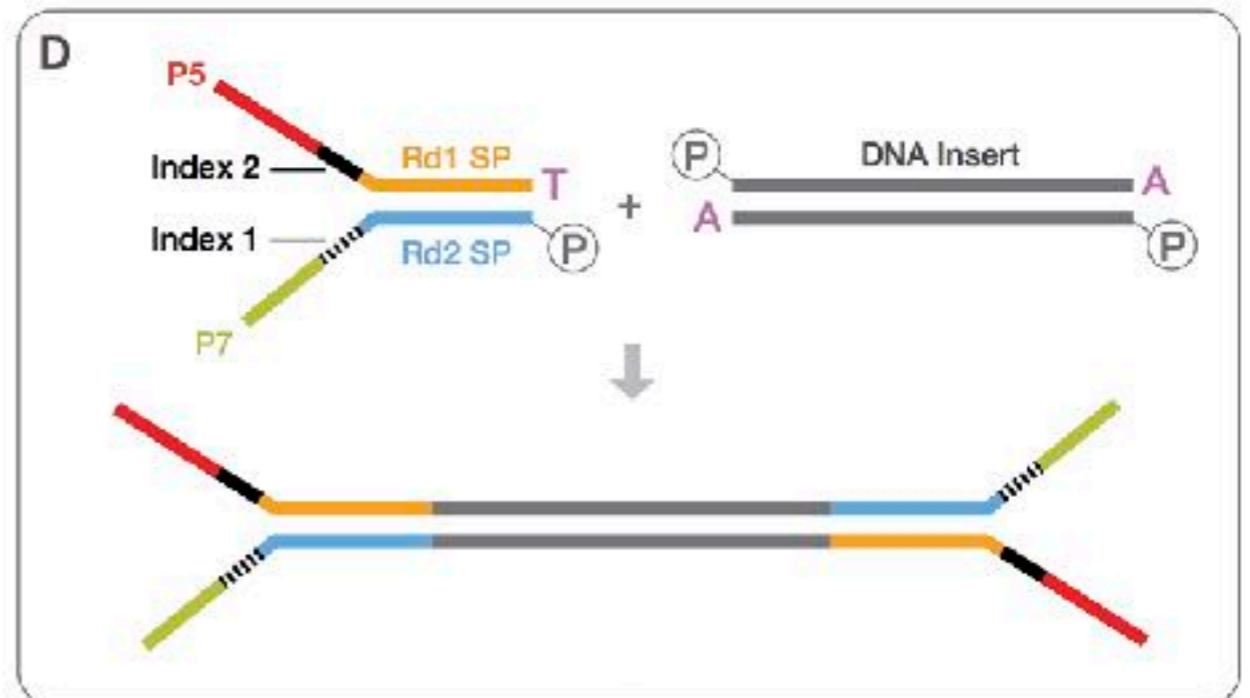
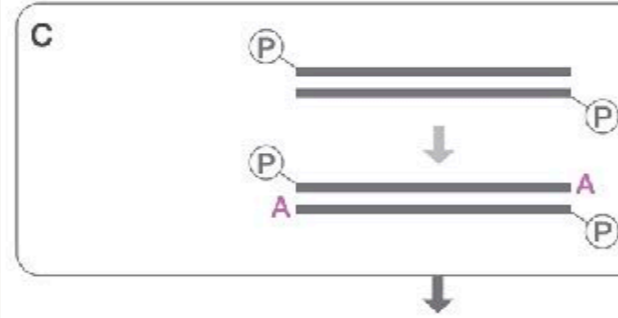
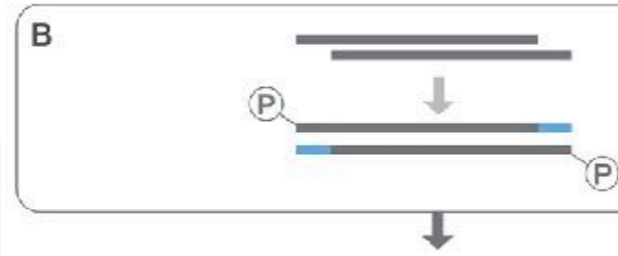
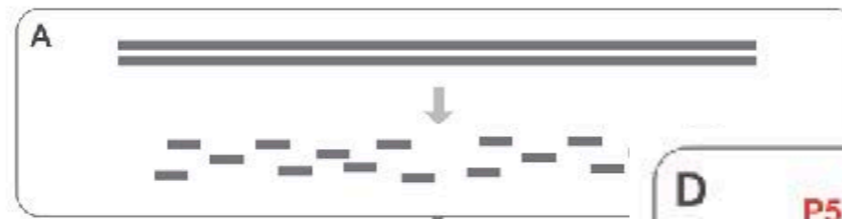
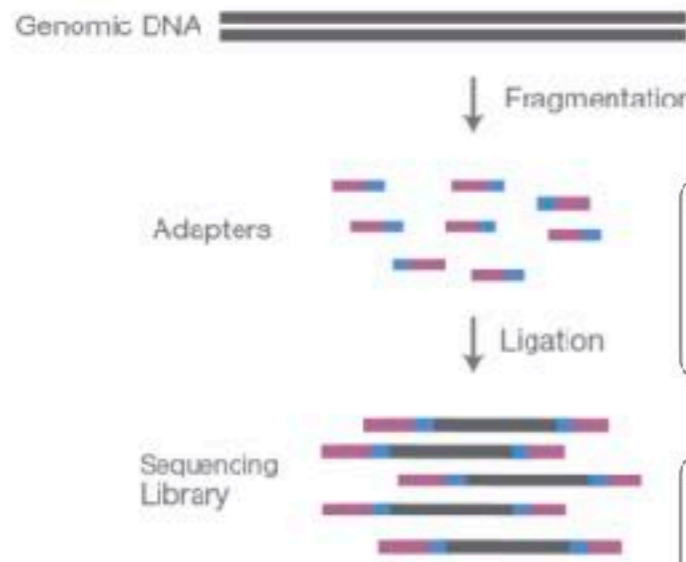
C. Sequencing



Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

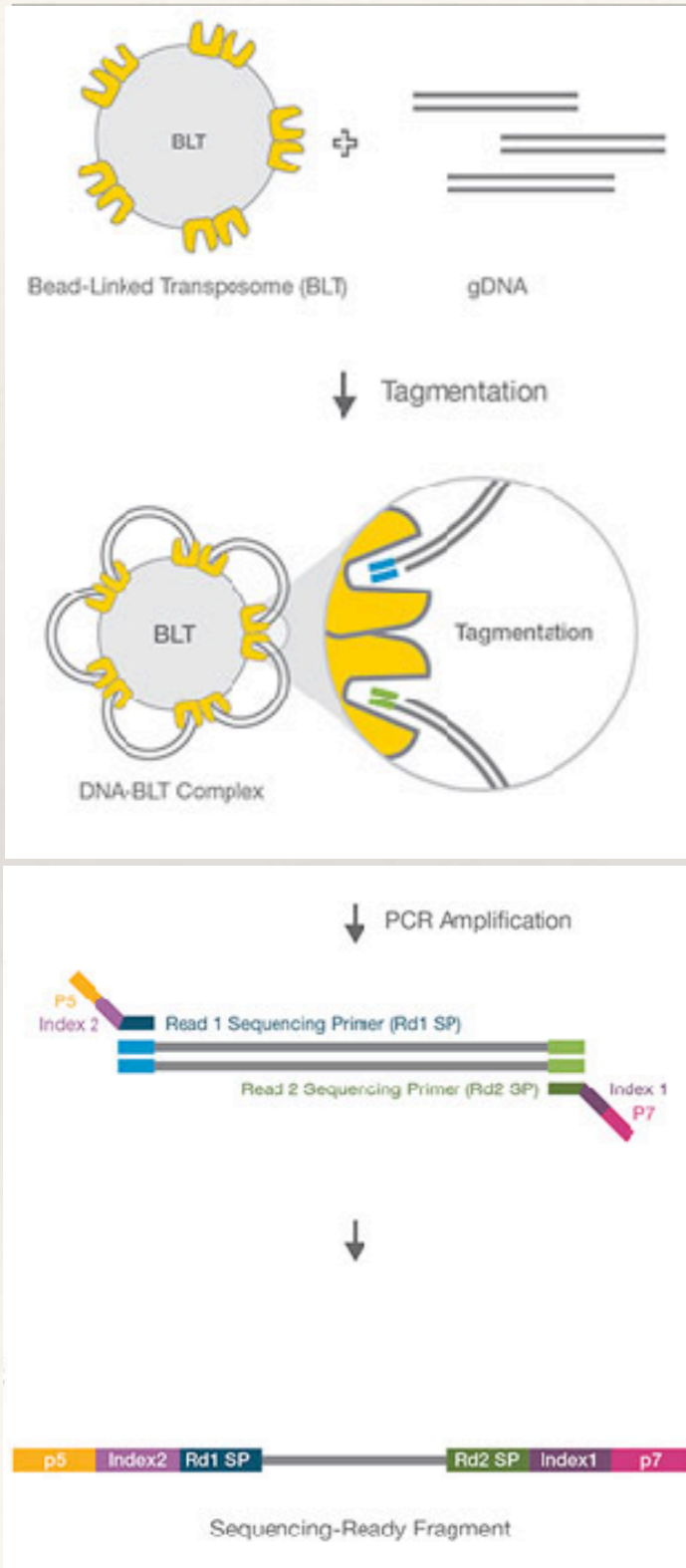
Library prep

A. Library Preparation



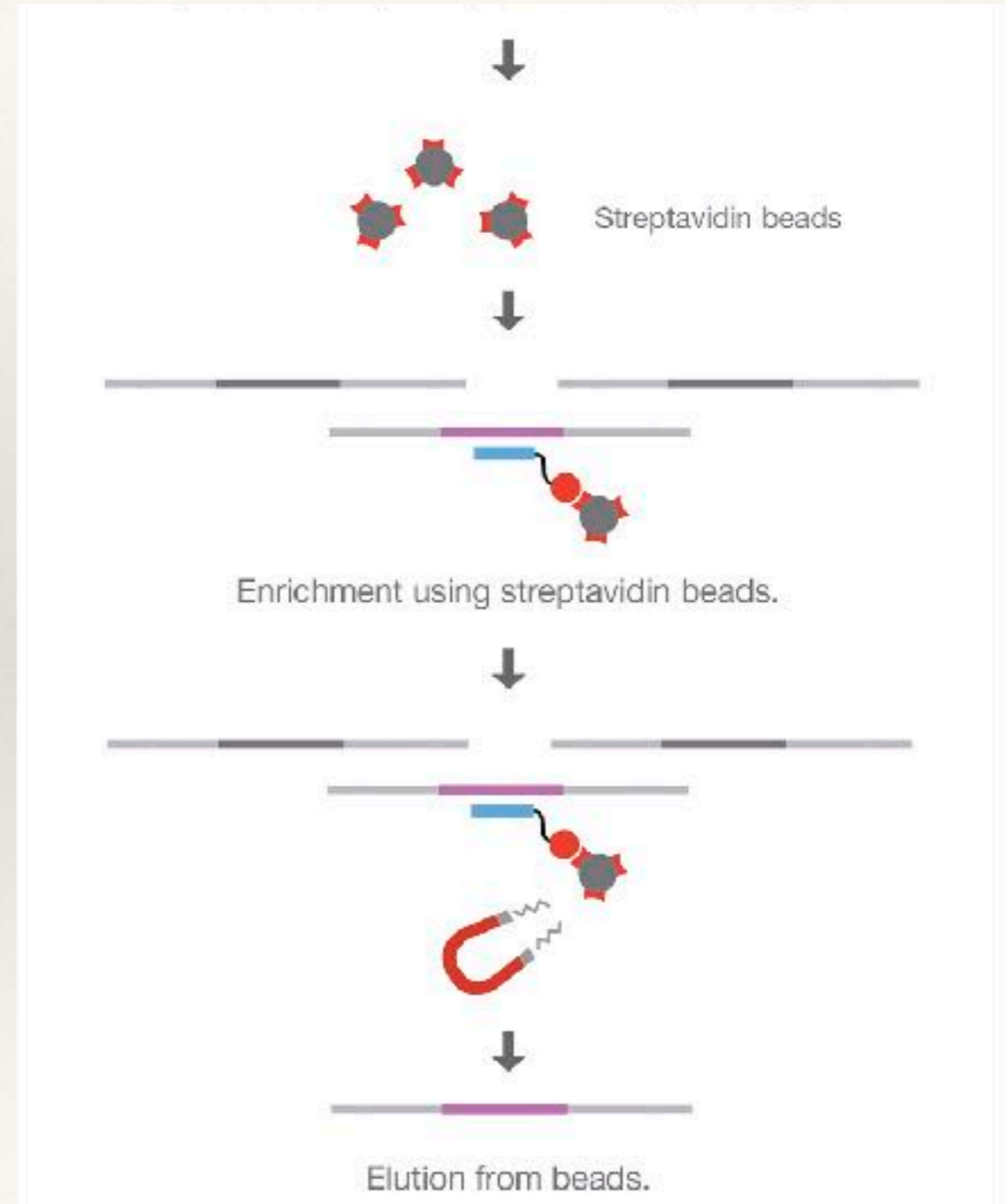
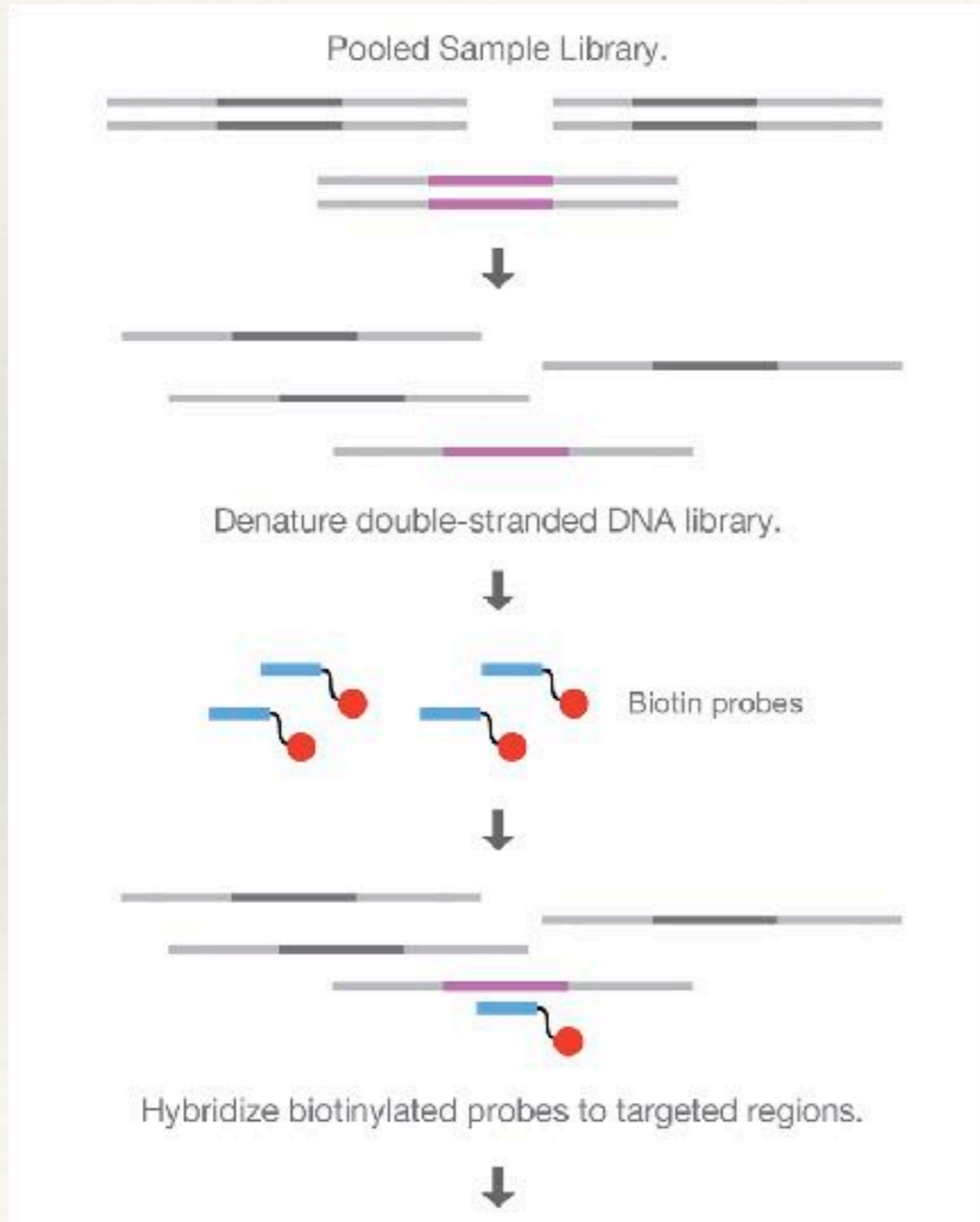
- A. Library construction begins with genomic DNA that is subsequently fragmented
- B. Blunt-end fragments are created
- C. A-base is added
- D. Dual-index adapters are ligated to the fragments*
- E. Final product is ready for amplification

On-bead tagmentation



- ❖ Bead-linked transposomes (BLT)
- ❖ Contains transposase-transposon complex
 - ❖ Cleaves DNA
 - ❖ Adds read 1 and read 2 sequencing primer
- ❖ Creates uniform insert size
- ❖ Integrated DNA normalisation
- ❖ Following post tagmentation cleanup
 - ❖ PCR amplification to incorporate the adapters
- ❖ Nextera XT, Illumina DNA prep and PCR-free prep

Target enrichment



Amplicons

Use DesignStudio Software to create custom oligo capture probes flanking each region of interest.



CAT probes hybridize to flanking regions of interest in unfragmented gDNA.



Extension/Ligation between Custom Probes across regions of interest.



PCR adds indexes and sequencing primers.



Uniquely tagged amplicon library ready for cluster generation and sequencing.



Amplicons

- ❖ Dual index possible
- ❖ Dual internal barcodes possible
 - ❖ multiplex up to 4000 samples.

Fadrosh et al. *Microbiome* 2014, 2:6
<http://www.microbiomejournal.com/content/2/1/6>

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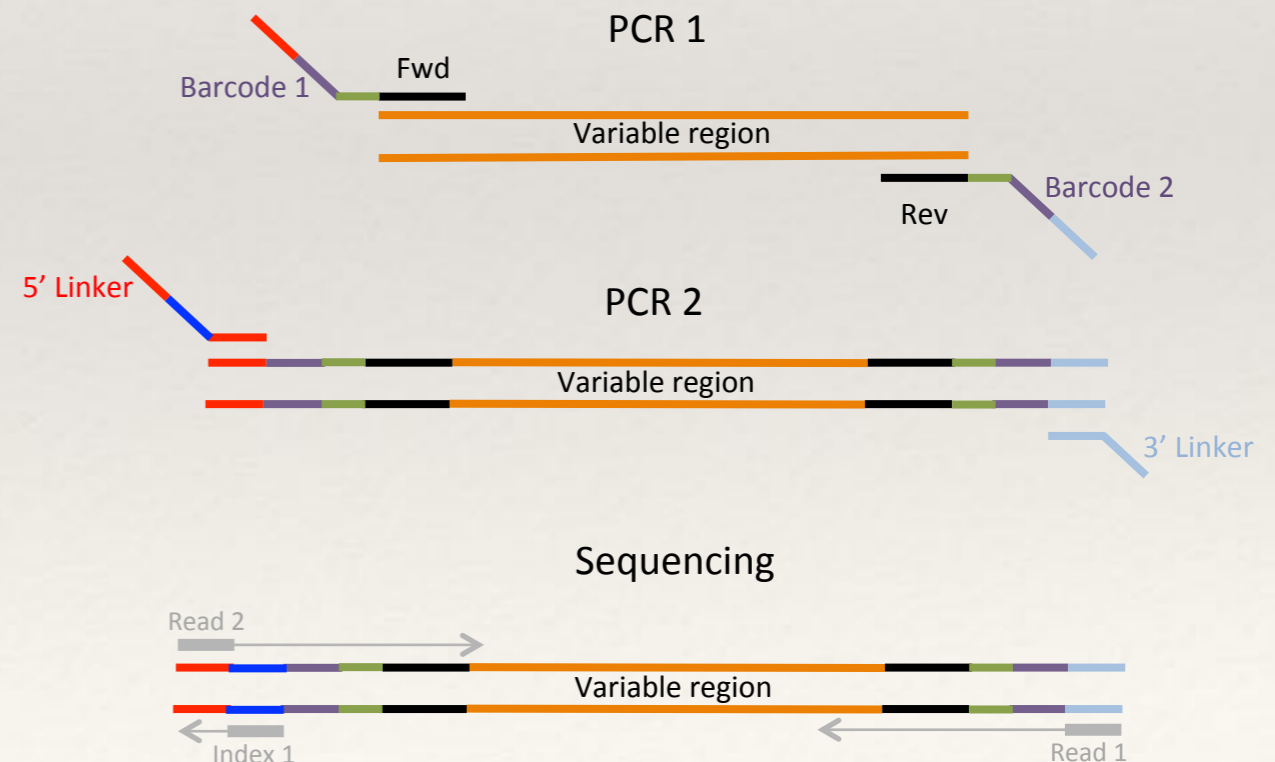
An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform

Douglas W Fadrosh^{1†}, Bing Ma^{1†}, Pawel Gajer¹, Naomi Sengamalay¹, Sandra Ott¹, Rebecca M Brotman² and Jacques Ravel^{1*}

Sequencing Cycle

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Sample 1	C	C	T	A	A	A	C	T	A	C	G	G	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A	G	C
Sample 2	G	T	G	G	T	A	T	G	G	G	A	G	T	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A	G
Sample 3	T	G	T	T	G	C	G	T	T	T	C	T	G	T	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A
Sample 4	A	C	A	G	C	C	A	C	C	C	A	T	C	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C
Sample 5	G	T	T	A	C	G	T	G	G	T	T	G	A	T	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G	G
Sample 6	T	A	C	C	G	G	C	T	T	G	C	A	T	G	C	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G
Sample 7	C	A	C	C	T	T	A	C	C	T	T	A	G	A	G	T	G	G	A	C	T	C	C	T	A	C	G	G	G	A
Sample 8	T	T	A	A	C	T	G	G	A	A	G	C	C	C	T	G	T	G	G	A	C	T	C	C	T	A	C	G	G	G

Index 1
Heterogeneity Spacer
16S rRNA gene sequence



Illumina library prep

- ❖ DNA sequencing
 - ❖ Whole genome
 - ❖ Targeted resequencing
 - ❖ ChIP seq
- ❖ RNA sequencing
 - ❖ Total RNA
 - ❖ mRNA
 - ❖ small RNA
 - ❖ Ribosome profiling
- ❖ Methylation sequencing

Check **Sequencing Method Explorer** at
<https://www.illumina.com/techniques/sequencing.html>

Illumina library prep

- ❖ TruSeq PCR-free DNA kit, TruSeq Nano DNA kit
- ❖ Nextera DNA kit, Nextera DNA XT kit
- ❖ Nextera Rapid Capture Exome kit
- ❖ Nextera Rapid Capture Expanded Exome kit
- ❖ TruSeq Stranded Total RNA kit
- ❖ TruSeq Stranded mRNA kit
- ❖ TruSeq small RNA kit
- ❖ TruSeq DNA Methylation kit

DNA prep poster:

<https://www.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/for-all-you-seq-dna.pdf>

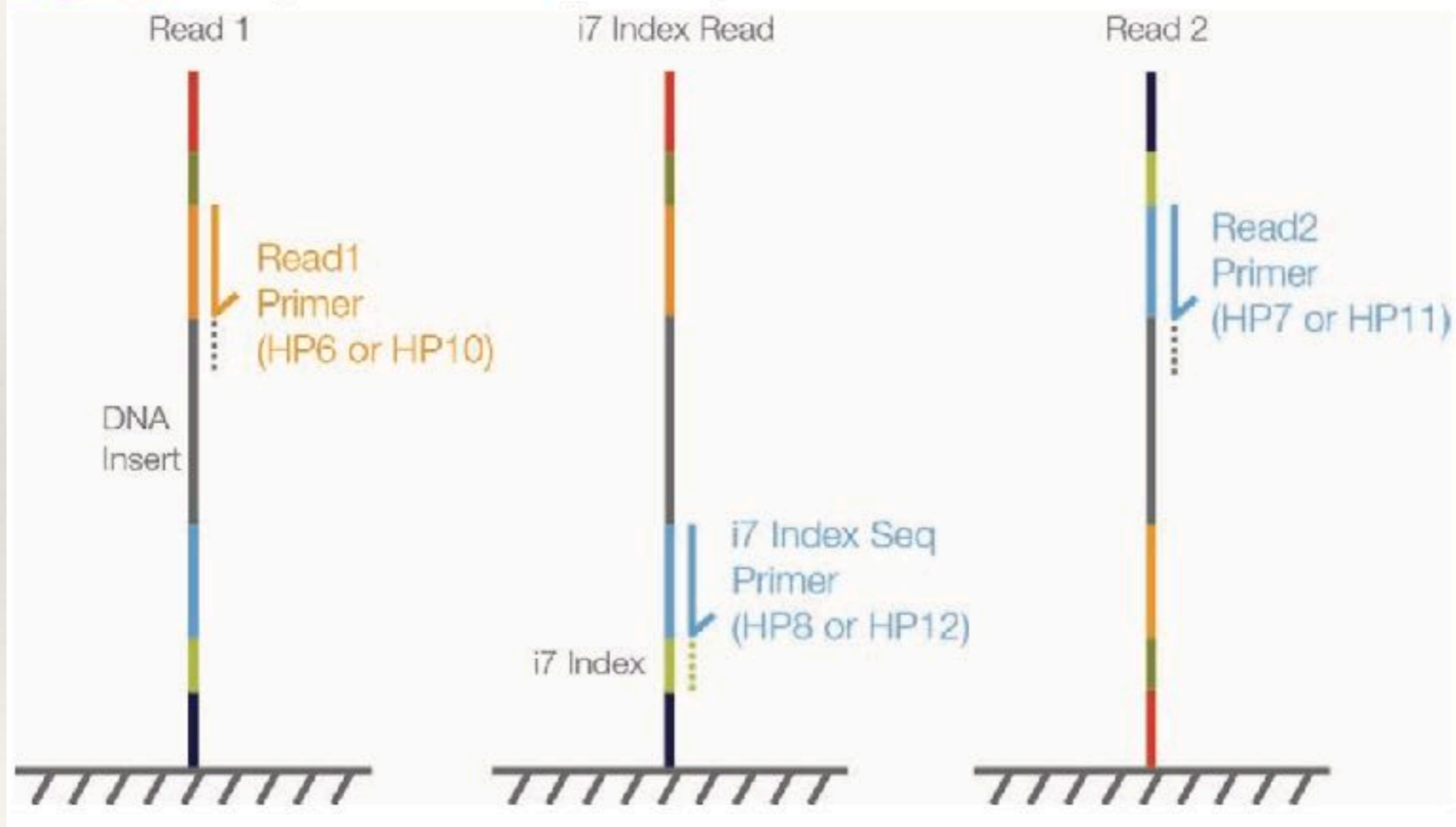
RNA prep poster:

<https://www.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/for-all-you-seq-rna.pdf>

Library prep video: <https://www.youtube.com/watch?v=-kTcFZxP6kM>

Sequencing

Figure 1 Single-Indexed Sequencing



Sequencing

