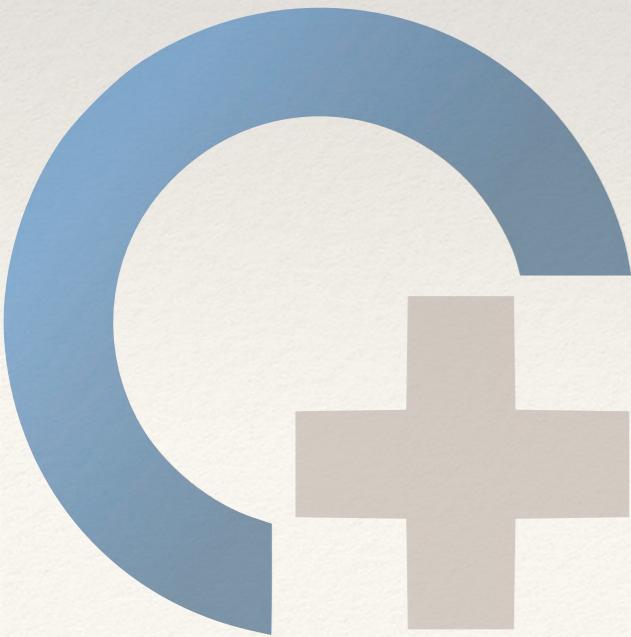


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HTS

Library prep

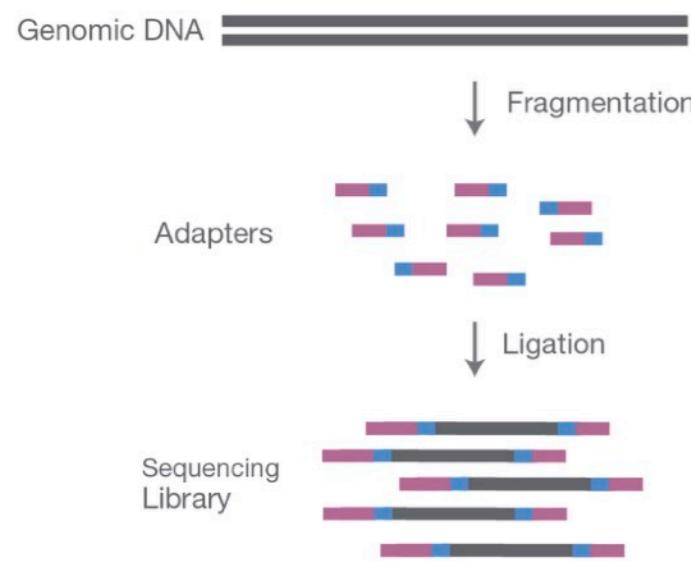


Arvind Sundaram
Oct 23, 2018

Norwegian Sequencing Centre
OUS, Ullevål, Oslo

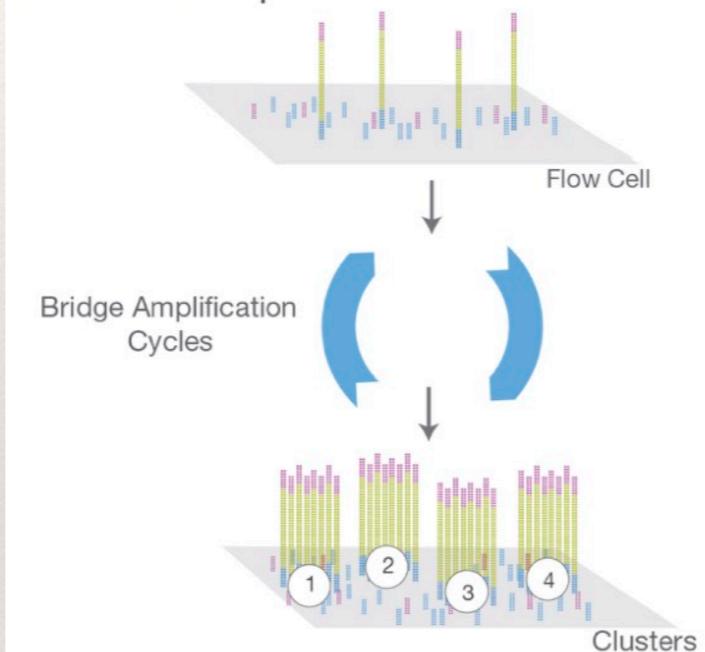
Sample to sequence data

A. Library Preparation



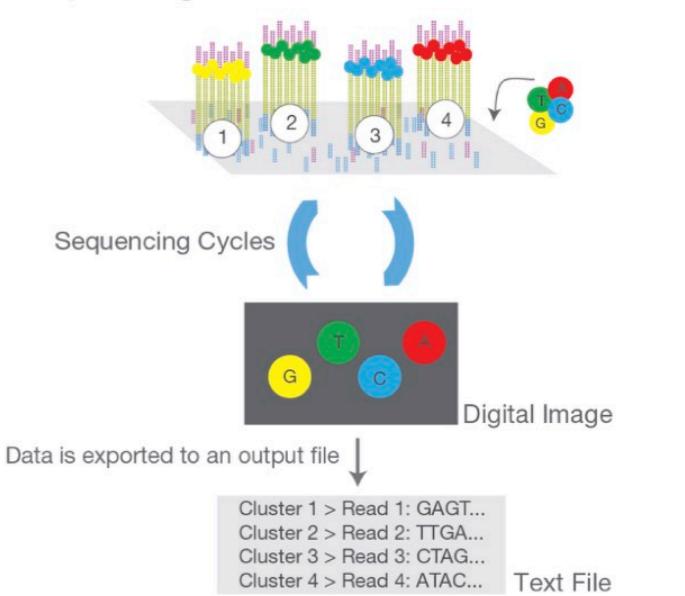
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

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.

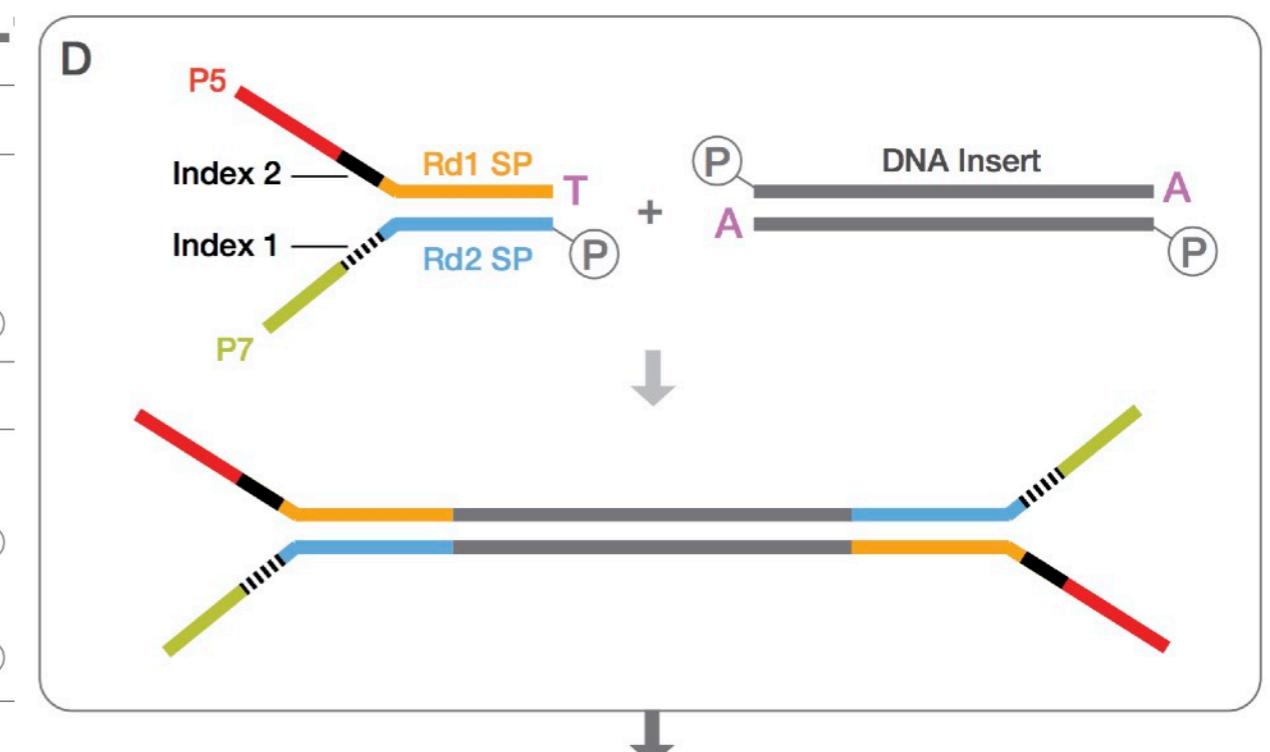
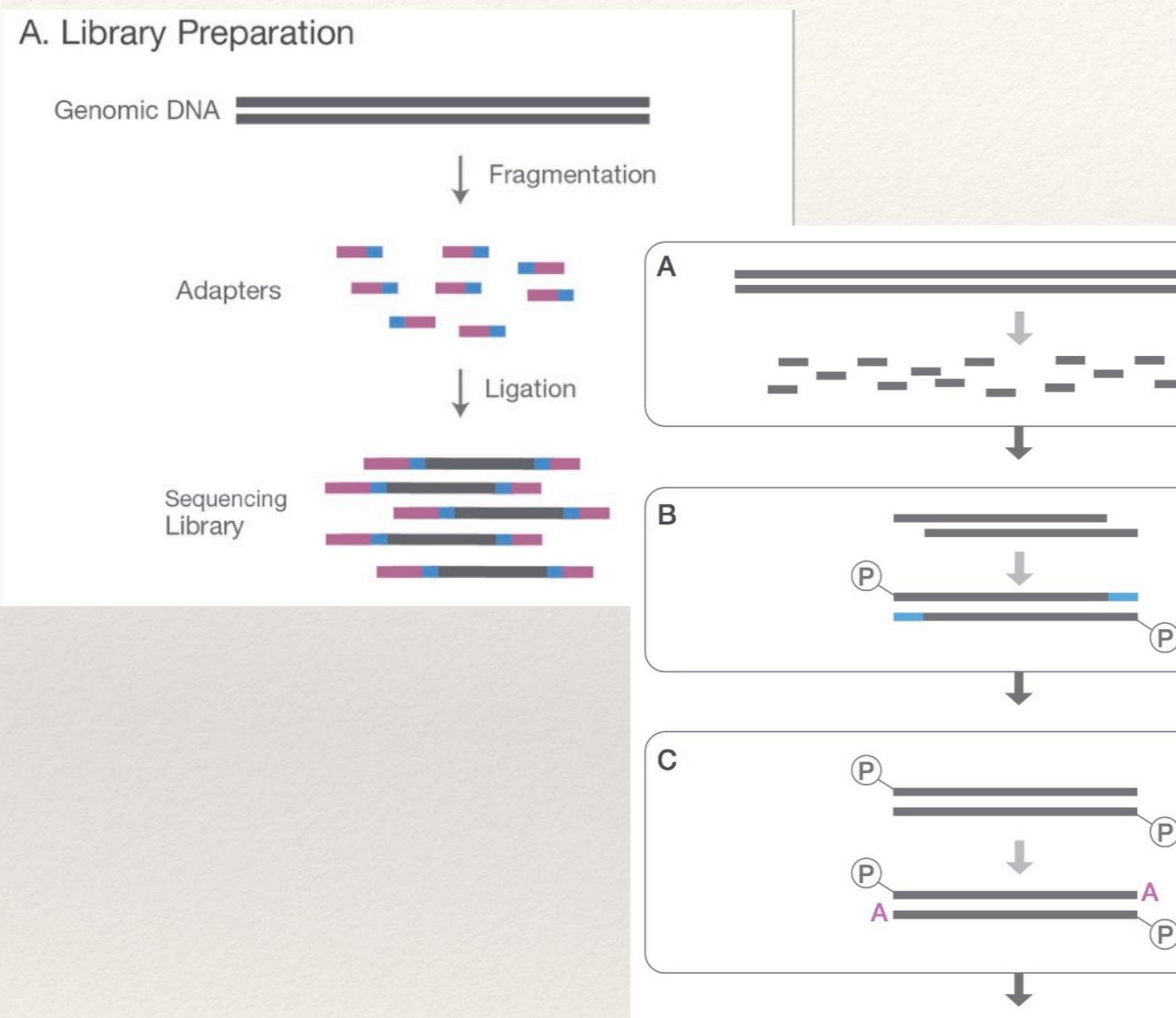
C. Sequencing



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.

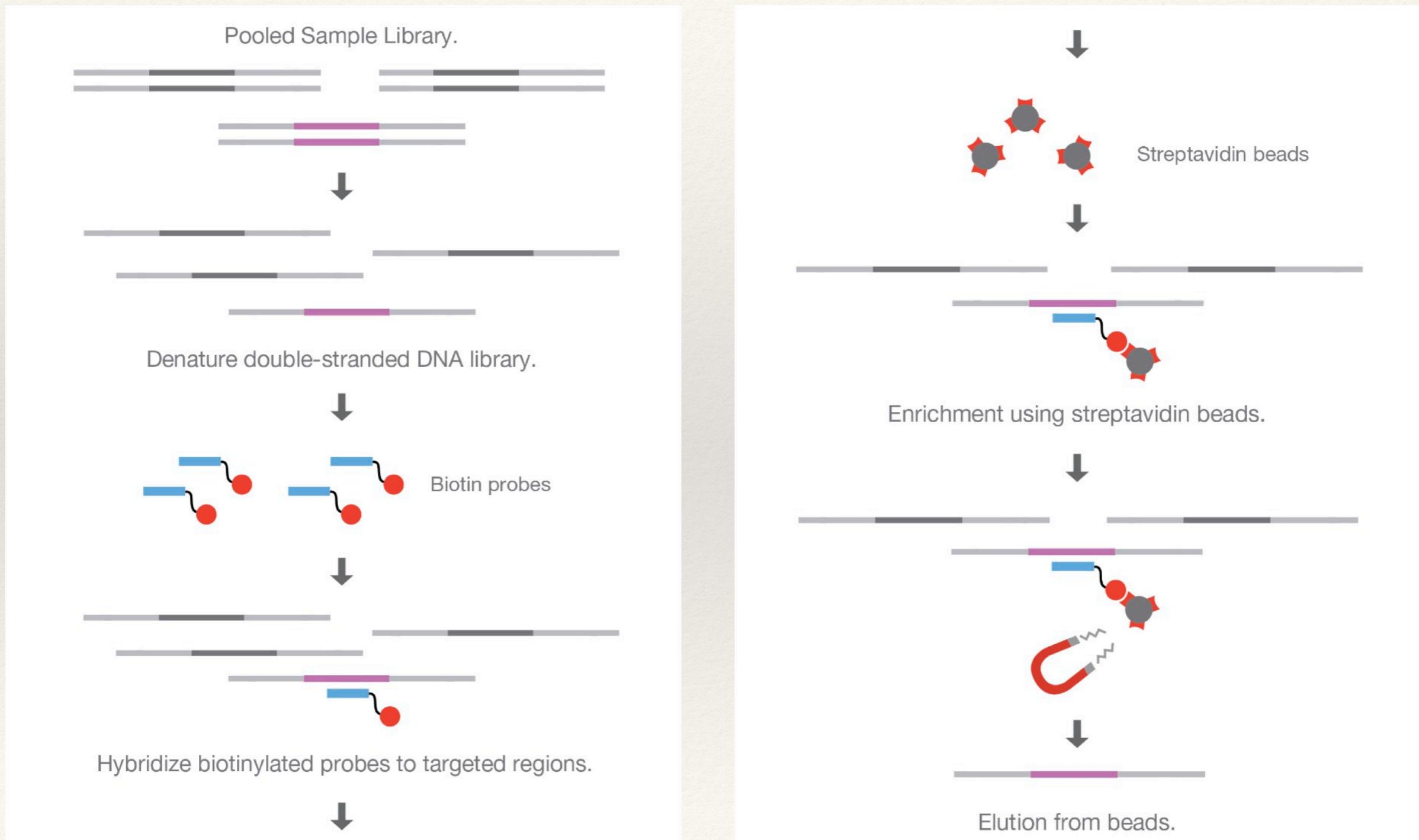
Library prep

A. Library Preparation



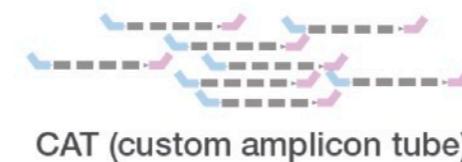
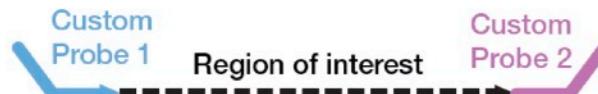
- A. Library construction begins with genomic DNA that is subsequently fragmented
- B. Blunt-end fragments are created
- C. A-base is added
- D. Dual-index adapters are ligated to the fragments*
- E. Final product is ready for amplification

Target enrichment



Amplicons

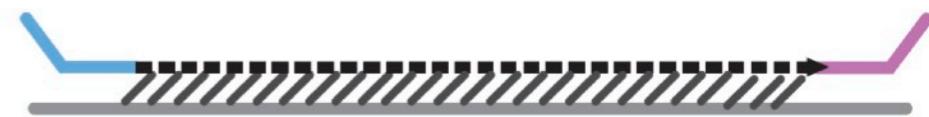
Use DesignStudio Software to create custom oligo capture probes flanking each region of interest.



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.

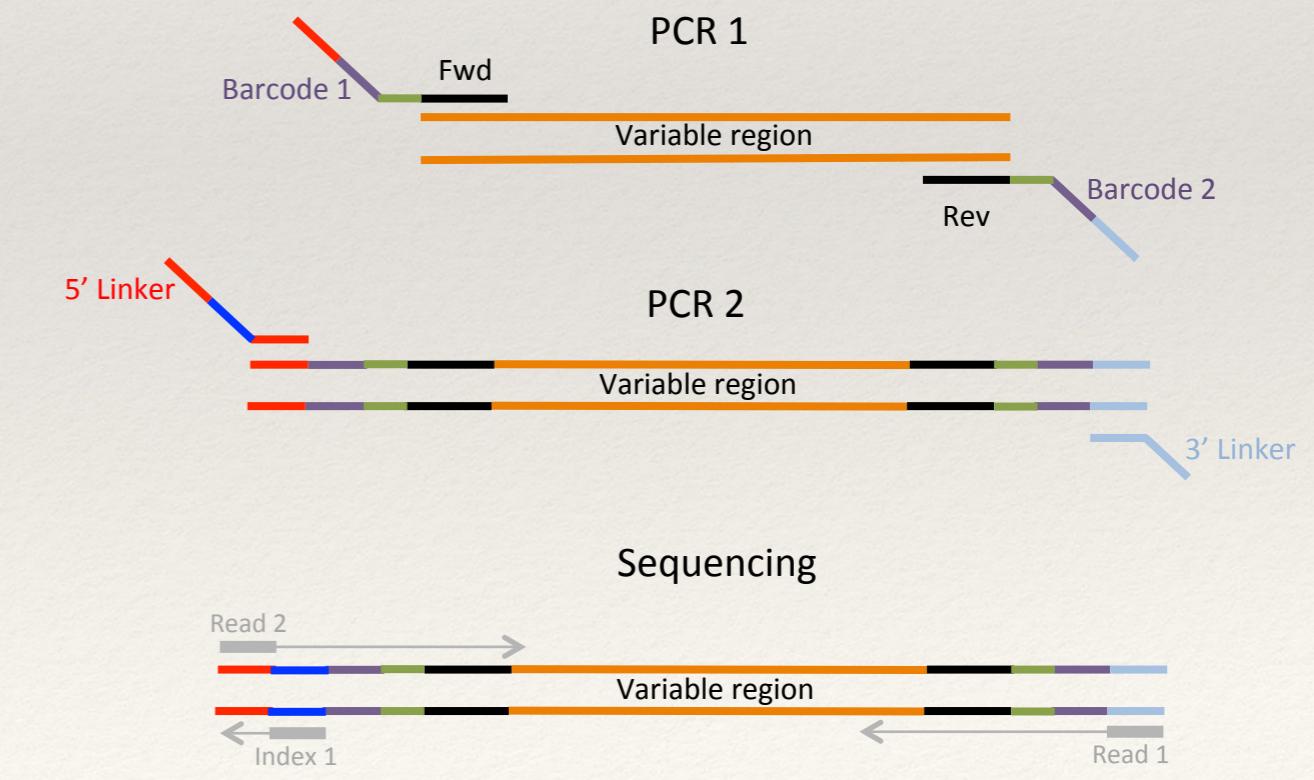
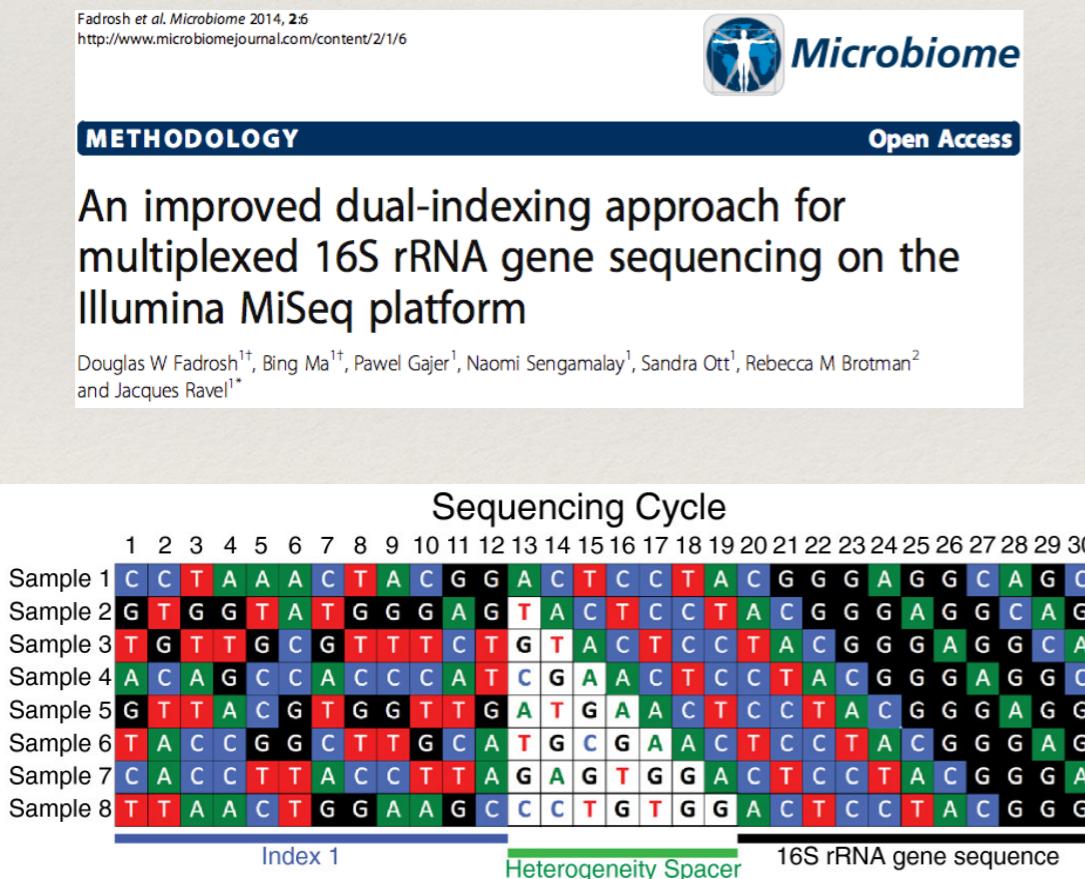


Uniquely tagged amplicon library ready for cluster generation and sequencing.



Amplicons

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



Illumina library prep

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

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

Illumina library prep

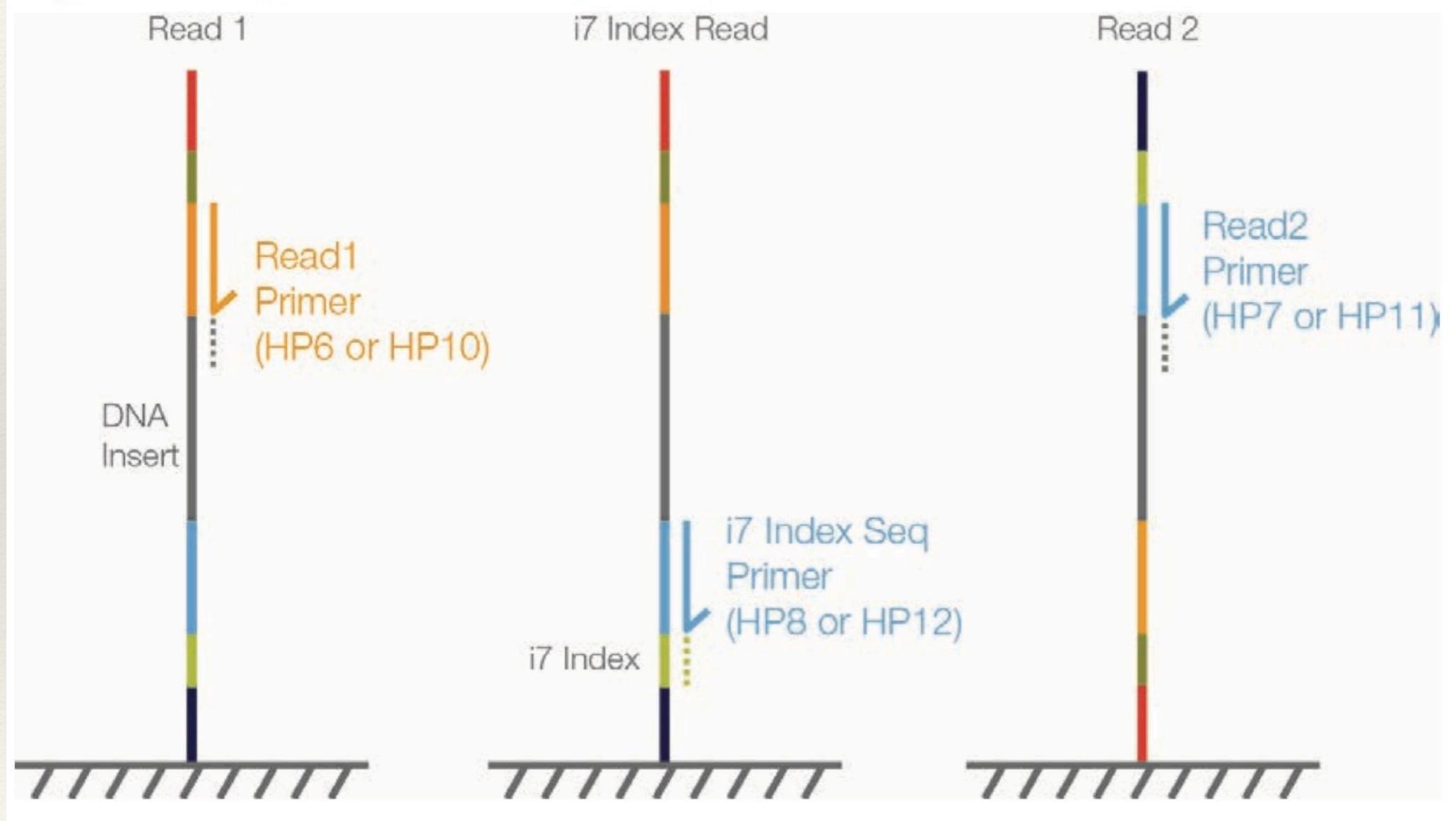
- ❖ TruSeq PCR-free DNA kit, TruSeq Nano DNA kit
- ❖ Nextera DNA kit, Nextera DNA XT kit
- ❖ Nextera Rapid Capture Exome kit
- ❖ Nextera Rapid Capture Expanded Exome kit
- ❖ TruSeq Stranded Total RNA kit
- ❖ TruSeq Stranded mRNA kit
- ❖ TruSeq small RNA kit
- ❖ TruSeq DNA Methylation kit

<https://www.illumina.com/library-prep-array-kit-selector.html>

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

Sequencing

Figure 1 Single-Indexed Sequencing



Sequencing

Figure 2 Dual-Indexed Single-Read Sequencing

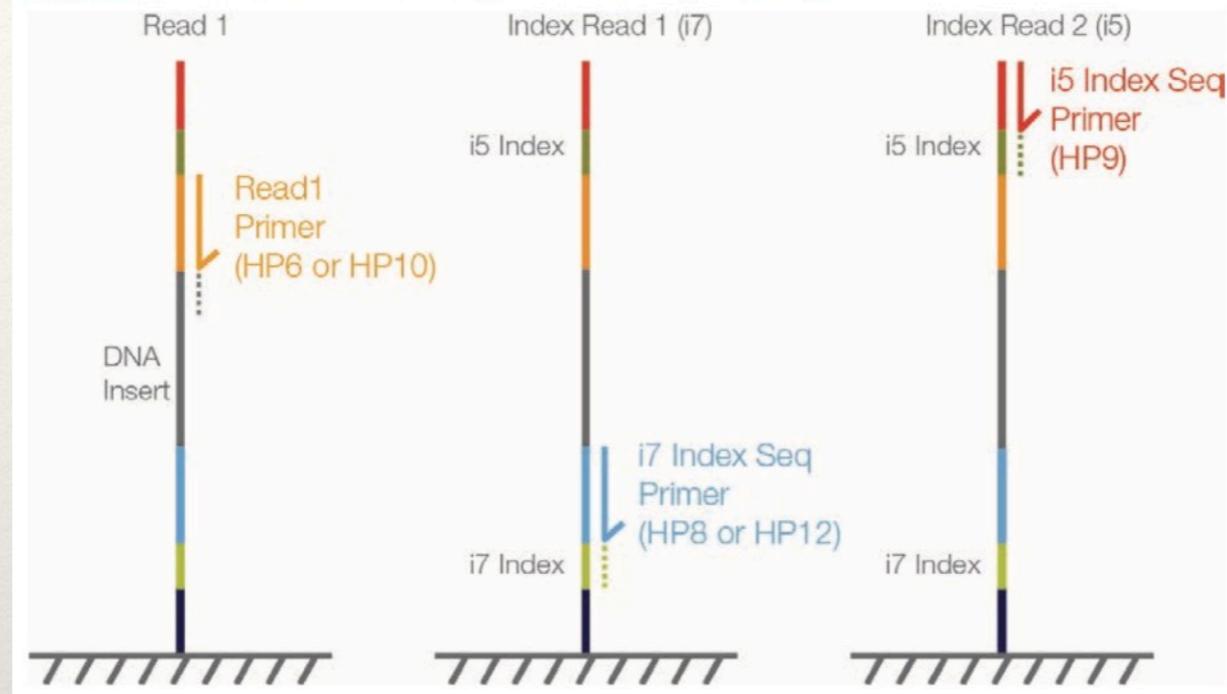
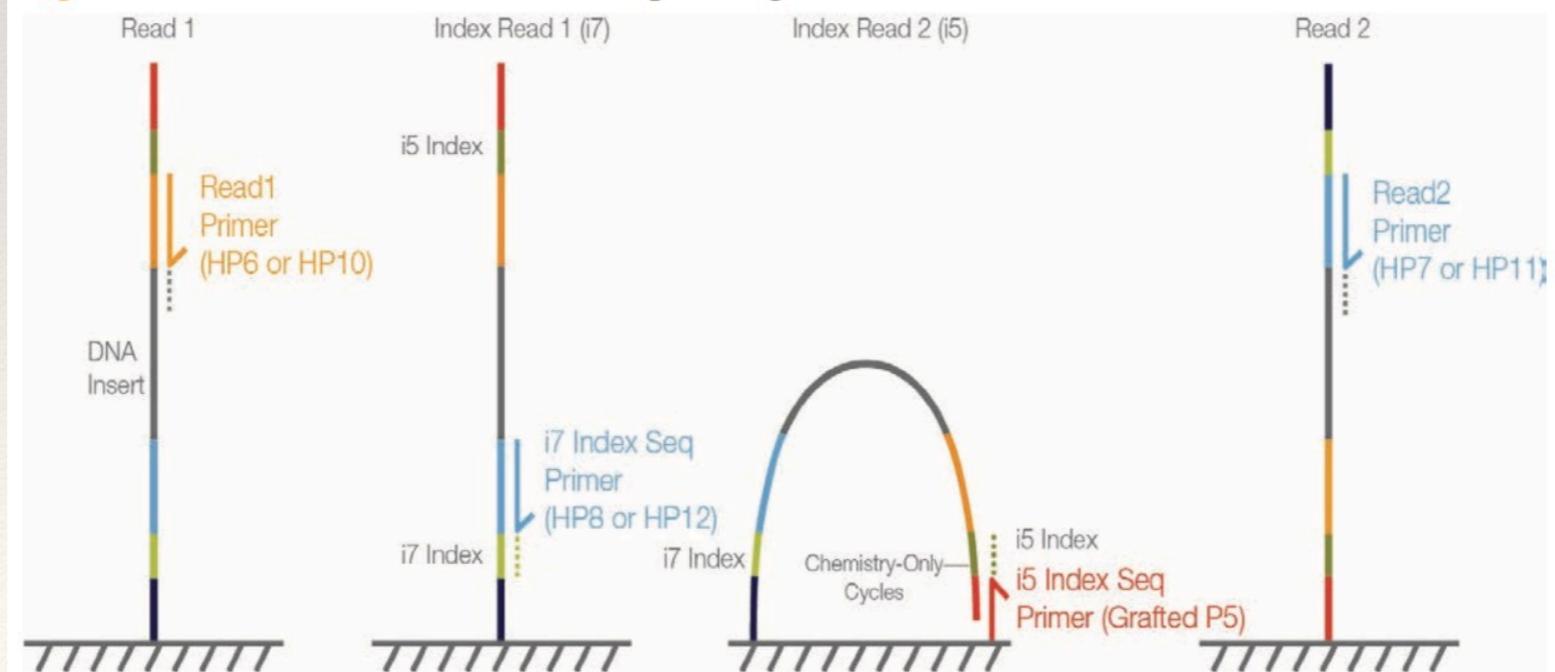
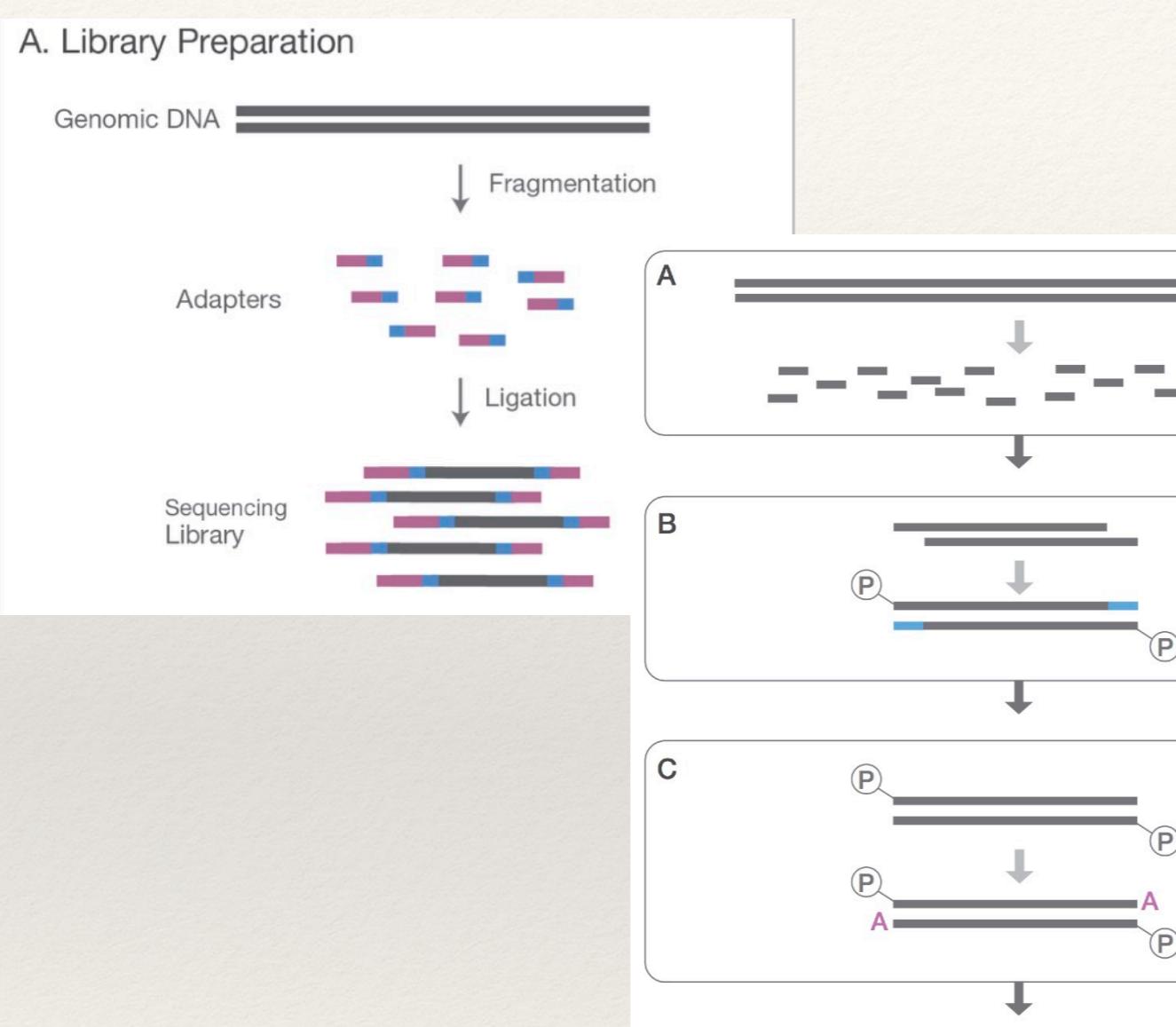


Figure 3 Dual-Indexed Paired-End Sequencing

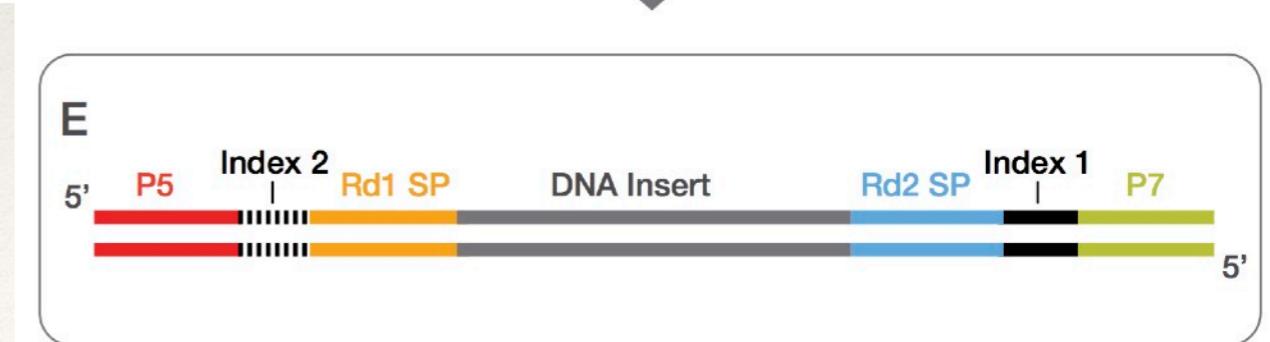
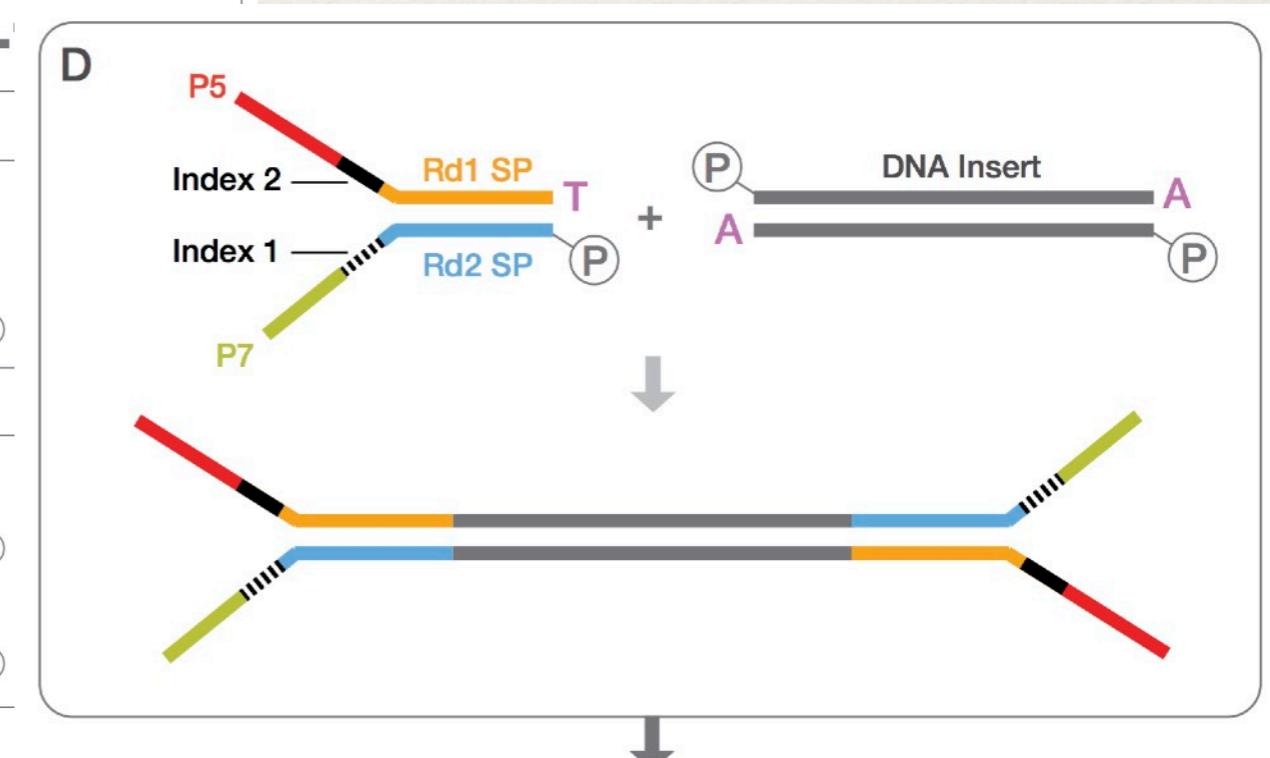


Library prep (Illumina)

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



Library prep (Illumina)

- ❖ RNA sequencing
 - ❖ Total RNA
 - ❖ mRNA
 - ❖ small RNA
 - ❖ Ribosome profiling
 - ❖ High quality and quantity of RNA
 - ❖ Do you want to sequence rRNA??
 - ❖ TruSeq Stranded Total RNA kit
 - ❖ TruSeq Stranded mRNA kit
 - ❖ TruSeq small RNA kit