IN-BIOS[9,5]000 2020

HTS

Library prep

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Oct 20, 2020

Norwegian Sequencing Centre
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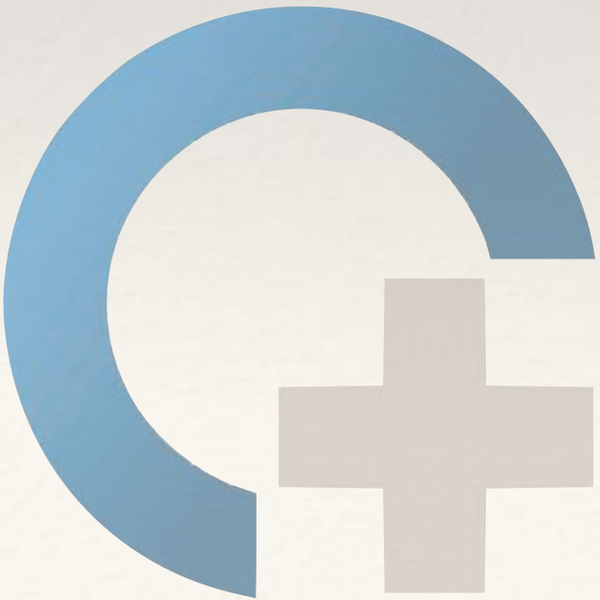
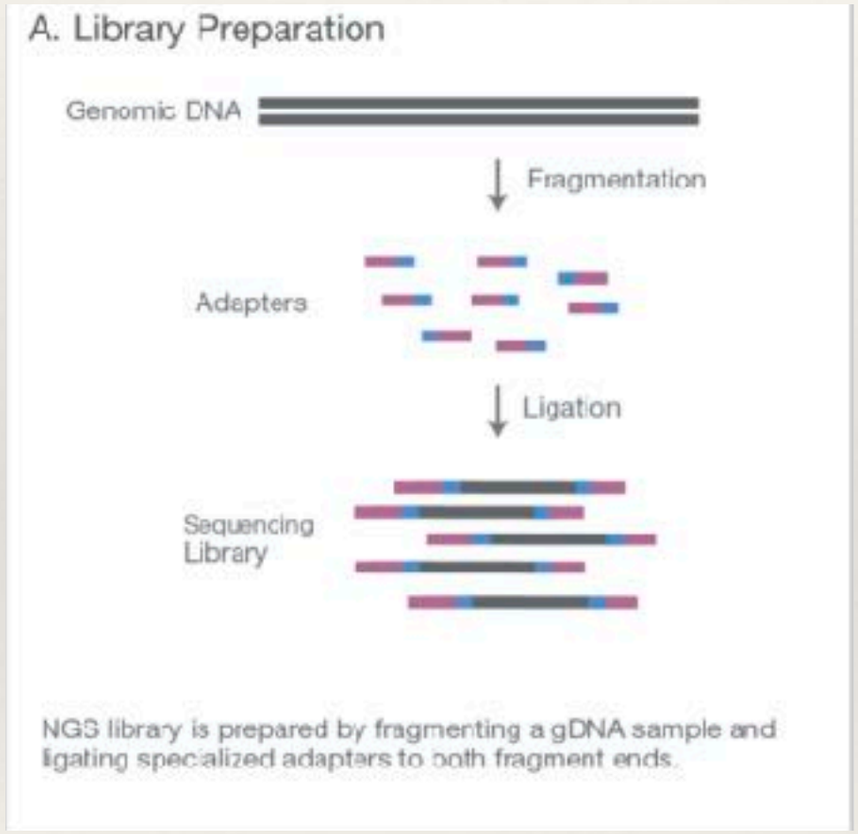
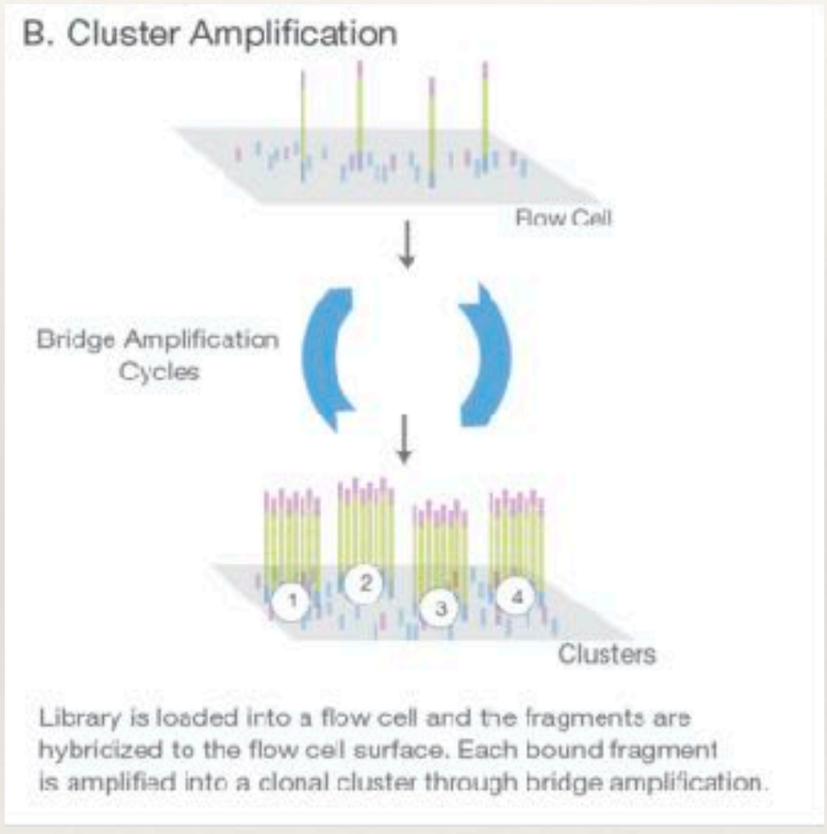
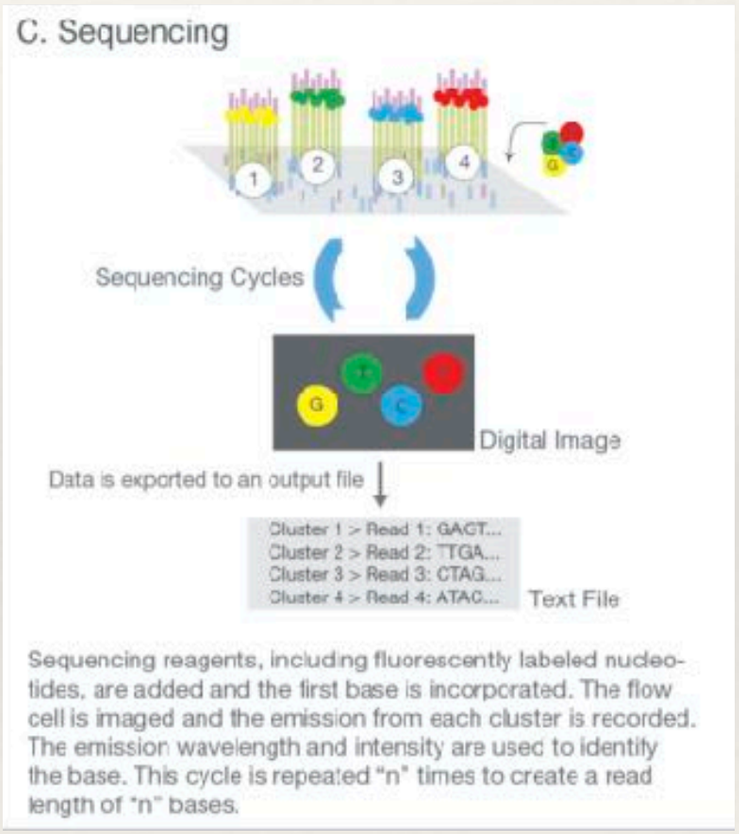


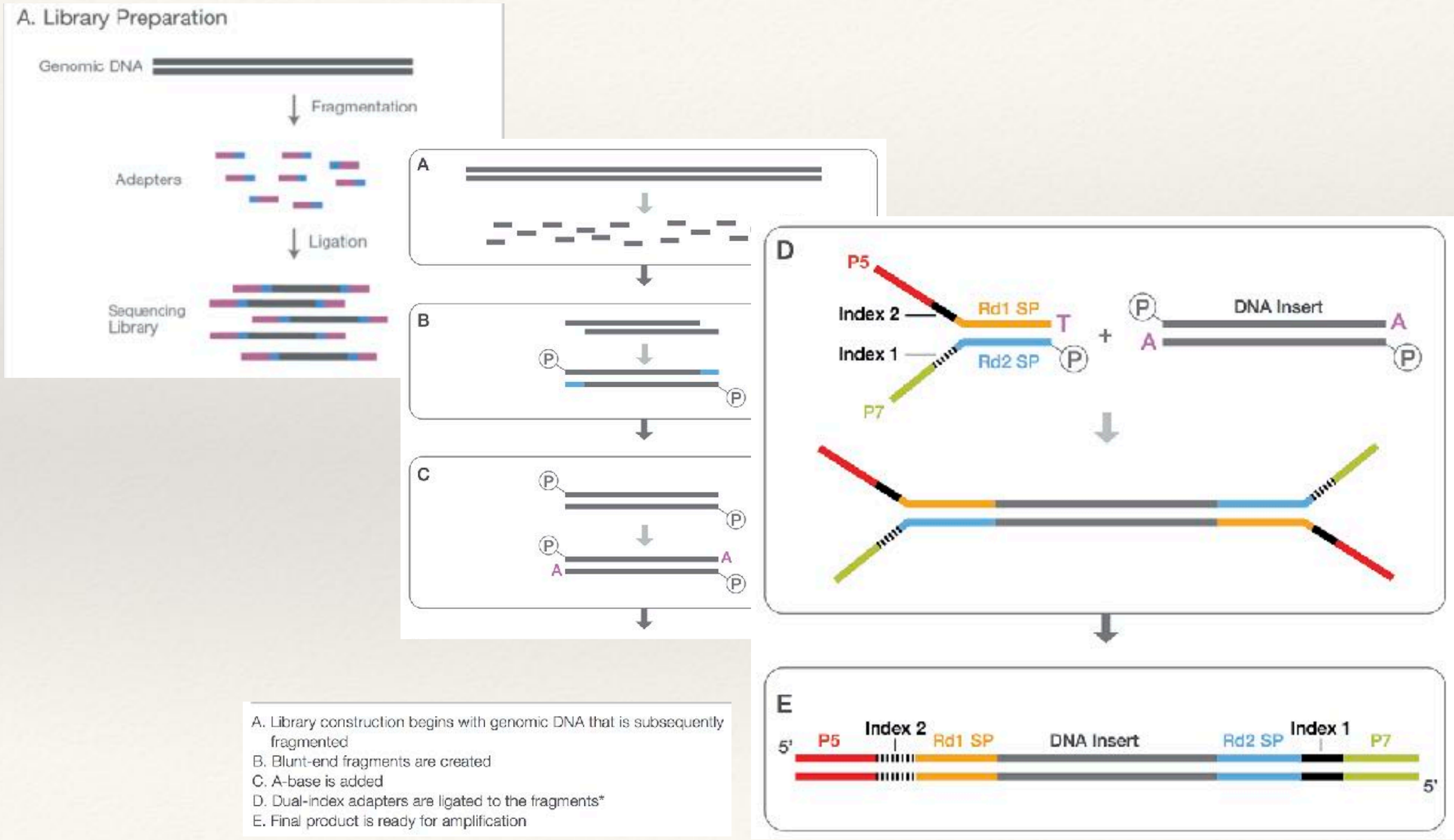
Sample to sequence data





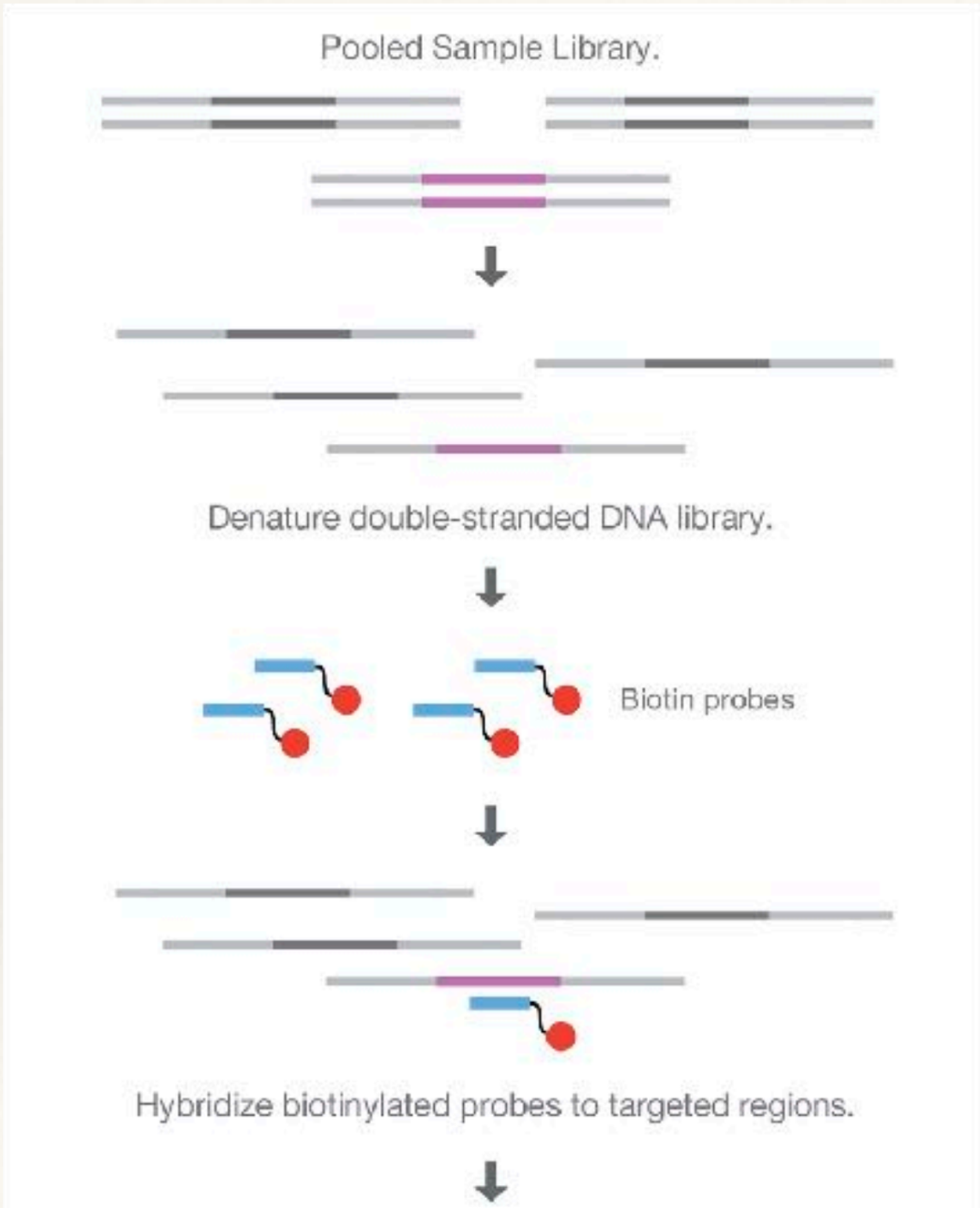


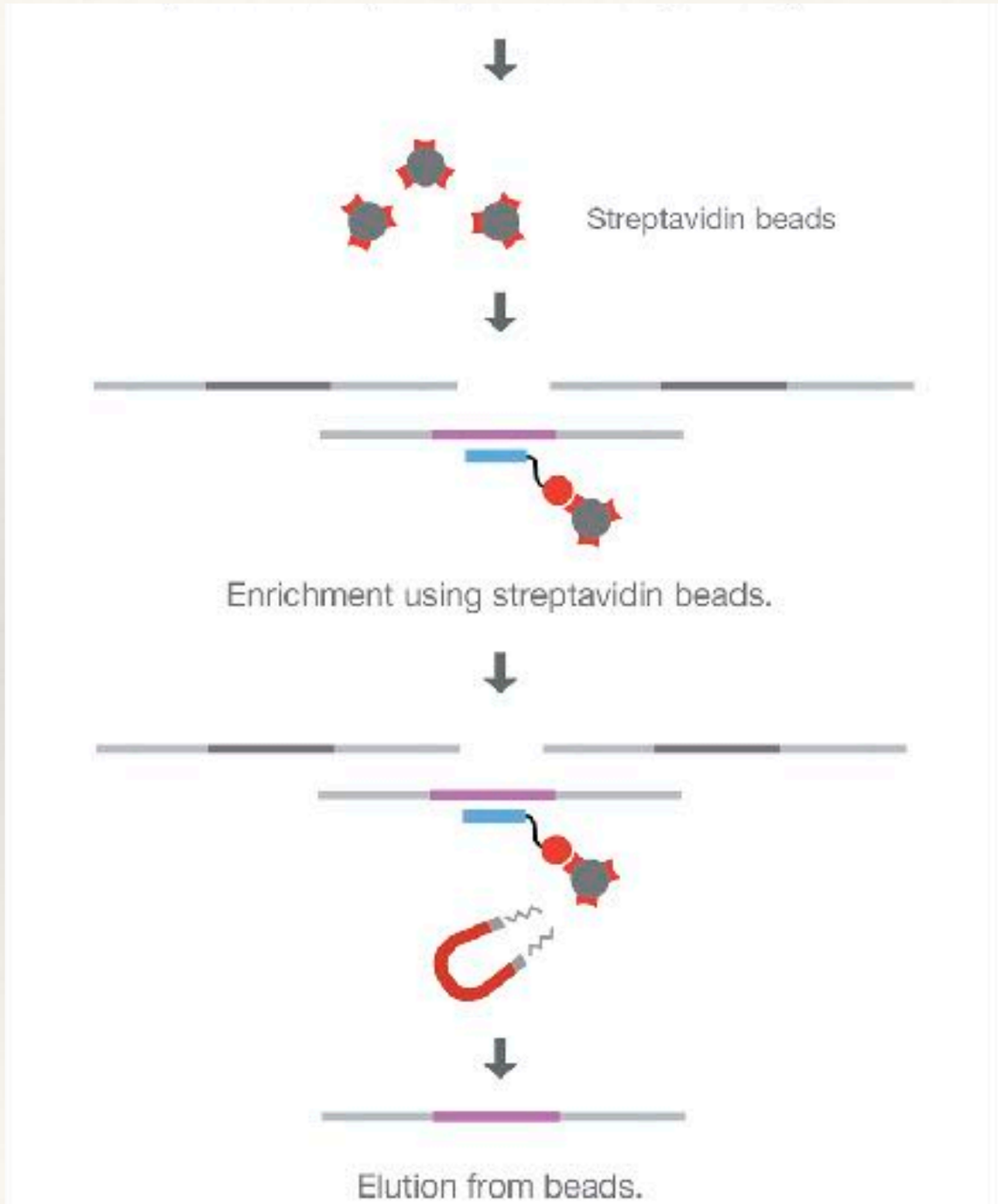
Library prep



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

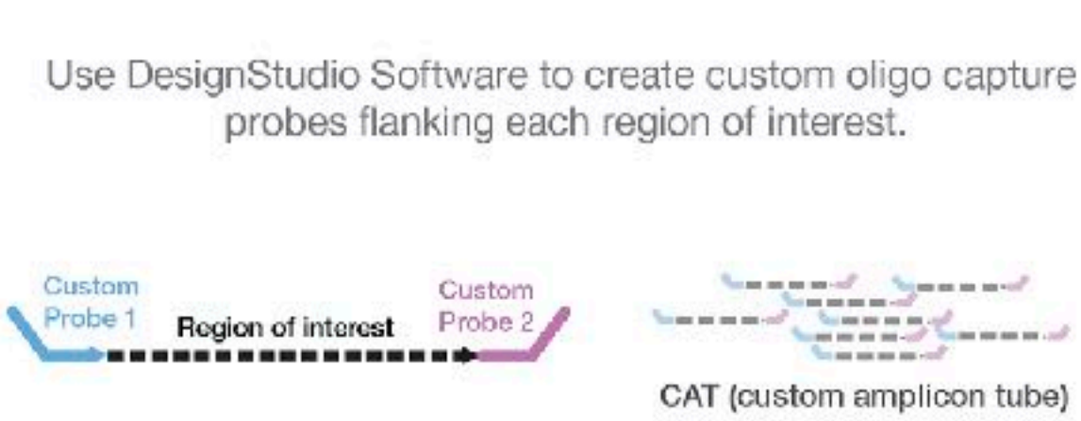
Target enrichment





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.

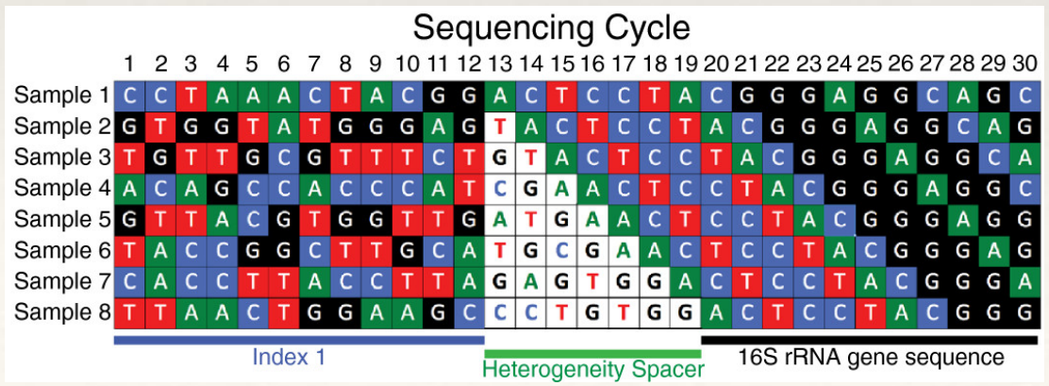
Fadrosh *et al. Microbiome* 2014, **2**:6
http://www.microbiomejournal.com/content/2/1/6

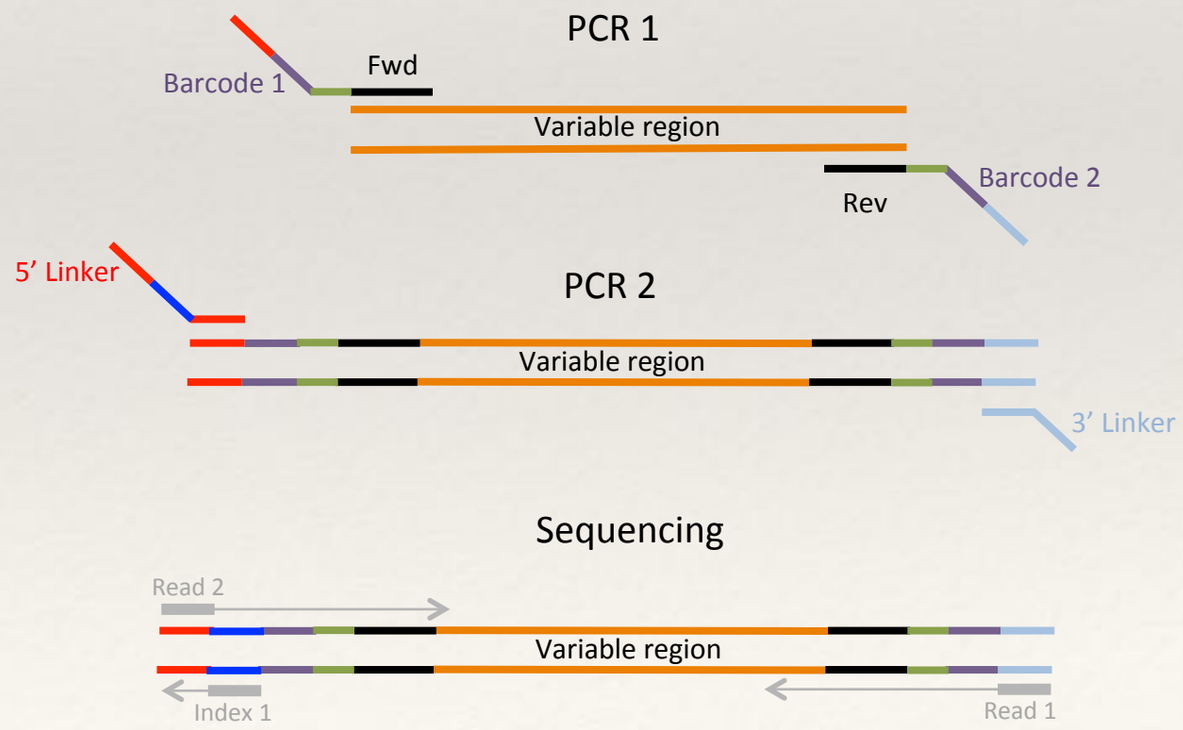
Microbiome

METHODOLOGY Open Access

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

Douglas W Fadrosh[1†], Bing Ma[1†], Pawel Gajer[1], Naomi Sengamalay[1], Sandra Ott[1], Rebecca M Brotman[2] and Jacques Ravel[1*]





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

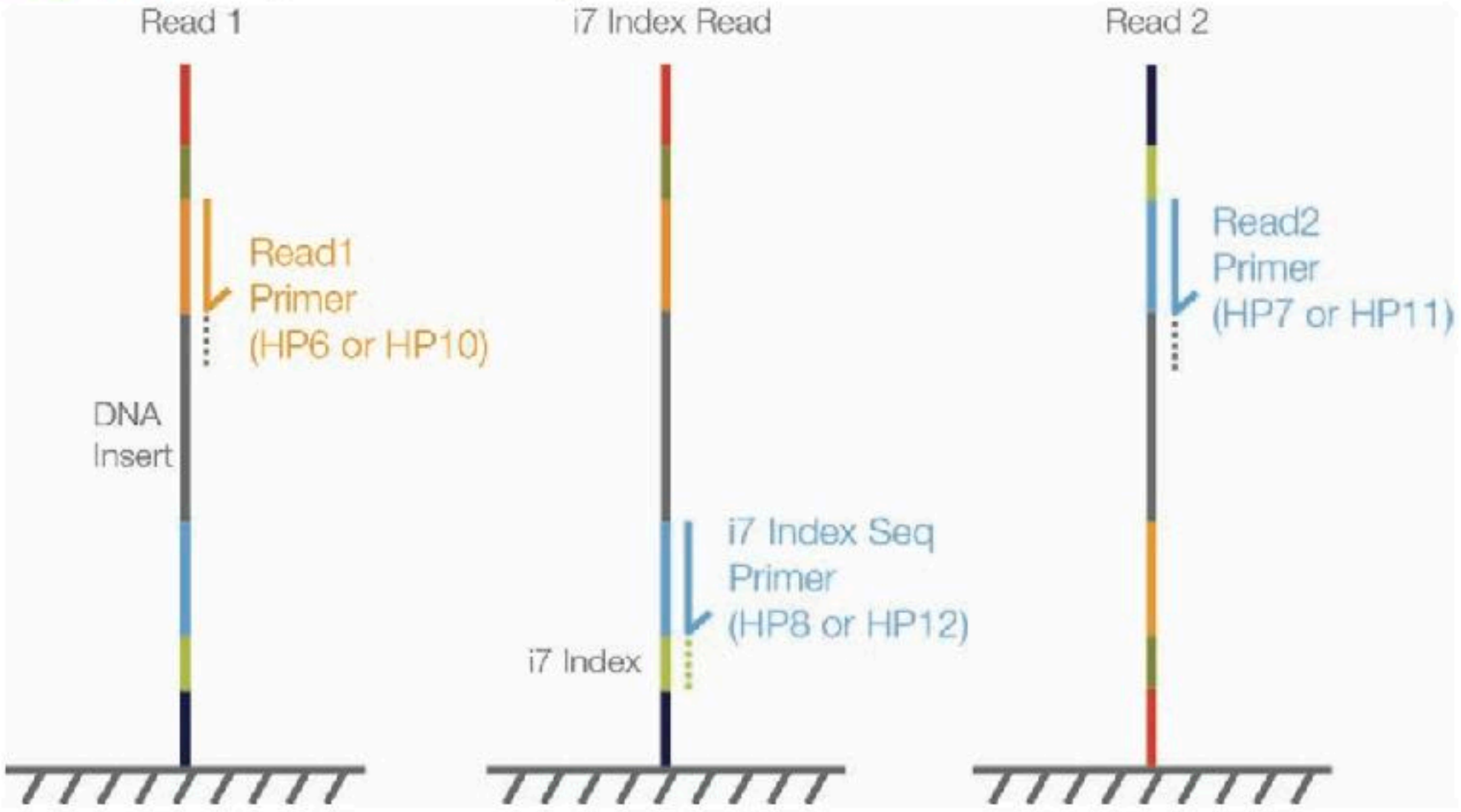
- TruSeq DNA Methylation kit

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

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

Sequencing

Figure 1 Single-Indexed Sequencing



Sequencing

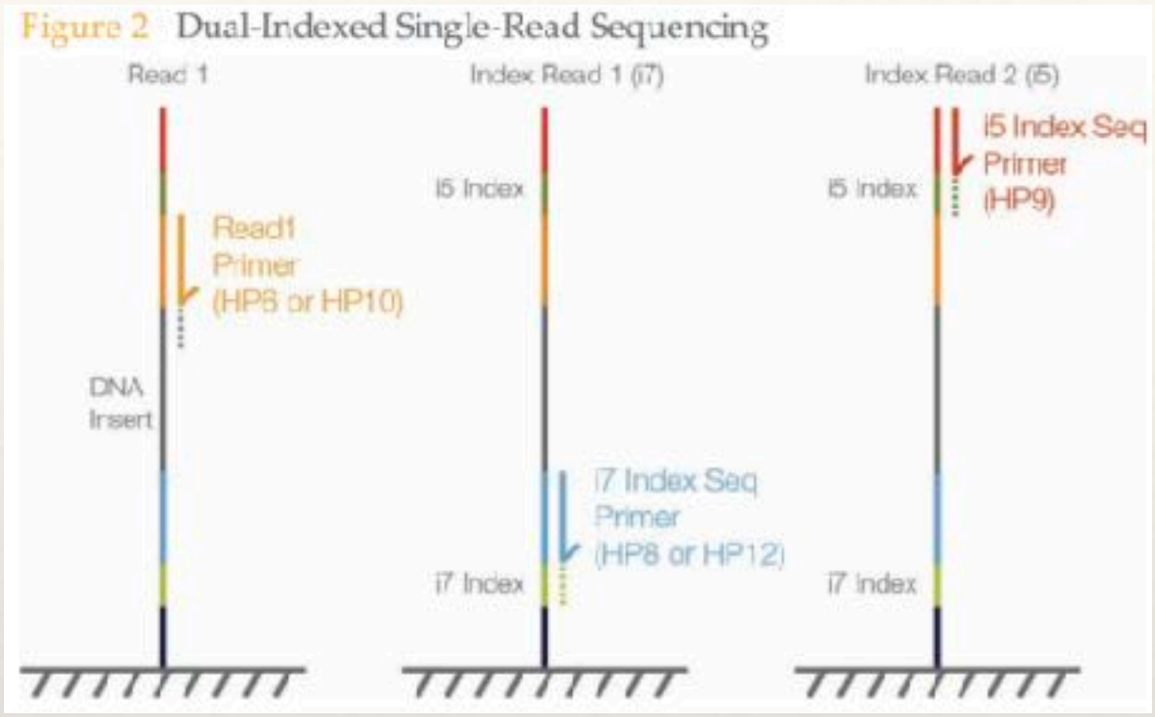


Figure 2 Dual-Indexed Single-Read Sequencing
Read 1
Index Read 1 (i7)
Index Read 2 (i5)
i5 Index Seq Primer (HP9)
i5 Index
Read1 Primer (HP6 or HP10)
DNA Insert
i7 Index Seq Primer (HP8 or HP12)
i7 Index
i7 Index

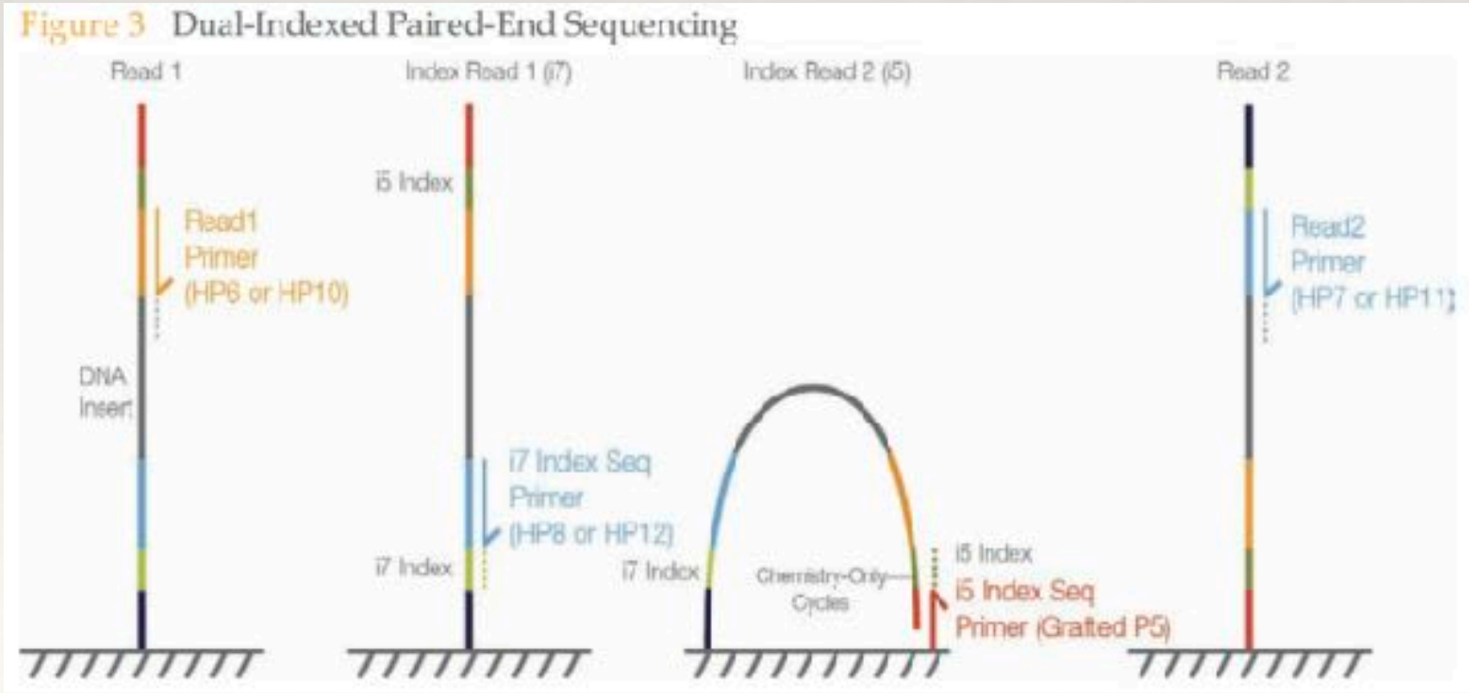


Figure 3 Dual-Indexed Paired-End Sequencing
Read 1
Index Read 1 (i7)
Index Read 2 (i5)
Read 2
i5 Index
Read1 Primer (HP6 or HP10)
Read2 Primer (HP7 or HP11)
DNA Insert
i7 Index Seq Primer (HP8 or HP12)
i7 Index
i7 Index
Chemistry-Only Cycles
i5 Index
i5 Index Seq Primer (Grafted P5)