

Sequencing Illumina

Library = Sample

Sequences = Reads = Clusters

Cycles = Bases

Index = Barcode

Illumina libraries

Single End Sequencing

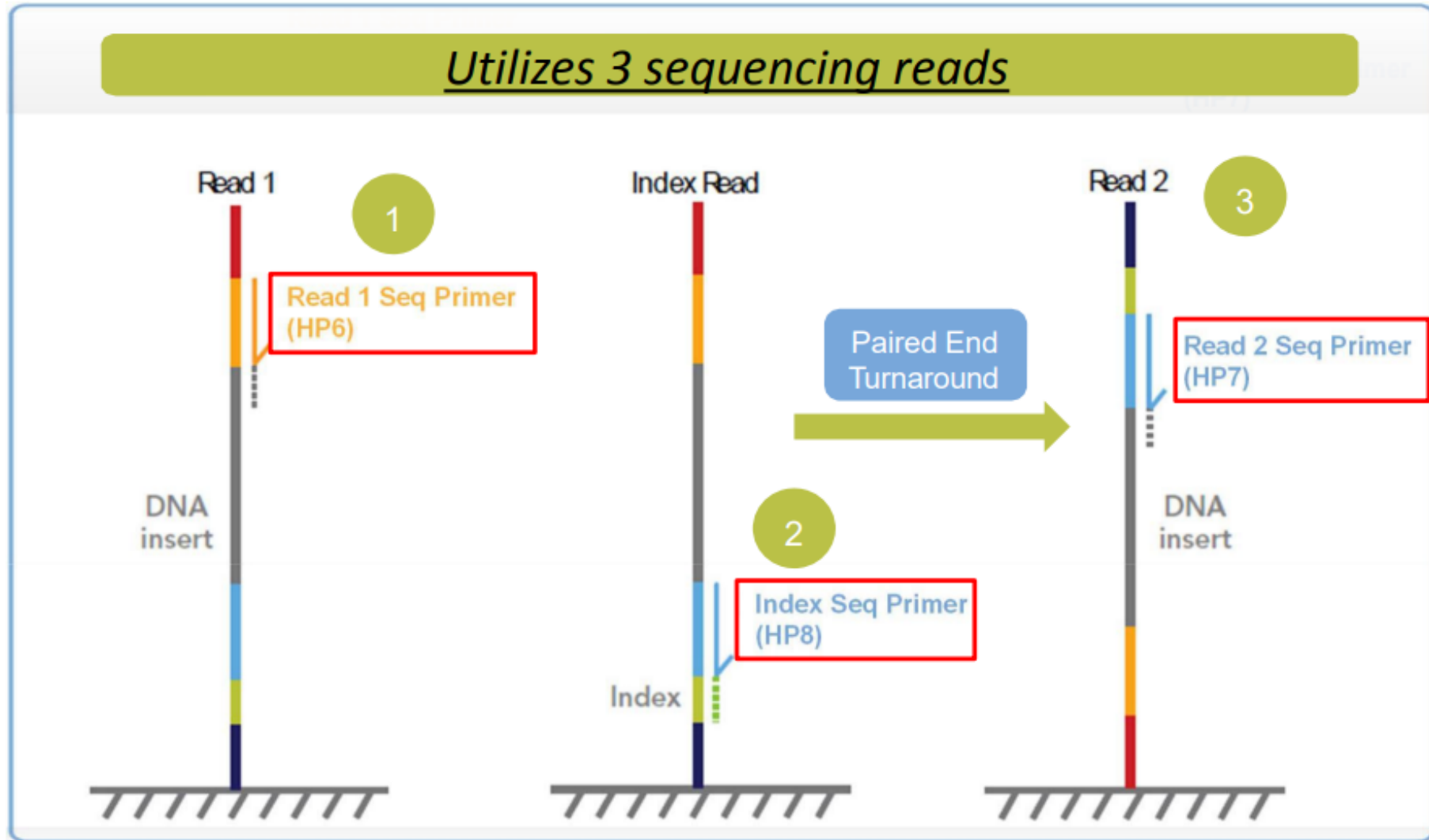


Paired End Sequencing

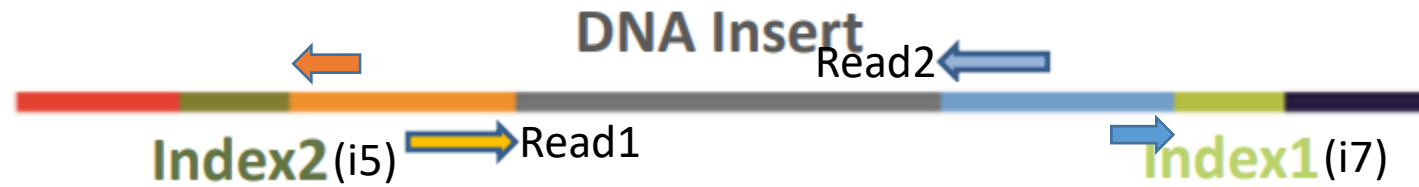


The indices have additional primers

Paired End Sequencing of Single-indexed libraries



Sequencing Paired End Libraries with Dual Index Read

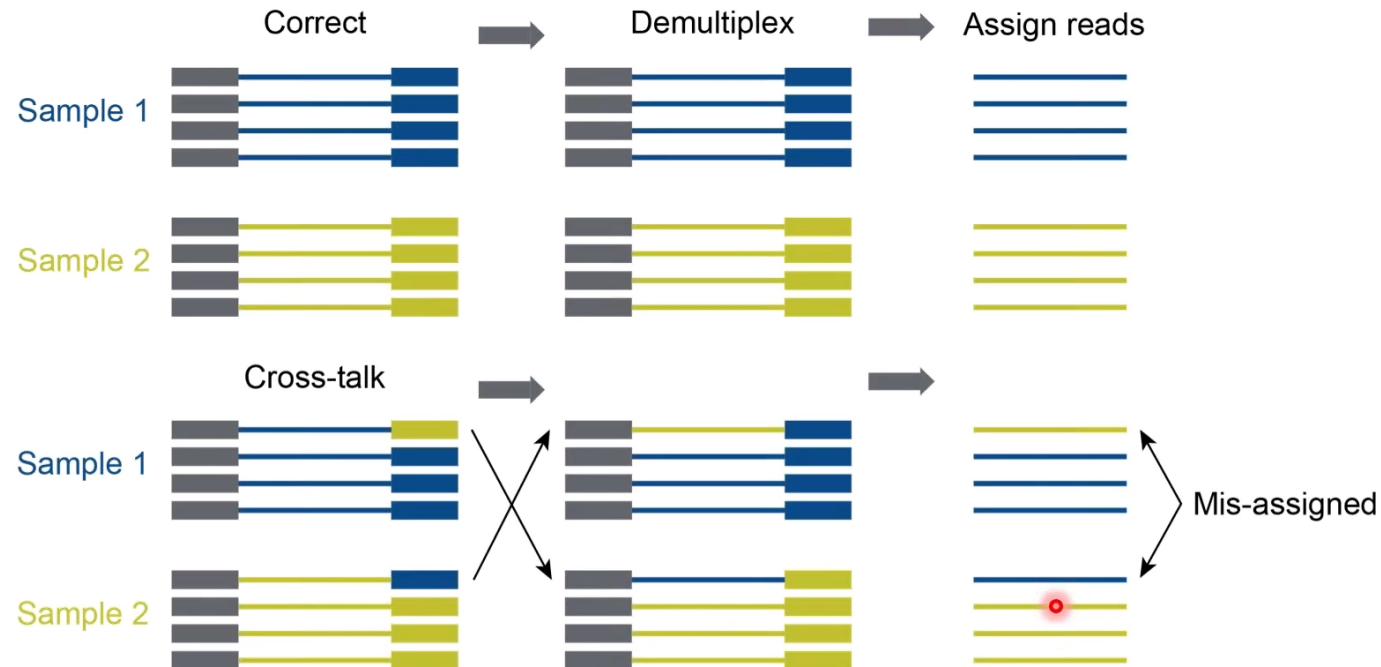


Utilizes 4 Sequencing reads:

- ❖ Read 1
- ❖ Index Read 1 (i7)
- ❖ Index Read 2 (i5)
- ❖ Read2

Explaining index hopping –cross talk Using only one index (i7)

What is sample cross-talk?



- Reads are assigned to the wrong sample



Applications that can be impacted by cross-talk

- **Low-frequency somatic variant detection**—false positives from other samples
- **Ancient DNA research**—a single sequence may support DNA survival or contamination
- **Viral detection**—false positives from other samples
- **Gene expression**—bleed over from one sample to another
- **Microbial profiling**—bleed over from one sample to another

Kircher M, Sawyer S, Meyer M. (2012) Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res*, 40(1):e3.

D'Amore R, Ijaz UZ, et al. (2016) A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics*, 17:55.

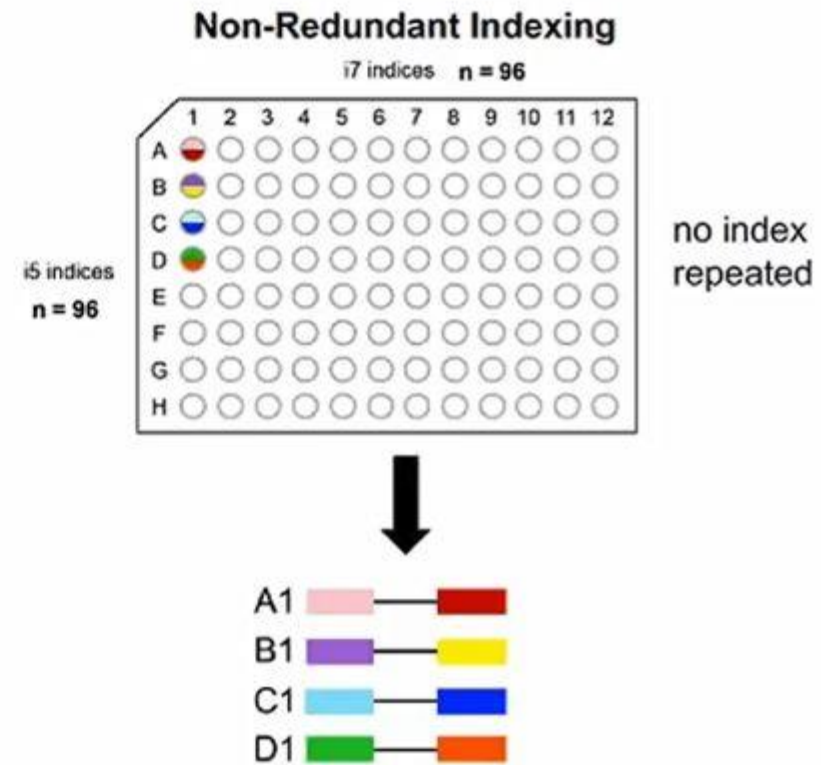
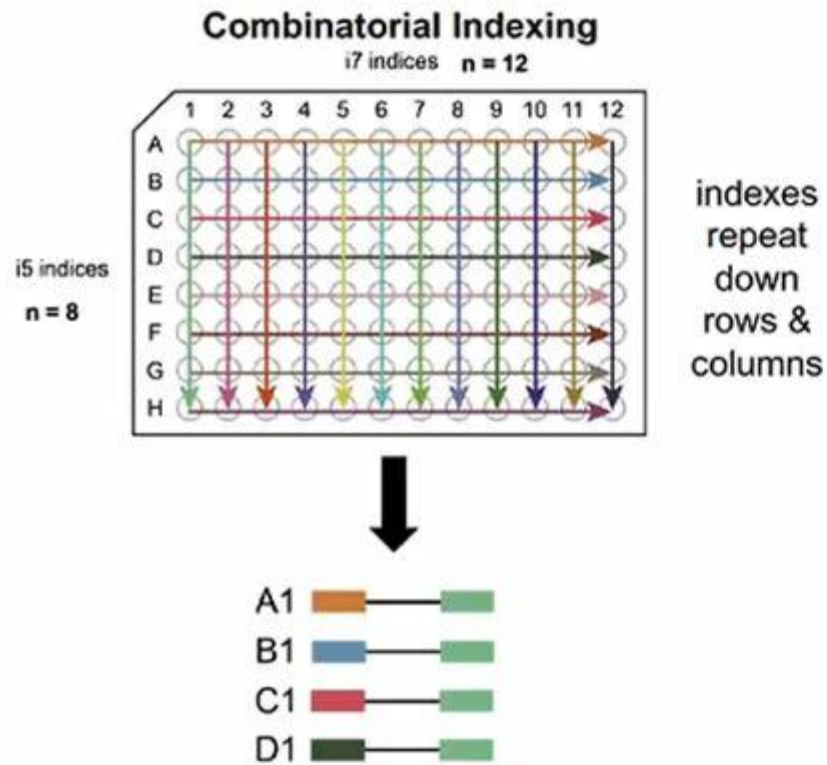
Unique dual indexing strategy

When preparing libraries for multiplexing, Illumina encourages customers to use unique dual indexing (UDI) whenever possible.

What you need to know about Unique Dual Indexing:

- ❖ Unique dual indexing is a sequencing strategy that has distinct, unrelated index sequences for the i5 and i7 Index Read.
- ❖ Unique dual indexing is a known mitigation for filtering index-hopped reads seen in downstream analyses. Misassigned reads will be flagged as “undetermined reads” and can be excluded from analysis. For more information about index hopping refer to the white paper [Effects of Index Misassignment on Multiplexing and Downstream Analysis](#).
- ❖ Unique dual indexes, or Non-Redundant Indexes, are used in the ligation step without any changes to the library preparation workflow.
- ❖ In contrast to combinatorial dual indexing, unique dual indexing has completely unique indexes (96 unique i5s and 96 unique i7s). With combinatorial dual indexing, there is a limit to 8 unique dual pairs, resulting in the majority of the libraries sharing common indexes on the i5 or i7 end.

Unique Dual Indexing



<https://emea.support.illumina.com/bulletins/2018/08/understanding-unique-dual-indices--udi--and-associated-library-p.html>

Unique Dual Index Kits

Product Name	Catalog Number	Indexes, Samples
IDT for Illumina - TruSeq DNA UD Indexes	20020590	24 Indexes, 96 Samples
IDT for Illumina - TruSeq DNA UD Indexes	20022370	96 Indexes, 96 Samples
IDT for Illumina - TruSeq RNA UD Indexes	20020591	24 Indexes, 96 Samples
IDT for Illumina - TruSeq RNA UD Indexes	20022371	96 Indexes, 96 Samples

Unique, dual indexes reduce contamination mis-assignment

