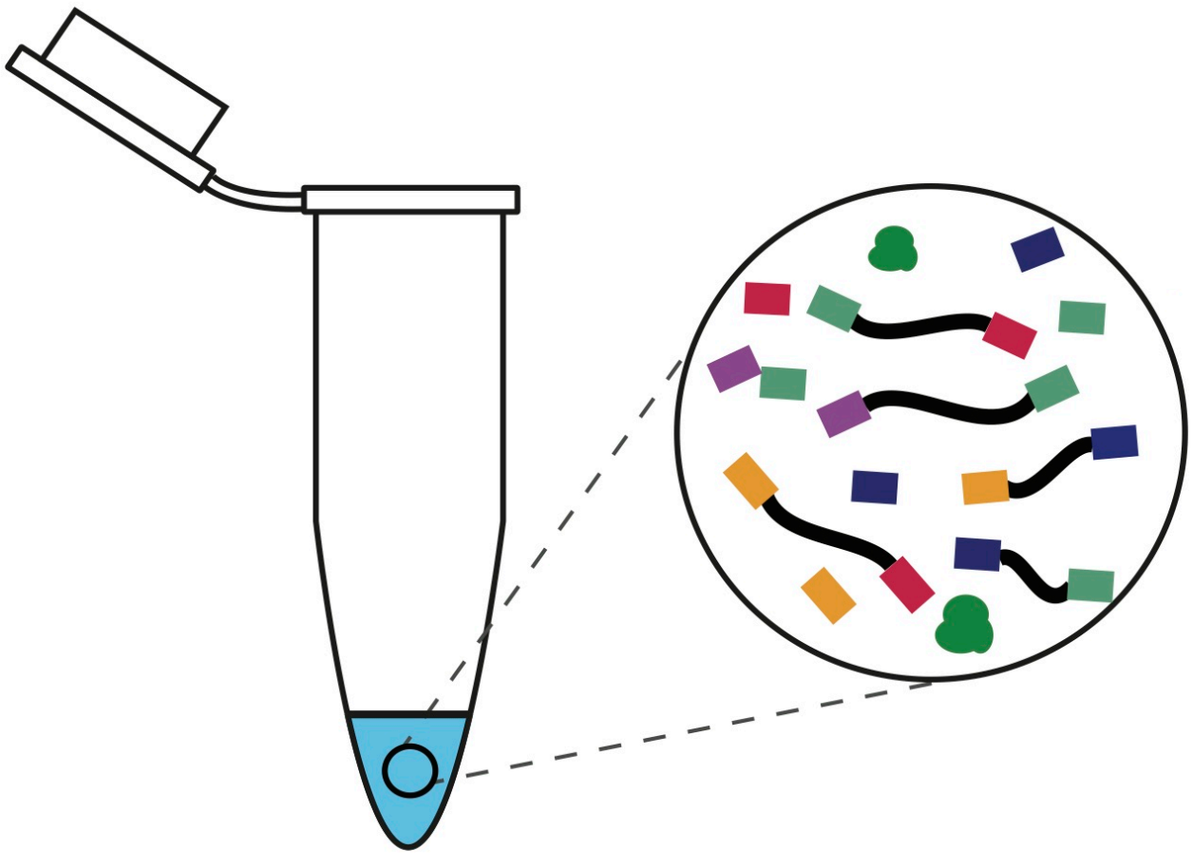
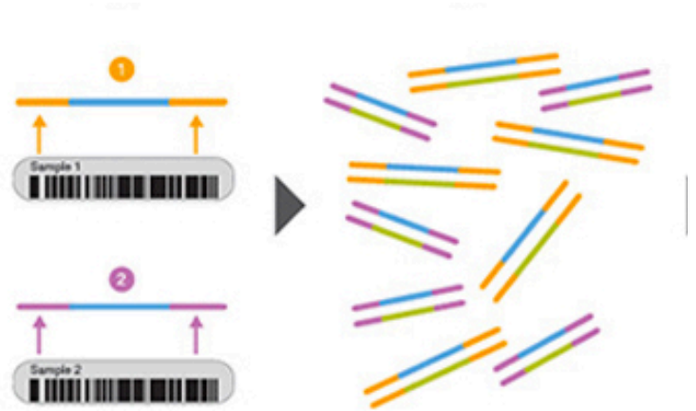
Multiplexing



Pooling Libraries from Different Samples

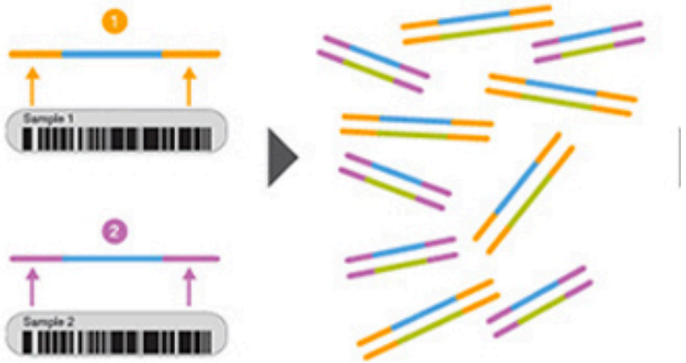
Index sequences are “barcodes” for multiplexing



- Multiplexing involves pooling libraries from different biological samples to be sequenced together on the same flow cell.
- The i5 and i7 index sequences are barcodes that are shared by all molecules from the same library so that libraries can be distinguished from each other during data analysis.

General Multiplexing Recommendations

- Incorporate library-specific barcodes. Avoid home-brew methods that add the barcode directly at the end of the DNA insert unless you know what you are doing.



- Be conservative about pooling
  - Not all libraries will be equally represented in your mix
  - Number of clusters may be lower than anticipated

- Consider how cross-contamination will affect your analysis and use redundant dual-indexing when possible.

Single vs Dual Indexing

Figure 1 Single-Indexed Sequencing

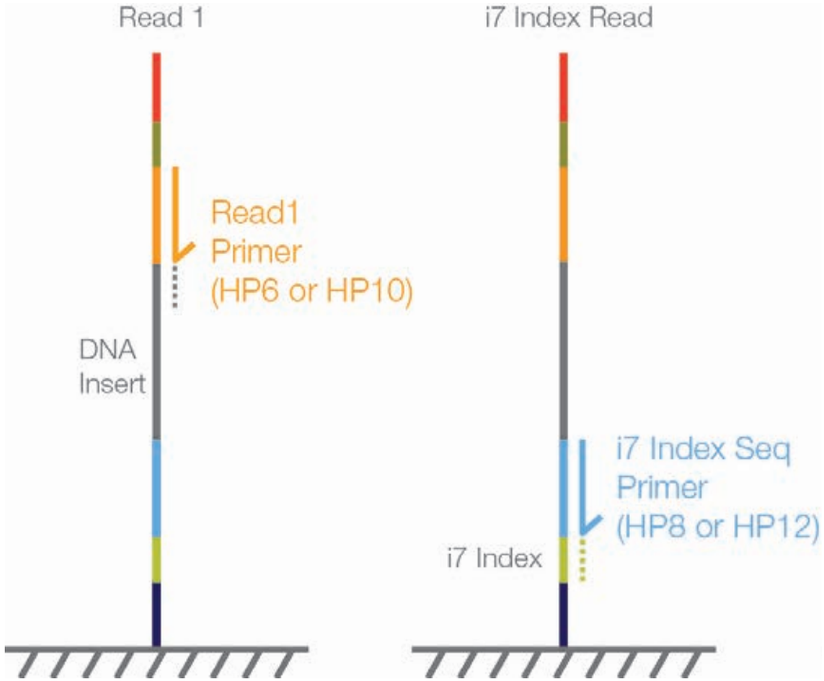
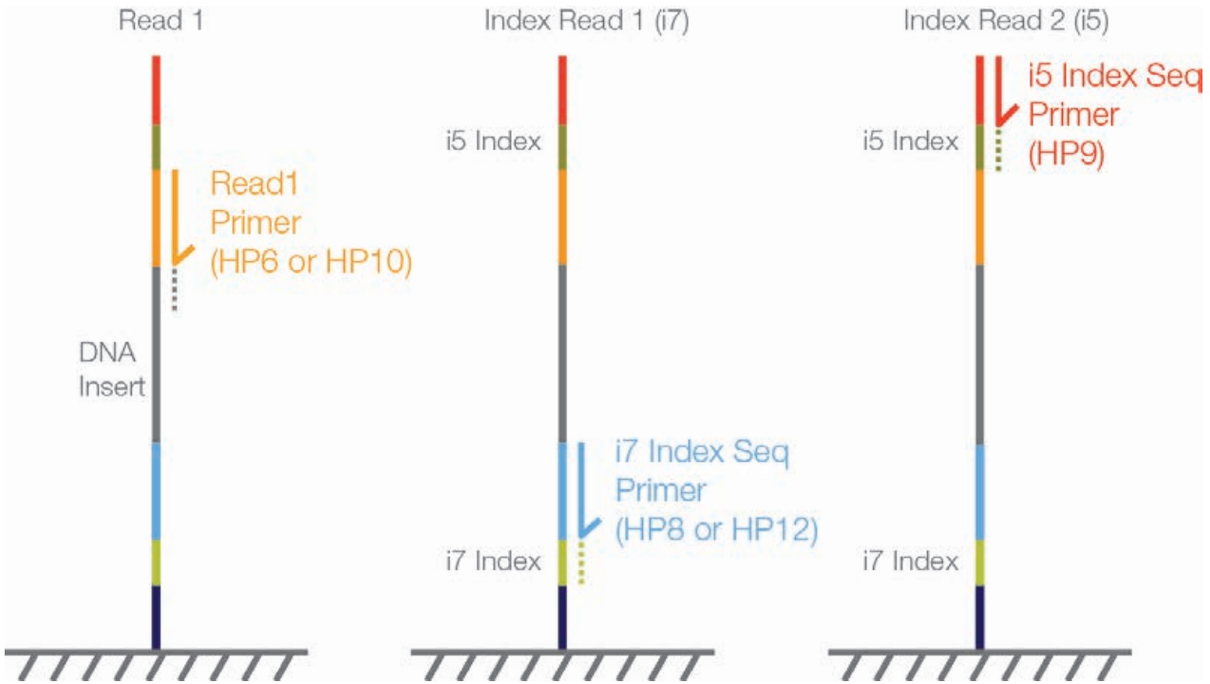


Figure 2 Dual-Indexed Single-Read Sequencing

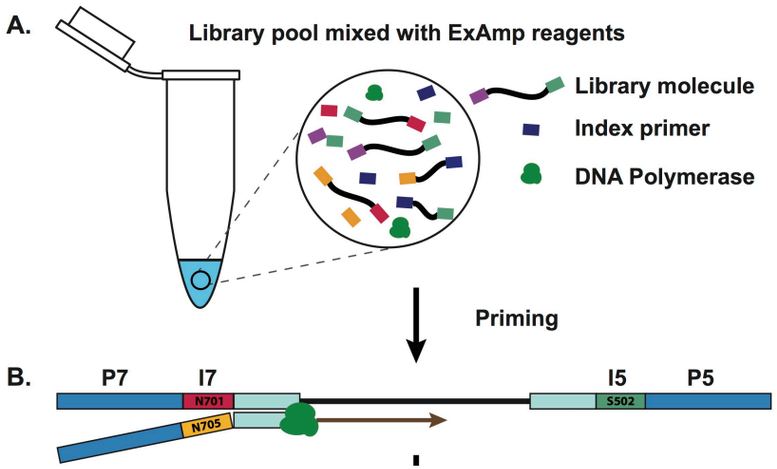


Dual indexing can either reduce library cross-contamination if indexes are used in a redundant fashion…

…or increase the degree of multiplexing if indexes are used in a combinatorial fashion.

Index “Hopping” and Library Cross-Contamination

Even with perfect lab technique, library contamination occurs on the flow cell.



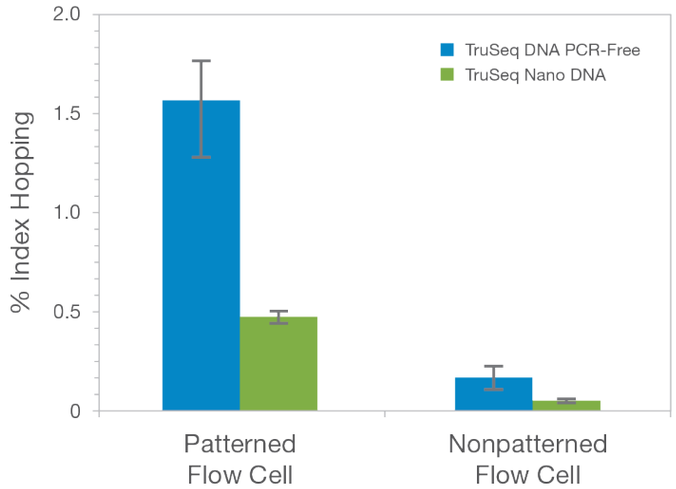


Table 1: Best Practices for Reducing Index Hopping

Mitigation/Recommendation	Benefit/Outcome
Prepare dual indexed libraries with unique indexes[a]	Converts index hopped reads to undetermined
Sequence one 30× human genome per lane[b]	Avoids pooling and index hopping
Remove adapters (cleanup, spin columns, etc)[c]	Reduces levels of index hopping
Store prepared libraries at recommended temperature of –20° C[c]	Reduces levels of index hopping
Pool similar RNA-Seq samples together	Reduces contamination between high and low-expressors

Image Sources: Illumina; Sinha et al. 2017 bioRxiv