ACCEL-NGS® UNIQUE DUAL INDEXING KITS

Less Sequencing Errors. Better Data Quality.

Highlights

- **Increased multiplexing capacity**
  Sequence up to 96-plexed libraries.
- **Improved target enrichment solutions**
  Reduce read misassignment due to PCR-induced chimerism during multiplexed hybrid capture.
- **Better data quality on patterned flow cells**
  Reduce read misassignment due to index hopping.

Introduction

Multiplexed sequencing relies on labeling genomic sequences from distinct samples with specific barcodes, also known as indices. The indices are short sequences, 6 to 8 nucleotides, that are incorporated into each DNA fragment during library preparation. This allows large numbers of libraries to be pooled and sequenced simultaneously during a single sequencing run. Gains in throughput come with an added layer of complexity, as sequencing reads from pooled libraries need to be accurately demultiplexed and assigned to each biological sample in the pool. Unfortunately, sample multiplexing suffers from the inherent risk of read misassignment, during which an index in the library pool is incorrectly assigned to a sequence read derived from a different sample in the pool.

To meet these challenges, Swift Biosciences developed a set of 96 indices, designed for use in a distinct manner in both the i7 and i5 positions, to form unique dual indices (UDIs). Swift's Accel-NGS Unique Dual Indexing Kits remove virtually all sequencing errors introduced by a variety of read misassignment mechanisms, including de-multiplexing errors, PCR-induced chimerism during multiplexed hybridization capture, and index hopping on patterned flow cells inherent to the function of sequencers.

The new Accel-NGS Unique Dual Indexing Kits can be used for a wide variety of applications, including whole genome sequencing, whole exome and epigenetic analysis. To make these improvements available to any laboratory, Swift Biosciences supports the new UDIs with Accel-NGS 2S Plus and Hyb DNA Library Kits, Accel-NGS 1S Plus and Methyl-Seq DNA Library Kits.

Design and Validation Parameters for Swift UDIs

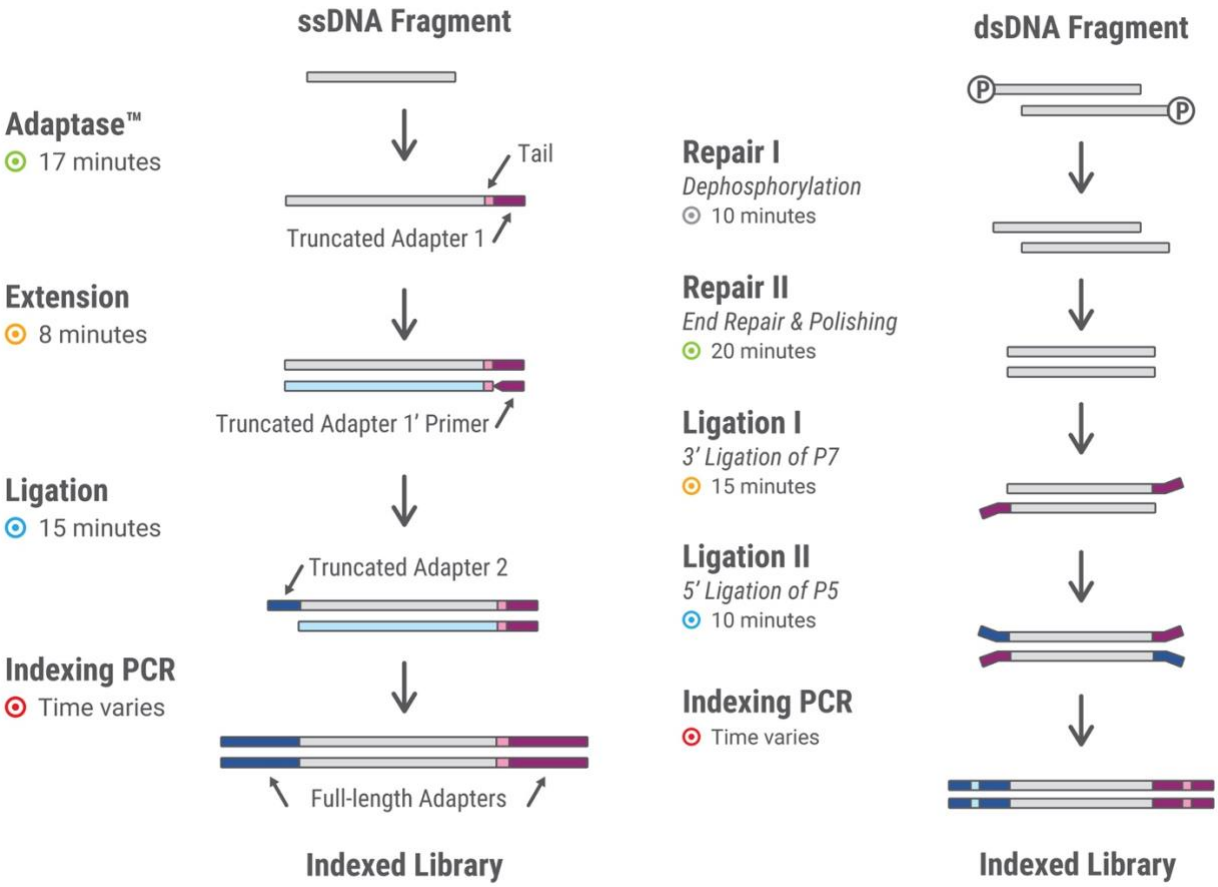
96 i7 index sequences were first validated as single indices for:

- Minimum edit distance of 3 to avoid index misassignment as the result of sequencing errors
- Index base composition with respect to instrument error profile
- Both 2-channel and 4-channel Illumina® instruments to exhibit misassignment rates < 0.1%

Swift 96 i7 single indices were then used to configure unique dual index combinations in a distinct manner while incorporating the design principles outline by Illumina bulletin for custom unique dual indices.

Workflows

There are no specific protocol recommendations for UDI usage with Accel-NGS 2S Plus and Hyb DNA Library Kits, Accel-NGS 1S Plus and Methyl-Seq DNA Library Kits. The only modification to the standard protocols is a second clean-up post-amplification to ensure optimal removal of carryover adapters. This modification only applies when sequencing libraries on patterned flow cells.



High Quality Data

Swift UDIs significantly reduce read misassignment due to index hopping on patterned flow cells.

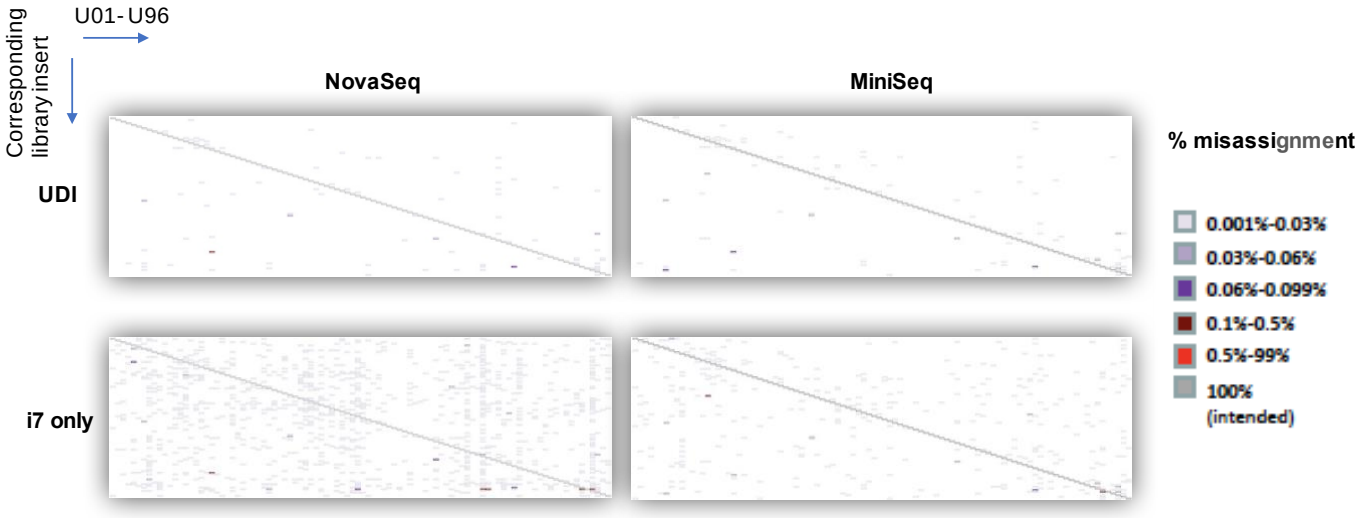


Figure 1: PCR-free libraries indexed with UDIs were constructed. The resulting libraries were co-sequenced on a MiniSeq and NovaSeq. De-multiplexing with the UDIs maintains low levels of misassignment, while single indexing (P7) analysis shows increased misassignment.

Swift UDIs eliminate read misassignment due to PCR-induced chimerism.

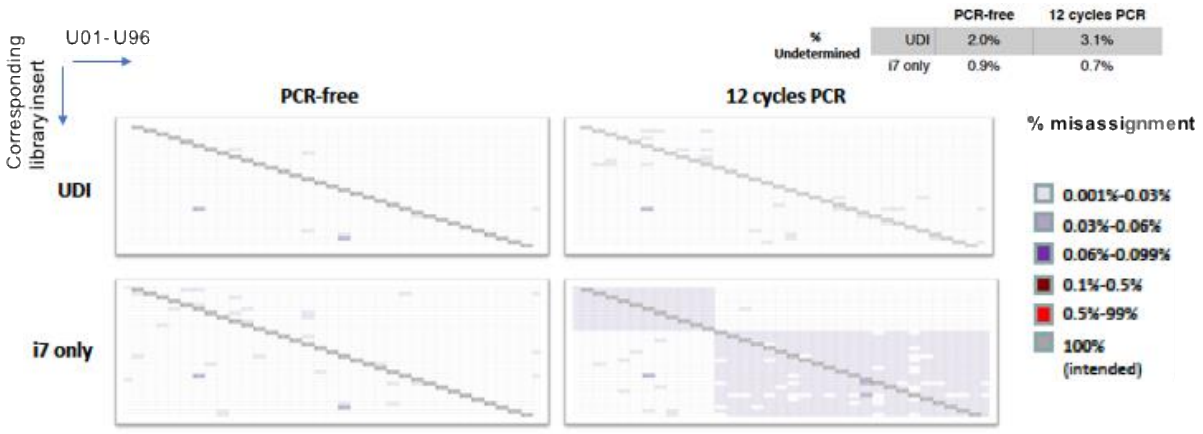


Figure 2: Pools of 12 and 24 libraries indexed with UDIs were subjected to 12 cycles of PCR to simulate a post-hybrid PCR condition. The resulting libraries were co-sequenced on a MiniSeq. Demultiplexing with the UDIs maintains low levels of misassignment, while single index (P7) analysis shows increased misassignment. The percent of reads that were undetermined increased slightly with UDI analysis, indicative of read being un-assigned instead of misassigned

Specifications

Specification	Feature
Format	96 unique dual Indices
Assay Compatibility	Accel-NGS 2S Library Kit: • 2S Plus • 2S Hyb Accel-NGS 1S Plus DNA Library Kit Accel-NGS Methyl-Seq DNA Library Kit
System Compatibility	2 and 4 channel Illumina sequencing instruments: • All Illumina sequencers
% Misassignment	< 0.1%
Configuration	Unique dual combinations – 24 or 96 dual indices (4 reactions each)
Kit Size	96 or 384 reactions

Ordering Information

Product Name	Reactions	Catalog No.
2S Unique Dual Indexing Kit	96 or 384	29096/290384
1S Plus Unique Dual Indexing Kit	96 or 384	19096/190384
Methyl-Seq Unique Dual Indexing Kit	96 or 384	39096/390384

Visit **www.swiftbiosci.com** for easy ordering.

References

- Illumina. Recommended strategies for unique dual index designs. Aug 2017.
- RoseFigura, et al. Improved Indices for High Fidelity De-Multiplexing on Illumina Instruments; Poster presented at Advances in Genome Biology and Technology; February 2018; Orlando, FL.

Swift Biosciences, Inc.
674 S. Wagner Road • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com