ULTRA-HIGH-THROUGHPUT MULTIPLEXED SEQUENCING WITH ACCEL-NGS® 2S INDEXED ADAPTERS

Multiplexed sequencing is enabled by the unique and correct assignment of sequencing reads to DNA fragments.

New sequencing platforms, such as the Illumina® NovaSeq™ and HiSeq®, with their unprecedented flexibility and performance, are driving demand for higher throughput multiplexed workflows.

The Accel-NGS 2S Indexed Adapters are a set of 96 single indices optimized specifically[1] to work in combination with either Illumina's universal P5 adapter for 96-plexing or with Illumina's eight TruSeq® HT i5 indices to provide up to 768 unique dual index combinations. These new indices were optimized specifically to provide greater flexibility to process between 12-768 uniquely tagged samples per run and to accommodate a wider range of projects with varying sequencing depth.

The Accel-NGS 2S Indexed Adapter Kits are available as individual sets (S1, S2, S3, S4) or as one complete set (S1-S4).

Highlights of Accel-NGS 2S Indexed Adapters

- **Fast, high-throughput strategy** – Automated sequencing up to 768 samples per flow cell.
- **Greater flexibility** – Provides more index combinations to maximize pooling strategy.
- **Lower cost per sample** – Multi-sample pooling reduces labor and reagent cost.
- **Higher quality data** – Accurately discriminate indices during de-multiplexing.
- **Flexible workflow** – Utilizes full-length adapter ligation during library construction to create a universal workflow for both PCR-free and library amplification-based approaches.

Cost-Effective High-Throughput Sequencing

High-throughput next-generation sequencing platforms, such as Illumina's HiSeq and NovaSeq, enable large scale whole genome sequencing projects, such as gene mapping, variant discovery, and profiling applications. However, more multiplexing tools are needed to increase throughput, lower per sample costs, and to maximize the amount of usable data per run.

To make high throughput multiplexed sequencing available to any laboratory, Swift Biosciences offers indexing kits for all Accel-NGS® 2S Library Kits (2S PCR-Free, 2S Plus, and 2S Hyb). Each index was designed to permit accurate de-multiplexing across samples, even if an index read contains an error.

High Quality Data

Some Illumina sequencing methods, library prep methods, and adapter sequences can create misassignment of sequencing reads. Misassignment can be caused by distinct factors, including index hopping[2, 3] on patterned flow cells, index sequencing errors, cross contamination of indices during reagent manufacturing, or run-to-run sample carry over. Using the Accel-NGS 2S library prep protocol in combination with the new P7 adapter indices, offers several advantages to minimize misassignment, such as:

Source	Cause	Solution
Index hopping[2, 3]	Index carryover into clustering reaction on patterned flow cells	Purification steps in Accel-NGS 2S workflow eliminates carryover
Index sequence error	Insufficient hamming distance or instrument error profile	Swift indices have hamming distance ≥ 3 and validated on both 2 and 4 channel instuments
Reagent cross-contamination	Manufacturing or library prep error	Swift implemented rigorous practices for reagent manufacturing and sample processing workflows
Sample carryover	Trace quantities of libraries from prior run	Illumina® sanctioned post-run instrument wash

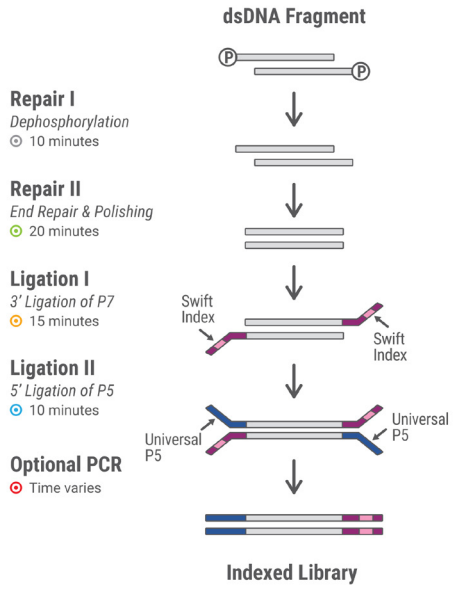
Easy Prep-to-Sequencing Workflow

The Accel-NGS 2S workflow is a standardized and robust protocol that requires as little as 10 pg of input DNA. The Accel-NGS 2S Single Indexed Adapters are fully compatible with the standard protocol and require no assay modification to incorporate it. During the Accel-NGS 2S Ligation I step (Figure A), Reagent Y2, a unique full length indexed adapter, is used to label each library.

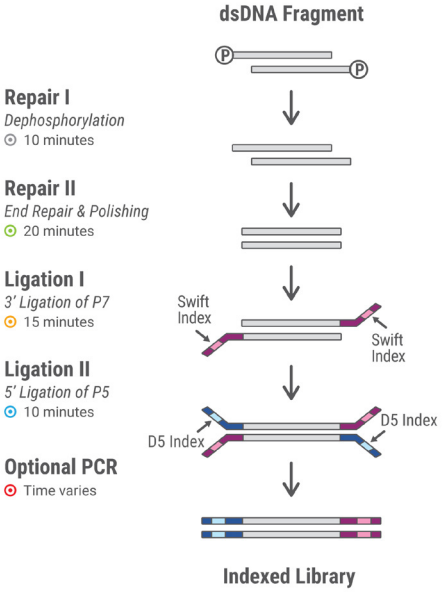
The Accel-NGS 2S Dual Indexed Adapter workflow contains two indexing steps (Figure B). During Ligation I step, Reagent Y2, a unique full length indexed adapter, is used to label each library. During the Ligation II step, Reagent B2, a full-length TruSeq HT P5 adapter is used to provide unique dual indexed combinations.

To sequence single and dual indexed libraries, create a sample sheet by modifying the TruSeq LT and TruSeq HT templates, respectively, and then save as a .CSV file. Using standard pipeline analyses, each index is then associated with a particular read-pair, identifying samples for downstream analysis. When performing dual indexing, the i5 indexed adapter sequence or its reverse complement is used depending on the sequencer of choice.

A. Single Indexed Library Workflow



B. Dual Indexed Library Workflow



Applications

High-throughput multiplexing for a variety of whole genome sequencing applications.

Applications	Needs
Genome-wide association studies of human disease	Highest coverage uniformity for sequencing of whole genomes or targeted regions across many individuals
Aneuploidy analysis	Non-invasive Prenatal Testing
Biomarker discovery	Fast, cost-effective analysis of germline variants
Liquid biopsy and tumor profiling	Fast, sensitive analysis of low frequency variants > 1% (somatic mutations)
Microbial sequencing	Characterize small, non-human genomes, i.e., when determining genetic variations between bacterial strains responsible for separate disease outbreaks
Epigenetics	In studies of gene structure and regulation, high-throughput multiplexed sequencing can be applied to chromatin immunoprecipitation (ChIP) experiments for fast, sensitive identification of genome-wide binding sites

Specifications

Specification	Feature
Format	96 Single i7 Indices
Assay Compatibility	Accel-NGS 2S Library Kits, including: • 2S PCR-Free • 2S Hyb • 2S Plus
Index Compatibility	• Illumina TruSeq HT i5 indices – enables up to 768 dual index combinations • Non-indexed P5 universal adapter – enables up to 96 single index plexing • Swift MID Indexed Adapter – enables up to 96 single index combinations with MID tagging

Specifications (continued)

Specification	Feature
System Compatibility and Multiplexing Formats	Two and four channel Illumina sequencing instruments: • NovaSeq, HiSeq 4000 – Up to 768-plex (2 or 4 channel) • HiSeq X Ten – Up to 96-plex (4 channel) • MiSeq®, HiSeq 2500, MiniSeq®, NextSeq® – Up to 768-plex (2 or 4 channel)
% Misassignment	< 0.1%
Configuration	Single Indexed Adapters – 24 or 96 indices (4 reactions each) Dual Combination – 192 (4 reactions each)
Kit Size	96 or 384 reactions

Ordering Information

Product Name	Reactions	Catalog No.
2S Set S1 Indexed Adapter Kit	24 indices, 96 rxns	26596
2S Set S2 Indexed Adapter Kit	24 indices, 96 rxns	26696
2S Set S3 Indexed Adapter Kit	24 indices, 96 rxns	26796
2S Set S4 Indexed Adapter Kit	24 indices, 96 rxns	26896
2S Set S1-S4 Indexed Adapter Kit	96 indices, 384 rxns	269384
2S Set S1 MID Indexed Adapter Kit	24 indices, 96 rxns	27596
2S Set S2 MID Indexed Adapter Kit	24 indices, 96 rxns	27696
2S Set S3 MID Indexed Adapter Kit	24 indices, 96 rxns	27796
2S Set S4 MID Indexed Adapter Kit	24 indices, 96 rxns	27896
2S Set S1-S4 MID Indexed Adapter Kit	96 indices, 384 rxns	279384
2S S1 Dual Indexed Adapter Kit	192 unique combinations	28596
2S S2 Dual Indexed Adapter Kit	192 unique combinations	28696
2S S3 Dual Indexed Adapter Kit	192 unique combinations	28796
2S S4 Dual Indexed Adapter Kit	192 unique combinations	28896
2S S1-S4 Dual Indexed Adapter Kit	768 unique combinations	289384

Required Accel-NGS® 2S Library Kits are sold separately.

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

References

1. Quail et al BMC Genomics 2014 15:110.
2. Effects of Index Misassignment on Multiplexing and Downstream Analysis. illumina.com, 2017.
3. Index Switching Causes "Spreading-Of-Signal" Among Multiplexed Samples In Illumina® HiSeq 4000 DNA Sequencing, Sinha R. et al., bioRXiv, April 2017.

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