SWIFT NORMALASE® KITS

Revolutionary NGS Library Normalization Technology

Highlights

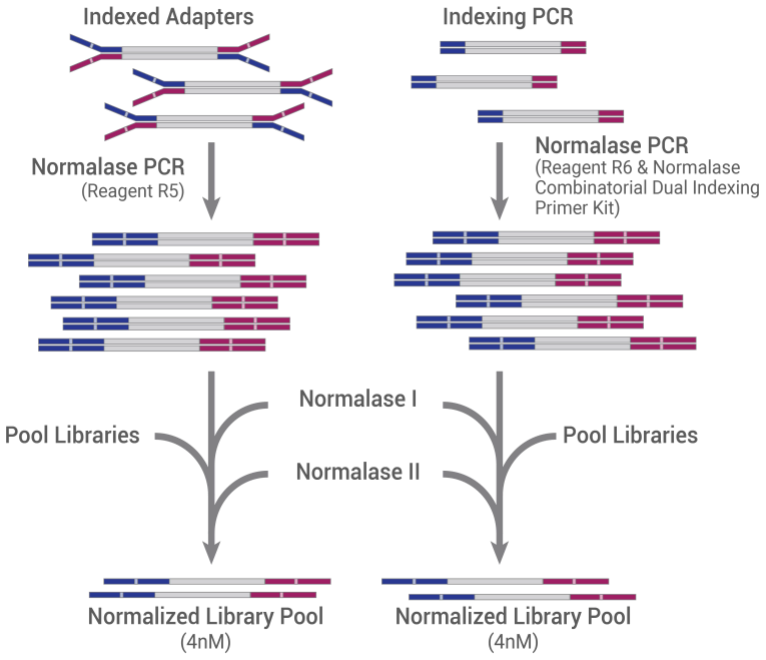
- **Saves time and increases throughput**
  Uniform sample processing to generate a balanced, multiplexed library pool
- **Reduces variability to save on sequencing costs**
  Better balanced pools allow higher multiplexing per run and fewer re-sequencing costs
- **Flexible design for many workflows**
  Compatible with diverse library preparation methods to produce evenly balanced sequence data

Introduction

The Swift Normalase Kits offer a novel enzymatic library normalization technology that consolidates DNA or RNA library normalization and pooling for loading on Illumina® systems. The Normalase workflow eliminates the need for library concentration adjustment prior to library pooling, resulting in optimal cluster density and library balance. The Swift normalization method can easily be integrated into standard library preparation protocols to improve turnaround time and loading accuracy for NGS laboratories. The library selection and enzymatic normalization steps of the Normalase workflow is designed for workflows that consistently produce amplified library yields 3x the target normalization amount following library amplification with Normalase primers (e.g., ≥6 nM or ≥12 nM yield in 20 µl volume to achieve 2 nM or 4 nM normalized library yield). This workflow does not introduce a second PCR; instead, it replaces the primers in conventional library amplification, either terminal or indexing. Swift Normalase Kits offer a fast, scalable library normalization workflow for high-throughput laboratories.

Normalase Workflow

Figure 1. *The Swift Normalase workflow begins after NGS library adapter ligation, using either full length indexed adapters or truncated adapters, were Normalase PCR primers are used to amplify the libraries to above the minimum threshold and condition the libraries for downstream Normalase enzymology. For full length indexed adapter libraries, Swift Normalase terminal primers are used, for truncated adapter libraries, Swift Combinatorial Dual Indexing Normalase primers are used. Amplified and conditioned NGS libraries are then individually incubated for 15 minutes with the Normalase I master mix to enzymatically select a specified molarity of each NGS library. After Normalase I, each library is pooled using equal volumes into a single tube and incubated for 15 minutes with the Normalase II Master Mix which enzymatically normalizes each NGS library to the specified selected molarity. The result of Normalase is a balanced multiplexed NGS library pool ready for sequencing.*



** ≥6 nM normalization workflow will result in a 2 nM final normalized library pool, which can be concentrated to achieve 4 nM. **

Highly Reproducible DNA Library Normalization and Robust Performance Across Variable Insert Sizes

Loading (pM)	Cluster Density (K/mm^2)	# of Libraries	Library Balance (CV%)	Insert Size (bp)
12	1370	6	9.7	150
12	1043	16	8.2	200
12	1157	6	5.4	350
12	1070	5	3.7	600

Table 1. *Expected and consistent cluster density generation using MiSeq® V2 chemistry at 12 pM from library pools normalized to 4 nM using Normalase.*

Better Normalization Compared to Conventional Methods

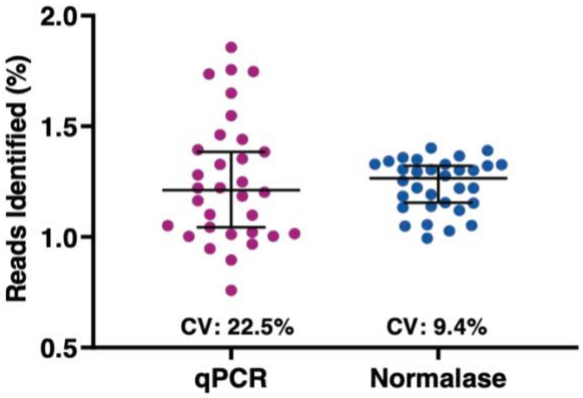


Figure 2. *Thirty-two Swift 2S Turbo libraries were generated with full- length indexed adapters between two users (n=16/user) with 1 to 250ng inputs of NA12878 gDNA. Post-Normalase PCR libraries were quantified with qPCR assuring the libraries met the minimum threshold. Libraries were either normalized and pooled and sequenced based on the qPCR quant or, using the same libraries, pooled and normalized using Normalase and sequenced to determine percent Reads Identified of each index (MiSeq V2 50 cycle Nano). The coefficient of variation (CV) for the qPCR pool was 22.5% across the two users, while the CV for the Normalase pool was 9.4%. Lines are median and 95% confidence interval.*

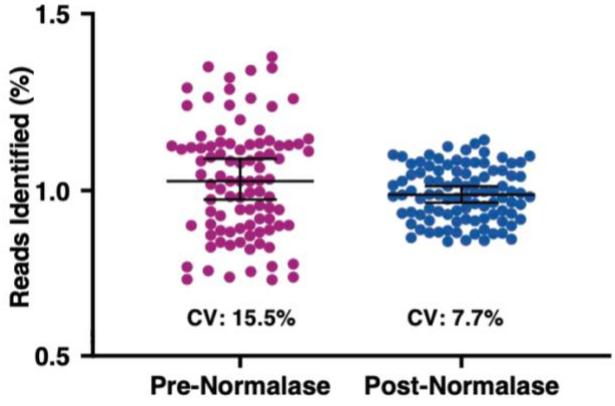


Figure 3. *Ninety-six Swift 2S Turbo libraries were generated with 10ng NA12878 gDNA and amplified with Swift Normalase Combinatorial Dual Indexing primers. Libraries were pooled, Pre-Normalase, using equal volumes and the pool quantified by Qubit for loading on the Illumina MiSeq V2 50 cycle Nano flow cell to obtain percent Reads Identified from each index. The pre- Normalase pool CV was 15.5%, demonstrating robust and reproducible amplification using the Normalase Indexing primers. The same libraries were subjected to Normalase and normalized to 4nM, Post-Normalase, and loaded on the Illumina MiSeq V2 50 cycle Nano flow cell. The Normalase pool CV was reduced to 7.7% demonstrating robust normalization of multiplexed library pools using Swift Normalase. Lines are median and 95% confidence interval.*

Better Normalization Compared to Conventional Methods

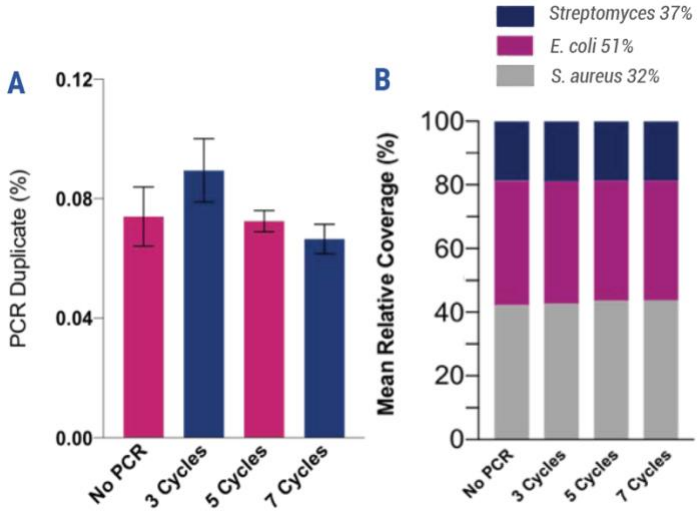


Figure 4. Swift 2S Turbo libraries made from 100ng of DNA consisting of a mix of three reference bacterial strains (*E.coli, S.aureus, Steptomyces*) mixed at unequal proportions. DNA fragmented to 350 bp were ligated with full-length indexed Y-adapters, and were either not amplified or amplified using 3, 5, and 7 cycles of PCR using the Swift HiFi Polymerase and Normalase terminal primers. All libraries were quantified and pooled based on their qPCR quantification. Libraries were sequenced on the Illumina MiSeq using 150PE sequencing. Across bacterial genomes with varying GC% content, there was no observed difference in A) the number of read duplicates or *B) genome coverage.*

Libraries Normalized Using Normalase Maintain High Quality Sequencing Coverage

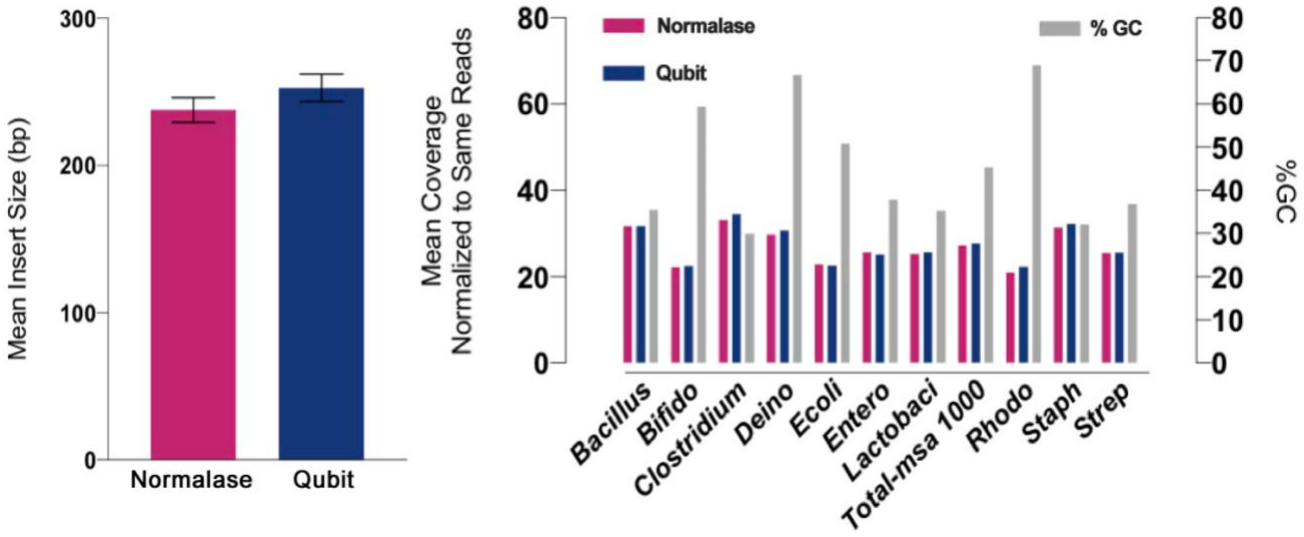


Figure 5. *Swift 2S Turbo libraries were made from 5ng of MSA-1000, an equal mass mix of 10 different bacterial strain genomes with varying GC%. Two libraries were either amplified and indexed with Swift CD Indexing Normalase primers (plum) or Swift CD Indexing primers (blue). Normalase conditioned libraries were normalized to 4nM and pooled using Normalase, while standard libraries were Qubit quantified and pooled at 4nM. Libraries were co-sequenced on the Illumina MiniSeq using High Output reagents and 150 PE sequencing. Across bacterial genomes with varying GC% libraries, Normalase treated libraries-maintained A) insert size and B) high quality genomic coverage.*

Normalase Preserves RNA-Seq Data Quality

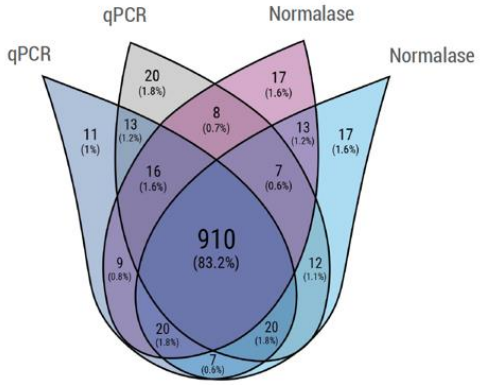
Sample Pooling	Exonic Rate (%)	Intronic Rate (%)	Unique Rate of Mapped (%)	Duplication Rate (%)	Estimated Library Size	Transcripts Detected	Genes Detected	Fragment Length Mean (bp)	Strandedness (%)
qPCR	54.57	36.22	95.50	4.50	41,387,519	94,895	17,061	162	97.33
qPCR	54.56	36.23	95.48	4.52	41,228,061	94,960	17,129	161	97.31
Normalase	54.50	36.32	95.58	4.42	42,333,797	94,608	17,091	159	97.28
Normalase	54.49	36.34	95.57	4.43	42,208,180	94,711	17,107	158	97.28

Table 2. *Four RNA-seq libraries were generated from 50ng human brain mRNA, amplified and indexed with either Swift Combinatorial Dual (CD) Indexing primers (n=2) or Swift Combinatorial Dual Indexing Normalase primers (CDI-N) using 9-cycles of PCR and the Swift HiFi Polymerase. CD libraries were pooled and normalized manually using library quants from the qPCR, while CDI-N libraries were subjected to Normalase to 4nM. The two pools were combined and sequenced on a MiniSeq High Output (2x150) run. Sequencing data was normalized to 4,011,853 paired-end reads and mapped using STAR and analyzed RNA-Seq metrics using Picard. Sequencing data was normalized to 4,011,853 paired-end reads to account for library pool variation and mapped using STAR and analyzed RNA-Seq metrics using Picard.*

Sample	Mean Per Base Cov.	Mean CV	No. Covered 5'	5' 200Base Norm	No. Covered 3'	3' 200 Base Norm	Num. Gaps	Cumul. Gap Length	Gap %
qPCR	273.90	0.90	860	0.27	948	0.45	669	63701	4.65
qPCR	278.39	0.90	864	0.26	954	0.45	639	62079	4.68
Normalase	277.33	0.91	859	0.26	944	0.45	661	64419	4.82
Normalase	275.19	0.91	866	0.27	944	0.45	650	61794	4.58

Table 3. *Coverage metrics of the top 1000 expressed transcripts demonstrating no differences between qPCR manual pooling and Normalase.*

Figure 6 (Right). *Venn diagram of the Top 1000 transcripts Detected in each sample demonstrating no significant differences in transcripts detected*



Specifications

Feature	Swift Normalase Kits
Post-library amplification molarity required prior to Normalase	3x target molarity in 20 µl
Normalized concentration post Normalase	4 nM (or 2 nM when using ≥ 6 nM normalization workflow option)
Library compatibility	• Libraries with full-length indexed adapters, Libraries constructed by indexing PCR • Libraries that have an amplified yield of consistently ≥ 6 nM or ≥ 12 nM in 20 µl volume • Libraries prepared for direct sequencing (i.e., whole genome, whole transcriptome) • Target enriched library pools post-hybridization capture that have indexed adapters
Library balance within a pool	Coefficient of variation ≤ 10%
Swift indexing compatibility	Swift Indexed Adapters for Accel-NGS 2S Indexed Adapters from Illumina or IDT Swift Normalase Combinatorial Duan Indexing Primers Swift Normalase Unique Dual Indexing Primers
System compatibility	Reproducible performance across Illumina sequencing instruments

Ordering Information

Workflow Component	Product Name	Catalog Number
Normalase Kits	Swift Normalase® Kit (96 rxns)	66096
	Swift Normalase® Kit (4x96 rxns Bundle)	660384
CDI Primers	Swift Normalase® Combinatorial Dual Indexing Primers (96-plex, 96 rxns)	68096
UDI Primers	Swift Normalase® Unique Dual Indexing Primer Plates (384-plex, 4x96 rxns Bundle)	X91384-PLATES
	Swift Normalase® Unique Dual Indexing Primer Plate (96-plex, 96 rxns, SU001-SU096)	X91096-1-PLATE
	Swift Normalase® Unique Dual Indexing Primer Plate (96-plex, 96 rxns, SU097-SU192)	X91096-2-PLATE
	Swift Normalase® Unique Dual Indexing Primer Plate (96-plex, 96 rxns, SU193-SU288)	X91096-3-PLATE
	Swift Normalase® Unique Dual Indexing Primer Plate (96-plex, 96 rxns, SU289-SU384)	X91096-4-PLATE
	Swift Normalase® Unique Dual Indexing Primer Plate (1536-plex, 16x96 rxns Bundle)	Please inquire

For technical support, please contact TechSupport@swiftbio.com

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