AGBT: #611

Turbolase: A Radically Streamlined High Throughput Sample Prep to Sequencing Workflow

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Abstract

Normalase is a novel enzymatic library normalization method that eliminates library quantification and manual concentration adjustment of each sample prior to library pooling. It does not use a limited capacity immobilization step which saves time and improves performance, while its uniform sample processing eliminates numerous error-prone pipetting steps. Normalase allows for > 10-fold variation in input quantity, while generating < 10% variation in sample representation within a pool resulting in optimal cluster density and sample balance for Illumina sequencing. When combined with Swift 2S Turbo rapid library kits, a highly streamlined 'Turbolase' workflow is created that is readily automated on the Hamilton Star and other platforms, where simple bulk processing improves throughput and reduces cost. Swift 2S Turbo kits comprise two enzymatic steps and a single purification that completes DNA fragmentation, end repair and adapter ligation. This is followed by standard library amplification using Normalase PCR primers to pre-condition the libraries and produce a required minimum yield in excess of the 4 nM final concentration. This is followed by a second purification and two Normalase steps that 1) enzymatically selects 4 nM of each library, and after pooling, 2) enzymatically normalizes each library to 4 nM within the pool. The pool can then be directly sequenced without further purification, and each library can be repeatedly pooled up to 5 times for flexibility in re-sequencing if needed. Because of the unique workflow, Normalase also mitigates index hopping for libraries sequenced on patterned flow cells. The 'Turbolase' workflow is compatible with full-length indexed adapters that have been added by ligation as well as workflows that require indexing PCR primers to complete library construction during library amplification. Normalase is also compatible with library preparation kits available from other vendors, and Normalase for PCR-free library construction is currently under development.

Introduction

Turbolase is a streamlined library generation and normalization workflow that combines the rapid and reproducible enzymatic shearing and adapter ligation provided by Swift 2S Turbo kits with the robust and precise normalization of Swift Normalase kits. The Turbolase workflow can be used with libraries either indexed with full-length indexed adapters or using Swift Combinatorial Dual indexing Normalase primers. The protocol is designed for workflows that produce consistent amplified library yields of 12nM or more following library amplification and generates a normalized library pool of 4nM for multiplexed direct sequencing or yields of 90nM or more to generate a normalized library pool of 30nM for multiplexed hybridization capture. The Turbolase workflow does not require quantification and is a bead-free normalization method where equal volumes of each library are pooled to simplify high throughput sequencing. Turbolase is compatible with multiple polymerases, including Swift Hifi, Kapa HiFi and NEB Q5.

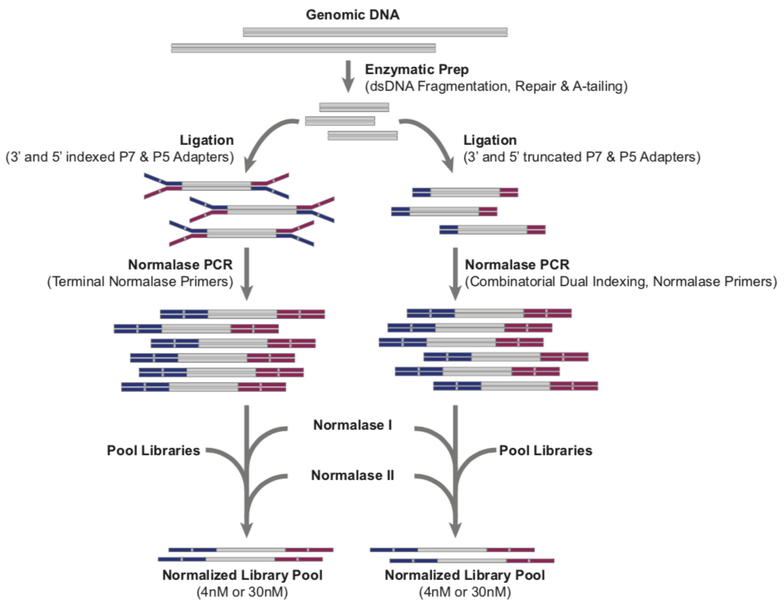

Figure 1) The Turbolase workflow begins at library preparation with Swift 2S Turbo Enzymatic Prep, which fragments dsDNA and performs end repair and A-tailing in a single step. Adapter ligation of either full-length indexed P7 and P5 adapters or truncated P7 and P5 adapters followed by a bead-based purification step. Libraries are then amplified by using either the Swift Terminal Normalase (N-primers) primers for libraries generated with indexed adapters or by Swift Combinatorial Dual Indexing Normalase (CDI-N) primers for libraries with truncated adapters followed by a bead-based purification step. Instead of traditional library quantification methods followed by manual library dilution and pooling, Normalase is performed and consists of two 15 minute incubations where equimolar amounts of libraries are enzymatically selected, pooled, and normalized yielding a single 4nM or 30nm library pool ready for multiplexed sequencing or for input into multiplexed hybridization capture, respectively.

12nM Direct Sequencing or 90nM Pre-Hyb Minimum Threshold

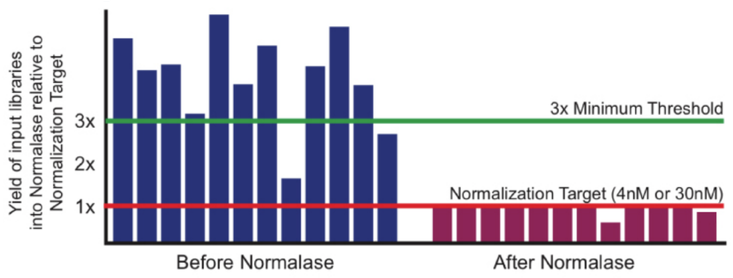

Figure 2) Library amplification with Normalase primers, either N-primers or CDI-N primers, increases the amount of libraries to ≥3x the target normalization amount, 12nM for 4nM normalization and 90nM for 30nM normalization, and conditions the libraries for Normalase I and II incubations. Following a standard purification, 4nM or 30nM of each library is enzymatically selected during the first step. After Normalase I, all libraries are combined into a single pool. The Normalase II step enzymatically normalizes all libraries within the pool to 4nM or 30nM. Without the need for further purification, the pool is ready for direct multiplexed sequencing applications, 4nM, or for input into multiplexed hybridization capture, 30nM.

Results

Uniform Library Balancing

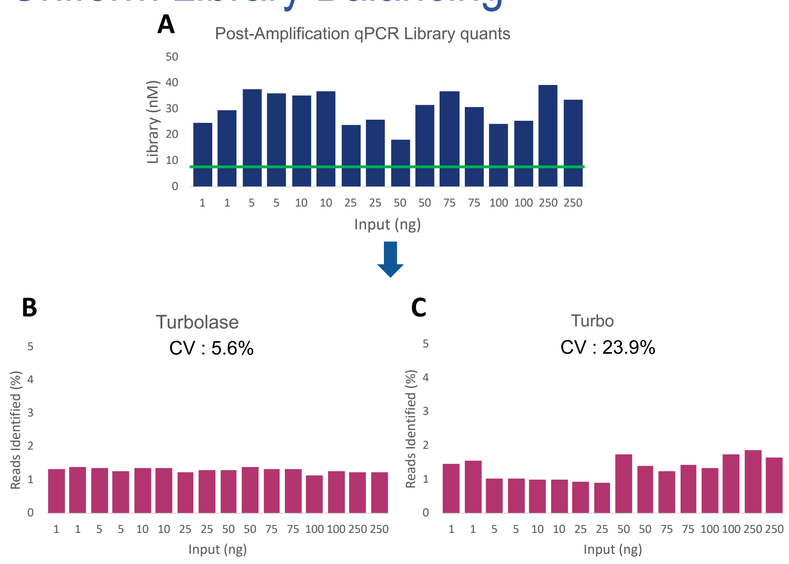

Figure 3) A. 16 Swift 2S Turbo Flexible libraries generated with 1 to 250ng of Coriell NA12878 DNA were amplified with N-primers with the recommended 10 to 3 PCR cycles to yield ≥12nM, confirmed by qPCR. Libraries were either normalized with Normalase (**B**) or manually diluted to 4nM based on the qPCR quant and pooled (**C**), both pools were sequenced on individual Illumina MiSeq® V2 Standard flowcells 1x50 cycles. **B.** The Normalase pooled libraries loaded at 12pM clustered at 892K/mm² and the index balance variation was calculated to be a CV of 5.6%. **C**. qPCR manually normalized libraries loaded at 10pM clustered at 848K/mm² and the index balance variation was calculated to be a CV of 23.9%. Both Library pools performed within specifications of MiSeq clustering, but Normalase provided 4-fold better library balancing.

Direct Sequencing Performance

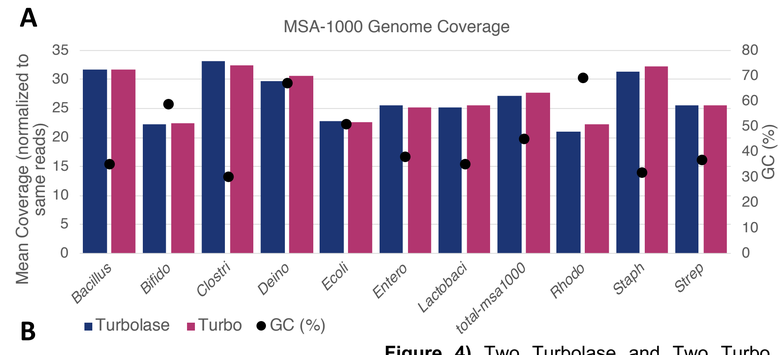

Figure 4) Two Turbolase and Two Turbo libraries made from 5ng MSA-1000 equal mass DNA mix of 10 reference bacterial strains. Turbolase libraries were amplified and Normalase conditioned with Swift CDI-N primers and Turbo libraries were amplified with Swift CD indexing primers. Turbolase libraries were run through Normalase to 4nM, while Turbo libraries were quantified and pooled by qPCR. The Turbolase pool and the Turbo libraries and sequenced on the Illumina MiniSeq using High Output reagents and 2x151 sequencing reads. Sequencing reads were aligned to the 10 bacterial reference genomes to assess mean genomic coverage (A) and median insert size (**B**). **A**) Across bacterial genomes with varying GC % levels (Black points) Turbolase libraries maintained high quality genomic coverage captured by Swift 2S Turbo libraries. **B)** Additionally, no significant change in median insert size was observed with Turbolase, suggesting robust library generation and sequencing performance of Turbolase libraries.

High Throughput Performance

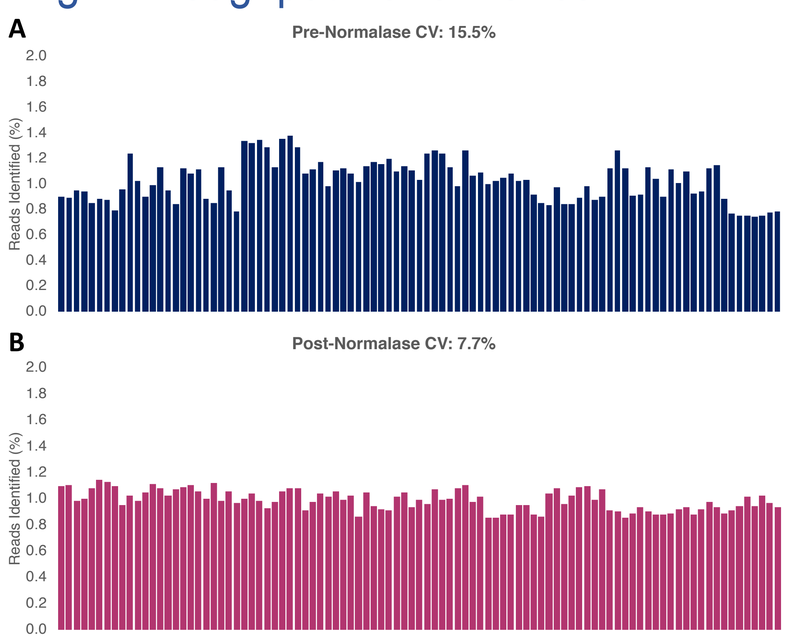

Figure 5) 96 Turbolase libraries were generated from 10ng NA12878 gDNA and amplified with Swift CDI-N primers and normalized down to 4nM using the Normalase workflow. **A**) Amplified and Purified libraries, >90nM, were first pooled in equal volumes, diluted to 4nM and sequenced on the Illumina MiSeq using 50 cycle reagents and Nano flowcell. The CV for this library pool was 15.5%, and demonstrated robust amplification of Turbo libraries with CDI-N primers. **B**) After the Normalase portion of the Turbolase workflow; the normalized library pool was sequenced on the MiSeq using 50 cycle reagents and a Nano flow cell. The CV for the normalized pool was 7.7% demonstrating outstanding normalization results using Turbolase.

Pre-Hyb Normalase

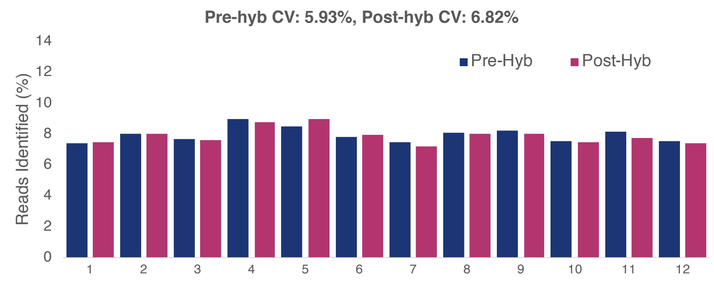

Figure 5) Twelve 200bp insert size Turbolase libraries were generated with 100ng NA12878 input, indexed and amplified with Swift CDI-N primers to ≥90nM. The Normalase portion of the Turbolase workflow normalized the libraries to 30nM, representing 200ng per library, in a single pool for IDT Exome multiplexed hybridization capture. Prior to hybridization capture, the pool was sequenced using MiSeq 50 cycle reagents and a Nano flowcell to determine index balance, which was observed to have a CV 5.9%. The pool was subjected to IDT Exome hybridization capture according to the IDT protocol. The post-hybridization capture pool was sequenced using MiSeq 50 cycle reagents and a Nano flowcell, and the observed index CV was 6.82%. These results demonstrate precise normalization of Turbolase libraries compatible with hybridization capture workflows to yield even representation of multiplexed libraries within a single hybridization capture pool.

Pre-Hyb Normalase Exome Capture

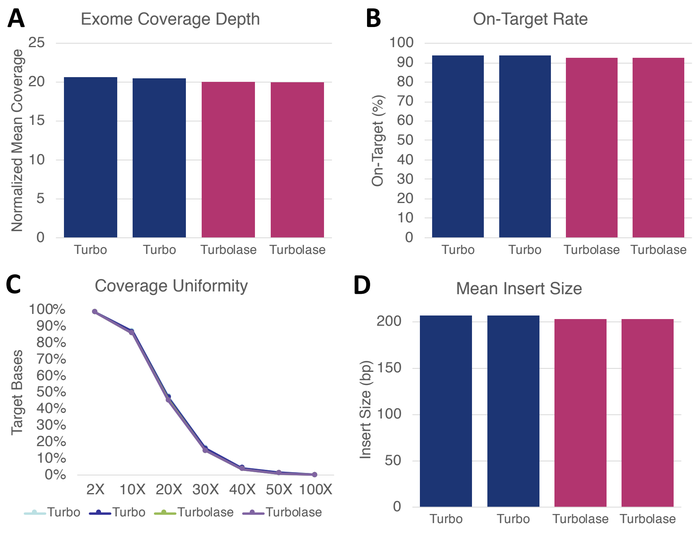

Figure 6) Two 200bp insert size Turbolase libraries and two Turbo libraries were generated with 100ng NA12878 input, indexed and amplified with Swift CDI-N primers to ≥90nM. The Normalase portion of the Turbolase workflow normalized the libraries to 30nM, representing 200ng per library, and combined with 200ng of each Turbo library in a single pool for IDT Exome multiplexed hybridization capture. **A)** Normalized mean Exome coverage demonstrating similar Exome coverage depth, ~20x, with Turbolase. **B)** On-Target rate of Turbo and Turbolase libraries demonstrating no differences in hybridization capture. **C)** Identical coverage uniformity across Target Bases of the IDT Exome. **D)** No difference in mean insert size. These results demonstrate similar Exome hyb-capture results with Turbolase as with Turbo.

Turbolase Compatible Workflows

- Libraries with full-length indexed adapters
- Libraries with truncated adapters indexed by PCR
- Libraries that have an amplified yields of consistently ≥12nM (20µL volume) for 4nM or ≥90nM for 30nM normalization
- Libraries prepared for multiplexed direct sequencing (i.e., whole genome, whole transcriptome)
- Libraries prepared for multiplexed hybridization capture
- Target enriched library pools post-hybridization capture

Future Developments

- Normalase for Swift targeted Accel-Amplicon panels
- PCR-Free Turbolase – Library synthesis and normalization without amplification

Conclusion

Turbolase is a novel NGS library preparation and normalization approach that produces libraries with specified molar concentration and the quantification is independent of library insert size

- Normalase employs two enzymatic incubations (15 minutes each) for robust normalization
- Minimal hands-on time
- Precisely and accurately normalizes NGS libraries from up to a 100 fold molar variation
- Eliminates concentration adjustments of each sample
- Ideal for automation

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