GENEWIZ
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

Client: Mark Johnson Brown University na na 60 Olive St. Providence, Rhode Island 02912 United States +1-4018633556 mark_johnson_1@brown.edu	**Tracking Number: 30-551599264** **Revision Number: R35** **Contact:** NGS@genewiz.com +1-877-GENEWIZ (436-3949) ext 1 Account Manager: Ann Robinson Account Manager Email: Ann.ronbinson@brooks.com

Library Preparation with polyA selection and HiSeq Sequencing

The RNA sample received was quantified using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked using TapeStation (Agilent Technologies, Palo Alto, CA, USA).

The RNA sequencing library was prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina using manufacturer's instructions (NEB, Ipswich, MA, USA). Briefly, mRNAs were initially enriched with Oligod(T) beads. Enriched mRNAs were fragmented for 15 minutes at 94 °C. First strand and second strand cDNA were subsequently synthesized. cDNA fragments were end repaired and adenylated at 3'ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by PCR with limited cycles. The sequencing library was validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA).

Text

The sequencing library was clustered on one lane of a flowcell. After clustering, the flowcell was loaded on the Illumina HiSeq instrument (4000 or equivalent) according to manufacturer's instructions. The sample was sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed using Illumina's bcl2fastq 2.17 software. One mismatch was allowed for index sequence identification.

NEBNext® Ultra™ II RNA Library Prep Kit for Illumina®

Note that Sample Purification beads are not included in this kit. If beads are required, we recommend ordering the NEBNext® Ultra™ II RNA Library Prep Kit for Illumina® (NEB #E7775).

Do you prefer to use non-strand-specific library prep methods, but need increased sensitivity and specificity from your RNA-seq experiments, from ever-decreasing amounts of input RNA? To address these challenges, our next generation of non-directional RNA library prep kit has been reformulated at each step, resulting in several fold higher yields of high quality libraries and enabling use of lower input amounts and fewer PCR cycles.

- Generate the highest yields of high quality libraries, with a broad range of input amounts
  - 10 ng – 1 µg Total RNA(polyA mRNA workflow)
  - 5 ng – 1 µg Total RNA (rRNA depletion workflow)
- Increase the complexity and transcript coverage of your libraries
- Optimize your time with streamlined workflows, reduced hands-on time, and automation compatibility