I. Introduction

This protocol describes the set-up for generating 96 uniquely-indexed Illumina® RNA-Seq libraries by PCR amplification of purified first-strand cDNA using the SMARTer Stranded RNA-Seq Kit HT (Cat. No. 634862). This protocol supplements the SMARTer Stranded RNA-Seq Kit User Manual Section V.C. The full protocol and additional information about the SMARTer Stranded RNA-Seq Kit HT can be found in the user manual.

II. Protocol for High-Throughput 96-Well PCR Amplification

1. Pick which combination of Forward PCR Primers HT and Reverse PCR Primers HT you will use. The combination of Forward and Reverse primers gives the unique index for each well of a 96-well plate.

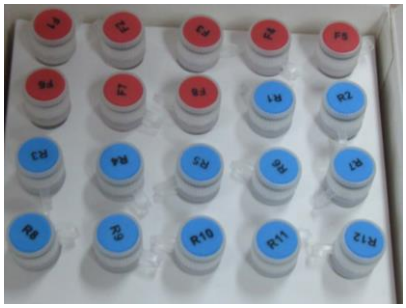

Figure 1. Forward PCR Primer HT and Reverse PCR Primer HT tubes.

2. Aliquot the Forward PCR Primers HT into 0.2 ml tubes in numerical order. Forward primers are in tubes with red caps labelled F1, F2, … F8. Be careful not to cross-contaminate the primers.

NOTE: If you are using less than the full 96 indexes, choose the subset of primers you would like to use and aliquot those into the appropriate number of 0.2 ml tubes, being careful to record which indexes are in which tube.

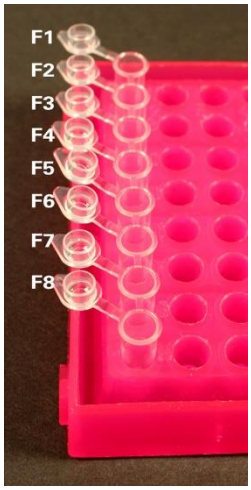

Figure 2. Forward PCR Primer HT aliquoted into tubes, ready to be added to first-strand cDNA.

3. Using an 8-channel pipette, add 1 µl of each Forward PCR Primer HT to the wells of the 96-well plate containing the bead-bound, purified DNA with PCR Master Mix. Be careful not to cross-contaminate the wells. Each well in a single row will have the same Forward PCR Primer HT Index.

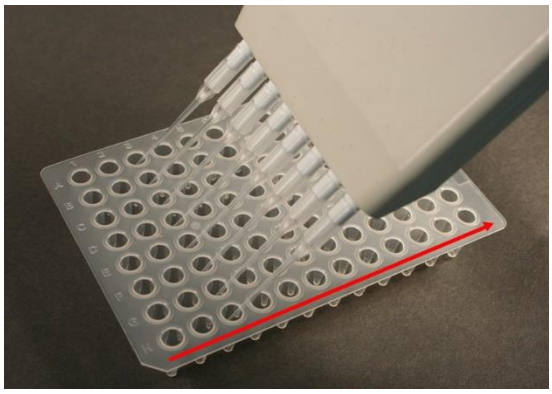

Figure 3. Addition of Forward PCR Primer HT

4. Aliquot the Reverse PCR Primers HT into 0.2 ml tubes in numerical order. Reverse primers are in tubes with blue caps labelled R1, R2, … R12. Be careful not to cross-contaminate the primers.

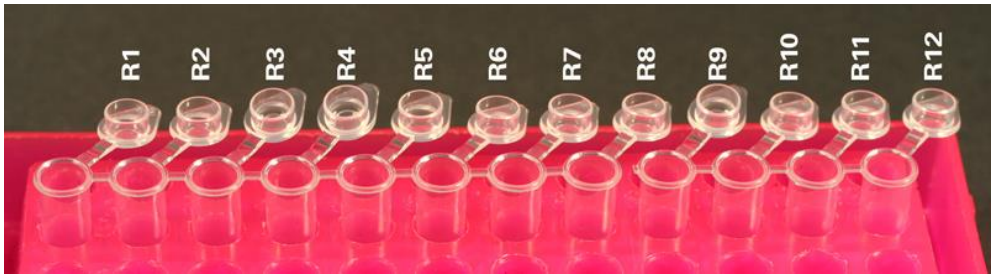

Figure 4. Reverse PCR Primer HT aliquoted in tubes, ready to be added to first-strand cDNA.

5. Using a 12-channel pipette, add 1 µl of each Reverse PCR Primer HT to the wells of the 96-well plate containing the bead-bound, purified DNA, PCR Master Mix and the Forward PCR Primer HT. Be careful not to cross-contaminate the wells. Each well in a single column will have the same Reverse PCR Primer HT Index. In this way, each well of the 96-well plate will have a unique index.

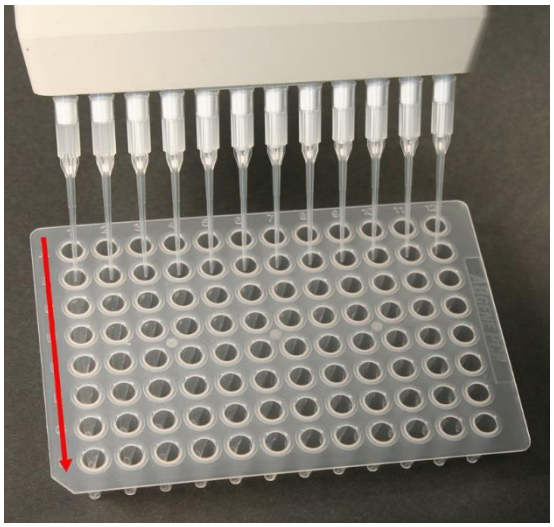

Figure 5. Addition of Reverse PCR Primer HT

6. Proceed with cDNA amplification as described in the SMARTer Stranded RNA-Seq Kit User Manual.

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