GeneLab Standard Operating Procedure: Normalizing TruSeq Stranded Total RNA Library

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Version 1.0

Document Revisions

Document Number	Revision Number	Date	Description of Changes
6.4	1	January 2021	Original

Scope and Purpose

This SOP describes the steps used by NASA GeneLab to normalize TruSeq Stranded Total RNA libraries.

Equipment and Consumables

1. TapeStation D1000

Reagents

1. DNase- and RNAse-free water
2. TruSeq Resuspension Buffer (RSB)
3. TruSeq Total RNA library
4. Ice
5. Ice bucket
6. Low bind microtube
7. 96-well sterile plate

Procedure

1. Obtain average library fragment size from TapeStation D1000 without adapter dimer if average size are within similar range -/+ 30 bp.
2. Convert each library concentration from PicoGreen measurement ng/uL to nM using average or individual fragment size bp. (Reference PicoGreen SOP)

   Equation for converting dsDNA:

   $$\text{conc nM} = \frac{(\text{conc ng/uL}) \times 10^6}{(660 \text{ g/mol} \times \text{library size bp})}$$

3. Dilute each library to 20nM in DNAse-/RNase-free water.
4. Store at -20°C for iSeq (GL-SOP-015.1) and NovaSeq run (GL-SOP-016.1).

Figure 1: Example dilution of a sample.

				20 nM	
Sample #	**Sample**	**Library PicoGreen conc. (ng/ul)**	**Libraries (nM)**	**Sample Vol. to 20 nM in 20ul**	**Water ul**
1	MGS_HLU IR_M9_D SKN_RNA_ALQ0	6.490	32.78	12.20	7.80