GeneLab Standard Operating Procedure:   
Normalizing TruSeq Stranded Total RNA Library

*January 2021*

*Version 1.0*

# 

Document Revisions

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# 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:

conc nM = (conc ng/uL) x 10^6

(660 g/mol x library size bp)

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

Figure 1: Example dilution of a sample.

