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| **GeneLab SOP for Normalizing TruSeq Stranded Total RNA Library** | Document No.: | GL-SOP-6.4 |
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| Last revised by: | Marie Dinh |

**Purpose/Scope:**

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

**Equipment:**

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).

