GeneLab SOP for Normalizing TruSeq Stranded Total RNA Library	Document No.:	GL-SOP-6.4
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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:

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

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).

				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