GeneLab Standard Operating Procedure: Normalizing sequencing library pools without the use of iSeq sequencing output

Sep 2021

Version 1.1

Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-6.4	1.0	Jan 2021	Original
GL-SOP-6.4	1.1	Sep 2021	Adjusted scope of procedure to incorporate non TruSeq libraries Changed procedure name to reflect that it can be used for various library kits Step – wording Added TapeStation, Picogreen and Qubit consumables to “Reagents” Added missing steps to combine individual libraries

Scope and Purpose

This SOP describes the steps taken by NASA GeneLab to normalize sequencing libraries in a pool.

Equipment and Consumables

1. DNase- and RNAse-free water
2. Ice
3. Ice bucket
4. low bind 1.5mL microtube
5. 96-well sterile plate with working capacity of 100-150u

Reagents

1. TapeStation reagents (as described in GL-SOP-6.3)
2. Qubit reagents (as described in GL-SOP-4.1)

   Or

3. Quant-iT PicoGreen reagents (as described in GL-SOP-6.2)

Procedure

1. Obtain average library fragment size from TapeStation D1000 following SOP GL-SOP-6.3. Check there is no and that the average size of libraries is within similar range -/+ 50 bp.
2. Convert each library concentration obtained from PicoGreen or Qubit measurement to molarity using average or individual fragment size in units of [bp].
   Equation for converting dsDNA:

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

3. Dilute each library to 20nM in DNAse-/RNase-free water.
4. Dilute further down to required molarity and combine equal volumes of each of the library in to a 1.5mL low bind tube to create a library pool.
5. Dilute the library pool to loading concentration or store at -20°C for iSeq and/or NovaSeq run (GL-SOP-7.1)

				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

Figure 1: Example dilution of a sample.