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

*Sep 2021*

*Version 1.1*

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Document Revisions

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| 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 |
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# 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

1. 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:

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

(660 **g/mol** x library size bp)

1. Dilute each library to 20nM in DNAse-/RNase-free water.
2. 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.
3. Dilute the library pool to loading concentration or store at -20°C for iSeq and/or NovaSeq run (GL-SOP-7.1)



Figure 1: Example dilution of a sample.