

NGS library quantitation  
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1. Make the following serial dilutions in TE/10 of a library of a known concentration: 200 pM, 20 pM, 2 pM, 0.2 pM. (See note #1 and #2)
2. Dilute your library 1:1,000 and 1:10,000.
3. Set up the following reactions using all prepared dilutions above in steps 1 and 2.
  - 4 µl diluted standards or diluted library
  - 1 µl TruSeq PCR primer cocktail (25 µM)
  - 5 µl H<sub>2</sub>O
  - 10 µl KAPA SYBER FAST 2x master mix
4. In plate set up window of the qPCR software insure that you designate your standards as 'standards' and enter their concentrations as 200, 20, 2 and 0.2. Your libraries to be quantitated mark as 'samples'.
5. Amplify with the following parameters:
  - a. 3 min at 95° C
  - b. 20 cycles of:
    - 30 sec at 95° C
    - 30 sec at 63° C
    - 30 sec at 72° C
  - plate read
6. The qPCR software calculates the efficiency of amplification of the standards, which should be between 95% and 105%. It also outputs the concentration of your diluted unnormalized libraries.
7. Calculate the actual concentration of your libraries using the following equation (see notes #3 and #4):
$$\text{concentration from qPCR software} \times \frac{\text{average size of your standards library}}{\text{average size of your library you are quantitating}} \times \text{dilution factor} = \text{actual library concentration}$$

Notes:

- 1) If you do not have a library of known concentration I would recommend buying the KAPA Biosystems library quantification kit.
- 2) If you are using your own standards, I recommend you make enough of the library so you do not have to make new standards for a long time. You do not want to have to recalibrate your standards with cluster formation.
- 3) a) 'Concentration from your qPCR software' is the starting quantity as stated by the qPCR software, e.g. 7.3 pM.  
b) 'Average size of your standards library' is the average size of the library as determined by running it on the Bioanalyzer, e.g. 275 bp.

c) 'Average size of the library you are quantitating' is the average size of the library as determined by running it on the Bioanalyzer, e.g. 275 bp.

d) 'Dilution Factor' is the amount the library was diluted, e.g. 1,000 or 10,000.

4) It is important that the average library sizes are determined from a non-over-amplified library, as over-amplified libraries do not run true to their size on the bioanalyzer.

### **TE/10**

10 mM Tris-HCl, pH 8.0

0.1 mM EDTA

### **TruSeq PCR Primer Cocktail**

1. Resuspend TruSeq PCR Primer 1 and TruSeq PCR Primer 2 in TE/10 to a concentration of 100  $\mu$ M.
2. In new tube mix:
  - 25  $\mu$ l TruSeq PCR Primer 1 (100  $\mu$ M)
  - 25  $\mu$ l TruSeq PCR Primer 2 (100  $\mu$ M)
  - 50  $\mu$ l H<sub>2</sub>O

### **Oligonucleotide Sequences**

\* = phosphorothioate bond

TruSeq PCR 1

AATGATACGGCGACCACCGA\*G

TruSeq PCR 2

CAAGCAGAAGACGGCATACGA\*G

**Note:** The PCR primer sequences are my best guess as to what Illumina is using in their PCR Primer Cocktail.