GeneLab Standard Operating Procedure:

qPCR quantification of Illumina sequencing libraries

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Version 1.0

Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-6.1	1.1	02_24_2020	Original document

Scope and Purpose

This SOP describes the steps used by NASA GeneLab for qPCR quantification of Illumina Sequencing libraries using a QIAgility.

Equipment and Consumables

1. Qiagen 990512 Conducting filter-tips, 50 uL (960)
2. 96-well semi-skirted plate
3. Tabletop centrifuge and vortex mixer

Reagents

1. Kapa KK4873 – *07960336001* ROX Low qPCR Master Mix *or*
   Kapa KK4824 – *07960140001* Universal qPCR Master Mix
2. These both contain DNA standards (1-6), Primer Mix (1 mL), KAPA SYBR® FAST qPCR Master Mix (5 mL). The universal contains ROX High and ROX Low to be added separately, the ROX Low has the ROX Low already added.
3. Library pool(s) diluted to 1:10,000, 1:20,000, 1:100,000, 1:200,000 according to GL-SOP-002.2 in 1.5 mL tapered tubes

For 1 library pool:
Requires 34, 50 uL tips
650 uL 2x Mix
70 uL water
41 uL each of 6 standards
30 uL each of 4 dilutions

For 2 library pools:
Requires 46, 50 uL tips
866 uL 2x Mix
70 uL water
41 uL each of 6 standards
30 uL each of 4 dilutions of each library

For 3 library pools:
Requires 58, 50 uL tips
1085 uL 2x Mix
70 uL water
41 uL each of 6 standards
30 uL each of 4 dilutions of each library

For 4 library pools:
Requires 70, 50 uL tips
1303 uL 2x Mix
70 uL water
41 uL each of 6 standards
30 uL each of 4 dilutions of each library

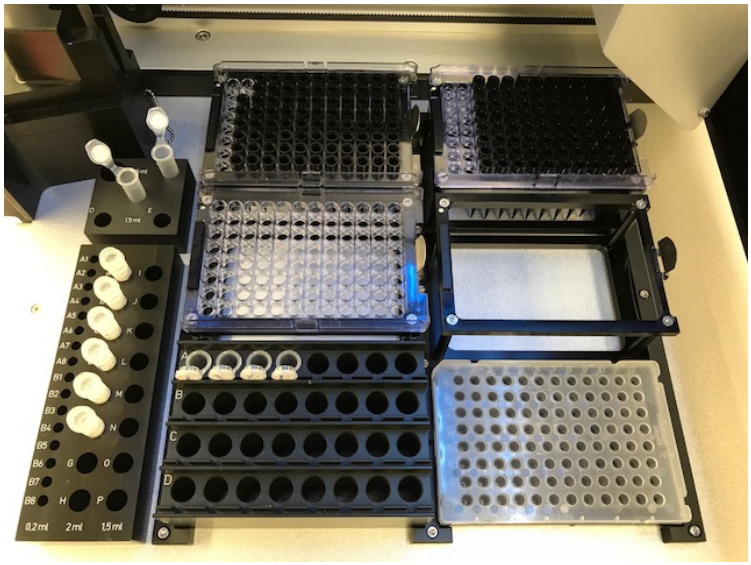
Dilutions

Serial dilution on both pools:

Dilution	Sample vol.	Buffer vol.
(1:10)	1	9
(1:100)	2	18
(1:1000)	2	18
(1:10,000)	10	90
(1:2)	20	20
(1:100,000)	10	90
(1:2)	20	20

Procedure

1. Thaw all components at room temperature for 15 min.
2. Vortex all components until well mixed.
3. Centrifuge until all droplets are collected.
4. Set-up QIAgility deck:



M1: Master Mix Block:

648 (or appropriate volume) 2x reaction mix in tapered tube in position B
70 ul water in tapered tube in position C

A1: 50 ul conductive tips
A2: (optional): 200 ul conductive tips
B1: (optional): 50 ul conductive tips
B2: (optional): 50 ul conductive tips

R1: Reagent block with 41 ul of each standard in position A - F

C1: Flip cap block with 30 ul of each library dilution at A1 - A4 (continuing in groups of 4 with more libraries)

C2: 96 sample qPCR plate

Set-up QIAgility

1. Start the QIAgility set-up manager.
2. Click on the appropriate template (e.g. 1 Lib Quant).
3. Click Assignment and select 3 for the number of replicates and then click assign.
4. Click Worktable.
5. Click each gold worktable box and confirm loading.
6. Click the green Start run button to begin the run.
7. Following the run, generate a report and save in the Reports folder.

Clean-up QIAgility

8. Remove samples, standards and reagents from QIAgility deck and store or dispose of them properly.
9. Close the lid.
10. Turn off QIAgility by clicking File > Exit and following prompts.
11. Turn off the computer.

Run qPCR

12. Seal the plate.
13. Run the qPCR.
14. Analyze the data.