Library Quantification Demo Kit

The purpose of this kit is to demonstrate Illumina® library quantification on the QuantStudio® 3D Digital PCR System.

Materials

The kit below has enough materials for 10 reactions.

Product	Cat. No.	Concentration	Vol (µL) Provided
TruSeq Control Template	N/A	2.5nM	7
TruSeq DNA/RNA Assay (Ac04364396_a1)	4331182	20X	10
QuantStudio® 3D Digital PCR Master Mix	4482710	2X	80

Methods

1. Use a serial dilution to make a 1 in 300,000 and a 1 in 600,000 dilution of the TruSeq control template using nuclease free water. Follow the table below to make your dilutions:

Dilution	TruSeq Control Template	Nuclease-Free Water
1 to 30	5 µL of stock library	145 µL
1 to 3,000	5 µL of 1 in 30 dilution	495 µL
1 to 300,000	5 µL of 1 in 3,000 dilution	495 µL
1 to 600,000	5 µL of 1 in 300,000 dilution	5 µL

2. Prepare a "super mix" containing master mix and assay sufficient for both dilutions to be run as described in the table below.

Component	Volume For 1 Chip	Volume For 2 Chips
2X master mix	7.25 µL	14.5 µL
20X assay	0.73 µL	1.46 µL
Nuclease Free Water	4. 52 µL	9.04 µL

3. Label a 0.5 mL tube for each dilution to be run.
4. Add 12.5 µL of the "super mix" into each 0.5 mL tube and then add 2 µL of each dilution into the appropriately labeled tube for a final volume of 14.5 µL per tube.
5. Load 14.5 µL of the PCR reaction mix on the chip followed by chip assembly.
6. Thermal cycle the chips according to the conditions provided in the tables below.

For the ProFlex: If not using "3D" default protocol from ProFlex v 1.1.4 firmware, user must create thermal protocol and verify the ramp rates.

	Stage 1	**Stage 2**		**Stage 3**		**Cover Temp.**	**Reaction Volume**
	96.0°C	55.0°C	98.0°C	55.0°C	10.0°C	70.0°C	1 nL (33 nL for firmware older than 1.1.4)
ramp rate	0.8 °C/sec	1.2 °C/sec	0.8 °C/sec	1.2 °C/sec	1.2 °C/sec		
	0:10:00	0:02:00	0:00:30	0:02:00	∞		
	1x (Hold)	39x (Cycles)		1x (Hold)			

For the GeneAmp® PCR System 9700: There is no need to adjust the cover temperature or the ramp rates.

Stage 1	**Stage 2**		**Stage 3**		**Reaction Volume**
96.0°C	55.0°C	98.0°C	55.0°C	10.0°C	20 µL
10 min	2 min	30 sec	2min	∞	
1x (Hold)	39x (Cycles)		1x (Hold)		

7. Exit the protocol allowing the thermal cycler to equilibrate to room temperature for 5 minutes to eliminate any chip condensation. See table below for stopping the run for each specific thermal cycler:

Thermal Cycler	**Procedure**
GeneAmp® 9700	Press **STOP** twice to stop the run, then **Exit**
Proflex™	Press **Stop Run**, then **OK** to stop the current run

Note: Any other condensation may be immersion fluid. Use lint-free wipes with Isopropanol or Ethanol to remove immersion fluid.

8. Read chips on the QuantStudio® 3D Digital PCR Instrument and upload results to AnalysisSuite™ Cloud Software.

9. Enter the appropriate dilution factor for each chip in “Define Chips” tab (Figure 1).

Sample	Dilution Factor to Add to Software
1 to 300,000	1 to 2.175E6
1 to 600,000	1 to 4.35E6

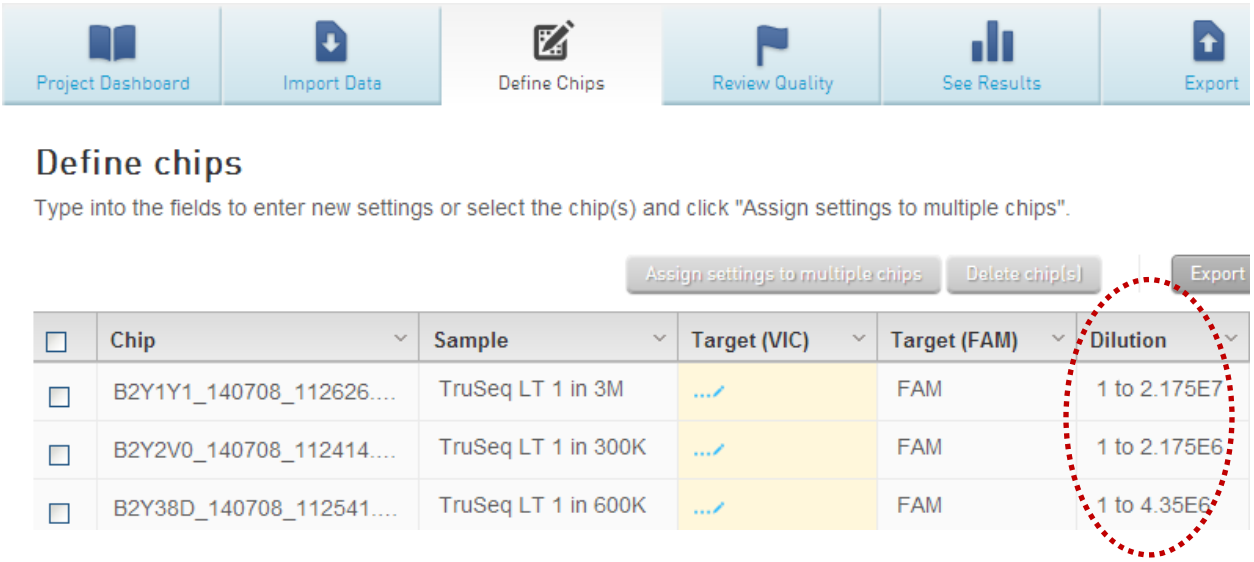

Figure 1: QuantStudio® 3D AnalysisSuite™ Cloud Software “Define Chips” tab displaying the final sample dilution factors highlighted by the red oval.

10. Obtain the copies/µL from the “Results” option in “See Results” tab (Figure 2).

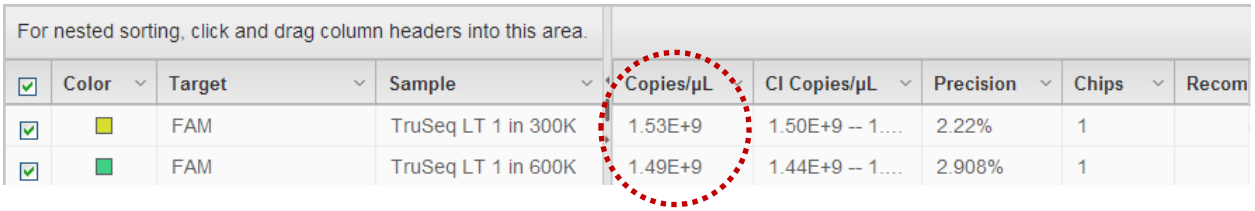

Figure 2: Final calculated copies/µL of library adjusts for sample dilution as defined in “Define Chips” tab and thus represents the concentration of library molecules in the starting sample prior to dilution.

11. Use the dPCR Library Quantification Calculator to convert the copies/µL to nM concentration (Figure 3).
    - Enter sample name, library size and sample concentration in the highlighted fields.
        i. The size of the control library is 465 bp
        ii. The expected library concentration is 2.5 nM
    - The calculator can be found at **lifetechnologies.com/dpcrlibquantcalc**

Sample ID	Lib size (bp)	Lib size (Mb)	cp/ul from digital PCR	ng/uL	nM
TruSeq 1 to 300K	465	0.000465	1.53E+09	7.7975E-01	2.541
TruSeq 1 to 600K	465	0.000465	1.49E+09	7.5936E-01	2.474

Figure 3: dPCR library quantification calculator. Target concentration should be close to 2.5 nM.

12. If you have any questions about library quantification, please refer to the Illumina protocol that can be found using the link below: http://abcommunity.lifetechnologies.com/community/digital_pcr/blog/2014/05/05/ion-torrent-and-illumina-library-quantification-with-quantstudio-3d-digital-pcr-system