Demonstration of automated KAPA Library Quantification Kits on firefly®

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Overview

In this poster, for the first time, we demonstrate that KAPA Library Quantification Kits protocols can be successfully automated on SPT Labtech's firefly® liquid handling platform, to increase throughput and efficiency while delivering consistent and reliable results with no detectable well-to-well contamination.

Library quantification is critical to achieving uniform sample pooling and optimal cluster density in next-generation sequencing (NGS) workflows, thereby ensuring the quality and quantity of data output. KAPA Library Quantification Kits contain all the reagents needed for accurate, reliable, and reproducible qPCR-based quantification of NGS libraries for Illumina sequencing, and cater to a wide range of library types, concentrations, fragment length distributions and GC content.

firefly is a comprehensive benchtop platform that combines all the technologies needed for complete automated qPCR setup, including 96/384-well pipetting and non-contact positive displacement dispensing.

Deck layout

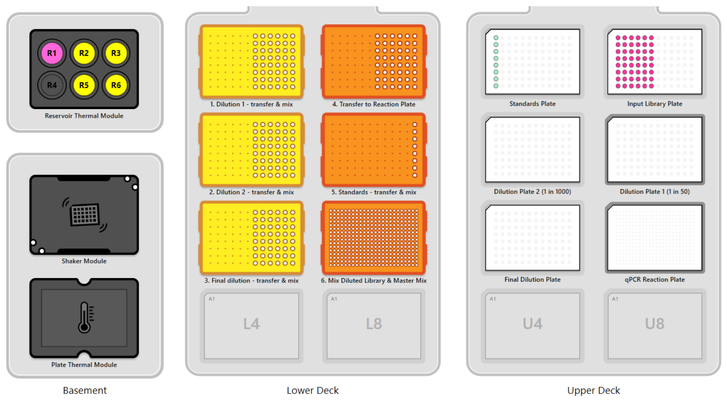
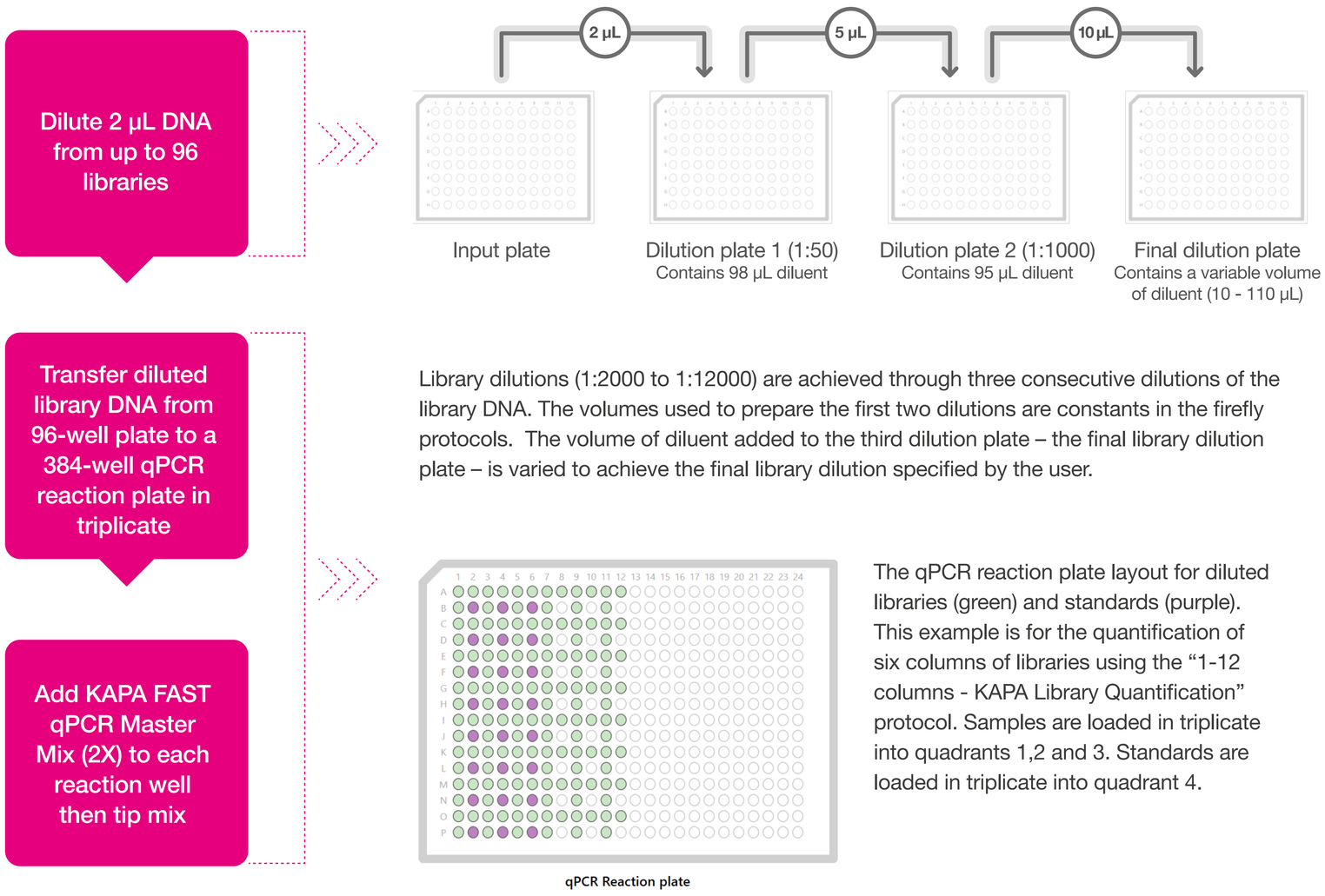


Figure 2. Starting deck layout for the "1-12 columns - KAPA Library Quantification" protocol – this example is for the quantification of six columns of libraries. Reservoir 1 (R1) contains the KAPA SYBR FAST qPCR Master Mix, reservoirs 2, 3, 5 & 6 (R2, R3, R5 & R6) contain the DNA dilution buffer. Lower deck contains 125 µL tips (yellow) and 50 µL tips (orange).

Workflow overview



Library dilutions (1:2000 to 1:12000) are achieved through three consecutive dilutions of the library DNA. The volumes used to prepare the first two dilutions are constants in the firefly protocols. The volume of diluent added to the third dilution plate – the final library dilution plate – is varied to achieve the final library dilution specified by the user.

The qPCR reaction plate layout for diluted libraries (green) and standards (purple). This example is for the quantification of six columns of libraries using the "1-12 columns - KAPA Library Quantification" protocol. Samples are loaded in triplicate into quadrants 1,2 and 3. Standards are loaded in triplicate into quadrant 4.

Figure 1. An overview of the steps performed on firefly to automate KAPA Library Quantification Kits. The output is a 384-well qPCR reaction plate that is ready to load onto a qPCR instrument.

Results

The data presented here was generated using Roche KAPA Library Quantification Kits (Complete kits, Universal, Cat. # 07960140001) and run on a LightCycler 480 System.

Standard Curve

firefly was used to prepare qPCR reactions for the DNA standards provided in the KAPA Library Quantification Kits. The Cq scores and standard curve demonstrate consistency between the technical replicates and show that the standards from the KAPA Library Quantification Kits exhibit the expected ΔCq and reaction efficiency when firefly is used to prepare the qPCR reactions.

DNA Standard	Concentration (pM)	Cq score			Average Cq	ΔCq*	Standard deviation	CV %
		1	2	3				
Standard 1	20	7.48	7.59	7.41	7.49	-	0.09	1.21
Standard 2	2	10.89	11.01	10.83	10.91	3.42	0.09	0.84
Standard 3	0.2	14.41	14.51	14.22	14.38	3.47	0.15	1.02
Standard 4	0.02	17.78	17.92	17.71	17.80	3.42	0.11	0.60
Standard 5	0.002	21.25	21.44	21.00	21.23	3.43	0.22	1.04
Standard 6	0.0002	24.61	24.78	24.35	24.58	3.35	0.22	0.88

Table 1. Example of the Cq scores for the six DNA standards to demonstrate the repeatability of technical replicates. *Expected ΔCq is 3.1-3.6 for a 10-fold dilution.

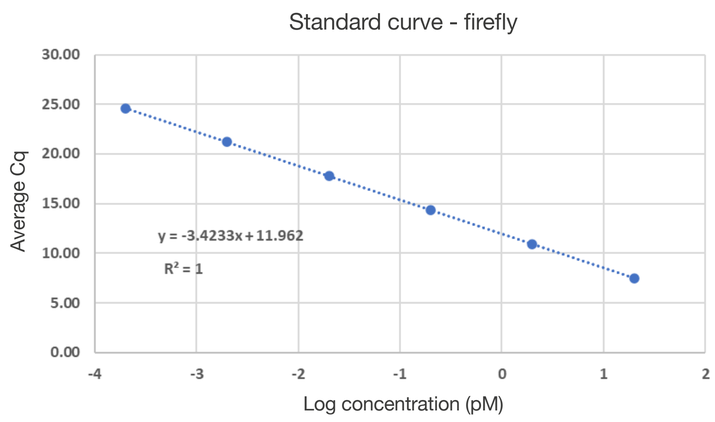


Figure 3. Standard curve generated with the KAPA Library Quantification Kits on firefly. Reaction efficiency = 95.9%.

Consistency of library dilutions

Consistency and accuracy of the library dilutions was assessed using 16 replicates of the same library and measuring the concentration of the library in each of the three dilution plates generated in the KAPA Library Quantification Kits protocol on firefly.

Automating the KAPA Library Quantification Kits protocol on firefly gives a consistent measure of sample concentration (%CV across 16 samples ≤3%). Concentration of the undiluted library varies by only 0.08 nM across the dilution range 1:50 to 1:12000.

Library dilution	Number of samples	Diluted library				Undiluted library	%CV
		Mean concentration (pM) size adjusted	Dilution factor	Expected concentration (pM)	% deviation from expected concentration	Mean concentration (nM) size adjusted	
1:50	16	19.74	n/a	n/a	n/a	0.99	2.2
1:1000	16	1.06	20	0.99	7.0	1.06	3.2
1:12000	16	0.09	12	0.09	1.2	1.07	2.6

Table 2. Dilution accuracy and variation in concentration across 16-replicates of the same sample. The expected concentration of the diluted library is based on the input concentration and the dilution factor.

Well-to-well contamination

The KAPA Library Quantification Kits protocol was run using an input plate containing 48 library samples and 48 no template controls (NTCs), arranged in a checkerboard pattern. There was no detectable well-to-well contamination between the library samples and the NTCs.

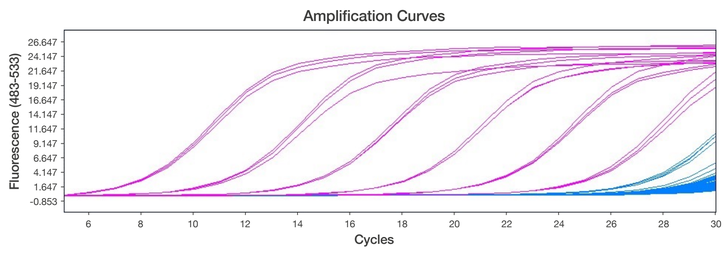


Figure 6. Amplification curves for KAPA qPCR standards 1 to 6 (pink) and NTCs (blue).

Conclusions

The KAPA Library Quantification Kits protocol has been successfully automated on firefly, enabling a 384-well qPCR reaction plate to be prepared with standards and up to 96 diluted samples, in just over 20 minutes. The qPCR reaction efficiency is as expected for the KAPA Library Quantification Kits, sample dilutions are both accurate and consistent, and no well-to-well contamination was detected. firefly provides accurate liquid handling and user-friendly software to remove the inconvenience and inconsistency of manual pipetting, enabling the fast, simple, high-throughput setup of qPCR reactions.

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