NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA Library Quantification kits (KK4827)
Manufacturer : KAPA BIOSYSTEMS
Application : Next-generation sequence library quantification by qPCR
– Comparison between fluorescent probe detection and SYBR Green I detection (KAPA LQ Kit) –
(Ion Torrent™ Ion AmpliSeq™ Cancer Panel Library)

This application note was provided by the courtesy of Dr. Tohru Niwa and Dr. Toshikazu Ushijima of Division of Epigenomics of National Cancer Center Research Institute.

Comparative study method

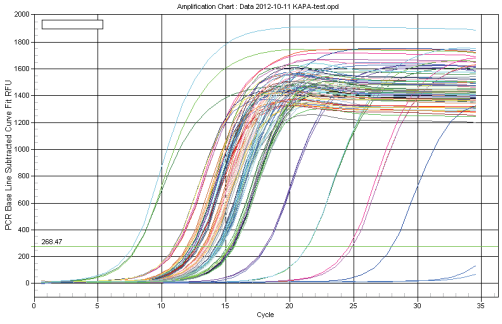
Six samples of Ion Torrent™ Ion AmpliSeq™ Cancer Panel library were previously quantified using a library quantification kit (fluorescent probe detection) from manufacturer A. Each sample was diluted 500-, 1,000-, 2,000- and 4,000-folds and quantified using the KAPA Library Quantification kit (SYBR Green I detection). Average values of the dilution factors were individually calculated and compared with the values obtained using the manufacturer A's kit.

qPCR system : Bio-Rad MyiQ™
Library quantification kit : Library quantification kit from manufacturer A — Fluorescent probe detection
KAPA Library Quantification kit (KK4827) from KAPA — SYBR Green I detection
Library sample : 6 samples of IonAmpliSeq™ Cancer Panel library

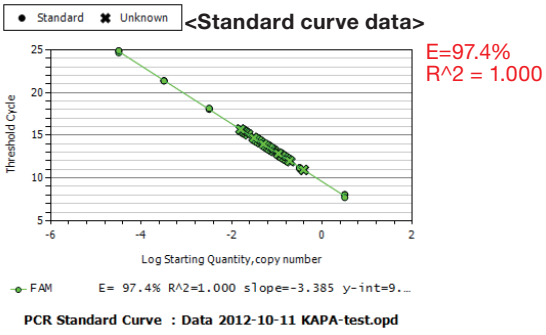
Results

(1) Data from qPCR using KAPA LQ Kit

<Amplification curve data>



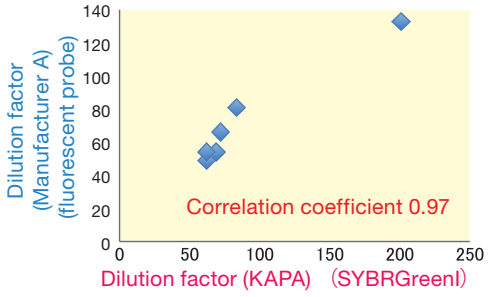
<Standard curve data>



(2) Comparison of calculated dilution factors

Sample No.	Calculated dilution factor	
	KAPA (SYBRGreenI)	Manufacturer A (fluorescent probe)
1	62	49
2	69	54
3	83	81
4	201	133
5	72	66
6	62	54

KAPA (SYBR Green I) vs. Manufacturer A (fluorescent probe)



<Customer's comments>

Compared to the manufacturer A's kit, KAPA kit tended to give higher dilution factors.
The correlation coefficient between the dilution factors obtained by the two kits (manufacturer A and KAPA) was high (0.97), so the difference in the dilution factor seems to reflect the difference in the standard DNA.
I cannot say which kit is better, but I can say that KAPA LQ Kit (SYBR Green I detection) was equally effective as the manufacturer A's kit (fluorescent probe detection) in terms of quantification.

Regarding the PCR amplification potential of the KAPA kit:
1) a high fluorescence value was achieved despite a small reaction volume (20μL);
2) the PCR efficiency calculated from the reaction pattern of the standard DNA was high (97.4%) and hardly showed any variation (R^2=1.000). I found that the enzyme and the reaction system were equipped with high amplification potentials despite high SYBR content.

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