Comparison of SYBR enzymes and standards in Illumina library quantification

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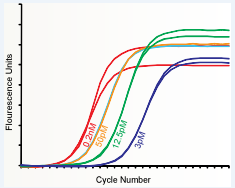
Objective:

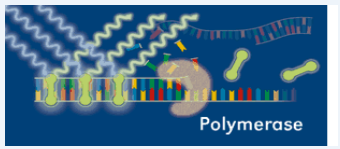
Comparing various SYBR enzymes and DNA standards for accurate quantification of libraries generated for Illumina next-generation sequencing.

Gene Expression Analysis
Genotyping
DNA Quantification
...More

SYBR



Success of qPCR library quantifications depend on the accuracy and reproducibility of standards used in addition to the ability of DNA polymerase to efficiently amplify all adaptor-flanked libraries. We compared the (i) Roche SYBR Green, (ii) KAPA SYBR Fast polymerases and (iii) Qiagen QuantiFast SYBR Green and the PhiX standards and KAPA pre-diluted standards to determine the relative importance of these two factors in library quantification by qPCR.



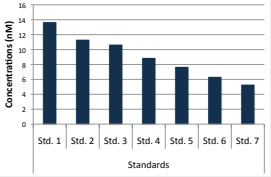
qRT-PCR: Confirm anchors and concentration

QIAGEN

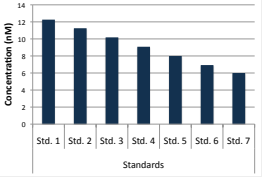
KAPABIOSYSTEMS

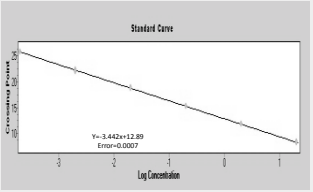
Roche

PhiX Standards - Plate 1



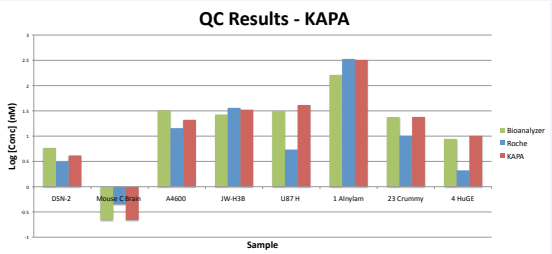
KAPA Standards - Plate 2





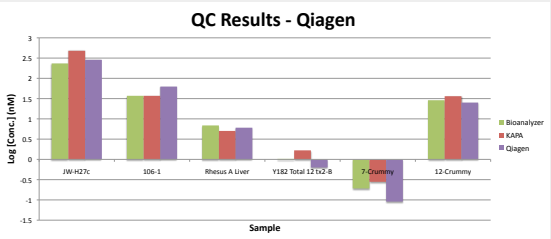
Comparison of Standards: Seven serial dilutions of PhiX were plated on plates 1 and 2 to test for reproducibility. A Third plate was run with pre-diluted KAPA DNA standards. Identical Illumina libraries were loaded on all plates in triplicates and run on the Roche 480 Lightcycler. The standards were used to generate a linear curve to determine the concentration of each library.

QC Results - KAPA

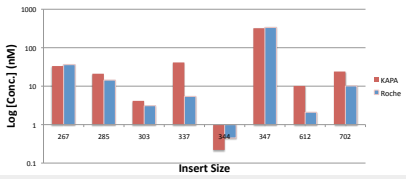


Comparison of enzymes: Three plates were loaded with Illumina libraries and PhiX standards. Both plates were amplified with three different enzymes: (1) Roche SYBR Green (2) KAPA SYBR FAST (3) Qiagen QuantiFast. Cluster counts from the Illumina sequencers were analyzed with respect to concentrations obtained from the enzymes.

QC Results - Qiagen

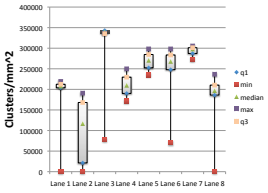


Concentration vs. Insert Size

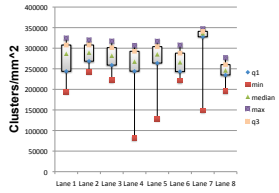


Efficiency of Enzymes: This data indicates that the KAPA enzymes has greater sensitivity with larger amplicons than Roche, resulting in accurate quantification of Illumina libraries of various sizes.

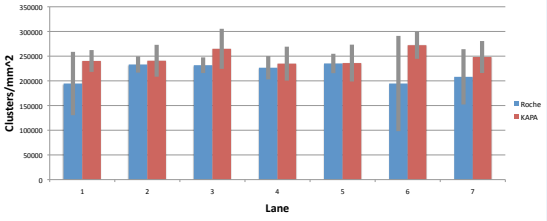
Clusters (PF) on GAIIx (Roche)



Clusters (PF) on GAIIx (KAPA)



Average Clusters

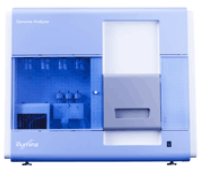


*Average clusters for six consecutive flowcells loaded with (i) Roche-generated concentrations and (ii) KAPA-generated concentrations.

Conclusion:

Our results indicate that the DNA polymerase from the KAPA SYBR Fast kit is better suited to library quantification applications. The Roche SYBR Green kit showed significant variability in respect to cluster generation using the obtained concentration from RT-qPCR, possibly related to sample type or insert size. Similar results were obtained with the Quantifast SYBR Green, in that libraries with atypical fragment sizes were inaccurately quantified. No differences were observed between the two types of standards used, making DNA polymerase the determining factor in the successful amplification of each library. This enzyme is also amenable to automated liquid handling, allowing high-throughput sample analysis, making it ideal for core laboratory settings.

ILLUMINA SEQUENCING



GAIIx

HiSeq2000

Sample Prep & Quality Control

SPRI-TE

Roche LC480

LabChip GX

MICROARRAYS

Affymetrix

Agilent

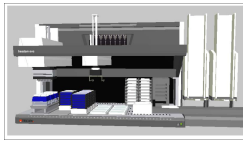
SERVICES:

	Affymetrix	Agilent
Prokaryote	*100ng*	*100ng*
3 prime	*50ng**	*50ng**
Exon	*50ng**	
miRNA		*100ng*
CGH		*3µg*

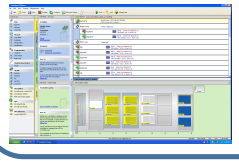
* *<1ng possible in some cases*

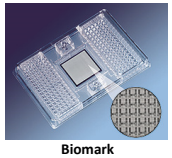
AUTOMATION

Tecan EVO150



Varioskan





Biomark