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TECHNICAL NOTE

QC of Chromium™ Genome Libraries: Quantitative Evaluation Using qPCR

INTRODUCTION

Chromium™ Genome Libraries are evaluated both qualitatively and quantitatively prior to preparing and loading for sequencing. While Bioanalyzer traces are used for qualitative assessment of libraries (see Technical Note CG00047), qPCR is the method of choice for quantitative measurements due to its sensitivity in measuring low DNA concentrations and its specificity for PCR-competent DNA molecules. It is crucial to accurately quantify Chromium™ Genome Libraries for optimal sample loading, clonal amplification and cluster density on an Illumina® flowcell. This Technical Note highlights qPCR quantification of Chromium™ Genome Libraries to evaluate the success of library generation and provide accurate quantitative information. A discussion on expected library yields is also included.

DISCUSSION

The KAPA Library Quantification Kit (Universal) is recommended for quantitative evaluation of Chromium™ Genome libraries to be sequenced on Illumina® platforms. To quantify the final library, appropriate dilutions (Table 1) are prepared with 1 µl of each Chromium™ Genome Library sample. Each prepared library will need 5 serial dilutions:

Step	Dilution	Cumulative Dilution
1	1 : 200	1 : 200
2	1 : 200	1 : 40,000
3	1 : 5	1 : 200,000
4	1 : 5	1 : 1,000,000
5	1 : 5	1 : 5,000,000

Table 1. Serial dilutions of constructed library for quantitation by qPCR

Figure 1 illustrates the serial dilutions (with PCR-grade water) of 8 Chromium™ Genome Library samples. Prepare the dilution fresh and use in qPCR quantification immediately, as dilute DNA is not stable in PCR-grade water. Alternatively, prepare the dilution in 10 mM Tris-HCl (pH 8.0) with 0.05% Tween-20.

The reagent preparation and master-mix assembly follows the KAPA Library Quantification Kit manual. Please refer to the manufacturer's protocol for the most up-to-date information. qPCR reaction setup and cycling conditions are found in Section 6.6 of the Genome Reagent Kit User Guide. Figure 2, below, illustrates the qPCR reaction setup plate layout for 8 libraries. Each library is plated with 4 serial dilutions (Dil. 2 to Dil. 5; note, Dil. 1 is not used) while the KAPA Standards 1-6 (KS1-KS6) are plated in duplicate.

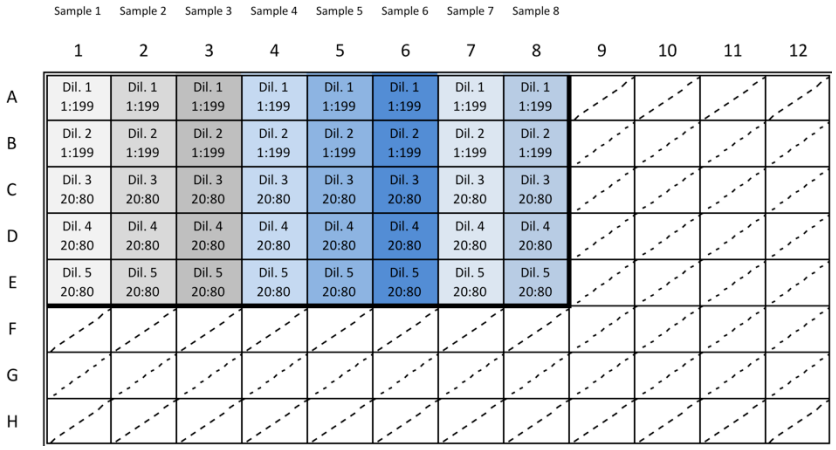

Fig. 1. 96-well dilution plate layout of 5 serial dilutions for each of 8 Chromium™ Genome Library samples.

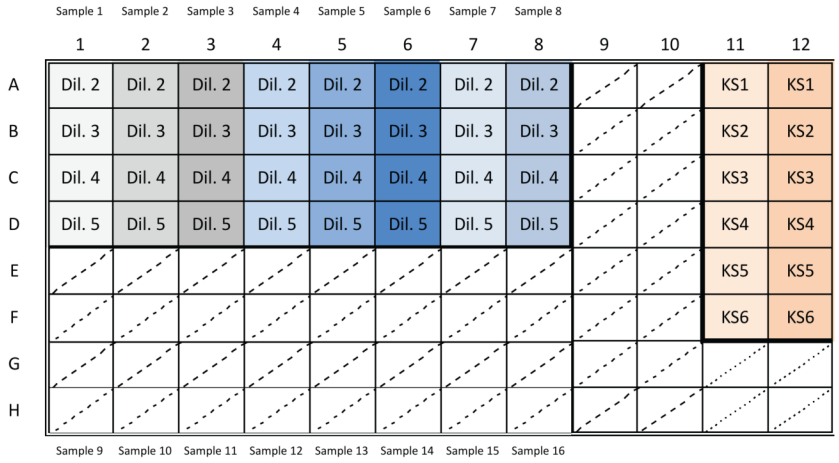

Fig. 2. 96-well dilution plate layout of qPCR reaction setup to quantify 8 Chromium™ Genome Library samples.

We recommend using the KAPA Library Quantification Data Analysis Template to generate the standard curve and for subsequent calculation of the library concentration. A fixed insert size of 550 bp is chosen to calculate library concentrations from the standard curve. We regularly obtain library yields that range between 20 nM and 50 nM. However, quantification results of a single library can be variable, caused by multiple factors including differences in sample handling between operators, and the tolerances of liquid handling tools. Therefore, the range specified above is to be used as a guideline. If the calculated library concentration is slightly outside of this range we encourage users to continue sequencing their samples and review the results. If the estimated library quantification lies significantly outside of this general range (e.g. <10 nM, >80 nM, or 3-fold above or below the typical yields): 1) review the calculations and check for errors in setup, 2) repeat the quantitation procedure or 3) review the library preparation protocol and remake the library.

CONCLUSION

The two QC tools mentioned in this Technical Note: Bioanalyzer traces for library insert sizes and qPCR for library quantification provide guidance to monitor effective library preparation. Reliable and accurate library quantification is required to determine overall library yield and to load precise amounts of library DNA onto an Illumina® flowcell for the highest quality sequencing data. We recommend following the guidelines discussed here and in the Genome Reagent Kit User Guide, as this will ensure the generation of optimal cluster densities, the equivalent representation of multiplexed libraries when pooling and ultimately high quality sequencing data of genomic libraries.

Notices

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