**Abstract**

The KAPA Biosystems Library Quantitation Kit for the Ion Torrent Personal Genome Machine (PGM) has helped us achieve a consistent template to sphere ratio and consistent equimolar barcode pooling when compared to other quantitative methods. A correct template to sphere ratio (also known as dilution factor) is essential for a successful sequencing run. If too much template is added during template preparation, the probability of polyclonal dilution factor is greatly increased. Conversely, if too little template is added the efficiency of PCR elongation is decreased. Therefore giving off a stronger signal.

**Introduction**

Ion Torrent's PGM is a massively parallel sequencing by synthesis (SBS) instrument. It operates by directly sensing (i.e. no enzymatic or chemical) the ions produced as DNA polymerase incorporates nucleotides to a sphere bound clonal template.

The PGM relies on a type of PCR to generate highly templated spheres. If a sphere has many copies of a single template covalently attached to it, the sphere is referred to as clonal. The more templates it has, the more ions will be released during polymerization, and the easier it will be for the base calling software to model the signal. Negative effects such as phasing will be less of an issue.

If a sphere has more than one template molecule attached to it is referred to as polyclonal. The signal modeling software will not be able to determine which template is being copied at any given incorporation event and therefore the entire read will be thrown out. Sometimes, one of the mixed molecules amplifies preferentially and therefore gives off a stronger signal. In this case the mixed signals act as background noise and drastically reduces the sequence quality and read length.

Prior publications have suggested that the ratio of mixed to clonal spheres as a function of input DNA follows a Poisson distribution. This suggests that if DNA quantitation was accurate enough optimal enrichment can easily be achieved. Therefore it is no longer an issue of trial and error titrations but of DNA quantitation accuracy. This is where quantitative PCR (qPCR) adds significant value.

**Experimental Design /Methods**

There are two types of qPCR, intercalator-based and probe-based. The probe based method uses a Taqman hydrolysis probe that is complementary to 1 of the ligated sequencing adaptors. The intercalator based probe method uses SYBR Green I which is a fluorescent dye that will bind to all dDNA in the sample well. Our lab decided to go with the intercalator based KAPA Biosystems NGS Library Quant Kit because of the lower cost and versatility.

qPCR is a subset of real time pcr that requires a specialized PCR instrument. Our lab uses a Roche LC480. These types of instruments use a charge-coupled camera in concert with a high intensity xenon lamp to collect fluorescent signals.

In the case of qPCR the fluorescent signal is collected at the end of each elongation step. After each consecutive PCR cycle the signal will build. Post PCR cycling the instruments software (using the 2nd derivative maximum analysis) will determine when the signal crossed a predetermined threshold and thus is able to back calculate the starting material to a very high level of accuracy.

Kapa's Ion PGM NGS Library Quant kit pairs well with the Fluidigm's Access Array technology we have in the lab. The lower throughput of the Agilent Bioanalyzer (11 samples per chip) cannot keep up with our Access Array output.

We have created a robotic method on a Biomek FX that does all prep for the Kapa assay including serial dilutions, master mix addition and sample/standard addition.

**Results**

Prior to qPCR implementation at our facility, barcode pooling was either done post library prep in a equi-volume or equi-molar (based on quantitation from Bioanalyzer). We found both methods to be suboptimal.

**Future Development**

In the future we believe Kapa Biosystems Quant Kits will have an important role in increasing our throughput by providing a fast (and easily automated) alternative to other inefficient methods and by removing the need for an amplification step at the end of library preparation. The dynamic range of qPCR is sensitive enough to determine a concentration for samples that can be detectable as an high sensitivity chip on a 316 chip allowing us to greatly reduce the amount of high sensitivity chips. Allowing our high libraries would previously have to be amplified.

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**References**
