Introduction

Accugen had developed a method for absolute quantification to all gene of interest without the use of reference genes and does not require multiple standard curves. As part of our R&D program, we will be giving qPCR users free samples to assist with their analysis.

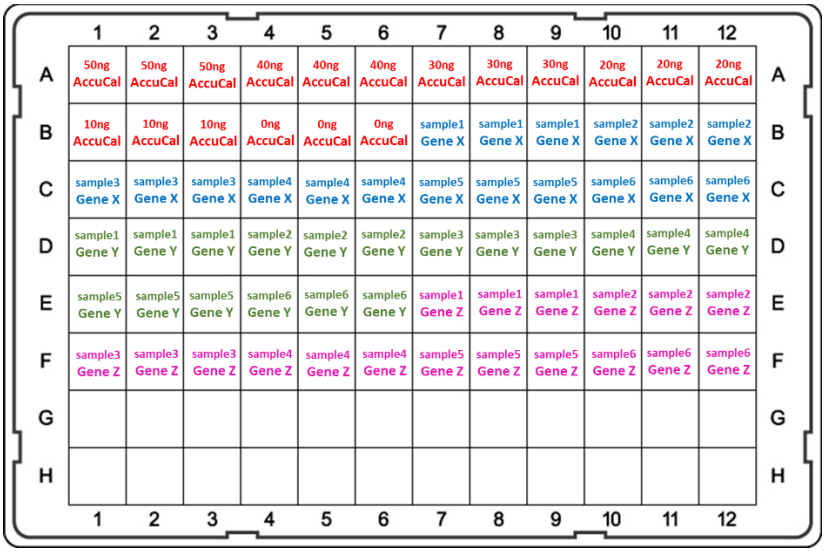
Background

Methods for quantification in qPCR experiments can be either relative or absolute, and both have fundamental problems. Relative quantification requires reference genes that should ideally have stable expression between the experimental groups and have similar amplification efficiency and abundance to the genes of interest (GOIs). The MIQE guidelines, introduced to facilitate standardization of the experimental and reporting practices in qPCR, recommend the use of 3-5 such reference genes, and in practice it is often impossible to identify suitable candidates. Often at times, researcher do not perform an internal validation to the existing reference gene that they are using.

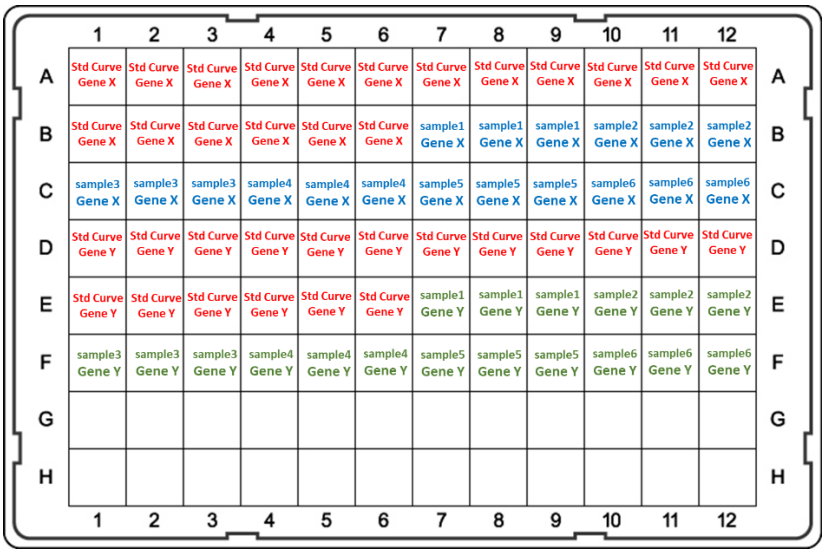
Absolute quantification is performed by constructing a standard curve for each GOI, and this is considered the gold standard for quantification in qPCR. However, each standard curve is only usable for the specific GOI, and constructing the curve is time-consuming and complex. Further, because the standards used are amplified, so too are any experimental errors. This is important as the standard curve for each GOI provides both the efficiency of the amplification primers and the amount of GOI in the unknown samples.

For absolute quantification users, Accugen product reduces the number of standard curves required.

***AccuCal standard curve* is universal across multiple genes**



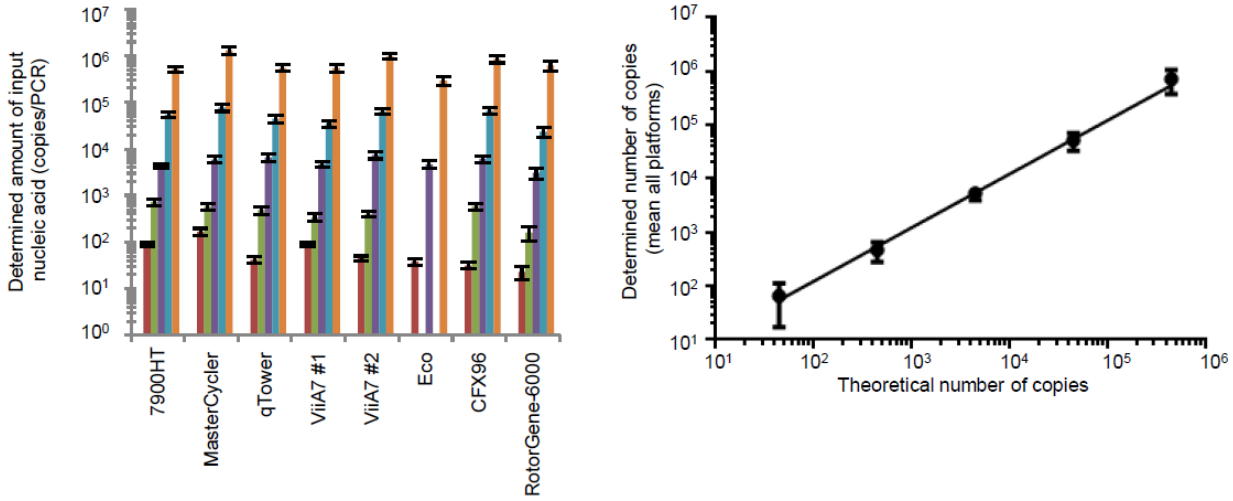
A *traditional standard curve* per gene of interest



How AccuCal works?

AccuCal-D™ calibrator was developed to overcome the shortcomings of using relative gene expression methods for qPCR. In contrast to relative quantification, the AccuCal-D™ method uses fluorescence data from a qPCR run to calculate the starting copy number of a gene of interest (GOI) in a sample to give called absolute quantification.

A lot of RnD was spent on optimizing the software for calculation, which involves the use of AccuCal standards and RealCount software. The protocol consists of the generation of a standard curve, followed by measuring the gene of interest against the standard curve. This method works accurately and consistently on any qPCR instrument using your choice of HRM or SYBR® Green dye containing master mix.



Dilutions of known quantity of cDNA were amplified using various qPCR platforms. The RealCount software had generated mean calculated amount and its plotted vs the theoretical amount of quantified cDNA. The method had shown consistency between different qPCR platforms.

The table below compares the requirement and capabilities of the 3 most commonly used DNA quantification methods – Digital droplet PCR, standard curve-based method and comparative Ct method with novel AccuCal™ method.

Parameters	AccuCal™	Digital PCR	Standard curve	ΔC_t method
Relative Quantification	✓*	✓*	✓*	✓
Absolute Quantification	✓	✓	✓	–
Use of gene standards	–	–	✓	–
Specialized equipment	–	✓	–	–
Use of reference genes	–*	–*	–*	✓
Automatically calculated amplification efficiency	✓	–	–	–

* - not required, but can be used in parallel

Benefits

Simplifies the qPCR workflow, eliminates the need in reference genes and GOI standard curves.

Alternate accurate method of analysis and save reagent cost.

Criteria

As part of our R&D program, we will be giving qPCR users who can find use of this technology free samples to assist with their analysis. The researcher must have a project using qPCR within the next 3 months and use qPCR as their primary method of analysis.

A collaborative output is possible for discussion. Please contact Terry Lee for further details.

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Reference

Paper: A simple, accurate and universal method for quantification of PCR