Antarctica - the source of *RNA*GEM™ (Camp, Ross Ice Shelf)

Sample normalisation with *RNA*GEM™ Tissue

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Introduction

When working with low cell counts or cell lysates, most rapid methods designed for estimating total RNA quantity (such as optical density or fluorescent dyes) are insufficiently sensitive or affected by material in unpurified lysates.

An alternative way to normalise samples is to rely on the cell numbers in the starting material. These can be measured with a cell counter or by using a microscope and haemocytometer. However, with low counts, small sample volumes or high-throughput analysis, direct cell counting is impractical and unreliable and so other methods must be used.

One solution is to use the DNA in the extracts. qPCR can precisely quantify DNA and this in turn can be used to estimate cell numbers. Ploidy and cell growth-phase need to be accounted for in calculating cell numbers from gene copy number, but in general, DNA quantification provides an accurate way to normalise samples - particularly for high-throughput systems using 96 or 384-well plates.

To use this approach, DNA must be present in the lysate in a readily amplifiable form and there must be a linear relationship between cell numbers and DNA yield over the expected working range.

*RNA*GEM and DNA extraction for cell number estimation

*RNA*GEM is a whole nucleic acid extraction kit that gives linear yields of DNA between <10 cells and ~50,000 cells when using the recommended method (figure 1). If larger samples are needed, then the method is easily scaled (see www.zygem.com for details).

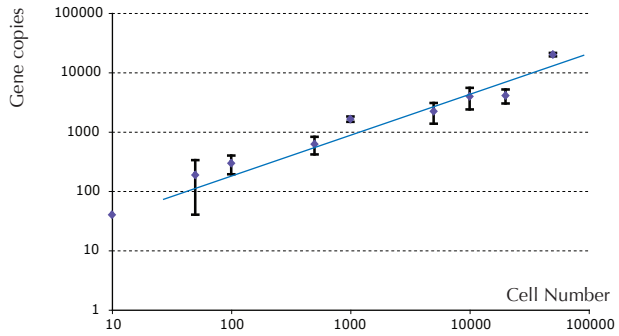

Figure 1: The number of BRCA1 gene copies detected from the gDNA co-extracted from HeLa cells using *RNA*GEM.

This linearity means that the DNA present in the lysate produced by *RNA*GEM can be used to determine the original cell number by qPCR.

Because different primers and PCR reagents have different amplification efficiencies, it is advisable to calibrate the DNA yields from a range of counted cells. Also, it is essential to consider the copy number of the gene, the presence of pseudogenes and the growth cell cycle all of which can affect the outcome.

Sample normalisation

When calculating the copy number of an mRNA, or using an analytical method with a narrow tolerance for sample variation, it is important to normalise samples before the analysis is performed. With normalised samples, failure rates are reduced and comparative measures are more reliable.

The amount of RNA in a sample can be normalised using an RT-qPCR. Either a reference housekeeping mRNA, rRNA or a synthetic molecule can be used to provide an estimate of the total RNA concentration. The advantages and disadvantages of these methods are reviewed in Hugget et al. 2005. However, if RNA and DNA are simultaneously co-extracted with similar efficiencies, then gDNA copies can also provide a simple and direct estimate of cell numbers which in turn provides a normalisation factor for total RNA quantity.

A prerequisite for using this approach is that the extraction efficiency of both DNA and RNA is consistent over the range of cell numbers likely to be encountered. Furthermore, this consistency must apply to both low and high copy mRNAs.

To demonstrate *RNA*GEM's ability to produce RNA and DNA in consistent, linear proportions, 10-10000 HeLa cells were extracted using *RNA*GEM Tissue. The mRNA was quantified by RT-qPCR using qScript cDNA Supermix. DNA was quantified using a qPCR withPerfeCTA® SYBR® Green FastMix®, ROX™ (Quanta Biosciences) - figure 2.

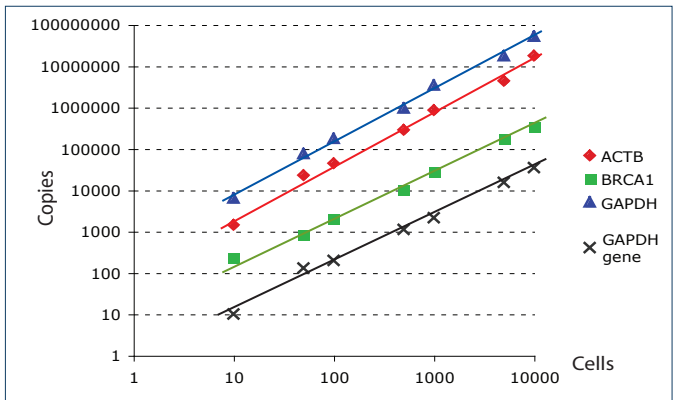

Figure 2: Copies of the human GAPDH gene detected by qPCR plotted alongside copies detected by RT-qPCR of three mRNAs. ACTB = 366 copies per cell; GAPDH = 2607 copies per cell; BRCA1 26 copies per cell.

Extraction linearity is seen for both low and high copy mRNA and also DNA. This ability means that it is possible to normalise *RNA*GEM extracts using a qPCR rather than an RT-qPCR - a simpler and less costly method.

Reference

Hugget et al. (2005) Real-time RT-PCR normalisation; strategies and considerations. Genes and Immunity. ***6****:279-284*