A novel Internal Control for monitoring DNA extraction processes in Real-Time PCR assays [1,2]

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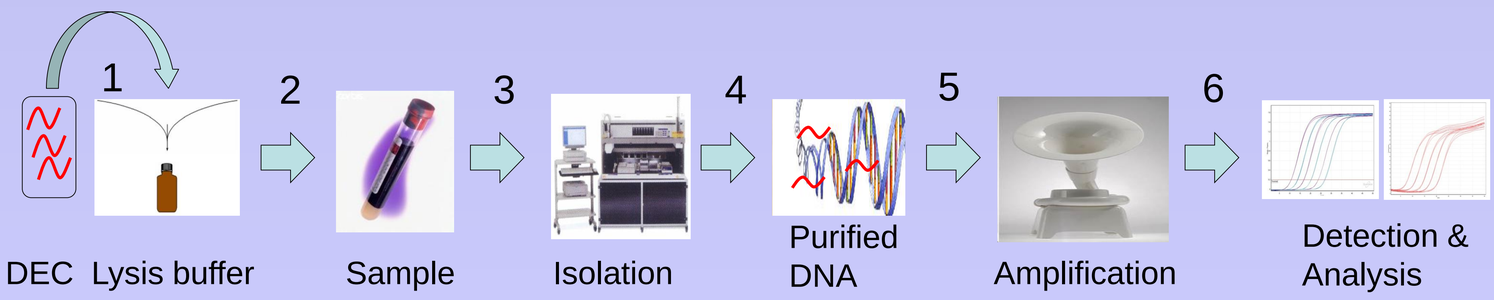
ABSTRACT

The use of Real-Time PCR or quantitative PCR (qPCR) has increased dramatically due to its high sensitivity, accuracy, and reliability, over a broad range of applications. These key attributes of a qPCR assay are heavily dependent on template quality whereby, reduced template quality results in significant reductions in the sensitivity and robustness of the qPCR assay. Also important are inhibitory components prevalent in biological samples and the reagents used during nucleic acid extraction. In summary, template quality and inhibitory components can result in false-negative results. A common practice is to spike a known amount of control DNA either before or after DNA extraction. Adding control DNA prior to extraction can result in degradation and loss of control DNA prior to the qPCR assay. However, adding control DNA after extraction allows for monitoring of inhibition within the assay, but has no value as an extraction control. The ideal situation is to have the test sample and internal control undergo the same processing prior to qPCR(1). We have developed a platform whereby the internal control (a living target) more closely mimics the test sample as compared to spike controls. The genetic material from the test sample and our internal control are simultaneously extracted by common extraction methods with our control being as sensitive to inhibition and extraction failure as the test sample. Our results present a clear advantage of implementing our internal control over spiked DNA controls by more accurately monitoring effectiveness of DNA extraction processes and inhibition within qPCR assays.

The DNA extraction Process

The DNA extraction control (DEC) consists of a unique probe-specific sequence harboured in a proprietary biological carrier



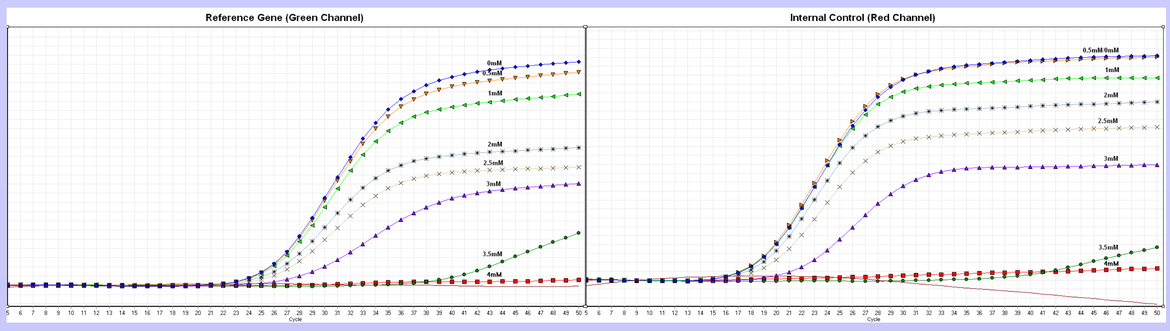
The steps in the DNA extraction process are labeled in numerical order.

1. Internal control (IC) mixed into lysis buffer. **2.** Added lysis buffer including the IC to all samples to be analysed. **3.** DNA extracted with commercial kit. **4.** Purified DNA consists of IC and sample DNA.. **5.** Extracted DNA subjected to qPCR amplification and multiplex assay reactions. **6.** The IC is detected on the Cy5 channel, and target(s) on other commonly used channel(s).

Advantages of the DNA Extraction Control

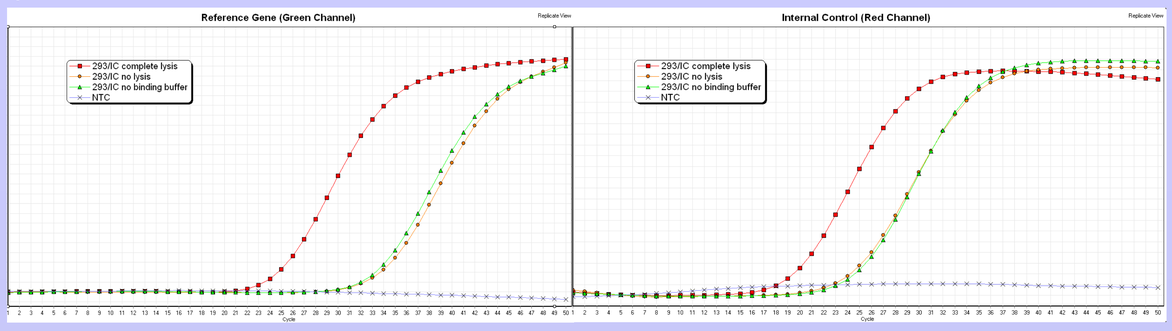
1. Monitor PCR reaction inhibition

Different concentrations of EDTA were added prior to elution, as an inhibitory agent to test the monitoring capability of the Internal Control.



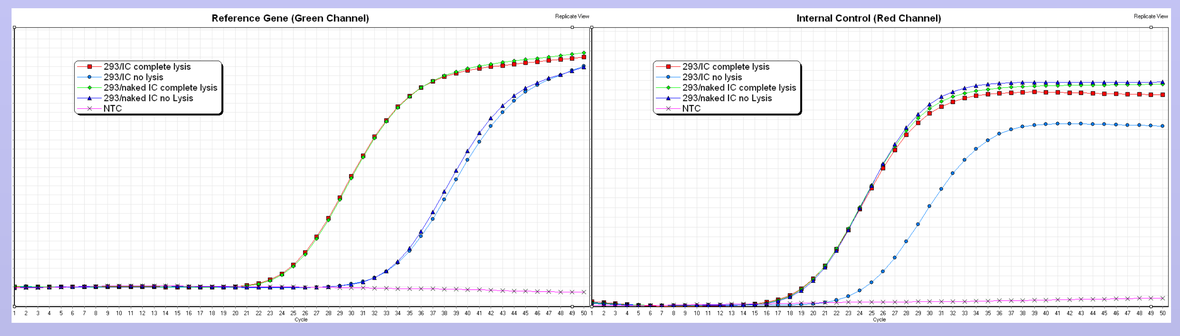
2. Inefficient DNA extraction

Bioline ISOLATE genomic DNA mini kit was used to extract DNA in parallel from a HEK293 cell line and spiked DNA extraction control. Inefficient DNA extraction was simulated by substitution of either the lysis buffer or binding buffer with PBS at the initial stages of the extraction procedure.



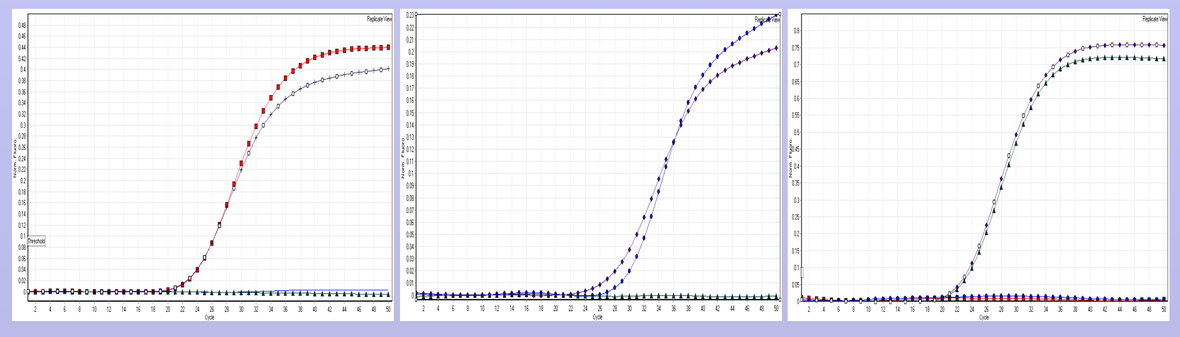
3. DEC vs naked DNA

DEC and equivalent amounts of IC DNA were spiked into a HEK293 cell resuspension. Extraction was carried out with or without lysis buffer in parallel using a Bioline ISOLATE genomic DNA kit.



4. Minimal interference with multiple target detection

The internal control sequence has been specifically engineered as a non-homologous sequence. The DNA extraction control was multiplexed with 2 of the HEK293 gDNA housekeeping genes.



Discussion

We have demonstrated that the DNA extraction control (DEC), not only monitors the co-purification of PCR inhibitors in a sample DNA isolation, but also serves as an indicator of the effectiveness of the extraction process. In monitoring inhibition in qPCR, the DEC exhibited a similar profile of inhibition to a control gene, both in Ct and in signal strength. More importantly, the DEC is also an indicator of sample loss during extraction step by shifting Ct in IC channel. In contrast, naked DNA spiked in to the lysis step has no value as an indicator of “cell lysis”, as the Ct does not change notably between “complete lysis” versus “no lysis”. The unique sequence used in the DEC minimizes the interference with target detection in a multiplex reaction; however, optimization and validation of multiplex assays is always recommended.

Various commercial extraction kits, as well as automated extraction systems, can also be used (data not shown). The compatibility of the control with different clinical samples, according to the pathology labs that have validated the DEC, is another yet crucial aspect of this internal control. Incorporating the DEC is very simple, and can be done utilizing a small number of experiments, to validate the concentration and any unforeseen interference with target assays. Finally, given the versatility of the DEC, it can be used as an universal qualitative control platform for a range of different applications in real time PCR assays.

REFERENCES

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2. Millar, B.C., Xu, J., and Moore, J.E. Molecular Diagnostics of Medically Important Bacterial Infections.*Curr. Issues Mol. Biol.* 9: 21–40. (2007)