DNA Extraction Control

QUALITY ASSURANCE

The use of real-time PCR has dramatically increased in recent years due to its high sensitivity, accuracy and reliability. These attributes are heavily dependent on template quality and the presence of inhibitory components. Subsequently, these factors impact the robustness of the assay and can also result in false-negative results.

A common practice is to spike a known amount of control DNA into the sample after DNA extraction. 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 real-time PCR (fig. 1). Bioline have developed a DNA Extraction Control (DEC) which more closely mimics the test sample, as compared to spike controls. The genetic material from the test sample and our DEC are simultaneously extracted by common extraction methods with the DEC being as sensitive to inhibition and extraction failure as the test sample.

The results present a clear advantage of implementing our internal control over spiked Internal Control DNA, by more accurately monitoring effectiveness of DNA extraction processes and inhibition within real-time PCR assays.

The DNA Extraction Control (DEC) consists of viable Alpha Select *E. coli* cells of a known concentration, containing plasmid pBR322 with the Internal Control DNA sequence (with no known homology to any organism). These cells are spiked into lysis buffer with the target sample, prior to DNA extraction. Following the extraction, Control Mix (primers and probe) is added to the reaction mix prior to amplification. Signal derived from the Internal Control DNA confirms the success of the extraction step.

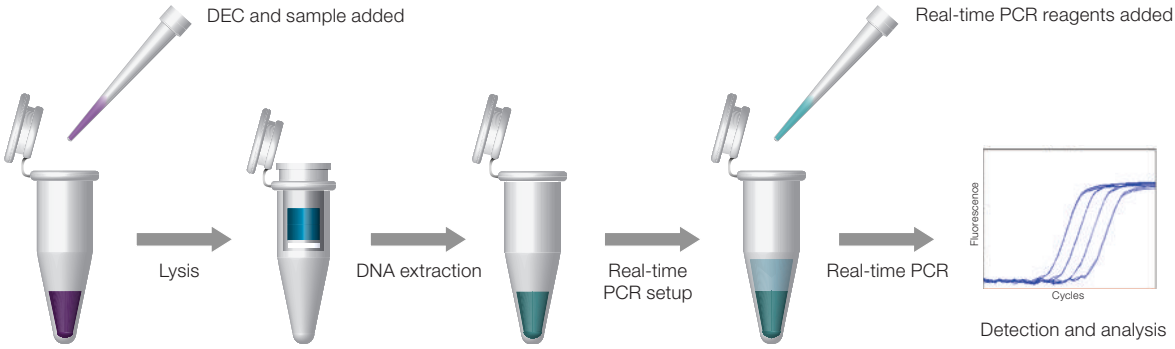

Fig. 1 Overview of the workflow
DEC assesses effects of extraction as well as PCR inhibition throughout the entire workflow.

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 (Fig. 2).

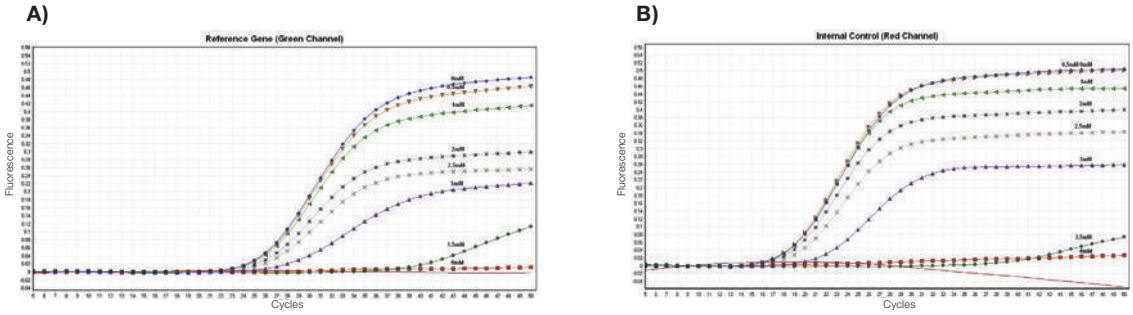

Fig. 2 DEC monitors PCR reaction inhibition
A) A fragment of the beta 2 microglobulin (*B2MG*) gene was amplified from human genomic DNA (green channel) and B) the internal control sequence was amplified from the DEC (red channel). Increasing concentrations of EDTA were included in the reaction (0mM, 1mM, 2mM, 2.5mM, 3mM, 3.5mM and 4mM respectively) to simulate increasing concentrations of an inhibitor. Conditions were 10min at 95°C followed by 50 cycles at 95°C 10s, 60°C 30s. The results illustrate that DEC gives the same pattern of inhibition as with the sample target, showing that inhibition of PCR reaction can be identified using DEC.

IDENTIFY INEFFICIENT DNA EXTRACTION

Bioline ISOLATE Genomic DNA Mini Kit (BIO-52032) was used to extract DNA in parallel from a HEK293 cell line and DNA extraction control (Fig. 3). 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.

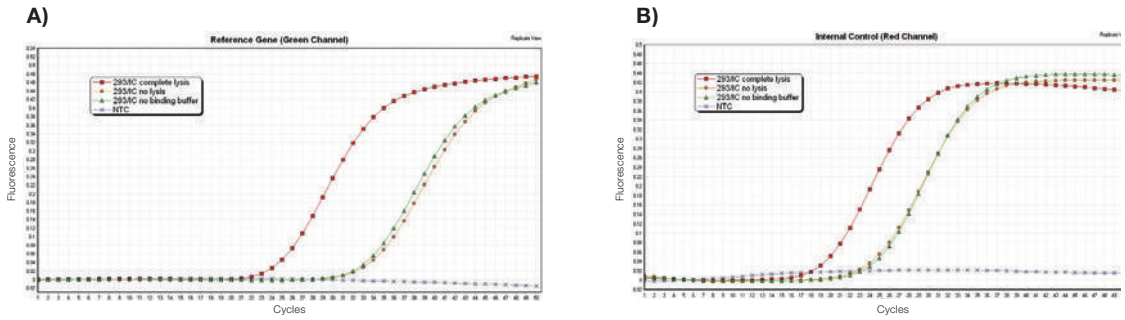

Fig. 3 DEC identifies inefficient DNA extraction
A) A fragment of the beta 2 microglobulin (*B2MG*) gene was amplified from HEK293 cells (green channel) and B) the internal control sequence was amplified from the DEC (red channel). ISOLATE Genomic DNA Kit was used, with the lysis buffer or binding buffer being substituted with PBS to simulate inefficient extraction. Conditions were 10min at 95°C followed by 50 cycles 95°C 10s, 60°C 30s. Complete lysis step (red) and the pattern of inhibition for no lysis (orange) and no binding buffer (green) are the same for the both, illustrating that the DEC can be used to monitor DNA extraction.

DEC VERSUS SPIKED INTERNAL CONTROL DNA

DEC and equivalent amounts of Internal Control DNA were added to a HEK293 cell resuspension. Extraction was carried out with or without lysis buffer in parallel (Fig. 4).

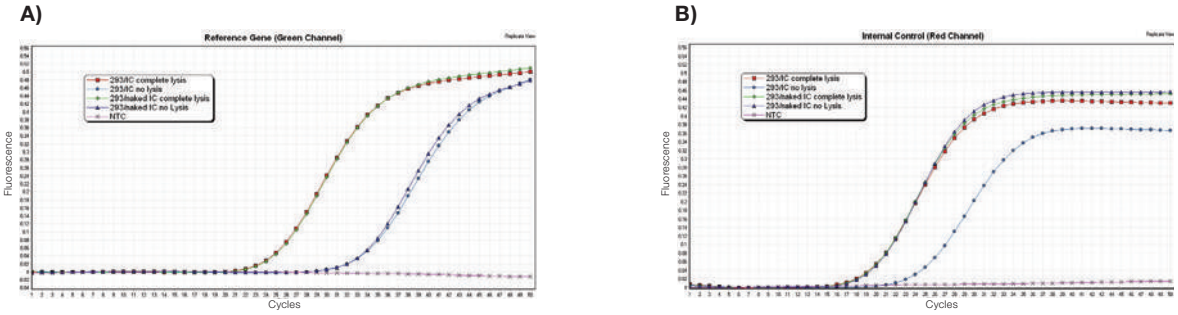

Fig. 4 DEC vs Internal Control DNA
A) Internal Control DNA (green channel) and B) DEC (red channel) were amplified from HEK293 cells. ISOLATE Genomic DNA Kit was used, with the lysis buffer or binding buffer being substituted with PBS to simulate inefficient extraction. Conditions were 10min at 95°C followed by 50 cycles at 95°C 10s, 60°C 30s. Complete lysis step (red) and the pattern of inhibition for no lysis (orange) and no binding buffer (green) are the same for the both, illustrating that the DEC can be used to monitor DNA extraction.

MINIMAL INTERFERENCE WITH MULTIPLE TARGET DETECTION

The internal control sequence has been specifically engineered as a non-homologous sequence. The DEC was used in a singleplex and triplex reaction with two HEK293 housekeeping genes to see if the DEC interferes with the real-time PCR assay (Fig. 5).

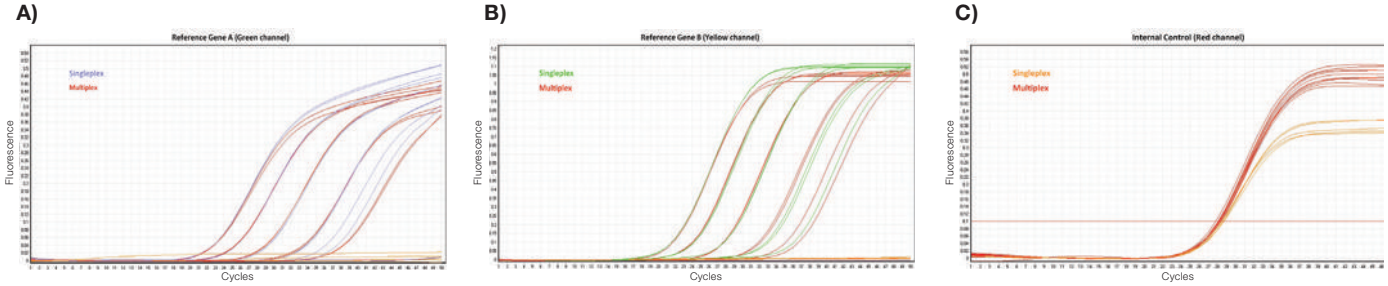

Fig. 5 DEC in multiplex real-time PCR
A) A fragment of the beta 2 microglobulin (*β2MG*) gene and B) A fragment of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene were amplified from HEK293 cells (green channel) and C) the internal control sequence was amplified from the DEC. Conditions were 10min at 95°C followed by 50 cycles 95°C 10s, 60°C 30s. The results illustrate that there is no difference in Cts between singleplexing and multiplexing, so therefore DEC does not interfere with the real-time PCR assay.

SUMMARY

We have demonstrated that the DNA extraction control (DEC), not only monitors the effectiveness of the extraction process but also serves as an indicator of the co-purification of PCR inhibitors in a sample DNA isolation. In monitoring inhibition in real-time PCR, 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 the internal control channel. In contrast, internal control (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 various 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 a universal qualitative control platform for a wide range of different applications in real-time PCR assays.

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