

A novel internal control for monitoring RNA extraction and RT-PCR inhibition in qPCR assays

T. Lin, L. Yang, C. Denver, and L. Lavulo

Bioline Australia, Suite 212A, NIC Building, 4 Cornwallis Street, Australian Technology Park, Eveleigh, NSW 2015

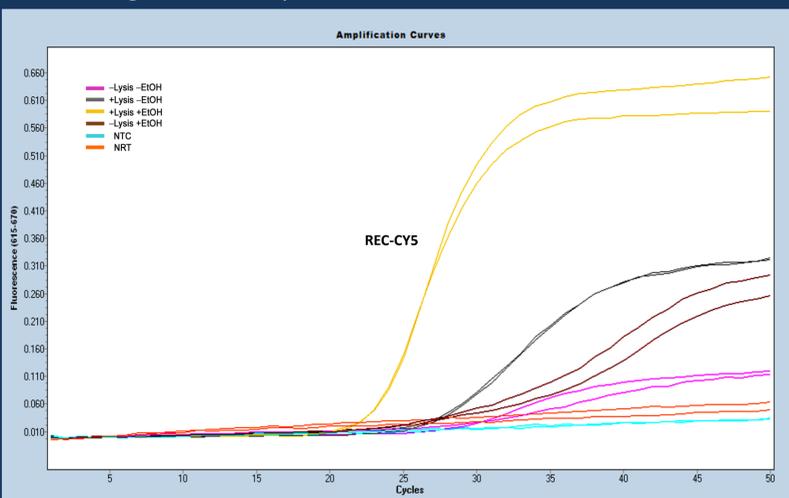
Introduction

Real-Time PCR (qPCR) is a sensitive, rapid, and robust tool that is widely used in research and diagnostic laboratories. At the same time, the output this technique delivers is only as good as the quality of the input material (extracted nucleic acid template). Current monitoring of nucleic acid extraction includes utilising “spiked” DNA controls prior to extraction. However, for an RNA based assay, “spiked” RNA is not suitable due to its instability. We have developed a novel RNA internal control for qRT-PCR. The internal control is a single stranded RNA encapsulated in an artificial cell that requires processing in much the same way a biological sample is processed prior to qRT-PCR. This control is easily incorporated into standard assay procedures by being spiked into the test sample prior to nucleic acid extraction. The genetic material from the test sample and our internal control are simultaneously extracted by common extraction methods in a single extraction. Our results present a clear advantage of implementing our internal control over “spiked” DNA controls by more accurately monitoring the effectiveness of the RNA extraction process and inhibition, within qRT-PCR assays.

Monitoring REC Extraction

Inefficient REC extraction

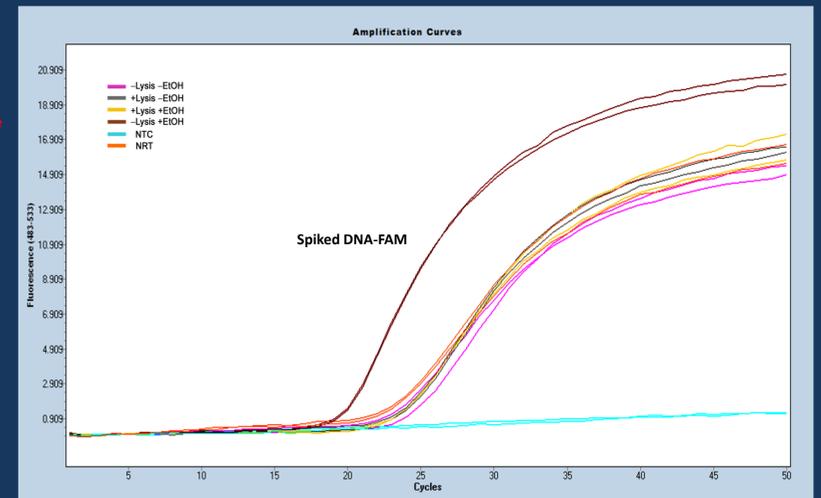
REC was extracted with a Bioline ISOLATE RNA mini kit. Total RNA extraction was carried out +/- lysis buffer or +/- EtOH (critical step in RNA Kit prep.). No DNase digestion was performed.



Only REC demonstrates the effect of extraction quality on the Ct

REC, spiked DNA and extraction control

Spiked DNA control and REC were co-extracted with a Bioline ISOLATE RNA mini kit. No DNase digestion was performed.

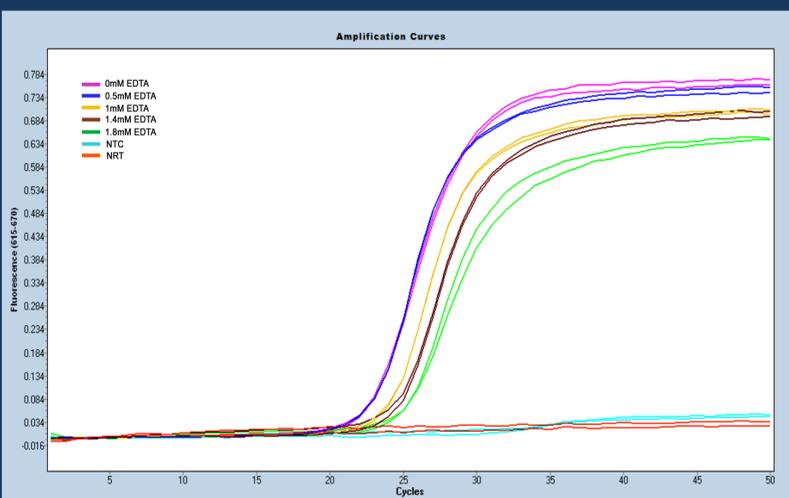


Spiked DNA control will always produce a positive signal

Spiked DNA control has no value as an extraction control

Monitor RT-PCR inhibition

REC samples were extracted with a Bioline ISOLATE RNA mini kit. Different concentrations of EDTA were added prior to elution, as an inhibitory agent to test the monitoring capability of the Internal Control.

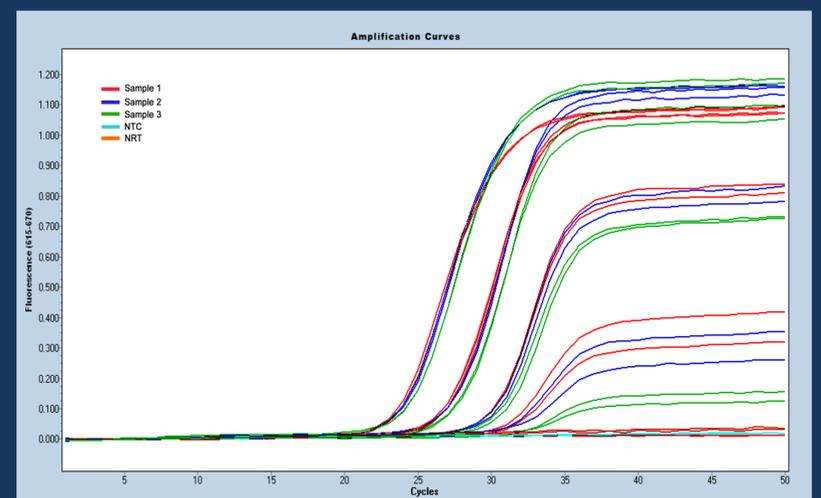


The presence of reverse transcription and PCR inhibitors result in a shift in Ct and decrease in fluorescence

Consistent reproducible results over several fold dilutions of template and number of extractions

REC provides reproducible results

Three REC samples were extracted in parallel with a Bioline ISOLATE RNA mini kit. The multiple extractions were performed and analysed by quantitative reverse transcription-PCR (qRT-PCR) to demonstrate reproducibility over several fold dilutions of template and number of extractions.



Discussion

Monitoring the process of nucleic acid extraction is important for reducing false negative results. This unique internal control, as demonstrated, allows specific monitoring of the extraction and analysis of RNA. The data shows how “spiked” DNA controls used in a RNA assay, are not effective or suitable for the monitoring of ribonucleic acid extraction and analysis. DNA will always provide a positive signal, unlike RNA which is heavily dependent on reverse transcription. We have shown that the RNA Extraction Control (REC) has a linear relationship with the efficiency of the extraction process. Furthermore, REC can also monitor the reverse transcription process. Finally, REC is not only an indicator of the presence of inhibitors in the extracted nucleic acid sample, but also provides reproducible results.

Conclusions

- Monitors extraction efficiency
- Monitors PCR inhibition
- True RNA control
- Easy to incorporate into qRT-PCR assays
- Provides reproducible results