

New! Sensitive qRT-PCR Obtained from the Lysates of 1 – 10 Cells Using the MessageBOOSTER™ cDNA Synthesis Kit for qPCR

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Introduction

The MessageBOOSTER™* cDNA Synthesis Kit for qPCR greatly improves qRT-PCR sensitivity, accuracy, and reproducibility of even low-abundance transcripts from as little as one cell.^{1,2} MessageBOOSTER reactions utilize a linear RNA amplification process that produces large amounts of anti-sense RNA (aRNA or cRNA) from a total RNA sample, and then converts the aRNA to single-stranded cDNA that is ready for qPCR (FIG 1). A MessageBOOSTER reaction preserves both the relative transcript abundance (gene expression profile) of the sample,³ and greatly increases the number of qRT-PCR reactions that can be performed from a very small number of cells.¹

Since the introduction of the MessageBOOSTER Kit, we have frequently been asked if a MessageBOOSTER reaction can be used to produce cDNA directly from cell lysates without the need for first purifying the total cellular RNA. This would be advantageous, because the initial purification of total cellular RNA can result in a diminished RNA yield. In this study, we demonstrate that a MessageBOOSTER reaction efficiently produces cDNA directly from cell lysates using just 1 and 10 cells, and that the cDNA produced enables sensitive qPCR detection of a medium- and low-abundance transcript.

Methods

Cell capture

Trypsinized HeLa cells were centrifuged, washed, and then resuspended in 1X PBS. A cytometer was then used to measure the number of cells/ml. Ten-cell samples were collected by serial dilution—the number of cells captured being verified using an inverted microscope. Single-cell samples were obtained by trapping one HeLa cell in a capillary (Wiretrol® II, Drummond Scientific). The trapped cell was then spun down in a microcentrifuge tube at 550 x g for 2 minutes.

Cell lysis and inhibition of cellular RNases

Cell lysates were produced by freezing the collected cell(s) and then thawing at room temperature. Ten units of

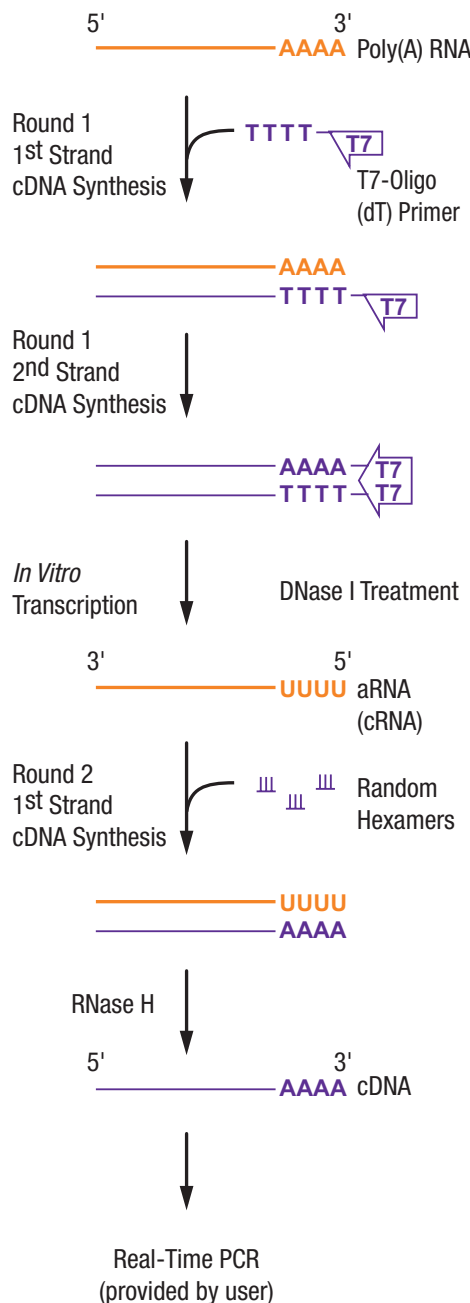


FIG 1. The MessageBOOSTER™ cDNA Synthesis Kit for qPCR procedure. A one-day MessageBOOSTER reaction first amplifies the poly(A) RNA from the total RNA of as little as one cell, and then converts the aRNA produced to cDNA that is ready, without further purification, for qPCR.

ScriptGuard™ RNase Inhibitor (EPICENTRE Biotechnologies) were added to each lysed sample. Since the MessageBOOSTER reaction incorporates a DNase treatment step, there was no need to treat the cell lysates to remove endogenous DNA.

Synthesis of cDNA using the MessageBOOSTER Kit

The MessageBOOSTER cDNA Synthesis reaction was carried out directly in the tubes containing the cell lysate(s) using the standard procedure described in the MessageBOOSTER product protocol. The single-stranded cDNA produced, MessageBOOSTER Lysate-cDNA, was used without further purification for qPCR.

Single-stranded cDNA produced by a MessageBOOSTER reaction using highly purified total RNA, has been shown to greatly improve the qPCR detection sensitivity of low-, medium-, and high-abundance transcripts.¹ As a positive control, cDNA was produced by a MessageBOOSTER reaction from 100 pg of total RNA (about the amount of total RNA in 10 cells) that had been previously purified using a commercial RNA purification kit. This cDNA was designated MessageBOOSTER Purified-cDNA.

Synthesis of cDNA from unamplified RNA

Single-stranded cDNA was also synthesized without the benefit of a MessageBOOSTER reaction using 10 pg and 100 pg of total RNA purified using a commercial RNA purification kit, or directly from 10-cell lysates. Five microliter reactions were set up containing MMLV Reverse Transcriptase (EPICENTRE) and random hexamer primers for 10 minutes at room temperature followed by 60 minutes at 37°C. These cDNAs were treated with RNase H, and designated Unamplified Purified-cDNA, and Unamplified Lysate-cDNA, respectively.

Quantitative PCR (qPCR)

PCR primers and sequence-specific fluorescent probes were synthesized (IDT) for two transcripts, porphobilinogen deaminase (PBGD) and beta-2-microglobulin (B2M). The probes incorporated the following 5'-fluorophores coupled

with 3'-BHQ® dark quenchers: 5'-FAM™/3'-BHQ1 (B2M) and 5'-Texas Red®/3'-BHQ2 (PBGD).

Quantitative PCR was performed in 25 µl reactions containing: 1X FailSafe™+ PROBES Real-Time PCR Optimization PreMix P3 (EPICENTRE), 12.5 pmole of forward and reverse PCR primers, 100 nM of the appropriate sequence-specific fluorescent probe, 1 µl of the appropriate dilution of MessageBOOSTER Lysate-cDNA; MessageBOOSTER Purified-cDNA, Unamplified Purified-cDNA or Unamplified Lysate-cDNA, and 1 U of FailSafe™ Real-Time PCR Enzyme Mix (EPICENTRE). PCR cycling conditions were: 95°C for 2 min, followed by 45 cycles of 94°C for 15 sec, and 60°C for 90 sec.

Results

cDNA produced by a MessageBOOSTER reaction improves qPCR sensitivity

cDNA produced by a MessageBOOSTER™ reaction directly from cell lysates, or from purified total RNA, yields significantly improved qPCR sensitivity when compared to results obtained using unamplified cDNA produced from either purified total RNA (Unamplified Purified-cDNA), or directly from cell lysates (Unamplified Lysate-cDNA), as measured by the reduction in the C_T values (FIG 2). These results confirm earlier findings that

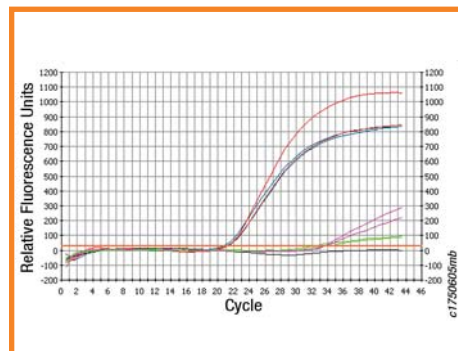


FIG 2. cDNA produced by a MessageBOOSTER™ reaction using purified total RNA, or directly from a HeLa cell lysate, greatly improves qPCR results. qPCR detection of B2M transcript using:

- cDNA produced using the MessageBOOSTER Kit from a 10-cell lysate and diluted 1:50 prior to qPCR.
- cDNA produced using the MessageBOOSTER Kit from 100 pg of purified total RNA and diluted 1:50 prior to qPCR.
- cDNA produced from a 10-cell lysate without benefit of a MessageBOOSTER reaction and diluted 1:50 prior to qPCR.
- cDNA produced from 100 pg of purified total RNA, without benefit of a MessageBOOSTER reaction and diluted 1:50 prior to qPCR.
- No template control.

a MessageBOOSTER reaction greatly improves qRT-PCR results from very small populations of cells.¹

MessageBOOSTER reactions efficiently produce cDNA directly from cell lysates

As shown in FIG 2, cDNA produced by a MessageBOOSTER reaction directly from a 10-cell lysate (MessageBOOSTER Lysate-cDNA), yielded nearly identical qPCR results when compared to cDNA produced by a MessageBOOSTER reaction using 100 pg (about the amount of total RNA in 10 cells) of purified total RNA (MessageBOOSTER Purified-cDNA). The possibility that the qPCR signal obtained using the MessageBOOSTER Lysate-cDNA was from contaminating genomic DNA was ruled out by running a *no reverse transcriptase control* MessageBOOSTER reaction.

Detection of a low-abundance transcript using MessageBOOSTER Lysate-cDNA produced directly from a single-cell lysate

As shown in FIG 3, the cDNA produced directly from the lysate of a single cell using the MessageBOOSTER Kit readily detected the low-abundance PBGD transcript (C_T=28). cDNA produced from 10 pg of purified total RNA, without the benefit of a MessageBOOSTER reaction, failed to detect the same transcript.

Conclusions

A MessageBOOSTER™ reaction greatly improves qRT-PCR detection of low-, medium-, and high-abundance transcripts when using total RNA purified from very small populations of cells.¹ These findings confirm our earlier work and the results recently reported by DeFazio, *et al.*² Additionally, we have now demonstrated that a MessageBOOSTER reaction will efficiently produce cDNA directly from the lysate of as little as one cell for sensitive qPCR detection of even low-abundance transcripts. These findings can significantly reduce the possibility of loss or degradation of a precious RNA sample that can occur during an RNA purification process. This will ultimately translate to reduced labor and handling time, and significantly improved results for those performing qRT-PCR from very small populations of cells.

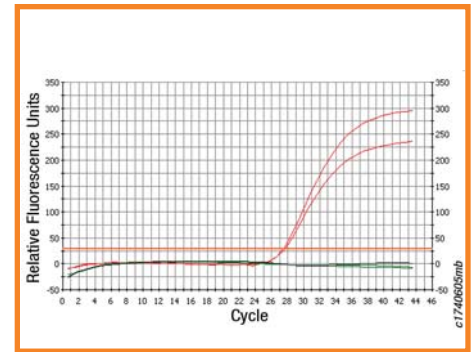


FIG 3. cDNA produced using the MessageBOOSTER™ Kit directly from a single-cell lysate readily detects the low-abundance PBGD transcript. qPCR detection of the low-abundance PBGD transcript using:

- cDNA produced using the MessageBOOSTER Kit from a single-cell lysate.
- cDNA produced from 10 pg of purified total RNA, without benefit of a MessageBOOSTER reaction.
- No template control.

References

1. Grunewald, H. *et al.*, (2006) *EPICENTRE Forum* 13(1), 7.
2. DeFazio, R.A. *et al.*, (2006) *J. Neurosci.* 26(15), 3971.
3. Meis, J.E. (2006) *EPICENTRE Forum* 13(2), 4.

www.EpiBio.com/messagebooster.asp

MessageBOOSTER™ cDNA Synthesis Kit for qPCR

MB060110	10 Reactions
MB060124	24 Reactions

ScriptGuard™ RNase Inhibitor

SRI6325	40 U/µl 2,500 Units
SRI6310K	40 U/µl 10,000 Units

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