New! MessageBOOSTER™ cDNA Synthesis Kit for Real-Time PCR Quantification of Low-Abundance Transcripts from as Little as One Cell

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Introduction

Ideally, gene expression studies by quantitative real-time RT-PCR (qPCR) analysis should be performed using RNA from homogeneous cell populations. Frequently, this means performing qPCR using picogram amounts of total cellular RNA and, in some instances, using total RNA from just a single cell (about 10 pg of total RNA). Detection of even high-abundance transcripts from such small samples is challenging, as evidenced by high C_T values and a lack of consistency between replicates; often low- and medium-abundance transcripts are not detected at all.

EPICENTRE Biotechnologies' new Message*BOOSTER*™† cDNA Synthesis Kit improves the sensitivity, accuracy, and reproducibility of qPCR, even for low-abundance transcripts from very small cell populations (1 to 50 cells or approximately 10 to 500 pg of total RNA). The Message*BOOSTER* Kit accomplishes this by amplifying the Poly(A)-containing mRNA in a total RNA preparation and then converting the amplified RNA to single-stranded sense cDNA that is ready for qPCR. Here we demonstrate the benefits of this new kit for the gene expression analysis of small samples by qPCR.

Methods

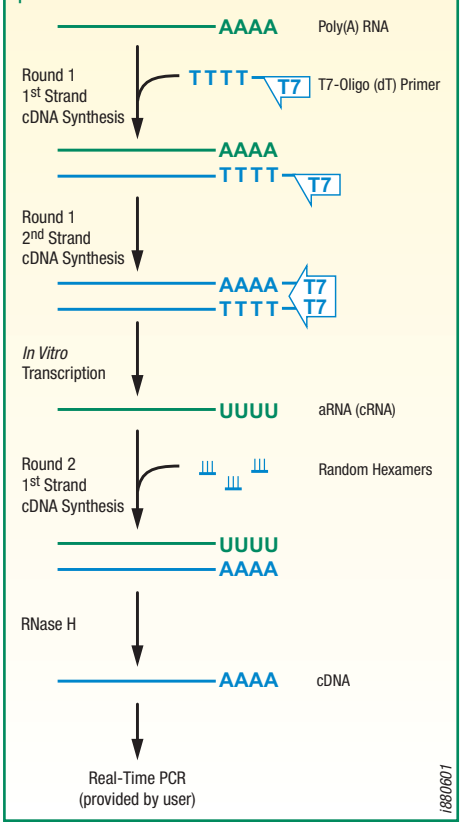
Synthesis of aRNA and single-stranded sense cDNA

An overview of the Message*BOOSTER* Kit RNA amplification and cDNA synthesis process is depicted in the illustration (center column). Briefly, 10 pg of total RNA purified from normal rat kidney (NRK) cells using EPICENTRE's MasterPure™ RNA Purification Kit were reverse transcribed using SuperScript™ III Reverse Transcriptase and an oligo(dT) promoter primer containing a phage T7 RNA Polymerase promoter sequence at its 5′-end [T7-Oligo(dT) Primer] for 30 minutes at 50ºC. The cDNA:RNA hybrid produced was digested by RNase H and second-strand cDNA was synthesized at 65ºC for 10 minutes. Antisense RNA (aRNA, also called cRNA) was then produced by *in vitro* transcription of the resulting double-stranded cDNA containing the T7 transcription promoter at 42ºC for 4 hours. The *in vitro* transcription reaction was treated with DNase I to remove any left over DNA template and the aRNA was purified by spin column chromatography. The purified aRNA was reverse transcribed using MMLV Reverse Transcriptase and random hexamer primers for 10 minutes at room temperature followed by 60 minutes at 37ºC. The resulting single-stranded sense cDNA was treated with RNase H to remove left over RNA template and used immediately for qPCR or stored at -20ºC. The final cDNA synthesis reaction volume was approximately 7 µl.

EPICENTRE's Message*BOOSTER*™ cDNA Synthesis Kit for qPCR uses a modified and improved "Eberwine-type" linear aRNA amplification process that enables sensitive qPCR of even low-abundance transcripts from as little as a single cell. The Message*BOOSTER* Kit produces cDNA starting from total RNA from 1-50 cells (10 pg to 500 pg total RNA) as follows:

1) Poly(A) RNA in the total RNA sample is converted to first-strand cDNA using reverse transcriptase and a T7-Oligo(dT) promoter primer.
2) The cDNA is converted to double-stranded cDNA.
3) aRNA is generated from the cDNA by *in vitro* transcription using EPICENTRE's high yield AmpliScribe™ T7 *in vitro* transcription technology.

After purification, the aRNA is converted to single-stranded sense cDNA which can be used for qPCR including multiplex qPCR without purification.



Synthesis of cDNA from unamplified RNA

Ten picograms of unamplified total NRK RNA were converted to cDNA using MMLV Reverse Transcriptase and random hexamer primers in a 5 µl reaction for 10 minutes at room temperature followed by 60 minutes at 37ºC. The cDNA was treated with RNase H, and then diluted to a final volume of 7 µl with double distilled H_2O to enable easy qPCR comparisons with cDNA produced using the Message*BOOSTER* kit.

Amount of cDNA used for PCR

One microliter of cDNA produced from the unamplified RNA or 1 µl of cDNA produced by the Message*BOOSTER* reaction was added to the appropriate 25 µl PCR reactions.

qPCR

PCR primers and sequence-specific probes for three transcripts, Porphobilinogen Deaminase (PBGD; a low-abundance transcript), pre-B-cell leukemia transcription factor 2 (PBX2; a low- to-medium-abundance transcript), and beta-2-microglobulin (B2M; a medium- to high-abundance transcript) were synthesized (IDT). These probes incorporated the following 5′-fluorophores and 3′-dark quenchers: 5′-FAM/3′-BHQ1 (B2M), 5′-Cy5/3′- BHQ2 (PBX2) and 5′-Texas Red/3′-BHQ2 (PBGD).

PCR was performed in triplicate 25 µl reactions using EPICENTRE's FailSafe™ PROBES Real-Time PCR PreMix 3, 12.5 pmoles of forward and reverse PCR primers and 100 nmoles of probe. All qPCR was performed using a Bio-Rad iCycler iQ® thermocycler with all

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Message*Booster*™ Kits—Cont'd from Page 7

reactions being run at 95ºC for 2 minutes followed by 50 cycles of 95ºC for 10 seconds and 60ºC for 1 minute.

Multiplex qPCR

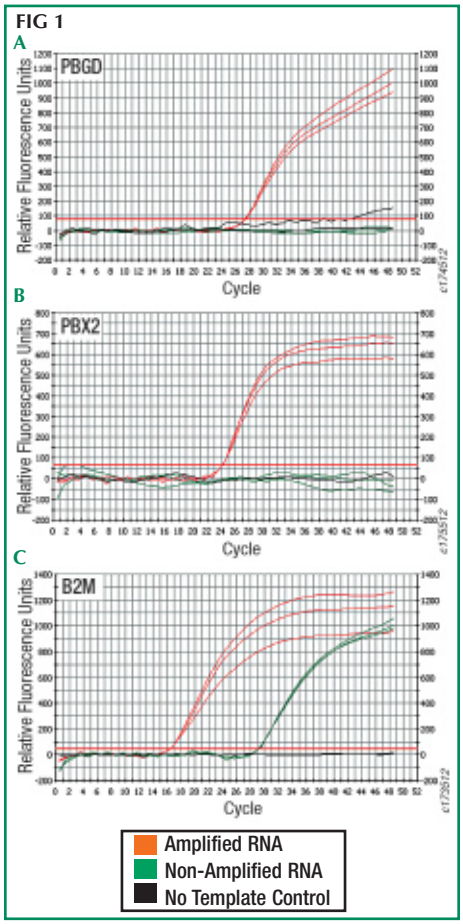
Multiplex qPCR of both B2M and β-actin target sequences in a single reaction tube were performed in triplicates using probes labeled with 5′-FAM/3′-BHQ1 and 5′-HEX/3′-BHQ1 for B2M and β-actin, respectively. The same reagents and cycling conditions described above for the PCR assays were used for the multiplex qPCR.

Results

FIG 1 (A, B and C) shows the quantification graphs obtained for the PCR reactions. Each graph compares the detection of transcript with and without poly(A) RNA amplification using the Message*BOOSTER*™ cDNA Synthesis Kit. In all three cases the cDNA produced using the Message*BOOSTER* kit resulted in significantly improved detection sensitivity as determined by a reduction in the C_T values compared to cDNA produced from unamplified RNA. The low-abundance transcript (PBGD; FIG 1A) and the low- to medium-abundance transcript (PBX2; FIG 1B), were not detected in cDNA prepared from unamplified mRNA but were readily detected using cDNA from amplified mRNA using the Message*BOOSTER* Kit. When 500 pg of total NRK RNA were reverse-transcribed into cDNA using oligo(dT) or random hexamer primers, the low- to medium-abundance transcripts PBGD and PBX2 were not detectable by qPCR. However, for the high-abundance transcript B2M, a slight increase of one C_T cycle was observed during qPCR when using cDNA synthesized from oligo(dT) primers versus random hexamer primers (data not shown). qPCR reactions utilizing SYBR® Green I dye detection chemistry provided comparable results to those using sequence-specific probes (data not shown).

FIG 2 demonstrates successful multiplex qPCR of the B2M and β-actin target sequences using cDNA produced from 10 pg of input total RNA (equivalent to the total RNA from a single cell) using the Message*BOOSTER* cDNA synthesis kit.

The number of qPCR reactions that can be obtained from one Message*BOOSTER* reaction was found to be dependent on two factors:



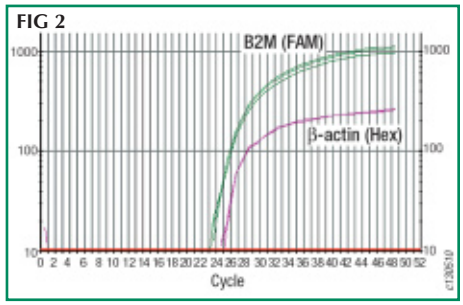


FIG 1. cDNA produced from 10 pg of total NRK RNA using the Message*BOOSTER*™ cDNA Synthesis Kit for qPCR significantly improves the sensitivity (lowers the C_T) of detecting low-, medium- and high-abundance transcripts compared to cDNA produced from 10 pg of unamplified RNA. A. Low-abundance transcript PBGD. **B**. Low- to medium-abundance transcript PBX2. **C**. Medium to high-abundance transcript B2M.

FIG 2. Multiplex qPCR of B2M and β-actin target sequences using cDNA produced from 10 pg of total RNA using the Message*BOOSTER*™ cDNA Synthesis Kit for qPCR. Multiplex qPCR was performed using EPICENTRE's FailSafe™ PROBES Real-Time PCR PreMix 3.

Amount of Total RNA in a Message*BOOSTER*™ Reaction	Number of Real-Time qPCR Reactions That Can Be Performed	
	Low- to Medium-Abundance Transcripts*	Medium- to High-Abundance Transcripts*
10 pg (~1 cell)	≥ 10	≥ 100
100 pg (~10 cells)	≥ 100	≥ 1,000
500 pg (~50 cells)	≥ 500-1,000	≥ 5,000-10,000

*Low-Abundance Transcripts = 1-1,000 copies per cell
Medium-Abundance Transcripts = 1,000-10,000 copies per cell
High-Abundance Transcripts = 10,000-100,000 copies per cell

Table 1. The number of qPCR reactions that can be performed using cDNA produced by a Message*BOOSTER*™ reaction is dependent on the amount of input total RNA and the abundance of the transcript(s) of interest.

1. The amount of total RNA added to the Message*BOOSTER* reaction.

2. The abundance of the transcript(s) of interest.

Table 1 summarizes our estimate of the number of qPCR reactions that can be obtained using cDNA produced from the Message*BOOSTER* reactions containing input total RNA from ~1, 10, and 50 NRK cells.

Conclusions

The Message*BOOSTER* cDNA Synthesis Kit produces abundant cDNA for qPCR (including multiplex qPCR) for sensitive, accurate and consistent detection of low- and medium-abundance transcripts, even from as little as 1 cell.

www.EpiBio.com/messagebooster.asp

Message*BOOSTER*™ cDNA Synthesis Kit for qPCR

MB060110	10 Reactions
MB060124	24 Reactions

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