EPICENTRE Forum

Tools and Techniques for Genomics and RNA Research

Volume 13-2

Featuring:
Products for In Vitro
Transcription and RNA Studies

Message*BOOSTER*™ cDNA Synthesis Kit for qPCR Preserves Transcript Abundance

Produce the Highest Possible Yields of 2'-Fluoro-Substituted RNA Aptamers

The ArrayPure™ Nano-scale RNA Purification Kit Produces Superior Quality and Yields of RNA

Get the Highest Yield of 5'-Capped RNA from an *In Vitro* Transcription Reaction

Cover Photo: A red Routemaster Bus in use around the streets of Central London
Photograph by: Simon T. M. Allard, EPICENTRE Biotechnologies, Madison, WI

With the 1992 introduction of the AmpliScribe™ T7 High Yield Transcription Kit, the first commercially available kit to utilize a unique high yield *in vitro* transcription technology, EPICENTRE Biotechnologies has been committed to providing the highest quality and broadest range of products for *in vitro* transcription and RNA studies. Since that time, our AmpliScribe Kits have become the standard *in vitro* transcription kit for many labs because of exceptional RNA yields, RNA quality and product value.

Today, our commitment to innovative, high quality, and high value *in vitro* transcription products continues with the recent introduction of the TargetAmp™ aRNA Amplification Kits, and the Message*BOOSTER*™ cDNA Synthesis Kit for qPCR.

This issue of our Forum newsletter is focused on kits, enzymes and reagents for RNA-based applications. Please take a few minutes to read about the broad range and high quality products that EPICENTRE has to offer. Also, visit our website (www.EpiBio.com) for additional products that are currently available, and for news about new and exciting products that we will be introducing in the future.

IN THIS ISSUE

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COMPLIMENTARY

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On the Cover:

EPICENTRE thanks editor Simon Allard for the use of his photograph of London. Taken in the summer of 2005, it shows one of the last red Routemaster Buses in use around the streets of Central London. The classic red double deck bus, officially named the Routemaster, is known around the world as one of the most enduring and best recognized symbols of London. Developed between 1947 and 1956 by a team led by Douglas Scott (1913-90) to replace the trolleybuses, the Routemaster was introduced in the 1950's, with the last new one taking to the roads in 1968. Sadly, Transport for London made the decision to remove all the Routemasters from the capital's streets by the end of 2005. However, two "heritage" routes have been introduced to keep the Routemaster Bus alive in London.

Hands on the Cover:

Judith E. Meis, EPICENTRE Biotechnologies Senior Application Scientist.

The New Message*BOOSTER*™ cDNA Synthesis Kit for qPCR Preserves Transcript Abundance

Judith E. Meis, EPICENTRE Biotechnologies

. . . it is clear that the Message*BOOSTER* reaction faithfully preserves the relative transcript level in a sample. Thus, in conjunction with previously reported data,[1] we have shown that the Message*BOOSTER* kit is ideal for linear amplification of poly(A) RNA from small samples, enabling accurate, sensitive, and reproducible qRT-PCR of low-abundance transcripts.

Introduction

In a previous report, we showed that EPICENTRE Biotechnologies' new Message*BOOSTER*™* cDNA Synthesis Kit for qPCR enables sensitive and reproducible real-time quantitative RT-PCR (qRT-PCR) detection of even low-abundance transcripts from as little as 1 cell.[1] A Message*BOOSTER* reaction amplifies the poly(A) RNA (mRNA) in a total RNA preparation from 1 to 50 cells (approximately 10 pg to 500 pg of total RNA), and then converts the amplified RNA to single-stranded sense cDNA (FIG 1). The cDNA produced by the Message*BOOSTER* Kit can be used for real-time quantitative PCR (qPCR) utilizing SYBR® Green I dye or fluorescent probes without further purification. The number of qPCR reactions that can be performed from cDNA produced in a Message*BOOSTER* reaction is dependent on the amount of total RNA used in the Message*BOOSTER* reaction, and the abundance of the transcript(s) of interest.

In this report, we show that the Message*BOOSTER* cDNA Synthesis Kit maintains the relative transcript abundance of the original sample. This was demonstrated by comparing the expression levels of 20 mRNA transcripts in Universal Human Reference RNA (Stratagene) and in adult skeletal muscle RNA before and after a Message*BOOSTER* reaction.

Methods

Poly(A) RNA amplification and cDNA synthesis using the MessageBOOSTER Kit

The poly(A) RNA (mRNA) in 500 pg of total Universal Human Reference RNA and 500 pg of adult skeletal muscle RNA was amplified and then converted to cDNA according to the Message*BOOSTER* protocol. Briefly, cDNA was synthesized using SuperScript™ III Reverse Transcriptase (Invitrogen Corp.) and an oligo(dT) primer containing a bacteriophage T7 RNA Polymerase promoter sequence at its 5'-end. The resulting cDNA:RNA hybrid was digested by RNase H and second-strand cDNA was synthesized. The double-stranded cDNA produced was used as template in an *in vitro* transcription reaction to generate high yields of aRNA. The *in vitro* transcription reaction was then treated with DNase I to remove the cDNA template, and the aRNA was purified by spin column chromatography. The purified aRNA was reverse transcribed using EPICENTRE's MMLV Reverse Transcriptase Plus (MMLV-RT Plus, provided in the kit) and random hexamer primers. After a brief RNase H treatment to remove the remaining aRNA, the resulting single-stranded sense cDNA (referred to here as "Message*BOOSTER* cDNA") was used directly in qPCR.

"Unamplified" cDNA was produced from both Universal Human Reference RNA and adult skeletal muscle RNA using MMLV-RT Plus and oligo(dT)$_{20}$ primers.

Real-time quantitative PCR

The abundance of beta-2 microglobulin mRNA (B2M) in both the Message*BOOSTER* cDNA sample and the unamplified sample was used for expression level normalization. The cDNA was diluted so that the threshold cycle (C_T) value for each B2M amplification was nearly identical. qPCR reactions were performed using EPICENTRE's TAQurate™ GREEN Real-Time PCR† MasterMix with optimized primer concentrations and cycling conditions in a BioRad iCycler iQ® Real-Time PCR Detection System.

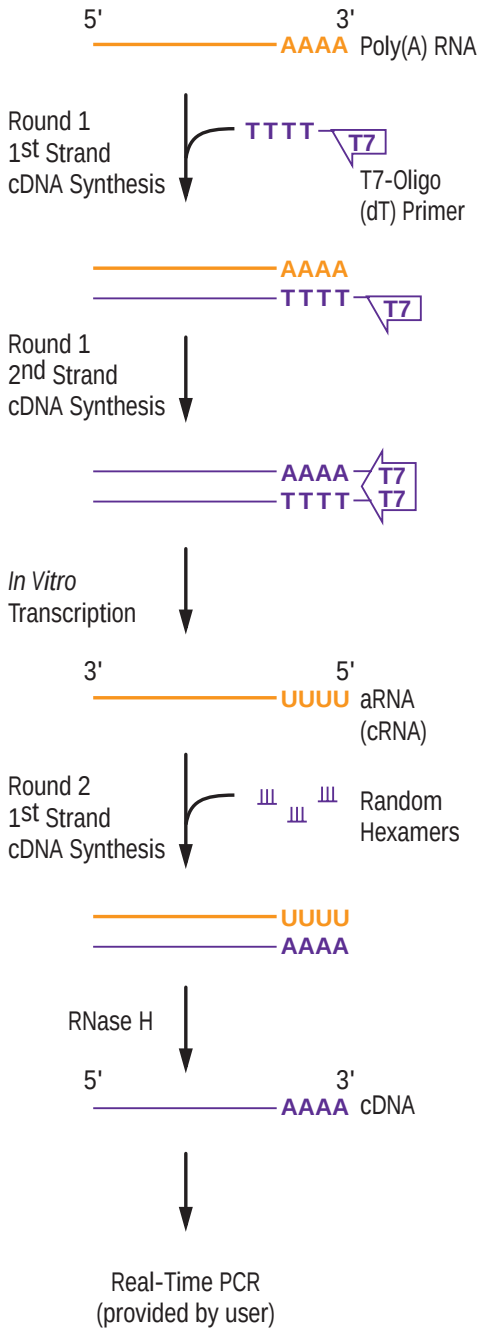

FIG 1. The Message*BOOSTER*™ cDNA Synthesis Kit for qPCR procedure. A one-day Message*BOOSTER* reaction first amplifies the poly(A) RNA from the total RNA of 1 to 50 cells and then converts the aRNA produced to cDNA that is ready, without further purification, for qPCR.

Three data points were averaged and normalized for each of the 20 transcripts tested. Simultaneous analysis on these samples was performed using control primers and test primers; non-template negative controls were included.

Results

The normalized difference in expression levels (calculated as the difference in C_T values between test amplifications and normalizer amplifications (ΔC_T)) between Universal Human Reference RNA and adult skeletal muscle RNA ($\Delta\Delta C_T$) for the 20 transcripts, tested both before and after the Message*BOOSTER*™ reaction, were plotted as a bar graph (FIG 2) and as a scatter plot (FIG 3). The high correlation coefficient of R^2 = 0.997 calculated from the scatter plot of FIG 3, demonstrates that the cDNA produced by a Message*BOOSTER* reaction retains the relative transcript abundance in the sample.

Conclusion

Based on the correlation coefficient of R^2=0.997 obtained with the 20 test transcripts, it is clear that the Message*BOOSTER* reaction faithfully preserves the relative transcript level in a sample. Thus, in conjunction with previously reported data,[1] we have shown that the Message*BOOSTER* kit is ideal for linear amplification of poly(A) RNA from small samples, enabling accurate, sensitive, and reproducible qRT-PCR of low-abundance transcripts.

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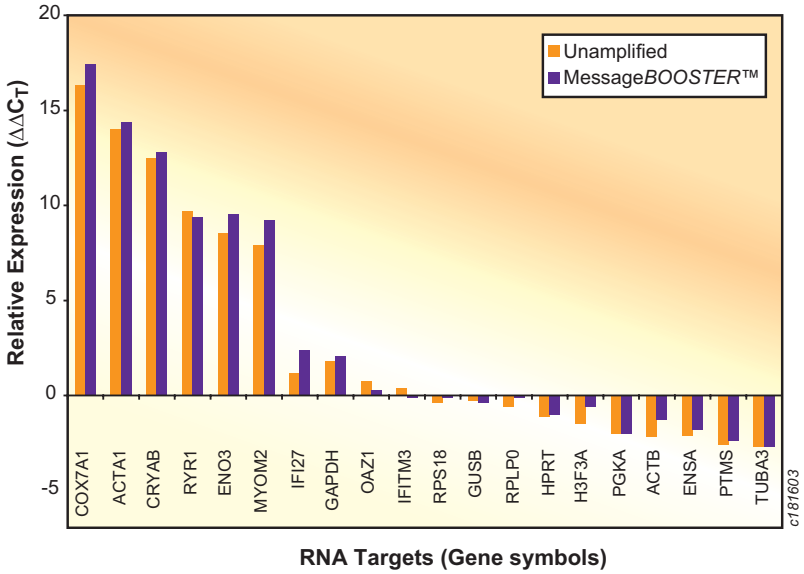

FIG 2. The normalized difference in expression levels between Universal Human Reference RNA and adult skeletal muscle RNA ($\Delta\Delta C_T$) for the 20 transcripts tested before and after the Message*BOOSTER*™ reaction. The differences in expression levels between RNA sample types are represented as the normalized ΔC_T in qPCR cycles ($\Delta\Delta C_T$).

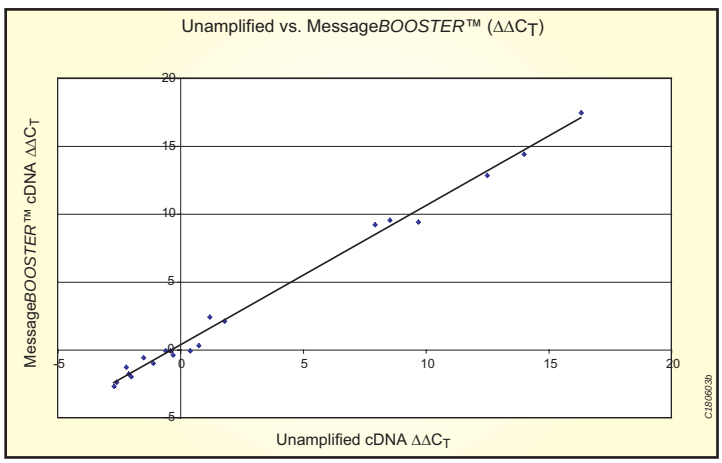

FIG 3. Scatter plot of the data from FIG 2. The differences in expression levels between RNA sample types are represented as the normalized ΔC_T in qPCR cycles ($\Delta\Delta C_T$). The differences in RNA expression levels detected in the Message*BOOSTER*™ cDNA were very consistent with the differences in expression with cDNA produced directly from total cellular RNA without amplification (R^2=0.997).

See *Product Data Sheet* on Page 10 and *Ask Frank* on Page 17 For More Information on The Message*BOOSTER*™ cDNA Synthesis Kit For qPCR

info

Produce High Yields of a High Affinity 2'-F-RNA Aptamer Using the DuraScribe® T7 Transcription Kit

Judith E. Meis, EPICENTRE Biotechnologies

Introduction

Current proteomics initiatives are focused on the production of high affinity ligands or probes that specifically target proteins. Particularly versatile probes with immense potential for use as affinity molecules are aptamers.[1] Aptamers are short single-stranded nucleic acid molecules (<100 bases) that have been selected from random pools based on their ability to bind other molecules. These high affinity molecules bind to proteins and allow detection of bound proteins in microarrays, or capture protein complexes for functional identification.[2] They can serve as probes or therapeutics by altering biological activity and inhibiting critical interactions by blocking access to active sites and interaction surfaces.[1] Traditionally, antibodies serve this function, but difficulties in selecting and producing antibodies in a high-throughput manner have sent researchers looking for alternatives. The most promising alternatives are aptamers, which are beginning to emerge as a class of molecules that rival antibodies in both therapeutic and diagnostic applications.[3]

Modification of pyrimidine nucleotides in RNA by substitution with fluoro (F) functional groups at the 2' position is sufficient to protect an RNA sequence from degradation by nucleases.[4] It has been shown that 2'-F-RNA ligands have high affinities (0.3-3 pM), bioactivities (K_i approximately 34 pM), and have extreme thermostabilities and specificities.[2]

Here we demonstrate the high yield synthesis of a biologically active 2'-F-RNA aptamer for streptavidin using the DuraScribe®* T7 Transcription Kit and a sequence selected and characterized by Tahiri-Alaoui *et al*.[5] The DuraScribe T7 RNA Polymerase provided in the kit, recognizes the same T7 transcription promoters as standard T7 RNA Polymerase, but is about 100-fold more active in the incorporation of 2'-fluorine-pyrimidines.[6] Using this mutant T7 RNA Polymerase in the DuraScribe T7 Enzyme Mix, which is formulated to utilize very high concentrations of nucleotides, produces the highest possible yields of 2'-fluoro-substituted RNA or DuraScript® RNA.

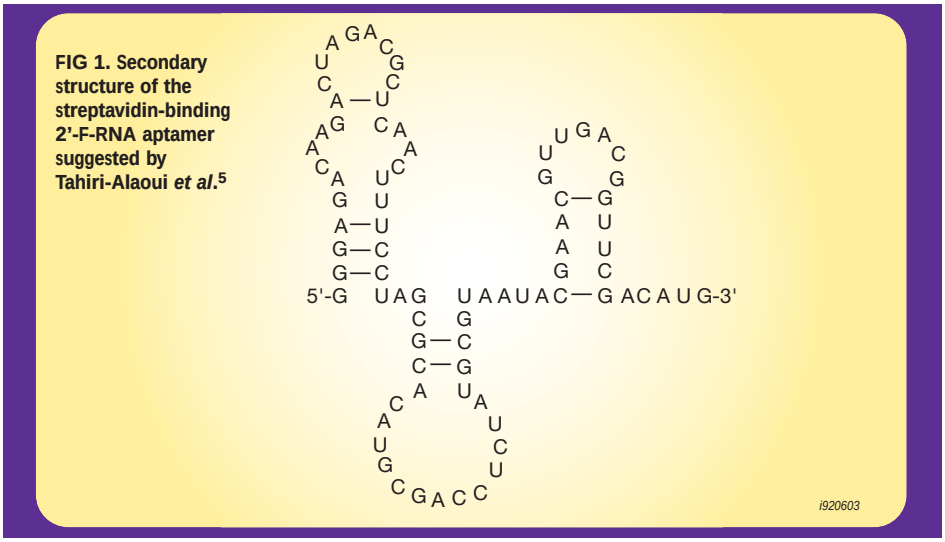

FIG 1. Secondary structure of the streptavidin-binding 2'-F-RNA aptamer suggested by Tahiri-Alaoui *et al*.[5]

Methods

Transcription template construction

The double-stranded transcription template was constructed by the overlap and extension of two oligodeoxynucleotides. The oligo containing the T7 RNA polymerase promoter sequence is 5'-TAA TAC GAC TCA CTA TA-3'. The streptavidin-aptamer oligonucleotide overlaps the T7 promoter oligonucleotide by 8 bases (underlined) 5'-CAT GTC GAA CCG TCA ACG TTC GTA TTA CGC ATA GAG GTC GCA TGT GCG CTA GGA AAG TTG AGC GTC TAG TCT TGT CTC CCT ATA GTG A-3'. It was designed to produce domains I and II of the streptavidin-specific aptamer selected by Tahiri-Alaoui *et al*.[5] A mutant, non-binding version of the aptamer was produced from a similar oligonucleotide containing a single base change, A to G (indicated in bold red) in the above sequence. An excess of T7 promoter oligo (75 picomoles) was annealed to 50 picomoles of streptavidin-aptamer primer in 5 mM Tris-HCl (pH 8.0) and 5 mM NaCl at 75°C for 2 minutes and cooled to room temperature. The overlapping single-stranded oligos were extended to form a blunt double-stranded transcription template with 20 units of Exo-Minus Klenow DNA Polymerase (EPICENTRE Biotechnologies) using standard conditions. The product was cloned into the vector pUC19, then plasmids were prepared, purified, and linearized with *Acc*65 I.

In vitro *transcription*

One microgram of linearized template was used in a standard 20 µl DuraScribe T7 Transcription reaction where the canonical CTP and UTP were replaced with 2'-F-dCTP and 2'-F-dUTP, respectively. Reactions were incubated at 40°C for 4 hours. The DuraScript RNA was precipitated and spin column purified, and yields were determined by spectrophotometry.

Gel mobility shift assays

The DuraScript RNA streptavidin-aptamer and the mutant non-binding aptamer were dephosphorylated, then end-labeled using T4 Polynucleotide Kinase (EPICENTRE) and γ-^{32}P-ATP under standard conditions. Each aptamer probe (10,000 cpm Cerenkov) was incubated in a binding reaction with 20 mM HEPES-NaOH, 100 mM NaCl, 50 mM KCl, 10 mM $MgCl_2$, 1 µg tRNA, and increasing amounts of streptavidin for 30 minutes at room temperature. Bound and free aptamers were then resolved on an 8% polyacrylamide native gel; the gel was then dried and exposed to film.

Results

Efficient in vitro *transcription of 2'-F-RNA aptamers with DuraScribe T7 Transcription Kit*

A double-stranded DNA transcription template containing the T7 promoter sequence and 80 bases of a streptavidin-specific aptamer sequence was produced. The product was cloned,

purified, and restricted to produce a linear transcription template 2.8 kb in length, which produces a run-off transcript of 86 bases (80 bases are aptamer sequence and 6 bases at the 3'-end are vector sequence). DuraScribe® T7 *in vitro* transcription reactions containing 1 µg (0.54 pmoles) of linear template produced 12.9 µg (~ 460 pmoles) of DuraScript® RNA wild-type streptavidin-aptamer, and 15.9 µg (~ 560 pmoles) of the mutant aptamer, which does not bind streptavidin. FIG 1 shows the secondary structure of the DuraScript RNA aptamer, with binding domains I and II of the previously described streptavidin-aptamer.[5] The *in vitro* transcription reactions resulted in specific transcripts of the expected size as determined by denaturing polyacrylamide gel electrophoresis (FIG 2).

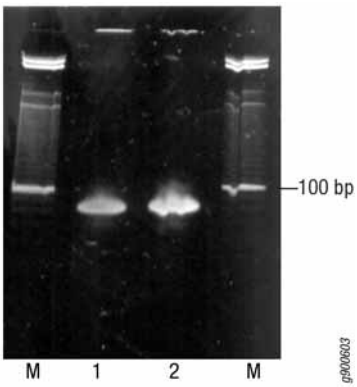

FIG 2. Uniform, full-length aptamer transcripts are produced with the DuraScribe® T7 Transcription Kit. DuraScript® streptavidin-aptamer (**Lane 1**) and mutant DuraScript aptamer (**Lane 2**) were analyzed by denaturing polyacrylamide gel electrophoresis; **Lane M**, 10 bp DNA ladder.

Specific binding of DuraScript RNA aptamer to streptavidin

A native gel mobility shift assay was used to detect the specific binding of the DuraScript aptamer to increasing amounts of streptavidin (FIG 3). The ^{32}P-labeled DuraScript RNA aptamer is smaller and therefore moves further in the gel when no streptavidin is present ("free" aptamer), but forms a slower migrating complex in the presence of 5 - 20 nM streptavidin ("bound" aptamer). No aptamer-streptavidin complex was formed when a mutant aptamer, containing a single-base change, was used in a similar binding reaction (FIG 4). The specificity of the binding was confirmed by adding excess wild-type unlabeled-streptavidin aptamer to the binding reaction. The unlabeled aptamer successfully competed with the labeled aptamer for the streptavidin, resulting in the loss of the bound complex (FIG 4).

FIG 3.

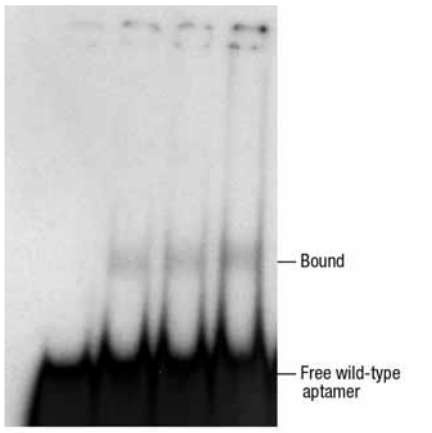

FIG 4.

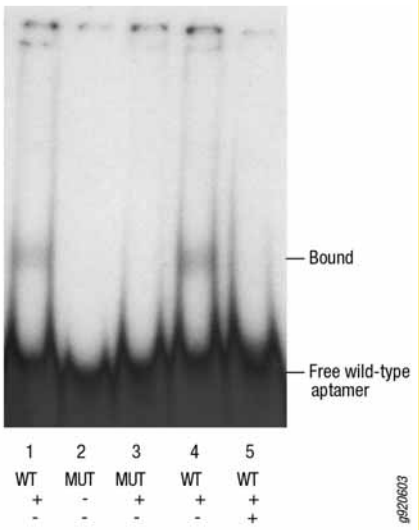

FIG 3. DuraScript® RNA aptamer complexes with streptavidin in a native gel mobility shift assay. Increasing amounts of streptavidin were incubated with 10,000 cpm of ^{32}P-labeled DuraScript 2'-F-RNA aptamer. The free aptamer migrates faster in the native 8% polyacrylamide gel than the protein-bound aptamer seen with 5 to 20 nM streptavidin in the binding reaction.

FIG 4. The DuraScript® 2'-F-RNA aptamer binds with high specificity to streptavidin. A native gel mobility shift assay for streptavidin binding was performed using both the DuraScript RNA aptamer and an aptamer containing a single-base mutation which disrupts binding, or using excess unlabeled-DuraScript RNA aptamer to effectively compete for the streptavidin binding activity. **Lane 1**, wild-type aptamer plus 20 nM streptavidin; **Lane 2**, free mutant aptamer; **Lane 3**, mutant aptamer plus 20 nM streptavidin; **Lane 4**, wild-type aptamer plus 20 nM streptavidin; and **Lane 5**, wild-type aptamer plus 20 nM streptavidin and excess unlabeled wild-type aptamer.

Conclusion

Unlike antibodies or canonical DNA or RNA aptamers, DuraScript RNA aptamers are resistant to both ribonuclease and deoxyribonuclease degradation,[7,8] making them ideal for both therapeutic and diagnostic applications. High yields of DuraScript RNA aptamer can be produced quickly and affordably by *in vitro* transcription with the DuraScribe T7 RNA Polymerase, which efficiently accepts non-canonical nucleotides as substrates.

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Control the Length of the 3'-Poly(A)-tail Added to Your RNA Using the A-Plus™ Poly(A) Polymerase Tailing Kit

Ron Meis, EPICENTRE Biotechnologies

EPICENTRE Biotechnologies' new A-Plus™ Poly(A) Polymerase Tailing Kit enables rapid, efficient, and controlled addition of a poly(A)-tail to the 3'-end of any RNA *in vitro*. The presence of a poly(A)-tail at the 3'-end of an RNA molecule can have an important and positive impact on studies requiring RNA including:

- Increased stability and enhanced translation after transfection or microinjection into eukaryotic cells.[1,2,3]
- Providing a priming site for synthesis of first-strand cDNA using a primer with poly(dT) on its 3'-end portion.
- Cloning of DNA encoding an RNA molecule or a mixture of RNA molecules of unknown or multiple sequences by adding a poly(A)-tail that can anneal to a T-tailed vector.
- 3'-End-labeling of RNA with radioactive ATP.[4]
- Quantifying mRNA.[5]

The A-Plus Poly(A) Polymerase uses ATP as a substrate for template-independent addition of adenosine monophosphate to the 3'-hydroxyl termini of RNA molecules.[6] In this report, we demonstrate that the length of the poly(A)-tail added to the 3'-end of an *in vitro* transcribed RNA can be easily controlled using the A-Plus Poly(A) Polymerase.

Methods and Results

In vitro *transcription and purification of a 5'-capped RNA template*

A 1760-base, 5'-capped RNA analog (m^7G[5']ppp[5']G) transcript was produced using a standard 20 µl AmpliCap-MAX™ T7 High Yield Message Maker Kit reaction (EPICENTRE; see p. 20) from a linearized DNA template. The completed AmpliCap-MAX reaction was treated with 1 U RNase-Free DNase I (EPICENTRE) to remove the DNA template, and the 5'-capped RNA was purified by addition of 20 µl 5M ammonium acetate followed by incubation on ice for 15 minutes and centrifugation at 10,000 x g for 15 minutes. The ammonium acetate selectively precipitates RNA while leaving most of the DNA, protein and unincorporated NTPs in the supernatant. The resulting pellet containing the capped-RNA transcript was washed with cold 70% ethanol, dried, and resuspended in 40 µl of Sterile RNase-Free Water (supplied). The yield of the RNA was measured at A_{260} and the quality of the 5'-capped RNA was confirmed by 1% denaturing agarose gel electrophoresis.

Poly(A)-tailing of RNA using the A-Plus Poly(A) Polymerase Kit

The standard 100 µl A-Plus Poly(A) Polymerase reaction containing 60 µg of the 5'-capped RNA, 1X Reaction Buffer (provided in the A-Plus Kit), 1 mM ATP and eight units of A-Plus Poly(A) Polymerase was incubated at 37°C. One microliter aliquots of the reaction were removed at 0, 10, 30 and 60 minutes, and 0.1 µg of RNA from each time point was loaded and run on a 1% denaturing agarose gel with RNA markers, followed by gel staining with ethidium bromide.

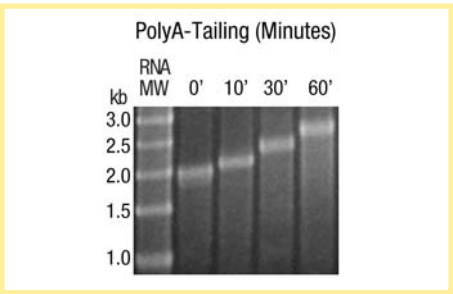

FIG 1. The length of the 3'-poly(A)-tail added to an RNA molecule can be readily controlled by altering the reaction time using the A-Plus™ Poly(A) Polymerase Tailing Kit. A 1760-base, 5'-capped RNA was incubated with 8 U of A-Plus Poly(A) Polymerase under reaction conditions described in the text.

FIG 1 shows the length of the 3'-poly(A)-tail added to the 1760 base RNA under standard reaction conditions after 10, 30, and 60 minutes. The un-tailed 1760 base RNA is shown in the zero minute gel lane. Incubation for 10 minutes yielded RNA with a poly(A)-tail length of approximately 200 bases; after 30 minutes the poly(A)-tail length was approximately 350 - 400 bases and after 60 minutes the reaction produced a poly(A)-tail length of >500 bases.

Other reaction conditions for the A-Plus Poly(A) Polymerase were investigated (data not shown). It was found that:

- The length of the poly(A)-tail was directly proportional to the amount of Poly(A) Polymerase in the reaction. The more Poly(A) Polymerase in the reaction, the longer the poly(A)-tail length.
- The length of the poly(A)-tail was inversely proportional to the molar amount of RNA in the reaction. The less RNA in the reaction, the longer the poly(A)-tail length.
- The length of the poly(A)-tail was inversely proportional to the reaction volume. Reducing the reaction volume produced RNA with longer poly(A)-tails.

Conclusion

We have demonstrated that the A-Plus Poly(A) Polymerase Tailing Kit can be used to rapidly and efficiently add a poly(A)-tail to the 3'-end of an *in vitro* transcribed RNA. The length of the poly(A)-tail can be controlled by altering the:

- Length of reaction time.
- Molar amount of RNA added to the reaction.
- Reaction volume.
- Amount of A-Plus Poly(A) Polymerase added to the reaction.

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www.EpiBio.com/aplus.asp

A-Plus™ Poly(A) Polymerase Tailing Kit

PAP5104H	400 Units

Contents: A-Plus™ Poly(A) Polymerase, A-Plus™ 10X Reaction Buffer, 10 mM ATP, Sterile RNase-Free Water.

RNase-Free DNase I

D9902K	2,500 Units
D9905K	5,000 Units
D9910K	10,000 Units

Supplied at a concentration of 1 U/µl.

Amplify mRNA from 1 Cell For Microarray Studies

TargetAmp™ 2-Round aRNA Amplification Kits

The TargetAmp™* 2-Round Aminoallyl-aRNA Amplification Kit 1.0 and TargetAmp™ 2-Round aRNA Amplification Kit 2.0 are ideal for amplifying poly(A) RNA using total RNA from 1 to 50 laser captured cells, FACS-sorted cells or other small samples for use with GeneChip® or other commercial or spotted-arrays.

TargetAmp™ 2-Round aRNA and Aminoallyl-aRNA Amplification Kits:

- Produce microgram amounts of aminoallyl-aRNA (AA-aRNA) or aRNA from total RNA of 1 to 50 cells (10 pg to 500 pg total RNA).
- Utilize an improved "Eberwine" linear RNA amplification process that preserves the relative transcript abundance of the sample and virtually eliminates nonspecific amplification products.
- Produce high quality microarray results as judged by % Present calls, 3'/5' ratios, correlation of differential gene expression and other important array parameters.
- TargetAmp™ 1-Round aRNA Amplification Kits that produce microgram amounts of aminoallyl-aRNA or aRNA from as little as 25 ng of total RNA are also available.

Total RNA in Sample	Rat Brain	HeLa RNA	Rat Kidney RNA
10 pg (1 cell)	3 µg	1.3 µg	1.4 µg
100 pg (10 cells)	54 µg	19 µg	15 µg
500 pg (50 cells)	161 µg	71 µg	72 µg

Fold Amplification of Poly(A) RNA	>5 x 10^6
Average 3'/5' ratio GAPDH	5.6 +/- 1.5
Average 3'/5' ratio β-actin	10.7 +/- 0.07
Average Present Calls	50.7 +/- 1%
Reproducibility (2 amplifications)	0.99

Table 1. AA-aRNA produced using the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0, and subsequently biotinylated, generated high quality GeneChip Arrays.

Ordering Information

TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0

TAA2R4910 10 Reactions
TAA2R4924 24 Reactions

TargetAmp™ 2-Round aRNA Amplification Kit 2.0

TAU2R5110 10 Reactions
TAU2R51224 24 Reactions

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qRT-PCR of Even Low-Abundance Transcripts From As Little as 1 Cell!

Message*BOOSTER*™ cDNA Synthesis Kit for qPCR

New!

The Message*BOOSTER*™* Kit greatly improves the qRT-PCR sensitivity, accuracy and reproducibility of even low-abundance transcripts from very small populations of cells.

A Message*BOOSTER* cDNA Synthesis Kit reaction:

- Greatly improves the sensitivity, accuracy and reproducibility of qRT-PCR for low-, medium-, and high-abundance transcripts from as little as 1 cell.
- Utilizes a linear RNA amplification process that preserves the relative transcript abundance (gene expression profile) of the sample.
- Significantly increases the number of qRT-PCR reactions that can be obtained from small samples.
- Enables sensitive and reproducible multiplex qRT-PCR using total RNA from as little as 1 cell.

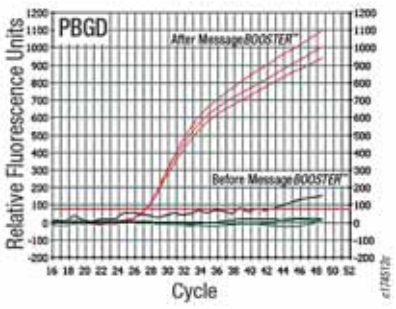


FIG 1. qPCR of the low abundance PBGD transcript using cDNA produced from 10 pg of total RNA before and after a Message*BOOSTER*™ cDNA Synthesis Kit for qPCR reaction.

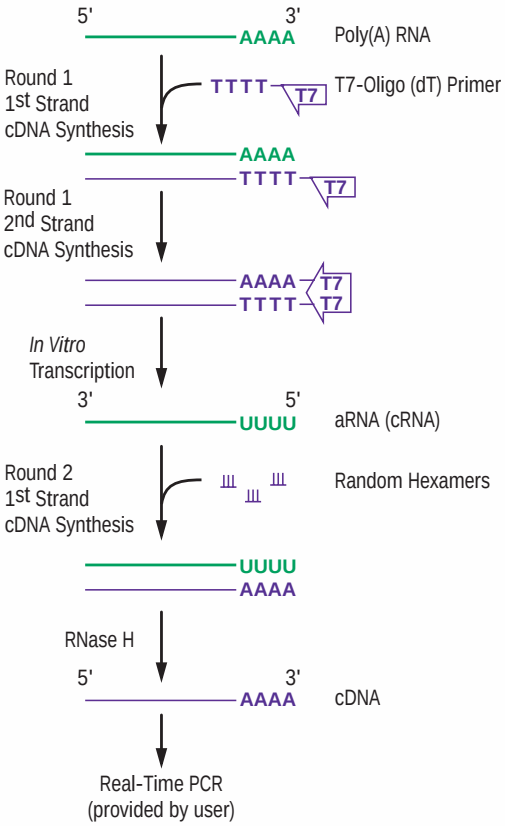


FIG 2. A Message*BOOSTER*™ reaction utilizes a linear RNA amplification process to amplify the poly(A) RNA (mRNA) from total RNA of as little as 1 cell and then converts the aRNA produced to cDNA that is ready for qPCR.

Ordering Information

Message*BOOSTER™ *cDNA Synthesis Kit for qPCR

MB060110	10 Reactions
MB060124	24 Reactions

EPICENTRE® Biotechnologies
www.EpiBio.com

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **MBDX1**

Make Circular ssDNA for Rolling Circle Transcription or Replication Assays

CircLigase™ ssDNA Ligase

CircLigase™* ssDNA Ligase is a thermostable, ATP-dependent ligase that catalyzes circularization of single-stranded DNA with 5'-phosphate and 3'-hydroxy termini. CircLigase does not require oligo "splints" or "bridges" and produces no detectable linear or circular concatamers, making the enzyme ideal for preparing circular ssDNA templates for rolling circle replication or rolling circle transcription.

Applications

- Rolling circle replication experiments.
- Rolling circle transcription experiments.
- RNA polymerase activity and inhibitor screening assays.

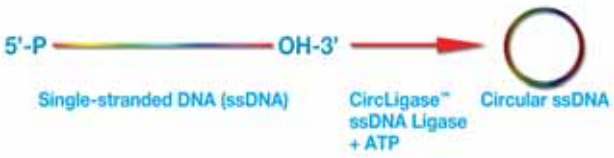


FIG 1. CircLigase™ ssDNA Ligase converts linear ssDNA to circular ssDNA.

Benefits

- Efficiently circularizes ssDNA of >30 bases.
- Produces no linear or circular concatamers.
- Does not require oligo "splints" or "bridges".

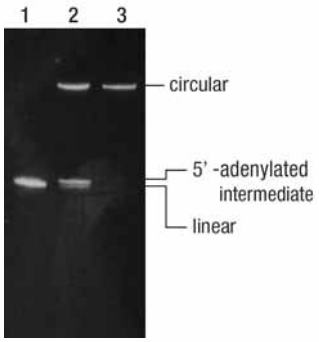


FIG 2. CircLigase™ ssDNA Ligase converts linear ssDNA to circular ssDNA. A 71-base ssDNA oligo **(Lane 1)** was incubated at 60°C for 1 hour in a reaction containing CircLigase ssDNA Ligase and ATP. A circular ssDNA and an adenylated intermediate were obtained (**Lane 2**). Exonuclease I digested the adenylated intermediate and starting linear ssDNA oligo, leaving only the circular ssDNA product (**Lane 3**).

Ordering Information

CircLigase™ ssDNA Ligase

CL4111K	1,000 Units
CL4115K	5,000 Units

Contents: CircLigase™ ssDNA Ligase, CircLigase™ 10X Reaction Buffer, ATP, $MnCl_2$, ssDNA Control, and Water.

**Patents pending. Sold under license to EPICENTRE.*

EPICENTRE® Biotechnologies
www.EpiBio.com

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **CLDX2**

RepliPHI™ Phi29 DNA Polymerase

www.phi29.com

RepliPHI™ Phi29 DNA Polymerase, derived from the *B. subtilis* phage phi29, is a highly processive enzyme with exceptional strand displacement activity. The enzyme also contains an inherent 3′→5′ exonuclease activity that enables proofreading capability.

Benefits

- Available at 1 µg/µl and 0.1 µg/µl.
- Free of detectable DNA contamination.
- Specific activity of 1 X 10^6 Units/mg.
- RepliPHI Phi29 Polymerase Dilution Buffer now available.

Unit Definition: One unit of RepliPHI Phi29 DNA Polymerase converts 25 pmoles of all deoxyribonucleotides into acid insoluble material in 30 minutes at 30ºC.

Quality Control: RepliPHI Phi29 DNA Polymerase is free of detectable endonuclease and RNase activities, and of detectable DNA contamination.

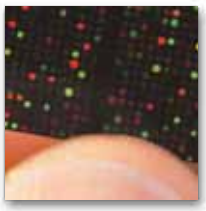
Ordering Information

RepliPHI™ Phi29 DNA Pol. 1 µg/µl (1,000 Units/µl)			*RepliPHI™ Phi29 DNA Pol. 0.1 µg/µl (100 Units/µl)*		
RepliPHI™ Phi29 DNA Pol. (Enzyme Only)			**RepliPHI™ Phi29 DNA Pol. (Enzyme Only)**		
PP031010	10 µg	(10,000 Units)	PP040110	10 µg	(10,000 Units)
RepliPHI™ Phi29 Reagent Set (Enzyme, dNTPs, Buffer, DTT)			**RepliPHI™ Phi29 Reagent Set (Enzyme, dNTPs, Buffer, DTT)**		
RH031110	10 µg	(10,000 Units)	RH040210	10 µg	(10,000 Units)

RepliPHI™ Phi29 Polymerase Dilution Buffer	
RPB04041	1 ml

EPICENTRE® Biotechnologies
www.EpiBio.com

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **PHDX2**



Do You Need to Purify RNA From 1 or More Cells?

ArrayPure™ Nano-scale RNA Purification Kit

The ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from a few hundred eukaryotic cells, a quantity typically obtained with Laser Capture procedures. The kit contains only aqueous reagents, requires no toxic organic solvents, and has been specifically designed for, and qPCR-tested on, 1 to 10,000 live eukaryotic cells. ArrayPure™ RNA from 20 HeLa cells has been used to produce µg amounts of RNA using 2-round RNA amplification techniques.

Applications

- RT-PCR.
- Amplified RNA.
- Laser Capture.

Benefits

- Isolate RNA from ≥ 1 cell.
- Purify RNA for RNA amplification.
- Avoid carrier RNA.

FIG 1.

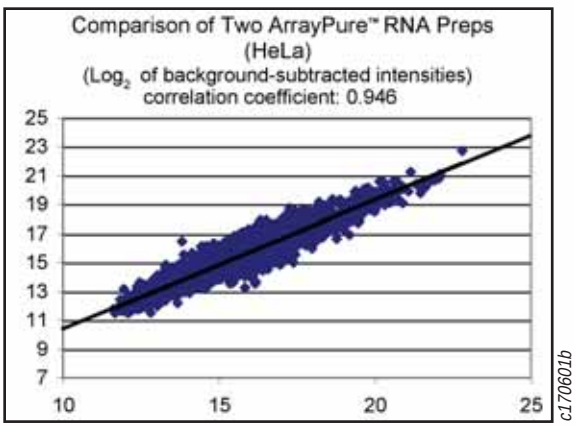


FIG 1. Comparison of Two ArrayPure™ RNA Preps. HeLa cell RNA was purified from two separate tissue culture flasks, labeled with Cy™3 or Cy5, and hybridized to a microarray: Operon's Array-Ready Human Oligo Set™ (70-mer).

FIG 2.

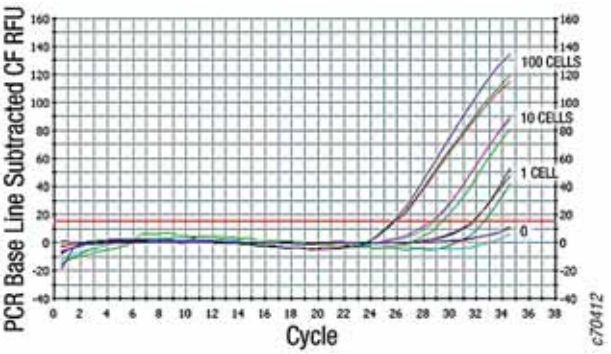


FIG 2. Quantitative Real-Time PCR Amplification Plot. HeLa cells were grown in tissue culture, aseptically diluted, and trapped inside sterile 5 µl microcapillary pipets. The number of cells isolated was verified by observation with an inverted microscope. Then, the cell(s) was/were eluted by centrifugation from the capillary pipet, washed with phosphate buffered saline, and the RNA purified by the ArrayPure™ Nano-scale RNA Purification Kit. Purified HeLa RNA was converted to cDNA using EPICENTRE's MMLV Reverse Transcriptase. The corresponding cDNAs were amplified using FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit (PreMix 3). Real-Time PCR results are shown in triplicate for RNA from an average of 100, 10, 1 and 0 (medium alone) cells.

Ordering Information

ArrayPure™ Nano-scale RNA Purification Kit

MPS04050 50 Purifications

EPICENTRE® Biotechnologies
www.EpiBio.com

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **ARDX2**

Human Genetic Studies Just Got Easier

BuccalAmp™ DNA Extraction Kit

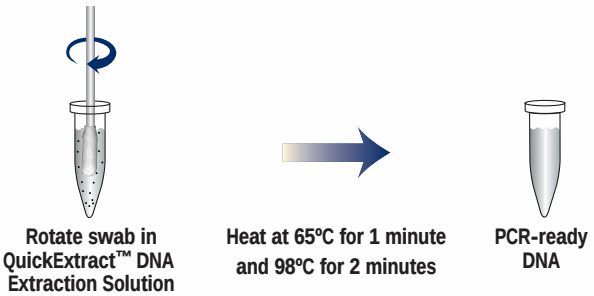
The BuccalAmp™ DNA Extraction Kit is a single-tube system for rapid preparation of DNA from buccal (cheek) swabs for use in PCR amplification assays. To obtain PCR-ready DNA, just rotate the buccal swab in one of these tubes, mix, and heat for 3 minutes. No centrifugation is needed.

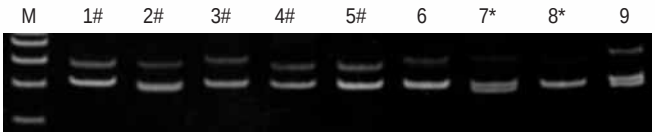
Applications

- ***Fastest available*** extraction of DNA from buccal swabs.
- Human or animal identity testing.
- SNP analysis.
- Assay for viruses, bacteria or other microorganisms.

Benefits

- Simple and rapid sample collection.
- Soft, gentle foam swab.
- 3 minute DNA extraction protocol.
- Safe - no toxic solvents.





PCR of polymorphic repeat sequences in DNA from buccal samples from 9 individuals obtained using the BuccalAmp™ DNA Extraction Kit. DNA was amplified using the FailSafe™ PCR System. **Lane M**, 100 bp marker.

remote-site collected sample * pediatric sample

Ordering Information

BuccalAmp™ DNA Extraction Kits

BQ0901S	1 Kit (15 Tubes & Swabs)
BQ0908S	8 Kits (120 Tubes & Swabs)
BQ0916S	16 Kits (240 Tubes & Swabs)

Contents per kit:

15 tubes (1 extraction per tube) of QuickExtract™ DNA Extraction Solution 1.0.

15 individually-packaged sterile Catch-All™ Swabs.

QuickExtract™ DNA Extraction Solution 1.0

QE09050 50 ml

Bulk solution, sufficient to perform 100 extractions.

Catch-All™ Sample Collection Swabs

QEC0925	25 Swabs
QEC091H	100 Swabs

EPICENTRE® Biotechnologies
www.EpiBio.com

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **BADX2**

RNA & DNA Purification

Successful PCR— *First Time and Every Time*

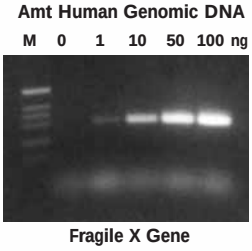
FailSafe™ PCR System

The FailSafe™* PCR System sets a new standard for PCR by combining a unique blend of thermostable enzymes and an extensively tested set of 2X PreMixes into a single system. The FailSafe PCR System provides dependable, consistent high-fidelity PCR results for every DNA template, regardless of its source or sequence.

Applications

- Multiplex PCR.
- Templates up to 85% G-C content.
- High sensitivity PCR.
- PCR any sequence up to 20 kb from any source.

(See www.EpiBio.com for additional details and data.)



FailSafe™ PCR of 85+%-GC human fragile X gene. Fragile X gene was amplified from as little as 1 ng of total human genomic DNA.

Benefits

- Generates PCR products suitable for both TA cloning and blunt-end cloning.
- Extremely simplified automatic PCR optimization.
- Successful PCR, first time and every time.
- High accuracy PCR using a unique enzyme blend with the lowest error rate.
- Extremely high sensitivity and specificity using the PCR Enhancer Technology.[a]
- No need for “hot-start” PCR techniques.

Ordering Information

FailSafe™ PCR PreMix Selection Kit
FS99060 60 Units
Contents: FailSafe™ PCR Enzyme Mix, 12 FailSafe™ PCR 2X PreMixes

FailSafe™ PCR System with PreMix Choice
FS99100 100 Units
Contents: FailSafe™ PCR Enzyme Mix, Choice of 1 FailSafe™ PCR 2X PreMix

FailSafe™ PCR System with PreMix Choice
FS99250 250 Units
Contents: FailSafe™ PCR Enzyme Mix, Choice of 2 FailSafe™ PCR 2X PreMixes

FailSafe™ PCR System with PreMix Choice
FS9901K 1,000 Units
Contents: FailSafe™ PCR Enzyme Mix, Choice of 8 FailSafe™ PCR 2X PreMixes

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FailSafe Enzyme Mix and PreMixes are available separately.

**EPICENTRE's PCR products are sold under licensing arrangements with F. Hoffmann-La Roche Ltd., Roche Molecular Systems, Inc., and Applied Biosystems. The products containing a thermostable DNA polymerase are accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler. Go to www.EpiBio.com website for complete license statements.*

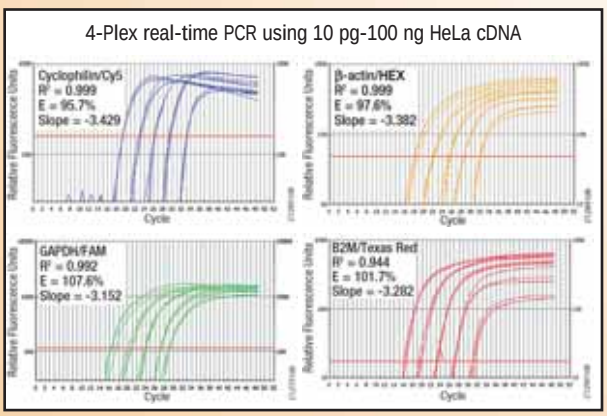
[a]Patents issued and pending on FailSafe™ PCR Enhancer.

Find this product web page using the QuickInfo search box! Go to **www.EpiBio.com** and enter this QuickInfo code: **FSDX1**

All-In-One Convenience

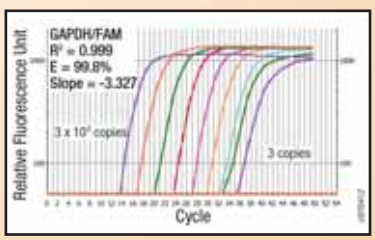
For Optimizing Singleplex and Multiplex Real-Time PCR

The **FailSafe™ PROBES Real-Time PCR System** is ideally suited for any probe-based assay, including challenging applications such as multiplex and high sensitivity real-time PCR. The kit contains all components needed for optimized real-time PCR results you can trust.



Advantages of the FailSafe™ PROBES Real-Time PCR System:

- *Convenient, simple and easy to use*—PreMixes are pre-optimized to save you time and energy.
- *Simple Optimization*—Robust enzyme mix and set of 2X optimization PreMixes contains all of the components necessary for successful PCR. It enables optimum amplification efficiency to generate high quality, trustworthy data.
- *Consistent results*—Enzyme and PreMix formulations do not change, which gives you consistent, error-free results every time.



First Time → and Every Time

For more information, go to **www.EpiBio.com** and enter this QuickInfo code: FPAX3

EPICENTRE® Biotechnologies
www.EpiBio.com

P-603FP-A

Ask Frank

by Fred and Hank


Fred Hyde


Hank Daum

Questions about EPICENTRE Biotechnologies' new Message*BOOSTER*™ cDNA Synthesis Kit for qPCR

Q. Why is the Message*BOOSTER*™ cDNA Synthesis Kit such a benefit for quantitative real-time PCR (qPCR)?

A. For a number of reasons: With even low-abundance transcripts the Message*BOOSTER* Kit can produce enough cDNA for sensitive and reproducible qPCR starting with total RNA from 1 to 50 cells (10 – 500 pg). It is important to note that the Message*BOOSTER* amplification process is linear, which means that the overall gene expression profile is preserved. Each reaction starting with total RNA from one cell, will produce enough cDNA for up to 10 qPCR experiments.

Q. How does the Message*BOOSTER* cDNA Synthesis Kit work?

A. Initially, poly(A) RNA in the total RNA sample undergoes a modified and improved "Eberwine-type" T7-based RNA amplification procedure to produce antisense RNA (aRNA, also called complementary RNA [cRNA]). The synthesized aRNA is then spin column purified and used in a cDNA synthesis reaction using EPICENTRE's MMLV (Moloney Murine Leukemia Virus) Reverse Transcriptase Plus (MMLV-RT Plus) with random primers. This creates a population of cDNA molecules that are an excellent, high-fidelity representation of the gene expression profile of the cell. The cDNA generated can then be used directly in qPCR applications with SYBR® Green I dye or fluorescent probe detection.

Q. Once I have synthesized the cDNA with the Message*BOOSTER* Kit, how much of it should I use in my qPCR?

A. The cDNA produced by a Message*BOOSTER* reaction is in a final volume of ~ 5.5 µl. The amount of cDNA to add to a qPCR reaction is dependent on the amount of starting total RNA in the Message*BOOSTER* reaction and on the abundance of the transcript of interest and should be determined empirically. For example, when starting with 10 pg of total RNA (about 1 cell equivalent), to detect low- or medium-abundance transcripts you would use 1 µl of the Message*BOOSTER* final reaction volume. For the detection of high-abundance transcripts, you would dilute the Message*BOOSTER* final reaction volume 1:10, and then use 1 µl in your qPCR assays. For more details see the article in EPICENTRE Forum 13.1.[1]

Q. In order to obtain optimal results with the Message*BOOSTER* cDNA Synthesis Kit, what are some of the important factors I should consider?

A. Before using up precious sample, familiarize yourself with the kit by running a control reaction. Use no more than 500 pg of total RNA (~50 cells) per reaction, and remember that the success of an RNA amplification reaction is strongly influenced by the quality of the input RNA.

Q. For best results, how should I design my primers for qPCR when using input cDNA synthesized by a Message*BOOSTER* reaction?

A. The amplified RNA used to make cDNA in a Message*BOOSTER* Kit reaction will have a 3-prime bias. For optimal qPCR sensitivity, we recommend selecting primers that prime within 500 bases of the 3'-end of the mRNA. Primers for sequences >500 bases from the 3'-end of the mRNA(s) may give reduced sensitivity.

Q. You are using a wild-type MMLV-RT in the second cDNA reaction, and then using RNase H to degrade the RNA portion of the reaction afterwards. Why is this? Doesn't wild-type MMLV have RNase H activity?

A. While EPICENTRE's MMLV-RT Plus does have RNase H activity, using additional RNase H after the reverse transcription reaction has been demonstrated to enhance the removal of the RNA from the RNA:DNA hybrid and yield more sensitive qPCR results.

Q. Does the cDNA produced in a Message*BOOSTER* reaction require additional cleanup after the RNase H treatment before use in qPCR?

A. No. The cDNA may be used directly as template in your qPCR reactions.

Reference

1. Grunenwald, H. *et al.*, (2006) EPICENTRE *Forum* **13**(1), 7.

See Page 4 - 5
For More Information on
The Message*BOOSTER*™ cDNA
Synthesis Kit for qPCR

info

The High Yield ArrayPure™ Nano-scale RNA Purification Kit Outperforms Kits from Two Other Major Suppliers

Bruce W. Jarvis and Haiying Grunenwald, EPICENTRE Biotechnologies

Introduction

The ArrayPure™ Nano-scale RNA Purification Kit provides all the reagents needed to purify total cellular RNA from one to 10,000 eukaryotic cells, including those cells obtained from laser capture microdissection procedures.

Obtaining high quality RNA in sufficient quantities is often the most crucial step in performing many downstream molecular biology applications. This step is even more critical when purifying RNA from a relatively limited number (10,000) of cells. In this report we demonstrate that the RNA yield produced by the ArrayPure™ Nano-scale RNA Purification Kit is two to three times greater than the yields produced by two other vendors' RNA purification kits. This conclusion is supported by data generated by two independent methods: fluorimetry with RiboGreen® fluorescent nucleic acid stain and quantitative RT-PCR (qRT-PCR).

Methods and Results

HeLa cells were grown under standard conditions and enumerated by microscopic observation using a hemacytometer. The ArrayPure™ Kit and RNA purification kits from vendors A and Q were used on replicate samples of 10,000 cells. The average RNA yields, as quantified by RiboGreen are shown in Table 1. The superior yield of the ArrayPure™ Kit is evident, possibly because unlike the ArrayPure™ Kit, the kits from vendors A and Q both use a column matrix to bind and then inefficiently release the RNA.

After the RiboGreen assay, RNA obtained from the three RNA purification kits was then compared by qRT-PCR. The volumes of the RNA samples were normalized, and an aliquot of the RNA purified by each of the three kits was reverse-transcribed into cDNA using EPICENTRE's MasterAmp™* High Fidelity RT-PCR Kit. The corresponding cDNAs were then assayed by qRT-PCR using primers and sequence-specific probes for cyclophilin A (peptidyl-prolyl cis-trans isomerase A) and B2M (beta-2-microglobulin). As shown in FIGS 1A and B, the cDNA from RNA purified using the ArrayPure™ Kit displayed the lowest threshold cycle (C_T) values, hence the highest amount of starting cDNA, indicating that the yield of ArrayPure™ RNA was the most abundant of the three kits tested. The cDNA made from RNA generated using the kit from vendor Q failed to amplify with the B2M primers, possibly due to the poor quality and/or size of the purified RNA.

	Yield (ng)	Technology
ArrayPure™ Kit	300	Precipitation
Vendor A	120	Spin Column
Vendor Q	95	Spin Column

Table 1. Average RNA yields produced by the ArrayPure™ Kit and kits from Vendors A and Q, as quantified by RiboGreen. Each kit was tested using 10,000 HeLa cells.

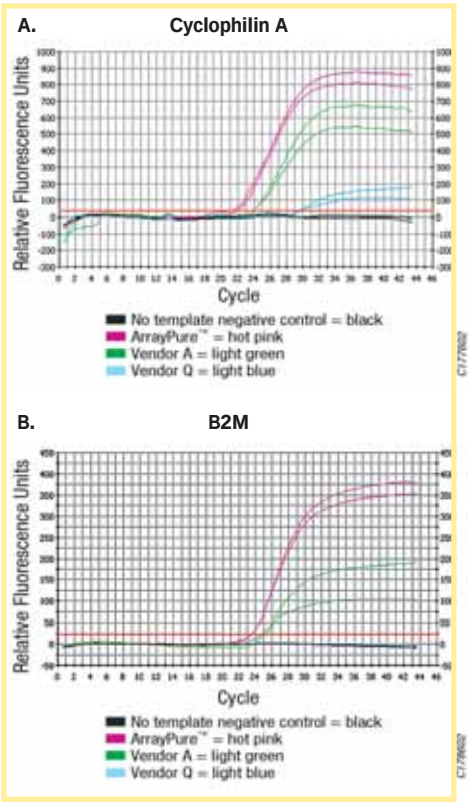


FIG 1. qRT-PCR using cDNA generated from RNA purified using the ArrayPure™ Kit and kits from Vendors A & Q. PCR reactions were carried out in 25 µl volume: 1X FailSafe™ PROBES PreMix Probe 3 (EPICENTRE), 12.5 pmole of forward and reverse primers, 100 nM probe, 1 U FailSafe™ Real-Time Enzyme Mix, 1 µl of appropriate cDNA. PCR cycling conditions: 95°C for 2 min, followed by 45 cycles of 95°C for 10 sec, and 60°C for 1 min. **A**. Sequence-specific probes for cyclophilin A were labeled with the fluorophore Cy™5. **B**. Sequence-specific probes for B2M were labeled with the fluorophore Texas Red®.

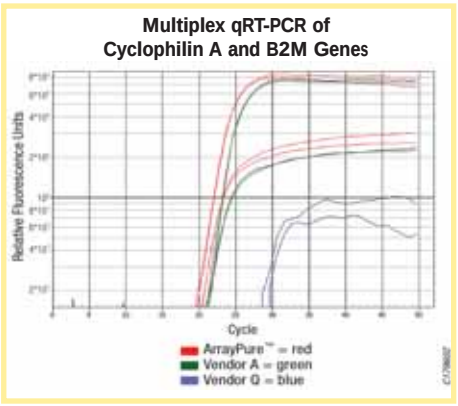


FIG 2. Multiplex qRT-PCR of cDNA made from three different RNA purification kits. Duplicate multiplex qPCR reactions in 25 µl volumes contained: 1X FailSafe™ PROBES PreMix Probe 3 (EPICENTRE), 12.5 pmole of forward and reverse primers, 100 nM of both probe, 1 U FailSafe™ Real-Time Enzyme Mix, 2.5 µl of appropriate cDNA. Sequence specific probes for B2M and cyclophilin A were 5'-labeled with Texas Red and Cy5, respectively. PCR cycling conditions: 95°C for 2 min, followed by 50 cycles of 95°C for 10 sec, and 60°C for 1 min. (No template [negative] and positive controls performed as expected, data not shown.)

We then used multiplex qRT-PCR to compare the RNA purified by the three kits. Multiplex qRT-PCR is the detection of multiple target sequences simultaneously using a single qRT-PCR reaction. Multiplex qRT-PCR makes quantification more feasible and economical since a normalizer or calibrator gene (required for accurate gene quantification) can be amplified in the same reaction as the gene of interest. However, the results of multiplex qRT-PCR will be meaningless if the template quality is poor. Therefore, obtaining a successful multiplex qRT-PCR profile is a very sensitive and effective test for evaluating the nucleic acid purification process.

FIG 2 shows the results of a multiplex qRT-PCR reaction amplifying both the B2M and cyclophilin A genes. Equal volumes of cDNA made from the HeLa cell RNA by the ArrayPure™ Kit, and kits

from vendors A and Q, were included in each qRT-PCR reaction to compare the abundance and quality of each cDNA sample. The consistently low C_T values achieved from multiplex qRT-PCR for both the cyclophilin A and the B2M gene targets further demonstrated the superior quality and quantity of the RNA purified using the ArrayPure™ Kit when compared to competitors A and Q. Both the ArrayPure™ and vendor A's cDNA demonstrated successful multiplex qRT-PCR of both gene targets; however, cDNA from vendor Q's kit failed to amplify the B2M target, again possibly due to the poor quality and/or size of the purified RNA.

Conclusion

EPICENTRE's ArrayPure™ Nano-scale RNA Purification Kit produces superior yields and quality of RNA when compared to kits from two other major manufacturers, and represents a more economical method for RNA purification.

**This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.*

See *Product Data Sheet* on Page 13
For More Information on ArrayPure™ Nano-scale RNA Purification Kit

info

A Rapid Method for Extraction of Rotavirus RNA from Fecal Samples and Genotyping of Rotavirus by Reverse Transcription-PCR

Ramesh Vaidyanathan and Bruce W. Jarvis, EPICENTRE Biotechnologies

Introduction

Rotavirus is the leading causative agent of severe diarrhea among children.[1] The rotavirus genome consists of 11 double-stranded RNA (dsRNA) segments enclosed in a double-shelled capsid. The outer shell is composed of a major glycoprotein (vp7), which defines the rotavirus serotype specificity. Nucleotide sequence analysis of vp7 cDNAs from different isolates indicates the presence of variable regions that are highly conserved within a given serotype, and distinct among different serotypes.[2] Gouvea *et al.*,[3] demonstrated a reverse transcription-PCR (RT-PCR) typing method in which each human serotype variant of the virus produced a characteristic fragment size, which were thus readily identifiable on agarose gels. The PCR typing method was applied to all known 6 human serotypes (1, 2, 3, 4, 8, and 9); an absolute correlation was found between molecular and traditional serologic rotavirus typing methods.

Methods

EPICENTRE Biotechnologies' Extract-Master™ Fecal DNA Extraction Kit was used to isolate rotavirus dsRNA from infant fecal samples. The inhibitor removal resin was placed in a glass container and spin columns wrapped in foil, then all were autoclaved for 10 min at 121°C using a liquid cycle to eliminate RNase contamination. Replicate samples, tested to compare the efficacy of other extraction methods, were also processed by phenol chloroform extraction and the nucleic acids recovered by ethanol precipitation. In all cases, the recovered nucleic acids were suspended in 100 µl TE Buffer.

Identification of rotavirus and its typing were accomplished by RT-PCR, using primer pairs that anneal to the conserved and variable regions of vp7 RNA.[3] RT-PCR was carried out in a one-tube format using EPICENTRE's MonsterScript™ Reverse Transcriptase and FailSafe™* PCR System. Rapid RT-PCR typing of the rotavirus was performed with a primer mix using both the MonsterScript and FailSafe PCR System, or using the single-enzyme MasterAmp™* RT-PCR Kit for High Sensitivity (EPICENTRE).

Results

Nucleic acids isolated with the ExtractMaster Kit yielded the expected 1062 bp RT-PCR amplicon corresponding to the full length vp7 RNA (FIG 1A, lanes 3 and 5), indicating a rotavirus-positive sample.[3] Fecal samples processed by phenol chloroform extractions did not give rise to any amplicon (FIG 1A, lanes 2 and 4). Thus, the ExtractMaster Kit yields

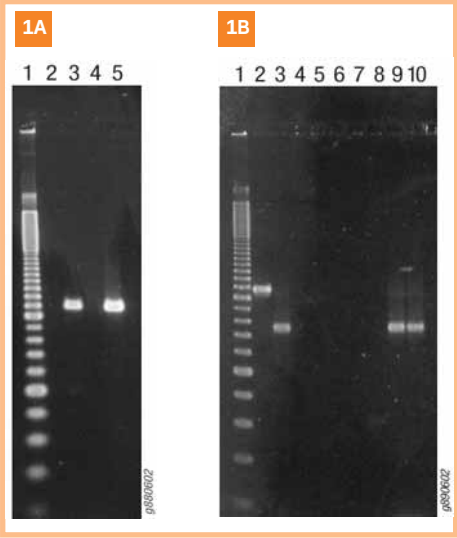


FIG 1. Agarose gel analysis of RT-PCR products. A, The positive identification of rotavirus. The 1062 bp amplicon that appears in lanes 3 and 5 (nucleic acids isolated by ExtractMaster™) corresponds to the full length vp7 RNA, and is a positive identification of rotavirus. **Lane 1**, 100 bp ladder; **Lanes 2 and 4**, Nucleic acids (1 µl) isolated by phenol chloroform extraction; **Lanes 3 and 5**, Nucleic acids isolated by ExtractMaster. All nucleic acids were used as templates for RT-PCR with MonsterScript™ Reverse Transcriptase and PCR with FailSafe™ Enzymes. **B**, The identification of rotavirus as serotype 1 using PCR typing. The gel shows an amplicon of size ~750 bp with serotype 1 specific primers (**Lane 3**), and none with the other serotype specific primers (**Lanes 4-8**). **Lane 1**, 100 bp ladder; **Lane 2**, ExtractMaster nucleic acid used in RT-PCR with primers against the full length vp7 RNA; **Lanes 3 to 8**, ExtractMaster nucleic acid used in RT-PCR with individual serotype-specific primers: types 1-4 and 8, 9 (**Lanes 3-6 and 7, 8** respectively). A mixture of six serotype-specific primers was used with MonsterScript and Failsafe **(Lane 9)** or with the MasterAmp™ RT-PCR Kit for High Sensitivity **(Lane 10**).

Continued on page 20

Get the Highest Yield of 5'-Capped RNA from an *In Vitro* Transcription Reaction

EPICENTRE Biotechnologies' AmpliCap™ T7 & SP6 High Yield Message Maker Kits, AmpliCap-MAX™ T7 & T3 High Yield Message Maker Kits, and MessageMAX™ T7 Capped Message Transcription Kit, are formulated to produce the highest yields of 5'-capped RNA from an *in vitro* transcription (IVT) kit for use in *in vitro* translation, microinjection and mRNA processing studies.

The AmpliCap and AmpliCap-MAX Kits feature an optimized PreMix solution of NTPs and the standard $m^7G[5']ppp[5']G$ cap analog commonly used in eukaryotic translation studies. The AmpliCap-MAX T7 reaction typically produces higher yields of 5'-capped RNA using a shorter reaction time (30 minutes) than an AmpliCap T7 reaction (Table 1).

Continued on next page

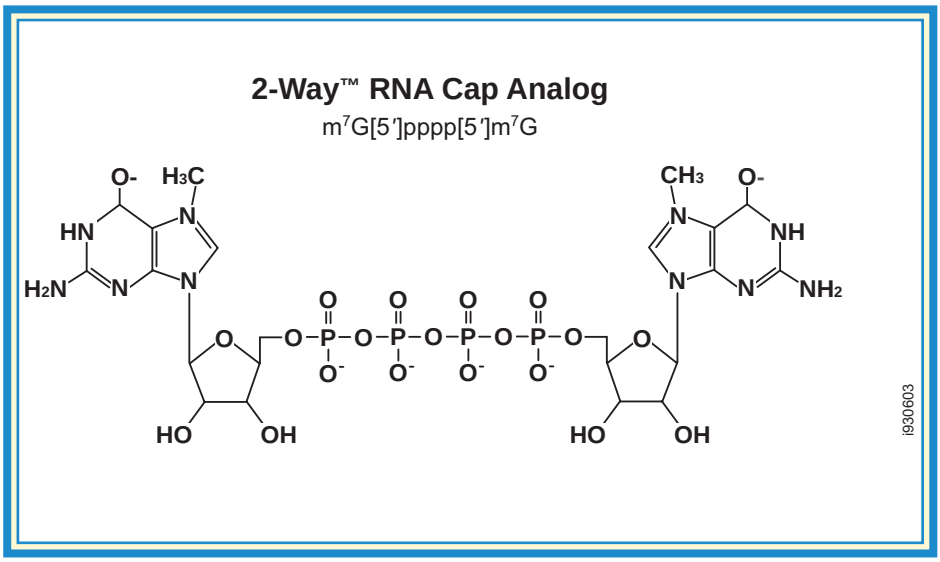


FIG 1. EPICENTRE's new symmetrical dimethylated 2-Way™ RNA Cap Analog $m^7G[5']pppp[5']m^7G$.

Extraction of Rotavirus RNA—Continued from page 19

high quality nucleic acids that can serve as templates in RT-PCR.

In order to determine the 'serotype' of the rotavirus strain by RT-PCR, six serotype-specific forward primers were used in individual RT-PCR molecular typing experiments in combination with a common reverse primer. The results showed an amplicon of size ~750 bp, with serotype 1 specific primers (FIG 1B, lane 3), and none with the other serotype specific primers (FIG 1B, lanes 4-8). A product of similar size was also seen in reactions that contained a mixture of six serotype-specific forward primers and a common reverse primer in the same tube (FIG 1B, lane 9). This demonstrates that molecular typing of rotavirus can be performed using one, single tube RT-PCR reaction.

The MasterAmp™ RT-PCR Kit for High Sensitivity also yielded a single product of ~750 bp in reactions that contained a mixture of six primers and a common reverse primer (FIG 1B, lane 10). As the RetroAmp™ RT DNA Polymerase (EPICENTRE) included in this kit is stable at high temperatures, one-step RT-PCR typing experiments can be performed on these samples.

Conclusion

EPICENTRE's ExtractMaster™ Fecal DNA Extraction Kit is effective for the isolation of dsRNA from fecal samples, enabling the rapid analysis of potential pathogens in disease outbreaks.

References

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** EPICENTRE's PCR products are sold under licensing arrangements with F. Hoffmann-La Roche Ltd., Roche Molecular Systems, Inc., and Applied Biosystems. The products containing a thermostable DNA polymerase are accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.*

Highest Yield of 5'-Capped RNA—continued from page 20

EPICENTRE's new MessageMAX™ T7 Capped Message Transcription Kit includes the new 2-Way™ Cap Analog ($m^7G[5']pppp[5']m^7G$). This 2-Way Cap Analog is a symmetrical dimethylated molecule (see FIG 1, p. 20), which means that it is always incorporated in the correct orientation during IVT. The 2-Way Cap Analog has also been demonstrated to translate *in vitro*-synthesized luciferase mRNA in a rabbit reticulocyte lysate system >3-fold more efficiently than the same mRNA capped with the standard $m^7G[5']ppp[5']G$ cap analog.[1]

Regardless of the cap analog incorporated, if the capped RNA is used for translation studies, translational efficiencies can be much higher if the mRNA is also 3'-polyadenylated. RNA can be easily and efficiently 3'-polyadenylated using EPICENTRE's A-Plus™ Poly(A) Polymerase Tailing Kit (p. 8).

The Largest Selection of Function-Tested Cap Analogs

EPICENTRE offers the largest selection of cap analogs for *in vitro* transcription of 5'-capped RNAs. Each cap analog is function tested as a substrate for capping an RNA transcript synthesized in an *in vitro* transcription reaction and provided as a 20 mM solution. The new anti-reverse cap analog (ARCA) 3'-O-methyl-$m^7G[5']ppp[5']G$, and the new symmetrical 2-Way Cap Analog, $m^7G[5']pppp[5']m^7G$, are unique in that 100% of the capped transcripts produced with these analogs have the cap in the proper orientation for maximum translation efficiency.[1,2]

References

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Table 1. Comparison of three of EPICENTRE Biotechnologies' *in vitro* transcription kits for producing 5'-capped mRNA. The yield of capped RNA was determined by transcribing 1 µg of the control DNA template provided in each kit.

Kit Features	AmpliCap™ T7 & SP6 High Yield Message Maker Kits	AmpliCap-MAX™ T7 & T3 High Yield Message Maker Kits	MessageMAX™ T7 Capped Message Transcription Kit
In vitro transcription promoter(s)	T7 or SP6	T7 or T3	T7
Cap analog	$m^7G[5']ppp[5']G$	$m^7G[5']ppp[5']G$	$m^7G[5']pppp[5']m^7G$ 2-Way™ RNA Cap Analog
Yield of 5'-capped RNA	45 µg from AmpliCap™ T7 reaction 35 µg from AmpliCap™ SP6 reaction	60 µg from both AmpliCap-MAX™ T7 and T3 reactions	60 µg
Reaction time	2 hours	30 minutes	30 minutes
Optimized NTP/Cap Analog PreMix included?	Yes	Yes	Yes

www.EpiBio.com/rna_caps_analogs.asp

Standard Cap Analog
$m^7G[5']ppp[5']G$ Solution
C31010 500 nmoles
C61025 1,250 nmoles

2-Way™ Cap Analog
$m^7G[5']pppp[5']m^7G$ Solution
C41210 500 nmoles

ARCA Cap Analog
3'-O-Methyl-$m^7G[5']ppp[5']G$ Solution
C50110 500 nmoles

Unmethylated Cap Analog
$G[5']ppp[5']G$ Solution
C32010 500 nmoles

Trimethyl Cap Analog
$m_3^{2,2,7}G[5']ppp[5']G$ Solution
C06005 250 nmoles

www.EpiBio.com/amplicap.asp

AmpliCap™ T7 High Yield Message Maker Kit
AC0707 25 Reactions

AmpliCap™ SP6 High Yield Message Maker Kit
AC0706 25 Reactions

www.EpiBio.com/acmax.asp

AmpliCap-MAX™ T7 High Yield Message Maker Kit
ACM04037 25 Reactions

AmpliCap-MAX™ T3 High Yield Message Maker Kit
ACM04033 25 Reactions

www.EpiBio.com/mmax.asp

MessageMAX™ T7 Capped Message Transcription Kit
MM50110 10 Reactions

Recent Publications Citing the TargetAmp™ aRNA Amplification Kits for Gene Expression Studies

In early 2005, EPICENTRE Biotechnologies introduced the TargetAmp™ aRNA Amplification Kits, which enable the amplification of RNA obtained from as little as a single cell for microarray studies.[1] Presented here are brief summaries of some of the recent publications citing the use of the TargetAmp Kits.*

Laser microdissection and microarray analysis of breast tumors reveal ER-α related genes and pathways.

Yang, F., Foekens, J. A., Yu, J., Sieuwerts, A. M., Timmermans, M., Klijn, J. G. M., Atkins, D., Wang, Y, and Jiang, Y. 2006. *Oncogene* **25**, 1413.

The authors compared gene expression profiles of estrogen receptor alpha (ER-α) positive and ER-α negative breast cancer cells, obtained by laser capture microdissection (LCM).

The poly(A) RNA in the total RNA sample obtained from the LCM cells was amplified with EPICENTRE's TargetAmp™ 2-Round aRNA Amplification Kit 2.0 and then biotin-labeled in the second *in vitro* transcription reaction (hereafter referred to as the "LCM-TargetAmp" protocol). The LCM-TargetAmp protocol produced an average of 60 µg of biotin-aRNA from approximately 1000 cells. Linear regression analysis of the gene expression data derived from replicate TargetAmp 2-Round amplification reactions produced an R^2 value of 0.96. To ensure that the amplification method preserved mRNA abundance in LCM-derived RNA samples, the expression levels of 21 constitutively expressed housekeeping genes were compared between LCM-procured samples and bulk tumor cells. No statistically significant differences were found between the expression levels of the 21 housekeeping genes from the LCM-TargetAmp samples and the corresponding bulk tumor samples. Also, global gene expression data from the LCM-TargetAmp protocol and bulk tumor samples were found to cluster similarly/identically by ER status, confirming that TargetAmp amplification accurately preserves the mRNA profile of the starting material.

From the LCM-TargetAmp samples, the authors identified 146 genes whose expression was influenced by the ER status; 85 of these could not be identified from the bulk tumor tissue. Several of these unique genes have been implicated in breast cancer previously, but their connection to ER signaling has not been known.

The authors concluded that the LCM-TargetAmp approach "has not only identified differentially expressed genes related to ER status but also provided new information in potential pathways associated with estrogen signaling and additional genes for further investigation."

Gene expression mapping of the rat dorsal root ganglia.

Scott Eastman, P. 2005. *Am. Biotechnol. Lab.* **23**(13), 22.

Dr. Scott Eastman's report demonstrates the use of EPICENTRE's TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 in combination with the Mosaic™ Gene Expression Assay System (Quantum Dot Corp) for performing gene expression analysis from single cells. Individual neurons were laser capture microdissected from freshly frozen rat dorsal root ganglia. The total RNA was extracted and amplified using EPICENTRE's TargetAmp™ Kit 101 in a standard 6-hour reaction. The Aminoallyl- aRNA produced by the TargetAmp Kit 101 was labeled using biotin-NHS and purified. The entire amount of biotin-aRNA produced from a single neuron was used to hybridize to a 60-plex panel of QDot® nanocrystal-coated beads for analysis. The resulting signal intensities were 100 to 1000-fold above the "water-only" amplification background. For some of the genes investigated, the expression level between individual cells was very similar. For other genes, the expression levels spanned a range and led the author to conclude that there were gene expression differences in individual neurons from the same tissue section. Additionally, the author noted that single neurons contain relatively large amounts of RNA compared to other cell types and, that some of the rare transcripts were not detected after one round of RNA amplification but were detected after 2-rounds of amplification.

References

1. Khanna, A. *et al.*, (2005) EPICENTRE *Forum* **12**(1), 4.

Tobacco Acid Pyrophosphatase Efficiently Removes the 5'-Cap Structure From Eukaryotic mRNAs

The 5'-termini of many natural RNA molecules, including most eukaryotic RNAs, viral RNAs and many small nuclear RNAs, have a 5'-terminal methylated guanine nucleotide structure called a "cap". Tobacco Acid Pyrophosphatase (TAP) hydrolyzes the phosphoric acid anhydride bonds in the triphosphate bridge of the cap structure, releasing the cap nucleoside and generating a 5'-phosphorylated terminus on the RNA molecule (FIG 1). The resulting "decapped" 5'-phosphorylated terminus may be ligated to a 3'-hydroxylated terminus using T4 RNA Ligase (EPICENTRE Biotechnologies) or dephosphorylated using APex™ Heat-Labile Alkaline Phosphatase (EPICENTRE) for end labeling. TAP also digests the triphosphate group at the 5'-end of prokaryotic transcripts, generating an RNA molecule with a 5'-phosphorylated terminus. TAP is function-tested in an RNA decapping assay,[1] and is free of detectable RNase activity.

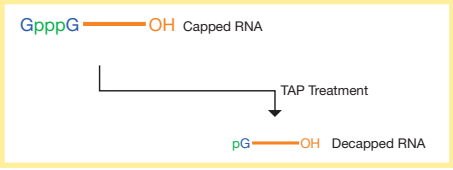

FIG 1. Tobacco Acid Pyrophosphatase removes the 5'-Cap structure from eukaryotic mRNAs generating a 5'-phosphorylated RNA.

Applications include:

- Preparation of templates for rapid amplification of cDNA ends (RACE).[2]
- Ligation of oligoribonucleotides to TAP-treated cellular RNA for construction of full-length cDNA libraries.[3]
- Mapping of transcription sites for eukaryotic[4] and prokaryotic[5] transcripts.
- Radiolabeling of RNA[6] for use in sequencing or as a hybridization probe.

References

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www.EpiBio.com/tap.asp

Tobacco Acid Pyrophosphatase

T19050	50 Units
T19100	100 Units
T19250	250 Units
T19500	500 Units

www.EpiBio.com/apex.asp

APex™ Heat-Labile Alkaline Phosphatase

AP49010	10 Reactions
AP49050	50 Reactions
AP49100	100 Reactions

www.EpiBio.com/t4_rna_ligase.asp

T4 RNA Ligase

LR5010	5 U/µl 1,000 Units
LR5025	5 U/µl 2,500 Units
LR5050	5 U/µl 5,000 Units

Includes 10X Reaction Buffer and a 10 mM ATP Solution.

Impressive Reproducibility of ArrayPure™ RNA Purifications

Bruce W. Jarvis and Agnes Radek, EPICENTRE Biotechnologies

Microarray technology offers an opportunity to assess the reproducibility of RNA preparations over the entire cellular transcriptome—the complete collection of all of a cell's mRNA molecules (transcripts). One possible approach is to convert two replicate preparations of RNA into microarray targets labeled with different fluorescent dyes, and hybridize them to the same array. The normalized fluorescence intensity of each spot is proportional to the abundance of a particular transcript in the RNA preparation. Therefore, a scatter plot displaying the Log_2 signal intensities for the two dyes provides information on how comparable the two RNA preparations are; the more similar they are, the more closely the plot approximates a straight line with a slope of 45°.

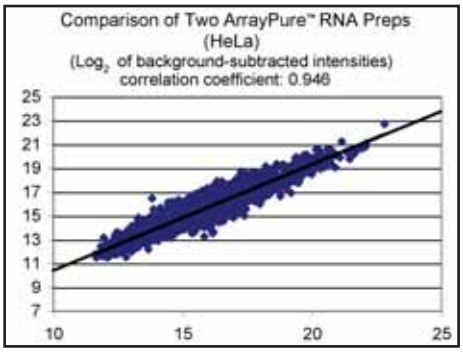

FIG 1. Scatter plot of microarray data indicates a high level of ArrayPure™ purification reproducibility. HeLa cell RNA was purified from two separate T25 tissue culture flasks, labeled with Cy™3 or Cy5, and hybridized to a microarray containing Operon Biotechnologies' Array-Ready Human Oligo Set™ (70-mer). Spotting, target labeling, and hybridizations were conducted by the University of Cincinnati Genomics and Microarray Laboratory.

FIG 1 shows such a scatter plot of microarray data from two HeLa cell RNA purifications prepared from two separate tissue culture flasks with a ten-fold scaled-up version of the ArrayPure™ Nano-scale RNA Purification Kit. A Pearson correlation coefficient of 0.946 indicates a high level of reproducibility of replicate ArrayPure™ RNA purifications.

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ArrayPure™ Nano-scale RNA Purification Kit

MPS04050	50 Purifications

EPI Announcements, etc.

Appointed as—

Key Account Manager

Gordy Hunter joined EPICENTRE Biotechnologies 3 years ago as Marketing Product Manager for PCR and cloning products. Recently, he moved into a new role as **Key Account Manager** to better serve the needs of EPICENTRE's customers in the US. Gordy has over 20 years of experience in sales and marketing of reagents and instruments for life science applications in industry, academia, government and other research institutes. Gordy has undergraduate and graduate degrees in Biology and Biochemistry from Southwestern College (Winfield, KS) and Northern Illinois University (DeKalb, IL).