

# EPICENTRE Forum

Tools and Techniques for Genomics and RNA Research

*Volume 12-1*

*Featuring:*

*TargetAmp™ aRNA  
Amplification Products*

## **TargetAmp™ Kits Improve on the Eberwine Procedure**

**Thermostable Reverse  
Transcriptase Prepares  
Full-Length cDNA**

**Optimize Real-Time PCR  
Using Probes in 1 Run**

**Extract PCR-Ready DNA  
from Fecal Samples**

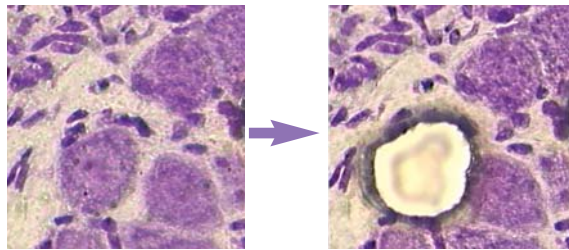


*Cover Photo: Ussing chamber  
Photograph by: Agata Nowinka  
University of Alberta, Edmonton, Alberta, Canada*

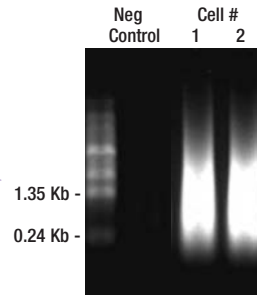
# Now You Can Amplify mRNA from 1 Cell

## Introducing EPICENTRE's New TargetAmp™ Aminoallyl-aRNA Amplification Kit 1.0<sup>a,b</sup>

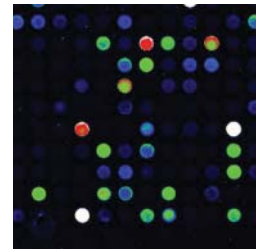
TargetAmp 2-Round AA-aRNA Amplification Kit 1.0 amplifies poly(A)-RNA from laser-captured cells, biopsy samples, cultured cells, or other minute cell or tissue samples by at least 5 million-fold using an improved Eberwine-type linear aRNA amplification method. The product is aminoallyl-labeled antisense RNA (AA-aRNA), also called "cRNA." After reacting the AA-aRNA with amine-reactive biotin or Cy<sup>®</sup>, Alexa<sup>®</sup> or other dye compounds, it can be used as labeled "target" for hybridizing to GeneChip<sup>®</sup>, CodeLink<sup>™</sup> or other commercial microarray chips or self-spotted slides.



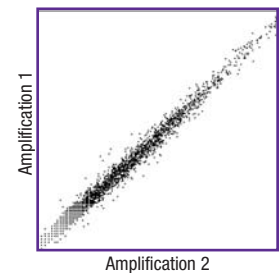
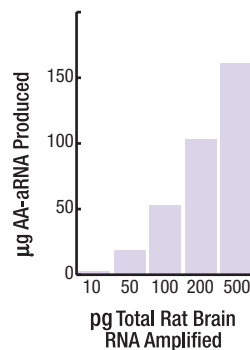
Laser capture of a single neuron from dorsal root ganglion section.



Gel of AA-aRNA obtained from 2 laser-captured cells using TargetAmp™ 2-Round AA-aRNA Amplification Kit 1.0.



Section of microarray hybridized to Cy3-labeled target aRNA amplified from 1 cell using TargetAmp™ Kit 1.0.



Reproducibility of amplification. The correlation coefficient was 0.9932.

### The New TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0

- Uses a simplified 2-day protocol that requires no cDNA cleanup and only ~4 hours of hands-on time.
- Amplifies mRNA from a single cell up to ~50 cells (~10 pg to ~500pg total RNA).
- Reproducibly amplifies mRNA at least 5 X 10<sup>6</sup>-fold.
- Gives low non-specific background amplification.
- Generates aminoallyl-aRNA that is quickly & easily labeled with biotin, Cy<sup>®</sup>, Alexa<sup>®</sup> or other dyes.
- Detects low abundance transcripts; e.g., ~83% "present" calls on a GE CodeLink<sup>™</sup> 10K rat array.
- Has been validated by the microarray group of a leading pharmaceutical company using SuperScript<sup>®</sup> II & III (not included in the kit).

NEW! For more information, go to [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **RNA14**

TargetAmp™ 1-Round aRNA Amplification Kits that amplify mRNA at least 5000-fold in <1 day using 10 ng to 500 ng of total RNA (≥1000 cells) are also available.

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### On the Cover:

EPICENTRE Biotechnologies thanks Agata Nowinka for the use of her photograph of the view from the laboratory of Dr. Marek Duszyk, Department of Physiology, University of Alberta, in Edmonton, Alberta, Canada. Agata worked as an undergraduate student in Dr. Duszyk's lab during the summers of 2003 and 2004. The Duszyk Lab studies the regulation of epithelial ion channels, focusing on the signal transduction pathways mediated by G-protein coupled receptors. The lab is also investigating the function of CFTR, a chloride channel that is mutated in persons with cystic fibrosis. Studies examine how CFTR's interactions with other cellular proteins affect its folding and translocation to the cell membrane. The lab employs various electrophysiological, biochemical, and molecular biology techniques. The Ussing chamber in the photograph is used for transepithelial studies.

### Hands on the Cover:

Rachel McDonald, EPICENTRE Biotechnologies Dispensing and Packaging Technician



# TargetAmp™ 2-Round Kits: An Improved RNA Linear Amplification System for Microarray Analysis from as Little as 1 Cell

Anupama Khanna, Agnes Radek, and Jim Pease, EPICENTRE Biotechnologies

The most commonly used microarray platforms require about 10 µg of labeled antisense RNA (aRNA or cRNA) for each hybridization. This poses a major obstacle for those working with small samples, for example laser-captured cells or biopsy samples. In response to this demand, EPICENTRE Biotechnologies has developed the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0, capable of generating microarray targets from very small biological samples. The TargetAmp Kit 1.0 produces microgram quantities of aminoallyl-aRNA (AA-aRNA) from the total RNA of a single cell (about 10 pg) – representing greater than 5x10<sup>6</sup>-fold amplification of the poly(A) RNA (mRNA). The aminoallyl-UTP used in the TargetAmp procedure is incorporated into the aRNA almost as efficiently as unmodified UTP, resulting in high yields of AA-aRNA. Following conjugation to an amine-reactive biotin- or fluorescent-NHS, the labeled-aRNA can be used to generate high quality DNA microarrays.

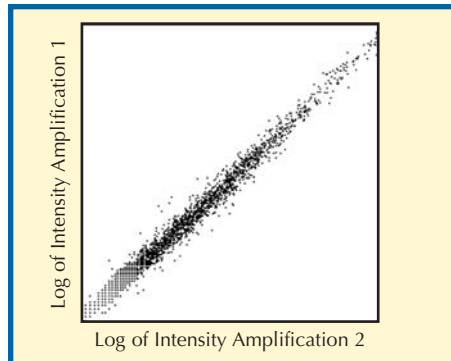
## Methods and Results

### AA-aRNA yield

The yield of AA-aRNA from a variety of cells was determined by performing TargetAmp 2-Round Amplification reactions using decreasing amounts of total RNA. The AA-aRNA produced was purified, and quantitated by UV spectroscopy. Table 1 shows the yield and fold-amplification of the poly(A) RNA contained in rat brain total RNA samples.

### Reduced non-specific amplification products

Baugh, *et al.*,<sup>1</sup> reports that most published Eberwine-based RNA amplification protocols, as well as commercial kits, produce a



**Figure 1. Two independent RNA amplification reactions, each using total RNA from a single laser captured rat dorsal root ganglion cell, amplified using the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0, and then labeled with Cy3-NHS, produced a correlation coefficient of 0.9932, indicative of highly reproducible RNA amplification.**

significant amount of template-independent reaction product (non-specific amplification product) in addition to the desired amplified aRNA. The TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0 utilizes a proprietary chemistry that virtually eliminates non-specific amplification products (see Row 1 of Table 1).

### Reproducible RNA amplification

Reproducible RNA amplification results are critical to the success of long-term gene expression studies using DNA microarrays. The RNA amplification reproducibility of the TargetAmp Kit 1.0 was demonstrated under the most extreme conditions – amplifying total RNA from a single laser-captured cell. Two samples of total cellular RNA, each isolated from a laser captured cell from a rat dorsal root ganglion section, were amplified inde-

pendently using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0. The resulting AA-aRNAs were labeled with Cy<sup>TM</sup>3-NHS and hybridized in parallel to rat arrays. A scatter plot of the fluorescence intensities of the genes detected on the two arrays is shown in Figure 1. The correlation coefficient is 0.9932, indicating highly reproducible amplification.

### Size distribution of the AA-aRNA

Total RNA (about 10 pg) from a single laser captured rat dorsal root ganglion cell, in duplicate, and 200 pg of total RNA from rat brain were amplified using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0. The size distribution of the resulting AA-aRNA produced from the rat dorsal root ganglion cells was analyzed by denaturing agarose gel electrophoresis (Figure 2A) and the AA-aRNA generated from the rat brain RNA was analyzed using an Agilent 2100 Bioanalyzer (Figure 2B). In both examples, the size distribution of the AA-aRNA is between 200 to 4000 bases, with an average size of about 700 bases.

### Quality assessment on GeneChip® Arrays

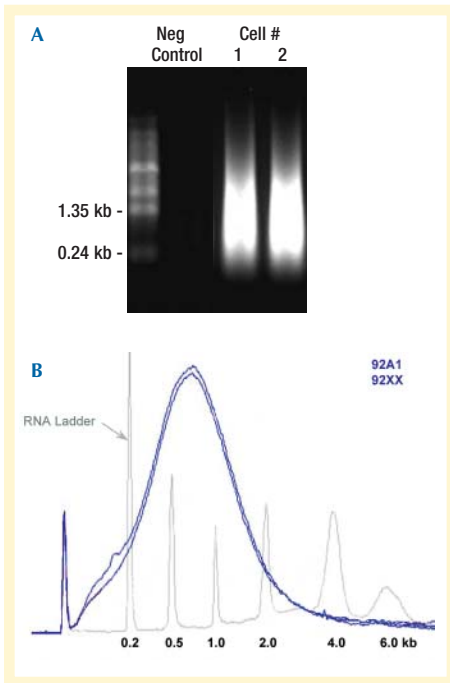
Performance of biotin-aRNA prepared using the TargetAmp 2-Round aRNA Amplification protocol was evaluated on the Affymetrix Genechip platform. Total RNA was isolated from 6-day and 12-day cultured mouse neural cells. The total RNA (400 pg) was amplified using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0 and the resulting AA-aRNA was labeled by conjugation to Biotin-X-X-NHS (See page 7) using a standard conjugation procedure. The resulting biotin-aRNA was fragmented

Rat Brain Total RNA	Rat Brain Poly(A) RNA (assume poly(A) RNA is 5% of total RNA)	AA-aRNA Yield	Fold-Amplification of Poly(A) RNA
0 pg	0 pg	0.6 µg	-----
10 pg	0.5 pg	3 µg	6.0 x 10 <sup>6</sup>
50 pg	2.5 pg	19 µg	7.6 x 10 <sup>6</sup>
100 pg	5 pg	53 µg	10.6 x 10 <sup>6</sup>
500 pg	25 ng	161 µg	6.4 x 10 <sup>6</sup>

**Table 1. Yield of AA-aRNA obtained using the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0.** Results are the average of multiple experiments using rat brain total RNA. Significantly less than 1 µg of non-specific amplification product was produced in the “no-RNA” control reaction.

Fold Amplification of Poly(A) RNA	>5 x 10 <sup>6</sup>
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 2. AA-aRNA produced using the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0, and subsequently biotinylated, generated high quality GeneChip Arrays.**

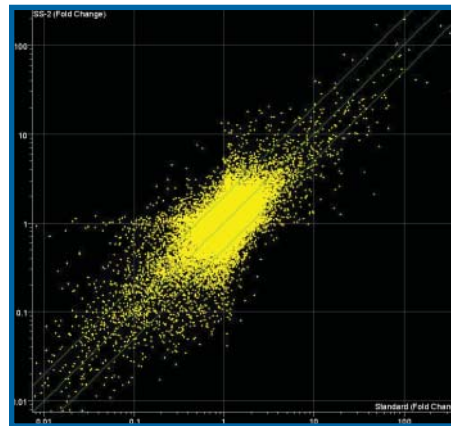


**Figure 2. The TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 produces AA-aRNA with a size distribution between 200 to 4000 bases. A.** Denaturing agarose gel analysis of AA-aRNA produced from 2 independent single-cell RNA amplification reactions. **B.** Agilent 2100 Bioanalyzer analysis of AA-aRNA produced from 200 pg of rat brain total RNA.

and hybridized to GeneChip Mouse Genome 430 2.0 Arrays, in triplicate, by the Gene Expression Center of the University of Wisconsin Biotechnology Center. The averages of the 3'-to-5' ratios and Present Calls were determined with the MAS 5 software (Affymetrix) and are shown in Table 2. The data demonstrates the high quality arrays that are obtained with biotin-aRNA target prepared using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0 and Biotin-X-X-NHS.

**Fidelity of amplification**

To assess the fidelity of the TargetAmp Kit 1.0 amplification process, biotin-aRNA was prepared from total RNA from 6-day and 12-day cultured mouse neural cells using the TargetAmp 2-Round protocol and the standard Affymetrix 1-Round labeling procedure. Each TargetAmp 1.0 amplification/labeling reaction started with 400 pg of total RNA and each Affymetrix labeling procedure started with about 5 µg of total RNA. The resulting biotin-aRNA samples were fragmented and hybridized to GeneChip Mouse Genome 430 2.0 Arrays, in triplicate. Differentially expressed genes were



**Figure 3. Correlation of differentially expressed genes identified by the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 protocol and the standard Affymetrix biotin-aRNA labeling procedure.** The 'Y' axis shows 12-day vs. 6-day cultured mouse neural cell gene expression ratios obtained with biotin-aRNA target produced by the TargetAmp 2-Round Kit 1.0 protocol. The 'X' axis shows the 12-day vs. 6-day gene expression ratios obtained with biotin-aRNA target produced by the Affymetrix standard procedure.

identified using the GeneSpring software package (Silicon Genetics). The expression ratios obtained with the TargetAmp Kit 1.0 protocol and with the Affymetrix protocol are plotted in Figure 3. A correlation coefficient of 0.89 demonstrates good agreement between differentially expressed genes identified using biotin-aRNA target produced by the TargetAmp Kit 1.0 and conjugated to Biotin-X-X-NHS and by the standard Affymetrix labeling method.

**Conclusion**

The TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0:

1. Amplifies poly(A) RNA at least 5x10<sup>6</sup>-fold, producing microgram amounts of AA-aRNA.
2. Minimizes the production of "Background aRNA".
3. Generates AA-aRNA that, once conjugated to Biotin-X-X-NHS or Cy-NHS, produces high quality array results on the GeneChip platform.

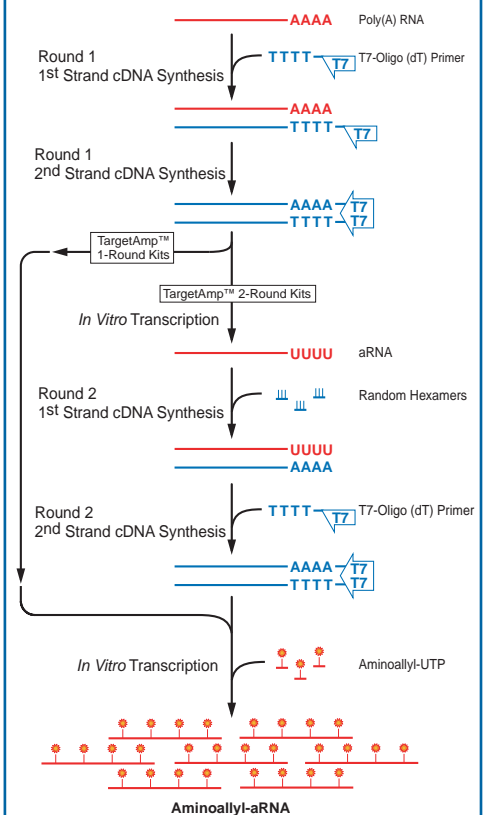
**Reference**

1. Baugh, et al. (2001) *Nucleic Acids Res.* **29**, e29.

Please see Page 7 for ordering information.

The TargetAmp™ 1-Round and 2-Round Aminoallyl-aRNA Amplification Kits utilize an improved "Eberwine" linear RNA amplification process (See Figure) to produce high yields of aminoallyl-aRNA (AA-aRNA). Improvements that EPICENTRE has incorporated into the TargetAmp aRNA Amplification process include:

- Fast procedures. Perform a TargetAmp 1-Round Kit 101 reaction in about 6 hours (less than 2 hours hands-on time). Perform a TargetAmp 2-Round Kit 1.0 reaction in 2 days (~4 hours hands-on time).
- Elimination of cDNA clean-up prior to the *in vitro* transcription reactions, as required by other kits.
- High temperature 1st-strand and 2nd-strand cDNA synthesis reactions to ensure the highest possible yields of full-length cDNAs.
- EPICENTRE's rapid, high-yield AmpliScribe™ *in vitro* transcription technology to maximize yields of AA-aRNA and reduce reaction times.
- Optimized for use with SuperScript™ II & III Reverse Transcriptases (provided by the user).
- Combined reagents to minimize pipetting steps.



**Figure. The TargetAmp™ 1-Round and 2-Round Aminoallyl-aRNA Amplification Kits produce high yields of AA-aRNA using an improved Eberwine linear RNA amplification procedure.**



## Eberwine-type Kits Generate High Yields of aRNA from 25 to 500 ng of Total RNA

Anupama Khanna, Agnes Radek, and Jim Pease, EPICENTRE Biotechnologies

EPICENTRE's new TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 enables the highest yields of aminoallyl-labeled antisense RNA (aRNA or cRNA), in the shortest time, for gene expression profiling of samples containing 25 to 500 ng of total RNA.

The kit uses an improved RNA amplification process (see insert on page 5) based on the method developed by Professor James Eberwine and colleagues.<sup>1</sup> The amplification protocol was improved by identifying better enzymes, optimizing enzyme reactions and reaction times, eliminating column cleanup of cDNA, and reducing the generation of "background RNA." The TargetAmp method uses a simple "One Tube" protocol that produces aminoallyl-aRNA (AA-aRNA). The resulting AA-aRNA can be efficiently biotinylated or dye-labeled using Biotin-X-X-NHS (see page 7) or a Cy™-dye NHS ester, respectively, for subsequent gene expression profiling on Affymetrix GeneChip® Arrays, GE CodeLink™ Arrays, or on other platforms, including self-spotted arrays.

The TargetAmp Kit 101 amplifies poly(A) RNA at least 5000-fold. Examples of AA-aRNA yields obtained from different starting amounts of total RNA from HeLa cells are presented in Table 1.

Here we demonstrate the high quality data that can be obtained using GeneChip Arrays and biotin-aRNA produced by biotinylation of AA-aRNA generated using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101.

HeLa Total RNA	HeLa Poly(A) RNA (assume poly(A) RNA is 2% of total RNA)	Aminoallyl-aRNA Yield	Fold-Amplification of Poly(A) RNA
25 ng	0.5 ng	2.9 µg	5800
50 ng	1.0 ng	5.2 µg	5200
100 ng	2 ng	11.4 µg	5700
500 ng	10 ng	69.9 µg	6990

**Table 1.** Yields of AA-aRNA obtained using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101. Results are the average of multiple experiments using HeLa Total RNA.

### Methods

Total RNA was isolated from mouse neural cells cultured for 6 or 12 days. For each sample, 200 ng were amplified using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 and purified by spin column chromatography. The AA-aRNA was biotin-labeled by conjugation with Biotin-X-X-NHS (EPICENTRE) and purified using the protocols supplied with the product. The resulting biotin-aRNA, as well as biotin-aRNA produced by the standard Affymetrix biotin-aRNA labeling procedure, was fragmented and hybridized to GeneChip Mouse Genome 430 2.0 Arrays, in triplicate.

### Results

Amplified labeled target RNA generated using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 was evaluated for gene expression profiling with Affymetrix GeneChip arrays based on four criteria: the size of AA-aRNA produced, 3'-to-5' ratios, % Present Calls, and the correlation of differential gene expression compared to the standard Affymetrix T7 RNA polymerase amplification protocol.

#### Size distribution of the AA-aRNA

The size of the AA-aRNA produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 was analyzed by denaturing agarose gel electrophoresis and with the Agilent Bioanalyzer. The TargetAmp Kit 101 produced AA-aRNA with a size distribution between 250 to 4000 bases, with an average size of about 1200 bases (data not shown).

#### 3'-to-5' ratios and % Present Calls

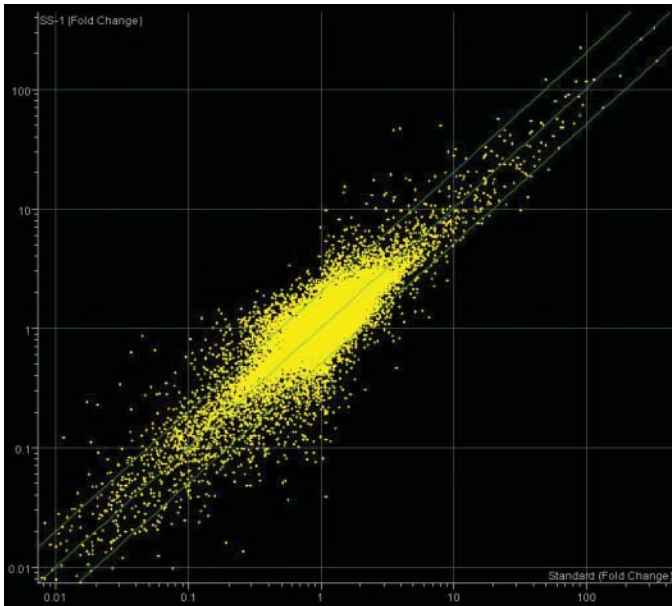
The average of the 3'-to-5' ratios and % Present Calls obtained using biotin-aRNA produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 and Biotin-X-X-NHS are shown in Table 2. These values are well within the quality assessment guidelines established by Affymetrix for target biotin-aRNA performance, and demonstrate that the TargetAmp Kit 101 yields high quality expression profile data using GeneChip Arrays.

<b>Fold Amplification</b>	>5000
<b>3'/5' ratio GAPDH</b>	1.05 +/- 0.05
<b>3'/5' ratio β-actin</b>	2.8 +/- 0.01
<b>% Present Calls</b>	56.3 +/- 2 %
<b>Reproducibility (2 amplifications)</b>	0.99

**Table 2.** AA-aRNA produced using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101, and subsequently biotinylated, produced high quality GeneChip Arrays as determined by Affymetrix quality assessment guidelines.

#### Correlation of differential gene expression

Chips hybridized to biotin-aRNA produced using the TargetAmp Kit 101 protocol were compared to chips hybridized to biotin-aRNA produced using the standard Affymetrix protocol, with respect to differentially expressed genes in mouse neural cells cultured for 6 or 12 days. Differentially expressed genes were identified and compared using the GeneSpring software package (Silicon Genetics). The 12-day vs. 6-day expression ratios obtained with target RNA prepared using the TargetAmp Kit 101 protocols versus the standard Affymetrix protocol are plotted in Figure 1. The correlation coefficient of 0.90 demonstrates good agreement between differentially expressed genes identified by the TargetAmp Kit 101 protocol compared to the standard Affymetrix T7 RNA polymerase protocol.



**Figure 1. Differentially expressed genes identified using biotin-aRNA produced by the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101, and subsequent biotinylation, and biotin-aRNA produced by the standard Affymetrix T7 RNA polymerase protocol gave a correlation coefficient of 0.90.** The 'Y' axis shows 12-day vs. 6-day gene expression ratios obtained using biotin-aRNA target produced by the TargetAmp 1-Round Kit 101 protocol. The 'X' axis shows the 12-day vs. 6-day gene expression ratios obtained using biotin-aRNA target produced by the Affymetrix standard procedure.

**Conclusion**

The TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 amplifies poly(A) RNA at least 5000-fold in 6 hours. This level of amplification results in sufficient AA-aRNA from 25 to 500 ng of total

RNA to produce high quality gene expression profiles using Affymetrix GeneChip or other microarray platforms.

**Reference**

1. Van Gelder, R.N. et al. (1990) *PNAS USA* 87, 663.

*Two TargetAmp 1-Round aRNA Amplification Kits are now available:*

[www.EpiBio.com/targetamp.asp](http://www.EpiBio.com/targetamp.asp)

**TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101\***

Produces **aminoallyl-labeled aRNA** from 25 to 500 ng of total cellular RNA.

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

---

**TargetAmp™ 1-Round aRNA Amplification Kit 103\***

Produces **unlabeled-aRNA** from 25 to 500 ng of total cellular RNA.

TAU1R5110	10 Reactions
TAU1R5124	24 Reactions

\* These kits have been optimized for use with SuperScript™ III (Invitrogen) Reverse Transcriptase, which is not included in the kits.

*Two TargetAmp 2-Round aRNA Amplification Kits are now available:*

[www.EpiBio.com/targetamp.asp](http://www.EpiBio.com/targetamp.asp)

**TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0\***

Produces **aminoallyl-labeled aRNA** from 10 to 500 pg (1 to 50 cells) of total cellular RNA.

TAA2R4910	10 Reactions
TAA2R4924	24 Reactions

---

**TargetAmp™ 2-Round aRNA Amplification Kit 2.0\***

Produces **unlabeled-aRNA** from 10 to 500 pg (1 to 50 cells) of total cellular RNA.

TAU2R5110	10 Reactions
-----------	--------------

\* These kits have been optimized for use with SuperScript™ II & III (Invitrogen) Reverse Transcriptases, which are not included in the kits.

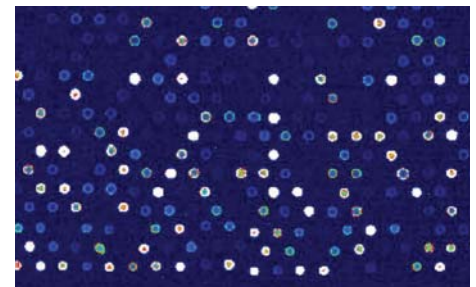
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**Biotin-X-X-NHS, for Biotin Labeling of Aminoallyl-RNA**

Biotin-X-X-NHS provides an efficient and cost-effective method for labeling aminoallyl-aRNA or aminoallyl-RNA produced using EPICENTRE's TargetAmp™ Aminoallyl-aRNA Amplification Kits (see Page 4) or AmpliScribe™ T7 Aminoallyl-RNA Transcription Kit, respectively.



**EPICENTRE's Biotin-X-X-NHS:**

- Contains a long spacer arm to maximize binding to avidin or streptavidin conjugates by minimizing steric hindrance between multiple biotinylated sites.
- Is supplied as a convenient 2.5 mg powder in an air-tight vial with a self-sealing septum.
- Is packaged under dry inert gas and is stable for at least one year when stored in powdered form at +4°C.
- Provides more cost-effective labeling than direct incorporation of biotin-UTP.

Biotin-X-X-NHS can also be used for labeling aminoallyl-cDNA and proteins.

[www.EpiBio.com/biotin.asp](http://www.EpiBio.com/biotin.asp)

**Biotin-X-X-NHS**

BXX51005	5X	2.5 mg vials
BXX51010	10X	2.5 mg vials

# Purify RNA from a Single Cell Using the ArrayPure™ Nano-scale RNA Purification Kit

Bruce W. Jarvis and Haiying Grunenwald, EPICENTRE Biotechnologies

The recently introduced ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from 1 to 10,000 eukaryotic cells, quantities typically obtained with laser-capture procedures.<sup>1,2</sup> Previously we showed that ArrayPure RNA from 20 HeLa cells could be successfully used in 1-round and 2-round RNA amplification procedures.<sup>3</sup> We also compared the real-time RT-PCR results for RNA purified from 10 to 10,000 HeLa cells by the ArrayPure Kit and another supplier's kit.<sup>3</sup> Here we present real-time RT-PCR data for RNA purified from 1, 10, and 100 cells, demonstrating the ability of the ArrayPure Kit to purify RNA from a single cell.

## Methods

HeLa cells were grown in tissue culture, aseptically diluted, and trapped inside sterile 5- $\mu$ l microcapillary pipets.<sup>4</sup> The number of cells isolated was verified by observation with an inverted microscope. Cells were eluted by centrifugation from the capillary pipets, washed with phosphate buffered saline, and RNA was purified using the ArrayPure Kit.

To compare the amount of RNA purified from varying numbers of cells, cDNA was prepared from the RNA using EPICENTRE's MMLV Reverse Transcriptase, and the human  $\beta$ -actin gene was amplified by real-time PCR using the FailSafe™ PROBES Real-Time PCR System (See page 18). Figure 1A shows the amplification plot of the cDNA from an average of 1, 10, and 100 cells. The negative control sample contained only tissue culture media that had gone through the purification procedure and that, as expected, did not amplify. Plotting the threshold cycle ( $C_T$ ) vs log of the average initial cell number gives a standard curve (Figure 1B) that indicates linearity of response over 3 orders of magnitude from 1 to 100 cells with a correlation coefficient of 0.992.

## Conclusion

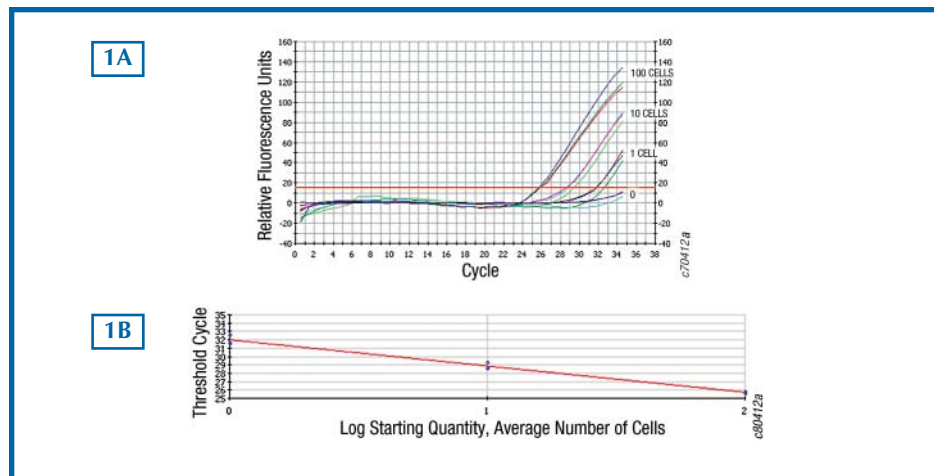
The new ArrayPure RNA Purification Kit successfully prepares RNA from a single eukaryotic cell, with the purity and quality necessary to prepare cDNA or to be amplified in 1-round and 2-round RNA amplification procedures.

## Acknowledgments

We wish to thank Ramesh Vaidyanathan, Anupama Khanna, and Judy Meis for technical discussions and preliminary data.

## References

- Emmert-Buck, M.R. *et al.* (1996) *Science* **274**, 998.
- Bonner, R.F. *et al.* (1997) *Science* **278**, 1481.
- Jarvis, B.W. and Meis, J.E. (2004) *EPICENTRE Forum* **11**(6), 5.
- Harbeck, M.C. and Rothenberg, P.L. pp. 27-33 in "Gene cloning and analysis by RT-PCR," eds. P.D. Siebert and J.W. Larrick, BioTechniques Books, Natick, MA, 1998.



**Figure 1. Real-time RT-PCR amplification data shows RNA purification from a single cell.**

RNA purified from HeLa cells using the ArrayPure™ Nano-scale RNA Purification Kit was converted to cDNA using EPICENTRE's MMLV Reverse Transcriptase. The corresponding cDNAs were amplified, in triplicate, using the FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit (PreMix 3). Real-Time PCR results were obtained using the Bio-Rad iCycler iQ™ Real-Time PCR Detection System and cycling conditions of 94°C (2 minutes) followed by 35 cycles of 95°C (10 seconds), 60°C (10 seconds), and 72°C (30 seconds). Primers used were for the human  $\beta$ -actin gene: 5'- TGG ACA TCC GCA AAG ACC TG and 5'- CCG ATC CAC ACG GAG TAC TT. The TET/BHQ1 dual-labeled fluorescent probe was 5'- TET-CAC CAC CAT GTA CCC TGG CAT TGC C-3'-BHQ1. **A.** The amplification plot for reactions containing cDNA prepared from an average of 100, 10, 1, and 0 (medium alone) cells. **B.** The standard curve produced by plotting the log of the average initial HeLa cell number against the cycle threshold ( $C_T$ ) is linear. Slope = -3.14; Correlation Coefficient = 0.992.

[www.EpiBio.com/arraypure.asp](http://www.EpiBio.com/arraypure.asp)

### ArrayPure™ Nano-scale RNA Purification Kit

MPS04050 50 Purifications

#### Contents:

Nano-scale Lysis Solution  
2X Nano-scale Lysis Solution  
MPC Protein Precipitation Reagent  
RNase-free DNase I  
1X DNase Buffer  
Proteinase K  
TE Buffer

[www.EpiBio.com/failsafeprobes.asp](http://www.EpiBio.com/failsafeprobes.asp)

### FailSafe™ PROBES Real-Time PCR Optimization Kit

FSP51048 48 25- $\mu$ l Reactions

#### Contents:

FailSafe™ PCR Enzyme Blend  
8 FailSafe™ PROBES Real-Time PCR  
2X PreMixes  
Passive Reference Dye  
Stabilizer




### FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit

FSP51200 200 25- $\mu$ l Reactions  
FSP5101K 5 x 200-Reaction Kits

#### Contents:

FailSafe™ PCR Enzyme Blend  
Passive Reference Dye  
Stabilizer  
Your choice of any two FailSafe™ PROBES  
Real-Time PCR 2X PreMixes per Kit

# TERMINATOR™ EXONUCLEASE

RNA Structure	Degraded	Examples
5'pN  OH <sup>3'</sup>	Yes	- Eukaryotic rRNA - Prokaryotic rRNA - RNA produced by RNase III processing
5'GpppN  OH <sup>3'</sup>	No	- Eukaryotic mRNA (capped)
5'pppN  OH <sup>3'</sup>	No	- Bacterial primary transcripts

Does not degrade double-stranded RNA or RNA with extensive secondary structure such as tRNA.

## Terminator™ 5'-Phosphate- Dependent Exonuclease

*Terminator™ 5'-Phosphate-Dependent Exonuclease is a processive 5'-to-3' exonuclease that digests RNA substrates with a 5'-monophosphate. RNA with a 5'-cap, a 5'-triphosphate, or a 5'-hydroxyl are resistant to degradation. Terminator Exonuclease is ideal for removing large ribosomal RNA (rRNA) from preparations of mRNA (patent pending).*

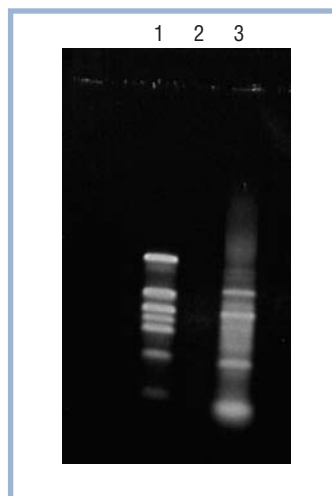
## RNA Applications

- \* Remove large rRNA (i.e., 18S and 28S) to enrich mRNA.
- \* Use for high-throughput isolation of eukaryotic mRNA, without using an oligo(dT) matrix.
- \* Isolate primary prokaryotic RNA transcripts.
- \* Improve the sensitivity of Northern blots.
- \* Active in the presence of RNase Inhibitors such as RNasin®.

## Terminator™ 5'-Phosphate-Dependent Exonuclease removes rRNA from total RNA

### Figure 1. Terminator™ 5'-Phosphate-Dependent Exonuclease removes rRNA from total RNA.

Total RNA was purified from green tobacco leaves and diluted in Terminator Exonuclease Reaction Buffer. An aliquot of the diluted total RNA was reserved as untreated RNA. Two units of Terminator Exonuclease were added to 9 µg of diluted total RNA and incubated at 30°C for 1 hour. After incubating, an aliquot of the reaction was reserved as Terminator-treated RNA and the remaining reaction was ethanol precipitated and resuspended in TE buffer to concentrate the reaction 40-fold. Samples were assayed on a denaturing agarose gel. **Lane 1**, 200 ng of untreated total RNA; **Lane 2**, 200 ng of Terminator-treated RNA; **Lane 3**, 40-fold concentration of the Terminator-treated RNA. Terminator Exonuclease removes rRNA from total RNA, leaving 5'-capped mRNA.



### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **TRD01**

#### Terminator™ 5'-Phosphate-Dependent Exonuclease

TER51020      1 U/µl      20 Units

Includes Terminator™ Exonuclease 10X Reaction Buffer.



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# In Vitro Transcription Product Selection Guide

*EPICENTRE is the leader in in vitro transcription technology, with more-innovative and higher-performing products for transcription than any other company. The guide below will help you to select the best products for your application.*

Product name	High-yield synthesis of RNA for work on RNA structure, processing, ribozymes, aptamers, RNAi or anti-sense	Synthesis of modified RNA resistant to RNase A	Synthesis of non-radioactive RNA probes for Northern, Southern & <i>in situ</i> hybridization	Synthesis of high-specific-activity radioactive RNA probes	Screening for RNA polymerase activity or for RNAP inhibitors (e.g., as potential antibiotics)	Transcription of 5'-capped RNA for <i>in vitro</i> translation & microinjection	RNA amplification for DNA microarray analyses	Synthesis of RNA using ssDNA templates & single-stranded promoters
AmpliScribe™ T7-Flash™ & T3-Flash™ Transcription Kits	+++++		++++			+++ (Cap analog sold separately)	++++	
AmpliScribe™ T7, T3 & SP6 High Yield Transcription Kits	++++		+++			+++ (Cap analog sold separately)	+++	
DuraScribe® T7 & SP6 Transcription Kits	+++	+++++				Modified RNA product not suitable for translation		
MessageMuter™ shRNA Production Kit	++++							
AmpliScribe™ T7 Aminoallyl-RNA Transcription Kit			+++++				++++	
RiboScribe™ T7, T3 & SP6 RNA Probe Synthesis Kits				+++++				
AmpliCap-MAX™ T7 & T3 High Yield Message Maker Kits						++++		
AmpliCap™ T7 & SP6 High Yield Message Maker Kits						+++		
MessageMAX™ T7 Capped Message Transcription Kit						+++++		
TargetAmp™ 1-Round aRNA Amplification Kits							+++++	
TargetAmp™ 2-Round aRNA Amplification Kits							+++++	
Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit					+++++			
Kool™ NC-45™ Universal RNA Polymerase Templates					+++++			
MiniV™ <i>In Vitro</i> Transcription Kit	++							+++++

# Simple and Rapid Plant Leaf DNA Purification



## MasterPure™ Plant Leaf DNA Purification Kit

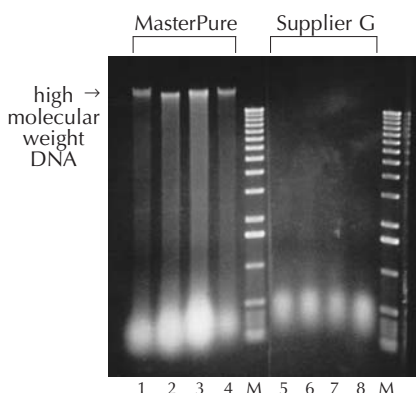
The MasterPure™ Plant Leaf DNA Purification Kit isolates DNA from 35-100 mg of fresh plant leaf tissue. The protocol can be completed in less than one hour and may be scaled up to obtain larger amounts of DNA. The purified DNA can be used for PCR amplification, restriction digestion, and Southern blotting.

### Applications

- \* Extract DNA from plant leaves high in polysaccharides
- \* Plants tested:
  - Grape
  - Apple
  - Tomato
  - Corn
  - Sugarcane
  - Sunflower
  - Fern
  - Quillwort
  - Pine
  - *Arabidopsis*

### Benefits

- \* Rapid: less than 1 hour
- \* High molecular weight DNA
- \* Simple and non-toxic
- \* Higher DNA integrity



**Figure 1. Higher yields of DNA isolated with the MasterPure™ Kit versus another supplier's kit.** 10 µl of each DNA preparation (either MasterPure or Supplier G) were separated by electrophoresis in a 0.7% agarose gel. Lanes M, 1-kb DNA ladder; Lanes 1-4, Pinot noir grape leaf DNA purified with the MasterPure Kit; Lanes 5-8, Pinot noir grape leaf DNA purified with Supplier G kit.

#### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **PLD01**

#### MasterPure™ Plant Leaf DNA Purification Kit

MPP92010      10 Purifications  
MPP92100      100 Purifications

#### Contents:

Plant DNA Extraction Solution  
Cleanup Solution  
TE Buffer

#### Related Products

SoilMaster™ DNA Extraction Kit  
WaterMaster™ DNA Purification Kit



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## SoilMaster™ DNA Extraction Kit

The SoilMaster™ DNA Extraction Kit provides a reliable, simple method for producing PCR-ready DNA from soil and sediment samples. This method is based on hot-detergent lysis and incorporates an inhibitor removal spin column. The SoilMaster™ Kit isolates more intact DNA than other soil DNA kits incorporating bead-beating or vortex mixing in the presence of beads.

### Applications

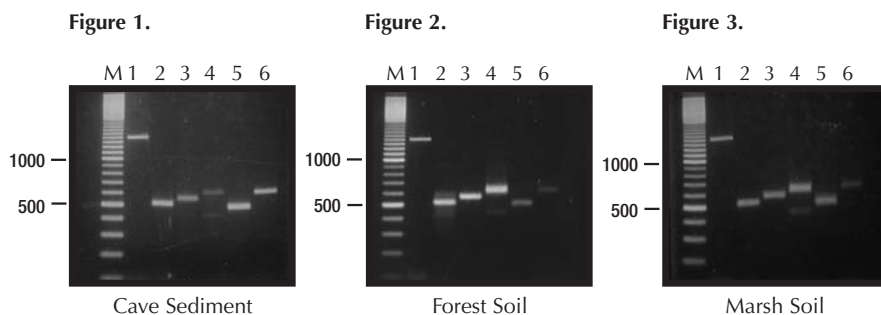
- ✦ Extraction of PCR-ready DNA from soil and sediment for PCR amplification of diverse organisms
- ✦ Humic acid and protein removal

### Benefits

- ✦ Extract PCR-ready microbial DNA from soil in less than 45 minutes
- ✦ No need for extra equipment or bead-beating
- ✦ Recover higher molecular weight DNA



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**Figures 1-3.** One microliter of extracted soil DNA (less than 1% of the total purified) was amplified using the FailSafe™ PCR System and primers to the following templates; **Lanes 1 and 2**, consensus bacterial primers to the 16S ribosomal RNA gene; **Lane 3**, Fungi, protists, and green algae primers; **Lane 4**, Plant primers NS3/NS4; **Lane 5**, High G+C gram positive bacterial primers; and **Lane 6**, *Bacillus* primers.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **SMD02**

#### SoilMaster™ DNA Extraction Kit

SM02050 50 Reactions

#### Contents:

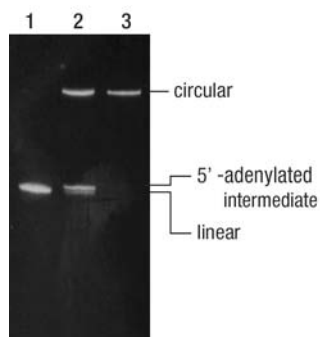
Soil DNA Extraction Buffer  
Proteinase K  
Soil Lysis Buffer  
Protein Precipitation Reagent  
Inhibitor Removal Resin  
Spin Columns  
DNA Precipitation Solution  
Pellet Wash Solution  
TE Buffer

#### Related Products

EasyLyse™ Bacterial Protein Extraction Solution  
MasterPure™ Gram Positive DNA Purification Kit  
MasterPure™ Yeast DNA Purification Kit  
MasterPure™ Yeast RNA Purification Kit

## Make Circular ssDNA

- ✓ Without “Bridges” or “Splints”
- ✓ Without T4 DNA Ligase
- ✓ Without Producing Concatamers



**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).

### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **CLD01**

#### CircLigase™ ssDNA Ligase

CL4111K 1,000 Units  
CL4115K 5,000 Units

#### Contents:

CircLigase™ ssDNA Ligase  
CircLigase™ 10X Reaction Buffer  
ATP  
MnCl<sub>2</sub>  
ssDNA Control  
Water

#### Related Products

Exonuclease I  
Kool™ NC-45™ Universal RNA Polymerase Template  
Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit  
*E. coli* RNA Polymerase Core Enzyme & Holoenzyme  
*Thermus* Thermostable RNA Polymerase  
Tagetin™ RNA Polymerase Inhibitor  
MiniV™ *In Vitro* Transcription Kit  
Phage T7, T3 & SP6 RNA Polymerase

## 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 virtually no linear or circular concatamers are produced, making the enzyme ideal for preparing circular ssDNA templates for rolling circle replication or rolling circle transcription.*

### Applications

Produce circular ssDNA templates for:

- \* Rolling circle replication experiments
- \* Rolling circle transcription experiments
- \* RNA polymerase activity and inhibitor screening assays (see reverse)

### Benefits

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



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# Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit

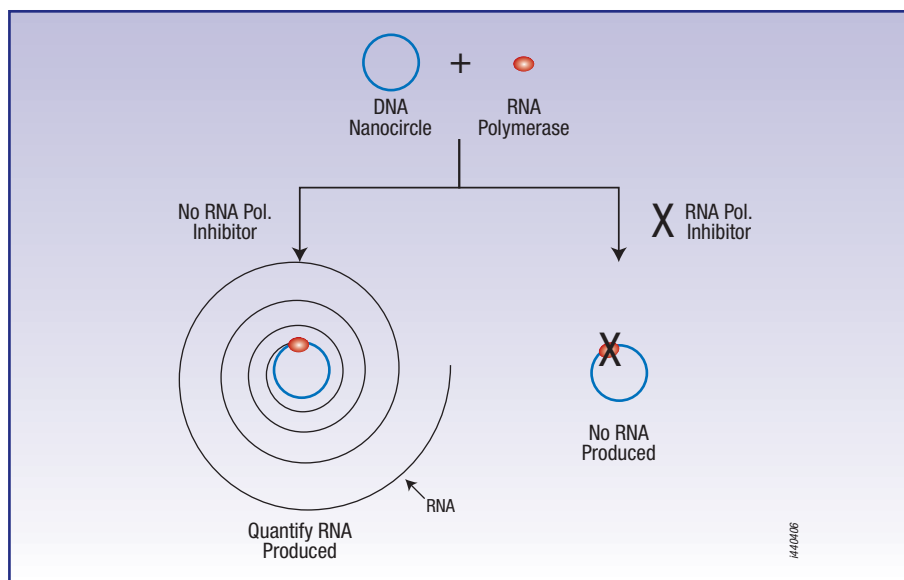
The Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit provides a rapid and sensitive assay for studying the activity of DNA-dependent RNA polymerases (RNAPs) and screening compounds for their ability to inhibit *E. coli* RNAP or an RNAP provided by the user.

## Applications

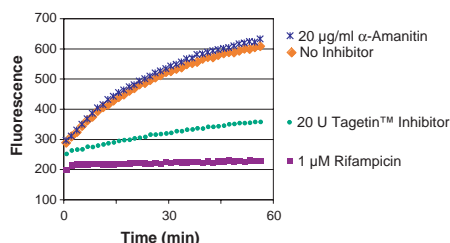
- ✦ Assay for DNA-dependent RNAP activity
- ✦ Screen compounds for RNAP inhibitor activity

## Benefits

- ✦ RNAP activity can be assayed without a promoter sequence
- ✦ High product-to-template ratio
- ✦ Compatible with a variety of detection methods
- ✦ Easy to automate for high-throughput screening



Based on patented technology developed by Dr. Eric Kool, the assay utilizes a 45-base circular single-stranded DNA molecule that functions as a template for rolling circle transcription. Transcription from this Kool™ NC-45™ Universal RNA Polymerase Template (also available separately) does not require a promoter sequence or primer, and bacterial RNAPs can transcribe Kool Templates in the absence of sigma factors. The Kool NC-45 Screening Kit includes SYBR® Green I Dye for real-time detection of RNAP activity, but alternative protocols are given for other real-time and end-point detection methods.



**Figure 1. Real-time detection of rolling circle transcription by *E. coli* RNAP in the presence and absence of inhibitors.** The Kool™ NC-45™ Template was incubated with *E. coli* RNAP Core Enzyme, SYBR® Green I dye, and the inhibitors indicated above. Strong inhibition by rifampicin, a known inhibitor of bacterial RNA polymerase, and partial inhibition by Tagetin™ Inhibitor can be detected, while α-amanitin, an inhibitor of eukaryotic RNA Polymerase II, has no effect.

### NEW! Easy Shortcut to Product Information

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#### Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit

(for Bacterial RNA polymerases)

KNK49025 25 Reactions

##### Contents:

Kool™ NC-45™ Template  
*E. coli* RNA Polymerase Core Enzyme  
 RNase Inhibitor  
 5X Reaction buffer  
 NTPs  
 DTT  
 SYBR® Green I Dye  
 Sterile Nuclease-free Water

#### Related Products

Kool™ NC-45™ Universal RNA Polymerase Template  
*E. coli* RNA Polymerase Core Enzyme  
*E. coli* RNA Polymerase Holoenzyme  
 Tagetin™ RNA Polymerase Inhibitor  
 NTPs  
 CirLigase™ ssDNA Ligase

**FIRST TIME**

Perform real-time PCR with your template and primers using the FailSafe™ GREEN Real-Time PCR Optimization Kit and select the optimal Real-Time PCR PreMix.

**and EVERY TIME**

For consistent PCR results, choose the PCR PreMix identified as optimal when you order the FailSafe™ GREEN Real-Time PCR PreMix-Choice Kit.

## FailSafe™ GREEN Real-Time PCR System

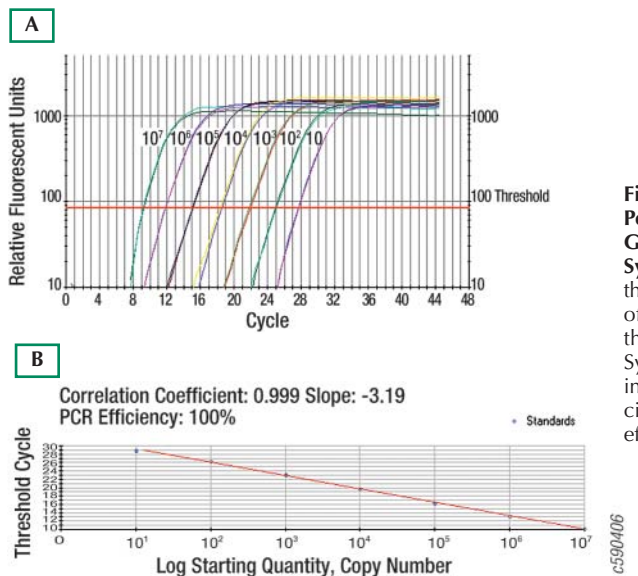
The FailSafe™ GREEN Real-Time PCR System uses the FailSafe™ PCR Enzyme Mix and an extensively tested set of 12 FailSafe™ GREEN PCR PreMixes (or 8 PreMixes with the Capillary Kit), representing a range of PCR conditions. These PreMixes contain everything you need for successful quantitative PCR: SYBR® Green I Dye, dNTPs, buffer, and varying amounts of MgCl<sub>2</sub> and EPICENTRE's patented FailSafe™ PCR Enhancer with Betaine\*.

### Applications

- ✧ Optimize real-time PCR conditions in one round of PCR. Then perform successful real-time PCR every time with the optimal PreMix and that template and primer pair.

### Benefits

- ✧ Real-Time PCR PreMixes contain all components for quantitative PCR using SYBR® Green I Dye.
- ✧ No specific probes required.
- ✧ Includes ROX, a passive reference dye.
- ✧ Kits available for both tube and capillary instruments.
- ✧ No hot-start necessary.



**Figure 1. Representative Performance of FailSafe™ GREEN Real-Time PCR System.** **A.** Real-Time PCR of the *cll* gene in 10-10<sup>7</sup> copies of lambda DNA using the Bio-Rad iCycler iQ™ System. **B.** Standard curve indicates a correlation coefficient of 0.999 and 100% PCR efficiency.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **FGD01**

#### FailSafe™ GREEN Real-Time PCR Optimization Kit

FSR0360 96 25-µl Reactions

##### Contents:

FailSafe™ PCR Enzyme Mix  
12 FailSafe™ GREEN Real-Time PCR 2X PreMixes  
Passive Reference Dye

#### FailSafe™ GREEN Real-Time PCR PreMix-Choice Kit

FSR03200 400 25-µl Reactions

##### Contents:

FailSafe™ PCR Enzyme Mix  
Passive Reference Dye  
Your choice of any two FailSafe™ GREEN Real-Time PCR 2X PreMixes

#### FOR CAPILLARY-BASED REAL-TIME PCR INSTRUMENTS

#### FailSafe™ GREEN Real-Time PCR Capillary Optimization Kit

FSRC3832 32 20-µl Reactions

##### Contents:

FailSafe™ PCR Enzyme Mix  
8 FailSafe™ GREEN Real-Time PCR Capillary 2X PreMixes

#### FailSafe™ GREEN Real-Time PCR Capillary PreMix-Choice Kit

FSRC3896 96 20-µl Reactions

FSRC38384 4 x 96 20-µl Reactions

##### Contents:

FailSafe™ PCR Enzyme Mix  
Your choice of one FailSafe™ GREEN Real-Time PCR Capillary 2X PreMix per Kit

\*Covered by issued and pending patents. 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.



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# FailSafe™ PROBES Real-Time PCR System

The FailSafe™ PROBES Real-Time PCR System enables rapid, precise optimization of PCR experiments that use target-specific probes for detection.

## Applications

- ✦ One-step optimization of PCR efficiency and specificity.
- ✦ Optimization of multiplex real-time PCR assays detected using target-specific probes.

## Benefits

- ✦ Simple, one-step optimization protocol.
- ✦ 8 PCR PreMixes contain all components for screening a complete range of real-time PCR conditions.
- ✦ Extremely high sensitivity, specificity, and PCR efficiency.
- ✦ FailSafe PROBES Real-Time PCR System is compatible with all real-time PCR instruments and fluorescent probes.
- ✦ No hot-start necessary.



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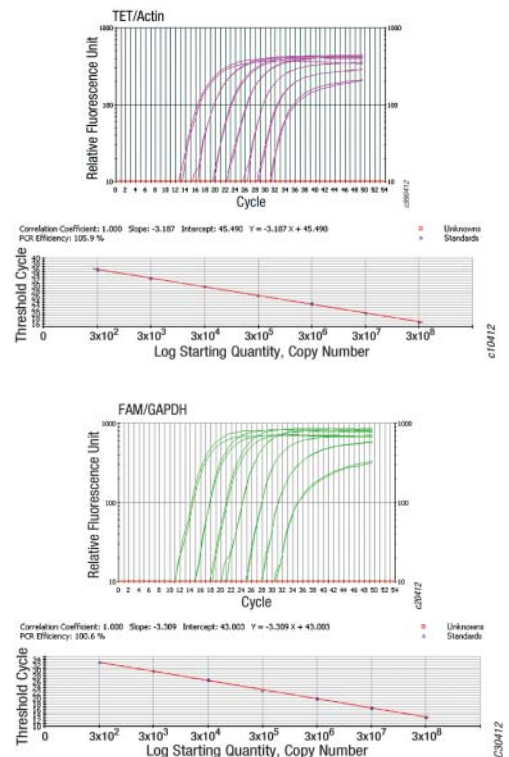
### FIRST TIME

Perform real-time PCR with your template, primers, and probes using FailSafe™ PROBES Real-Time PCR Optimization Kit and select the optimal Real-Time PCR PreMix.

### and EVERY TIME

Get the selected Real-Time PCR PreMix with FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit and use it for consistent PCR with the same template, primers, and probes.

**FIG 1. Excellent multiplexing can be achieved with FailSafe™ PROBES Real-Time PCR System.**  $3 \times 10^2$  to  $3 \times 10^8$  copies of each plasmid, containing either a fragment of human  $\beta$ -actin or GAPDH gene, were amplified in 25- $\mu$ l reactions using the FailSafe PROBES Real-Time PCR PreMix P3.  $\beta$ -actin probe was labeled with TET and the GAPDH probe was labeled with FAM. Highly consistent duplicates showed good linearity with a correlation coefficient of 1.000 for both genes and excellent PCR efficiency of 105.9% and 100.6% for actin and GAPDH, respectively, over a broad 7-log dynamic range.



### NEW! Easy Shortcut to Product Information

Go to our website at [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **FPD01**

#### FailSafe™ PROBES Real-Time PCR Optimization Kit

FSP51048 48 25- $\mu$ l Reactions

##### Contents:

- FailSafe™ PCR Enzyme Blend
- 8 FailSafe™ PROBES Real-Time PCR 2X PreMixes
- Passive Reference Dye
- Stabilizer

#### FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit

FSP51200 200 25- $\mu$ l Reactions  
FSP5101K 5 x 200-Reaction Kits

##### Contents:

- FailSafe™ PCR Enzyme Blend
- Passive Reference Dye
- Stabilizer
- Your choice of any two FailSafe™ PROBES Real-Time PCR 2X PreMixes per Kit

# Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

## Questions about TargetAmp™ Aminoallyl-aRNA Amplification Kits

**Q.** When should I use the 1-Round vs. the 2-Round TargetAmp™ aRNA Amplification Kit?

**A.** Use the TargetAmp 1-Round aRNA Amplification Kit to produce micrograms of antisense RNA (aRNA) from as few as 1000 cells or 25 ng total cellular RNA. The TargetAmp 2-Round Kit efficiently amplifies RNA from one cell, or as little as 10 to 500 pg of total cellular RNA. For more specific yield data, please see the Tables on pages 4 and 6. If you are planning to compare multiple samples, be sure that all of the samples are prepared by the same procedure. (i.e., all 1-Round or all 2-Round)

**Q.** What is the best way to preserve samples and purify RNA for use with the new TargetAmp™ aRNA Amplification Kits?

**A.** Any RNA Purification Kit that produces highly purified, full-length RNA will work fine. We recommend our ArrayPure™ RNA Purification Kit, which can purify RNA from as little as one cell (see page 8), or our MasterPure™ RNA Purification Kit for >10,000 cells. When using any salt-based extraction system, like the ArrayPure and MasterPure Kits, we advise not using RNAlater®-Ice to preserve the samples prior to extraction, because it may interfere with the proteinase K digestion step. Instead, use a snap-freeze technique with a dry ice/ethanol bath followed by storage at -80°C until RNA Purification. The purified RNA should be resuspended in water, and be free of salts, ethanol, and genomic DNA.

**Q.** In the TargetAmp procedure, the second-strand cDNA synthesis reaction (first round) is incubated at 65°C. Why is that? Other procedures incubate this reaction at 16°C.

**A.** The TargetAmp Kits use a unique, proprietary thermostable DNA polymerase that works optimally at 65°C. This enzyme contributes significantly to the very high aRNA yields obtained with the TargetAmp Kits.

**Q.** What amount of RNA amplification can I expect with the TargetAmp 1-Round and 2-Round aRNA Amplification Kits?

**A.** With the 1-Round Amplification Kit, you can expect at least 5000-fold amplification of the poly(A) RNA; with the 2-Round Kit, at least  $5 \times 10^6$ -fold amplification of the poly(A) RNA is typical.

**Q.** How can I calculate the fold-amplification of aRNA that I get from my starting total RNA?

**A.** Depending upon the tissue or cell type being used, the poly(A) RNA fraction can be from 1% to 5% of the total RNA in a cell. Thus, when calculating the fold-amplification of a TargetAmp aRNA amplification, consider the amount of the poly(A) RNA, not the total RNA. From 100 pg of total RNA, let's estimate that the starting poly(A) RNA is approximately 3 pg (3%). If amplification produces 20 µg of RNA, the procedure resulted in  $\sim 7 \times 10^6$ -fold amplification of the poly(A) RNA.

We strive to provide our customers with the most accurate technical support – both in selecting an appropriate product and answering your technical questions. If you need more information about the TargetAmp products, or any EPICENTRE products, please contact us.  
Fred and Hank

**Q.** Why is the non-specific background amplification so low with the TargetAmp 2-Round aRNA Amplification Kit?

**A.** We use a proprietary background reduction chemistry to greatly reduce, or even eliminate, this problem.

**Q.** Can I use the TargetAmp Kits to make biotin-aRNA for use with Affymetrix GeneChip® arrays?

**A.** Yes, two TargetAmp Kits produce aminoallyl-aRNA, which can be readily conjugated to Biotin-X-X-NHS to produce biotin-aRNA (See page 7). The biotin-aRNA can be used as target on GeneChip® arrays.

**Q.** Can I quantitate my aminoallyl-labeled RNA by real-time RT-PCR?

**A.** Yes, see the article on page 20 for more information on this technique.

FIND OUT MORE ABOUT THE TARGETAMP™ 1-ROUND ARNA AMPLIFICATION KITS ON PAGE 6 AND THE TARGETAMP™ 2-ROUND KITS ON PAGE 4.

info



# FailSafe™ PROBES Real-Time PCR System Enables Easy, Reliable Optimization of Allelic Discrimination Assays

Anne E. Jedlicka and Margaret Mintz

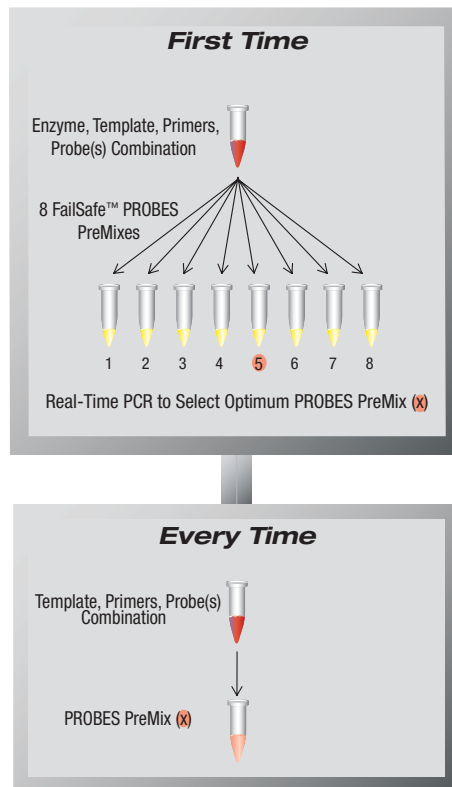
Malaria Research Institute, Gene Array Core Facility, Johns Hopkins Bloomberg School of Public Health

Quantitative real-time PCR is widely used to measure copy number, viral load, and gene expression (including microarray validation) and to detect mutations (e.g., SNPs or allelic discrimination) and microbiological contamination.<sup>1</sup> Assays are relatively easy to perform, capable of high throughput, and can combine high sensitivity with reliable specificity.<sup>2</sup> However, the optimization of reaction conditions is critical for successful quantification by real-time PCR. Lack of optimization can lead to problems ranging from no detectable PCR product to poor amplification efficiency, resulting in inaccurate quantification. Here EPICENTRE's FailSafe™ PROBES Real-Time PCR System was used by the Malaria Research Institute at the Johns Hopkins Bloomberg School of Public Health to optimize an allelic discrimination clustering assay designed to genotype the ApoE Arg112Cys single-nucleotide polymorphism (SNP).<sup>3</sup>

The FailSafe PROBES Real-Time PCR System has two components: an Optimization Kit and a PreMix-Choice Kit. The System provides a fast and economical method to optimize real-time PCR experiments in which the product is detected with fluorescent-labeled probes. Optimum real-time PCR conditions for virtually any template(s), primers, and probe(s) combination can be achieved in just one round of PCR experiments. This new real-time PCR system provides both high specificity and high sensitivity, ensuring successful PCR the first time and every time.

### First time – determine optimum PreMix

Initially, use the FailSafe™ PROBES Real-Time PCR Optimization Kit to determine the optimal PCR PreMix for an individual or multiplex PCR reaction. This FailSafe Kit contains the FailSafe™ PCR Enzyme Blend and a set of 8 optimized FailSafe™ PROBES Real-Time PCR 2X PreMixes, which represent a complete range of real-time PCR conditions. PreMixes contain everything needed for successful PCR, including dNTPs, buffer, varying levels of a magnesium salt, and EPICENTRE's PCR Enhancer with Betaine\* for optimal PCR



efficiency, specificity, and sensitivity. Just add your template, primers, and probe(s) to each PreMix, combined with the FailSafe Enzyme Blend, and run the thermocycler. At least one FailSafe PROBES PreMix will provide optimal reagent conditions.

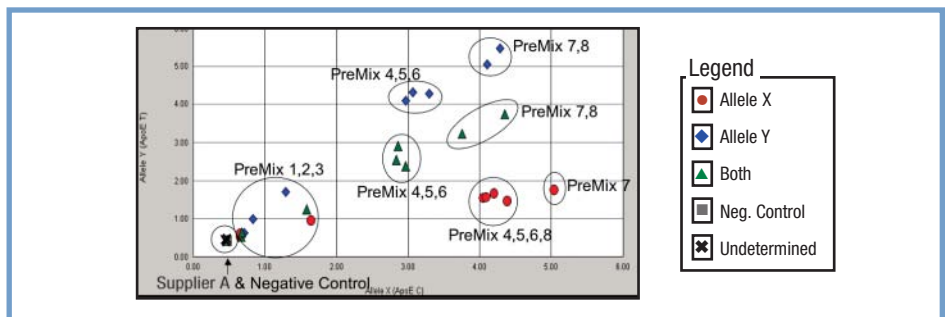
### Every time – consistent optimal PCR

Use the PreMix determined to be optimal with the FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit to obtain consistent, reliable results in all subsequent real-time PCR assays with the same template, primers, and probe(s).

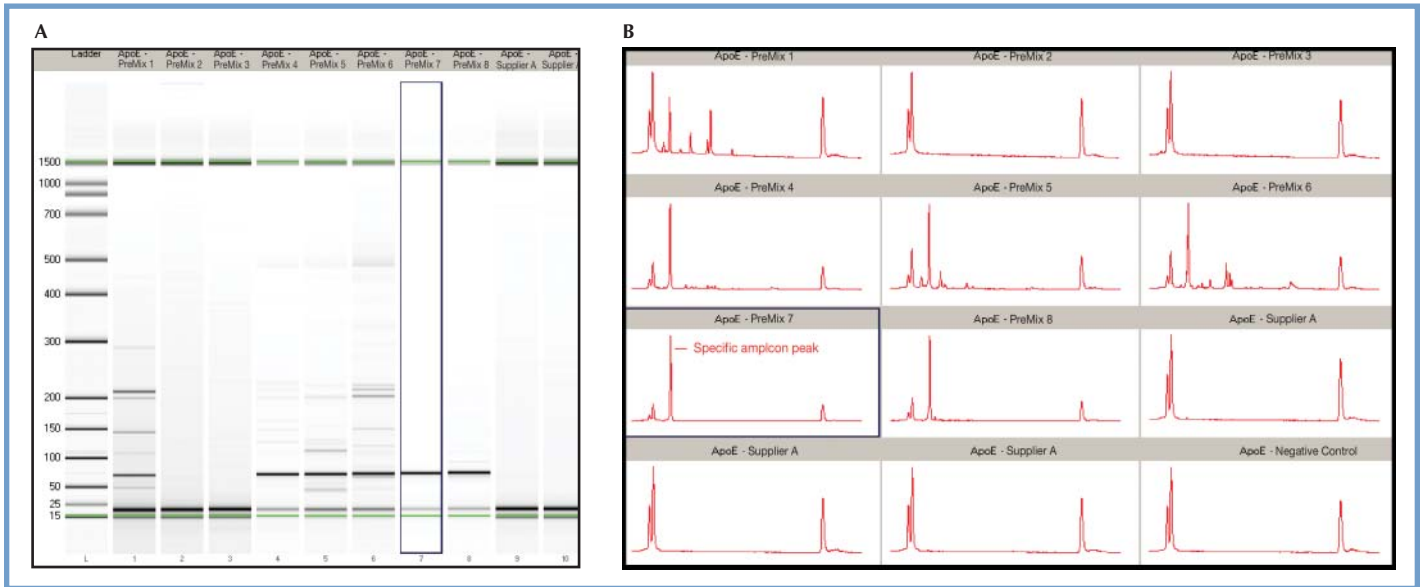
The robust FailSafe PCR Enzyme Blend, combined with the specifically formulated PreMixes, allows room temperature reaction setup and does not require hot-start PCR. The FailSafe PROBES Real-Time PCR System is compatible with all real-time PCR platforms and types of fluorescent probes.

### Results

Scientists at the Malaria Research Institute at Johns Hopkins were unable to genotype a population of individuals using a fluorescent probes custom assay for Apolipoprotein E (ApoE) allelic discrimination obtained from Supplier A. After failing to resolve this problem using other methods, they tested the FailSafe PROBES Real-Time PCR System. They were able to quickly optimize real-time PCR for this fluorescent probes allelic discrimination clustering assay using the FailSafe PROBES Real-Time PCR Optimization Kit. Data presented here compares the real-time PCR results obtained using the FailSafe Kit with those obtained using Supplier A's custom assay mix.



**Figure 1. Apolipoprotein E (ApoE) Arg112Cys Allelic Discrimination Clustering with the FailSafe™ PROBES Real-Time PCR Optimization Kit and a custom assay mix.** Three individuals representing each of the ApoE genotypes were used to determine the optimum real-time PCR reaction conditions with the 8 FailSafe™ PROBES Real-Time PCR PreMixes from EPICENTRE and with a custom assay mix from Supplier A. Reactions were performed on a Prism 7000 Sequence Detection System (Applied Biosystems), using the reaction conditions recommended by Supplier A. PreMix 7 provided optimum clustering of the ApoE genotypes and good amplification and fluorescence detection. Supplier A's custom mix resulted in amplification and fluorescence detection comparable to the negative control (no template).



**Figure 2: Evaluation of amplification quality between the FailSafe™ PROBES Real-Time PCR Optimization Kit and a custom assay mix.** Amplicons for one individual from the optimization experiment in Figure 1 were analyzed using the Agilent Bioanalyzer 2100 with DNA 1000 LabChips. Consistent with data in Figure 1, most PreMixes from the FailSafe™ PROBES Real-Time PCR System generated appropriate amplicons (peak at the correct migration time), with PreMix 7 yielding the cleanest products (with no additional products **A**. bands, **B**. peaks). Supplier A's custom mix yielded no products and is comparable to the negative control. **A**. Gel view of amplicons and **B**. Electropherogram view.

Figure 1 shows real-time PCR results of the ApoE allelic discrimination assays using the FailSafe PROBES Real-Time PCR Optimization Kit compared to Supplier A's custom mix. Based on the level of fluorescence and the single, clean, robust product obtained, FailSafe PreMix 7 was determined to provide optimum reaction conditions for this assay. Supplier A's custom mix gave results comparable to the negative control (no template). Figure 2A presents a gel view and Figure 2B presents a peak view showing the quality of the amplicons generated using each of the FailSafe PROBES Real-Time PCR PreMixes and Supplier A's custom mix for one individual. FailSafe

PreMix 7 was chosen as an optimal PreMix based on the specificity and cleanliness of PCR amplicons. Figure 3 shows genotyping of 24 individuals and demonstrates that ApoE allelic clustering could be assigned for all individuals tested using the FailSafe System with PreMix 7.

**Conclusion**

The FailSafe PROBES Real-Time PCR System enabled easy, accurate optimization of an ApoE allelic discrimination assay by doing only one round of real-time PCR using each of the 8 carefully formulated FailSafe PROBES Real-Time PCR PreMixes. Using the optimal FailSafe

PreMix, it was possible to cluster the ApoE alleles for all individuals tested, which had not been possible using the custom assay from Supplier A.

**References**

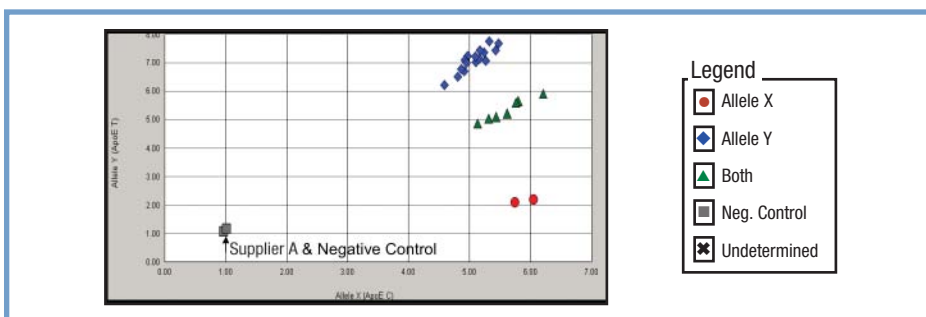
1. Gloffke, W. (2003) *The Scientist* April 21, 41.
2. Bustin, S.A. (2002) *J. Mol. Endocrinol.* **29**, 23.
3. Emi, M et al. (1988) *Genomics* **3**(4), 373.

**FailSafe PROBES Real-Time PCR System**

[www.EpiBio.com/failsafeprobases.asp](http://www.EpiBio.com/failsafeprobases.asp)

**FailSafe™ PROBES Real-Time PCR Optimization Kit**  
 FSP51048 48 25-µl Reactions  
**Contents:**  
 FailSafe™ PCR Enzyme Blend  
 8 FailSafe™ PROBES Real-Time PCR 2X PreMixes  
 Passive Reference Dye  
 Stabilizer

**FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit**  
 FSP51200 200 25-µl Reactions  
 FSP5101K 5 x 200-Reaction Kits  
**Contents:**  
 FailSafe™ PCR Enzyme Blend  
 Passive Reference Dye  
 Stabilizer  
 Your choice of any two FailSafe™ PROBES Real-Time PCR 2X PreMixes per Kit



**Figure 3. Apolipoprotein E (ApoE) Arg112Cys Allelic Discrimination Clustering using the FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit with PreMix 7 or a custom assay mix.** Using the FailSafe™ Real-Time PCR Enzyme Blend with PreMix 7, which had been determined to be the optimal PreMix for this assay, a random group of 24 individuals were genotyped for ApoE. Supplier A's custom mix was used for comparison, along with a negative control (no template). PreMix 7 successfully genotyped the samples, as demonstrated by the allelic discrimination clustering. Supplier A's custom mix again gave results comparable to the negative control.

\* The use of Betaine in DNA or RNA polymerase reactions is covered by patent rights exclusively licensed to EPICENTRE Technologies. Purchase of EPICENTRE's products for use under these rights is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for non-commercial life science research.

New!

# TargetAmp™ Aminoallyl-aRNA is a Suitable Template for cDNA Synthesis

Judith E. Meis and Anupama Khanna, EPICENTRE Biotechnologies

T7-based, linear RNA amplification methods are used to increase the small amount of RNA obtained from limited samples so that the RNA can be used for microarray analysis. When sufficient RNA is available, a portion of the cDNA produced early in the RNA amplification procedure can be saved and used later in real-time RT-PCR to validate gene expression differences. However, with rare or precious RNA samples, using the abundant antisense RNA (aRNA) product of the amplification process is preferable. Frequently aminoallyl-UTP (AA-UTP) is incorporated into the aRNA during the amplification procedure to produce aminoallyl-aRNA (AA-aRNA), which can be labeled and used as target for microarray studies. To verify that AA-aRNA is a suitable template for real-time RT-PCR, we tested AA-aRNA samples produced using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 (See page 6). Here we report that AA-aRNA can be used effectively as a template for real-time RT-PCR to identify differences in gene expression or to validate microarray results.

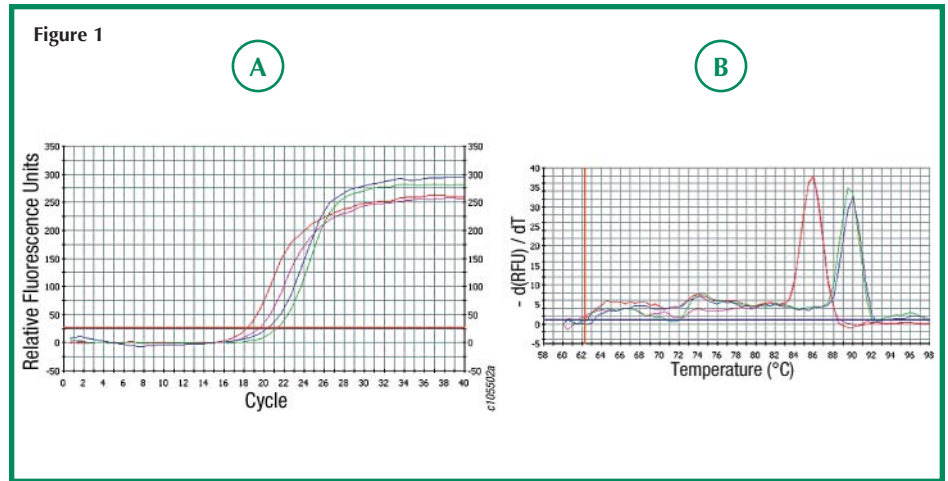
## Methods

### MonsterScript™ 1st-Strand cDNA Synthesis

Total cellular rat RNA from heart and kidney was amplified using the TargetAmp Aminoallyl-aRNA Amplification Kit 101. For comparison, heart and kidney RNA samples were also amplified without the incorporation of AA-UTP to produce unmodified aRNA. First-strand cDNA was synthesized from 200 ng of AA-aRNA (rat heart and kidney) and aRNA (rat heart and kidney) using random nonamer primers and EPICENTRE's new MonsterScript 1st-Strand cDNA Synthesis Kit (See page 22).

### TAQurate™ GREEN Real-Time PCR

One microliter from each 20- $\mu$ l cDNA synthesis reaction was used in a TAQurate GREEN Real-Time PCR reaction containing 1X TAQurate™ GREEN Real-Time PCR MasterMix, and 20 pmoles of each message-specific primer.<sup>1</sup> Rat messages detected were:  $\beta$ -2-microglobulin (B2M), a ubiquitously expressed housekeeping gene; protease serine 15 (Prss15), a mitochondrial ATP-dependent protease; myomegalin, a phosphodiesterase D4



**Figure 1. MonsterScript™ 1st-Strand cDNA, made from both AA-aRNA and unmodified aRNA, amplifies efficiently by real-time PCR using TAQurate™ GREEN Real-Time PCR Master Mix.** Real-time PCR was performed, as described in the text, using AA-aRNA and aRNA. **A.** PCR amplification plot of B2M (AA-aRNA, pink; aRNA, red) and Prss 15 (AA-aRNA, green; aRNA, blue). Primers used for B2M were: 5' CGT GCT TGC CAT TCA GAA AAC 3' and 5'-TCT GAG GTG GGT GGA ACT GAG 3', and for Prss15 were: 5' GTC ACA TCC CAC ATC CAC CTG C 3' and 5' GTG ATG AAG GGA GCC AAG TCT GA 3'. **B.** Melt curve analysis of the PCR products indicated a specific product for each primer pair tested.

interacting protein found to be highly expressed in rat heart and skeletal muscle,<sup>2</sup> with lower expression in other cell types; and kallikrein, a serine protease found to be highly expressed in rat kidney, with lower expression in other cell types.<sup>3</sup> Real-time PCR was performed and results were analyzed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad) using the following cycling profile: 95°C (2 minutes), followed by 45 cycles of 92°C (20 seconds), 64°C (30 seconds), and 72°C (30 seconds).

## Results

### B2M and Prss15

The AA-aRNA and aRNA samples produced similar results by real-time PCR analysis for the expression of B2M and Prss15. The threshold cycles ( $C_T$ ) for the AA-aRNA samples demonstrated a slight delay in amplification, as indicated by an increase in  $C_T$  of about one cycle, compared to the aRNA samples, for both heart and kidney (Figure 1A and data not shown). The slight increase in  $C_T$  appears to be consistent among AA-aRNA PCR amplified samples and is probably due to less efficient reverse transcription of the AA-aRNA template. Therefore, for accu-

rate and meaningful comparisons, relative (comparative) real-time RT-PCR should be performed using either AA-aRNA samples or aRNA samples, but not a combination of both. No differences between PCR products were observed by melt curve analysis (Figure 1B).

### Myomegalin and kallikrein

The expression levels of both rat myomegalin and kallikrein in heart and kidney were compared by relative real-time PCR using both AA-aRNA and aRNA templates. Real-time RT-PCR results confirm that expression of myomegalin is high in rat heart tissue and low in rat kidney as demonstrated by a  $C_T$  difference of approximately 5 cycles, for both AA-aRNA ( $\Delta C_T=5.0$ ) and aRNA ( $\Delta C_T=5.4$ ) samples (Figure 2A). Kallikrein expression is very low in heart and high in kidney as shown by a  $C_T$  difference of approximately 9 cycles, for AA-aRNA ( $\Delta C_T=9.1$ ) and aRNA ( $\Delta C_T=9.7$ ) samples (Figure 3A).

For myomegalin and kallikrein, the  $C_T$  values from the AA-aRNA samples again showed a slight delay in amplification, as demonstrated by an increase of up to one cycle, when compared to the aRNA samples (Figures 2A, 3A). Melt curve analysis

of the PCR products showed only the expected, specific products with the myomegalin primers (Figure 2B). In heart samples, where the kallikrein message was extremely limited, some primer-dimer products were synthesized in addition to the specific products, for both the AA-aRNA and the aRNA. The negative control, which contained no cDNA produced only primer-dimers.

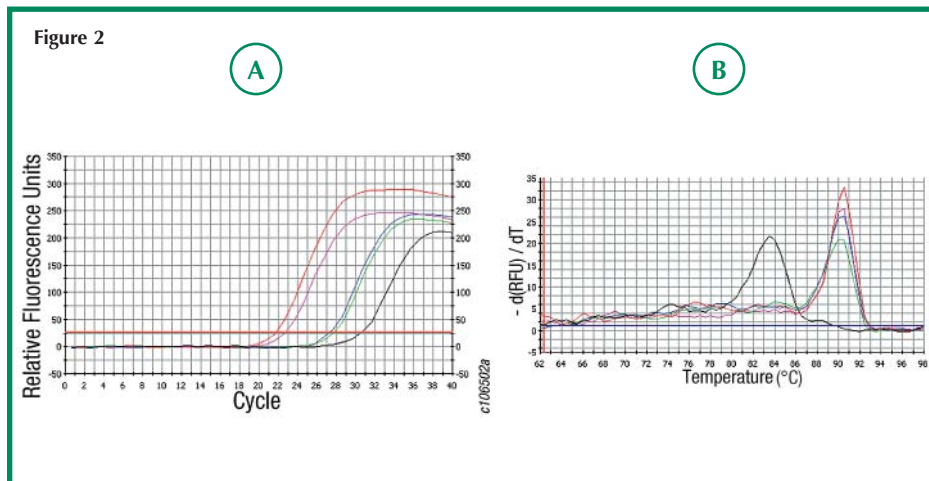
**Conclusions**

Aminoallyl-aRNA, produced using the TargetAmp Aminoallyl-aRNA Amplification Kits, is a suitable substrate for real-time RT-PCR to assay gene expression and validate microarray results. For relative real-time PCR, we recommend that all samples, including target and reference genes, are PCR amplified from the same type of starting RNA; either unamplified,

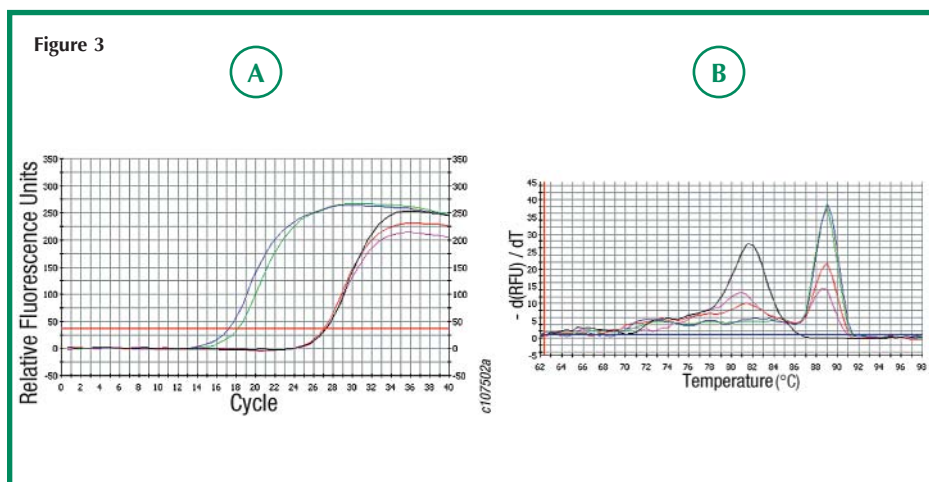
amplified without aminoallyl-labeling, or amplified with aminoallyl-labeling, to obtain the most accurate expression comparisons.

**References**

1. Grunenwald, H. *et al.* (2004) *EPICENTRE Forum* **11**(5), 9.
2. Verde, I. *et al.* (2001) *J. Biol. Chem.* **276**(14), 11189.
3. Saed, G.M. *et al.* (1990) *Circ. Res.* **67**, 510.
4. Heil, S.G. *et al.* (2003) *BioTechniques* **35**, 502.



**Figure 2. Myomegalin expression level differences in rat heart and kidney RNA were determined by real-time RT-PCR using both AA-aRNA and aRNA.** Real-time RT-PCR was performed, as described in the text, from rat heart and kidney RNA. **A.** PCR amplification plot for myomegalin reactions (heart AA-aRNA, pink; heart aRNA, red; kidney AA-aRNA, green; kidney aRNA, blue; no cDNA, black). Myomegalin primers were 5' ACA GCA GCA GAT TGG GGA AGG G 3' and 5' GGG ACA CTT GGG CTC GCA GGT 3'. **B.** Melt curve analysis shows only specific myomegalin products for all reactions containing cDNA and only a primer-dimer peak in the control sample containing no cDNA.



**Figure 3. Kallikrein expression level differences in rat heart and kidney RNA were determined by real-time RT-PCR using both AA-aRNA and aRNA.** Real-time RT-PCR was performed, as described in the text, from rat heart and kidney RNA. **A.** PCR amplification plot for kallikrein reactions (heart AA-aRNA, pink; heart aRNA, red; kidney AA-aRNA, green; kidney aRNA, blue; no cDNA, black). Kallikrein primers were 5' CTG CCC ACT GAG GAG CCC AA 3' and 5' TAA GTT TGG TGT AGA TGG CTG GC 3'. **B.** Melt curve analysis shows specific kallikrein PCR products in the kidney samples, where kallikrein expression is high. In the heart, where kallikrein expression is very low, both specific products and primer-dimers were made. Only a primer-dimer peak is seen in the control sample containing no cDNA.

[www.EpiBio.com/targetamp.asp](http://www.EpiBio.com/targetamp.asp)

**TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101**

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

[www.EpiBio.com/monsterscript.asp](http://www.EpiBio.com/monsterscript.asp)

**MonsterScript™ Reverse Transcriptase**

MSTA5110	10 Reactions
MSTA5124	24 Reactions

Includes MonsterScript™ 5X Reaction buffer

**MonsterScript™ 1st-Strand cDNA Synthesis Kit**

MS040910	10 Reactions
MS041050	50 Reactions

**Contents:**  
 MonsterScript™ Reverse Transcriptase (with RNase Inhibitor)  
 MonsterScript™ PreMix Solution (with Mg<sup>2+</sup>, dNTPs and Betaine\*)  
 V<sub>3</sub>-Oligo(dT)<sub>21</sub> Primer  
 Random Nonamer Primer  
 Sterile RNase-Free Water

[www.EpiBio.com/taqurate.asp](http://www.EpiBio.com/taqurate.asp)

**TAQurate™ GREEN Real-Time PCR MasterMix**

TM049096	96 25-µl Reactions
TM046400	400 25-µl Reactions

**Contents:**  
 TAQurate™ Real-Time PCR Enzyme Blend  
 TAQurate™ GREEN Real-Time 2X PCR MasterMix  
 Passive Reference Dye  
 Stabilizer

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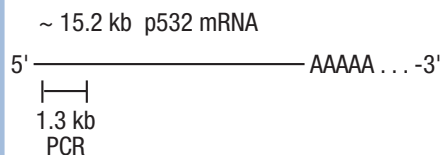
## Full-Length Reverse Transcription of >15-kb mRNA Using MonsterScript™ Reverse Transcriptase

Ramesh Vaidyanathan, EPICENTRE Biotechnologies

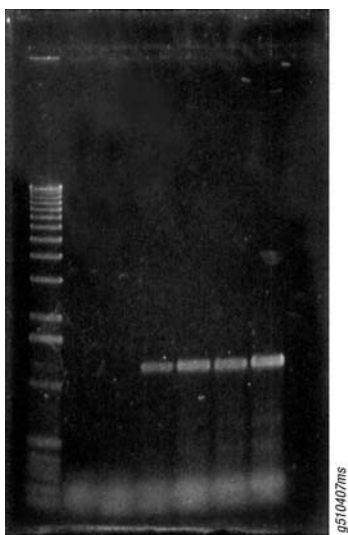
EPICENTRE's MonsterScript™ Reverse Transcriptase is a highly processive, thermostable enzyme that completely lacks RNase H activity. MonsterScript Reverse Transcriptase retains full activity up to 65°C, enabling reverse transcription through regions of high GC-content or difficult secondary structure. The enzyme is capable of synthesizing full-length cDNA from RNA templates greater than 15 kb. In addition to the enzyme, the MonsterScript™ 1st-Strand cDNA Synthesis Kit is also available.

In this article we demonstrate the use of MonsterScript Reverse Transcriptase for full-length cDNA synthesis from the approximately 15-kb human guanine nucleotide exchange factor, p532, mRNA<sup>1</sup> [Entrez: U50078].

A



B



**Figure 1. MonsterScript™ Reverse Transcriptase produces full-length cDNA from mRNA greater than 15 kb.** Total RNA isolated from HeLa cells was reverse transcribed and the cDNA was amplified as described in the text. **A.** Detection of the 1.3-kb PCR product demonstrates full-length reverse transcription of p532 mRNA. **B.** Agarose gel analysis of the PCR reaction shows the 1.3-kb amplicon from the 5'-end of the mRNA.

### cDNA synthesis

Total RNA was isolated from HeLa cells using EPICENTRE's MasterPure™ RNA Purification Kit. For cDNA synthesis, 1, 10, 100, or 200 ng of total RNA was combined with a T7 Oligo(dT) primer in 10 µl of water, heated at 65°C for 1 minute, and cooled on ice. Fifty units of MonsterScript Reverse Transcriptase, 5X reaction buffer (provided with the enzyme), and water were added to a final reaction volume of 20 µl. The cDNA synthesis reaction was incubated at 42°C for 5 minutes, then at 50°C for 40 minutes, and was terminated by heating at 90°C for 5 minutes.

### Detection of full-length cDNA

Synthesis of full-length cDNA was demonstrated by PCR using primers complementary to a 1.3-kb region of the cDNA, within 68 bases of its 3'-end (5'-end of the mRNA), as shown in Figure 1A. Using these primers (5'-CACGACTAATGGCTGAAGGA-3' and 5'-TCCAGCTTCAATGTCTGTG-3'), the 1.3-kb PCR product will be produced only if the cDNA synthesized is greater than 15 kb. Each 50-µl PCR reaction contained 2 µl of the cDNA reaction, 0.25 µM of each PCR primer, FailSafe™ PCR 2X PreMix D and 2.5 units of FailSafe™ PCR Enzyme Mix. Cycling conditions were 40 cycles of 94°C (30 seconds), 55°C (30 seconds) and 72°C (1 minute). For analysis, 5 µl of the PCR reaction was electrophoresed through a 1% agarose gel and visualized by staining with SYBR® Gold (Figure 1B).

### Conclusion

As shown in Figure 1B, MonsterScript Reverse Transcriptase successfully reverse transcribed full-length cDNA from the approximately 15-kb p532 mRNA, demonstrating the ability of the enzyme to synthesize full-length cDNA from very long mRNA templates. This experiment also shows that the MasterPure RNA Purification Kit purifies intact, high molecular weight RNA.

### Acknowledgement

We would like to thank Judith Meis for technical discussions, HeLa cell RNA, and the p532 1.3-kb amplicon primers.

### Reference

1. Rosa, J.L. *et al.* (1996) *EMBO J.* **15**(20), 5738.

### Benefits of MonsterScript™ Reverse Transcriptase

- Synthesizes full-length cDNA, >15 kb.
- Thermostable, permitting reverse transcription of mRNA containing high GC-content or secondary structure.
- RNase H minus.
- Improves the sensitivity of RT-PCR. MonsterScript Reverse Transcriptase produces cDNA from picogram amounts of total RNA, and MonsterScript reaction buffer is compatible with PCR reactions, so more of the RT reaction can be used in the PCR reaction.

### The MonsterScript™ 1st-Strand cDNA Synthesis Kit

- Provides a convenient PreMix Solution containing Mg<sup>2+</sup>, dNTPs, optimized buffer, and Betaine\*, which reduces pausing and stops by the reverse transcriptase through difficult sequences.
- Includes both V<sub>3</sub>-Oligo(dT)<sub>21</sub> and Random Nonamer primers.
- Contains a potent RNase Inhibitor.

[www.EpiBio.com/monsterscript.asp](http://www.EpiBio.com/monsterscript.asp)

### MonsterScript™ Reverse Transcriptase

MSTA5110	10 Reactions
MSTA5124	24 Reactions
Includes MonsterScript™ 5X Reaction Buffer	

### MonsterScript™ 1st-Strand cDNA Synthesis Kit

MS040910	10 Reactions
MS041050	50 Reactions

### Contents:

MonsterScript™ Reverse Transcriptase (with RNase Inhibitor)  
 MonsterScript™ PreMix Solution (with Mg<sup>2+</sup>, dNTPs, and Betaine\*)  
 V<sub>3</sub>-Oligo(dT)<sub>21</sub>  
 Random Nonamer Primer  
 Sterile RNase-Free Water

\* The use of Betaine in DNA or RNA polymerase reactions is covered by patent rights exclusively licensed to EPICENTRE Technologies. Purchase of EPICENTRE's products for use under these rights is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for non-commercial life science research.

\* SYBR is a registered trademark of Molecular Probes, Invitrogen Detection Technologies.

New!

## Fecal DNA is PCR-Ready in 50 Minutes Using the ExtractMaster™ Fecal DNA Extraction Kit

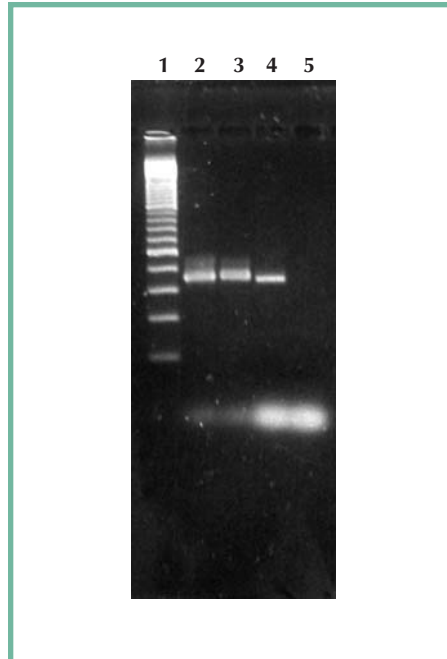
### Introduction

The New ExtractMaster™ Fecal DNA Extraction Kit is specifically designed to recover PCR-ready DNA from the feces of a variety of animals, including bird, cat, cow, human, and rat. The kit uses a detergent lysis process to extract DNA from small fecal samples (50 mg or less) in about 50 minutes. A simple spin column chromatography step removes enzymatic inhibitors (i.e., heme, bilirubin, bile salts) that often co-extract with DNA from fecal samples.<sup>1</sup> Extracted DNA is PCR-ready, and can be used with the FailSafe™ PCR System to amplify bacterial, protist, or animal DNA templates.

DNA obtained from fecal samples provides a window into animal pathophysiology. For example, changes in the methylation patterns in fecal DNA may be a promising marker for human colorectal cancer screening.<sup>2</sup> In rural watersheds, *E. coli* typing can help track the animal sources of fecal water pollution.<sup>3</sup> PCR can be used to detect intestinal protozoan infections in avian wildlife populations<sup>4</sup> or in humans.<sup>5</sup> Fecal DNA also provides a non-invasive method for genotyping animals, which is especially useful when studying species in their natural habitat.<sup>6</sup>

### Eubacterial 16S rRNA amplification

The ExtractMaster DNA Extraction Kit was used to extract DNA from human, rat, and rooster fecal samples. Figure 1 shows the PCR amplification products from the ExtractMaster DNA template for a eubacterial 16S rRNA gene. Bacterial DNA was detected by PCR using eubacterial rRNA gene primers<sup>7</sup> (5'-CTG CTG CCT CCC GTA GGA GT and 5'-AGA GTT TGA TCC TGG CTC AG), the FailSafe™ PCR Enzyme Mix and FailSafe™ PCR PreMix B. Cycling conditions were 96°C (3 minutes), followed by 24 cycles of 96°C (30 seconds), 54°C (30 seconds), and 72°C (45 seconds). After 24 cycles, the samples were diluted ten-fold into fresh reaction mix and amplified for an additional 3 cycles. Because the PCR products obtained could be derived from one or more bacterial species, this two-stage PCR is used to reduce heteroduplex formation between 16S rRNAs from dif-



**Figure 1. Amplification of a eubacterial 16S rRNA gene obtained from the fecal samples of several animals using the ExtractMaster™ Fecal DNA Extraction Kit. Lane 1, 100-bp ladder; Lane 2, human; Lane 3, rat; Lane 4, rooster; Lane 5, no template. The amplicon is approximately 350 bp long.**

ferent species.<sup>8</sup> To further identify the specific bacteria, amplicons would be sequenced and compared to a database of known bacterial 16S rRNA genes.

### Conclusion

Fecal samples could provide a non-invasive source of DNA for a variety of analyses, but, until recently, extracting quality DNA from these samples was difficult. Now the ExtractMaster Fecal DNA Extraction Kit provides a rapid method to process multiple samples and obtain PCR-ready DNA.

### References

1. Thornton, C.G. and Passen, S. (2004) *J. Microbiol. Meth.* **59**, 43.
2. Muller, H.M. *et al.* (2004) *Lancet* **363**, 1283.
3. Johnson, L.K. *et al.* (2004) *Appl. Environ. Microbiol.* **70**, 4478.
4. Xiao, L. *et al.* (2004) *Appl. Environ. Microbiol.* **70**, 4211.
5. Pontes, L.A. *et al.* (2002) *Am. J. Trop. Med. Hyg.* **66**, 157.
6. Wehausen, J.D. *et al.* (2004) *J. Hered.* **95**(6), 503.
7. Brow, M.A.D., *et al.* (1996) *J. Clin. Microbiol.* **34**, 3129.
8. Thompson, J.R. *et al.* (2002) *Nucleic Acids Res.* **30**, 2083.

[www.EpiBio.com/extractmaster.asp](http://www.EpiBio.com/extractmaster.asp)

#### ExtractMaster™ Fecal DNA Extraction Kit

FD05005 5 Purifications  
FD05025 25 Purifications

#### Contents:

Fecal DNA Extraction Buffer  
Proteinase K (50 µg/µl)  
Protein Precipitation Reagent

#### 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 PreMixes

#### FailSafe™ PCR System with PreMix Choice

FS99250 250 Units

#### Contents:

Includes FailSafe™ PCR Enzyme Mix  
Choice of 2 FailSafe PCR 2X PreMixes

#### FailSafe™ PCR System with PreMix Choice

FS9901K 1,000 Units

#### Contents:

Includes FailSafe™ PCR Enzyme Mix  
Choice of 8 FailSafe PCR 2X PreMixes

#### FailSafe™ Enzyme Mix Only<sup>+</sup>

FSE51100 100 Units  
FSE5101K 1,000 Units

<sup>+</sup> Note: We can only guarantee the failsafe nature of this system if the FailSafe Enzyme Mix is used with a FailSafe PCR 2X PreMix that is selected using the FailSafe PCR PreMix Selection Kit.

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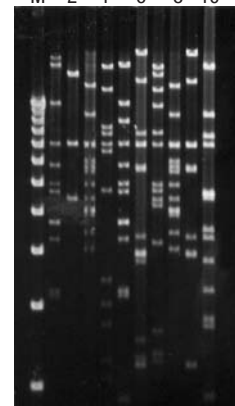
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