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Volume 13-4

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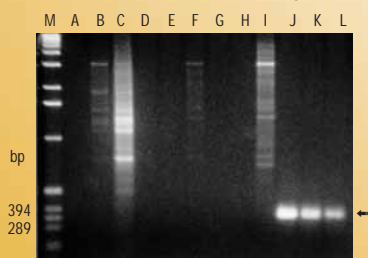
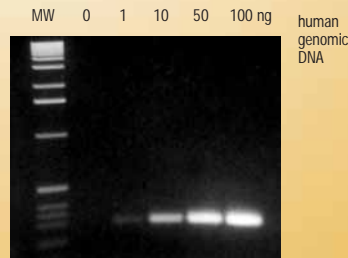


Figure 1

Amplification of an 80-85% GC-rich region of the human fragile X gene. PCR was performed using the FailSafe PCR System. Lanes A-L show the amplification products resulting from PCR using the 12 FailSafe PCR PreMixes. M, molecular weight marker. Optimal amplification was obtained with FailSafe PCR PreMix J.

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EPICENTRE Biotechnologies thanks Haiying Grunenwald for the use of her photo of Navy Pier, Chicago. Located on Lake Michigan near Streeterville, close to Chicago's downtown, construction started in May 1914 and in 1916 it was opened to the public. At the time it was the world's largest pier, 292 ft wide and 3000 ft long. In 1927, the pier was renamed Navy Pier in honor of World War I veterans. Following renovation of the pier between 1992-4, it became a very successful recreational center with many attractions and 50 acres of parks and gardens; it currently attracts more than 8 million visitors each year.

### Hands on the Cover:

Joanne Decker, EPICENTRE Biotechnologies Senior R&D Scientist

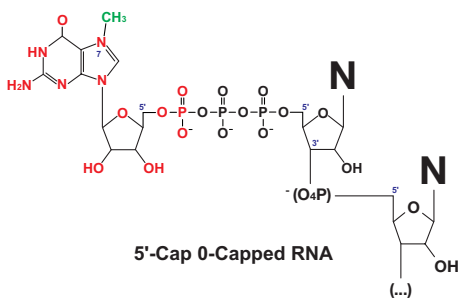


# Achieve 100% Capping Efficiency with the NEW ScriptCap™ m<sup>7</sup>G Capping System

Ronald Meis and Judith E. Meis, EPICENTRE Biotechnologies

## Introduction

Eukaryotic mRNA contains a “cap structure” on its 5′-end, which plays a crucial role in maintaining the stability and translational efficiency of the mRNA *in vivo*. mRNA caps are composed of a guanosine, which is methylated at the N7 position and linked to the 5′-end of the RNA by a triphosphate bridge in a 5′ to 5′ manner (FIG 1); this is referred to as a Cap 0 structure.



**FIG 1. 5′-Cap 0-Capped RNA.** Red atoms are derived from GTP, green atoms are derived from SAM.

Traditionally, *in vitro* 5′-Cap 0-capped RNA is synthesized by carrying out an *in vitro* transcription (IVT) reaction where  $\geq 80\%$  of the GTP added to the reaction is substituted with a dinucleotide cap analog (i.e., m<sup>7</sup>G[5′]ppp[5′]G) in order to drive transcription initiation with the cap analog.<sup>1</sup> This approach has several drawbacks. First, the yield of RNA in a capping-IVT reaction is greatly reduced ( $\leq 33\%$ ) compared to a standard IVT reaction due to the limiting amount of GTP present in the reaction. Second, the theoretical maximum percentage of capped RNA produced is equal to the ratio of cap analog to cap analog plus GTP present in the reaction. Consequently, capping efficiencies can never reach 100%, and are usually lower than the theoretical maximum.<sup>2,3</sup> This can complicate the interpretation of downstream applications. Third, some cap analogs can be incorporated bidirectionally. The percentage of reverse orientation incorporation events (~33%) effectively reduces the amount of properly-capped RNA produced in the reaction.<sup>4,5</sup> And fourth, cap analogs are a costly reagent.

A solution to these problems would be to enzymatically build Cap 0 structures onto the RNA transcripts using a “capping enzyme”, GTP and S-adenosyl-methionine (SAM; methyl group donor). Traditionally, capping enzymes have been used for 5′-end labeling of capped RNA, but not for quantitative production of capped RNA for use in *in vitro* and *in vivo* translational studies. While most characterized eukaryotic capping systems are multienzymatic,<sup>6</sup> the Vaccinia Virus capping enzyme contains all three enzymatic activities (mRNA triphosphatase, guanylyltransferase and guanine-7-methyltransferase) necessary to build 5′-Cap 0 structures *in vitro*.<sup>7-10</sup> In this article, we introduce EPICENTRE Biotechnologies’ new ScriptCap™ m<sup>7</sup>G Capping System featuring Vaccinia Virus Capping Enzyme (VCE; ScriptCap™ Capping Enzyme) as part of a convenient, highly efficient, single-enzyme capping system, which is ideally suited for quantitative production of Cap 0-capped RNA.

## Methods and Results

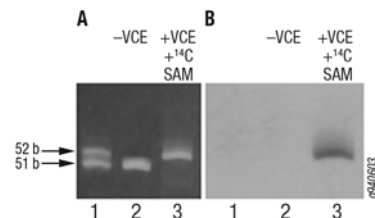
Quantitative mRNA triphosphatase and guanylyltransferase activities of VCE are demonstrated in FIG 2. To ease visualization, 5  $\mu$ g of a 51 nucleotide transcript (untreated: lane 2) was incubated with VCE and GTP in a standard 30 minute reaction (treated: lane 3), producing a 52 nucleotide product. Approximately 100% of the 51 nucleotide RNA was capped under these conditions. Reactions can be



**FIG 2. The mRNA triphosphatase and guanylyltransferase activities of VCE completely cap an *in vitro* transcript.** A total of 0.1  $\mu$ g of RNA from each treatment was run on a denaturing polyacrylamide gel and stained with ethidium bromide. The 52 and 51 nucleotide RNA markers are shown in Lane 1.

scaled up to completely cap any amount of RNA (i.e., 60  $\mu$ g RNA in 30 minutes; data not shown). To demonstrate that VCE-capped RNA can be further manipulated, the 52 nucleotide capped RNA was subsequently treated with either Tobacco Acid Pyrophosphatase (TAP) [which removes 5′-cap structures] (lane 4) producing a 51 nucleotide product, or with A-Plus™ Poly(A)-Polymerase (PAP) [which adds a 3′-poly(A) tail] (lane 5) producing a large size product. Both the 5′ and 3′ ends of VCE-capped RNA could be enzymatically treated.

The guanine-7-methyltransferase activity of VCE is demonstrated qualitatively in FIG 3 and quantitatively in FIG 4. In FIG 3, a 51 nucleotide transcript (untreated: lane 2) was incubated with VCE, GTP, and <sup>14</sup>C-methyl-SAM (treated: lane 3), producing an N7-[<sup>14</sup>C]-methylated capped 52 nucleotide product. The 52 nucleotide capped RNA was exclusively labeled. Control reaction products lacking VCE or GTP were not radio-labeled (data not shown).



**FIG 3. Guanine-7-methyltransferase activity of VCE.** A total of 0.1  $\mu$ g of RNA from each treatment was run on a denaturing polyacrylamide gel. **A.** Stained with ethidium bromide. **B.** Dried and subjected to autoradiography. The 52 and 51 nucleotide RNA markers are shown in Lane 1.

In FIG 4, a 335 nucleotide transcript was capped with VCE and  $\alpha$ -[<sup>32</sup>P]-GTP in the absence (lane 3), or presence (lane 4) of SAM, producing methylated or unmethylated <sup>32</sup>P-cap-labeled products. The RNA was digested to completion with RNase I and APex™ Heat-Labile Alkaline Phosphatase. With this treatment, only the 5′-cap dinucleotide will be RNase resistant due to its 5′ to 5′ linkage. The lack of a spot in lane 4 on the autoradiogram (FIG 4A) co-migrating with the G[5′]ppp[5′]G standard, indicates that the RNA was completely N7-methylated by VCE.

Finally, the ability of VCE-capped RNA to be translated was examined. RNA encoding *Renilla* luciferase was produced via IVT using an AmpliScribe™ T7-Flash™ Transcription Kit. The RNA was capped with VCE in the absence or presence of SAM, poly(A)-tailed with A-Plus™ Poly(A) Polymerase, and used to transfect HeLa cells in culture. Twenty-four hours post transfection, the cells were harvested and lysed. Lysates were

assayed for luciferase activity and normalized to total protein content. The relative translation efficiencies are shown below in Table 1. Maximum signal intensity was produced with cap 0-capped, poly(A)-tailed RNA.

**Conclusion**

EPICENTRE's new ScriptCap™ m<sup>7</sup>G Capping System featuring Vaccinia Virus Capping Enzyme (ScriptCap™ Capping Enzyme), provides a convenient, highly efficient, single-enzyme capping system to quantitatively produce Cap 0 structures on any amount of RNA *in vitro*.

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**Table 1. Translation efficiency of RNA capped with VCE.**

Post-transcription Treatment	Relative Translation Efficiency
No RNA	0%
Poly(A)-tailed, non-capped	~0%
Poly(A)-tailed, capped without SAM	10%
Poly(A)-tailed, capped with SAM	100%

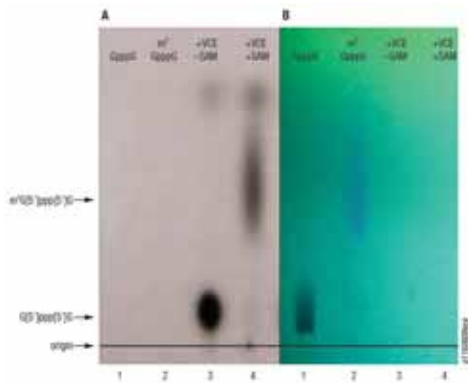
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- ✓ A-Plus™ Poly(A)-Polymerase
- ✓ RNase I
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[www.EpiBio.com/scriptcapvce.asp](http://www.EpiBio.com/scriptcapvce.asp)

**ScriptCap™ m<sup>7</sup>G Capping System**

SCCE0625      25 Reactions  
 Contents: ScriptCap™ Capping Enzyme, 10X Capping Buffer, ScriptGuard™ RNase Inhibitor, 20 mM SAM, 10 mM GTP, RNase-Free Water



**FIG 4. Complete guanine-7-methyl transfer activity of VCE.** Digestion products were subjected to thin layer chromatography on PEI-F cellulose plates developed in 0.4 M LiCl and 1 M Formic acid. The plates were visualized under short-wave UV light (B) and then subjected to autoradiography (A). Cap analog standards G[5']ppp[5']G (Lane 1) and m<sup>7</sup>G[5']ppp[5']G (Lane 2) were also run. The cap analog m<sup>7</sup>G[5']ppp[5']G appears as a smear with blue fluorescence under these conditions.



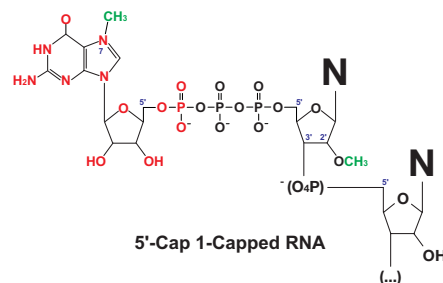
## Improve the Translation Efficiency of any 5'-Capped mRNA with the NEW ScriptCap™ 2'-O-Methyltransferase

Ronald Meis and Judith E. Meis, EPICENTRE Biotechnologies

**Introduction**

Various classes of eukaryotic cap structures, differentiated by their state of methylation, have been identified. Cap 0 structures contain an N7 methyl group on the capping guanosine nucleotide (see previous article) and are found in lower eukaryotes (m<sup>7</sup>GpppNpNpN...)<sup>1</sup> Cap 1 structures contain an additional methyl group at the 2'-O position of the penultimate nucleotide (m<sup>7</sup>Gppp[m<sup>2'-O</sup>]NpNpN...) (FIG 1), while Cap 2 structures contain yet an additional methyl group at the 2'-O position of the antepenultimate nucleotide (m<sup>7</sup>Gppp[m<sup>2'-O</sup>]Np[m<sup>2'-O</sup>]NpN...). Cap 1 and Cap 2 structures are found in higher eukaryotes.<sup>1</sup>

Distinct 2'-O-methyltransferases are



**FIG 1. 5'-Cap 1-Capped RNA.** Red atoms are derived from GTP, green atoms are derived from SAM.

responsible for the methylation of the penultimate and antepenultimate nucleotides of the cap structures. These are sometimes referred to as Cap 1 methyltransferase and Cap 2 methyltransferase.<sup>2</sup> Although the exact function of these additional methylation events is

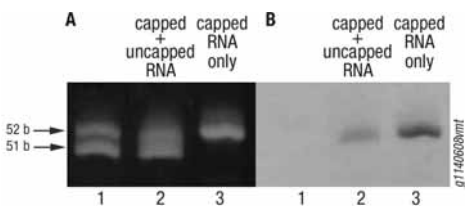
still unclear, a growing body of evidence suggests that the Cap 1 methylation serves, at least in part, to increase the translation efficiency of the mRNA.<sup>3</sup> Cap 1-capped RNA cannot be produced through the use of a dinucleotide cap analog in an *in vitro* transcription system.<sup>4</sup> In this article, we introduce EPICENTRE Biotechnologies' new ScriptCap™ 2'-O-Methyltransferase derived from Vaccinia Virus Cap 1 methyltransferase<sup>5-7</sup> (VMT) for the preparation of completely Cap 1-capped RNA from any source of Cap 0-capped RNA, whether it be from the ScriptCap m<sup>7</sup>G Capping System (see previous article; EPICENTRE), or cap analog-based transcription kits such as the AmpliCap-Max™ High Yield Message Maker Kits (EPICENTRE).

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Improve Translation Efficiency. . . Cont'd from page 5

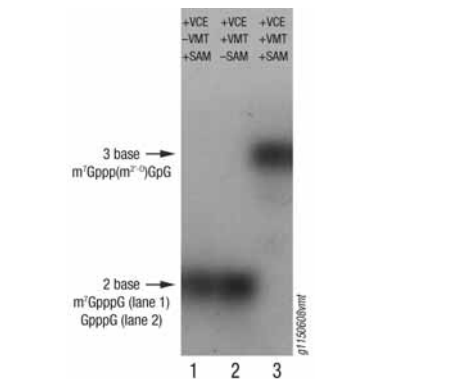
**Methods and Results**

FIG 2 shows both the substrate specificity and qualitative 2'-O-methyltransferase activity of VMT. A 51 nucleotide RNA transcript was either untreated or cap 0-capped using the ScriptCap™ m7G Capping System (producing a 52 nucleotide RNA product). A mixture of uncapped and cap 0-capped RNA (lane 2), or just cap 0-capped RNA (lane 3), was incubated with VMT and 14C-methyl-S-adenosyl-methionine (SAM; methyl group donor). Only cap 0-capped RNA served as a substrate for VMT.



**FIG 2. Substrate specificity and qualitative 2'-O-methyltransferase activity of VMT.** A total of 0.1 µg of RNA from each treatment was run on a denaturing polyacrylamide gel. **A.** Stained with ethidium bromide. **B.** Dried and subjected to autoradiography. The 52 and 51 nucleotide RNA markers are shown in Lane 1.

Complete 2'-O-methylation by VMT is demonstrated in FIG 3. A 335 nucleotide transcript was capped by simultaneous treatment with both the ScriptCap m7G Capping System and ScriptCap™ 2'-O-Methyltransferase with α-[32P]-GTP in the absence (lane 2) or presence (lane 3) of SAM. A control ScriptCap m7G Capping System reaction which lacked VMT is shown in lane 1. After capping, the RNA was digested to completion with RNase I and APex™ Heat-Labile Alkaline Phosphatase. The only RNase-resistant products are either the 5'-cap dinucleotide, due to its 5' to 5' linkage, or the 5'-cap trinucleotide, due to the 5' to 5' linkage and the 2'-O-methylation of the penultimate nucleotide. The lack of an RNase-resistant dinucleotide in lane 3 indicates that the RNA was completely 2'-O-methylated by VMT. Reactions can be scaled up to quantitatively treat any amount of cap 0-capped RNA (data not shown).



**FIG 3. Complete 2'-O-methyltransfer by VMT.** Digestion products were run on a denaturing polyacrylamide gel, dried and subjected to autoradiography.

**Table 1. Translation efficiency of cap 1-capped RNA produced with VMT.**

Means of Cap 0 Production	ScriptCap™ 2'-O-Methyltransferase Treatment	Final mRNA Cap Structures Formed	Translation Efficiency relative to non-treated mRNA
No RNA	no	none	0%
ScriptCap™ m7G Capping System (Capping Enzyme)	no	m7GpppN (Cap 0)	100%
ScriptCap™ m7G Capping System (Capping Enzyme)	yes	m7Gppp[m2-9]N (Cap 1)	147%
AmpliCap-Max™ High Yield Message Maker Kit (Standard Cap Analog)	no	m7GpppN * (Cap 0)	100%
AmpliCap-Max™ High Yield Message Maker Kit (Standard Cap Analog)	yes	m7Gppp[m2-9]N * (Cap 1)	148%
AmpliScribe™ T7-Flash™ Transcription Kit (ARCA Cap Analog)	no	m2,3-0GpppN * (Cap 0)	100%
AmpliScribe™ T7-Flash™ Transcription Kit (ARCA Cap Analog)	yes	m2,3-0Gppp[m2-9]N * (Cap 1)	128%

\* Represents the predominant cap structure formed in the reaction (see previous article).

Since cap 0-capped mRNA can be produced post-transcriptionally with a capping enzyme, or co-transcriptionally with a cap analog-based transcription kit, we examined the translation efficiency of mRNA produced by both means after subsequent conversion to cap 1-capped mRNA with VMT. Cap 0-capped *Renilla* luciferase mRNA was produced, 2'-O-methylated, poly(A)-tailed with A-Plus™ Poly(A) Polymerase, and used to transfect HeLa cells in culture. Twenty-four hours post transfection, the cells were harvested and lysed. Lysates were assayed for luciferase activity and normalized to total protein content. The means of cap 0 production, final cap structures produced, and relative translation efficiencies are shown in Table 1. Treatment with ScriptCap 2'-O-Methyltransferase improved the luciferase expression irrespective of the means of cap 0-cap production.

**Conclusion**

EPICENTRE's new ScriptCap™ 2'-O-Methyltransferase provides a convenient and effective method for preparing cap 1-capped RNA from any source of cap 0-capped RNA. Treatment may be done simultaneously with the ScriptCap™ m7G Capping System, or sequentially following cap analog-based transcription reactions.

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EPICENTRE Products Utilized in this Work (see [www.EpiBio.com](http://www.EpiBio.com) for more details).

- ✓ ScriptCap™ 2'-O-Methyltransferase
- ✓ ScriptCap™ m7G Capping System
- ✓ AmpliCap-Max™ High Yield Message Maker Kits
- ✓ RNase 1
- ✓ APex™ Heat-Labile Alkaline Phosphatase
- ✓ A-Plus™ Poly(A)-Polymerase
- ✓ AmpliScribe™ T7-Flash™ Transcription Kit
- ✓ ARCA Cap Analog

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

**ScriptCap™ 2'-O-Methyltransferase**

SCMT0625 25 Reactions  
 Contents: ScriptCap™ 2'-O-Methyltransferase, 10X Capping Buffer, 20 mM SAM

# High Sensitivity RT-PCR from as Little as 1 Cell

In previous issues of the EPICENTRE Forum newsletter, we have demonstrated that EPICENTRE Biotechnologies' new MessageBOOSTER™ cDNA Synthesis Kit for qPCR greatly improves quantitative real-time RT-PCR (qRT-PCR) sensitivity, accuracy, and reproducibility of even low-abundance transcripts from as little as one cell.<sup>1,2,3</sup> Here, we summarize the key benefits that this kit offers for RT-PCR studies using very small populations of cells.

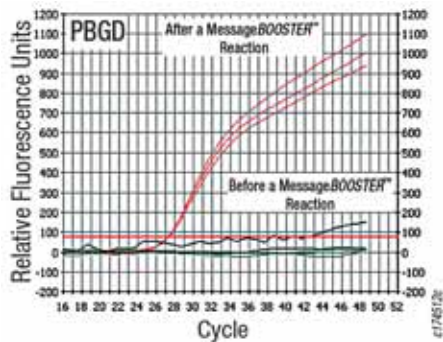


FIG 1. Detection by qPCR of the low-abundance PBGD transcript before and after cDNA production using the MessageBOOSTER™ cDNA Synthesis Kit for qPCR.

### Significantly improve the sensitivity and reproducibility of qPCR from small populations of cells

A MessageBOOSTER reaction amplifies the poly(A)-containing mRNA present in a total RNA preparation, and then converts the amplified RNA to single-stranded sense cDNA, which is ready for PCR or qPCR. FIG 1 shows the quantification graph obtained for the low-abundance porphobilinogen deaminase (PBGD) transcript using cDNA produced from 10 pg of purified total RNA (the approximate amount of total RNA in 1 cell) before and after a MessageBOOSTER reaction. The results show that the PBGD cDNA produced from a MessageBOOSTER reaction significantly improves the

detection sensitivity of this low-abundance transcript, as determined by a reduction in the  $C_T$  value, when compared to the PBGD cDNA produced without the MessageBOOSTER poly(A) RNA amplification reaction.

### Unbiased RNA amplification

The relative abundance of 20 transcripts in both Universal Human Reference RNA (Stratagene) and adult skeletal muscle RNA was compared by qPCR before and after a MessageBOOSTER reaction (FIG 2).<sup>2</sup> The high correlation coefficient obtained ( $R^2 = 0.997$ ) demonstrates that the cDNA produced by a MessageBOOSTER reaction faithfully preserves the relative transcript abundance of the sample.

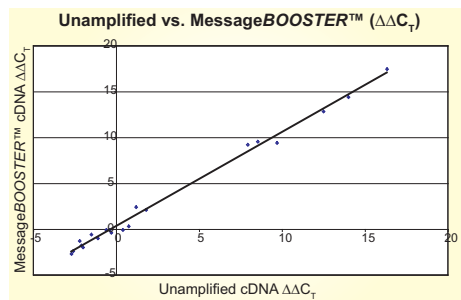


FIG 2. The normalized difference in expression levels ( $\Delta\Delta C_T$ ) between Universal Human Reference RNA and adult skeletal muscle RNA for the 20 transcripts tested before (unamplified) and after a MessageBOOSTER™ reaction. The differences in expression levels between RNA sample types are represented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

### Perform a MessageBOOSTER™ reaction directly from a whole-cell lysate. . . without having to purify total RNA

The qPCR results using MessageBOOSTER cDNA produced directly from the lysate of a single cell were compared to results obtained using MessageBOOSTER cDNA produced from 10 pg of highly purified total RNA. Published data show that the cDNA produced by a MessageBOOSTER

reaction directly from cell lysates yields essentially identical results to those obtained using purified total RNA.<sup>3</sup>

### Greatly increase the number of RT-PCR reactions obtained from very small samples

The number of RT-PCR or qRT-PCR reactions that can be completed using the cDNA produced by a MessageBOOSTER reaction (see Table 1) is dependent on two main factors:

1. The amount of total RNA added to the MessageBOOSTER reaction.
2. The abundance of the transcript(s) of interest.

### References

1. Grunenwald, H. et al., (2006) EPICENTRE Forum 13(1), 7.
2. Meis, J.A. (2006) EPICENTRE Forum 13(2), 4.
3. Grunenwald, H. et al., (2006) EPICENTRE Forum 13(3), 22.
4. Yang, F. et al., (2006) Oncogene 25(9), 1413.

### Note added in proof:

A recent publication demonstrates the existence of two distinct functional classes of taste cells—receptor cells and presynaptic cells.<sup>4</sup> The two cell classes were identified, in part, by single-cell RT-PCR enabled by the MessageBOOSTER™ cDNA Synthesis Kit for qPCR.



Table 1. The number of qPCR reactions that can be performed using cDNA produced by a MessageBOOSTER™ reaction.

Amount of Total RNA in a MessageBOOSTER™ Reaction	Number of qRT-PCR Reactions That Can Be Performed	
	Low- / Medium- Abundance Transcripts*	Medium- / High- Abundance Transcripts*
10 pg (~1 cell)	>10	>100
100 pg (~10 cells)	>100	>1,000
500 pg (~50 cells)	>500-1,000	>5,000-10,000

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

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

**MessageBOOSTER™ cDNA Synthesis Kit for qPCR**

MB060110	10 Reactions
MB060124	24 Reactions

\*MessageBOOSTER is a trademark of EPICENTRE Biotechnologies, Madison, WI. This product is covered by intellectual property licensed to EPICENTRE Technologies Corporation from Johnson & Johnson Pharmaceutical Research & Development, L.L.C. See [www.EpiBio.com](http://www.EpiBio.com) website for complete license statements.

# Direct Genomic DNA Sequencing for Rapid Fungal Identification

Bruce W. Jarvis,<sup>1</sup> Brian L. Wickes,<sup>2</sup> and Les M. Hoffman<sup>1</sup>  
<sup>1</sup>EPICENTRE Biotechnologies, and <sup>2</sup>Advanced Nucleic Acids Core Facility,  
 University of Texas Health Science Center, San Antonio, TX

## Introduction

There are several methods available for taxonomic classification of clinically occurring fungi. Chief among these are methods based on biochemical tests<sup>1</sup> and those based on DNA sequence identity. Among the DNA sequencing methods are Pyrosequencing™\*,<sup>2</sup> PCR-based technologies<sup>3</sup>, and Random Amplified Polymorphic DNA (RAPD).<sup>4</sup> We have found that direct sequencing of genomic DNA purified with EPICENTRE Biotechnologies' MasterPure™ Yeast DNA Purification Kit provides nine-fold longer read lengths than pyrosequencing with only a 1 hour increase in time. Genomic DNA (gDNA) sequencing can be performed on standard capillary-based instruments commonly found in molecular biology laboratories. In contrast, the PCR-based methods require several more hours of time to provide equivalent sequence read lengths, and pyrosequencing requires highly specialized equipment. Fungal identification by direct genomic DNA sequencing centers on the highly repetitive ribosomal

gene (rDNA) tandem repeats (FIG 1), which are repeated up to 200 times in fungal genomes.<sup>5,6</sup> We have discovered that multiple copies of this region provide enough template for direct sequencing.

## Methods

Genomic DNA was extracted from yeast, filamentous fungi, and mushrooms using EPICENTRE's MasterPure Yeast DNA Purification Kit according to the kit protocol. Optimal sequencing was obtained with 1/4X BigDye® sequencing reactions (ABI, Foster City, CA) and 100 ng of gDNA templates. Primer sequences were: LROR (5'-ACC CGC TGA ACT TAA GC), NL-1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG), NL-4 (5'-GGT CCG TGT TTC AAG ACG G), and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC). Reactions were ethanol precipitated and sequenced on an ABI PRISM® 310 Genetic Analyzer.

## Results and Conclusions

The MasterPure™ Yeast DNA Purification Kit provides impressive yields of high molecular weight DNA (>25 kb) from

yeasts and filamentous fungi,<sup>7</sup> which can be used in direct sequencing. As shown in Table 1, direct sequencing with the ITS4 primer enabled positive identification of each of the ten clinical fungal isolates and one environmental isolate. Thus, direct sequencing of DNA purified from fungal cultures using the MasterPure Kit offers a rapid, convenient, and relatively inexpensive method for identifying medically significant fungi. The additional time and expense of PCR are also avoided. The multiple copies (50-200) of fungal rDNA genes<sup>6</sup> provide a sufficient homogeneous template for direct sequencing without amplification. Sequencing with commonly available instrumentation yields read lengths nine-fold longer than those obtained from typical pyrosequencing. As a result, the accuracy of BLAST sequence alignments is improved.

## References

1. Ciardo, D.E. *et al.*, (2006) *J. Clin. Microbiol.* **44**(1), 77.
2. Ahmadian, A. *et al.*, (2006) *Clin. Chim. Acta* **363**(1-2), 83.
3. van Burik, J-A, *et al.*, (1998) *J. Clin. Microbiol.* **36**(5), 1169.
4. Aoki, F.H. *et al.*, (1999) *J. Clin. Microbiol.* **37**(2), 315.
5. Xu, J. *et al.*, (2005) *New Microbiol.* **28**(2), 135.
6. Rustchenko, E.P. *et al.*, (1993) *J. Bacteriol.* **175**(22), 7189.
7. EPICENTRE Forum (2002), **9**(3), 11.

## Acknowledgments

This article is based on a poster presented at the ASM Meeting, May, 2006, Orlando, FL. The authors wish to thank Julie Capadona for her graphic design work.

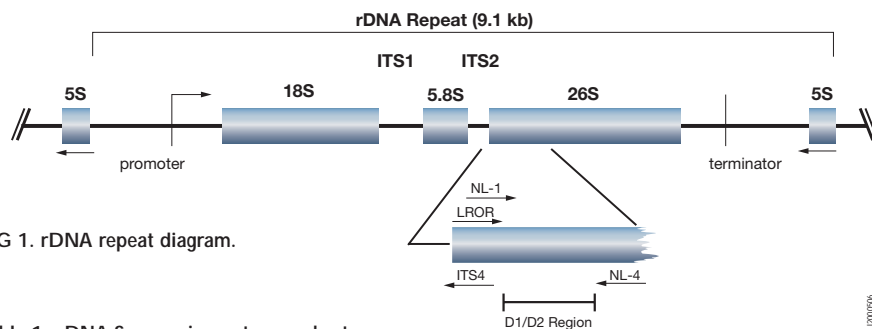


FIG 1. rDNA repeat diagram.

Table 1. gDNA Sequencing outcome chart.

Sample Identity	[gDNA] ng/μl	Primers							
		LROR	e value	NL-1	e value	NL-4	e value	ITS4	e value
<i>Cryptococcus neoformans</i>	39	yes	4e-91*	yes	0.0	yes	0.0	yes	0.0
<i>Yarrowia (Candida) lipolytica</i>	46	no		yes	0.0	yes	4e-9	yes	2e-156
<i>Candida tropicalis</i>	43	no		no		yes	2e-32	yes	2e-29
<i>Candida parapsilosis</i>	34	yes	7e-110	no		no		yes	0.0
<i>Cryptococcus laurentii</i>	116	yes	0.0	no		no		yes	8e-100
<i>Clavispora (Candida) lusitanae</i>	37	no		no		yes	2e-29	yes	3e-173
<i>Cryptococcus neoformans</i>	25	yes	3e-119	yes	0.0	yes	3e-96	yes	0.0
<i>Aspergillus fumigatus</i>	48	no		yes	2e-63	yes	8e-14	yes	6e-61
<i>Candida albicans</i>	25	nd		nd		nd		yes	0.0
<i>Candida glabrata</i>	20	nd		nd		nd		yes	0.0
<i>Coprinus comatus</i>	50	nd		nd		nd		yes	0.0

nd = not determined

\*Expect Value

"... E value describes the random background noise that exists for matches between sequences. For example, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. This means that the lower the E-value, or the closer it is to "0" the more "significant" the match is." See

[http://www.ncbi.nlm.nih.gov/blast/blast\\_FAQs.shtml](http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.shtml)

[www.EpiBio.com/masterpure\\_yeast.asp](http://www.EpiBio.com/masterpure_yeast.asp)

**MasterPure™ Yeast DNA Purification Kit**

MPY80010 10 Purifications  
 MPY80200 200 Purifications

Contents: Yeast Cell Lysis Solution, MPC Protein Precipitation Reagent, TE Buffer and RNase A.

\* Pyrosequencing is a trademark owned by Biotage AB. Pyrosequencing technology is covered by patents and patent applications owned by Biotage AB.



# Stretch the limits— Reliable and Consistent Extra-Long PCR

## MasterAmp™ Extra-Long PCR Kit

The MasterAmp™ Extra-Long PCR Kit allows successful and accurate one-step amplification of sequences from 20 kb to 40 kb in an easy to use optimization format. The kit contains the MasterAmp™ Extra-Long DNA Polymerase mix along with 9 MasterAmp™ Extra-Long PCR 2X PreMixes containing dNTPs, buffer, varying amounts of MgCl<sub>2</sub>, and the MasterAmp™ PCR Enhancer (with betaine\*). For each new set of template and primers perform a PCR with the MasterAmp™ Extra-Long DNA Polymerase mix and 9 PreMixes to find the optimal PreMix, and then perform subsequent amplifications with the same template and primer pair using that PreMix.

### Applications

- ✦ Determination of optimal conditions for extra long PCR in a single experiment.
- ✦ Amplification of PCR sequences for 20 kb to 40 kb.

### Benefits

- ✦ One-Step Optimization of Extra-Long PCR reactions.
- ✦ High fidelity MasterAmp™ Extra-Long DNA Polymerase mix ensures PCR products with the lowest error rate.
- ✦ No hot-start necessary.

#### STEP 1

Perform PCR with your template and primers using MasterAmp™ Extra-Long DNA Polymerase Mix and the 9 PreMixes supplied in the MasterAmp™ Extra-Long PCR Kit (FIG 1) to find the optimal PreMix.

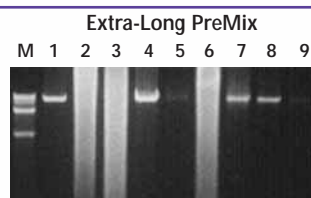


FIG 1. Amplification of a 20 kb region of lambda DNA using MasterAmp Extra-Long PCR 2X PreMixes (1-9). MasterAmp Extra-Long PCR PreMix 4 produced optimal results. Lane M = DNA size marker.

#### STEP 2

Continue to use the optimal MasterAmp™ Extra-Long PCR PreMix chosen in Step 1 to obtain reliable and consistent PCR results using the same template and primer pair to amplify the same sequence in subsequent samples (FIG 2).



FIG 2. Eight individual amplifications of a 20 kb region of lambda DNA using MasterAmp™ Extra-Long PCR PreMix 4. Subsequent PCR results are consistent and reliable using the PreMix identified in Step 1.

### Ordering Information

#### MasterAmp™ Extra-Long PCR Kit

MHF9220 50 Reactions

Contents: MasterAmp™ Extra-Long PCR PreMixes 1-9, MasterAmp™ Extra-Long DNA Polymerase Mix, Control Lambda DNA/Primers.

#### MasterAmp™ Extra-Long DNA Polymerase Mix (Enzyme Mix Only)

QU92125 125 Units

QU92500 500 Units

QU9201K 1000 Units

Contents: MasterAmp™ Extra-Long DNA Polymerase Mix at 2.5 U/μl.

#### MasterAmp™ Extra-Long PCR 2X PreMixes

PreMix 1 - MHF925A 5 ml

PreMix 2 - MHF925B 5 ml

PreMix 3 - MHF925C 5 ml

PreMix 4 - MHF925D 5 ml

PreMix 5 - MHF925E 5 ml

PreMix 6 - MHF925F 5 ml

PreMix 7 - MHF925G 5 ml

PreMix 8 - MHF925H 5 ml

PreMix 9 - MHF925I 5 ml



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\*Use of Betaine for DNA Polymerase Reactions, including, but not limited to, use for PCR or DNA Sequencing, is covered by U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1, and other issued or pending applications in the U.S. and other countries that are either assigned or exclusively licensed to EPICENTRE. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased products solely for life science research. Contact EPICENTRE for information on licenses for uses in diagnostics or other fields.



# Successful PCR— First Time and Every Time

## FailSafe™ PCR System

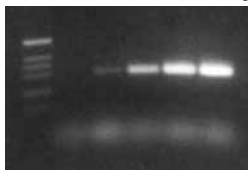
The FailSafe™\* PCR System sets a new standard for PCR by combining a unique blend of thermostable enzymes and an extensively tested set of 2X PreMixes into a single system. The FailSafe PCR System provides dependable, consistent high-fidelity PCR results for every DNA template, regardless of its source 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](http://www.EpiBio.com) for additional details and data.)

Amt Human Genomic DNA  
M 0 1 10 50 100 ng



Fragile X Gene

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.<sup>a</sup>
- ✧ 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 Enzyme Mix and PreMixes are available separately.

#### 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



\*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](http://www.EpiBio.com) website for complete license statements.

<sup>a</sup>Patents issued and pending on FailSafe™ PCR Enhancer.

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# High-Throughput Fosmid and BAC Purification



## BACMAX96™ DNA Purification Kit

Purify 384 fosmids or BACs in less than four hours with EPICENTRE's BACMAX96™ DNA Purification Kit manual protocol. We've modified our popular BACMAX™ Purification Kit to provide you with the high-throughput, high quality purification you asked us for. The purified DNA performs excellently in downstream applications such as fingerprinting, end-sequencing, and transposon insertion sequencing.

### Yield

Whether the vector is single-copy or one of EPICENTRE's induced CopyControl™ systems, BACMAX96™ consistently delivers enough DNA for a fingerprint and two end-sequences.

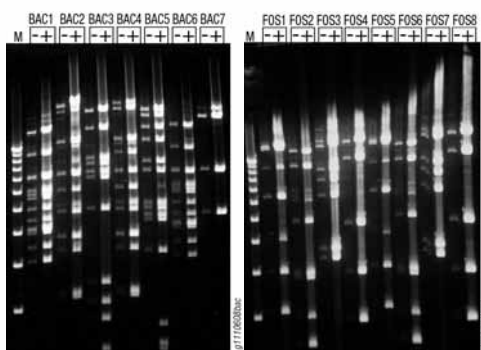
Yield	Single-Copy	Induced CopyControl™
Fosmid	600-900 ng	2.1-2.5 µg
BAC	600-900 ng	2.0-2.5 µg

Typical yield of a 1.2 ml culture for the BACMAX96™ DNA Purification Kit.

### Induction

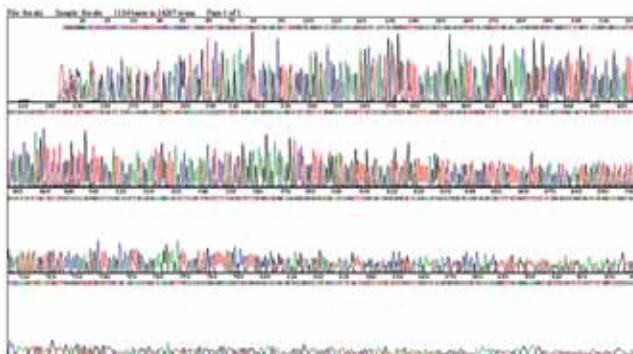
When used with an EPICENTRE CopyControl™ vector, a simple hands-off auto induction step increases plasmid yield without time-consuming subculturing.

*Hind* III digests of uninduced, single-copy (-) and induced (+) CopyControl™ Fosmids and BACs. Digests are performed on one third (8µl) of the sample.



### Purity

The addition of Plasmid-Safe™ ATP-Dependent DNase selectively removes any genomic DNA contamination, a common problem in other high-throughput methods.



Using the rest of the uninduced sample, two end-sequencing reactions can be performed with excellent results. Sequencing reads at 1000 bp were consistently obtained using an ABI 3730 DNA Analyzer using Big Dye Terminator.

**A fingerprint and two sequences from every prep, every time.**

## Ordering Information

### BACMAX96™ DNA Purification Kit

BAC96066 1 Kit

Kit contains: BACMAX96™ Solutions 1,2, and 3, BACMAX96™ RNase Blend, Plasmid-Safe™ ATP-Dependent DNase, Plasmid-Safe™ 10X Buffer, 100 mM ATP Solution, TE Buffer, and BAC and Fosmid Autoinduction Solutions.

Kit reagents are sufficient for 4 X 96 well plate purifications.



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# EPICENTRE Simplifies BAC and Fosmid Library Construction

CopyControl™ BAC Cloning Kits—CopyControl™ Fosmid Library Production Kit

EPICENTRE's CopyControl™ Systems\* provide researchers the quality reagents they need to build complete BAC and fosmid libraries.

## CopyControl™ BAC Kits:

**Cloning Efficiency**—Vectors are pre-cut, dephosphorylated, and highly purified to provide maximum cloning efficiency with very few empty vectors.

**Construction Time**—The ligation and insert size-screening processes have both been streamlined, shortening the library construction time by at least three days.

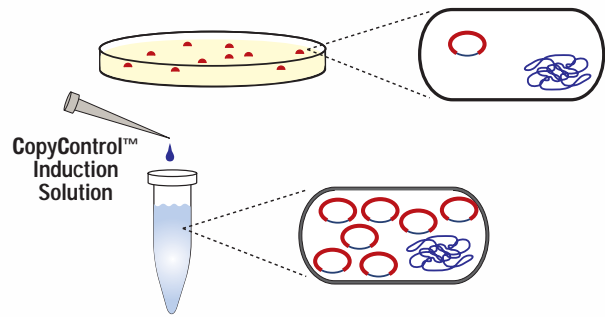
**DNA Yield**—The CopyControl™ feature allows yields of over 2.5µg of DNA to be obtained from 1.2mls of culture using the BACMAX96™ DNA Purification Kit.

## CopyControl™ Fosmid Kits:

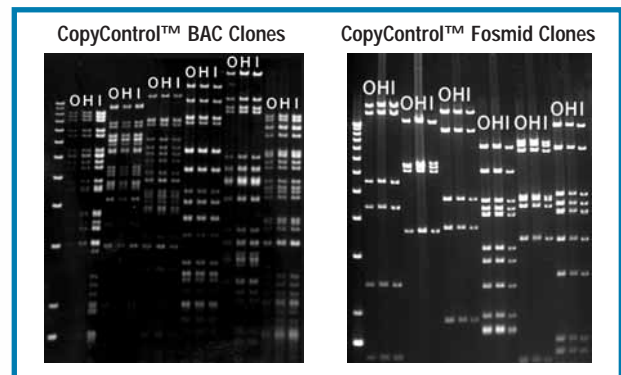
**Unbiased Library**—Using randomly sheared genomic DNA creates a complete library without restriction site biases inherent to many organisms.

**Throughput**—The CopyControl™ HTP Fosmid Library Production Kit has been optimized for high-throughput sequencing, with very short vector-sequence reads.

**Sample DNA Preparation**—Obtaining 40 kb DNA pieces for fosmids does not require PFGE or partial restriction digests, removing difficult steps in the library construction process.



CopyControl™ technology allows the researcher to maintain single-copy clones to ensure stability. A simple induction step raises the copy number, increasing DNA yields during purification.



Consistent *Hind* III restriction patterns indicate that CopyControl™ clones are stable after 100 generations of growth (Lane H) or following a 2 hr induction (Lane I) when compared to a 0 generation control (Lane O).

\*CopyControl™ Products are covered by U.S. Patent No. 5,874,259 licensed to EPICENTRE and by other patents pending and assigned to EPICENTRE for specific CopyControl vectors and/or cell lines.

## Ordering Information

### CopyControl™ BAC Cloning Kits

<b>BamH I</b>	CCBAC1B	1 Kit
<b>EcoR I</b>	CCBAC1E	1 Kit
<b>Hind III</b>	CCBAC1H	1 Kit

### CopyControl™ HTP Fosmid Library Production Kit

CCFOS059	1 Kit
----------	-------

### CopyControl™ Fosmid Library Production Kit

CCFOS110	1 Kit
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# Obtain *much higher* yields of yeast and fungal genomic DNA

## MasterPure™ Yeast DNA Purification Kit

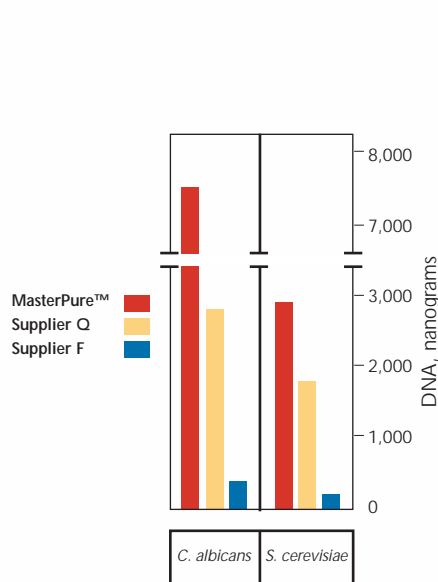
The MasterPure™ Yeast DNA Purification Kit enables efficient, high-yield purification of high molecular weight DNA from yeast and filamentous fungi. No lyticase or proteolytic enzymes are used in the procedure. Yeast genomic DNA yields using the MasterPure Kit are much higher than yields obtained with other commercially available kits (FIG 1). The protocol can be easily adjusted for larger or smaller samples, including single yeast colonies.

### Applications

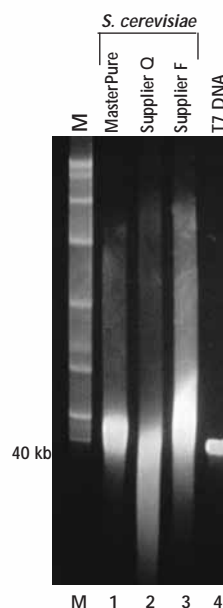
- ✧ PCR amplification, restriction endonuclease digestion, Southern blotting, and genomic library preparation.
- ✧ Identification and typing of fungi.

### Benefits

- ✧ Higher yields of yeast chromosomal DNA.
- ✧ Fungi tested:
  - *Candida*
  - *Saccharomyces*
  - *Pichia*
  - *Schizosaccharomyces*
  - *Aspergillus*
  - *Penicillium*
- ✧ Rapid – purify DNA in less than 40 minutes.
- ✧ Simple and safe – eliminates the need for enzymatic lysis, columns, and phenol.



**FIG 1. The MasterPure™ Yeast DNA Purification Kit gives higher yields of DNA than other kits.** The data represent the average DNA yields determined by fluorometry from two experiments with *S. cerevisiae* and *C. albicans*. The MasterPure Kit produced up to 17 times more DNA from *C. albicans* and 12 times more DNA from *S. cerevisiae* than other kits.



**FIG 2. Yeast DNA purified using the MasterPure™ Kit has a higher molecular weight.** 500 ng of purified yeast DNAs were analyzed by pulsed field gel electrophoresis on a 1% agarose gel, then stained with ethidium bromide. Lane M, lambda DNA ladder; Lane 4, T7 DNA (40 kb).

### Ordering Information

#### MasterPure™ Yeast DNA Purification Kit

MPY80010 10 Purifications  
MPY80200 200 Purifications

Contents: Yeast Cell Lysis Solution, MPC Protein Precipitation Reagent, TE Buffer, and RNase A.



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# Hot Acid Phenol is a Thing of the Past

## MasterPure™ Yeast RNA Purification Kit

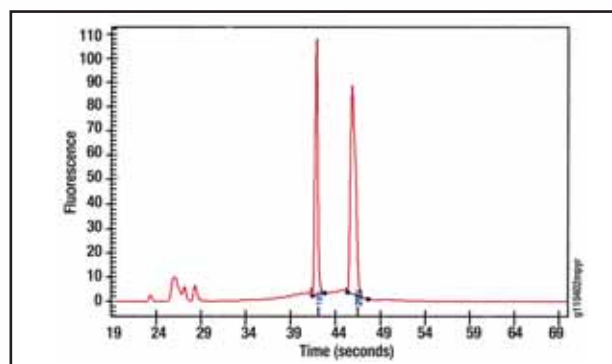
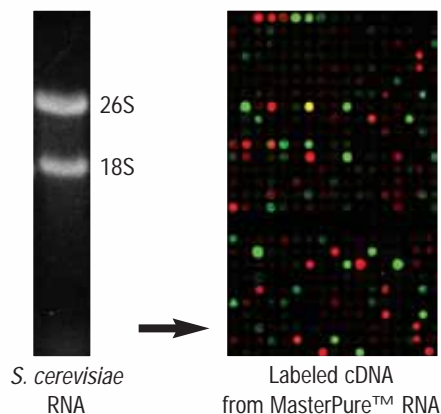
The MasterPure™ Yeast RNA Purification Kit provides all of the reagents needed to purify RNA from cell types including: *Candida*, *Saccharomyces*, *Schizosaccharomyces*, and *filamentous* fungi. The kit utilizes a rapid desalting process to remove contaminating macromolecules, avoiding toxic organic solvents, bead-beating, and spheroplasting.

### Applications

- \* Obtain RNA suitable for cDNA synthesis and microarray gene expression analysis.

### Benefits

- \* Avoid hot acid phenol.
- \* Faster than spheroplasting.
- \* Higher quality than bead-beating.
- \* No extra enzyme or equipment to purchase.
- \* Also extract RNA from *Aspergillus fumigatus*.



Electrophoretogram of the *S. cerevisiae* RNA.

### Ordering Information

#### MasterPure™ Yeast RNA Purification Kit

MPY03010	10 Reactions
MPY03100	100 Reactions

Contents: Extraction Reagent for RNA, MPC Protein Precipitation Reagent, TE Buffer (in 100 rxn kit only), Proteinase K (50 µg/µl), RNase-Free DNase I (1 Unit/µl), 10X DNase Buffer, and 2X T & C Lysis Solution.



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# Get High Yields of Biotin-aRNA for GeneChip® and Illumina® Arrays



## TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104

The TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 produces high yields of biotin-aRNA from small samples sizes for microarray analysis. The kit includes Biotin-UTP for easy and rapid direct labeling of target anti-sense RNA (aRNA; also called cRNA).

### TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104:

- ✦ Generates microgram amounts of biotin-aRNA from as little as 25 ng of total RNA.
- ✦ Includes Biotin-UTP/UTP PreMix for optimal labeling and signal intensity.
- ✦ Produces high quality microarray results using Affymetrix GeneChip, Illumina, GE CodeLink™, and other arrays utilizing biotin-aRNA target.
- ✦ Is Fast. Produce high yields of biotin-aRNA in 1 day.
- ✦ Delivers reproducible amplification and labeling results.

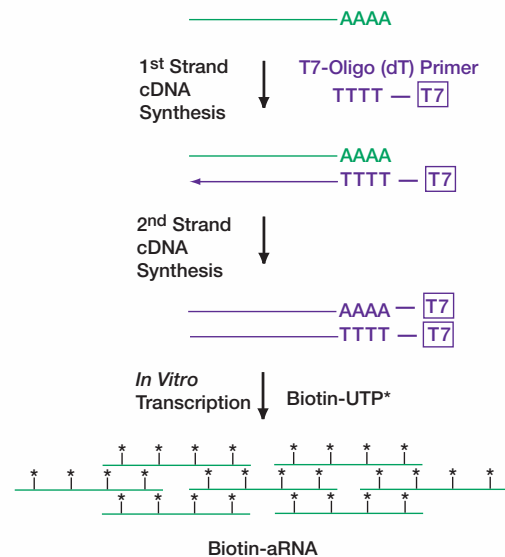


FIG 1. The TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 includes an optimized Biotin-UTP / UTP PreMix for optimal labeling and signal intensity.

Table 1. The TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 produces high yields of biotin-aRNA from small amounts of input RNA.

Input Total RNA	Yield (fold-amplification)
25 ng	4.1 µg (7300)
100 ng	13.7 µg (7300)
500 ng	64.9 µg (6300)

Biotin-aRNA synthesis kit	Amount of total RNA	% Present Calls
TargetAmp™ Kit 104	200 ng	70%
Competitor A	1000 ng	55%

FIG 2. Biotin-aRNA produced by the TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 yields higher Present Calls than a competitor's kit on a CodeLink™ UniSet Human I Bioarray.

### Ordering Information

#### TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104

TAB1R6910	10 Reactions
TAB1R6924	24 Reactions



Find this product web page using the QuickInfo search box! Go to [www.EpiBio.com](http://www.EpiBio.com) and enter this QuickInfo code: **T4DX1**

# Make RNA Transcripts That Are Completely Resistant to RNase A

DuraScribe® T7 Transcription Kit – DuraScribe® SP6 Transcription Kit

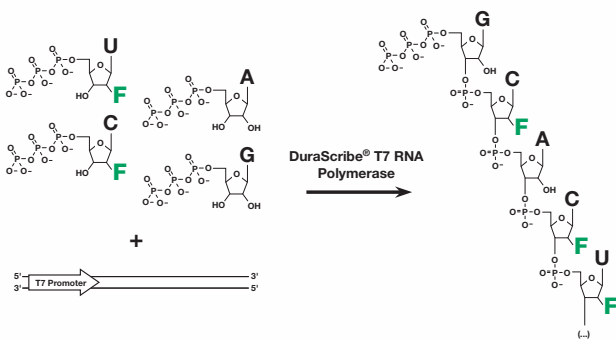
The DuraScribe® T7 RNA Polymerase efficiently incorporates 2'-Fluorine-dCTP (2'-F-dC) and 2'-Fluorine-dUTP (2'-F-dU), as well as ATP and GTP, into full-length DuraScript® RNA. The presence of the 2'-F-dC and 2'-F-dU renders DuraScript RNA completely resistant to the ubiquitous RNase A.

## Applications

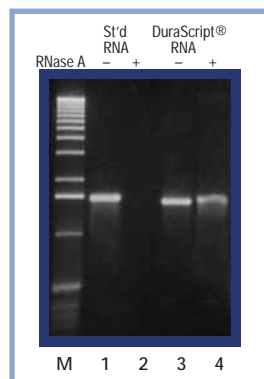
- ✧ Aptamer generation.
- ✧ Ribozyme studies.
- ✧ Anti-sense studies.
- ✧ RNase protection studies.

## Benefits

- ✧ DuraScript® RNA is completely resistant to RNase A.
- ✧ Make up to 50 µg DuraScript RNA per reaction.
- ✧ Use templates with standard T7 promoters.
- ✧ DuraScript RNA retains sensitivity to RNase T1, RNase H, and human Dicer.



**FIG 1.** DuraScribe® T7 RNA Polymerase efficiently incorporates 2'-F-dCTP and 2'-F-dUTP as well as ATP and GTP into full length DuraScript® RNA. The presence of the 2'-F on the C and U nucleotides prevents RNase A digestion.



**FIG 2.** DuraScript® RNA is completely resistant to RNase A digestion.

Lane M, Size ladder;  
Lane 1, Standard RNA transcript  
Lane 2, Standard RNA after RNase A treatment  
Lane 3, DuraScript RNA  
Lane 4, DuraScript RNA after RNase A treatment.

## Ordering Information

### DuraScribe® T7 Transcription Kit\*

DS010910 10 Reactions  
DS010925 25 Reactions

### DuraScribe® SP6 Transcription Kit\*

DS041010 10 Reactions

*Contents:* DuraScribe® T7 or SP6 Enzyme Mix, DuraScribe® T7 or SP6 10X Reaction Buffer, ATP, GTP, 2'-F-dCTP and 2'-F-dUTP, DNase I, DTT, DuraScript® RNA Control Template, and Sterile RNase-Free Water.



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**Biotechnologies**

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\*DuraScribe T7 and SP6 RNA Transcription Kits to synthesize nucleic acids with non-canonical bases or for partial ribosubstitution is covered by U.S. patents 5,849,546; 6,107,037 or 6,596,494 and other patents issued or pending. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for life science research. Contact EPICENTRE concerning licenses for other uses.

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# Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

## Questions about Labeling anti-sense RNA (aRNA; also called cRNA) using EPICENTRE Biotechnologies' TargetAmp™ aRNA Amplification Kits

**Q.** Is it possible to incorporate biotin-UTP directly into the aRNA produced by the TargetAmp™ kits?

**A.** Yes. Both the TargetAmp™ 1-Round & 2-Round™ aRNA Amplification Kits can be modified for direct incorporation of biotin-UTP (provided by the user). Additionally, EPICENTRE's new TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 provides a UTP/biotin-UTP PreMix for optimized labeling and signal intensity. It will produce microgram amounts of biotin-aRNA from as little as 25 ng of total RNA for use on Affymetrix® GeneChip® arrays, Illumina® BeadChips, and other microarray platforms.

**Q.** Can you double-label aRNA with biotin-UTP and biotin-CTP?

**A.** Yes. Data in the literature indicate that the contribution of biotin-CTP to the overall signal intensity is much lower than that of biotin-UTP; however, incorporation of biotin-CTP may have some benefits for the detection of low-abundance targets. In addition to using biotin-UTP, 25-30% of the CTP can be substituted with biotin-16-CTP without decrease of yield in the 1-round amplification reactions.

**Q.** What is the recommended ratio of UTP to biotin-UTP?

**A.** The best ratio of UTP to biotin-UTP can vary a bit based on the nucleotide concentration and the GC content of the transcription templates. To achieve good overall signal intensity without compromising aRNA yield and length, between 25% to 40% of the UTP can be replaced with biotin-16-UTP (corresponding to

ratios of UTP/biotin-16-UTP of between 3:1 and 1.5:1) in the 1-round TargetAmp protocol.

**Q.** What are the advantages of aminoallyl-labeling over direct incorporation of a labeled-NTP?

**A.** The aminoallyl method for indirect labeling of the target nucleic acid has become increasingly popular because it has important advantages over direct incorporation of a biotin- or dye-labeled NTP. Aminoallyl-UTP is more efficiently incorporated into the aRNA during the *in vitro* transcription reaction than labeled nucleotides. Additionally, conjugation of an amine-reactive N-hydroxysuccinimide (NHS) ester of biotin (e.g., Biotin-X-X-NHS; EPICENTRE), Cy-NHS or other fluorescent dye-NHS to aminoallyl-aRNA (AA-aRNA) is a much less expensive way to label the target compared to direct incorporation of labeled nucleotides.

**Q.** What are the advantages of direct labeling of aRNA?

**A.** Labeling by direct incorporation is faster. Conjugation of aminoallyl-aRNA to aminoreactive biotin- or dye-derivatives after *in vitro* transcription requires a 1 hour incubation, and an additional clean-up step compared to direct incorporation. Furthermore, labeling aRNA by direct incorporation does not require the use of toxic reagents (such as dimethyl sulfoxide), which are used in the indirect labeling protocol.

**Q.** Can I use the TargetAmp Kits for producing Cy-labeled aRNA?

**A.** Yes. The TargetAmp™ 1-Round & the TargetAmp™ 2-Round Aminoallyl-aRNA

Amplification Kits produce AA-aRNA, which can be readily labeled with Cy-NHS or other types of fluorescent dye-NHS (provided by the user).

**Q.** What is the best method to clean up the labeled aRNA?

**A.** aRNA labeled by direct biotin-UTP incorporation is usually cleaned up on silica spin-columns. After coupling AA-aRNA to biotin- or dye-NHS, the labeled RNA can be cleaned-up using a spin-column, or with standard ion-exchange or gel filtration methods. Another common approach is to use microconcentrators.

**Q.** Are there any specific precautions one must take to ensure the efficiency of the aminoallyl/N-hydroxysuccinimide coupling reaction?

**A.** Biotin-X-X-NHS is readily hydrolyzed by water and can react with nucleophilic compounds, for example the amino groups of Tris buffers. Biotin-X-X-NHS should be dissolved in dry dimethyl sulfoxide (DMSO) as close to the time of use as possible. Once dissolved in DMSO or other solvent, its stability is entirely dependent on the continued absence of water or other nucleophilic compounds. Since DMSO is extremely hygroscopic and quickly takes up water vapor from the air, we recommend using Biotin-X-X-NHS that has been freshly dissolved in dry DMSO. If the Biotin-X-X-NHS has been dissolved and stored in DMSO, the stability of the Biotin-X-X-NHS should be validated prior to using it for biotinylation of aminoallyl-aRNA from a rare or precious sample.

# PCR Amplification of a High-GC Region of the Glucocorticoid Receptor Gene Using the FailSafe™ PCR System

Richard S. Smith and Gregory A. Hawkins, Center for Human Genomics, Wake Forest University School of Medicine

## Introduction

A key component in any genetic linkage or association study is identifying validated polymorphic markers. This can be accomplished through either database searches or by re-sequencing genomic DNA. Since many single nucleotide polymorphisms (SNPs) reported in databases are not validated, are very rare, or are found in specific racial or ethnic groups, re-sequencing a subset of a study population is mandatory to identify and validate useful SNPs. One of the primary areas of research in our laboratory is determining which genetic factors increase an individual's risk for asthma and severe asthma. Therefore, before a gene is studied in one of our asthma populations, we routinely re-sequence candidate genes in a panel of 96 DNA samples collected from two asthma studies<sup>1,2</sup> consisting of 24 Caucasians, 24 African Americans, 24 Hispanics, and 24 Dutch Caucasians, with each population subset divided into 16 individuals with asthma and 8 unaffected controls.

Our general strategy for identifying SNPs is to generate 600- to 800-bp PCR products that include all exons, exon-intron junctions, and ~1 to 3 kb of the putative promoter sequence. Since introns are excellent sources for SNPs, up to 300 bases of intronic sequence, which flank exons, are normally screened. Small introns are completely sequenced. Because our laboratory performs high throughput DNA sequencing, standard PCR protocols using *Taq* polymerase are used to streamline the PCR process. To improve the success of PCR reactions using *Taq* polymerase, we routinely include EPICENTRE Biotechnologies' MasterAmp™ PCR\* Optimization Kit in our optimization process to increase our PCR success rate.

The glucocorticoid receptor gene (*NR3C1*) has important pharmacogenetic implications in the treatment of asthma and severe asthma. *NR3C1* consists of twelve exons<sup>3</sup> that are differentially spliced to produce three 80 kDa isoforms: GR $\alpha$ , GR $\beta$ , and GR $\gamma$ .<sup>4,5</sup> *NR3C1* has three forms of exon 1: 1A, 1B, and 1C, each with its own promoter region.



Gregory A. Hawkins, Ph.D.

Exons 1B and 1C lie about 1 kb apart in a very GC-rich region of *NR3C1*. In our effort to re-sequence *NR3C1*,<sup>6</sup> one segment of exon 1B was consistently resistant to PCR amplification using standard PCR techniques and multiple primer re-designs.

Based on our tiling path for re-sequencing *NR3C1*, we identified a 131 bp region (89.3% GC content) in exon 1B that appeared to cause our PCR difficulty (FIG 1A). Based on DNA folding analysis using the program Mfold,<sup>7</sup> this 131 bp region was capable of forming a thermo-

dynamically stable stem-loop structure (FIG 1B). Several attempts were made to amplify a clean PCR product of this region through the use of different types of PCR, denaturing agents, and primer design. In this report, we show that after exhaustive attempts to PCR amplify this region using standard PCR methods, we were only able to obtain a clean PCR product using EPICENTRE's FailSafe™ PCR System.

## Methods and Results

PCR amplification was carried out in a 20  $\mu$ l volume using 20 ng DNA. The primer pair used (Forward: 5'-GAG GTA GCG AGA AAA GAA ACT GG-3'; Reverse: 5'-CCC GTC ACA GAC ACG AGC T-3') was designated GRlex1b-9 and produced a 490 bp PCR product. Cycling conditions were: denaturation at 95°C (5 minutes) followed by 35 cycles of 95°C (1 minute), 55°C (1 minute), 72°C (1 minute), and a final extension at 72°C (7 minutes). The PCR product was purified using a 96-well PCR purification kit and sequenced. DNA sequencing data was aligned and polymorphisms identified using Sequencher™ DNA analysis software (Gene Codes Corporation, Ann Arbor, MI).

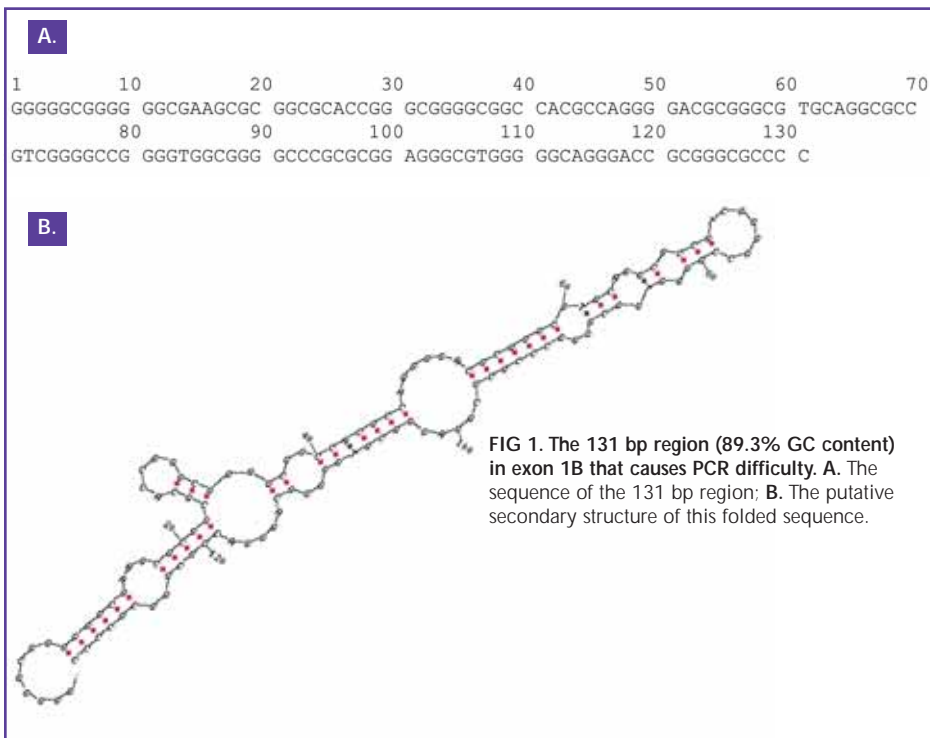
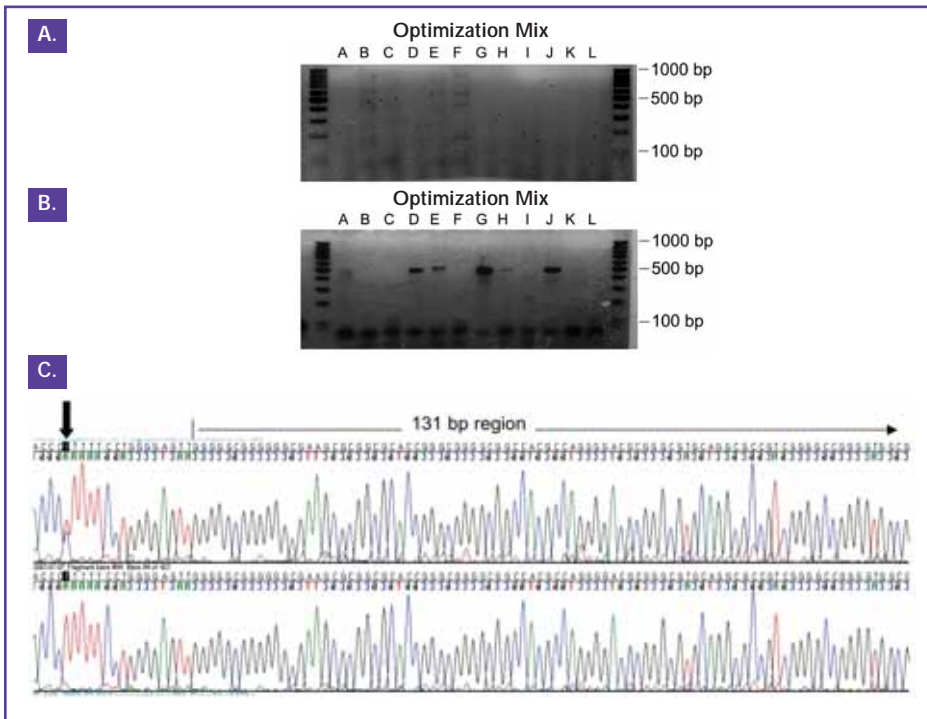


FIG 1. The 131 bp region (89.3% GC content) in exon 1B that causes PCR difficulty. A. The sequence of the 131 bp region; B. The putative secondary structure of this folded sequence.



**FIG 2. PCR and sequencing results.** A. PCR using standard *Taq* polymerase and MasterAmp™ Optimization mixes; B. PCR using the FailSafe™ PCR System; C. Sequencing results for 131 bp region. Arrow shows T>C polymorphism identified 5' of 131 bp region.

FIG 2A shows one of the many attempts to amplify the exon 1B region using the GRLex1b-9 primers with standard *Taq* polymerase and EPICENTRE's MasterAmp™ PCR Optimization PreMixes A-L. FIG 2B shows a first attempt PCR using identical PCR conditions with the FailSafe™ PCR System. FailSafe PreMix G was chosen to amplify the complete screening plate of 96 samples. FIG 2C shows sequence data through a portion of the 131 bp region, including a polymorphism that was identified 16 bp 5' of the start of the 131 bp region.

**Conclusion**

In the past 4 1/2 years, our lab has sequenced more than 100 candidate genes in order to identify and validate genetic polymorphisms for various genetic linkage and association studies. On first pass PCR optimization, using standard PCR conditions and *Taq* polymerase, our PCR success rate was 70-80%. However, by including the MasterAmp Optimization Kit and the FailSafe PCR System in our PCR optimization process, our PCR success rate was >95% for most genes. Many of the remaining PCR problems can be traced to poor primer design. Thus, the MasterAmp Optimization Kit and the FailSafe PCR System are, and will remain, an integral part of our high throughput sequencing process.

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[www.EpiBio.com/masteramp\\_pcr.asp](http://www.EpiBio.com/masteramp_pcr.asp)

**MasterAmp™ PCR Optimization Kit WITHOUT Ammonium Sulfate**

MOS001	20 Templates
MO7201	60 Templates

For MasterAmp™ *Taq* and AmpliTherm™ DNA Polymerases. Contains 12 MasterAmp™ PCR PreMixes (A-L), 0.5 ml or 1.5 ml each for 20 or 60 templates, respectively.

**MasterAmp™ PCR Optimization Kit WITH Ammonium Sulfate**

MOS02N	20 Templates
MO751N	60 Templates

For MasterAmp™ *Tfl*, *Tth*, and proofreading DNA Polymerases. Contains 12 MasterAmp™ Ammonium Sulfate PCR PreMixes (AN-LN), 0.5 ml or 1.5 ml each for 20 or 60 templates, respectively.

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

**FailSafe™ PCR PreMix Selection Kit**

FS99060	60 Units
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Contents: FailSafe™ PCR Enzyme Mix and **all 12 FailSafe™ PCR 2X PreMixes.**

**Note:** Each PreMix volume has been modified to match the Enzyme Mix volume.

**FailSafe™ PCR System with PreMix Choice**

FS99100	100 Units
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Contents: Includes FailSafe™ PCR Enzyme Mix and choice of **one** FailSafe™ PCR 2X PreMix.

**FailSafe™ PCR System with PreMix Choice**

FS99250	250 Units
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Contents: Includes FailSafe™ PCR Enzyme Mix and choice of **two** FailSafe™ PCR 2X PreMixes.

**FailSafe™ PCR System with PreMix Choice**

FS9901K	1000 Units
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Contents: Includes FailSafe™ PCR Enzyme Mix and choice of **eight** FailSafe™ PCR 2X PreMixes.

\* EPICENTRE Biotechnologies' 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](http://www.EpiBio.com) for complete license statements.

## 3'/5' Ratio Analysis of FFPE RNA Purified by the MasterPure™ RNA Purification Kit

Bruce. W. Jarvis and Haiying Grunenwald, EPICENTRE Biotechnologies

### Introduction

Archived formalin-fixed, paraffin-embedded (FFPE) tissues are the most widely available specimens for providing a retrospective source of RNA, which can be used for gene expression analysis.

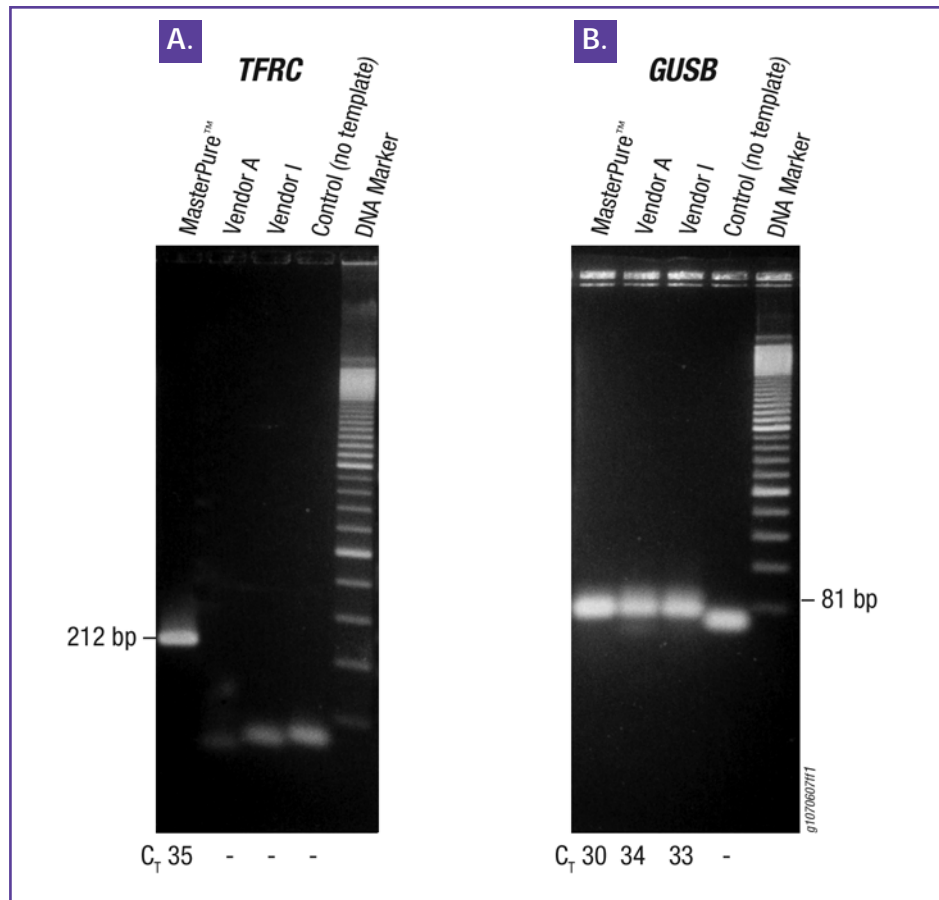
Unfortunately, RNA from FFPE tissue is of notoriously low quality due to a number of factors. These include RNA backbone fragmentation, cross-linkage between nucleic acids and proteins, and the covalent modification of RNA by mono-methylol (-CH<sub>2</sub>OH) addition to the bases.<sup>1</sup> Recently, improved buffers have been developed that protect nucleic acid molecules during fixation,<sup>2</sup> but these methods have not been used for the vast majority of archived tissue sections. RNA damage accumulates over time, with more degradation in evidence after longer periods of archival storage. Nonetheless, RNA fragments purified with the MasterPure™ RNA Purification Kit are of sufficient length and quality for real-time PCR analysis, as demonstrated here.

### Methods and Results

RNA was purified from two 10 µm X 2 cm<sup>2</sup> thin sections of mixed human FFPE tissues (Sigma H-2286) using three kits: EPICENTRE Biotechnologies' MasterPure RNA Purification Kit, and those of two additional vendors labeled A and I. The MasterPure FFPE RNA purification method is as follows:

1. Remove section of tissue using a clean microtome blade—if possible trim excess paraffin.
2. Place 2–30 mg of 10–35 µm thick paraffin sections into an appropriate RNase-free tube.
3. Add 300 µl of the T & C Lysis Solution containing Proteinase K (contained in the kit) to the sample and mix thoroughly.
4. Incubate at 65°C for 30 minutes.
5. Place the samples on ice for 3–5 minutes and then proceed with RNA precipitation.

Total time from FFPE to pure RNA is less than 2 hours.



**FIG 1. Agarose gel analysis of real-time PCR amplification of cDNA reverse-transcribed from FFPE RNA.** **A.** Amplification with *TFRC* primers, amplicon size is 212 bp. **B.** Amplification with *GUSB* primers, amplicon size is 81 bp. Duplicate 25 µl reactions were set up containing: 1X FailSafe™ GREEN Real-Time PCR PreMix E (EPICENTRE), 12.5 pmole of forward and reverse primers, 1 U FailSafe™ Real-Time Enzyme Mix and 1 µl of appropriate cDNA. PCR cycling conditions were: 95°C for 2 minutes, followed by 45 cycles of 95°C for 10 seconds, and 60°C for 1 minute.

The purified FFPE RNA was converted to cDNA by reverse-transcription with EPICENTRE's MMLV-RT-Plus using random hexamer primers. The resulting cDNA was subsequently used in real-time PCR analyses without further purification. In order to assess the quality and quantity of each cDNA sample, real-time PCR of two human housekeeping genes, transferrin receptor (*TFRC*) and β-glucuronidase (*GUSB*), was carried out using SYBR® Green I dye detection (with EPICENTRE's FailSafe™ GREEN Real-Time PCR PreMix E).

As demonstrated in FIG 1B, cDNA obtained from all three vendors' FFPE RNA was amplified with the *GUSB* primers. MasterPure cDNA gave the

lowest C<sub>T</sub> value and, hence, had the highest starting template amount (data not shown). However, for the *TFRC* target (see FIG 1A), only the cDNA reverse transcribed from FFPE RNA purified by the MasterPure Kit gave an amplification product.

RNA integrity is often determined by assessing the 3'/5' ratio of a specific transcript. In this report, two sets of β-actin specific primers, one set of primers close to the 3' end of the message, and the other set closer to the 5' end, were used to amplify cDNA obtained from MasterPure FFPE RNA (14 µg of FFPE RNA was reverse-transcribed into 50 µl of cDNA solution from which 1 µl [~3 ng-worth of mRNA] was used in each real-

time PCR reaction). A standard curve for each set of primers was generated using serially-diluted intact total HeLa RNA reverse-transcribed into cDNA. The absolute quantity of the 3' and 5' end of the  $\beta$ -actin message was calculated using the corresponding standard curve. The ratio of the 3' to 5' message abundance was used to evaluate the quality and integrity of the FFPE RNA (see Table 1).

**Table 1. RNA 3'/5' Ratio Analysis of  $\beta$ -Actin Gene Amplification.** The 3'/5' ratio for  $\beta$ -actin gene amplification is 1.88, which indicates high quality RNA. \* $\beta$ -actin primer sequences obtained from a commercial kit used to assess FFPE RNA quality:

- 3' - End Forward:  
5'-TCCCCCAACTTGAGATGTATGAAG-3'
- 3' - End Reverse:  
5'-AACTGGTCTCAAGTCAGTGACAGG-3'
- 5' - End Forward:  
5'-ATCCCCCAAAGTTCACAATG-3'
- 5' - End Reverse:  
5'-GTGGCTTTTAGGATGGCAAG-3'

Primer*	C <sub>T</sub>	Quantity cDNA Present	3'/5' Ratio
3'	23.6	133 pg	-
5'	27.6	71 pg	1.88

**Conclusion**

The quality of FFPE RNA is crucial for downstream molecular analysis, such as real-time PCR RNA amplification, and microarray analysis. Therefore, it is important to assay the integrity of such RNA by determining the 3'/5' ratio of a specific gene target. A 3'/5' ratio of less than 6 has been considered to indicate high quality RNA.<sup>3</sup> From Table 1, the 3'/5' ratio for  $\beta$ -actin gene was 1.88, which is well within the acceptable range.

Thus, we have shown that RNA purified from FFPE tissue by the MasterPure™ RNA Purification Kit yields high quality RNA suitable for applications such as real-time PCR analysis.

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[www.EpiBio.com/masterpure\\_complete.asp](http://www.EpiBio.com/masterpure_complete.asp)

**MasterPure™ RNA Purification Kit (for isolating RNA only)**

MCR85102 100 Purifications  
 For a small size kit, see the small MasterPure Complete Kit, catalog #MC89010.  
 Contents: Red Cell Lysis Solution, Tissue and Cell Lysis Solution, MPC Protein Precipitation Reagent, 2X T&C Lysis Solution, TE Buffer, RNase-Free DNase I, Proteinase K, and 1X DNase Buffer.

[www.EpiBio.com/masteramp\\_high.asp](http://www.EpiBio.com/masteramp_high.asp)

**MasterAmp™ High Fidelity RT-PCR Kit**

RF91025 25 Reactions  
 RF910100 100 Reactions  
 Contents: MMLV-RT Plus, MasterAmp™ TAQurate™ DNA Polymerase Mix, MasterAmp™ 2X RT-PCR PreMix (includes dNTPs), MasterAmp™ 10X PCR Enhancer, Random Nonamer Primer, Oligo (dT)18 Primer, Control Template and Primer Mix, and Sterile Water.

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

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

FSR0360 96 25- $\mu$ l Reactions  
 Contents: FailSafe™ PCR Enzyme Mix, 12 FailSafe™ GREEN Real-Time PCR 2X PreMixes, and Passive Reference Dye.

*What purification product fits your application?*

**DNA from:**

- Blood, bone marrow, packed cells, buffy coat
- Buccal cell DNA for PCR
- Buccal cell DNA for cloning, sequencing, etc.
- Plant
- Yeast
- Gram positive bacteria
- Gram negative bacteria
- Tissue, mouse tail DNA for PCR
- Tissue, mouse tail DNA for cloning, sequencing
- FFPE tissue DNA

**EPICENTRE Kit:**

- MasterPure™ DNA Purification Kit
- BuccalAmp™ DNA Extraction Kit
- MasterPure™ DNA Kit & Catch-All™ Swabs
- MasterPure™ Plant Leaf DNA Kit
- MasterPure™ Yeast DNA Purification Kit
- MasterPure™ Gram Positive DNA Kit
- MasterPure™ DNA Purification Kit
- QuickExtract™ DNA Extraction Solution
- MasterPure™ DNA Purification Kit
- QuickExtract™ DNA Extraction Solution

**RNA from:**

- Blood, cultured cells (> 10,000), bacteria
- Cultured cells (1 – 10,000)
- Yeast
- FFPE tissue RNA

**EPICENTRE Kit:**

- MasterPure™ RNA Purification Kit
- ArrayPure™ Nano-scale RNA Purification Kit
- MasterPure™ Yeast RNA Purification Kit
- MasterPure™ RNA Purification Kit

# Rapid and Highly Sensitive Screening of Bacterial RNA Polymerase Activity and Inhibitors Using the Kool™ NC-45™ Universal RNA Polymerase Templates

Agnes Radek, EPICENTRE Biotechnologies

Kool™ Universal RNA Polymerase Templates are small (28 – 150 nucleotide) circular single-stranded DNA molecules (ssDNA nanocircles). As observed in the laboratory of Dr. Eric Kool, DNA nanocircles can be efficiently transcribed *in vitro* by DNA-dependent RNA polymerases (RNAP) by a rolling circle transcription (RCT) mechanism\* without the requirement for canonical promoter sequences or a primer.<sup>1,2,3</sup> The product of a Kool RCT reaction can be detected by a variety of methods, including radioactive or non-radioactive end-point detection, or real-time monitoring using fluorescent dyes or molecular beacons.<sup>3</sup>

The Kool™ NC-45™ Universal RNA Polymerase Template (included in the Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit and also sold separately) is a 45 base ssDNA nanocircle that efficiently functions as a template for *in vitro* transcription by bacterial and bacteriophage RNA polymerases, such as *E. coli* RNA polymerase (both core and holo-enzyme), and phages T7, T3, SP6 and N4. In addition, the fact that RCT from a Kool NC-45 Template does not require canonical promoter sequences makes it possible to utilize Kool NC-45 Templates for detecting RNA polymerases. The system is also suitable for the high-throughput screening of potential RNAP inhibitor compounds (see FIG 1).

Here we demonstrate the use of the Kool NC-45 Template as a tool for *E. coli* RNAP inhibition studies (FIG 2).

## Method and Results

### Real-Time detection of inhibitors of *E. coli* RNA polymerase (core enzyme)

To verify the suitability and specificity of the Kool NC-45 Template for screening RNAP inhibitors, the activity of *E. coli* RNAP (Core enzyme; EcRNAP) was tested in the presence and absence of RNAP inhibitors using the Kool RCT assay. The RNAP inhibitors chosen were: rifampicin (a strong inhibitor of EcRNAP),  $\alpha$ -amanitin (an inhibitor of eukaryotic RNA polymerase II), and Tagetin™ RNA Polymerase Inhibitor (EPICENTRE

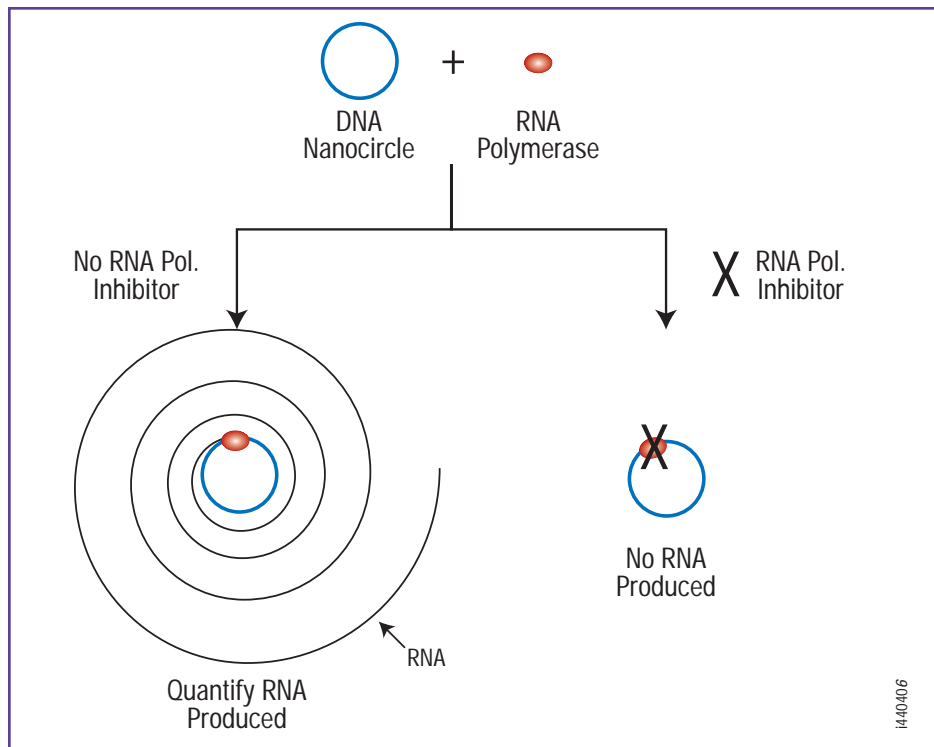


FIG 1. An outline of the Kool™ rolling circle transcription (RCT) assay for RNA polymerase inhibition.

Biotechnologies; the only compound known to potently and selectively inhibit RNA Polymerase III from a variety of eukaryotic organisms, including mammalian cells).

Half a unit of EcRNAP was pre-incubated at 37°C for 10 minutes with or without each inhibitor (25 picomoles of rifampicin, 20 U of Tagetin, or 0.5 ng  $\alpha$ -amanitin) in the presence of *E. coli* RNA Polymerase Reaction Buffer, DTT, and 20 U of RNase Inhibitor. Following incubation, 2.5  $\mu$ l of SYBR® Green I dye

(1:150 dilution of the 10% stock solution provided in the Kit), and 2 picomoles of Kool NC-45 template were added to the reaction. The reaction was initiated by addition of a mixture of ATP, CTP, GTP, and UTP. The reaction contained 0.5mM NTPs, 8 mM DTT, 40 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM MgCl<sub>2</sub>, and 0.01% Triton®-X 100 (final concentration), plus the proteins and inhibitors, in a 25  $\mu$ l volume. RNAP activity was monitored by following the increase of SYBR Green I dye fluorescence at 490

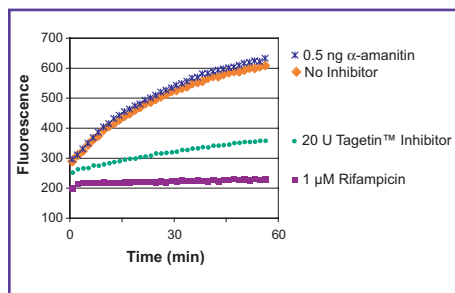


FIG 2. Inhibitors of bacterial RNA polymerases can be rapidly screened using the Kool™ NC-45™ RNA Polymerase Template and the Kool™ NC-45™ RNAP Activity & Screening Kit. The activity of *E. coli* RNA polymerase (Core Enzyme; EcRNAP) was assayed in the presence of the RNAP inhibitors rifampicin,  $\alpha$ -amanitin, and Tagetin™ RNA Polymerase Inhibitor, using the Kool NC-45 Template in the Kool Rolling Circle Transcription assay with fluorescent SYBR Green I dye detection. See text for details.

and 530 nm excitation and emission wavelengths using a Bio-Rad iCycler iQ® Real-Time PCR Detection System. For high throughput screening, fluorescence can be measured using 96-well plates and a microplate fluorimeter. As shown in FIG 2, strong inhibition of EcRNAP activity by rifampicin, a known inhibitor of bacterial RNA polymerase,<sup>4</sup> and partial inhibition by Tagetin<sup>5</sup> can be detected, while  $\alpha$ -amanitin, an inhibitor of eukaryotic RNA polymerase II, has no effect.

**Conclusions**

The Kool™ NC-45™ Universal RNA Polymerase Template and the Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit enable rapid and sensitive real-time screening of inhibitors of *E. coli* RNA polymerase.

**References**

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\* Use of Kool™ Templates in Rolling Circle Transcription reactions is covered by U.S. Patent Nos. 5,714,320; 6,077,668; 6,096,880; 6,368,802; and other patents pending in the U.S. and foreign countries, licensed or assigned to EPICENTRE Biotechnologies. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for life science research. Contact EPICENTRE concerning licenses for other uses.

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

<b>Kool™ NC-45™ RNA Polymerase Template</b>	
KN411100	100 pmoles
<b>Kool™ NC-45™ RNAP Activity &amp; Inhibitor Screening Kit</b>	
KNK49025	25 Reactions

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

<b><i>E. coli</i> RNA Polymerase Core Enzyme</b>	
C90100	100 Units
C90250	250 Units
C90500	500 Units
<b><i>E. coli</i> RNA Polymerase Sigma-Saturated Holoenzyme</b>	
S90050	50 Units
S90100	100 Units
S90250	250 Units

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

<b>Tagetin™ RNA Polymerase Inhibitor</b>		
T9705H	20 U/μl	500 Units
T9701K	20 U/μl	1,000 Units
T9702K	20 U/μl	2,500 Units

## Obtain Higher Molecular Weight Soil DNA without Bead-Beating

Bruce W. Jarvis, EPICENTRE Biotechnologies

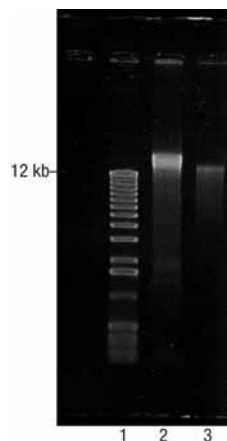
*The SoilMaster™ Kit is ideal for extracting high quality PCR-ready soil DNA without bead-beating.*

DNA fragment size, yield, and quality, are important considerations in research. Consequently, when working with soil DNA, it is essential to choose an extraction procedure that does not include bead-beating, which can shear DNA into smaller fragments. EPICENTRE Biotechnologies' SoilMaster™ DNA Extraction Kit is ideal for extracting high quality PCR-ready soil DNA without bead-beating.

FIG 1 shows the effect of bead-beating on soil DNA fragment length. Lanes 2 and 3 were loaded with DNA extracted from an equivalent amount of the same garden soil. In lane 3, the soil DNA was extracted using another vendor's kit (vendor M), which includes bead-

beating. As can be seen, the soil DNA fragment size in lane 3 has been reduced significantly. In contrast, the DNA extracted using the SoilMaster DNA

Extraction Kit in lane 2 is much larger and more typical of the size used to make fosmid libraries (additional field inversion gel electrophoresis data not shown). Fosmid libraries are used for soil metagenomics work—the study of genomes recovered from environmental samples as opposed to those from pure cultures. Furthermore, the results show that the SoilMaster DNA Extraction Kit provides a greater yield of DNA than the kit from vendor M. High yield is important for obtaining representative DNAs from a mixed soil microbial population.



**FIG 1.** Agarose gel (1%) of DNA extracted from replicate 33 mg samples of garden soil. Lane 1, molecular weight markers; Lane 2, DNA extracted using EPICENTRE's SoilMaster™ DNA Extraction Kit; Lane 3, DNA extracted using vendor M's kit.

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

<b>SoilMaster™ DNA Extraction Kit</b>	
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 and TE Buffer.	

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EPICENTRE's **ScriptCap™ 2'-O-Methyltransferase** allows you to convert Cap 0 transcripts into Cap 1 mRNA, improving *in vivo* translation efficiency by up to 50%. Combine the 2'-O-Methyltransferase with the ScriptCap™ m<sup>7</sup>G Capping System, and you can make Cap 1 mRNA in only 30 minutes.

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## EPI Announcements, etc.

### Appointed as— Marketing Product Manager

**John Luecke** joined EPICENTRE Biotechnologies this summer as a Marketing Product Manager for Genomic Cloning, Transposomics, and Sequencing products. Previously, he gained research experience in both academic and core laboratories at the University of Wisconsin-Madison. John has an undergraduate degree in Microbiology from Miami University (Oxford, Ohio), and both a MS in Bacteriology and MBA in Strategic Management in Life and Engineering Sciences from the University of Wisconsin-Madison.



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