

# EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

## EZ::TN<sup>TM</sup> Transposomes<sup>TM</sup> :

### A Novel System for Identifying Genes Involved in Bacterial Pathogenesis

Magalie R. Guilhabert<sup>1</sup>, Les M. Hoffman<sup>2</sup>, David A. Mills<sup>3</sup> and Bruce C. Kirkpatrick<sup>1</sup>.  
<sup>1</sup>Department of Plant Pathology, University of California, Davis, Davis CA, <sup>2</sup>EPICENTRE,  
<sup>3</sup>Department of Viticulture and Enology, University of California, Davis, Davis CA

#### Introduction

Pierce's disease (PD), a lethal disease of grapevine, is caused by *Xylella fastidiosa*, a Gram-negative, xylem-limited bacterium, transmitted from plant to plant by xylem feeding insects.<sup>1</sup> Strains of *X. fastidiosa* have also been associated with diseases that cause tremendous losses in many other economically important plants including citrus.<sup>2</sup> Although the complete genome sequence of *X. fastidiosa* has recently been determined, the inability to genetically manipulate *X. fastidiosa* has been a major impediment to understanding pathogen, plant and insect vector interactions.

Transposable elements have become valuable mutagenic tools for genetic and molecular analysis in many different bacteria.<sup>3</sup> The most widely used transposon in Gram-negative bacteria is Tn5, which transposes at high frequency, has relatively little target sequence specificity, and does not share homology with genomic sequences of most bacterial species.<sup>4,5</sup> However, transposon mutagenesis can have the following technical limitations: (i) the transposase gene must be expressed in the target host, (ii) the transposon must be introduced into the host on a suicide vector and (iii) the transposase gene on the transposon can be expressed in subsequent generations, which may result in genetic instability.

Recently, the EZ::TN<sup>TM</sup> Transposome<sup>TM</sup> system was developed to overcome the above problems. The EZ::TN Transposome is an association between a hyperactive Tn5-derived EZ::TN<sup>TM</sup> Transposase and a linearized EZ::TN<sup>TM</sup> Transposon that

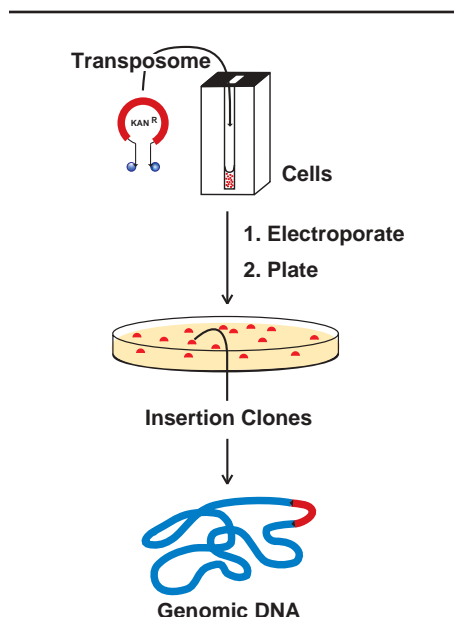


Figure 1. Use of the EZ::TN<sup>TM</sup> Transposome<sup>TM</sup>.

contains a selectable marker flanked at each end by modified 19-bp transposase recognition sequences of Tn5.<sup>6</sup> In the absence of magnesium ions, the DNA:protein complex is stable and may be electroporated into bacterial cells and the EZ::TN Transposon can subsequently undergo transposition *in vivo* (Figure 1).<sup>7,8</sup> Because the EZ::TN Transposase is degraded in the cell, no subsequent transposition occurs. In this paper we describe the successful use of an EZ::TN Transposome to mutagenize *X. fastidiosa*. EZ::TN Transposome insertions have been demonstrated for a number of species, including *Escherichia*, *Proteus*, *Salmonella*, *Pseudomonas*, *Mycobacterium*, and *Saccharomyces*.<sup>7,8</sup> To our knowledge, however, this is the

first report of a transposition system for *X. fastidiosa* and the first use of a Transposome to mutate a plant pathogenic bacterium.

... continued on page 2

Visit EPICENTRE  
(Booth No. 557)  
at ASM in Orlando, FL

#### In This Issue

- 1 A Novel System for Identifying Genes Involved in Bacterial Pathogenesis
- 4 Direct PCR from Dried Blood Without DNA Extraction
- 6 High Fidelity PCR Amplification of DNA up to >40 Kb
- 7 Cloning and Identification of Plant Defense Genes by RT-PCR Using Degenerate Primers
- NEW!** 8 A Revolutionary Method for Buccal Sample Collection and Processing
- 9 Completely Remove Oligonucleotides and Single-Stranded DNA from Your Reaction Mixes
- NEW!** 9 Produce Single-Stranded PCR Product for SSCP or Sequencing
- 10 Consistent Production of the Highest RNA and 5'-Capped RNA Yields from Transcription Reactions
- 12 Gentle and Quantitative Recovery of Large Genomic DNA Fragments from Agarose Gels
- 12 The Highest Transformation Efficiency Electrocompetent Cells
- NEW!** 13 Produce Unbiased Cosmid and Fosmid Libraries
- 14 Use of the EZ::TN<sup>TM</sup> Transposon Insertion System for High Throughput Sequencing at the NIH
- 15 New Applications for the EZ::TN Transposon Insertion System
- 16 Perform DNA Ligations in 5 Minutes!

**Materials and Methods**

**Preparation of electrocompetent *X. fastidiosa* cells.**

Because *X. fastidiosa* cells stored at  $-80^{\circ}\text{C}$  grow poorly when directly seeded into liquid culture, the cells were initially plated on PD3 agar plates. After incubating for 10-12 days at  $28^{\circ}\text{C}$ , a piece of agar medium containing *X. fastidiosa* cells was cut from a plate and used to inoculate 35 ml of liquid PD3 medium. These liquid cultures were allowed to grow for 7-10 days at  $28^{\circ}\text{C}$  and then the cell density was adjusted to  $10^6$  cells/ml ( $\text{OD}_{600}=0.0025$ ) using fresh PD3 medium. One hundred  $\mu\text{l}$  of the adjusted cell culture were used to inoculate each of six PD3 agar plates. After 6 days at  $28^{\circ}\text{C}$ , the cells were gently washed off each plate with 2-3 ml of PD3 medium. The cells were harvested by centrifugation ( $5,000\times g$ ) at  $4^{\circ}\text{C}$  for 5 min, washed in 10 ml of cold, sterile 10% glycerol, concentrated by centrifugation and suspended in 1 ml of cold 10% glycerol. The suspension was centrifuged at  $5,000\times g$  for 5 min at  $4^{\circ}\text{C}$ , resuspended in 10% glycerol to a final concentration of approximately  $10^9$  cells/ml and held on ice until electroporated with the transposon constructs.

***X. fastidiosa* electroporation protocol.**

One microliter of an EZ::TN <KAN-2> Tnp Transposome (20 ng of transposon DNA) or 100 ng of plasmid DNA was mixed with 20  $\mu\text{l}$  of electrocompetent cells and placed in a 0.15 cm gap electroporation chamber (Gibco BRL). A single high-voltage pulse (10 kV/cm for 5 ms with a resistance value of 4 k $\Omega$  and a



**Figure 2. Southern blot analysis of independent EZ::TN™ <KAN-2> Transposon insertion clones of *X. fastidiosa*.** Genomic DNA from *X. fastidiosa* was digested with *EcoR* I/ *Eag* I restriction enzymes and probed with the EZ::TN <KAN-2> Transposon. Lane 1, *EcoR* I/*Eag* I digested DNA isolated from *X. fastidiosa* cells that were not electroporated with the Transposome; Lanes 2 –11, individual transposition clones of *X. fastidiosa*; Lane 6, the clone did not initially hybridize to the probe, however Southern analysis of a second restriction digest yielded positive hybridization results (data not shown); Lane 12, EZ::TN <KAN-2> Transposon positive control.

capacitance of 330  $\mu\text{F}$ ) was applied across the chilled suspension with the Cell-Porator electroporation system (Gibco BRL). After the pulse delivery, the cells were immediately removed from the electroporation chamber and inoculated into 1 ml of liquid PD3 medium without antibiotics. The cells were incubated for 24h at  $28^{\circ}\text{C}$  with constant shaking (100 rpm) to allow expression of

antibiotic resistance. After incubation, the putative transformants were selected on PD3 agar plates containing 5, 10, or 5  $\mu\text{g/ml}$  of kanamycin, tetracycline or chloramphenicol respectively, depending on the antibiotic marker of the construct. The 1-ml of liquid PD3 medium containing the electroporated sample was entirely plated on 10 plates of selective PD3 medium.

**Cosmid cloning of Kan<sup>R</sup> tagged regions of *X. fastidiosa* DNA.**

Cosmid libraries were prepared from each of the 10 *X. fastidiosa* insertion mutants. Briefly, genomic DNA was end-repaired and ligated into a linearized, blunt and dephosphorylated cosmid vector and packaged using MaxPlax™ Lambda Packaging Extracts. Cosmids carrying the <KAN-2> Transposon region were selected on LB plates containing chloramphenicol and kanamycin (25  $\mu\text{g/ml}$  each).

**Sequence analysis of cosmids containing *X. fastidiosa* transposon DNA.**

Cosmid DNA was used as template in “2X” Big Dye Terminator sequencing reactions according to the manufacturer’s protocols (Applied Biosystems) using the forward (KAN-2 FP-1) and reverse (KAN-2 RP-1) primers supplied with the EZ::TN <KAN-2> Tnp Transposome. Samples were injected into an ABI 310 Genetic Analyzer (Applied Biosystem) and analyzed with ABI version 3.3 sequence analysis software. The location of the transposon inserts was determined by comparing the sequence of regions flanking the element with the genome

**Table 1. Sequence analysis and putative function of *X. fastidiosa* DNA flanking EZ::TN Transposon insertions in the Fetzer grapevine strain.**

Insertion Clone	Sequence of <i>X. fastidiosa</i> DNA flanking the insertion <sup>a</sup>	ORF designation <sup>b</sup>	Location on map of <i>X. fastidiosa</i> CVC strain <sup>a</sup>	Putative gene function <sup>c</sup>
FB1	gltttatggctcaCACCGTGGC <KanR> CACCGTGGCtctgtgccc	Xf2752	(2,643,701-2,643,709)	Unknown <sup>d</sup>
FB2	ggacatcacacaGCATGAAGG <KanR> GCATGAAGGtatcgccagatt	--	(943,970-943,978)	Intergenic insertion
FB3	tgccggtgtattGCTATCACA <KanR> GCTATCACAttaatacagcag	Xf1854	(1,770,398-1,770,406)	Unknown
FB9	ttccttctagcaGGTTTTTTG <KanR> GGTTTTTTGtttcccatcag	Xf0070	(64,213-64,371)	Unknown
FC2	ttgttccagtgaCTGCAACAC <KanR> CTGCAACACcgtaggcactcg	Xf1903	(1,810,187-1,810,195)	Potassium transport protein
FD5	ggttttgaagcaGCATTGTGT <KanR> GCATTGTGTtccgccagaagt	Xf1056	(1,015,537-1,015,545)	Unknown
FD6	tgcttcaaaaccGTAACA <KanR> GTAACAAGtgccttaag	Xf1919	(1,824,956-1,824,964)	Similar to human K <sup>+</sup> channel protein
FD7	agagcattggcaGTACATGAAC <KanR> GTACATGAACcgtaccgccctt	Xf0156	(159,967-159,976)	Putative cysteine protease
FD10	ttgactggaictGTCATAAAC <KanR> GTCATAAACacaacctgcat	--	(1,895,553-1,895,561)	Intergenic insertion
FE1	agtaaacctcgaGGTGAGGAT <KanR> GGTGAGGATtgcatttata	Xf2677	(2,551,921-2,551,929)	L-ascorbate oxidase

a. Numbers indicate the position of the EZ::TN Transposon in the genomic sequence of the citrus variegated chlorosis (CVC) strain of *X. fastidiosa*.<sup>9</sup>  
 b. Identification number of open reading frame (ORF) in CVC strain of *X. fastidiosa*.<sup>9</sup>  
 c. Putative function of ORF based on homology with other gene sequences.  
 d. Function of ORF is unknown.  
 e. Bases in capital letters indicate duplicated EZ::TN Transposon insertion site.

sequence of the citrus strain of *X. fastidiosa* (<http://onsona.lbi.ic.unicamp.br/xfi/>).<sup>9</sup>

## Results and Discussion

We evaluated the ability of four different suicide vectors, as well as the EZ::TN Transposome to transpose *X. fastidiosa*. The four suicide vectors tested (pBSL181 (Cm<sup>R</sup>), pBSL346 (Cm<sup>R</sup>), pSUP2021 (Nm<sup>R</sup>), and pSUP102Cm::Tn5 (Tc<sup>R</sup>) failed to produce any detectable transposition events. In contrast, when the EZ::TN Transposome was electroporated into the Fetzer strain of *X. fastidiosa*, 115 Kan<sup>R</sup> colonies were obtained in the first experiment. Controls in which no Transposome complex was added failed to yield any Kan<sup>R</sup> colonies on selective PD3 agar plates, indicating no spontaneous production of Kan<sup>R</sup> colonies. No Kan<sup>R</sup> colonies were obtained when the cells were electroporated with transposon DNA only, indicating that the transposition event is transposase-dependent.

Previous work in other prokaryotes has demonstrated that Tn5 insertions will occur at unique sites in the chromosome.<sup>7,8</sup> To confirm this in *X. fastidiosa*, genomic DNA was isolated from 10 transposon mutants and subjected to Southern hybridization analysis using the <KAN-2> Transposon as a probe. The results indicate that the insertions were single and independent events (Figure 2). Randomness was further verified by sequencing the DNA flanking each of the insertion sites. Cosmid clones carrying the region of genomic DNA that was disrupted by the transposon were selected on kanamycin and then sequenced bidirectionally using primers homologous to either end of the transposon. All 10 insertion sites were unique. A BLAST homology search against the genome sequence of the citrus strain of *X. fastidiosa* indicated that two insertions are in intergenic regions, and eight are in recognized or hypothetical open reading frames (Table 1).

Sequence analysis also indicated nine inserts generated 9 bp duplication at the boundaries, as expected for a Tn5-transposase-mediated transposition.<sup>6</sup> One product (FD7) showed an exceptional insertion of 10 bp duplications at the boundaries (Table 1), which has been observed with *in vitro* insertion of Tn5 transposons (I. Goryshin, personal communication), but not with *in vivo* Tn5 transpositions. The EZ::TN Transposon inserted in both orientations with respect to *X. fastidiosa* open reading

frames indicating that transcription of the kanamycin resistance gene is under control of the Tn903 promoter and not a *X. fastidiosa* promoter.

Because a transformation system can be highly strain-dependent, EZ::TN Transposome electroporation experiments were performed with a second *X. fastidiosa* strain termed Temecula. Following electroporation seventy-four Kan<sup>R</sup> Temecula strain colonies grew on PD3 medium supplemented with 5 µg/ml of kanamycin indicating that the Temecula, like the Fetzer, *X. fastidiosa* strain is amenable to transposition using the Transposome system. We found that for both strains the number of insertion mutants obtained is highly dependent on optimizing the transformation efficiency.

The ability to maintain a transposon insertion without selective pressure was demonstrated when seventy of the transposed products did not lose their kanamycin resistance when grown on medium lacking antibiotic (data not shown). Genetic stability is expected since the EZ::TN <KAN-2> Transposon used in this study lacks the transposase gene and can therefore no longer transpose once it is inserted in the chromosome.<sup>7</sup>

The Transposome strategy should allow the identification of *X. fastidiosa* genes that mediate plant pathogenicity and insect transmission. Our *X. fastidiosa* transposition mutants are currently being inoculated into grapevines in order to identify genes that allow *X. fastidiosa* cells to colonize, move and cause disease in grapevines. Hopefully, mutagenesis studies, in conjunction with the additional genome sequence of the Temecula grapevine strain of *X. fastidiosa*, will lead to a better understanding of the molecular mechanisms of *Xylella fastidiosa* pathogenicity and perhaps suggest novel treatments for Pierce's disease.

## Acknowledgments

This work was supported by the American Vineyard Foundation and the Viticulture Consortium Grants Program of the USDA/ARS. We wish to thank Valley Stewart for his helpful advice and critical review of the manuscript.

## References

1. Frazier, N. W. (1965) Proc. Int. Conf. Virus vector on perennial hosts, with special reference to *Vitis*, ed. WB Hewitt, 91-99. Univ. Calif. Davis.

2. Purcell, A. H. (1997) *J. Plant Pathol.* **79**, 99.
3. Voelker, L. L. and Dybvig, K. (1998) *Methods Mol. Biol.* **104**, 235.
4. Reznikoff, W. S. (1993) *Annu. Rev. Microbiol.* **47**, 945.
5. Goryshin, I. Y. *et al.* (1998) *Genetics* **18**, 10716.
6. Goryshin, I. Y. and Reznikoff, W. S. (1998) *J. Biol. Chem.* **273**, 7367.
7. Goryshin, I. Y. *et al.* (2000) *Nature Biotechnology* **18**, 97.
8. Derbyshire, K. M. *et al.* (2000) *EPICENTRE Forum* **7** (2), 1.
9. Simpson, A. J. G. *et al.* (2000) *Nature* **406**, 151.

### EZ::TN™<KAN-2>Tnp Transposome™ Kit

TSM99K2 10 Reactions

### EZ::TN™<DHFR-1>Tnp Transposome™ Kit

TSM99D1 10 Reactions

### EZ::TN™<R6K<sub>Yori</sub>/KAN-2>Tnp Transposome™ Kit

TSM08KR 10 Reactions

Each Transposome™ kit contains the specific Transposome™ complex and two unlabeled sequencing primers.

## Make Your Own Transposon and Transposome

A custom EZ::TN Transposon containing any DNA sequence of interest (e.g. selectable marker, control element, gene, cDNA) can be quickly and easily prepared using the EZ::TN™ pMOD™-2 <MCS> Transposon Construction Vector. To prepare your transposon, clone the DNA of interest into the multiple cloning site and then release the transposon by PCR or by digestion with *Pvu* II or *Psh* A I. The transposon can be used for *in vitro* insertion into any target DNA, or it can be incubated with EZ::TN Transposase to form an EZ::TN Transposome for random insertion into the genomic DNA of living cells. Your custom transposon will also include primer binding sites at either end for bidirectional sequencing. No need to design your own primers.

See page 15 of this FORUM for ordering information.

# Direct PCR from Dried Blood without DNA Extraction Using the FailSafe™ PCR System

Haiying Grunenwald, EPICENTRE

The FailSafe™ PCR System enabled consistent and reproducible PCR amplification of six different gene targets directly from dried blood without prior nucleic acid extraction of the genomic DNA template.

## Introduction

Dried blood spots collected on Specimen Collection Cards, commonly called “Guthrie cards” (Figure 1), have been used widely as valuable resources for genetic studies, such as, determination of hereditary diseases like phenylketonuria and congenital hypothyroidism. The ease of storage and transportation of these cards with blood spots also lends them to a variety of large field studies, especially in remote areas. For example, over 15,000 Guthrie cards with dried blood spots were collected during a pre-natal HIV transmission study in South Africa.<sup>1</sup>

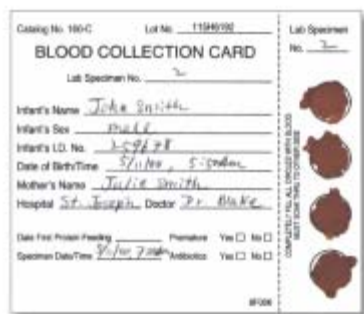


Figure 1. An example of a Guthrie card.

Polymerase chain reaction (PCR) is commonly used when evaluating Guthrie card blood spots. Unfortunately, amplification of DNA from dried Guthrie card blood spots presents considerable technical difficulties due to the presence of natural PCR inhibitors, e.g., protein, heavy metals, heme, and heme degradation products,<sup>2</sup> and the fact that the amount of genetic material is limited. A number of specialized protocols have been developed to effectively recover DNA from Guthrie card blood spots,<sup>3,4</sup> although they still prove to be time consuming, cumbersome, and expensive, especially for high throughput studies.

In a previous report,<sup>5</sup> we demonstrated that the FailSafe PCR System enabled direct PCR amplification using whole

Gene Region amplified	PCR Cycling conditions	Expected size of PCR product
<i>apoE</i>	94°C 5 minutes	268 bp
	95°C 30 seconds	
	60°C 30 seconds	
	72°C 1 minute	
	x 35	
CFTR (cystic fibrosis transmembrane conductance regulator) exon 11	94°C 2 minutes	233 bp
	94°C 10 seconds	
	53°C 10 seconds	
	74°C 10 seconds	
	74°C 5 minutes	
	x 30	
Human microsatellites: DXS6789 DX7132 GATA31E08 GATA175D03	94°C 2 minutes	118 - 150 bp 283 - 299 bp 226 - 254 bp 170 - 186 bp
	94°C 1 minute	
	55°C 1 minute	
	72°C 1 minute	
	74°C 4 minutes	
	x 30	

**Table 1. PCR cycling parameters and expected product size from each of the six primer pairs tested.** Each 50 µl FailSafe PCR reaction contained 25 µl of the appropriate FailSafe PCR 2X PreMix, 50 pmoles of each of the respective primers and 2.5 U of FailSafe PCR Enzyme Mix. One nanogram of purified human genomic DNA was used to identify the optimal FailSafe 2X PreMix for each primer pair.

blood that had been preserved under various conditions, without prior purification of the DNA template. This article reports a fast and easy way to obtain consistent, reproducible PCR amplification directly from dried blood spots collected on Guthrie cards or glass slides, without the need for DNA extraction.

## Materials and Methods

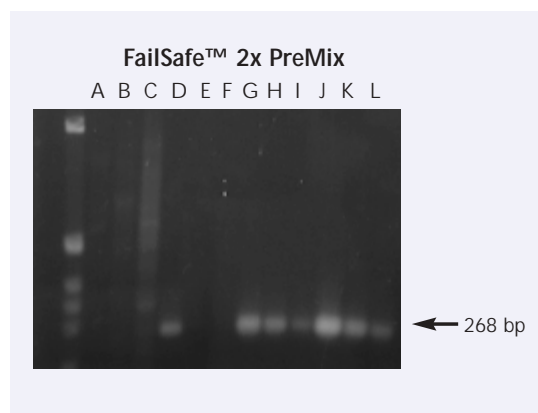
### Identifying optimal PCR conditions

Primer pairs for six different gene targets were tested independently with each dried blood sample. First, the optimal PCR reaction conditions for each primer pair were identified using the FailSafe PCR PreMix Selection Kit and purified human genomic DNA as a template. The appropriate optimal FailSafe PCR PreMix was then used for all subsequent amplifications with each primer pair using dried

blood samples. For example, FailSafe PCR PreMix J was identified as optimal for the *apoE* gene amplification (Figure 2). Similarly, FailSafe PreMix C was chosen for amplification of CFTR exon 11, and PreMix G was chosen for amplification of the four human microsatellite markers (DXS6789, DXS7132, GATA31E08, and GATA175D03). The primer pairs, PCR amplification conditions, and PCR product sizes for the 6 primer pairs are presented in Table 1.

### Methods for processing dried blood samples

Fifty microliters of freshly drawn whole blood was spotted onto Guthrie cards and glass slides. Blood spots were allowed to dry for at least 48 hours before use. Three different methods were used to process the dried blood samples for use in PCR (Figure 3).



**Figure 2. Identification of the optimal FailSafe 2X PreMix for amplification of the *apoE* gene.** The *apoE* gene was amplified from 1 ng of purified human genomic DNA using all 12 FailSafe PCR 2X PreMixes (A-L) contained in the FailSafe PreMix Selection Kit. Amplification conditions were as described in Table 1. PCR products were electrophoresed on a 2% agarose gel and visualized with SyberGold™ (Molecular Probes). FailSafe 2X PreMix J produced optimal results for this template/primer pair.

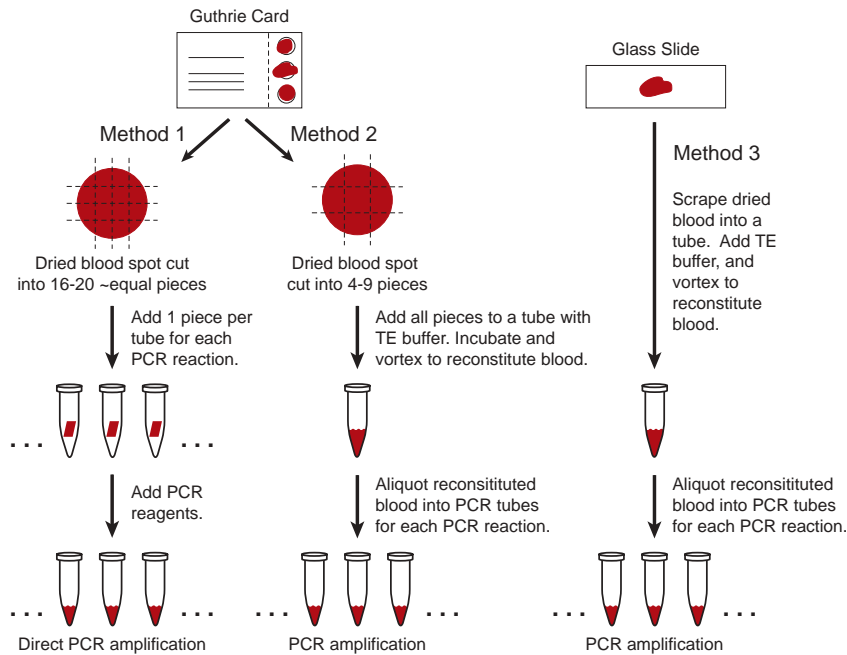


Figure 3. Summary of the three dried blood sample methods used for direct PCR.

In Method 1, the sample used for FailSafe PCR was a piece of a dried blood spot on a Guthrie card. Thus, a Guthrie card circle, completely filled with 50  $\mu$ l of dried blood, was cut into 20 pieces of similar size (each piece > 2mm x 2mm), and stored in a sterile tube at room temperature until used. A single piece of the cut up Guthrie card, corresponding to about 2.5  $\mu$ l of whole blood, was used for analysis by FailSafe PCR.

In Method 2, the sample used for FailSafe PCR consisted of reconstituted blood samples obtained by elution of dried blood spots from Guthrie cards. Guthrie card blood spots were cut into approximately 10 pieces and combined in a sterile 1.5 ml tube. Seventy-five microliters of TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA) was added, and the solution was incubated at room temperature for 10 minutes, and then vortexed vigorously. The reconstituted blood solutions were stored at 4°C until used. One microliter of the resulting reconstituted blood, corresponding to about 0.67  $\mu$ l of the original whole blood sample, was used for analysis by FailSafe PCR. In another experiment using reconstituted blood samples prepared according to Method 2, Guthrie cards were prepared for two individuals using spots containing only 5  $\mu$ l of blood. Each dried blood spot was then eluted with 10  $\mu$ l of TE buffer and FailSafe PCR of human microsatellite marker GATA31E08 was performed using 1  $\mu$ l of the resulting reconstituted

blood solution, which corresponds to 0.5  $\mu$ l of the original whole blood sample.

In Method 3, the sample used for FailSafe PCR was reconstituted blood from dried spots on glass slides. Thus, 50  $\mu$ l samples of fresh blood were dried on a glass slide for 72 hours at room temperature. Then, dried blood samples were individually scraped into a sterile microcentrifuge tube, reconstituted with 75  $\mu$ l of TE buffer, vortexed vigorously, and incubated at room temperature for 10 minutes. The reconstituted blood solutions were stored at 4°C until used. One microliter of the resulting reconstituted blood was used for analysis by FailSafe PCR, which corresponds to about 0.67  $\mu$ l of the original whole blood sample.

#### FailSafe PCR

FailSafe PCR was performed using the amounts and kinds of samples as

described in the section above entitled "Methods for processing dried blood samples," and other reaction components and conditions as described in Table 1 for each of the six primer pairs. Prior to PCR, the reaction tubes containing Guthrie card pieces were incubated for 5 minutes at room temperature and vortexed. Then, PCR reactions were incubated using the cycling conditions described in Table 1 and analyzed on 2% agarose gels visualized with SyberGold™ (Molecular Probes).

#### Results

Figure 4 shows PCR results obtained using samples processed according to Method 1. Using dried blood spot pieces from Guthrie cards for three different individuals, all amplifications were successful with all six primer pairs tested. These amplifications were successfully repeated three times to confirm the consistency and reliability of this direct PCR method.

FailSafe PCR amplifications were also successful with all six primer pairs and all reconstituted blood samples tested using samples prepared by Method 2. As an example, the *apoE* amplification from reconstituted dried blood from Guthrie cards is shown in Figure 5, Lane 4. PCR amplifications using each of the six different primer pairs were successfully repeated three times, confirming the consistency and reproducibility of PCR using samples prepared using this method. Fibers from the Guthrie cards that were present in the reconstituted blood samples did not appear to inhibit FailSafe PCR amplification.

As observed with the other two methods, FailSafe PCR was also successful with all six primer pairs using reconstituted blood samples prepared according to Method 3 from dried blood spots on glass slides. As an example, the *apoE* amplification from reconstituted blood from glass slides is

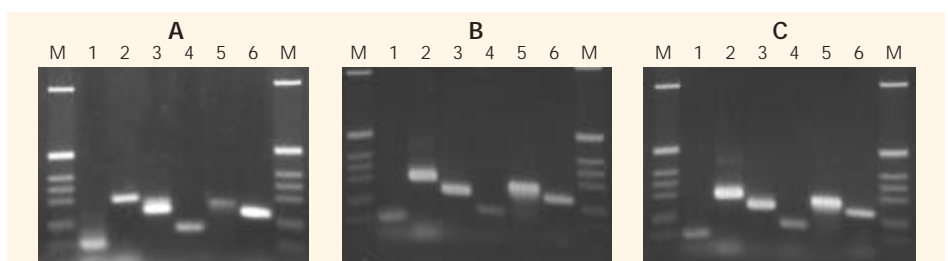
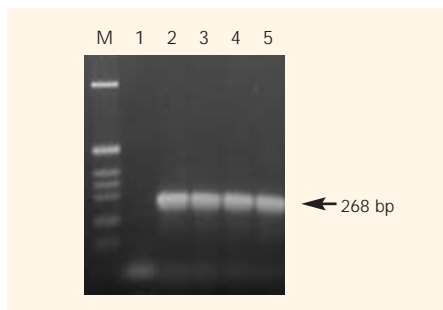


Figure 4. Direct PCR amplification of dried blood from cut up Guthrie card pieces using six different primer pairs and blood from three different individuals. Six different PCR amplifications were carried out using Guthrie card pieces from the dried blood of three individuals (A,B,C). M, DNA size standards; Lane 1, Human microsatellite DXS6789; Lane 2, Human microsatellite DXS 7132; Lane 3, Human microsatellite GATA31E08; Lane 4, Human microsatellite GATA175D03; Lane 5, *apoE*; Lane 6, CFTR exon 11. PCR products were electrophoresed on a 2% agarose gel and visualized with SyberGold™ staining.

shown in Figure 5, Lane 5. The six different amplifications were also successfully repeated three times.

Figure 5 summarizes the direct PCR amplification results obtained with the *apoE* primer pair using all three dried blood preparation methods.



**Figure 5. Amplification of the *apoE* gene by direct PCR of dried blood samples prepared by three different methods.** M, DNA size standards; Lane 1, Negative control; Lane 2, Positive control using 1 ng of purified human genomic DNA; Lane 3, Method 1; Lane 4, Method 2; Lane 5, Method 3.

**Conclusion**

The FailSafe PCR System permits consistent and reproducible direct amplification of genomic DNA targets in samples of dried whole blood on Guthrie cards or glass slides without any prior DNA extraction. Thus, the FailSafe PCR System provides a simple, economical and sensitive method for analysis of DNA templates in blood samples, both for small numbers of samples or for high throughput applications.

**References**

1. Briggar, R. J., et al. (1997) *J. Acqui. Immune Defic. Syndr. Hum. Retrovirol.* **14**, 368.
2. Makowski, G. S., et al. (1996) *Ann. Clin. Lab Sci.* **26**, 458.
3. Polski, J. M., et al. (1998) *Mol. Pathol.* **51**, 215.
4. Iovannisci, D. M., et al. (2000) *EPICENTRE Forum* **7**(1), 6.
5. Grunenwald, H. (2000) *EPICENTRE Forum* **7**(4), 10

**FailSafe™ PCR PreMix Selection Kit**  
FS99060 60 Units

**Contents:**

60 units FailSafe™ PCR Enzyme Mix and 12 FailSafe™ PCR 2X PreMixes.

See the center insert for additional information on the FailSafe PCR System.

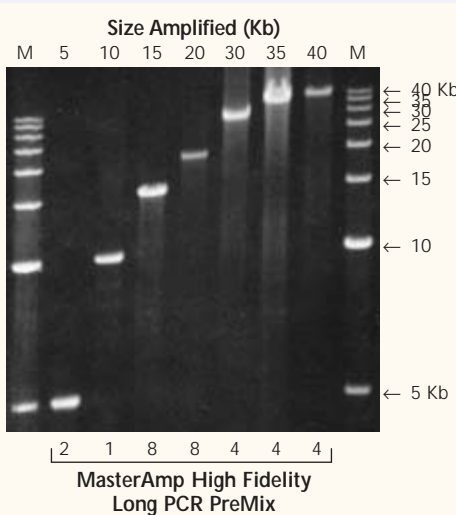
# High Fidelity PCR Amplification of DNA from 20 Kb to >40 Kb Using the MasterAmp™ Extra-Long PCR Kit

The FailSafe™ PCR System is ideal for consistent and accurate amplification of any template up to about 20 Kb, whatever its sequence and without need for “hot start” techniques. However, for sequences up to >40 Kb, the MasterAmp™ Extra-Long PCR Kit enables consistent and accurate amplification (see Figure below for lambda DNA regions). “Hot start” techniques are typically not required when using the MasterAmp Extra-Long Kit.

The MasterPure™ Extra-Long DNA Polymerase contained in the kit combines MasterAmp™ Taq DNA Polymerase with a proprietary 3' → 5' proofreading enzyme to achieve PCR fidelity at least three times better than Taq DNA Polymerase alone. The kit

includes MasterAmp Extra-Long DNA Polymerase and nine different Extra-Long PCR 2X PreMixes for convenient and fast PCR set-up. The nine Extra-Long PCR PreMixes each contain buffer, dNTPS and differing amounts of both Mg<sup>2+</sup> and MasterAmp™ PCR Enhancer (with betaine\*). Once the optimal PreMix is identified for a particular template/primer combination, consistent amplification of the template will be achieved using the same PreMix.

\* Patents issued and pending.



**Figure 1. Amplification of 5, 10, 15, 20, 30, 35, and 40 Kb sequences from lambda DNA.** One nanogram of lambda DNA was used to amplify 5, 10, 15, 20, 30, 35, and 40 Kb sequences. Lane M, 5 Kb DNA ladder. Results were analyzed on a 0.5% agarose gel run at 30 V for 20 hours.

**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

**Individual Extra-Long PCR 2X PreMixes**

- MasterAmp™ Extra-Long PCR 2X PreMix 1**  
MHF925A 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 2**  
MHF925B 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 3**  
MHF925C 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 4**  
MHF925D 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 5**  
MHF925E 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 6**  
MHF925F 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 7**  
MHF925G 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 8**  
MHF925H 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 9**  
MHF925I 5 ml

**MasterAmp™ Extra-Long DNA Polymerase Mix**

- QU92125 125 U
- QU92500 500 U
- QU9201K 1,000 U

# Cloning and Identification of Expressed Plant Defense Genes Following RT-PCR Using the MasterAmp™ High-Fidelity RT-PCR Kit and Degenerate Primers

Daniel Bergey, Rebecca Bargabus, and Maria Hernandez  
Department of Plant Sciences & Plant Pathology, Montana State University

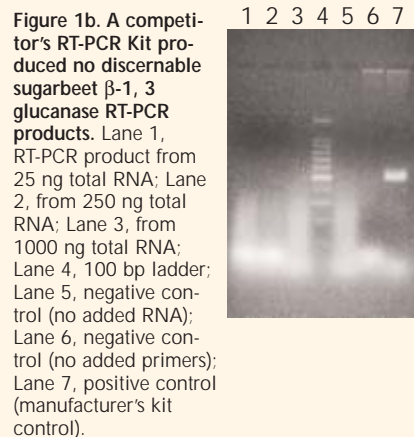
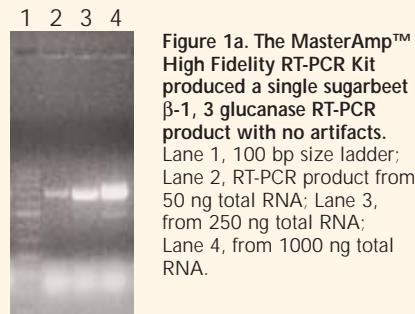
Pathogen infection can activate several classes of pathogenesis-related (PR) genes in a wide range of plant species. However, very little is known about pathogen-induced defense responses in sugarbeet plants. In a collaborative effort with Barry Jacobsen's group (Montana State University) we are seeking to characterize the molecular and biochemical basis for induced defense responses against pathogens in sugarbeet leaves. Since very few genes have been isolated from sugarbeet, a necessary first step toward this end entailed isolating and cloning, by RT-PCR, a battery of PR gene transcripts from sugarbeet leaves to use as molecular probes to monitor defense response activation. Chitinase, peroxidase, and  $\beta$ -glucanase genes were initially selected as candidate markers because they have been previously demonstrated as reliable markers for induced defense responses in other plant species. The MasterAmp™ High Fidelity RT-PCR Kit was chosen because of its excellent sensitivity, and its utilization of a blend of high fidelity PCR enzymes to ensure accurate sequence of the RT-PCR products.

Sugarbeet leaves were treated with either the plant defense response elicitor benzothiadiazole (BTH) or water (negative control). Total RNA was isolated by a modified phenol-SDS procedure followed by LiCl precipitation. This RNA was used as template for RT-PCR.

## RT-PCR primer design

The degenerate primer (poly-T lok) consisting of 20 T residues ending in either A, C, or G at its 3'-end was used as the reverse transcription (RT) primer. Since very little sequence information is available for sugarbeet genes, PCR primers for each RNA transcript were designed by first comparing amino acid sequences of comparable known genes from diverse plant species. We identified conserved domains for each of the three model response markers and then made internal degenerate oligonucleotide primers for the subsequent PCR step. The degree of degeneracy for the internal primers ranged from 128X-512X.

**Figure 1. Comparison of the sugarbeet  $\beta$ -1, 3 glucanase RT-PCR product produced using the MasterAmp™ High Fidelity RT-PCR Kit and a competitor's Kit.** RT-PCR was performed using highly degenerate primers and total RNA purified from benothiadiazole-treated sugarbeet leaves.



## RT-PCR Method

RT-PCR was performed according to the standard one-step, one-tube continuous RT-PCR protocol described in the MasterAmp High Fidelity RT-PCR Kit product literature. First, 300 ng of total RNA from BTH-treated plants, 150 pmoles (3  $\mu$ M final concentration) of poly-T lok primer, and an added RNase inhibitor were combined and heated to 65° C for 5 minutes followed by immediate immersion in an ice-water bath. After 3-5 minutes on ice, the remainder of the RT-PCR reagents (MasterAmp 2X RT-PCR PreMix, MMLV-RT Plus, MasterAmp TAQurate™ DNA Polymerase Mix and the transcript-specific PCR Primer 2) were added. Optimum first strand synthesis (reverse transcription) for our model plant system was performed at 45° C for 40-45 minutes. The reaction was then transferred to a 95° C heat block for 2 minutes followed by

36-38 PCR amplification cycles using cycling conditions optimized for each primer pair. The final RT-PCR products were cloned and the DNA sequences of each determined.

## Results

Figure 1a shows a typical RT-PCR result obtained using EPICENTRE's MasterAmp High Fidelity RT-PCR Kit. Even when performing RT-PCR with degenerate primers, the MasterAmp High Fidelity Kit produced complete RT-PCR product without artifacts. In contrast, using the same primers and template, an RT-PCR kit from another supplier did not produce any identifiable RT-PCR product except in the control reaction (Figure 1b). The identities of the cloned RT-PCR products produced using the MasterPure High Fidelity RT-PCR Kit were confirmed by DNA sequencing. Sequence comparison of the cloned sugarbeet genes to related homologues from other plant species revealed a surprising degree of divergence in the sugarbeet sequences.

We used the cloned sequences as probes in Northern analysis and confirmed that the three marker genes were induced systemically after treatment of sugarbeet leaves with BTH (data not shown). In contrast, no induction of the marker genes was seen in leaves treated with water. Complete results of this work will be published elsewhere.

### 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)<sub>18</sub> Primer, Control Template and Primer Mix, Sterile Water

# New BuccalAmp™ DNA Extraction Kit Revolutionizes Buccal Sample Collection and Processing

EPICENTRE's new BuccalAmp™ DNA Extraction Kits are single-tube systems for rapid preparation of DNA from buccal (cheek) samples for use in PCR amplification assays. BuccalAmp Kits incorporate a new QuickExtract™ DNA Extraction Solution that permits processing of samples using a simplified new protocol (Figure 1).

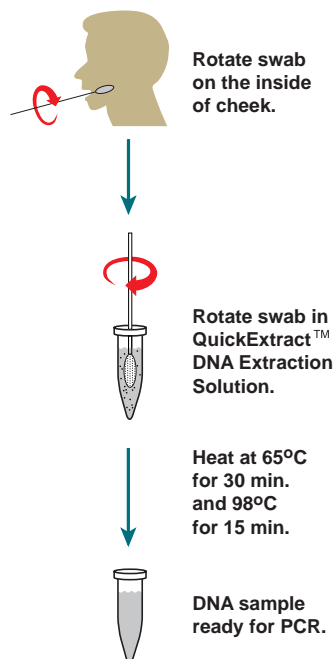


Figure 1. Procedure for obtaining PCR-ready DNA using the BuccalAmp™ DNA Extraction Kit.

The QuickExtract Solution is pre-allocated into individual sample tubes. To obtain PCR-ready DNA, just rotate the buccal sample swab in one of these tubes, mix and heat. No centrifugation step is needed, making it easy to process one to hundreds of samples in less than an hour-without phenol, chloroform, or other toxic organic solvents.

## Buccal samples are easy to collect and transport

BuccalAmp Kits include Catch-All™ Sample Collection Swabs—a soft foam swab on a soft, flexible plastic handle (Figure 2). Catch-All Swabs provide gentle, safe buccal sample collection, even for infants, and the porous foam on these swabs catches more of the sample

than buccal brushes. The use of buccal (cheek) samples eliminates the time and discomfort required for sample collection by blood draws and minimizes exposure to harmful pathogens. Nor does sample collection require a health care professional.

Catch-All Swabs are provided individually packaged in sterile hard-pack plastic cylinders. After collecting the sample, return the sample swab to the cylinder package, for safe, secure storage and transport from the collection site to the analysis site.

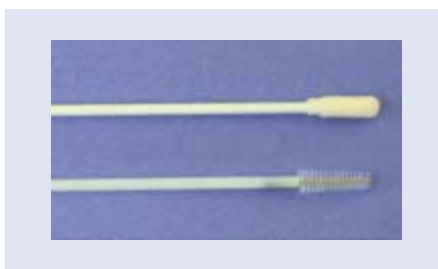


Figure 2. Catch-All Sample Collection Swabs (upper) are gentle and effective - even for pediatric sampling. The porous swab improves DNA yields by absorbing more sample than standard buccal brushes (lower).

## High yields of PCR-ready genomic DNA

Buccal DNA yield per individual ranges from 1-7 µg - more than other methods and enough to perform at least 100 PCR assays. The DNA obtained is suitable for PCR amplification of even difficult sequences, including both GC-rich sequences (Figure 3) and long amplifications (Figure 4).

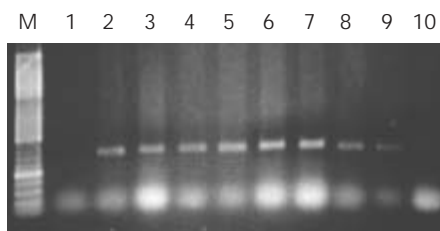


Figure 3. PCR amplification of the GC-rich tumor necrosis factor gene from DNA obtained from 9 individuals using the BuccalAmp™ DNA Extraction Kit. The 740-bp product was amplified using the FailSafe™ PCR System. Lane M, 100 bp ladder; Lane 1, negative control.

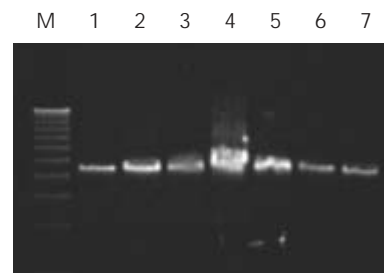


Figure 4. PCR amplification of a 5.5 Kb sequence from DNA obtained from 7 individuals using the BuccalAmp™ DNA Extraction Kit. DNA was amplified using the FailSafe™ PCR System. Lane M, Kb ladder.

## Useful for many applications

BuccalAmp DNA Extraction Kits and Catch-All Sample Collection Swabs are useful for any application benefiting from rapid, easy extraction of PCR-ready DNA from Buccal samples. Examples include human or animal identity testing, SNP analysis, and assays for viruses, bacteria, and other microorganisms.

Since no centrifugation step is used, BuccalAmp DNA extraction is easily automated in a 96-well format. QuickExtract DNA Extraction Solution and Catch-All Sample Collection Swabs are available separately and in bulk for high throughput applications.

<b>BuccalAmp™ DNA Extraction Kit</b>	
BQ0901S	1 Kit
BQ0908S	8 Kits
BQ0916S	16 Kits
<b>Contents:</b>	
15 tubes (1 extraction/tube) of BuccalAmp™ QuickExtract™ Solution 1.0.	
15 individually-packaged sterile Catch-All™ Swabs.	
<b>QuickExtract™ DNA Extraction Solution 1.0</b>	
QE09050	50 ml
Bulk solution, sufficient to perform 100 extractions.	
<b>Catch-All™ Sample Collection Swabs</b>	
QEC091H	100 swabs
100 individually-packaged swabs in sterile hard-pack plastic cylinders.	
Contact EPICENTRE or your local distributor to discuss discounts for larger quantities, special packaging, or standing orders for any of the above items.	

HIV *E. coli* Yeast

HCV Enterovirus HPV

*B. pertussis* Mammalian

*M. tuberculosis* RSV

**Purify *DNA or RNA*  
from any sample,  
every time.**

***We guarantee it.***

Paraffin Tissues Liver

Saliva Serum/Plasma

Whole Blood Mouse Tail

Buccal Cells Kidney Soy

Urine Sputum Maize

Tissue Culture Cell Lines

## **MasterPure™** *DNA & RNA Purification Kits*

Whatever the specimen,  
whatever its size or scarcity,  
**MasterPure™ DNA & RNA  
Purification Kits** give  
consistent quality and yield —  
**your PCR will never suffer  
from poor sample preparation.**

The MasterPure Kit has been used for almost every type of sample you can imagine: dried and fresh whole blood, plasma, serum, fresh and paraffin-embedded tissues, mouse tail snips, buccal cells, saliva, urine — you name it — purifying for HIV, HCV, HPV, *E. coli*, yeast, *M. tuberculosis*, human or mammalian genomic (among others). **No matter what the source, MasterPure gives efficient isolation of DNA and RNA — even from the smallest of samples — every time.**

MasterPure uses a simple salt precipitation protocol that takes less than an hour. It uses no filters, no spin columns, no membranes, no phenol, no chloroform — nothing to harm you or cut yields. Yet you'll get extreme purity, with O.D.<sub>260/280</sub> ratios between 1.8 and 2.0.

**We guarantee that MasterPure will work for you.** Try a MasterPure DNA or RNA Purification Kit at no risk. If you're not completely satisfied, you pay nothing.



[www.epicentre.com](http://www.epicentre.com)  
800-284-8474

# MasterPure™

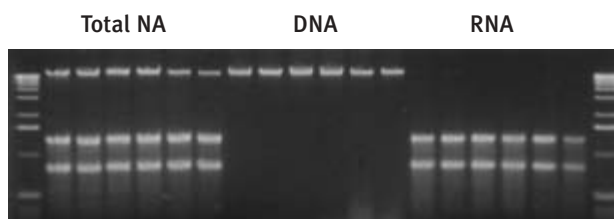
## DNA & RNA Purification Kits

Now you can purify DNA or RNA from any sample, every time!



## Consistent Purity

Consistently obtain DNA or RNA free of protein.



Total nucleic acid, DNA and RNA were purified from a liquid *E. coli* culture sample

## Extensive Sample Range

Using an extremely simple salt precipitation protocol, DNA or RNA can be isolated from virtually any source in less than 1 hour.

### Examples of Targets Analyzed

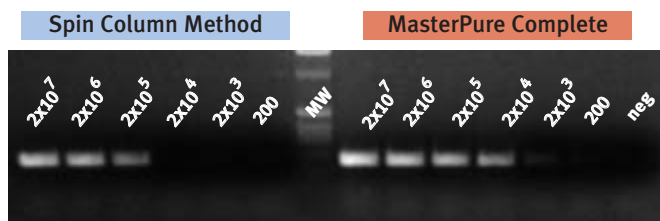
Mammalian Systems  
HIV  
*E. coli*  
HCV  
*B. pertussis*  
RSV  
Yeast  
*M. tuberculosis*  
Enterovirus  
HPV  
Soy  
Maize  
Insect Tissues

### Examples of Samples Extracted

Serum  
Plasma  
Whole Blood  
Buccal Cells  
Liver  
Mouse Tail  
Kidney  
Saliva  
Urine  
Sputum  
Tissue Culture Cell Lines  
Cervical Cells  
Paraffin Tissues

## High Sensitivity

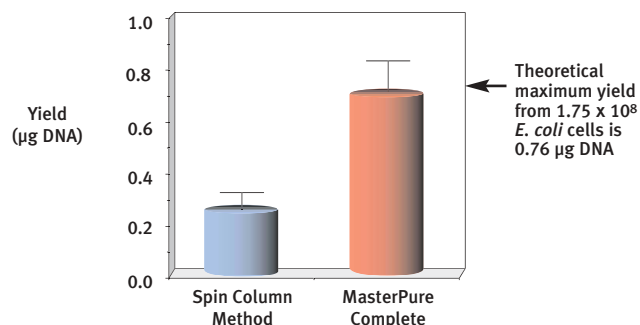
The MasterPure co-precipitant greatly improves sensitivity.



PCR amplification after extraction from the indicated number of *E. coli* cells

## Greater Yields

Get higher yields with the MasterPure Complete DNA and RNA Purification Kit than with spin column-based methods.



DNA was purified from 1.75 x 10<sup>8</sup> *E. coli* cells and quantified by fluorometry

## Safe and Easy to Use

- No caustic solvents
- No cumbersome columns

## Ordering Information

### MasterPure™ Complete DNA and RNA Purification Kit

(for isolating TNA, DNA, or RNA)

MC89010	10 Purifications
MC85200	200 Purifications

### MasterPure™ DNA Purification Kit

(for isolating TNA or DNA)

MCD85201	200 Purifications
----------	-------------------

### MasterPure™ RNA Purification Kit

(for isolating RNA only)

MCR85102	100 Purifications
----------	-------------------

# Sign Up Now to Become Eligible to Win!

Simply complete the attached postage-paid card (including e-mail, phone, fax, & address) & mail to become eligible to win 1 of 3 DVD players. Visit EPICENTRE's web site for details:

[www.epicentre.com](http://www.epicentre.com)

Winners will be announced in the *EPICENTRE Forum* newsletter.



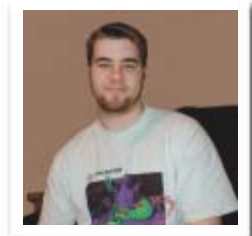
## Congratulations to our 3 Digital Cameras Winners!



**Maria Fedorova**  
University of Minnesota  
St. Paul, MN



**Raymond Cologna**  
Southwest Foundation for  
Biomedical Research  
Department of Virology &  
Immunology  
San Antonio, TX



**Matthew Eckman**  
HHMI - MIT CCR  
Department of Biology  
Cambridge, MA

## Congratulations to Our T-shirt Winners!

William Itterly, Greensboro, NC  
Amanda Saklad, Tucson, AZ  
Phillippa Neville, Cleveland, OH  
Muxiang Zhou, Atlanta, GA  
Jianxin Hu, Bethesda, MD

Stephen D. Torosin, Durham, NH  
Christine Snyder, Fort Detrick, MD  
Lalit Kumar, Boston, MA  
John Ong, Los Angeles, CA  
Ying Wang, Los Angeles, CA

Please tear along the perforated lines and return.

## KEEP UP WITH WHAT'S NEW!

Please,

- Send me a copy of the *EPICENTRE Catalog*.
- Add me to your mailing list for the *EPICENTRE Forum*.
- I would like to receive information by E-mail:

E-mail address: \_\_\_\_\_

- |  |   |                                       |
|--|---|---------------------------------------|
| <input type="checkbox"/> Mutation Detection/<br>DNA Typing | <input type="checkbox"/> cDNA / RT-PCR          | <input type="checkbox"/> Non-PCR      |
| <input type="checkbox"/> Sequencing                        | <input type="checkbox"/> DNA/RNA Purification   | <input type="checkbox"/> DNA Repair   |
| <input type="checkbox"/> Molecular Cloning                 | <input type="checkbox"/> In Vitro Transcription | <input type="checkbox"/> Protein      |
| <input type="checkbox"/> PCR                               | <input type="checkbox"/> Transposon Tools       | <input type="checkbox"/> Cell Biology |

I'm interested in:

- Send more information about the following products:

\_\_\_\_\_

- I have these new product suggestions:

\_\_\_\_\_

- Change my address, my previous zip code was:

Name \_\_\_\_\_

Position \_\_\_\_\_

Institution \_\_\_\_\_

Dept. \_\_\_\_\_

Address \_\_\_\_\_

City/State/Zip \_\_\_\_\_

Phone \_\_\_\_\_ Fax \_\_\_\_\_

Website: \_\_\_\_\_



Having used the FailSafe PCR PreMix Selection Kit for the first time this week I wanted to say thank you. It is fantastic! I have been trying unsuccessfully to get a PCR to work from *Streptomyces* (a GC-rich soil bacterium) for 3 months using a range of enzymes from other manufacturers. Your FailSafe kit worked the first time and PreMix J gave a superbly clear SINGLE band at THE EXPECTED SIZE.

Karen Jolly  
School of Biology  
University of Leeds, Leeds, UK

By the way - I LOVE this  
FailSafe PCR system - it works beautifully  
on 4 different and annoying PCR's of mine.  
It's wonderful - thank you!  
- Jess

Jessica Otte  
Center for Neurovirology & Cancer Biology  
Temple University, Philadelphia Pennsylvania

From: Moises Hernandez, CDC  
To: EPICENTRE

Identification of *Mycobacterium tuberculosis* complex from cerebral spinal fluid is very difficult, especially when sample volume is low, yet using the FailSafe PCR System, I identified six samples I could not otherwise amplify.

Moises Hernandez  
Centers for Disease Control and Prevention  
Atlanta, Georgia

Never fail at *PCR* again.  
**We Promise.**

I've recently started  
using Fail Safe and  
it is GREAT!  
Thank you.  
Regina

Regina Harlow  
Fralin Biotechnology Center  
Virginia Tech, Blacksburg, Virginia



**EPICENTRE®**

# FailSafe™ PCR System: A New Standard in

## A COMPLETE SYSTEM OF ENZYME MIX AND REAGENTS

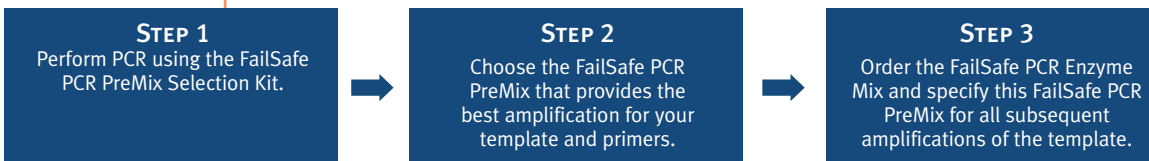
The FailSafe PCR System sets a new standard for PCR by combining a unique blend of thermostable enzymes and an extensively tested set of reaction “PreMixes” into a single system. This system contains everything you need (including dNTPs) for successful PCR. The FailSafe PCR System is designed to solve all your PCR problems.

## THE FAILSAFE PCR PREMIX SELECTION KIT — SUCCESSFUL PCR WITH ANY TEMPLATE

Don't spend days or weeks optimizing or troubleshooting your PCR reaction. Instead, start with the FailSafe PCR PreMix Selection Kit. This kit contains everything you need for successful PCR: the FailSafe PCR Enzyme Mix and carefully designed FailSafe PCR PreMixes with dNTPs, buffer, and various amounts of  $MgCl_2$  and FailSafe PCR Enhancer

(with betaine).\* Simply add a cocktail with your primers, template and the enzyme mix to each of the twelve FailSafe PCR PreMixes and amplify. At least one FailSafe PreMix is guaranteed to amplify your template. Then...order additional FailSafe PCR Enzyme Mix and specify the FailSafe PCR PreMix(es) that works best for you.

\*Patents issued and pending.



## Consistent and Reliable PCR Results

The FailSafe PCR System assures successful and optimal PCR *every time*. This increased consistency is due in part to the FailSafe PCR PreMixes that contain the FailSafe PCR Enhancer (with betaine).

As shown in figure 5, FailSafe PCR PreMix J was able to consistently amplify a GC-rich region of human fragile X gene.

## GREATER ACCURACY AND SENSITIVITY

Accurate amplification is critical for many applications. The FailSafe PCR Enzyme Mix includes a 3'→5' proofreading enzyme that delivers fidelity significantly greater than Taq (fig.1). EPICENTRE's patented PreMix component also insures that

your reactions will suffer no loss in sensitivity, as often occurs with other “high fidelity” systems. As shown in figures 2 & 3, the FailSafe PCR System amplifies challenging templates with as little as 1 ng of human genomic DNA.



PCR Mutation Frequency (errors per 10<sup>6</sup> base-pairs)(lower value represents higher fidelity)

FIGURE 1  
Fidelity comparison of FailSafe PCR Enzyme Mix with other supplier's enzymes.

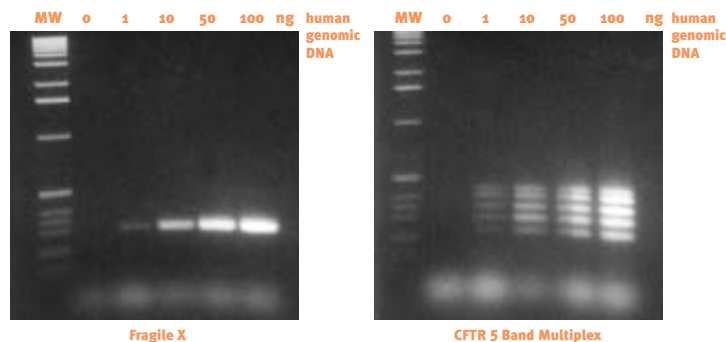


FIGURE 2 & 3  
High sensitivity with the FailSafe PCR Enzyme System. Fragile X FMR1 (containing 80-85% GC-rich regions) and 5 exons of CFTR were amplified with various amounts of human genomic DNA (0 to 100 ng).

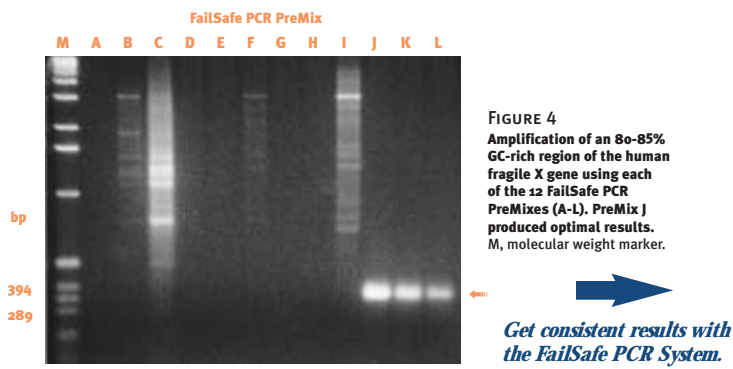
# Performance and Reliability

**“Difficult Amplifications” are No Longer Difficult with the FailSafe PCR System**

## GC-RICH TEMPLATE AMPLIFICATION MADE EASY

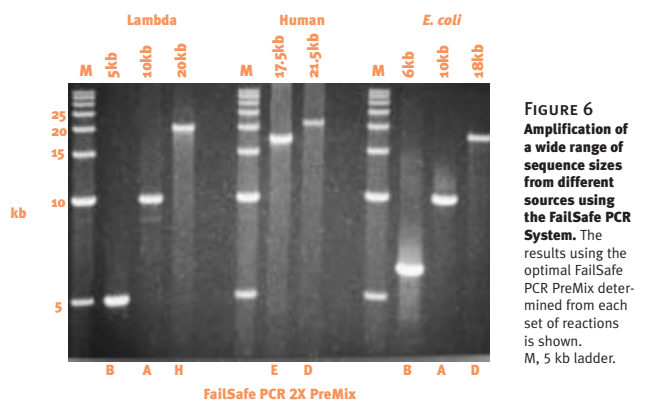
Do you have any templates you’ve never been able to PCR? Try them again! The FailSafe PCR System can handle the toughest amplifications. Templates with high GC content and templates with extensive secondary structure have been amplified successfully and consistently. Specifically, a 250-350 bp region of the fragile X gene (human FMR1 gene) with a GC content of 80-85% was

initially amplified using the FailSafe PCR PreMix Selection Kit. The optimal PreMix for amplification was easily determined to be FailSafe PCR PreMix J (fig. 4). Consistent results were then obtained for amplification of this high GC region region of the human fragile X gene from 4 separate individuals using FailSafe PCR PreMix J (fig. 5).



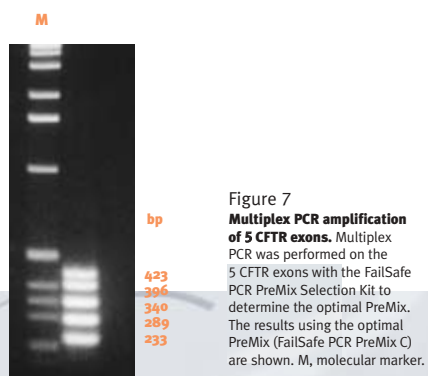
## AMPLIFY TEMPLATES AT LEAST 20 KB IN LENGTH

Amplifying long templates used to require tedious, repetitive rounds of optimization. With the FailSafe PCR System, your optimization time is minimized to a single reaction set and your reaction yield is maximized. The FailSafe PCR System can easily amplify templates up to 20 kb in length (fig. 6).



## SUCCESSFUL MULTIPLEX AMPLIFICATION ON THE FIRST TRY

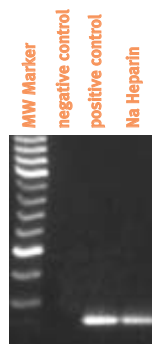
The FailSafe PCR System also works well for multiplex amplification. For example, figure 7 shows the amplification results for 5 exons from the cystic fibrosis transmembrane conductance regulator (CFTR) gene using the optimal FailSafe PCR PreMix identified with the FailSafe PCR PreMix Selection Kit. This multiplex analysis resulted in the correct size PCR product for each exon.



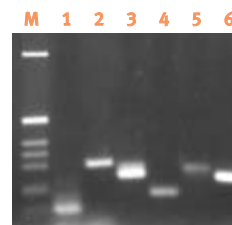
## DIRECT PCR FROM WHOLE OR DRIED BLOOD

Using the FailSafe PCR System, you can perform PCR using fresh or sodium heparin-preserved whole blood or blood dried on Guthrie cards or

glass slides...*without* the need to first purify the genomic DNA.



**FIGURE 8**  
Amplification of a 200 bp region of human hemochromatosis gene directly from blood stored at 4°C in sodium heparin using FailSafe PCR PreMix D. M, molecular weight marker; Lane 1 negative control; Lane 2, PCR using purified human genomic DNA as a positive control; Lane 3, PCR using whole blood preserved with sodium heparin.



**FIGURE 9**  
FailSafe PCR amplification of regions of 6 different genes directly from human blood dried on Guthrie cards. Lane 1, PCR of microsatellite DXS6789; Lane 2, PCR of microsatellite DXS7132; Lane 3, PCR of microsatellite GATA31E08; Lane 4, PCR of microsatellite GATA175D03; Lane 5, PCR of apoE; Lane 6, PCR of CFTR exon 11; M, molecular weight marker.

## FailSafe™ PCR System Ordering Information

### FailSafe™ PCR PreMix Selection Kit

Cat. No.	Size
FS99060 - B2	60 UNITS

Contains FailSafe PCR Enzyme Mix and the 12 FailSafe PCR 2X PreMixes (200 µl each).

First, use the FailSafe PCR PreMix Selection Kit to identify the optimal FailSafe PCR PreMix for your template/primer pair.

### FailSafe™ PCR System

Cat. No.	Size	No. of FailSafe PCR 2X PreMixes Included (2.5 ml ea.)
FS99100	100 UNITS	CHOICE OF 1
FS99250	250 UNITS	CHOICE OF 2
FS9901K	1,000(4 X 250) UNITS	CHOICE OF 8

Order additional FailSafe PCR Enzyme Mix and specify the FailSafe PCR PreMix(es) that work best for you.

### Individual FailSafe™ PCR 2X PreMixes\*

Cat. #	FailSafe PCR 2X PreMix											
	A	B	C	D	E	F	G	H	I	J	K	L
FSP995A	FSP995B	FSP995C	FSP995D	FSP995E	FSP995F	FSP995G	FSP995H	FSP995I	FSP995J	FSP995K	FSP995L	
Size	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml

\*Contain dNTPs, buffer, and various amounts of MgCl<sub>2</sub> and FailSafe™ PCR Enhancer (with betaine\*\*)

\*\*Patents issued and pending on FailSafe PCR Enhancer.

### Please contact EPICENTRE at:

Toll-free in the USA: 800-284-8474  
Phone: 608-258-3080  
Fax: 608-258-3088

web site: [www.epicentre.com](http://www.epicentre.com)  
e-mail: [techhelp@epicentre.com](mailto:techhelp@epicentre.com)  
Outside the USA: please contact your local EPICENTRE distributor

©2001 EPICENTRE Technologies. All rights reserved. EPICENTRE is a registered trademark and FailSafe and MasterAmp are trademarks of EPICENTRE Technologies. AmpliTaq is a trademark of The Perkin-Elmer Corp. Expand is a trademark of Boehringer Mannheim Corp. LA Taq is a trademark of Takara Shuzo Co., Ltd. This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler. FailSafe PCR Enzyme Mix is made by EPICENTRE using a licensed technology covered under U.S. Patent No. 5436149 and other patents pending worldwide owned by TaKara Shuzo Co., Ltd.



These companies are authorized  
to distribute EPICENTRE products.

#### AUSTRALIA

**Astral Scientific Pty. Ltd.**  
Unit 39/ Block C  
1-3 Endeavour Rd.  
Caringbah NSW 2229  
Australia  
Tel: 61-2-9540-2055  
Fax: 61-2-9540-2051  
astralscientific@compuserve.com

#### AUSTRIA

**Biozym Diagnostik GmbH**  
Niederlassung Osterreich  
Wehlstrasse 27b  
A-1200 Wein  
Austria  
Tel: 43-1-334 0156 0  
Fax: 43-1-334 0156 88  
support@biozym.com  
www.biozym.com

#### BENELUX

**Biozym Nederland B.V.**  
Rotscherweg 61  
6374 XW Landgraaf  
The Netherlands  
Tel: 31-45-532-7755  
Fax: 31-45-532-7733  
info@biozym.nl  
www.biozym.nl

#### CANADA

**InterScience, Inc.**  
169 Idema Road  
Markham Ontario  
Canada L3R 1A9  
Tel: 800-661-6431  
Fax: 888-673-3148  
info@interscience.com  
www.interscience.com

#### DENMARK

**Boule Nordic AB**  
Branch Office Denmark  
Egensevej25  
2770 Kastrup  
Tel: 45-32-52-88-00  
Fax: 45-32-52-88-79  
info@boule-nordic.dk  
www.boule-nordic.dk

#### FINLAND

**Immuno Diagnostic Oy**  
Turuntie 8 L 1  
FIN-13130 Hämeenlinna  
Finland  
Tel: 358-3-6822-758  
Fax: 358-3-6822-039  
immuno.diagnostic@immuno.htk.fi  
www.immunodiagnostic.fi

#### FRANCE

**TEBU International**  
39, rue de Houdan BP15  
Le Perray en Yvelines  
F-78610 France  
Tel: 1-30-46-39-00  
Fax: 1-30-46-39-11  
fr@tebu-bio.com  
www.tebu-bio.com

#### GERMANY

**Biozym Diagnostik GmbH**  
P.O. Box 180  
D-31833 Hessisch-Oldendorf  
Germany  
Tel: 49-5152-9020  
Fax: 49-5152-2070  
support@biozym.com  
www.biozym.com

#### GREECE

**P. Bacacos S.A.**  
3, Meg. Alexandrou Str.  
104 37 Athens, Greece  
Tel: 301-5230-109  
Fax: 301-5229-141  
bacacos@hol.gr

#### HONG KONG/CHINA

**Gene Company, Ltd.**  
Unit A, 8/F,  
Shell Industrial Bldg.  
12 Lee Chung Street  
Chai Wan, Hong Kong  
Tel: 852-2896-6283  
Fax: 852-2515-9371  
info@genehk.com

#### HUNGARY

**Kvalitex Kft.**  
Pannonia u. 7  
H-1136 Budapest, Hungary  
Tel: 36-1-340-4700  
36-30-509-618  
Fax: 36-1-339-8274  
kvalitex@mail.mata.vu  
www.kszsz.hu/kvalitex.htm

#### INDIA

**TechnoConcept**  
824/3, Sukhdav Nagar  
Kotla Mubarakpur  
New Delhi - 110003  
India  
Tel: 91-11-4636224/ext.5  
Fax: 91-11-4636223  
technoconcept@vsnl.com  
www.technoconcept-india.com

#### ISRAEL

**Rhenium, Ltd.**  
Bet Nekofa #84  
Jerusalem 91035  
Israel  
Tel: 972-2-5335599  
Fax: 972-2-5335590

#### ITALY

**SPA-BioSPA Division**  
Via Biella 8  
20143 Milano, Italy  
Tel: 39-2-891-391  
Fax: 39-2-813-2983  
miranda@spaspa.it  
www.spaspa.it/biospa.htm

#### JAPAN

**AR Brown Co., Ltd.**  
Daini-Marutaka Bldg.  
13-8, Ginza 7-Chome, Chuo-Ku  
Tokyo 104-0061  
Japan  
Tel: 03-3545-5720 or  
06-6441-5103  
Fax: 03-3543-8865 or  
06-6441-5095  
scg@arbrown.com or  
seg@arbrown.com  
www.sci.arbrown.com

#### KOREA

**LRS Laboratories, Inc.**  
Song Buk-Ku  
Shin Bldg. 11, Rm. 501  
102-1 BoMun-Dong  
4-GA  
Seoul, Korea 137-130  
Tel: 82-2-924-8697  
Fax: 82-2-924-8696

#### PureTech Company, Ltd.

Rm 301, Han Joong Bldg  
264-1Yangjae-dong  
Seocho-ku  
Seoul, Korea 137-130  
Tel: 82-2-576-2545-6  
Fax: 82-2-576-2547  
puretech@netsgo.com

#### MEXICO

**Control Tecnico Y Rep.**  
Ave. Lincoln PTE. 3410  
Monterrey, N.L. C.P. 64320  
Mexico  
Tel: 52-8-371-60-50  
Fax: 52-8-371-21-80  
controltec@infosel.net.mx

#### NORWAY

**Boule Nordic AS**  
Eilit Erievik  
Lototgt. 2  
N-0458 Oslo  
Norway  
Tel: 47-22-37-3255  
Fax: 47-22-37-3691

#### POLAND

**AKOR Laboratories Sp. z o.o.**  
ul. Kielnienska 68  
PL 80-299 Gdańsk  
Poland  
Tel: 48-58-522-9210  
Fax: 48-58-522-9000  
akor@sprintnet.pl

#### PUERTO RICO

Please contact EPICENTRE

#### SINGAPORE

**SciMed (Asia) Pte Ltd**  
Blk 196 Pandan Loop  
#07-11  
Pantech Industrial Complex  
Singapore 128384  
Tel: 65-779-3388  
Fax: 65-266-3086  
www.scimed@singnet.com.sg

#### SOUTH AFRICA

**Separations**  
E1 Mikro Industrial Park  
17-19 Hammer Ave.  
Strijdompark, South Africa 2193  
Tel: 27-11-792-3428  
Fax: 27-11-792-1043  
sepapaul@iatria.com  
www.separations.co.za

#### SPAIN

**Ecogen S.R.L.**  
Passatge Dos de Maig  
9-11 entlo. 2a  
08041 Barcelona  
Spain  
Tel: 34-93-4502601  
Fax: 34-93-4560607  
ecogen@ecogen.com  
www.ecogen.com

#### SWEDEN

**Boule Nordic AB**  
Lunastigen 3  
S-141 22 Huddinge  
Sweden  
Tel: 46-8-608-8630  
Fax: 46-8-746-8496  
info@boule.se  
www.boule.se

#### SWITZERLAND

**Inotech AG**  
Kirchstrasse 1  
CH-5605 Dottikon  
Switzerland  
Tel: 41-56-624-01-00  
Fax: 41-56-624-29-88  
info@inotech.ch  
www.inotechintl.com

#### TAIWAN

**EASTYU Company, Ltd.**  
No. 22-2 Wan-Ching Street, 2nd  
Fl.  
Ching-Mei, Taipei,  
Taiwan R.O.C.  
Tel: 886-2-2932-1528  
Fax: 886-2-2242-2430  
eastyu@hotmail.com

#### Unimed Healthcare Inc.

3F No 74, Song-Te Rd.  
Taipei Taiwan R.O.C.  
10522  
Tel: 886-2-2720-2215  
Fax: 886-2-2723-3666  
info@unimed.com.tw  
www.unimed.com.tw

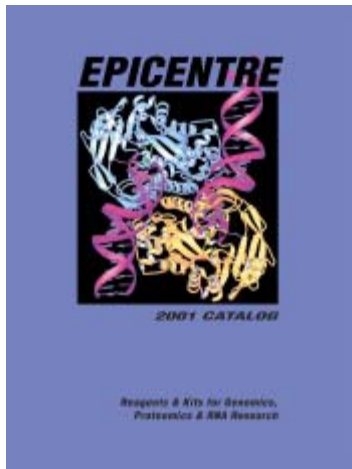
#### TURKEY

**Ay-Ka Ltd. Sti.**  
Ziya Gokalp Caddesi  
24/82  
Kizilay/Ankara 06420  
Turkey  
Tel: 90-312-435-0775  
Fax: 90-312-435-0427  
aykald@tr-net.net.tr

#### UNITED KINGDOM

**Cambio, Ltd.**  
34 Newnham Road  
Cambridge CB3 9EY  
England  
Tel: 44-1-223-366500  
Fax: 44-1-223-350069  
support@cambio.demon.co.uk  
www.cambio.co.uk

Visit us online at  
**www.epicentre.com**



Request a copy of the  
**2001 EPICENTRE Catalog**  
 or upcoming issues of the  
**EPICENTRE Forum** on the  
 Web at  
**www.epicentre.com**



# Completely Remove Oligonucleotides and Single-Stranded DNA From Your Reaction Mixes Using Exonuclease I

Exonuclease I specifically digests single-stranded DNA, containing a 3'-OH, in a 3'→5' direction. Although the enzyme requires Mg<sup>2+</sup> for activity, it is active in a wide variety of buffers and can be added directly into most reaction mixes. Exonuclease I can be heat inactivated by incubation at 80°C for 15 minutes.

## Applications

Removal of residual single-stranded DNA and oligonucleotides from reaction mixes.

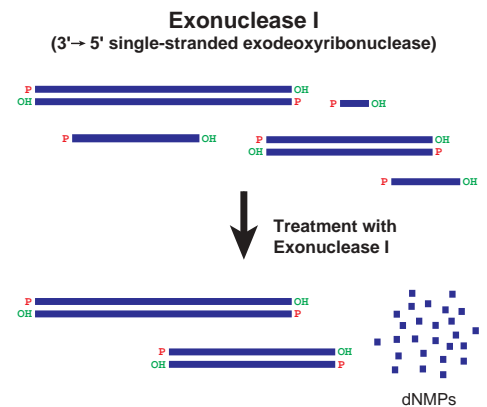
Selective removal of single-stranded DNA from nucleic acid mixtures.

## Quality Control

Exonuclease I is tested for selective degradation of single-stranded DNA and is free of detectable RNase, endonuclease and double-stranded exonuclease activities.



**Figure 1. Specificity of Exonuclease I for single-stranded substrates.** 200 ng of pUC19 DNA linearized with EcoR I and 1 µg of a 100-mer single-stranded oligonucleotide were mixed in 1X TA Buffer (33 mM Tris-acetate, pH 7.8, 66 mM potassium acetate, 10 mM magnesium acetate, and 0.5 mM DTT) and incubated at 37°C for 20 minutes in the absence or presence of 10 U of Exonuclease I (Exo I). Reaction products were separated by electrophoresis on a 1% agarose gel. Lane 1, molecular weight markers; Lane 2, minus Exo I treatment; Lane 3, plus Exo I treatment. Exonuclease I completely digested the linear single-stranded oligonucleotide while leaving the linear double-stranded plasmid DNA intact.



**Figure 2. Exonuclease I selectively digests single-stranded DNA with a 3'-OH in a 3'→5' direction.** Exonuclease I can be used to completely remove oligonucleotides and single-strand DNA from reaction mixes and nucleic acid preparations.

### Exonuclease I, *E. coli*

X40501K	20 U/µl	1,000 U
X40505K	20 U/µl	5,000 U
X40520K	20 U/µl	20,000 U

Exonuclease I is also available in bulk. Please inquire.

NEW  
PRODUCT!

## Produce Single-Stranded PCR Product for SSCP or Sequencing Using Lambda Exonuclease

Lambda Exonuclease is a highly processive 5'→3' exodeoxyribonuclease that selectively digests the phosphorylated strand of double-stranded DNA. The preferred substrate is blunt-ended, 5'-phosphorylated double-stranded-DNA. The enzyme has reduced activity against nicked DNA and against single-stranded DNA and gapped DNA<sup>1</sup>.

## Applications

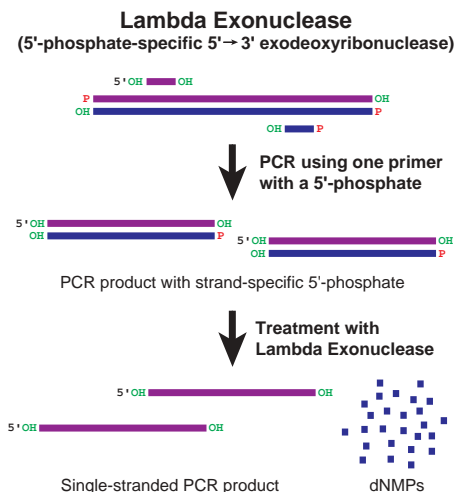
SSCP (single-strand conformation polymorphism) analysis of PCR product<sup>2,3</sup>.

Generate single-stranded DNA sequencing template from PCR product.

**Unit Definition:** One unit will produce 10 nmoles of acid soluble deoxyribonucleotides from double-stranded DNA template in 30 minutes at 37°C in 1X Lambda Exonuclease Reaction Buffer.

**Quality Control:** Lambda Exonuclease is function-tested to ensure complete and preferential degradation of PCR product produced using 5'-phosphorylated primers. PCR product made using primers

containing 5'-OH ends is not digested. Lambda Exonuclease is tested to be free of contaminating endonuclease activities.



**Figure:** Lambda Exonuclease selectively digests the strand of a PCR product produced using a PCR primer with a 5'-phosphate. The resulting single-strand PCR product can be used for SSCP analysis or sequencing.

## References

- Mitsis, P.G. and Kwag, J.G. (1999), *Nucl. Acid. Res.* **27**(15):3057
- Schwieger, F. and Tebbe, C.C (1998), *App. And Environ. Microb.* **64**(12):4870
- Schwieger, F. and Tebbe, C.C (2000), *App. And Environ. Microb.* **66**(8):3556

### Lambda Exonuclease

LE015H	500 Units	10 U/µl
LE012K	2,500 Units	10 U/µl

Each includes 10X Reaction Buffer  
Lambda Exonuclease is also available in bulk. Please inquire.

# Consistent Production of the Highest RNA Yields with AmpliScribe™ High Yield Transcription Kits

Judith E. Meis, EPICENTRE

## Introduction

EPICENTRE'S AmpliScribe™ T7, T3, and SP6 High Yield Transcription Kits have been specially formulated to utilize high concentrations of NTPs that are inhibitory to other kits and conventional, *in vitro* transcription systems. Yields of up to 150 µg of full length RNA per µg of DNA template in a standard 20 µl reaction can be consistently realized.

Here we compare the performance of the AmpliScribe T7 High Yield Transcription Kit with *in vitro* transcription kits from two other suppliers and with a conventional T7 RNA Polymerase transcription reaction. Comparative analyses of *in vitro* transcription reaction products were based on both the yield and the integrity of the RNA produced.

## Methods

Linear DNA templates were generated by restriction enzyme digestion of plasmid DNA. The digested DNA was treated with 200 µg/ml Proteinase K and 0.5% SDS for 30 minutes at 50°C to minimize nuclease contamination. Plasmids were then purified by phenol/chloroform extraction, ethanol precipitated, and resuspended in TE buffer. The AmpliScribe linear control DNA was used as supplied in the kit.

Standard 20 µl *in vitro* transcription reactions were performed according to the protocol provided in the kits tested. Each 20 µl reaction contained 1 µg of linearized DNA template and was incubated at 37°C for 2 hours, unless otherwise indicated. The conventional transcription reaction contained 10 U of T7 RNA Polymerase, 0.5 mM each NTP, in 1X transcription buffer with 10 mM DTT as described in reference 1.

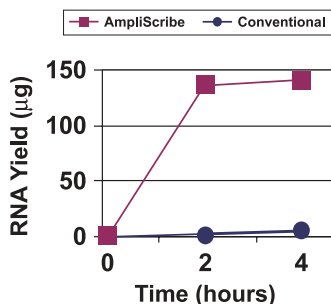
Transcription reactions were stopped and RNA transcripts were purified from unincorporated NTPs by the addition of an equal volume of cold 5 M NH<sub>4</sub> OAc. The samples were chilled on ice for 10 minutes and the RNA was pelleted in a microcentrifuge for 10 minutes at full speed. The RNA samples were resuspended in TE, quantitated by spectrophotometry, and analyzed for integrity by electrophoresis on native agarose gels.

## Results

**AmpliScribe Kits produce >20 fold more RNA than conventional methods**

Transcription reactions were performed for 2 hours and 4 hours using the AmpliScribe T7 High Yield Transcription Kit and a conventional T7 RNA Polymerase method.<sup>1</sup> Figure 1 shows that the AmpliScribe T7 Transcription Kit produced > 20 fold more full length 1.4 Kb RNA transcript than the conventional reaction.

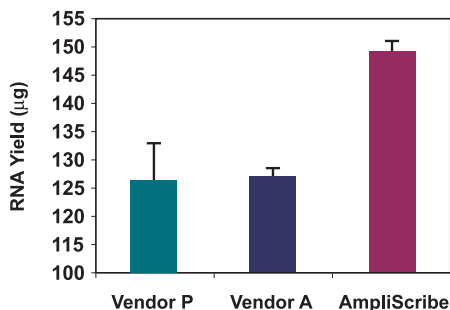
Figure 1. The AmpliScribe™ T7 High Yield Transcription Kit consistently produced >20 fold more of a 1.4 Kb RNA than a conventional T7 RNA Polymerase reaction.



**AmpliScribe T7 High Yield Transcription Kits consistently produce the highest yield of long RNA**

Yields of a 1.8 Kb RNA transcript produced using the AmpliScribe T7 High Yield Transcription Kit and transcription kits from two other suppliers were compared. The AmpliScribe High Yield Kit consistently produced more of the 1.8 Kb RNA in a standard 20 µl, 2-hour reaction than the other kits (Figure 2).

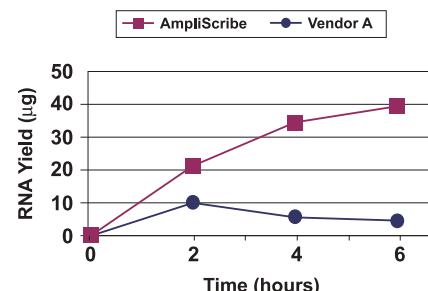
Figure 2. AmpliScribe™ T7 High Yield Transcription Kit consistently produced the highest yield of a 1.8 Kb RNA. Up to 150 µg of RNA was produced using the AmpliScribe™ T7 High Yield Kit in a standard 20 µl, 2-hour reaction.



**The AmpliScribe Kit produces the highest yields of short (<300 bases) RNA**

Producing large quantities of a short transcript requires more transcription initiation events than with production of a larger (e.g., 1 Kb) transcript. The AmpliScribe T7 High Yield Transcription Kit was compared to Vendor A's "short transcription" kit specifically designed for producing short RNA. As shown in Figure 3, the AmpliScribe Kit produced twice as much of a 63 base RNA transcript in a standard 2-hour reaction than the competitor's kit designed for producing short transcripts. Lengthening the reaction incubation to 6 hours increased

Figure 3. The AmpliScribe™ T7 High Yield Transcription Kit produces higher yields of short (<300 base) RNA than a competitor's kit specifically designed for short templates. The yield of a 63 base RNA was compared at 2, 4, and 6 hour time points.



the yield from the AmpliScribe Kit to as much as 4 times the amount produced using Vendor A's specialized kit. Some RNA degradation was also detected with Vendor A's kit after a 6 hour reaction, while the AmpliScribe Kit continued to accumulate full-length RNA transcripts. Note that although the number of micrograms of the 63 base RNA produced is small compared to the yield of the 1.8 Kb RNA, the number of moles of 63-base RNA produced is greater.

**Excellent RNA integrity with the AmpliScribe T7 Kit**

The AmpliScribe RNA Polymerases contain an added RNase inhibitor. We analyzed the integrity of RNA transcripts produced using the AmpliScribe T7 Kit by agarose gel electrophoresis. Figure 4 presents agarose gel analysis of a 1.4 Kb RNA transcript produced by the

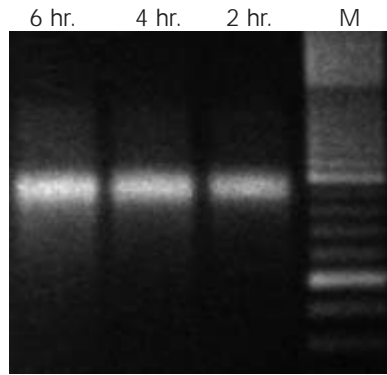
AmpliScribe T7 Kit after 2, 4 and 6 hour reactions. All time points yielded high quality, full-length RNA.

### Summary

The AmpliScribe T7 High Yield Transcription Kit consistently produces the highest RNA yields of any *in vitro* transcription system available using a broad range of DNA templates.

### References

- Melton, D. *et al.* (1984) Nucl. Acids Res. **12**, 7035.



**Figure 4.** RNA with excellent integrity is produced with the AmpliScribe™ T7 High Yield Transcription Kit. Agarose gel electrophoresis of the 1.4 Kb transcript produced from 2, 4, and 6 hour transcription reactions with the AmpliScribe T7 Kit. M, DNA ladder.

#### AmpliScribe™ T7 High Yield Transcription Kit

AS2607	25 Reactions
AS3107	50 Reactions

#### AmpliScribe™ T3 High Yield Transcription Kit

AS2603	25 Reactions
AS3103	50 Reactions

#### AmpliScribe™ SP6 High Yield Transcription Kit

AS2606	25 Reactions
AS3106	50 Reactions

Each kit includes RNA Polymerase (with added RNase inhibitor), 10X AmpliScribe Reaction Buffer, 100 mM each NTP, RNase-free DNase I, DTT, and Control DNA template.

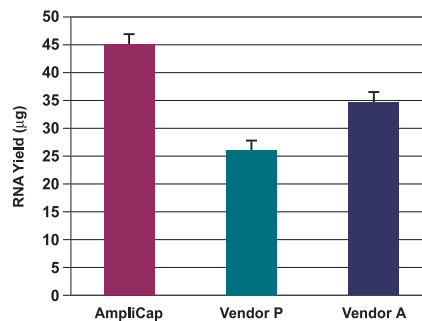
Quantity discount on AmpliScribe Kits is available. Please inquire.

## Highest Yield of 5'-Capped RNA from an *In Vitro* Transcription Reaction with AmpliCap™ High Yield Message Maker Kits

EPICENTRE's AmpliCap™ T7, T3 and SP6 High Yield Message Maker Kits are specially formulated to produce the highest yield of 5'-capped RNA from an *in vitro* transcription reaction. A Cap/NTP PreMix containing optimal concentrations of m<sup>7</sup>G[5']ppp[5']G Cap analog and NTPs, is provided to maximize capping efficiency and RNA yield.

The new AmpliCap™ T7, SP6 and T3 High Yield Message Maker Kits feature:

- Capped RNA yields of up to 45 µg from the T7 and T3 kits and up to 35 µg using the SP6 kit.
- Up to 80% of the RNA is capped using all three kits.
- An optimized m<sup>7</sup>G[5']ppp[5']G Cap/NTP PreMix solution is provided for ease of use and highest yields of capped RNA transcripts.
- A separate vial of GTP for efficient production of long, 5'-capped RNA.



**Figure.** AmpliCap™ T7 High Yield Message Maker Kit consistently produces the highest yield of 5'-capped RNA transcript. A standard 20 µl reaction produces more 5'-capped transcript than the competitor's kits.

#### AmpliCap™ High Yield Message Maker Kits

T7	
AC0707	25 Reactions
T3	
AC0703	25 Reactions
SP6	
AC0706	25 Reactions

Each kit contains the respective RNA Polymerase (including RNase inhibitor), Cap/NTP PreMix, 20 mM GTP 10X AmpliCap™ Transcription Buffer, 100 mM DTT, RNase-free DNase I, Control template DNA, RNase-free water

## Gentle and Quantitative Recovery of Large Genomic DNA Fragments from Agarose Gels for Genomic Cloning

Gentle, rapid and efficient recovery of even minute amounts of size-separated genomic DNA fragments from low-melting point (LMP) agarose gels can be accomplished using GELase Agarose Gel-Digesting Preparation. GELase Preparation provides for quantitative recovery of large (up to >2 Mb) DNA fragments without the loss or shearing that can occur with electroelution or column purification methods. The DNA recovered from the agarose is often concentrated enough to be used directly for ligation into the BAC, cosmid or fosmid cloning vector. Additional information on using GELase Preparation for genomic cloning is presented in EPICENTRE's product literature for the pWEB™ Cosmid Cloning Kit, the pWEB::TNC™ Cosmid Cloning Kit and the EpiFOS™ Fosmid Library Production Kit.

Here are a few of the many citations describing use of GELase Preparation to recover size-selected genomic DNA fragments from LMP agarose gels for subsequent BAC cloning.

1. Brosch, R. *et al.* (1998) *Infection and Immunity* **66**(5), 2221.
2. Buchrieser, C. *et al.* (1999) *Infection and Immunity* **67**(9), 4851.
3. Clemson University Genomics Institute at [www.genome.clemson.edu/protocols/](http://www.genome.clemson.edu/protocols/)
4. Texas A&M University BAC Center at <http://hbx.tamu.edu/bacindex.html>
5. Rondon, M.R. *et al.* (1999) *Proc. Nat'l Acad. Sci., USA* **96**(11), 6451.
6. Birren, B. *et al.* (1999) *Bacterial Artificial Chromosomes in Genome Analysis: A Laboratory Manual v.3*, 241, Cold Spring Harbor Press.

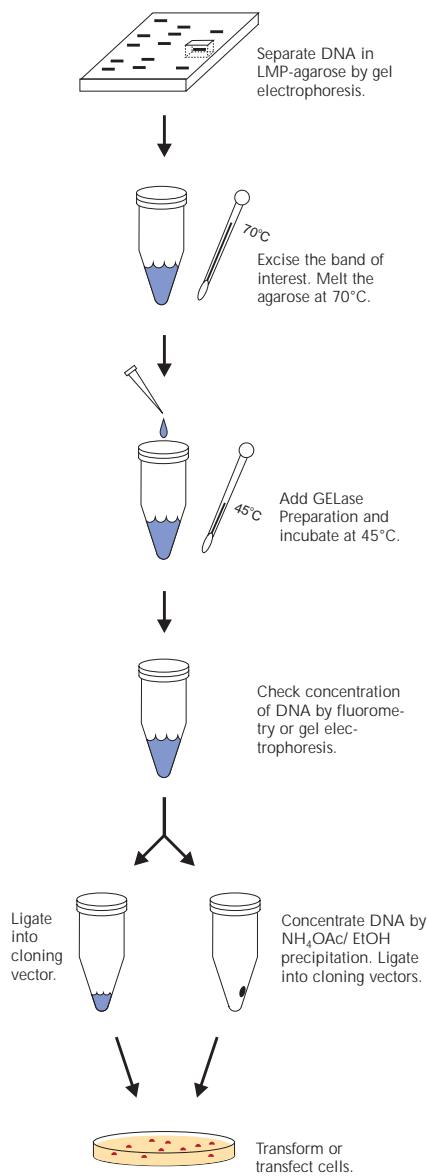


Figure: GELase™ Agarose Gel-Digesting Preparation provides a rapid and gentle method for quantitative recovery of genomic DNA fragments from low melt point (LMP) agarose gels following gel electrophoresis.

## The Highest Transformation Efficiency Electrocompetent *E. coli* Cells

With their high efficiency of transformation and lack of size bias against large inserts, TransformMax™ EC100™ Electrocompetent *E. coli* are ideal for producing Bacterial Artificial Chromosome (BAC) libraries as well as for routine cloning and subcloning applications.

### Genotype

F<sup>-</sup> *mcrA* Δ(*mrr-hsdRMS-mcrBC*)  
 ϕ80*dlacZ*ΔM15 Δ*lacX74* *recA1* *endA1*  
*araD139* Δ(*ara, leu*)7697 *galU* *galK* λ<sup>-</sup>  
*rpsL* *nupG*

### Important Phenotypes & Applications

- Readily take up large clones for unbiased BAC library production.
- Supports blue/white screening of vectors, including Cloning-Ready pIndigoBAC-5 vectors.
- Restriction minus for efficient cloning of methylated DNA (e.g. mammalian genomic DNA).
- Endonuclease minus (*endA1*) to ensure high yields of clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

### Transformation Efficiency of TransformMax™ EC100™ Electrocompetent *E. coli* Versus Three Leading Competitors

	Transformation Efficiency (cfu/μg DNA)*
TransformMax™ EC100™ <i>E. coli</i>	9.2 X 10 <sup>9</sup>
Competitor S	5 X 10 <sup>9</sup>
Competitor I	4 X 10 <sup>9</sup>
Competitor L	3 X 10 <sup>9</sup>

\* Average of eight independent transformations using consistent conditions with a pUC vector. Efficiencies vary with electroporator and conditions such as voltage and pulse time. Under optimal conditions, TransformMax™ EC100™ Cells can yield efficiencies >10<sup>10</sup> cfu/μg.

### TransformMax™ EC100™ Electrocompetent *E. coli*

EC10005	5 X 100 μl (10 Electroporations)
EC10010	10 X 100 μl (20 Electroporations)

Each includes pUC19 control DNA.

### GELase™ Agarose Gel-Digesting Preparation

G09050	50 Units	1 U/μl
G09100	100 Units	1 U/μl
G09200	200 Units	1 U/μl

# pWEB™ and pWEB::TNC™ Cosmid Cloning Kits & EpiFOS™ Fosmid Library Production Kit

The pWEB™ and pWEB::TNC™ Cosmid Cloning Kits and the EpiFOS™ Fosmid Library Production Kit facilitate rapid and efficient construction of unbiased cosmid or fosmid libraries. The pWEB and pWEB::TNC cosmid vectors and the pEpiFOS-5 fosmid vector provided in the respective kits are provided linearized and dephosphorylated and ready for ligation of blunt-end DNA fragments of about 40 Kb. The cosmid and fosmid kits contain reagents and protocols to prepare approximately 10 complete and unbiased cosmid or fosmid libraries.

## The Cosmid Cloning and Fosmid Library Production Process

The kits utilize a novel strategy (figure) of cloning randomly sheared and end-repaired genomic DNA. Shearing the DNA into approximately 40-kb fragments leads to generation of highly random fragments, in contrast to the conventional approach of generating DNA fragments by partial restriction endonuclease digestion. The result is a complete and unbiased cosmid or fosmid library.

### pWEB™ Cosmid Cloning Kit

The pWEB Cosmid Cloning Kit contains EPICENTRE's original cosmid cloning vector. Like the pWEB::TNC vector, the pWEB vector has been linearized at its *Sma* I site that is flanked by pairs of *Bam*H I, *Eco*R I and *Not* I sites to aid in the excision and mapping of the cloned insert.

### pWEB::TNC™ Cosmid Cloning Kit

The pWEB::TNC Cosmid Cloning Kit contains the pWEB::TNC Cosmid Vector. Cosmid clones produced in this vector can be used for subsequent generation

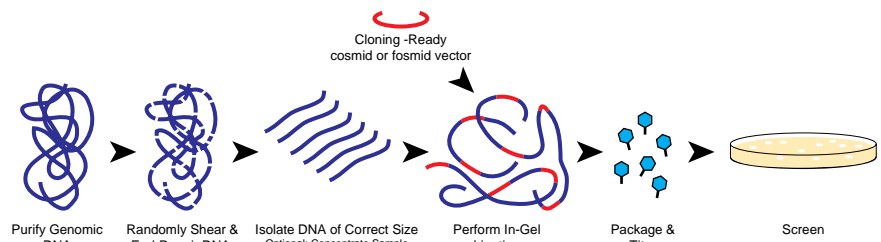


Figure. Process for preparing cosmid or fosmid libraries using the pWEB™ and pWEB::TNC™ Cosmid Cloning Kits or the EpiFOS™ Fosmid Library Production Kit. The kits feature a novel strategy of cloning randomly sheared and end-repaired genomic DNA fragments to better insure production of complete and unbiased cosmid and fosmid libraries.

of a random unidirectional deletion library from a chosen cosmid clone when used in the conjunction with the pWEB::TNC™ Deletion Cosmid Transposition Kit (available separately).

## EpiFOS™ Fosmid Library Production Kit

Fosmid vectors<sup>1,2</sup> provide an improved method for cloning and stably maintaining cosmid-sized (about 40 kb) libraries in *E. coli*. The pEpiFOS-5 Fosmid Vector provided in the kit is derived from the single copy F-factor of *E. coli*. Thus, the cosmid-sized clones produced using pEpiFOS-5 are propagated as a single copy in the cell to improve their stability compared to conventional cosmid libraries.

## References

- Kim, UJ. *et al.* (1992) *Nucl. Acid Res.* **20**, 1083.
- Birren, B. *et al.* (1999) *Construction of Bacterial Genomic Libraries in Genome Analysis: A Laboratory Manual v.3*, 24.

Table. A comparison of the pWEB™ and pWEB::TNC™ Cosmid Cloning Kits and the EpiFOS™ Fosmid Library Production Kit.

Library	EPICENTRE vector	Cloning Capacity	Introduce Into <i>E. coli</i> by	Copies per cell
Cosmid	pWEB* and pWEB::TNC**	≈40 Kb	λ packaging	5 - 50
Fosmid	pEpiFOS-5***	≈40 Kb	λ packaging	1 - 2

\* Provided in the pWEB Cosmid Cloning Kit.

\*\* Provided in the pWEB::TNC Cosmid Cloning Kit.

\*\*\* Provided in the EpiFOS Fosmid Library Production Kit.

### pWEB™ Cosmid Cloning Kit

PC8805 1 Kit

For producing up to 10 complete and unbiased cosmid libraries.

### pWEB::TNC™ Cosmid Cloning Kit

WEBC931 1 Kit

For producing up to 10 complete and unbiased cosmid libraries.

### EpiFOS™ Fosmid Library Production Kit

FOS0901 1 Kit

For producing up to 10 complete and unbiased fosmid libraries.

Kits include: pWEB™, pWEB::TNC™ Cosmid Vector or pEpiFOS™-5 Fosmid Vector, End-repair Enzyme Mix, End-repair 10 X Buffer, dNTP Mix, Fast-Link™ DNA Ligase, Fast-Link™ 10X Ligation Buffer, ATP Solution, GELase™ Gel-digesting Preparation, GELase™ 50X Reaction Buffer, MaxPlax™ Lambda Packaging Extracts, Ligated Lambda Control DNA, Control DNA, EPI100™ Plating strain, Control Lambda Plating strain.

# Use of the EZ::TN™ Transposon Insertion System for High Throughput Full-Length cDNA Sequencing at the NIH Intramural Sequencing Center (NISC)

Yuriy Shevchenko, Robert Blakesley, Gerry Bouffard, Keith Wetherby, Eric Green and Jeffrey Touchman  
NIH Intramural Sequencing Center, National Institutes of Health, Gaithersburg, MD

## Introduction

In addition to producing over two million genomic sequence reads per year, the NIH Intramural Sequencing Center (NISC) also participates in the sequencing program of the Mammalian Gene Collection (MGC) project. Briefly, the goal of this project is to generate collections of human and other mammalian full-length cDNAs that are sequenced to high accuracy, then to make the clones freely available to the scientific community.<sup>1</sup> This article provides a brief description of the high throughput full-length cDNA sequencing activities at NISC. Results of these efforts will be published elsewhere. Progress reports are also available through the MGC Web site (<http://www.ncbi.nlm.nih.gov/MGC>).

## Strategy and Methods

Sequencing of MGC cDNA clones, whose inserts range in size from 300 bp to over 6 kb and are relatively high in GC content, represents a challenge. After evaluating several available methods, including primer walking,<sup>2</sup> concatenated cDNA sequencing<sup>3</sup> and multiple transposon-based systems, we decided to use the Tn5-based EZ::TN™ Transposon Insertion System because of its high performance in our setting. This system is robust, gives a reasonably uniform distribution of random transposon insertions, is easily adaptable to 96-well format, and is compatible with antibiotic selection in the MGC cDNA vector.

## Generation *In Vitro* of cDNA Insertion Subclones

Transposon insertion reactions using the EZ::TN System are simultaneously set up for all cDNA clones in a 96-well reaction tray. First, a transposon "brew" containing EZ::TN Reaction Buffer, EZ::TN <KAN-2> Transposon DNA, and EZ::TN Transposase is dispensed into each well of the tray. Next, purified cDNA clones (target DNAs) are transferred into separate wells of the tray using a Microlab 4200 pipetting robot (Hamilton). Transposon insertion reactions are carried out by incubating the tray in a thermal cycler (MJ Research) for 2 hours at 37°C. Reactions are terminated by the addition of EZ::TN Stop Solution, followed by incubation at 70°C for 10 minutes.



The EZ::TN™ Transposon Insertion System is an effective component of a high throughput sequencing pipeline at the NIH Intramural Sequencing Center.

Aliquots of the completed insertion reactions are then used for transformation of chemically competent *E. coli*. First, insertion reactions are diluted by a constant factor to eventually achieve about 100 colonies per plate. Next, transformation reactions are performed in a 96-well format with an aliquot of the diluted DNAs. Last, the entire volume of each transformation is plated on a its own 90-mm Petri dish containing appropriate antibiotics. Despite the variability in DNA concentration and insert size between samples, the EZ::TN System produces fairly uniform numbers of colonies for each cDNA clone using our optimized conditions.

## Sequencing of cDNA Insertion Subclones

EZ::TN Transposon-containing insertion subclones are entered into our standard sequencing pipeline, thereby benefiting from considerable economies of scale. Colonies are picked with a QPix automated picker (Genetix) and transferred to medium in 96-well growth blocks. After overnight growth, DNAs are isolated in 96-well format using the Concert96 Plasmid Purification System (Life Technologies) and a second Microlab 4200 pipetting robot. For each subclone DNA, paired sequencing reactions containing BigDye Terminator chemistry (Applied Biosystems) are set up with the first pipetting robot. Each reaction of the pair utilizes a primer complementary to one or the other

transposon end, facing into the inserted DNA. The cycling parameters are: 96°C for 1 min, followed by 35 cycles of 96°C for 10 sec; 55°C for 10 sec; and 60°C for 4 min. Completed reaction products are purified, and then analyzed on AB3700 capillary electrophoresis instruments (Applied Biosystems). Success rates for sequencing reactions with transposon-specific insertions are similar to those obtained with other samples in our production pipeline.

All the sequencing data for each cDNA clone are assembled with the use of the conventional program, phrap. We usually see a uniform coverage of the entire cDNA insert with individual reads from the ends of transposon inserts.

## Project Status

The ability of the EZ::TN System to be used for processing multiple DNA samples in a batch mode makes it an effective component of a high throughput sequencing pipeline. This transposon-based sequencing system permits parallel independent processing of a large number of clones and is easily scalable to meet the growing demands of the project.

At present, we have successfully integrated transposon-based full-length cDNA sequencing into the main NISC sequencing pipeline and our MGC effort is on track to generate 4000-5000 high-quality cDNA sequences in the coming year.

## References

1. Strausberg *et al.* (1999) *Science* **286**, 455.
2. Yu *et al.* (1997) *Genome Res* **7**(4), 353.
3. Andersson *et al.* (1997) *DNA Seq* **7**(2), 63.

### EZ::TN™ <KAN-2> Insertion Kit

EZI982K 10 Reactions

### EZ::TN™ <TET-1> Insertion Kit

EZI921T 10 Reactions

### EZ::TN™ <DHFR-1> Insertion Kit

EZI912D 10 Reactions

Each kit contains the specific EZ::TN™ Transposon, EZ::TN™ Transposase, Buffers and two unlabeled sequencing primers.

Contact EPICENTRE or your local distributor to discuss discounts for bulk quantities.

# The Most Versatile Transposition System

EPICENTRE offers a choice of EZ::TN™ Insertion Kits because sequencing is often just part of your research project. In addition to primer binding sites and a selectable marker each of the EZ::TN Transposons included in these kits contain features that can be used in gene analysis, proteomics or RNA research. But that's not all. You can also make your own transposon using the EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector.

## Find Functional Domains or Epitopes of Proteins

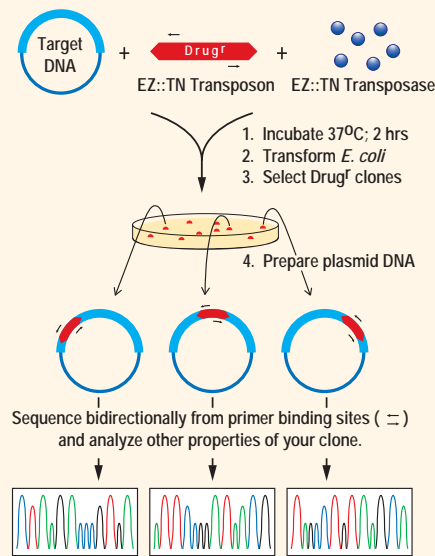
The EZ::TN™ In-Frame Linker Insertion Kit was designed to rapidly and easily produce random 19-amino acid (19 codon; 57-nucleotide) in-frame insertions into genes of expressed proteins for protein engineering, functional analysis, and domain or epitope mapping. The kit features the EZ::TN™ <Not I/KAN-3> Transposon, which contains a kanamycin resistance marker flanked by *Not I* restriction sites. Kanamycin-resistant insertion clones are digested with *Not I*, ligated, and re-transformed into *E. coli*. Since each resulting clone contains a random 19-codon insertion that can be read in all three reading frames the protein retains its original amino acid sequence on both sides of the insertion site.

## Synthesize RNA from Any Region of Your Cloned DNA

The EZ::TN™ <T7/KAN-2> Promoter Insertion Kit provides an easy and reliable method to randomly insert a phage T7 RNA polymerase promoter into any target DNA. The transposon does not have a transcription termination sequence so RNA can be produced from chosen insertion clones by *in vitro* transcription using an AmpliScribe™ T7 High Yield Transcription Kit, or *in vivo* after transformation of *E. coli* having an inducible T7 RNA polymerase gene.

## Insert a Conditional Origin of Replication

The EZ::TN™ <R6K $\gamma$ ori /KAN-2> Insertion Kit allows you to randomly insert the *E. coli* R6K $\gamma$  conditional origin of replication into target DNA *in vitro*. The target can then be propagated as independently replicating DNA in *E. coli* hosts expressing the *pir* gene product such as TransforMax™ *pir*<sup>+</sup> or TransforMax™ *pir*-116 Electrocompetent *E. coli*.



The process for generating insertion clones for sequencing and a myriad of other applications using an EZ::TN™ Insertion Kit or your own custom EZ::TN™ Transposon. The EZ::TN Transposon insertion reaction is a simple, one-step enzymatic reaction that randomly inserts an EZ::TN Transposon into your plasmid, cosmid or BAC clone. Transform *E. coli* (e.g., TransforMax™ EC100™ Electrocompetent *E. coli*) with an aliquot of the reaction and select for EZ::TN Transposon insertion clones. Prepare sequencing template from randomly chosen clones and sequence each bidirectionally using a single set of sequencing primers that are homologous to the ends of the inserted EZ::TN Transposon. Continue to analyze your insertion clone using the other unique features contained on the transposon.

## Make Your Own Transposon

A custom EZ::TN Transposon containing any DNA sequence of interest (e.g. selectable marker, control element, gene, cDNA) can be quickly and easily prepared using the EZ::TN™ pMOD™-2 <MCS> Transposon Construction Vector. To prepare your transposon, clone the DNA of interest into the multiple cloning site and then release the transposon by PCR or by digestion with *Pvu II* or *PshA I*. The Transposon can be used for *in vitro* insertion into any target DNA, or it can be incubated with EZ::TN™ Transposase to form an EZ::TN™ Transposome (see pages 1-3 of this Forum), for random insertion into the genomic DNA of living cells. Your custom transposon will also include primer binding sites at either end for bidirectional sequencing. No need to design your own primers.

### EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector

MOD0602 20 µg

Includes: pMOD™-2<MCS> Vector and the Forward and Reverse PCR Primers

### EZ::TN™ Transposase

TNP92110 10 Units

### pMOD™<MCS> Forward Sequencing Primer

MODFSP201 1 nmole

### pMOD™<MCS> Reverse Sequencing Primer

MODRSP202 1 nmole

### EZ::TN™ In-Frame Linker Insertion Kit

EZI04KN 10 Reactions

For sequencing cloned DNA then generating random 19 amino acid in-frame insertions into the encoded protein.

### EZ::TN™ <T7/KAN-2> Insertion Kit

EZI03T7 10 Reactions

For random insertion of a T7 transcription promoter.

### EZ::TN™ <R6K $\gamma$ ori /KAN-2> Insertion Kit

EZI011RK 10 Reactions

For random insertion of the *E. coli* R6K $\gamma$  origin of replication.

### TransforMax™ EC100™ Electrocompetent *E. coli*

EC10005 5 x 100 µl  
(10 electroporations)

EC10010 10 x 100 µl  
(20 electroporations)

TransforMax™ EC100™ Electrocompetent *E. coli* have the highest transformation efficiency available and are function tested for optimal EZ::TN Insertion reaction results.



# DNA Ligation in 5 Minutes!

## At room temperature

Lab Tested ...  
Scientist Approved.

Visit [www.biowire.com](http://www.biowire.com) and search for "Fast-Link" to view comments from users of EPICENTRE's Fast-Link DNA Ligation Kits.

EPICENTRE's Fast-Link™ DNA Ligation kits are specially formulated to provide fast and efficient DNA ligations in as little as 5 minutes at room temperature for both routine and high throughput cloning.

### Fast and Efficient DNA Ligations.

- Ligate cohesive-end DNA in 5 minutes at room temperature.
- Ligate blunt-end DNA in 15 minutes at room temperature.
- Ligate PCR product with A-overhangs in 1 hour or less at 16°C.

### Transform Cells Without Desalting the Ligation Reaction.

Desalting the Fast-Link DNA ligation reaction prior to electroporation of competent cells is not necessary. Aliquots (1 - 2 µl) of a Fast-Link ligation reaction may be used directly in electroporation.

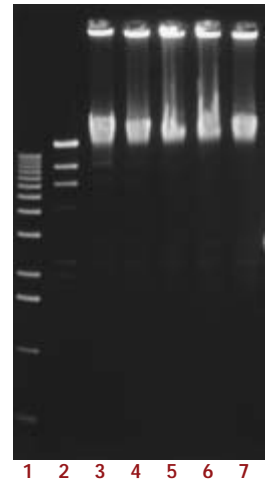
### Representative Transformation Results with Fast-Link Ligation Products\*

	Ligation Time	% White Colonies	Recombinants per µg DNA
Cohesive ends	5 min.	>90%	2.0 x 10 <sup>6</sup>
Blunt ends	15 min.	>95%	5.0 x 10 <sup>5</sup>

\* Cohesive-end ligation results were obtained by ligating *Hind* III-digested *E. coli* chromosomal DNA into EPICENTRE's pIndigoBAC-5 (*Hind* III-Cloning Ready) BAC vector. Blunt-end ligation results were obtained by ligating a 1.2 Kb blunt-ended fragment into a blunt-end dephosphorylated pUC-based plasmid vector. One microliter from each ligation reaction was used to transform TransforMax™ EC100™ Electrocompetent *E. coli*.

Figure 1. Time course for cohesive-end ligation using the Fast-Link™ DNA Ligation Kit. Lambda Hind III markers were ligated in a standard Fast-Link reaction using 2 U of Fast-Link DNA Ligase (Lanes 3-7). Lane 1, 1 kb ladder; Lane 2, no enzyme.

Ligation Time in Minutes  
5 15 30 60 120



[www.epicentre.com/catalog/fastlink.htm](http://www.epicentre.com/catalog/fastlink.htm)

### Fast-Link™ DNA Ligation Kits

**NEW!** LK0750H 50 ligations  
LK6201H 100 ligations

Includes Fast-Link™ DNA Ligase, Fast-Link™10X Ligation Buffer, ATP

### HOW TO CONTACT US AT EPICENTRE

Toll-free in the USA: **800-284-8474**  
Tel: 608-258-3080 Fax: 608-258-3088  
Web site: [www.epicentre.com](http://www.epicentre.com) E-mail: [techhelp@epicentre.com](mailto:techhelp@epicentre.com)  
Outside the USA: contact your local EPICENTRE Distributor.

### EPICENTRE FORUM

Editors MERRIANN CAREY  
JIM PEASE  
Graphic Designer BRENDA HOUSE

© 2001 EPICENTRE All rights reserved. Publication date: April, 2001. Printed in USA.

