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In This Issue

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RECENT RELEASE

Using the EZ::TN™ Transposome™ for Transposon Mutagenesis in *Mycobacterium smegmatis*

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Mycobacterium smegmatis is the organism of choice for genetic studies of mycobacteria because the strain is fast growing, non-pathogenic, and DNA can be introduced into it by transformation (electroporation), transduction and conjugation.[1-3] As such it is used as the genetic testing ground for its more pathogenic cousins *M. tuberculosis* and *M. leprae.* The development of additional genetic tools to facilitate the study of this fascinating group of organisms is important, especially as until recently they have proven to be refractory to genetic analyses. This article describes the application of a Tn5-based transposome system[4] (Figure 1) to *M. smegmatis* and it is the first description of its use in a gram-positive organism. These results therefore underline the potential of this system as a mutagen for many bacterial species.

Transposons have proven to be one of the most versatile and useful genetic tools for molecular genetic research. They can be used to generate marked gene knockouts, create gene fusions, and act as mobile priming sites for DNA sequence analyses.[5] More recently, the repertoire of transposon applications has expanded dramatically with the development of *in vitro* transposition systems.[6-10] These systems have had great impact on the ability to generate insertion mutations and to provide sequencing priming sites by taking advantage of a more efficient biochemical reaction that allows insertion of the transposon to cloned regions of interest.

For many less-characterized organisms, there is still a need for the development of *in vivo* transposon mutagenesis systems to facilitate genetic studies.

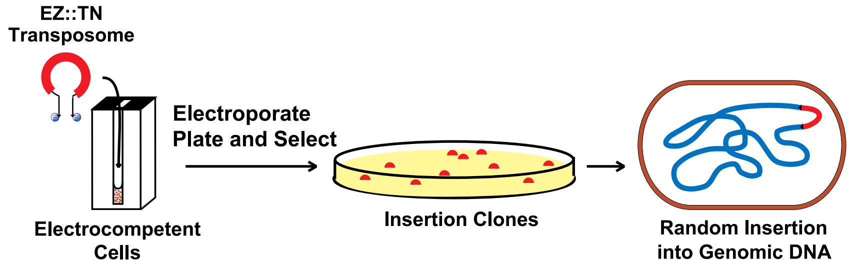

Figure 1. Editor's Note: Overview of the process for generating EZ::TN Transposome-mediated insertions into living cells.

continued

This requires both an efficient transposition system for the host organism and a method to deliver the transposon. Most transposon vectors are based on a conditionally replicating phage or plasmid that is used to deliver the transposon into the host under permissive conditions and provide a window of time for transposition to occur. Transposon insertions are then selected with an appropriate antibiotic, while growing the cells under non-permissive conditions eliminates the vector. Unfortunately, for many of these organisms functional transposons, and plasmid or phage vectors have still to be described.

Recently, a simple and elegant *in vivo* transposon delivery and transposition system has been described that is destined to become the transposon mutagenesis method of choice for such organisms.[4] The only requirement is that DNA can be introduced into the host by electroporation; no vector delivery system is required. Its simplicity relies on the ability to generate a stable EZ::TN Transposome (Tn*5* transposon intermediate) that can be electroporated into the bacterial host. Tn*5* transposes by a simple insertion mechanism,[6] and one of the key intermediates in this pathway is the transposome: an excised transposon with the transposase protein bound to the inverted repeats (Mosaic Ends) found at the ends of the transposon. The transposome can be generated *in vitro* using purified transposase protein and a DNA fragment flanked by the inverted repeats. The fact that any gene flanked by the inverted repeats can be used to form this intermediate endows the system with great flexibility, as it can be tailored to the organism of choice. The procedure relies on the fact that transposomes are extraordinarily stable DNA complexes[11,12] and, in particular, the EZ::TN (Tn*5)* complex has been shown to maintain its integrity throughout the electroporation process.

Methods

One microliter of the preformed EZ::TN <Kan-1>Tnp Transposome (EPICENTRE) was introduced into *M. smegmatis* strains, mc^{2}155[13] and mc^{2}874,[14] by electroporation. Electrocompetent cells were prepared and used according to previously described methods.[3] Immediately after pulsing, 1 ml of trypticase-soy broth (TS) containing 0.05% Tween 80 was added, and the cell suspension was incubated for 4-6 hrs before plating on TS media containing kanamycin at 10 µg/ml. Plates were incubated for 4-5 days at 37°C to allow colony formation. No transformants were detected in mock electroporations. The auxotrophic screen was performed by growing Kan^r transformants in microtitre plates for 3 days at 37°C before spotting 5 µl onto either TS media or Middlebrook 7H10 media plus ADC[3] containing kanamycin.

Chromosomal DNA for Southern analysis was obtained from strains of *M. smegmatis* using established procedures.[15] The Kan^r gene was labeled with [α-^{32}P] dATP by random priming, and used as a probe of Southern blots of *Eco*RI- and *Pst*I- digested chromosomal DNA. There are no *Eco*RI or *Pst*I sites in the EZ::TN <KAN-1> Transposon.

Results and Discussion

Electroporation of the EZ::TN <KAN-1>Tnp Transposome into both strains of *M. smegmatis* generated between 1-5 x 10^2 Kan^r colonies. This is in contrast to plasmid electroporation, which yields approximately 10^5 transformants/µg of plasmid DNA. To confirm that these were *bone fide* insertions, chromosomal DNA was isolated from 16 transformants and subject to Southern hybridization analysis. Two different enzymes were used to digest the DNA, and a segment of the Kan^r gene was used as a probe (Figure 2). The analysis shows that none of the hybridizing bands co-migrate in both of the restriction analyses. This demonstrates unequivocally that the insertions are on different DNA fragments and are

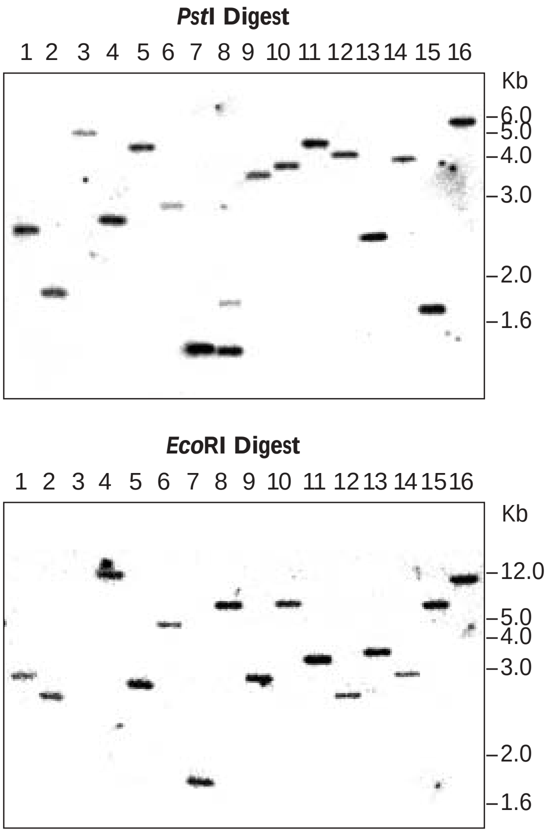

Figure 2. A Southern blot analysis showing random insertion of the EZ::TN <KAN-1> Transposon into the mycobacterial chromosome. *Chromosomal DNA from sixteen Kan^r colonies from an electroporation was digested with either PstI (top panel), or EcoRI (bottom panel), before agarose gel electrophoresis. The DNAs were then probed with the Kan^r gene contained within the EZ::TN <KAN-1> Transposon. All insertion sites are different. Any two hybridizing fragments that might comigrate in the PstI digest do not in the EcoRI digest. Each clone contains a single insertion, since only one hybridizing band is detected per lane. The second fainter band in lane 8 is not observed in the EcoRI digest or other analyses (data not shown). Size markers are shown at the side of each panel and are indicated in kb.*

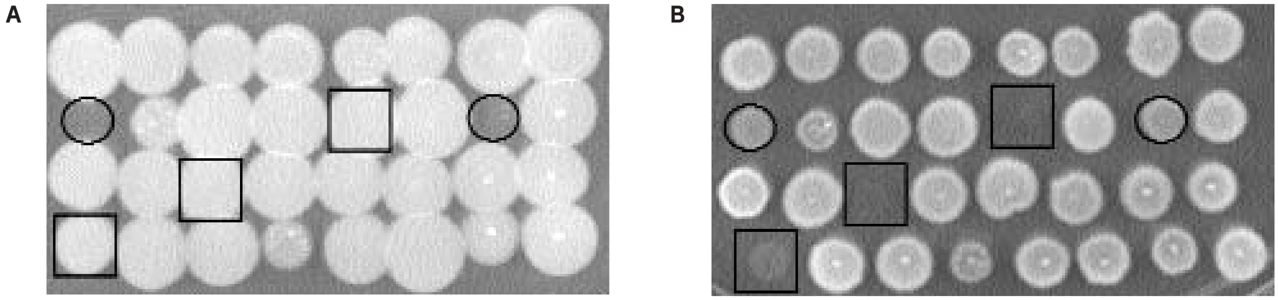

Figure 3. A representative sample of EZ::TN <KAN-1> Transposon insertion mutants used to screen for growth deficiencies. *Aliquots of broth cultures were spotted onto either rich media (A), or minimal media (B). Mutants unable to grow on minimal media are highlighted in a box, mutants defective for growth on rich media are circled.*

therefore independent. Furthermore, it indicates that the insertion sites are randomly distributed around the genome. None of the isolates contained more than one hybridizing DNA fragment, demonstrating that each Kan^r colony contains a single insertion. This is important as it allows any phenotype to be attributable directly to a single insertion.

To demonstrate that the transposon insertions can be used to isolate mutants, a screen for auxotrophic mutants was performed. Small aliquots of broth cultures were screened for their ability to grow on rich or minimal medium (Figure 3). Out of 400 insertion mutants screened seven failed to grow on minimal media suggesting a deficiency in amino acid biosynthesis. In addition, two mutants could not grow on rich media. This latter class of mutants was unexpected and interesting, and may reflect an inability to grow at the faster growth rate on rich media. For example, *polA* mutants of *Escherichia coli* are unable to grow on rich medium.[16] Importantly, they highlight the unexpected benefits of a random mutagenesis.

This work describes the application of a new transposon system to mycobacteria. There are several features of this system that make it extremely attractive to the mycobacteriologist and also to those working on other bacterial systems. The first is simplicity: insertions are directly selected after electroporation and all insertion events are independent. The system does not require the initial establishment of conditionally replicating vectors. Two temperature-sensitive transposon delivery vectors have been described for mycobacteria that are derived from the phage, TM4[17] and the plasmid, pAL500.[18] Unfortunately, a distinct disadvantage with the plasmid system is that many of the insertions are siblings (Takacs and Derbyshire, unpublished results). This is presumably because of the extended period of time required to establish the transposon delivery plasmid in the host (5-6 days for colony formation on plates at 30°C). Any cell in which transposition takes place at an early stage of growth will continue to divide and form siblings.

The second advantage of the EZ::TN Transposome system is that the transposon is not native to mycobacteria. This allows for easy detection, by Southern analysis or PCR, and ensures that the insertion is stable, as it will not be activated by trans-acting transposases. The two transposon systems most commonly used in mycobacteria are native elements, IS*1096* and Tn*611*, although more recently the eukaryotic transposon *Himar 1* has been used in *M. smegmatis.*[19] IS*1096* is found in many copies in most of the common laboratory strains of *M. smegmatis*. Thus it is possible for transposon insertions to be trans-activated. However, in our experience a bigger problem is that integration of the marked IS*1096* can occur by both transposition and homologous recombination into existing elements.[20] Clearly, these latter recombinants will not result in insertion mutants. Tn*611* transposes by a replicative mechanism and thus insertion results in integration of both the transposon and vector into the target genome.[18] This is undesirable as it complicates analyses and leads to unstable insertions.

When using the EZ::TN Transposome system in *M. smegmatis* only a limited number of transformants were obtained in each experiment (1-5 x 10^2), which is mainly due to the low efficiency of electroporation of mycobacteria. Thus it would be necessary to carry out multiple electroporations to ensure saturation of a genome. However, given the simplicity of the experiment this need not be construed as a disadvantage, and in fact ensures multiple independent mutant populations.

Note: *The EZ::TN <KAN-1>Tnp Transposome used in this study has been replaced by the smaller, but functionally equivalent EZ::TN <KAN-2>Tnp Transposome.*

Acknowledgements

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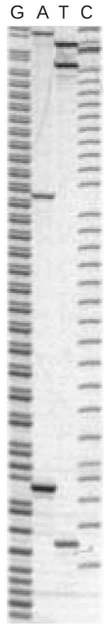

Figure. Sequencing through a GC-rich trinucleotide repeat. *Supercoiled plasmid template containing $(CGG)_{23}$ was sequenced using the SequiTherm EXCEL II isothermal sequencing protocol.*

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EZ::TN™ Transposome™ Frequently Asked Questions

In the previous article Dr. Keith Derbyshire *et. al.* demonstrate the use of an EZ::TN Transposome to generate auxotrophic mutations in mycobacteria. EZ::TN Transposomes, which can be used to create gene knockouts in living cells and to facilitate direct sequencing of bacterial genomic DNA without cloning, have generated a great deal of interest among scientists working with a variety of organisms. Here, we provide answers to some of the most frequently asked questions received by our Technical Consultants.

What is an EZ::TN Transposome?

An EZ::TN Transposome is the stable complex formed between an EZ::TN Transposon–containing any DNA sequence of interest–and the hyperactive EZ::TN Transposase in the absence of Mg^{2+}. An EZ::TN Transposome is so stable that it can be electroporated directly into cells where it is activated by the intracellular Mg^{2+}. Once activated, the transposome randomly inserts its transposon component into the host's genomic DNA.

How efficient is an EZ::TN Transposome?

The most critical parameter affecting transposition efficiency is the transformation efficiency of the cell. The higher the transformation efficiency of the cell, the more clones will be produced. Electroporation of competent cells using an EZ::TN Transposome is less efficient than transformation with a small plasmid. Therefore, use cells with the highest transformation efficiency possible.

Additionally, the selectable marker in the transposon must be expressed in the cell to a high enough level to confer resistance to the insertion clones. Therefore, it may be necessary to plate electroporated cells on media containing different amounts of the antibiotic or other selection agent to detect the insertion clones.

Table 1. Number Kan^R transposon insertion clones generated by electroporation of 1 µl of EZ::TN <KAN>Tnp Transposome.

E. coli	*Salmonella typhimurium*	*Proteus vulgaris*	*Pseudomonas sp.*	*Mycobacteria smegmatis*
1-5 x 10^5	1-5 -x 10^4	1-5 x 10^3	1-5 x 10^2	1-5 x 10^2

How random is an EZ::TN Transposon insertion?

The Tn*5* transposition system, upon which the EZ::TN system is based, is a highly random transposon system. However, keep in mind that transposon insertions into essential host genes are likely to be lethal and thus will not be represented in the insertion clone library produced.

Can I make a custom EZ::TN Transposon containing a DNA sequence of my own design?

Yes. Custom EZ::TN Transposons containing virtually any DNA sequence of interest (e.g., species-specific selectable markers, control elements, cDNA) can be constructed using EPICENTRE's **EZ::TN™ pMOD<MCS> Transposon Construction Vector.** *For a special offer on this vector see page 4.*

Can EZ::TN Transposomes be used to transform gram-positive microorganisms, yeast or mammalian cells?

In theory, yes. However the transformation efficiency of the cell and availability of a selectable marker expressed in the host will be important parameters to success. Again, the higher the transformation efficiency of the cell, the more clones that will be generated. EZ::TN Transposons containing species-specific selection markers and control elements can be constructed using the EZ::TN pMOD<MCS> Transposon Construction Vector.

> *"I consider the EZ::TN Transposome one of the most important new tools for genetic manipulation of non-E. coli bacteria that has come along in the last 5 years."*
>
> **— Dr. Barry Hall,**
> *Univ. of Rochester*
> *Biology Dept.*

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

Judith T. Schanke, EPICENTRE Technologies

Introduction

In vitro transcription of DNA templates by a phage RNA polymerase is a commonly used, efficient method for generating specific RNA transcripts. A phage RNA polymerase directs the synthesis of RNA from a cloned, linearized DNA template under the control of a specific phage promoter. RNA produced by *in vitro* transcription is used in a variety of applications including *in vitro* translation, ribozyme synthesis[1], splicing or processing studies, structural determinations, and the creation of non-radioactive RNA probes.[2-4] RNA transcripts produced by transcription reactions are also often microinjected into cells[5] for anti-sense experiments, or used for cellular localization of RNA.[4]

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. The result is the highest possible yield of RNA transcripts, which can range in size from 25 bases to several kilobases.[6] Using AmpliScribe's T7 RNA transcription reaction can generate up to 150 µg of high quality, full-length RNA transcript (1.4 Kb) per µg of template DNA. Thus, up to 90% of the NTPs present are incorporated into RNA product.

Here the performance of the AmpliScribe T7 Transcription Kit was compared with a conventional T7 RNA polymerase transcription reaction[7] as well as similar transcription kits from two leading suppliers. Comparative analyses of *in vitro* transcription reaction products were based on both the yield and the integrity of the RNA produced.

Materials and Methods

Preparation of DNA Templates

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.

Transcription Reactions

AmpliScribe T7 transcription reactions were performed according to the protocol provided in the kit. Briefly, 20 µl reactions contained 1X Reaction Buffer, 10 mM DTT, 7.5 mM each NTP, 1 µg linearized DNA template, and 2 µl AmpliScribe T7 Enzyme Solution. Reactions were incubated at 37°C for 2 hours, unless otherwise indicated.

The transcription reactions performed with other manufacturers' kits were performed according to the protocols provided. Each 20 µl reaction contained 1 µg 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.[7]

RNA Quantitation and Integrity Analysis

Transcription reactions were stopped by the addition of an equal volume of cold 5 M NH_4OAc. 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 using standard methods.

Results and Discussion

The AmpliScribe High Yield Transcription Kits produced >20 fold more RNA than conventional methods

Timed transcription reactions were performed using the AmpliScribe T7 High Yield Transcription Kit and a conventional T7 RNA polymerase method[7] in order to produce a 1.4 Kb transcript. Reactions were incubated for 2 or 4 hours at 37°C and stopped by ammonium acetate precipitation. The RNA yields produced, as determined by spectrophotometry, are depicted in Figure 1.

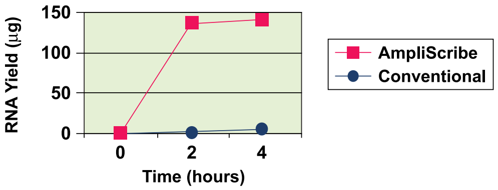

Figure 1. The AmpliScribe High Yield Transcription Kit produced >20 fold more RNA than conventional methods. *Spectrophotometry was used to determine the yield of RNA produced from a conventional T7 RNA polymerase transcription reaction and an AmpliScribe T7 transcription reaction using the AmpliScribe Kit control DNA as template. The data represent the average of duplicate reactions.*

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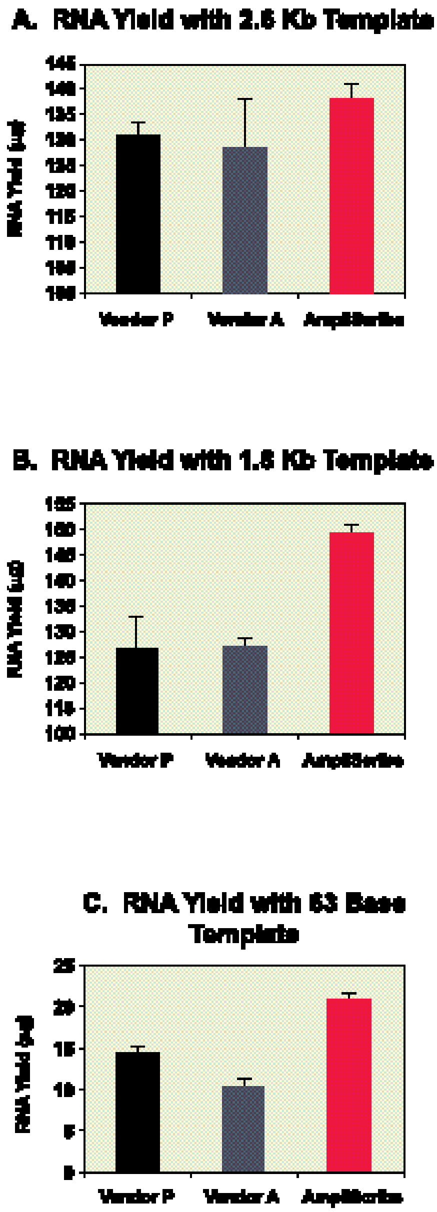

Figure 2. AmpliScribe High Yield Transcription Kits consistently produce the highest possible RNA yields. *Spectrophotometry was used to determine the yield of RNA produced by 2-hour transcription reactions with the AmpliScribe T7 Kit and the kits of two competitors. The data represent the average of duplicate reactions with standard errors. The transcripts produced were A) 2.6 kb, B) 1.8 kb, and C) 63 bases long from different DNA templates.*

The AmpliScribe T7 transcription reaction produced > 20 fold more full-length RNA transcript than the conventional reaction.

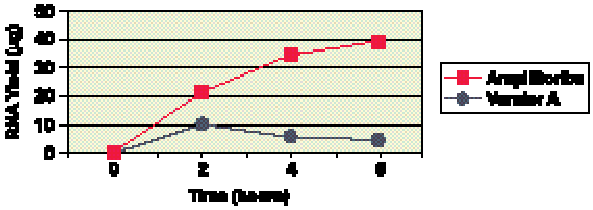

Figure 3. The AmpliScribe T7 High Yield Transcription Kit produces higher yields of short (<300 base) RNA than a transcription kit specifically for short templates. *The yield of a 63 base RNA was compared at 2, 4, and 6 hour time points. The average yield of duplicate reactions is shown.*

AmpliScribe High Yield Transcription Kits consistently produce the highest possible RNA yields

Comparisons were performed with the AmpliScribe T7 High Yield Transcription Kit and high yield transcription kits from two other leading suppliers. Two-hour transcription reactions were performed on three linear DNA templates varying from 63 bases to 2.6 kilobases in length, following the manufacturers' recommended protocols. Figure 2 shows that the AmpliScribe Kit consistently produced more transcripts than either of the other high yield transcription kits (e.g., 150 µg of the 1.8 Kb RNA in 2 hours versus less than 128 µg for vendors A and P).

The AmpliScribe T7 High Yield Transcription Kit produces higher yields of short (<300 bases) RNA

Producing large quantities of a short transcript requires more transcription initiation events than with a standard (e.g., 1 Kb) template. Figure 2C shows that the amount of a short (63 base) transcript produced with the AmpliScribe T7 High Yield Transcription Kit exceeded the amount produced with high yield kits from vendors A and P. Vendor A also markets a T7 transcription kit specifically designed for producing short (<300 base) RNA. In order to determine how the AmpliScribe T7 High Yield Transcription Kit compared with this suppliers "short transcription" kit, comparative reactions were performed.

As shown in Figure 3, the AmpliScribe Kit clearly produced more of the 63 base transcript than the kit designed for short templates. The standard two-hour AmpliScribe transcription reaction produced approximately twice as much RNA as the other system. Lengthening the reaction incubation to 6 hours increased the yield to as much as 4 times the amount using vendor A's specialized kit. Note that although the number of micrograms of short RNA produced is small compared to the yield of > 1 Kb RNA, the number of moles of short RNA produced is greater than for a >1 Kb RNA. Some RNA degradation was also detected with vendor A's kit after 6 hours, while the AmpliScribe Kit continued to accumulate full-length RNA transcripts.

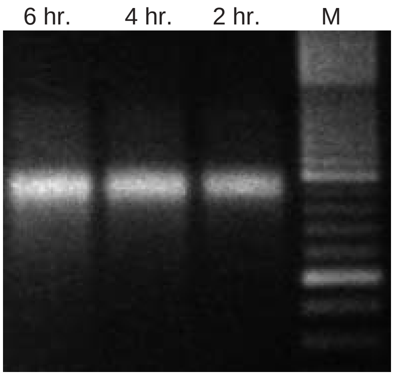

Figure 4. RNA with excellent integrity is produced with the AmpliScribe T7 High Yield Transcription Kit. *Agarose gel electrophoresis of the 1.4 kb transcript produced from 2, 4, and 6 hour transcription reactions with the AmpliScribe T7 Kit. M, DNA ladder.*

Excellent RNA Integrity with the AmpliScribe T7 High Yield Transcription Kit

Ideally *in vitro* transcription reactions should yield full-length, intact RNA transcripts. In order to compare the integrity of RNA transcripts produced, agarose gel electrophoretic analyses were performed on transcription products made using the AmpliScribe T7 Kit and high yield kits from vendors A and P. All of the transcripts produced by the kits tested produced primarily high-quality, full-length, 1.8 Kb RNA transcripts (data not shown). In the time course experiment shown in Figure 4, the integrity of the 1.4 Kb transcript produced by the AmpliScribe T7 Kit is shown 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 possible RNA yields of any *in vitro* transcription system available. The AmpliScribe Kit efficiently uses a broad range of DNA templates, to synthesize reproducibly large amounts of full-length RNA transcript.

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AS2607C5	25 Reactions

SP6 Polymerase Kit

AS2606C5	25 Reactions

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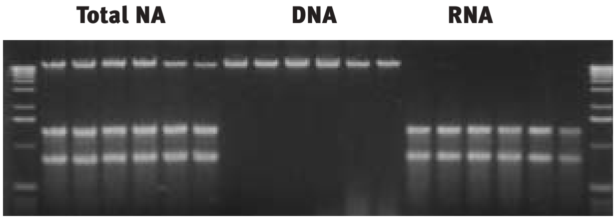


Total nucleic acid, DNA and RNA were purified from a liquid *E. coli* culture sample

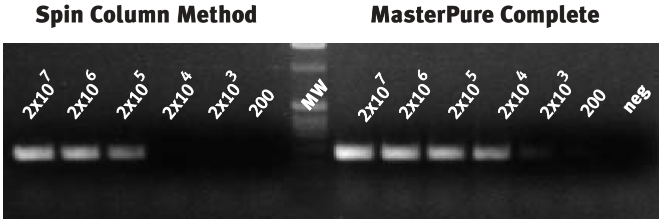
Extensive Sample Range

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	*Examples of Samples Extracted*
Mammalian Systems	**Serum**
HIV	**Plasma**
E. coli	**Whole Blood**
HCV	**Buccal Cells**
B. pertussis	**Liver**
RSV	**Mouse Tail**
Yeast	**Kidney**
M. tuberculosis	**Saliva**
Enterovirus	**Urine**
HPV	**Sputum**
Soy	**Tissue Culture Cell Lines**
Maize	**Cervical Cells**
Insect Tissues	**Paraffin Tissues**

High Sensitivity

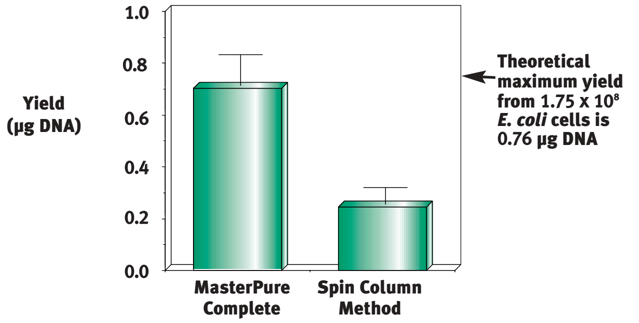
The MasterPure co-precipitant greatly improves sensitivity.



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 x10^8 *E. coli* cells and quantified by fluorometry

Safe and Easy to Use

- **No caustic solvents**
- **No cumbersome columns**

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> *School of Medicine*

> ***"I consider the EZ::TN Transposome one of the most important new tools for genetic manipulation of non-E.coli bacteria that has come along in the last 5 years."***
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The *In Vitro* Insertion Strategy:

One Simple Reaction Generates All DNA Templates for Complete Sequencing of Cloned DNA

An EZ::TN Transposon insertion reaction is a one-step, enzymatic reaction that randomly inserts an EZ::TN Transposon, containing a selectable marker and sequencing primer binding sites into plasmid or cosmid clones. Reaction conditions are optimized to maximize insertion efficiency. A single, 2-hour reaction using 0.2 µg of target DNA generates up to >10^6 independent sequencing templates, each containing a single, randomly inserted EZ::TN Transposon. Choose clones and sequence each bidirectionally using a **single set of sequencing primers** (provided in the kits) homologous to the ends of the inserted EZ::TN Transposon.

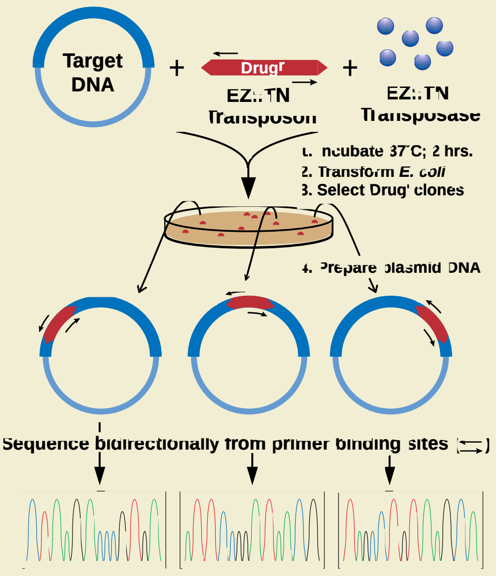
Typical *In Vitro* EZ::TN Transposon Insertion Results

Insertion efficiency = 0.5 - 10%
Insertion clones / µg target DNA = 5 x 10^5 - 2 x 10^8
Insertion clones / reaction = 1 x 10^5 - 1 x 10^7

Completely Sequence Large Clones and Challenging Templates without Subcloning or Primer Walking

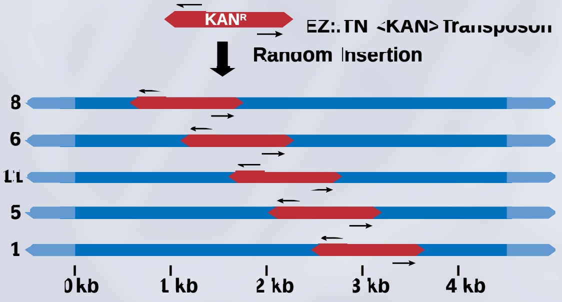
EZ::TN Transposon insertions are highly random even into sequences that are difficult to subclone or to sequence by primer walking. This ensures sequencing priming sites are distributed throughout your clone to facilitate *complete* sequencing of the clone using a single set of sequencing primers. Never make sequencing primers again! Sequence clones by radioactive or non-radioactive methods.

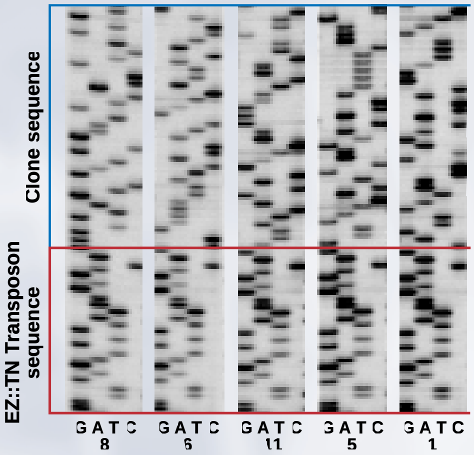
EZ::TN *In Vitro* Insertion Reaction



Sequencing a 3.2 Kb Plasmid Clone Using the EZ::TN <KAN> Insertion Kit

The EZ::TN <KAN> Insertion Kit was used to randomly insert an EZ::TN Transposon containing a kanamycin resistance marker into a 3.2 Kb DNA cloned into pUC19. EZ::TN <KAN> Transposon insertion clones were selected on Kan plates. Transposon insertion efficiency was 5.7%, producing 2.2 x 10^6 Kan^R colonies/µg of DNA. Kan^R clones were randomly chosen and the Transposon insertion sites mapped. Five mapped clones were subsequently used to sequence the entire 3.2 Kb insert using a single set of sequencing primers.





EZ::TN Transposon insertion map and sequence analysis of 5 pUC19 clones. The region outlined in red is a 19 bp EZ::TN Transposon sequence found at the junction between the EZ::TN Transposon and the target DNA of all EZ::TN Transposon insertion clones. This sequence is used as a landmark to distinguish EZ::TN Transposon sequence from target sequence.

Applications of the *In Vitro* Insertion Strategy

- Generate DNA sequencing template.
- Randomly insert a new selectable marker into a cloning vector.
- Introduce insertional mutations into cloned genes and cDNA.
- Insert any DNA sequence of interest into cloned DNA .

Products for The *In Vitro* Insertion Strategy

EZ::TN™ <KAN-2> Insertion Kit Cat. No. EZI982K 10 Reactions
For random insertion of Tn903 kanamycin-resistance marker and primer sites into cloned DNA. Kit contains all reagents and two unlabeled sequencing primers.

EZ::TN™ <TET-1> Insertion Kit Cat. No. EZI921T 10 Reactions
For random insertion of tetracycline-resistance marker and primer sites into cloned DNA. Kit contains all reagents and two unlabeled sequencing primers.

EZ::TN™ <DHFR-1> Insertion Kit Cat. No. EZI912D 10 Reactions
For random insertion of dihydrofolate reductase (trimethoprim selection) and primer sites into cloned DNA. Kit contains all reagents and two unlabeled sequencing primers.

The Transposome Strategy:

Create Gene "Knockouts" In Living Cells and Sequence the Genes without Cloning

An EZ::TN Transposome is the stable complex formed between the EZ::TN Transposase and an EZ::TN Transposon in the absence of Mg^{2+}. EZ::TN Transposomes are so stable that they can be electroporated into living cells. Once activated by Mg^{2+} in the host's intracellular environment, the Transposon component of the Transposome is randomly inserted into the host's genomic DNA[1,2].

References 1. Goryshin, I.Y., et. al. (2000) *Nature Biotechnology* **18**: 97

2. Hoffman, L. and Jendrisak, J. (1999) *EPICENTRE Forum* **6:3** 1

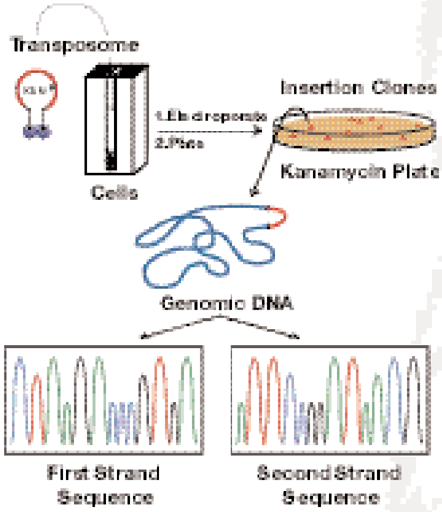
Average Number of Transposon Insertion Clones Generated by Electroporation of EZ::TN <KAN>Tnp Transposome.

Values are Kan^R colonies per µg of EZ::TN Transposon DNA.

E. coli	*Salmonella typhimurium*	*Proteus vulgaris*	*Pseudomonas sp.*
8.4×10^6	5.6×10^5	1.0×10^5	5×10^3

Random insertion of the EZ::TN Transposon creates gene "knockouts" and phenotypic changes. Up to 1000 bases of bacterial genomic DNA surrounding the EZ::TN Transposon insertion site can be sequenced directly using primers homologous to the ends of the inserted EZ::TN Transposon without cloning or shearing of the DNA. EZ::TN Transposome insertions have been demonstrated for a number of species, including *E. coli, Proteus vulgaris, Salmonella typhimurium, Pseudomonas sp., Mycobacteria smegmatis, Saccharomyces cerevisiae* and more are being added regularly.

Direct Sequencing of Insertion Clones



Create Your Own EZ::TN Transposome

An EZ::TN Transposon is any DNA contained between the Mosaic End (ME) EZ::TN Transposase recognition sequences. Create an EZ::TN Transposome from any DNA of interest such as genetic control elements, species-specific selectable marker, cDNA or gene using pMOD™<MCS> Transposon Construction Vector and EZ::TN Transposase.

Preparation of a Custom EZ::TN Transposome

1. Clone your DNA sequence into the multiple cloning site of pMOD<MCS>.
2. Prepare the custom EZ::TN Transposon by Pvu II digestion or by PCR amplification.
3. Purify the EZ::TN Transposon.
4. Incubate the newly prepared EZ::TN Transposon with EZ::TN Transposase in the absence of Mg^{2+} to make the EZ::TN Transposome.

Applications of the Transposome Strategy

- Create gene "knockouts" in living cells.
- Sequence bacterial genomic DNA without cloning.
- Insert any DNA of interest into living cells.
- Introduce a selectable marker into living cells.

Products for the Transposome™ Strategy

EZ::TN™ <KAN-2>Tnp Transposome™ Cat. No. TSM99K2 10 Reactions

Pre-formed Transposome containing the Tn903 kanamycin-resistance marker that is functional in many gram-negative bacteria. The kit includes two unlabeled primers.

EZ::TN™ <DHFR-1>Tnp Transposome™ Cat. No. TSM99D1 10 Reactions

Pre-formed Transposome containing dihydrofolate reductase gene (trimethoprim selection). The kit includes two unlabeled primers.

pMOD™<MCS> Transposon Construction Vector Cat. No. MOD9201 20 µg

Construct a custom EZ::TN Transposon containing any DNA sequence of interest.

EZ::TN™ Transposase Cat. No. TNP92110 10U

Prepare an EZ::TN Transposome by incubating a purified EZ::TN Transposon with EZ::TN Transposase in the absence of magnesium. Protocol provided.

The *In Vitro* Deletion Strategy:

Find Precise Coding Sequences of Functional Genes, Prepare Nested Deletion Sequencing Templates or Map Epitopes

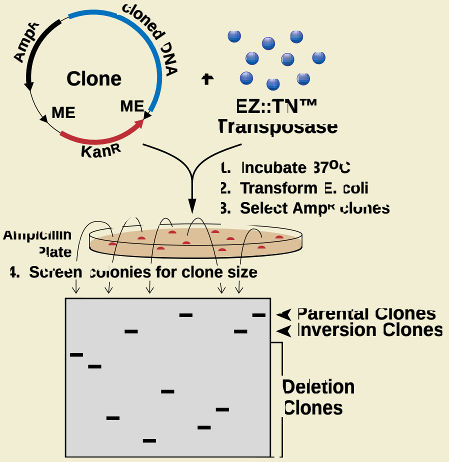
Unidirectional deletion libraries can be now generated in plasmid or cosmid clones easily and reliably by the EZ::TN *in vitro* Deletion reaction. The deletion reaction generates up to 10^6 independent DNA deletion clones per µg DNA in a simple, one-step reaction.

Create Unidirectional DNA Deletions Easier and More Reliably than by Nuclease Digestion

Clone your DNA into one of the specially constructed pPDM™ deletion plasmid vectors or pWEB::TNC™ deletion cosmid vector. Then, incubate 0.2µg DNA from a single clone with EZ::TN Transposase. The ensuing reaction generates a population of independent clones, each containing a random deletion or inversion.[1] The deletion process is highly random to ensure production of a complete population of deletion clones. Transform *E. coli* and select transformants. Choose the desired deletion clones by agarose gel electrophoresis, for DNA sequencing or other application.

References 1. York, D. et. al. (1998) Nuc. Acid. Res. **26**: 1927

Preparing and Selecting DNA Deletion Clones



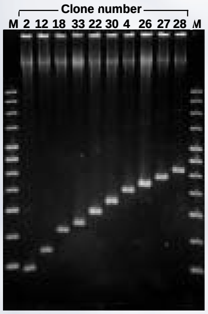
Localizing the C-terminal End of a Cloned Gene Using the *In Vitro* Deletion Strategy

A fragment of bacterial DNA containing a functional phosphatase gene was cloned into pPDM-2 Deletion Plasmid Vector. The deletion reaction was performed, and *E. coli* transformed. Amp^R colonies were chosen and their clones sized by agarose gel electrophoresis. Appropriate sized deletion clones were identified and the phosphatase activity expressed from each was determined. The C-terminus of the gene was localized between deletion clones #4 and #30, and precisely identified by sequencing clone #4.

Phosphatase Activity of 10 Deletion Clones

Clone #	Phosphatase Activity	Clone #	Phosphatase Activity
2	—	30	—
12	—	4	+
18	—	26	+
33	—	27	+
22	—	28	+

Ordering and enzymatic activity profile of ten deletion clones obtained from a phosphatase gene cloned into pPDM-2.



M = size marker

Applications of the *In Vitro* Deletion Strategy

- Generate DNA sequencing template from plasmid or cosmid clones.
- Identify the coding sequence of cloned genes.
- Epitope mapping.
- Create truncated proteins from cloned genes and cDNA.

Products for the *In Vitro* Deletion Strategy

EZ::TN™ Plasmid-Based Deletion Machine Cat. No. DPM9401 10 Reactions
Create a population of deletion clones. The Kit includes pPDM-1 and pPDM-2 Deletion Plasmid Vectors, EZ::TN Transposase, Buffers, unlabeled sequencing primers and a Control Plasmid clone.

pWEB::TNC™ Deletion Cosmid Cloning Kit Cat. No. WEBC931 10 Reactions
Prepare cosmid libraries for subsequent generation of deletions and inversions. Kit includes reagents and enzymes to prepare and clone high MW DNA, pWEB::TNC™ Deletion Cosmid Cloning Vector, MaxPlax™ Lambda Packaging Extracts, E. coli EPI305™ plating cells and control DNAs.

pWEB::TNC™ Deletion Cosmid Transposition Kit Cat. No. WEBC942 10 Reactions
Create a population of deletions in DNA previously cloned into pWEB::TNC Deletion Cosmid Vector. Kit contains EZ::TN Transposase, unlabeled sequencing primer, Buffers and a Control Cosmid clone.

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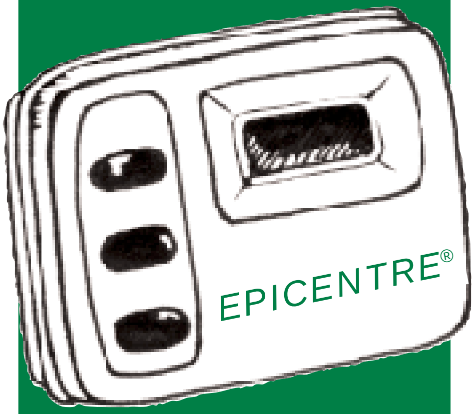

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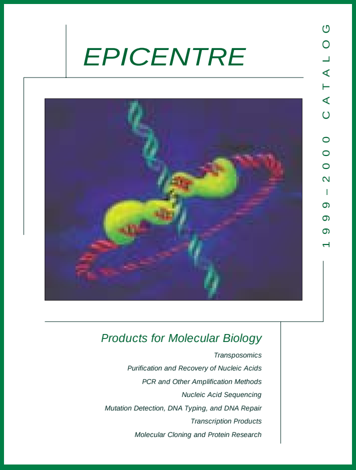

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ailSafe™ PCR: A New System For Reliable and Consistent Amplification of Both Routine and Challenging Templates

Haiying Grunenwald, EPICENTRE Technologies

Introduction

Editor's note: This article is being reprinted due to the popularity of the FailSafe PCR System.

Successful PCR depends on a variety of factors including the quality of the template, choice of enzyme, and primer design, as well as the amplification conditions used. The ideal PCR system would be able to: 1) consistently amplify a wide variety of templates including difficult (e.g., high GC content or secondary structure) and long sequences, 2) amplify with high fidelity, and 3) achieve amplification with little optimization. While enzymes for high fidelity PCR exist, as do methods for difficult and long PCR, no one system exists that addresses all of these factors in an easy-to-use format.

Here, we introduce FailSafe™ PCR. The FailSafe PCR System ensures successful and consistent PCR results with both routine and challenging templates, including long templates (up to approximately 20 kb in length) and templates with high GC content (>80% GC). The FailSafe PCR System consists of two components. The FailSafe PCR Enzyme Mix is a unique enzyme blend containing a 3'→5' proofreading enzyme for high fidelity. PCR products generated by the FailSafe PCR Enzyme Mix are readily cloned with high efficiency in TA or blunt-end vectors. The second component of the FailSafe PCR System is a set of FailSafe PCR PreMixes. The FailSafe PCR PreMixes contain buffer, dNTPs, and various amounts of $MgCl_2$ and FailSafe PCR Enhancer (with betaine).* The user simply adds template, primers, and the FailSafe PCR Enzyme Mix to the FailSafe PCR PreMixes and amplifies. This single-step protocol is used for all templates, no tedious optimization is required. The FailSafe PCR Enhancer included in the PreMixes increases PCR specificity, sensitivity, and consistency.

In this article, we compare the fidelity of the FailSafe PCR System to other commercially available PCR enzymes and enzyme blends. We also demonstrate amplification of long and difficult templates and multiplex PCR using the FailSafe PCR System.

Methods and Results

Fidelity of the FailSafe PCR Enzyme Mix

Applications of PCR such as cloning, expression, mutation analysis, and long amplification require the use of enzymes with low error rates. We compared the fidelity of the FailSafe PCR Enzyme Mix with enzymes and enzyme blends from other suppliers using a PCR-based forward mutation assay. The method is similar to that used by Cline *et al.*[1] and measures PCR fidelity by amplifying the α-complementing portion of the lacZ gene and assessing its sequence integrity using a blue/white colony screening assay. Amplification reactions were performed using the reagents and protocols supplied by each respective manufacturer and fidelity assays were performed side by side. As shown in Figure 1, the fidelity of FailSafe PCR Enzyme Mix was at least three times better than both standard Taq polymerases tested and was equivalent to or better than the other high fidelity enzyme blends tested. Although the FailSafe PCR Enzyme Mix exhibits slightly lower fidelity than has been reported for Pfu DNA polymerase, the FailSafe PCR System is much more robust and is able to achieve more specific and consistent amplification of difficult templates (e.g., with high GC content) and long templates on which Pfu fails.

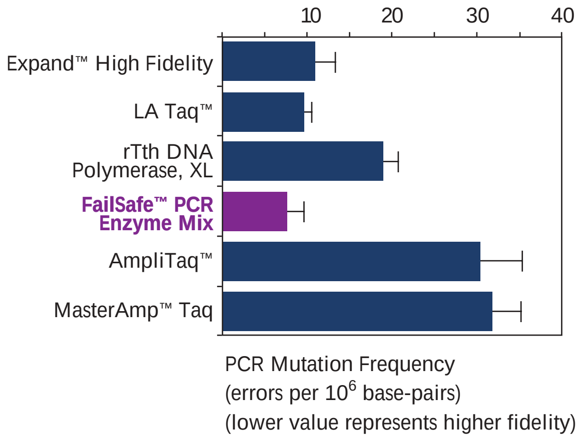

Figure 1. Fidelity comparison of FailSafe PCR Enzyme Mix with other suppliers' enzymes or enzyme blends.

Amplification of long sequences using the FailSafe PCR System

Amplification of long templates often requires tedious optimization of reaction conditions including the addition of PCR additives. To demonstrate that the FailSafe PCR System amplifies long templates, as well as standard templates,

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**Patents issued and pending.*

without tedious optimization of individual reaction components, we amplified lambda, human, and *E. coli* genomic DNA targets ranging in size from 5 kb to 21.5 kb.

For each template/primer pair combination, PCR reactions were performed using the FailSafe PCR PreMixes. Each 50 µl reaction contained 1-500 ng of genomic DNA (depending on the template), 10-50 pmoles of each primer (depending on the template), 2.5 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix (A-L). The lambda template was amplified with the following cycling profile: 94°C for 1 minute, followed by 20 cycles at 98°C for 20 seconds, 56°C for 1 minute (5 kb and 10 kb only), and 68°C for 5 minutes (5 kb), 10 minutes (10 kb), or 20 minutes (20 kb). The *E. coli* genomic DNA was amplified with the following cycling profile: 94°C for 1 minute, followed by 20 cycles (except 30 cycles for the 6 kb template) of 98°C for 20 seconds and 68°C for 5 minutes (6 kb), 10 minutes (10 kb), or 20 minutes (18 kb). The human genomic DNA was amplified with the following cycling profile: 94°C for 1 minute, followed by 14 cycles of 98°C for 20 seconds and 68°C for 20 minutes, and then another 10 cycles where the extension time is lengthened by 15 seconds for each subsequent cycle.

Figure 2 shows the PCR products amplified using the optimal FailSafe PCR PreMix for each template/primer pair combination. All PCR products were amplified in high yield and with high specificity.

Amplification of GC-rich templates using the FailSafe PCR System

As with long PCR, amplification of difficult templates, such as those with high GC content or secondary structure, often requires extensive optimization. To demonstrate that the FailSafe PCR protocol can be used for amplification of GC-rich templates without extensive optimization, we amplified a 250-350 bp region of the human FMR1 gene, which has a GC content of 80-85%.[2] An expansion of a triplet repeat (CGG) region in this gene is associated with fragile X syndrome. Human genomic DNA was purified from blood with the MasterPure™ DNA Purification Kit (Epicentre). PCR reactions were performed using the FailSafe PCR PreMixes. Each 50 µl reaction contained 50 pmoles of each primer, 100 ng of genomic DNA, 1.25 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix (A-L). After an initial denaturation at 94°C for 2 minutes, the Enzyme Mix was added and the reaction was amplified at 94°C for 4 minutes, followed by 30 cycles of 98°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. Using a single set of 12 reactions, FailSafe PCR PreMix J was determined to be optimal for amplification of the high GC region of the FMR1 fragile X gene (Figure 3).

Amplification of this region of the fragile X gene from four separate individuals was performed with FailSafe PCR PreMix J and the FailSafe PCR Enzyme Mix. The results are shown in Figure 4, p. 8. As seen in the Figure, the FailSafe PCR System consistently amplified this 80-85% GC-rich region. Because the number of CGG repeats varies among different individuals, the size of the resulting PCR product varies slightly, ranging between 250 bp and 350 bp.

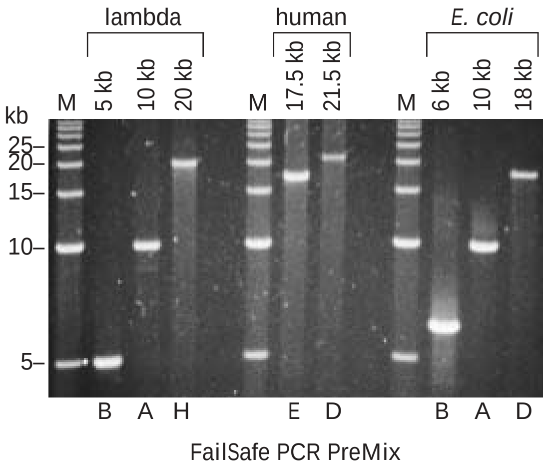

Figure 2. Amplification of various sequence lengths using the FailSafe PCR System. *PCR amplification was performed as described in the text. The results using the optimal FailSafe PCR PreMix determined from each set of reactions is shown. M, 5 kb ladder.*

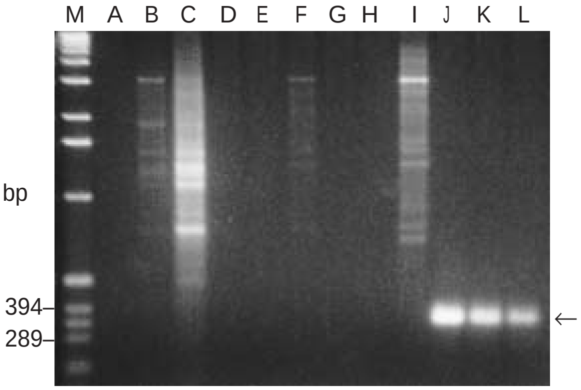

Figure 3. FailSafe PCR amplification of an 80-85% GC-rich region of the human FMR1 gene. *PCR was performed as described in the text. Lanes A-L show the amplification products resulting from PCR using the 12 FailSafe PCR PreMixes. M, molecular weight marker. Optimal amplification was obtained with FailSafe PCR PreMix J.*

continued

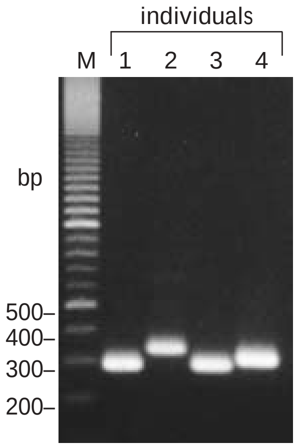

Figure 4. FailSafe PCR amplification of the FMR1 region from four different individuals. *PCR was performed using FailSafe PCR PreMix J as described in the text. M, 100 bp ladder.*

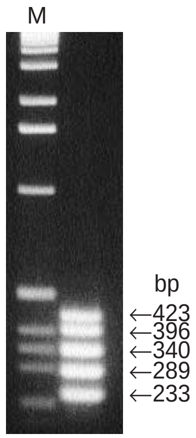

Figure 5. Multiplex PCR amplification of the CFTR gene. *Multiplex PCR was performed as described in the text. The results using FailSafe PCR PreMix C are shown. M, molecular weight marker.*

Multiplex amplification using the FailSafe PCR System

The FailSafe PCR System was also tested for multiplex amplification. Five exons from the cystic fibrosis transmembrane conductance regulator (CFTR) gene[3] were amplified using the FailSafe PCR PreMixes. The 50 µl multiplex PCR reactions contained 25 pmoles of each primer,[2] 500 ng of human genomic DNA, 2.5 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix. After an initial denaturation at 94°C for 2 minutes, the Enzyme Mix was added and the reaction was amplified for 30 cycles at 94°C for 10 seconds, 53°C for 10 seconds, 74°C for 10 seconds, followed by a final extension step at 74°C for 5 minutes.

The sizes of the PCR products from the CFTR gene exons 4, 10, 11, 20, and 21 are 423, 340, 233, 289, and 396 bp respectively.[2] Optimal amplification was achieved using FailSafe PCR PreMix C (Figure 5). The multiplex analysis resulted in the correct size PCR product for each exon. The 5-band multiplex PCR from the CFTR gene was successfully obtained with one set of reactions using the FailSafe PCR System.

Summary

FailSafe PCR ensures successful PCR results for a variety of applications including amplification of templates at least 20 kb in length, amplification of GC-rich templates, and multiplex PCR. The high fidelity of the FailSafe PCR Enzyme Mix is important for making PCR products for cloning, expression, and mutation analysis. The convenient kit format enables easy, single-step amplification of any template without tedious optimization, and no change in protocol is required for different templates. These advantages make the FailSafe PCR System suitable for both routine and challenging PCR amplifications.

References

1. Cline, J. *et al.* (1996) *Nucl. Acids Res.* **24** (18), 3546.
2. Fu, Y.H. *et al.* (1991) *Cell* **67** (6), 1047.
3. Richards, B. *et al.* (1993) *Human Molecular Genetics* **2** (2), 159.

FailSafe™ PCR PreMix Selection Kit

FS99060

Contains the FailSafe™ PCR Enzyme Mix and the 12 FailSafe™ PCR PreMixes.

FailSafe™ PCR System

FS99100	100 Units*
FS99250	250 Units**
FS9901K	1,000 Units*** (4x250 U)

*Includes your choice of one FailSafe™ PCR 2X PreMix (2.5 ml).

**Includes your choice of two FailSafe™ PCR 2X PreMixes (2.5 ml each).

***Includes your choice of eight FailSafe™ PCR 2X PreMixes (2.5 ml each).

For more information, please circle reader service number N724 on the reply card found in the center insert or visit our website at www.epicentre.com/catalog/failsafe.htm

FailSafe™ PCR System

Never fail at *PCR* again.

Three easy steps to solving your worst PCR problems—

Step 1
Perform PCR
with the FailSafe PCR PreMix Selection Kit.

The *FailSafe PCR PreMix Selection Kit* is the starting point for using this system. This kit contains the *FailSafe PCR Enzyme Mix* and all twelve *FailSafe PCR 2X PreMixes* (with dNTPs, *FailSafe PCR Enhancer*, $MgCl_2$ and buffer included in each PreMix).

Your first step is to perform PCR with your template/primer pair using each of the twelve PreMixes. This is quicker than it sounds — you need only add a master mix of the DNA polymerase and your template primer pair to each PreMix. Furthermore, you will save ample time by succeeding at your amplification on your first attempt (avoiding lengthy reworks).

Step 2
Select *the best FailSafe PCR 2X PreMix for your template/primer pair.*

After performing PCR, at least one of the twelve *FailSafe PCR 2X PreMixes* will effectively amplify your template/primer pair. Select the PreMix that provides the best amplification (see Figure).

We highly recommend a separate evaluation with the *FailSafe PCR PreMix Selection Kit* for each unique template/primer pair to assure optimal results.

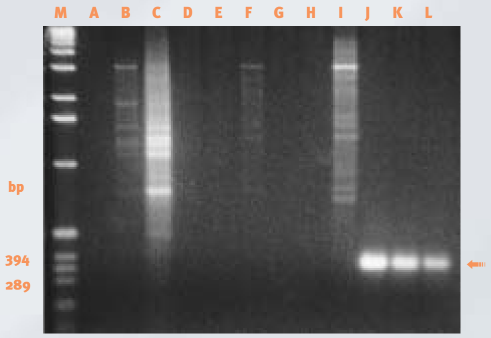

Figure. PCR results for amplification of a GC-rich region of the human fragile X gene. For this template/primer pair, *FailSafe PCR 2X PreMix* J was the optimal PreMix. (Please note that other GC-rich templates may require a different *FailSafe PCR 2X PreMix*.)

Step 3
Use *the selected PreMix with the FailSafe PCR Enzyme Mix for consistent amplification of your template/primer pair.*

Simply use the PreMix you chose in Step 2 (along with the *FailSafe PCR Enzyme Mix*) for continued success amplifying your current template/primer pair. You receive your choice of *FailSafe PCR 2X PreMixes* for free when ordering more enzyme. (PreMixes come with each order of the 100, 250 or 1000 Unit size of the *FailSafe PCR System*, and are also available separately.)

For each new template/primer pair you wish to amplify, simply return to Step 1 and perform PCR with the *FailSafe PCR PreMix Selection Kit*. As before, choose the best PreMix for consistent amplification of each template/primer pair.

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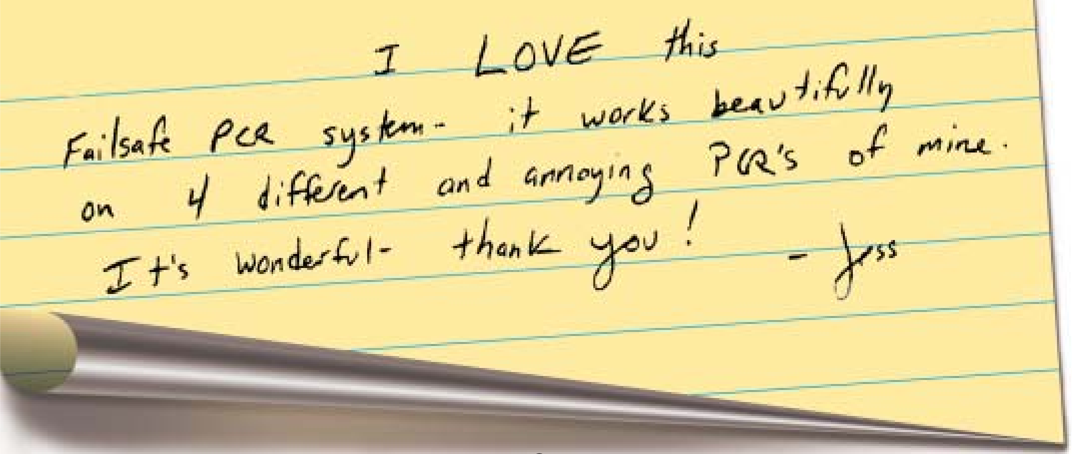
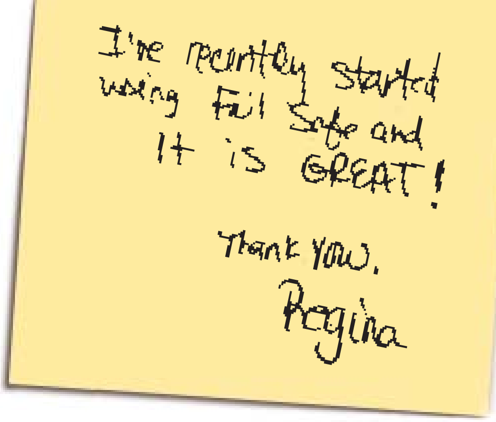
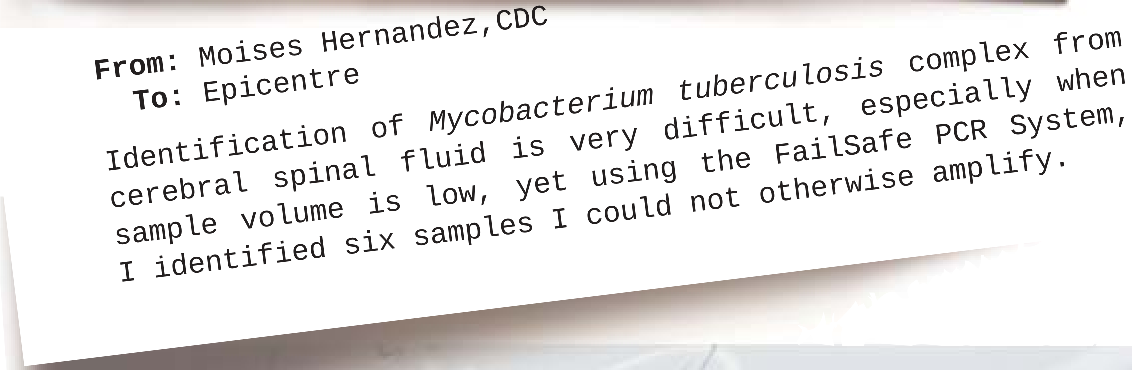
www.epicentre.com


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


I've recently started
using Fail Safe and
It is GREAT!

Thank You.
Regina


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.

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

Regina Hanlon
Fralin Biotechnology Center
Virginia Tech, Blacksburg, Virginia

Moises Hernandez
Centers for Disease Control and Prevention
Atlanta, Georgia

FailSafe™ PCR System Ordering Information

FailSafe™ PCR PreMix Selection Kit

Cat. No.	Size
FS99060	60 Units

Contains FailSafe PCR Enzyme Mix and the 12 FailSafe PCR 2X PreMixes.

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

Individual FailSafe™ PCR 2X PreMixes*

FailSafe PCR 2X PreMix

	A	B	C	D	E	F	G	H	I	J	K	L
Cat. #	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 $MgCl_2$ and FailSafe™ PCR Enhancer (with betaine)*

***Patents issued and pending on FailSafe PCR Enhancer (with betaine).*

EPICENTRE®
800-284-8474 (U.S. only)
www.epicentre.com

EZ::TN™ Transposon Tools

Generate RNA Transcripts From *Any* Region of Your Plasmid or Cosmid Clone

EZ::TN™ <T7/KAN-2> Promoter Insertion Kit

The **EZ::TN™ <T7/KAN-2> Promoter Insertion Kit** randomly inserts a single EZ::TN Transposon containing the phage T7 promoter and a kanamycin resistance marker into your cloned DNA to facilitate transcription of RNA from any region of the clone.

- **Randomly insert a phage T7 transcription promoter into plasmid or cosmid clones.**

  The EZ::TN T7 Promoter Insertion Kit is based on a highly random Tn*5* transposition system. This ensures that the T7 promoter is inserted in a different location in each clone.

- **Generate a population of >10^5 T7 promoter insertion clones.**

  A single 2-hour *in vitro* enzymatic reaction produces >10^5 clones–each with a single, randomly inserted T7 promoter.

- **Transcribe high yields of RNA *in vitro* or *in vivo*.**

  High yields of RNA can be generated *in vitro* using the AmpliScribe™ T7 High Yield Transcription Kit, or *in vivo* after transformation of *E. coli* harboring an inducible T7 RNA polymerase gene (e.g., BL21(DE3)pLysS).

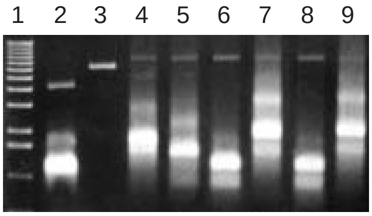


Figure 1. Agarose gel analysis of RNA produced from 6 randomly chosen T7 Promoter insertion clones that were linearized and transcribed using AmpliScribe™ T7 High Yield Transcription Kit. *Lane 1, DNA size marker; Lane 2, 1.4 kb positive control DNA from the AmpliScribe™ Kit; Lane 3, negative control pUC19 DNA without T7 promoter; lanes 4-9, T7 promoter insertion clones.*

EZ::TN™ <T7/KAN-2> Promoter Insertion Kit

EZI03T7 10 Reactions

Kit contains EZ::TN <T7/KAN-2> Transposon, EZ::TN Transposase, Reaction Buffer, Stop Buffer, two unlabeled Sequencing Primers, Control DNA and Water.

Generate Random In-Frame 19-Codon Insertions into Genomic or cDNA Clones

EZ::TN™ In-Frame Linker Insertion Kit

The **EZ::TN™ In-Frame Linker Insertion Kit** is a transposon-based linker insertion system that facilitates rapid and easy production of random 19-codon (57-nucleotide) linker insertions into cloned DNA *in vitro.* Use the Kit to identify permissive and non-permissive insertion sites in proteins, to map domains and epitopes or to disrupt genetic control regions.

- **Make random 19-codon insertions into all three reading frames of your cloned DNA.**

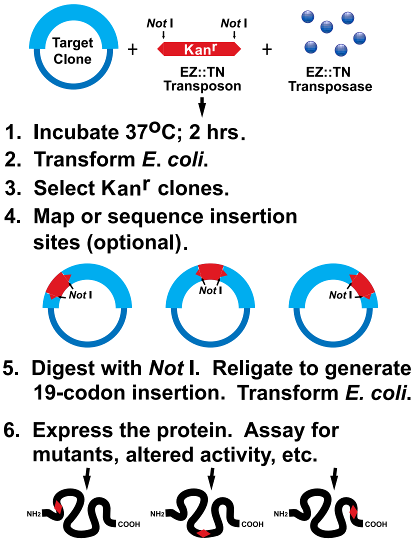
  The EZ::TN <*Not* I/Kan-3> Transposon was designed to preserve all three reading frames in the final linker insertion construct. The result is that your protein is unchanged except for the random insertion of 19 amino acids.

- **Generate a complete population of in-frame insertion clones.**

  The EZ::TN™ In-Frame Linker Insertion Kit is based on the highly random Tn*5* transposition system. A single *in vitro* reaction generates >10^4 insertion clones–each containing a different, single, random transposon insertion.

- **More versatile than linker scanning mutagenesis.**

  Because the insertion reaction is random, the 19-codon insertions are not limited to pre-existing restriction endonuclease sites in the cloned DNA as is the case with traditional linker scanning mutagenesis.



1. **Incubate 37°C; 2 hrs.**
2. **Transform *E. coli*.**
3. **Select Kan^r clones.**
4. **Map or sequence insertion sites (optional).**
5. **Digest with *Not* I. Religate to generate 19-codon insertion. Transform *E. coli*.**
6. **Express the protein. Assay for mutants, altered activity, etc.**

Figure 1. Process for making random, in-frame 19-codon insertions.

EZ::TN™ In-Frame Linker Insertion Kit

EZI04KN 10 Reactions

Kit includes EZ::TN <*Not* I/KAN-3> Transposon, EZ::TN Transposase, Reaction Buffer, Stop Buffer, two unlabeled Sequencing Primers, Control DNA and Water.

Recover Intact DNA Up to >2 Mb in Length

GELase™ Agarose Gel-Digesting Preparation

GELase Agarose Gel-Digesting Preparation is a unique enzyme solution developed at EPICENTRE for quantitative recovery of intact DNA from low melting point (LMP) agarose gels following electrophoresis in TAE, TBE, MOPS, or phosphate buffers. Excised gel bands can be digested in the above-mentioned buffers, or for higher activity, GELase Buffer may be added to or exchanged with those buffers.

Applications:

Recover high molecular weight nucleic acids from low melting point (LMP) agarose gels for use in:

- Preparation of YAC, BAC, cosmid, and plasmid vectors
- Subcloning from YACs, BACs, and cosmids
- Microinjection
- Size selection of genomic DNA for subsequent cloning
- Restriction mapping
- PCR

Benefits:

- Gentle procedure - purify multi-megabase DNA that is intact and biologically active
- Recoveries of DNA consistently approach 100%
- Protocol requires minimal hands-on time
- High activity - GELase Preparation is more active than other gel-digesting enzymes*
- Cost effective - GELase is priced well below spin column or other gel-digesting methods*

GELase™ Agarose Gel-Digesting Preparation

1 U/ul

G09050	50 U
G09100	100 U
G09200	200 U

Includes GELase™ 50X Reaction Buffer

**One unit of GELase Preparation is equivalent to approximately three units of other gel-digesting enzymes.*

Completely Remove Contaminating Chromosomal DNA from your Plasmid, Cosmid, and BAC Vector Preparations

Plasmid-Safe™ ATP-Dependent DNase

Preparations of plasmid, cosmid, and BAC vector preparations are frequently contaminated with fragments of bacterial genomic DNA generated during alkaline lysis. Other purification options, such as spin-columns or even CsCl centrifugation, do not effectively remove these contaminants. Contaminating DNA fragments left behind by these methods ultimately become ligated into your cloning vector, resulting in false positives and high backgrounds.

Plasmid-Safe ATP-Dependent DNase digests linear double-stranded DNA to deoxynucleotides at slightly alkaline pH and, with lower efficiency, closed-circular and linear single-stranded DNA. The enzyme has no activity on nicked or closed-circular dsDNA or supercoiled DNA. Therefore, Plasmid-Safe is ideal as the final purification step for plasmid, cosmid, and BAC vectors (up to 8 kb).

Benefits

- Minimizes the possibility of cloning or sequencing contaminating chromosomal DNA from your plasmid, cosmid, or BAC vector (up to 8 kb).
- Fast and easy protocol with minimal handling time.
- Complete protocols for miniprep, midiprep, and maxiprep plasmid, cosmid, and BAC DNA purifications using Plasmid-Safe DNase are provided.
- Plasmid-Safe may also have potential uses purifying DNA vaccines.

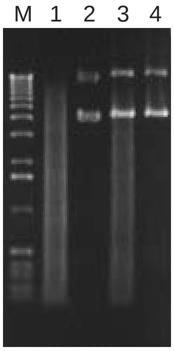


Figure 1. Use of Plasmid-Safe ATP-Dependent DNase to remove contaminating linear DNA from plasmids. *Lane 1, 3 µg of Sma I-digested bacterial chromosomal DNA; Lane 2, 500 ng of uncut plasmid DNA; Lane 3, mixture of 3 µg of digested bacterial chromosomal DNA and 500 ng of uncut plasmid before Plasmid-Safe DNase treatment; Lane 4, mixture of chromosomal DNA and plasmid DNA after Plasmid-Safe DNase treatment (incubated with Plasmid-Safe DNase for 30 minutes at 37°C); M, kb ladder.*

Plasmid-Safe™ ATP-Dependent DNase

E3101K	10 U/µl	1,000 U
E3105K	10 U/µl	5,000 U
E3110K	10 U/µl	10,000 U

Includes Plasmid-Safe™ 10X Reaction Buffer and 25 mM ATP Solution.

PRODUCT REVIEW

The Highest Efficiency Packaging of DNA for Genomic and cDNA Library Construction

Using MaxPlax™ Lambda Packaging Extracts

Producing complete primary genomic or cDNA libraries requires use of lambda packaging extracts with the highest possible efficiency. EPICENTRE's MaxPlax™ Lambda Packaging Extracts provide the maximum *in vitro* packaging efficiencies of both methylated and unmethylated DNA for lambda or cosmid library production.

MaxPlax™ Lambda Packaging Extracts:

- **Have high packaging efficiency.** 1-3 X 10^9 pfu/μg of DNA using lambda DNA and >3 x 10^7 cfu/μg for cosmid clones (Table 1).
- **Package methylated DNA as efficiently as unmethylated DNA.** Successful construction of lambda or cosmid genomic libraries from higher eukaryotes (i.e. highly methylated DNA) requires use of lambda packaging extracts devoid of restriction activities (e.g. *Mcr* and *Mrr*) that specifically digest the methylated DNA. EPICENTRE's MaxPlax™ Lambda Packaging Extracts are derived from *E. coli* BHB2688 and a restriction-minus K-12 strain, NM759 to maximize the size of your genomic library. MaxPlax™ Extracts package highly methylated DNA as efficiently as unmethylated DNA (Table 1).
- **Are individually dispensed extracts.** Just add the substrate DNA (in a volume of 1-10 μl) to the packaging reaction.
- **Are available now.** Don't let product backorders slow your research. MaxPlax™ Lambda Packaging Extracts are never on backorder.
- **Are the best value in lambda and cosmid packaging.** Compare our efficiency. Compare our price. You'll agree that MaxPlax™ Lambda Packaging Extracts are the best value in lambda and cosmid packaging.

Table 1. Packaging of cosmid clones containing methylated and unmethylated T7 DNA using MaxPlax™ Lambda Packaging Extracts.

Cosmid	Plaques per plate	Pfu/μg DNA
T7 DNA	140	3.5 x 10^7
Methylated T7 DNA	155	3.9 x 10^7

MaxPlax™ Lambda Packaging Extract

The best value in lambda and cosmid packaging

MP5105	5 Extracts
MP5110	10 Extracts
MP5120	20 Extracts

Each contains Extracts (individually dispensed), Control Lambda DNA, Control E. coli host cells.

Extracts are available in bulk quantity. Please inquire.

See the center insert for more information about ***pWEB::TNC™ Deletion Cosmid Cloning Kit;*** *a complete kit, including MaxPlax™ Extracts, for cosmid cloning and library production.*

EPICENTRE TECHNOLOGIES

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E-mail: techhelp@epicentre.com

Outside the USA: contact your local Epicentre Distributor.

EPICENTRE FORUM

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