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New Tools & Techniques for Molecular Biology

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Volume 7 Number 3 2000

## High Throughput Screening of Single Nucleotide Polymorphisms (SNPs) Using the BESS-T&G™ Base Reader Kit and the ABI PRISM® 3700 DNA Sequencer

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University of Nebraska Medical Center, Omaha, NE

### Introduction

With the near completion of the human genome sequencing project, there is increasing interest in identifying single nucleotide polymorphisms (SNPs) that can be linked to phenotypes of clinical significance. The ability to screen large numbers of samples is essential both for SNP discovery and for screening to establish linkage relationships.

In the past, screening for mutations involved the use of rudimentary techniques, such as single strand

conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and other inefficient processes. Such inaccurate and labor-intensive techniques are not viable for handling large numbers of samples due to reproducibility, sensitivity and data management issues. Thus, a simple, accurate, and robust SNP detection technique, coupled with a rapid analysis system, is needed for discovery and scoring of specific SNPs in a high-throughput screening environment.

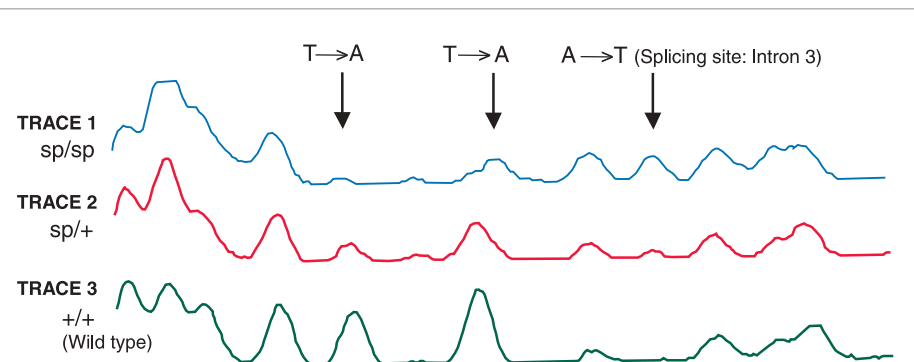
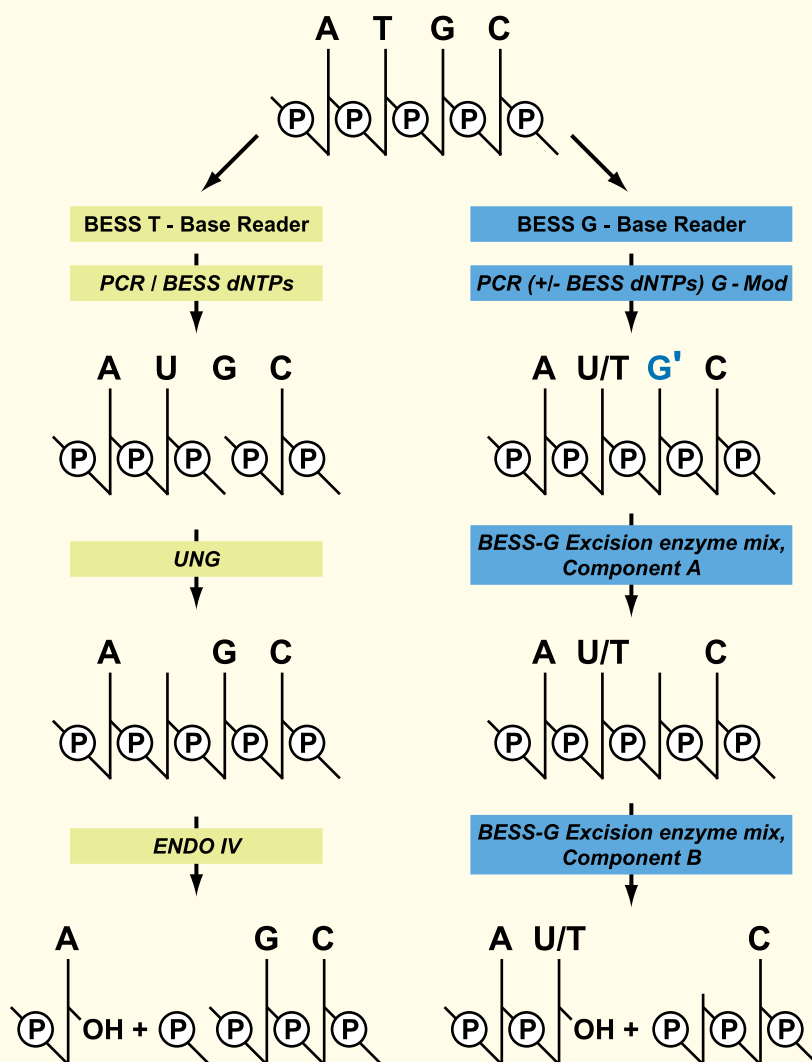


Figure 1. Sample electropherograms showing the presence of mutations in the Pax-3 gene in mice obtained using the BESS-T & G™ Base Reader Kit and the ABI Prism® 3700 DNA Sequencer. Trace 1 shows BESS-T data for the homozygous mutant, with arrows indicating the locations of mutations of interest. Trace 2 is for the heterozygous condition, which shows a smaller T base peak at the splicing site for intron 3. Trace 3 shows the wild-type homozygous condition, with strong T peaks at arrows 1 and 2 and no detectable T peak at the intron 3 splice junction.

continued

## How Does BESS Work?

Base Excision Sequence Scanning (BESS)\* is the easiest, fastest, least expensive, most sensitive and most accurate method to generate T- and/or G-lane sequence data from either one or both strands of a PCR product made using labeled primers— without dideoxy sequencing. Unlike dideoxy sequencing, the sequence-determining nucleotides for BESS (dUMP for T or a modified dGMP for G) are not terminators, permitting the full length of PCR product to be generated. However, BESS generates fragments similar to those obtained in dideoxy sequencing reactions using a brief (15-30 minutes) enzymatic treatment of the PCR product. The BESS reaction products can be analyzed using a capillary or gel just like dideoxy sequencing reactions. The mechanisms of BESS reactions are presented below.



\* U.S. Patent No. 6,048,696 & other patents pending.

The ABI PRISM® 3700 DNA sequencer (PE Biosystems, Foster City, CA) is a new sequencing instrument that is capable of analyzing up to 96 samples simultaneously. This device uses capillary electrophoresis to resolve DNA fragments and uses Windows NT-based versions of the GeneScan™ and Genotyper™ fragment analysis software.

Base Excision Sequence Scanning (BESS) has been used previously to identify mutations that correlate to a number of medical conditions.<sup>1,2,3</sup> These reports demonstrated that BESS products were readily analyzed using ABI 310 and other sequencers<sup>2,3</sup> and thus, this technique was potentially adaptable to high-throughput mutation scanning applications.

In this report, we describe the application and optimization of BESS on the ABI 3700 sequencer. We selected the BESS-T&G™ Base Reader Kit for analyzing SNPs on this instrument because of its ability to locate and specifically identify all base sequence changes in a PCR-amplified DNA fragment and the ease of optimizing the BESS technique for use on a multiple-channel DNA sequence analyzer (Figure 1).

### Methods

To test the BESS-T&G Base Reader technology in a high throughput application, we looked at a specific A→T transversion in intron 3 of the Pax-3 gene of the Splotch mouse.<sup>4</sup> Genomic DNA was isolated from Splotch mouse cells and an approximately 244 bp fragment of the Pax-3 gene was amplified using the BESS PCR Optimization Kit (BESS 2X PreMix B, EPICENTRE), and 1.25 U of Taq Polymerase. Pax-3 amplification reactions were performed in a 25 µl reaction volume, using the following primers: UP primer: 5' FAM-TAG GGA GAG GGT TGA GTA CG-3';

DN primer: 5'-CTC GCT CAC TCA GGA TGC C-3'. Thermal cycling conditions were as follows: denaturation for 5 minutes at 95°C, then 35 cycles at 95°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute. A final 10-minute extension step at 72°C completed the process. BESS-T&G Base Reader reactions were performed as described in the protocol. After completing the excision/cleavage reaction, 1 µl of the reaction product was added directly without further purification to 19 µl deionized formamide. Finally, 0.8 µl of GeneScan™ 400HD Internal Lane Size Standard (PE Biosystems) was added, and 20.8 µl of each sample was applied to the ABI PRISM® 3700 DNA Sequencer in 96-well plates. Resolution of BESS-T or BESS-G excision products was accomplished by creating a run module for each 96-well plate using a run-time of 6000 seconds, a sheath flow of 12000, run temperature 50°C, cuvette temperature of 40°C, full analysis range, peak threshold of 50, and Local southern size calling. The polymer used in the capillaries was Performance Optimized Polymer (POP) 6.

## Results and Discussion

The Splotch mouse contains a mutation in intron 3 of the Pax-3 gene that is a valuable model to study defects in neural tube closure.<sup>4</sup> Pax genes encode a series of DNA-binding transcription factors whose expression has been shown to occur in distinct regions of developing mouse embryos. Human syndromes associated with defects in Pax-3 are Waardenburg syndromes type 1 and 2, which include various defects, e.g., deafness, pigmentary deficiency or dystopia canthorum. The mutation in intron 3 results in aberrant splicing of the Pax-3 transcript, resulting in production of a non-functional protein. The BESS-T&G Base Reader readily located and

identified the mutation in intron 3 of the Pax-3 gene. In addition, heterozygotes were detected by observing a decrease in peak height of the affected base in the forward strand of the PCR product, which was verified by detecting a T→A transition opposite the mutated base on the reverse strand. A partial electropherogram of the A→T transversion from intron 3 of the Pax-3 gene is shown in Figure 1. While strong signal intensities were achieved in all 96 samples, the laser detection system showed signal variability depending on the capillary being analyzed. A comprehensive review of the data generated for the Pax-3 gene using the BESS-T&G Base Reader and ABI 3700 will be presented elsewhere.

The BESS system was previously demonstrated to work well with the ABI PRISM® 310 automated DNA sequencer, which is able to sequentially process up to 48 samples. The ABI 3700 is able to process 96 samples concurrently using GeneScan™ software in a Windows NT operating environment, thus enabling the use of BESS in a high-throughput mode.

## Conclusion

The BESS-T&G Base Reader Kit, used in conjunction with the ABI 3700 automated DNA sequencer, is an excellent tool for high throughput mutation discovery or scoring of SNPs in PCR-amplified DNA. The strong signal intensities generated using samples obtained from the simple BESS protocol permits generation of SNP or mutation data on PCR products of at least 600 bases.

## References

- Hawkins, GA, et al. (1999) *Electrophoresis* **20**, 1171.
- Brieger, A, et al. (1999) *Clin. Chem.* **45** (9), 1564.
- Brieger, J; et al. (1999) *Clin. Genet.* **56**, 210.
- Epstein, DJ, et al. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 532.

### BESS-T&G™ Base Reader Kit (also called BESS MutaScan™)

BTG8520  
40 BESS-T™ Rxns & 40 BESS-G™ Rxns  
BTG85100  
200 BESS-T™ Rxns & 200 BESS-G™ Rxns

*Includes BESS dNTP Mix, BESS-G™ Modification Reagent, BESS-G™ Excision Enzyme Mix, BESS-T™ Excision Enzyme Mix, BESS Excision Enzyme Buffer, Stop/Loading Buffer, Control Primer (forward), Control Primer (reverse), and Control Template.*

*Note: PCR enzyme not included*

### BESS-T™ Base Reader Kit (also called BESS T-Scan™)

BN712100  
200 BESS-T™ Rxns

### BESS-G™ Base Reader Kit (also called BESS G-Tracker™)

GT85100  
200 BESS-G™ Rxns

### BESS PCR Optimization Kit

BP8020 20 Templates

*Contains 12 BESS 2X PCR PreMixes (A-L), 0.5 ml each.*

*Each BESS 2X PCR PreMix contains a buffered salt solution with all four dNTPs and a limiting amount of dUTP, a range of magnesium ions and MasterAmp™ PCR Enhancer (with betaine)\*.*

### MasterAmp™ Taq DNA Polymerase

Q82250N 5 U/µl 250 U

Enzyme only.

*\* Patents issued and pending.*

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

# Protein Modification Using the New EZ::TN™ In-Frame Linker Insertion Kit

Les M. Hoffman and Kathryn B. Loomis, EPICENTRE

## Introduction

Conserted genome sequencing projects have elucidated the coding sequence of a myriad of genes from many different organisms. An important next step is to determine the structural and functional relationships of the proteins encoded by these genes. Recently, EPICENTRE introduced the EZ::TN™ In-Frame Linker Insertion Kit to speed up and simplify protein modification and facilitate determination of key regulatory, binding and catalytic regions of cloned proteins.

The EZ::TN In-Frame Linker Insertion Kit was designed to randomly insert a 19 codon (57 nucleotide) “linker” into cloned genes. The kit features the EZ::TN <Not I/KAN-3> Transposon which contains a kanamycin-resistance (Kan<sup>R</sup>) marker flanked by Not I restriction sites. The process for introducing random 19-codon insertions into cloned DNA is shown in Figure 1. A simple *in vitro* enzymatic reaction randomly inserts a single EZ::TN <Not I/KAN-3> Transposon into each clone and produces thousands of Kan<sup>R</sup> insertion mutants. Insertion clones for further analysis can be identified by gene functional analysis or by restriction or DNA sequencing analysis of the transposon insertion site. Once clones are chosen, the Kan<sup>R</sup> gene is excised from the transposon by Not I digestion (Figure 2). Each Not I digested clone is then

religated and retransformed into *E. coli*. Since the transposon mosaic ends have been modified to eliminate translational stops, the resulting clones each contain a random 19-codon insertion that can be read in all three reading frames. And, the protein will retain its original amino acid sequence on both sides of the insertion site.

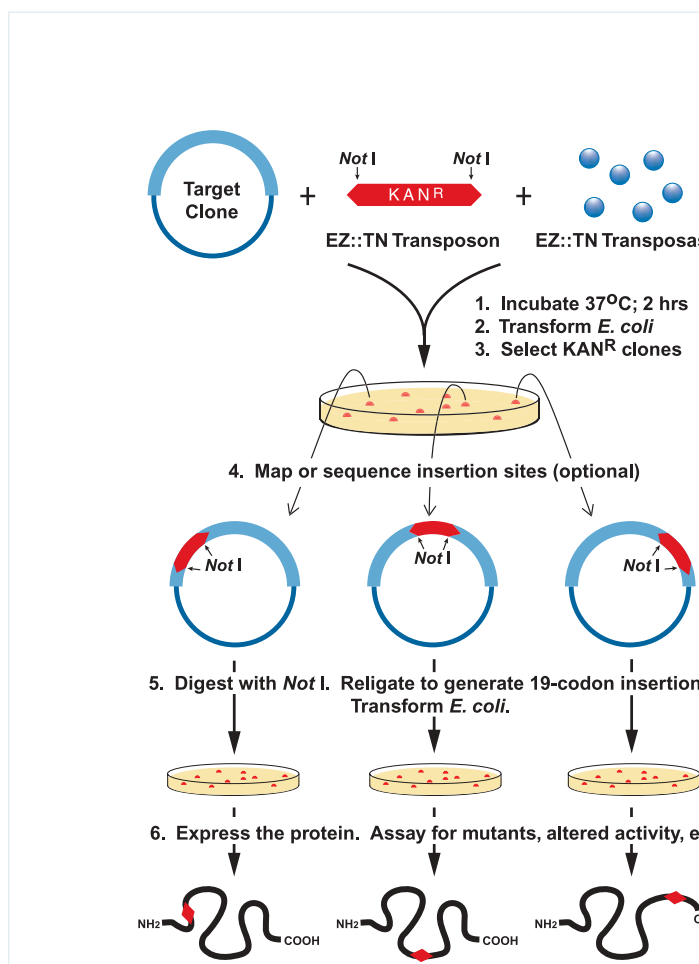
In this report we demonstrate the utility of the EZ::TN™ In-Frame Linker Insertion Kit by generating and analyzing the effects of random 19-codon insertions into the tetracycline/H<sup>+</sup> antiporter (tetracycline resistance, Tet<sup>R</sup>) and β-lactamase (ampicillin resistance, Amp<sup>R</sup>) genes of pBR322.

## Materials and Methods

### *In vitro* insertion reactions with EZ::TN <Not I/KAN-3> Transposon

The *in vitro* insertion reaction utilizes 0.2 μg of target DNA (pBR322 in this example), an equimolar amount of EZ::TN <Not I/KAN-3> Transposon, 1 U of EZ::TN Transposase, and a Mg<sup>2+</sup>-containing buffer. The reaction is performed for 2 hours at 37°C as described in the EZ::TN™ In-Frame Linker Insertion Kit literature.

One microliter of reaction mix was electroporated into 50 μl of TransformMax™ EC100 electrocompetent *E. coli* (EPICENTRE). After overnight selection on kanamycin (50 μg/ml) randomly chosen Kan<sup>R</sup> colonies were replica plated onto both ampicillin- and tetracycline-containing plates. Colonies with either a Amp<sup>R</sup>/Tet<sup>S</sup> or Amp<sup>S</sup>/Tet<sup>R</sup> phenotype were selected for further analysis.



**Figure 1.** The EZ::TN In-Frame Linker Insertion Kit is based on the highly random Tn5 transposition system. A single *in vitro* reaction generates thousands of insertion clones- each containing a different transposon insertion.

### Mapping and sequencing the EZ::TN Transposon insertion sites

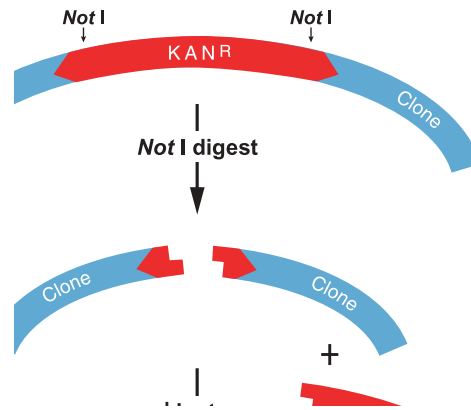
The approximate EZ::TN <Not I/KAN-3> Transposon insertion site of each Amp<sup>R</sup>/Tet<sup>S</sup> clone or Amp<sup>S</sup>/Tet<sup>R</sup> clone was mapped by restriction endonuclease digestion using *Eco* RI and *Xho* I or *Eco* RI and *Xba* I, respectively. The precise transposon insertion site was determined by DNA sequencing using the primers provided in the EZ::TN In-Frame Linker Insertion Kit, which are homologous to the ends of the EZ::TN <Not I/KAN-3> Transposon.

### Removal of the kanamycin resistance cassette from insertion clones

Five hundred nanograms of each pBR322 insertion clone was digested to completion with *Not* I and reaction products were electrophoresed on a 1% low melting point agarose gel. The larger fragment (i.e., plasmid minus 1.1 kb Kan<sup>R</sup> gene) was cut from the gel, melted at 70°C for 10 minutes, and 10 microliters were placed in a microcentrifuge tube prewarmed to 42°C. The vector *Not* I ends were then religated in-gel using Fast-Link™ DNA Ligase (EPICENTRE). After heat inactivation at 70°C for 15 minutes, one microliter was used to electroporate TransforMax™ EC100 electrocompetent *E. coli* cells as described above. Transformants were plated on tetracycline or ampicillin plates as appropriate for growth and then the target gene was screened for restored activity.

### Results and Discussion

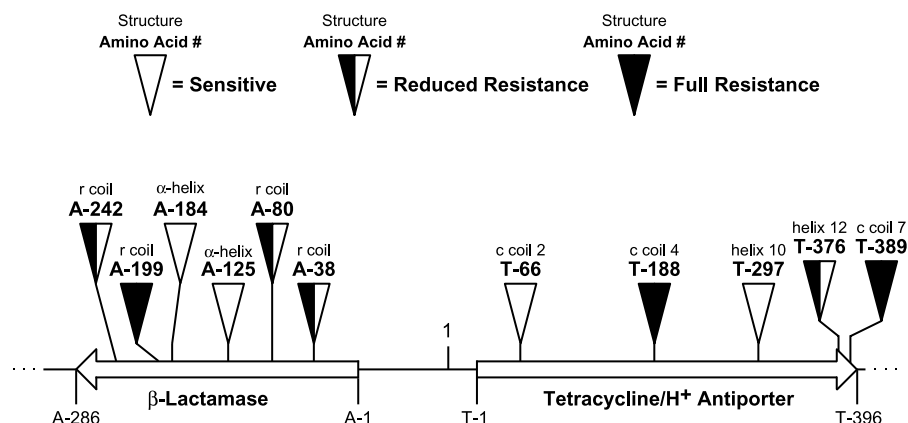
The EZ::TN In-Frame Linker Insertion Kit provides several advantages for generating protein modifications. The *in vitro* EZ::TN <Not I/KAN-3> Transposon insertion reaction is very efficient (greater than 10<sup>6</sup> clones per reaction) and minimizes multiple insertion events. In addition, the general location of each transposon insertion was readily determined by restriction data and more precisely defined



**Figure 2.** The EZ::TN <Not I/KAN-3> Transposon contains a kanamycin resistance gene flanked by *Not* I restriction sites. A 19-codon insertion that can be read in all three reading frames is generated following *Not* I digestion and religation.

by sequence analysis using the primers provided in the kit. Finally, as shown in Figure 3, insertions appeared to be random throughout each antibiotic resistance gene.

Excision of the kanamycin resistance gene from the EZ::TN <Not I/KAN-3> Transposon by *Not* I digestion and religation generated a 57-bp (19-codon) insertion in the gene, which could be read in all three reading frames (Figure 4). Since EZ::TN Transposons are hyperactive



**Figure 3.** Random, 19-codon insertions into the tet/H<sup>+</sup> antiporter and  $\beta$ -lactamase genes of pBR322 resulted in a range of antibiotic resistance levels. *r coil*, random coil; *c coil*, cytoplasmic coil.

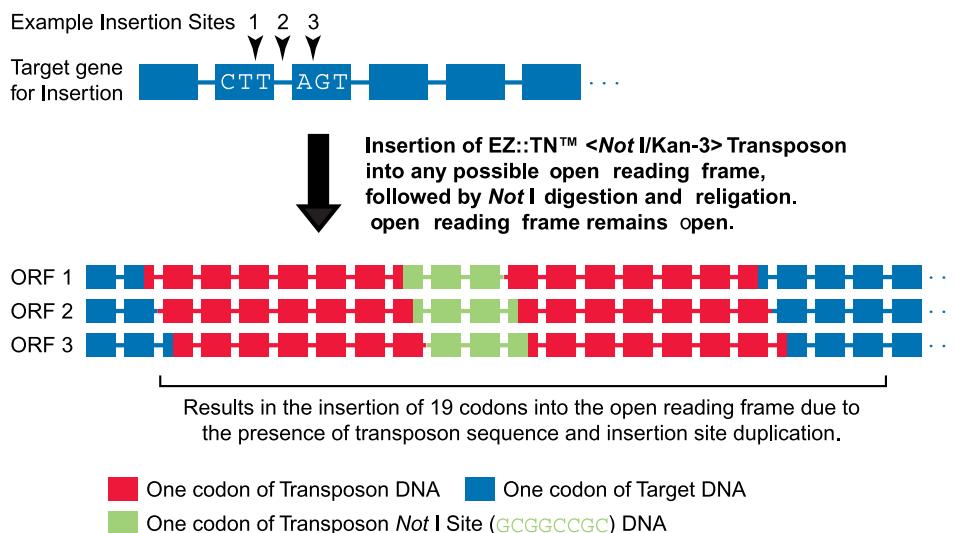


Figure 4. Excision of the kanamycin resistance gene from the EZ::TN <Not I/KAN-3> Transposon generates a 57 bp (19 codon) insertion. Insertions in each of the three reading frames remains open (i.e., does not contain a nonsense (stop) codon) and varies in composition. The amino acid sequence on both sides of the insertion remains unaltered.

forms of Tn5 transposons, 9 of the 57 bp are the result of a 9-bp duplication of target DNA that occurs during the insertion process.<sup>1</sup> However, the number of duplicated amino acid residues flanking transposon sequences can be two or three, depending upon the specific codon into which the transposon inserts.

The tetracycline/H<sup>+</sup> antiporter (Tet<sup>R</sup>) and β-lactamase (Amp<sup>R</sup>) genes of pBR322 were chosen to demonstrate the utility of the EZ::TN In-Frame Linker Insertion Kit because these two antibiotic gene targets are readily detectable and the products of these genes have distinct cellular locations and modes of action.<sup>2,3</sup> At this point, it is difficult to theoretically predict how the position and composition of a specific 19-amino acid insertion will affect protein activity. Nevertheless, some conclusions could be made from the data.

For example, Figure 3 shows the insertion site in 5 randomly chosen Amp<sup>R</sup>/Tet<sup>S</sup> clones and the effect of the 57-nucleotide insertion on each clone's ability to confer resistance to tetracycline. Because the Tet<sup>R</sup> gene is a membrane protein with twelve putative transmembrane segments, insertions within those regions affect its topology and function as a tetracycline antiporter.<sup>2</sup> Surprisingly, in the majority of cases (clones T-188, T-376, T-389) the 19- amino acid insertions had little to no effect on the ability of the clones to grow on tetracycline.

Also, as expected, the 19-amino acid fusions in β-lactamase had varying effects. For example, the insertion in clone A-80 interrupted a random coil domain and reduced drug resistance. Similarly, the 19-amino acid insertion in clone A-184 resulted in loss of activity presumably because a crucial alpha-helical domain was interrupted. The insertion in clone A-199, however, did not appreciably affect the ampicillin resistance of bacteria harboring the plasmid and indicated a permissive region of the protein.

## Conclusion

The EZ::TN In-Frame Linker Insertion Kit is a fast and efficient method for randomly inserting 19-amino acid peptides in-frame into the proteins encoded by a cloned DNA for a variety of applications. These include: 1) structure and function analysis of the protein encoded by a cloned DNA; 2) identifying permissive insertion sites for protein engineering; and 3) epitope or domain mapping of proteins.

Recently, Biery et al.<sup>4</sup> described a transposon-based linker insertion method using a modified Tn7 transposon system. However, as opposed to the three open reading frames provided by the EZ::TN system described here, the Tn7 system contains a nonsense (stop) codon in one of three reading frames on each strand.

## References

- Goryshin, I. Y and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 7367.
- Allard, J.D. and Bertrand, K.P. (1992) *J. Biol. Chem.* **267**, 17809.
- Strynadka, N.C.J. et al. (1992) *Nature* **359**, 700.
- Biery, M.C. et al. (2000) *Nucleic Acids Res.* **28**, 1067.

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

EZ104KN 10 Reactions  
Kit includes EZ::TN <Not I/KAN-3> Transposon, EZ::TN Transposase, Reaction Buffer, Stop Solution, two unlabeled Sequencing Primers, Control DNA and Water.

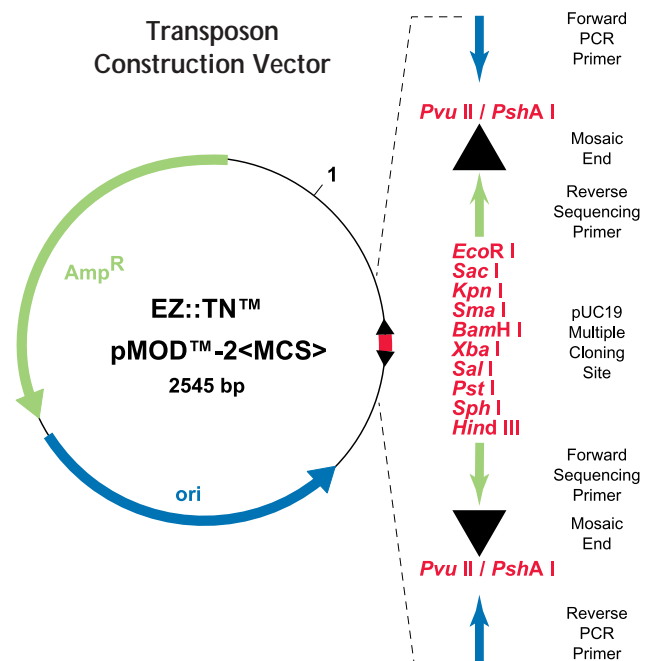
## New Transposon Construction Vector for Constructing a Custom EZ::TN™ Transposon

EZ::TN pMOD™-2<MCS> is a pUC-based vector containing a new transposon construction module for preparation of custom EZ::TN Transposons containing virtually any DNA sequence of interest (e.g. species-specific selectable markers, genetic control elements, cDNA). The new EZ::TN pMOD-2<MCS> features primer binding sites within the transposon for bidirectional sequencing from the insertion site of any custom EZ::TN Transposon. No need to design your own primers.

### How to prepare a custom EZ::TN Transposon and EZ::TN Transposome™ using pMOD-2<MCS>

1. **Clone your DNA into pMOD-2<MCS>.** pMOD-2<MCS> contains the multiple cloning site from pUC19 between the hyperactive 19-bp Mosaic Ends (ME) that are specifically and uniquely recognized by EZ::TN Transposase.
2. **Isolate your custom EZ::TN Transposon.** There are three options for preparing an EZ::TN Transposon from pMOD-2<MCS> -digestion with either *Pvu* II or *Psh* A I to release the transposon from the vector backbone or PCR amplification of the transposon region using the Forward and Reverse PCR Primers included with the vector.
3. **Use the newly prepared EZ::TN Transposon** for random, *in vitro* insertion into a plasmid or cosmid clone using EZ::TN Transposase or prepare an EZ::TN Transposome by incubating with EZ::TN Transposase in the absence of Mg<sup>2+</sup> for *in vivo* insertions. Sequence from the transposon insertion site bidirectionally using the Forward and Reverse Sequencing Primers (available separately).

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

MOD0606 20 µg  
Includes Forward and Reverse PCR Primers

### pMOD™<MCS> Forward Sequencing Primer

MODFSP201 1 nmole

### pMOD™<MCS> Reverse Sequencing Primer

MODRSP202 1 nmole

### EZ::TN™ Transposase

TNP92110 10 Units

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*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.

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- Gentle procedure - purify multi-megabase DNA that is intact and biologically active.
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- 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

\*Note: One unit of GELase Preparation equals three or more units of most other gel-digesting enzymes.

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### Highest Activity, Available Now!

Exonuclease I specifically digests *single-stranded* DNA in a 3'-5' direction and is active under a variety of buffer conditions. Add Exonuclease I directly to your reaction mix. Incubate 30 minutes to completely digest the single stranded DNA and oligonucleotides. Then, the enzyme can be inactivated by heating at 80°C for 15 minutes. Exonuclease I is tested to be free of RNase, endonuclease and double-strand exonuclease activities.

#### Application

- Removal of residual single stranded DNA and oligo nucleotides from reactions and nucleic acid preparations.

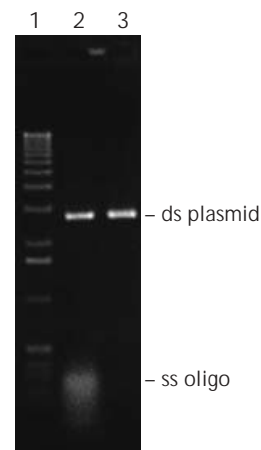
#### Exonuclease I

X40501K	1000 U	20 U/μl
X40505K	5000 U	20 U/μl
X40520K	20,000 U	20 U/μl

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#### Figure. Specificity of Exonuclease I (Exo I) for single strand DNA.

200 ng of *Eco* RI-linearized pUC19 DNA and 100 μg of a 100-mer single strand oligo nucleotide were mixed and incubated at 37°C for 20 minutes in the presence and absence of 10 U of Exo I. 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 oligo nucleotide while leaving the linear double-stranded plasmid DNA intact.



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HCV Enterovirus HPV  
B. pertussis Mammalian  
M. tuberculosis RSV

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every time.**

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The MasterPure Kit has been used for almost every type of sample you can imagine: dried and wet 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).  
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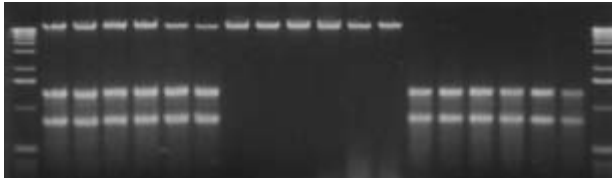
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**ON TRIAL SIZE**  
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## Consistent Purity

Consistently obtain DNA or RNA free of protein.

Total NA      DNA      RNA



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

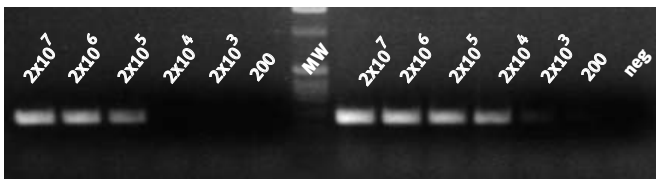
### Examples of Samples Extracted

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

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The MasterPure co-precipitant greatly improves sensitivity.

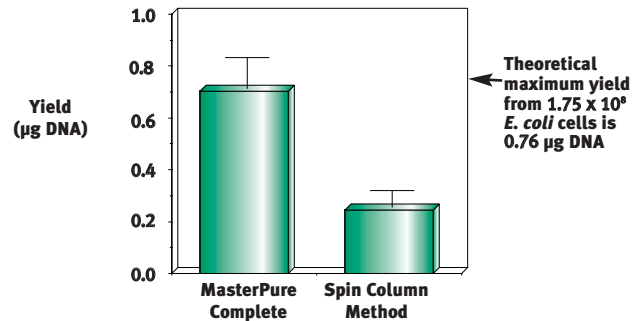
Spin Column Method      MasterPure Complete



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  $1.75 \times 10^8$  *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

\*Offer Ends Sept. 15, 2000. Limit one kit per customer.

### MasterPure™ DNA Purification Kit

(for isolating TNA or DNA)

MCD85201      200 Purifications

### MasterPure™ RNA Purification Kit

(for isolating RNA only)

MCR85102      100 Purifications

# Completely Sequence Plasmid and Cosmid Clones Without Subcloning or Primer Walking

## EZ::TN Transposon Insertion Kits

An EZ::TN Transposon™ insertion kit facilitates complete sequencing of cloned DNA faster, more economically and more reliably than by primer walking or subcloning.

The EZ::TN insertion reaction randomly inserts a single EZ::TN Transposon containing sequencing primer binding sites and a selectable marker (Kan<sup>R</sup>, Tet<sup>R</sup> or DHFR gene) into your plasmid or cosmid clone.

**Reduce your sequencing time by 10-fold or more.**

A single 2-hour reaction generates >10<sup>6</sup> independent sequencing templates – enough to completely sequence even the largest clones – thus saving you the time usually spent subcloning or designing and synthesizing sequencing primers.

**Never synthesize sequencing primers again.**

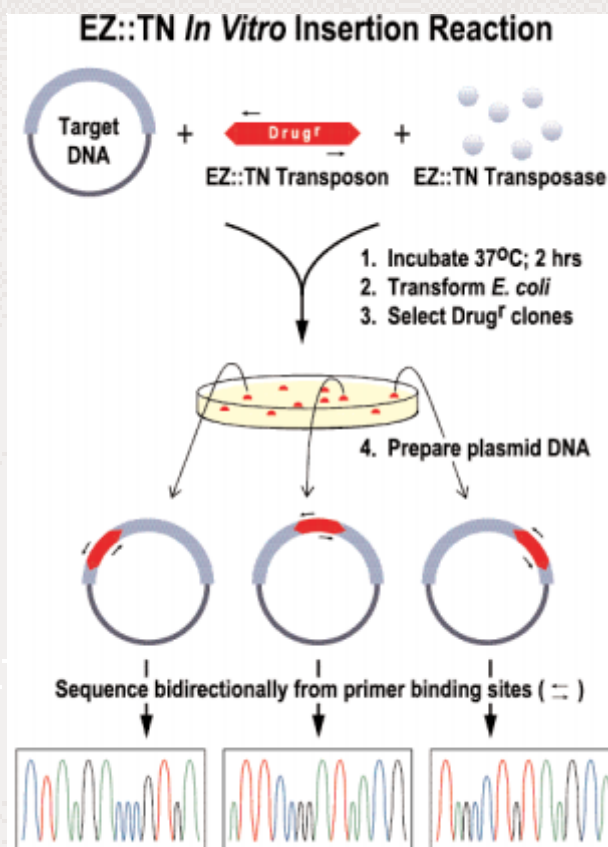
Sequence all clones bidirectionally using a single set of sequencing primers that are homologous to the ends of the inserted EZ::TN Transposon. Unlabeled primers are provided in the kits.

**Challenging templates are no problem.**

EZ::TN Transposon insertions are highly random - even into regions that are difficult to subclone or to sequence by primer walking. This ensures sequencing primer binding sites are distributed throughout your clone to facilitate complete sequencing of the clone.

"Using the EZ::TN <KAN-2> Insertion Kit we reduced the time of our sequencing project from 6 months to 12 days."

*Harold Sims, Washington University School of Medicine*



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 respective EZ::TN Transposon, EZ::TN Transposase, Reaction buffers and two unlabeled sequencing primers.

Contact EPICENTRE for a copy of the new EZ::TN Transposon Tools brochure.

# Create Gene Knockouts in Living Cells & Sequence the Genes *Without Cloning*

## EZ::TN™ Transposomes™

An EZ::TN Transposome - the stable complex formed between an EZ::TN Transposon and EZ::TN Transposase - can be electroporated into many living cells that can be transformed by electroporation. The Transposome is activated by Mg<sup>2+</sup> in the cell and the transposon is randomly inserted into the cell's genomic DNA to...

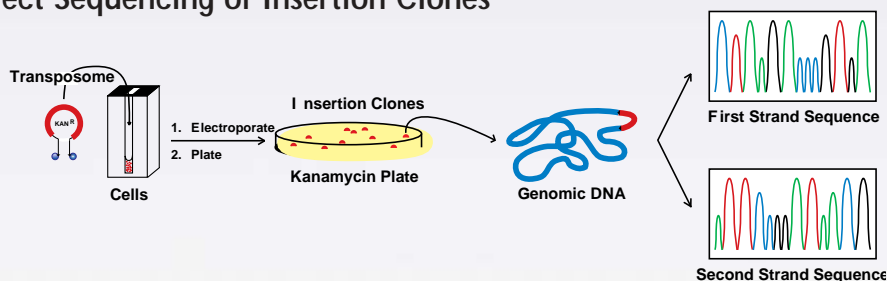
### Make Gene Knockouts Rapidly & Easily...

without the need for cell conjugation, suicide vectors or mini-transposons. Simply electroporate the EZ::TN Transposome into the cell. EZ::TN Transposomes have already been used successfully by scientists working with a variety of organisms.

Number of Kan<sup>r</sup> transposon insertion clones produced from electroporation of 1 µl of EZ::TN <KAN>Tnp Transposome.

<i>E. coli</i>	>10 <sup>5</sup>	<i>Proteus vulgaris</i>	>10 <sup>3</sup>
<i>Salmonella ty.</i>	>10 <sup>4</sup>	<i>Mycobacterium smegmatis</i>	>10 <sup>2</sup>
<i>Pseudomonas sp.</i>	>10 <sup>2</sup>		

### Direct Sequencing of Insertion Clones



Once a gene knockout has been selected, then...

### Sequence the Gene in Bacterial Genomic DNA *Without Cloning*.

Rapidly identify the affected gene by direct genomic DNA sequencing. Use a MasterPure™ (EPICENTRE) prep of bacterial genomic DNA as template and primers homologous to the ends of the inserted EZ::TN Transposon.

### Construct a Custom EZ::TN Transposome (see page 7 of this Forum)

Pre-formed EZ::TN Transposomes are available. A custom EZ::TN Transposome containing virtually any DNA sequence can be prepared using pMOD-2™<MCS> Transposon Construction Vector and EZ::TN Transposase.

#### EZ::TN™ <KAN-2>Tnp Transposome™

TSM99K2 10 Reactions

Pre-formed Transposome containing kanamycin selection marker. Two unlabeled sequencing primers are included.

#### EZ::TN™ <DHFR-1>Tnp Transposome™

TSM99D1 10 Reactions

Pre-formed Transposome containing dihydrofolate reductase gene for selection with trimethoprim. Two unlabeled sequencing primers are included.

#### pMOD™-2<MCS>

MOD0602 20 µg

#### EZ::TN™ Transposase

TNP92110 10 Units

Contact EPICENTRE for a copy of the new EZ::TN Transposon Tools brochure.

Saccharomyces cerevisiae

Salmonella typhimurium

E. coli

Mycobacterium tuberculosis

Pseudomonas

Mycobacterium smegmatis

Proteus vulgaris

# *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.

M A B C D E F G H I J K L

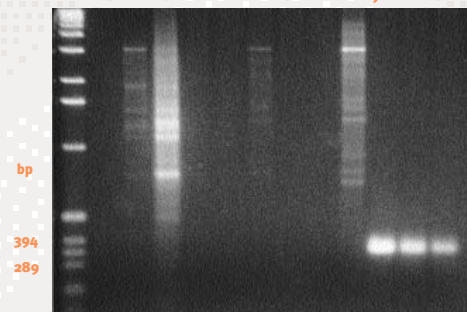
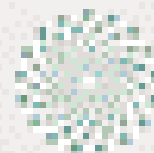


Figure. PCR results for amplification of a GC-rich region of the human fragile X gene. For this template/primer pair, *FailSafe PCR 2X PreMix J* was the optimal PreMix. (Please note that other GC-rich templates may require a different *FailSafe PCR 2X PreMix*.)

**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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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											
Cat. #		A	B	C	D	E	F	G	H	I	J	K	L
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
		FSP995A	FSP995B	FSP995C	FSP995D	FSP995E	FSP995F	FSP995G	FSP995H	FSP995I	FSP995J	FSP995K	FSP995L

\*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 (with betaine).

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- N733 FailSafe™ PCR System
- N734 MasterAmp™ RT-PCR Kits (for High Fidelity or High Sensitivity)
- N735 MasterPure™ Complete DNA and RNA Purification Kits
- N736 Other\_\_\_\_\_

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- Typing or fingerprinting
- Automated DNA sequencing
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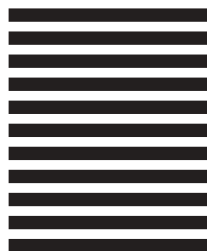
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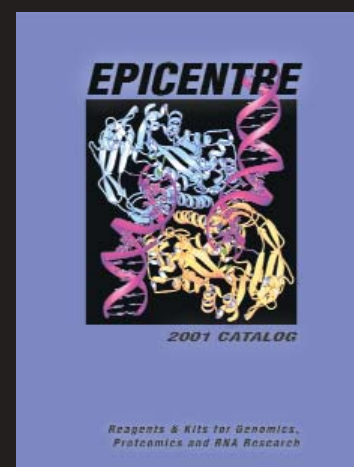
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# Using the FailSafe™ PCR System to Construct Molecular Tools for the Functional Characterization of a *Saccharomyces cerevisiae* Gene

Thierry Brulier and Marie-Claire Daugeron

Institut de Génétique et Microbiologie, Université de Paris Sud, Orsay, France

## Introduction

The functional characterization of a new gene requires the construction of several molecular tools which very often includes PCR amplification steps. Thus, having an efficient, easy handling and faithful PCR system represents a real gain of time and can greatly facilitate the first steps toward the elucidation of the *in vivo* function of a gene.

We are interested in the transcriptional control of ribosome biogenesis in *Saccharomyces cerevisiae*, and we started the study of a new gene (Our Favourite Gene, *OFG*) potentially involved in this process.<sup>1,2</sup> First, we wanted to determine the cellular location of the *OFG* protein product and to assess the impact of a chromosomal deletion of *OFG* on the cell physiology. To do so, we needed to tag the protein with an influenza hemagglutinin (HA) epitope and create an *ofg::kanMX4* disruption cassette. We successfully generated these two DNA constructs using the FailSafe™ PCR System.

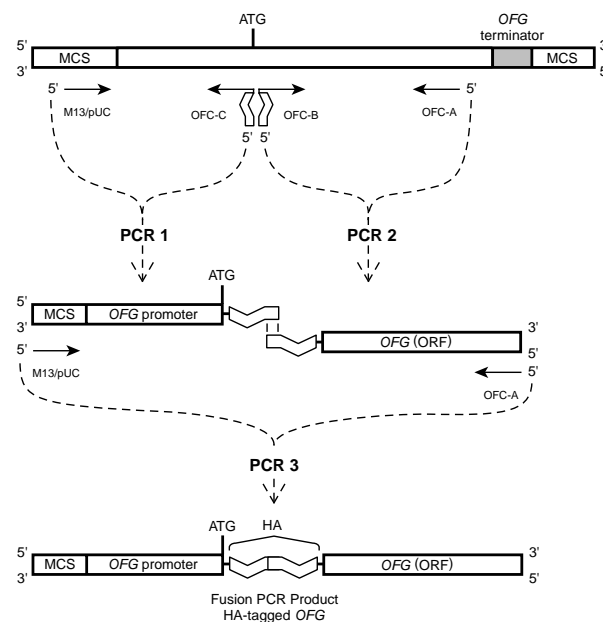
## Materials and Methods

### Construction of a plasmid-borne HA-epitope tagged *OFG* allele by fusion PCR

In order to immunodetect the protein product of *OFG*, we introduced by fusion PCR an *in-frame* HA-epitope tag encoding sequence just after the start codon of a plasmid-borne *OFG* allele (Figure 1). The modified *OFG* allele is controlled by its own promoter so that expression

is driven in a physiological manner. A portion of the promoter region of *OFG* (PCR 1) was amplified from plasmid YCplac33-*OFG* using primers OFG-C (5'**GTC ATA GGG ATA GCC CGC ATA GTC AGG AAC ATC GTA TGG GTA TGC** CAT CAA AGG CGT CGG TAT TG 3') and the M13/pUC sequencing primer (-20, BioLabs). The N-terminal region of *OFG* (PCR 2) was amplified from the same template using primers OFG-B (5'**GCG GGC TAT CCC TAT GAC GTC CCG GAC TAT GCA** TTC ATC AAA CAG TCT GAA AAA 3') and OFG-A (5' TTC GAC GGA ATC ATG AGA T 3'). Bolded nucleotides encode the HA-epitope; overlapping bases between OFG-C and OFG-B are underlined.

PCR reactions were performed using the FailSafe PCR Enzyme Mix and a set of 12 FailSafe PCR PreMixes (A-L). The PreMixes contain a buffered salt solution with all four deoxyribonucleotides, and various amounts of MgCl<sub>2</sub> and the FailSafe PCR Enhancer (with betaine). Each 50 µl reaction contained 200 ng of the DNA template, 0.5 µM of each primer, less than 1.25 U of the FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix. The cycling profile for PCR 1 and PCR 2 was one cycle at 94°C for 2 minutes followed by 30 cycles of 94°C for 45 seconds, 52°C for 45 seconds, 72°C for 1.2 minutes, and a final extension at 72°C for 5 minutes. Five microliters of each PCR reaction was further analysed by electrophoresis on a 1% agarose gel, the expected size for PCR 1 and 2 products is 0.9 kb and 1 kb, respectively



**Figure 1. Scheme of the fusion PCR procedure.** The 5' region of primer OFG-C, used in the amplification of PCR product 1, is complementary to a segment of PCR product 2 (i.e., the 5' region of primer OFG-B). The HA sequence is therefore created when this overlap is extended and co-ordinately amplified with the inclusion of outside primers M13/pUC and OFG-A.

(Figure 2). A third PCR corresponding to the fusion PCR was performed using PCR products 1 and 2 as DNA template and the two oligonucleotides OFG-A and M13/pUC. A 50  $\mu$ l reaction contained ~300 ng of gel-purified PCR 1 product (buffer D) and ~300 ng of gel-purified PCR 2 product (buffer D), 0.5  $\mu$ M of each primer, 1.25 U of FailSafe PCR Enzyme Mix, and 25  $\mu$ l of FailSafe PCR PreMix D. The cycling profile was one cycle at 94°C for 2 minutes followed by 30 cycles of 94°C for 45 seconds, 51°C for 45 seconds, 72°C for 2.5 minutes, and a final extension at 72°C for 5 minutes. Two microliters of the PCR reaction (Figure 3) were further analysed by electrophoresis on a 1% agarose gel, the expected size of the fusion PCR product is 1.9 kb. After ethanol precipitation, the fusion PCR product was digested with *EcoR* I, gel purified and further subcloned into a *EcoR* I-restricted YCplac33-*OFG* plasmid. The final plasmids, YCplac33-HA-*OFG*, fully complemented an *ofg*-disrupted strain.

#### Generation of an *ofg::kanMX4* disruption cassette

To perform the chromosomal disruption of *OFG*, a *kanMX4* cassette harbouring *OFG* short flanking homology regions was generated using the FailSafe PCR System with the plasmid pFA6a-*kanMX4* as the DNA template and OFG-P1 (5' GGC TTT TGT TCT TTG ATG TTA ATT CGG CAA TAC CGA CGC CTT TGC GTA CGC TGC AGG TCG AC 3') and OFG-P2 (5' TGT TAT GAA GCT ATA TGG TAA AGA ATA CAT GGT GTC ATA TAG ATT CGA TGA ATT CGA GCT CG 3') as primers. PCR reactions were set up with the 12 PCR 2X PreMixes as described above for PCR 1 and 2 (data not shown). The cycling profile for the PCR reaction was one cycle at 94°C for 2 minutes followed by 30 cycles of 94°C for 45 seconds, 50°C for 45 seconds, 72°C for 1.5 minutes, and a final extension at 72°C for 5 minutes. After concentration by ethanol precipitation, the *ofg::kanMX4* product (1.5kb) was used to transform the yeast strain W303.

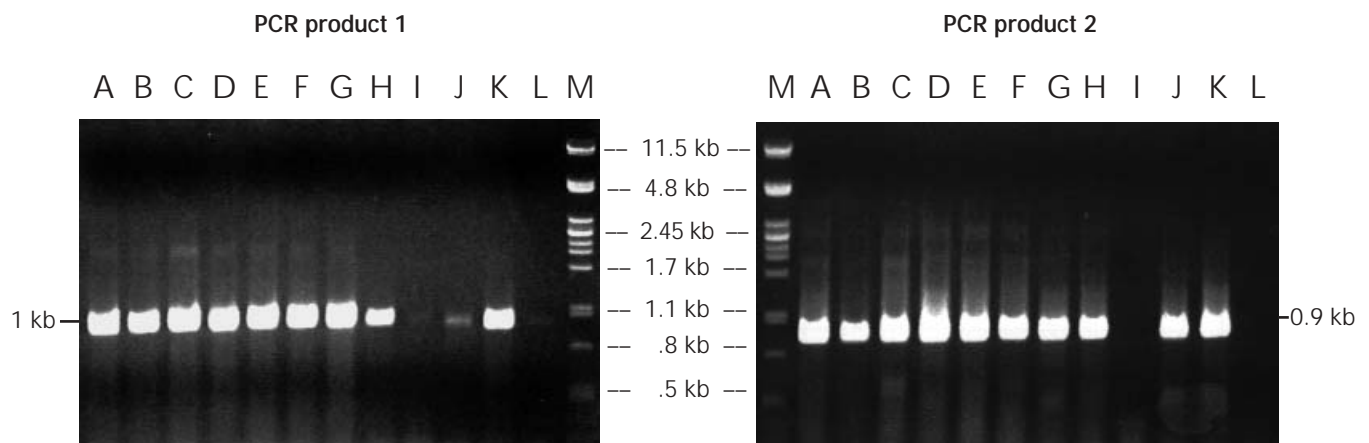
Transformants were selected on YPD plates containing 200 mg/ml G418.<sup>3</sup>

#### Results and Discussion

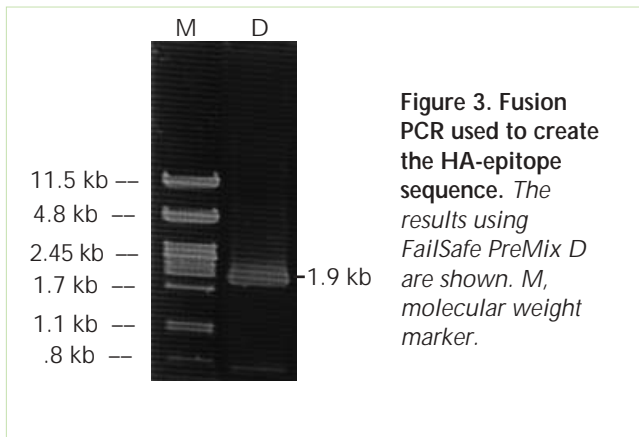
With each PCR reaction performed with the FailSafe PCR System, we succeeded on the first try, recovering the expected product in sufficient amounts for the following steps and in most cases sufficiently pure to avoid any further gel-purification steps. A clear advantage of the FailSafe PCR System lies in the 12 PreMixes which allow a direct, easy, and quick determination of the best amplification conditions for each primer pair and DNA template. For the construction of an HA epitope-tagged *OFG* allele, PCR 1 and 2 employed primer pairs highly heterogeneous in size (17 and 65 bases; 19 and 54 bases) with, in both cases, the longest primer having a long 3' non-hybridizing tail. As shown in Figure 2, large amounts of the expected products were obtained with several of the PreMixes but not with all of them, emphasizing the importance of the buffer conditions in the PCR reaction. The amplifications were highly specific in all cases since no significant aberrant products were generated.

Similarly, the PCR reaction yielding the 1.5 kb *ofg::kanMX4* disruption cassette employed a pair of long primers (> 60 bases) with a long non-hybridizing tail (~40 bases). We also obtained high amounts of a highly specific product with 10 of the 12 PreMixes tested (data not shown). As in the previous PCR series, the poorest results were obtained with PreMixes I, J and L.

In the case of the fusion PCR, potential difficulty resides in the nature of the DNA template which consists of two DNA molecules of ~ 1kb (PCR products 1 and 2) having only a very short overlapping region (18 bp) (Figure 1). When the assay was performed with DNA template that was not purified and two different PreMixes, three products (0.9, 1.0 and 1.9 kb) were amplified in



**Figure 2.** FailSafe PCR amplification of the promoter (PCR1) and N-terminal (PCR2) regions of *OFG*. Lanes A-L show the amplification products resulting from PCR using the 12 FailSafe PCR PreMixes. M, molecular weight marker.



**Figure 3. Fusion PCR used to create the HA-epitope sequence.** The results using *FailSafe PreMix D* are shown. *M*, molecular weight marker.

comparable amounts (data not shown). The unexpected amplification of the PCR 1 and 2 products was due to traces of primers OFG-A and OFG-B. This was easily overcome by using gel-purified PCR 1 and 2 products as DNA template (Figure 3). We did not sequence the plasmid-borne HA-*OFG* alleles recovered after subcloning of the fusion PCR product, but the fact that they fully complemented the *ofg*-disrupted strain argues in favor of the high fidelity of the *FailSafe* PCR Enzyme Mix.

#### References

1. Warner, J. R. (1999) *Trends Biochem. Sci.* **24** (11), 437.
2. Mizuta, K. et al. (1997) *Gene* **187** (2), 171.
3. Gietz, D. et al. (1992) *Nucleic Acids Res.* **20**, 1425.

#### FailSafe™ PCR PreMix Selection Kit

FS99060                      60 Units

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

#### FailSafe™ PCR System

FS99100                      100 Units\*  
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\* 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).

Individual *FailSafe*™ PCR 2X PreMixes are also available separately. Please see the center insert for more ordering information.

For more information, please circle reader service number N733 on the reply card found in the center insert or visit our website at



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

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"I know of no other sequencing method that resolves GC compressions better than *SequiTherm EXCEL™ II*."

- Glenn Dawes, Affymax Research Institute, *The Scientist*, Vol. 12, #6, 1998, pg. 13

*SequiTherm EXCEL II* resolved a "2-year-old puzzle" due to "an unreadable hairpin loop" for the Washington University genome center.

- *Science*, Vol. 280, 8 May 1998, pg. 816

Sequence through hairpin loops, regions of high GC or AT content, areas of interstrand reannealing, and inverted or direct repeats. Using end labeled primers, *SequiTherm EXCEL II* DNA Sequencing Kits provide complete, clear sequencing data when all other methods fail.

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SE9202LC                      (100 Sequences)

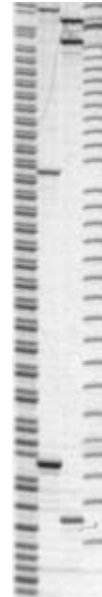
#### *SequiTherm EXCEL™ II* Long-Read™ DNA Sequencing Kit - ALF™ For ALF DNA Sequencers

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SEM79020                      20 Sequences  
 SEM79050                      50 Sequences  
 SEM79100                      100 Sequences

G A T C



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

EPICENTRE offers two different kits for RT-PCR. The MasterAmp RT-PCR Kit for High Fidelity, which utilizes an enzyme blend with proofreading activity, should be used for generating cDNA for cloning and expression. On the other hand, the MasterAmp RT-PCR Kit for High Sensitivity should be used for detection of small quantities of RNA.

## The MasterAmp™ RT-PCR Kit for High Sensitivity Outperforms Other Kits for Sensitive Detection of RNA

Judith T. Schanke, EPICENTRE

### Introduction

Standard RT-PCR protocols recommend the use of up to one microgram of RNA template per reaction. In reality, researchers often have only a few nanograms or picograms of RNA with which to work. The amplification of these RNAs can be further complicated by the secondary structure of the RNA sequence of interest.

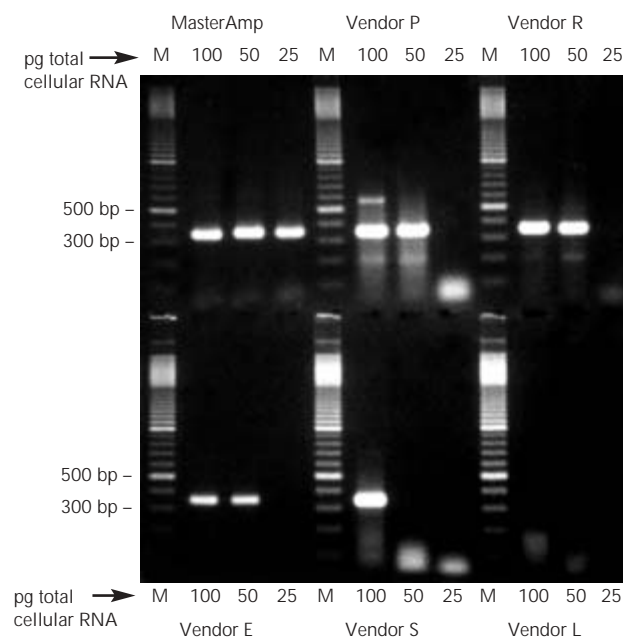
Thus, in order to achieve the highest sensitivity, both reverse transcription and PCR reactions must be optimized to strike the best balance between maximizing denaturation of RNA secondary structure, primer binding and primer extension, and minimizing high temperature RNA template degradation and enzyme inactivation.<sup>1,2</sup> These variables are optimized for the MasterAmp RT-PCR Kit for High Sensitivity by incorporating several advances that permit greater sensitivity in a single RT and PCR reaction mixture. This report compares the MasterAmp Kit for High Sensitivity with RT-PCR kits from six other suppliers for its ability to detect a small amount of a specific mRNA in 25-100 picograms of total cellular RNA.

### Methods

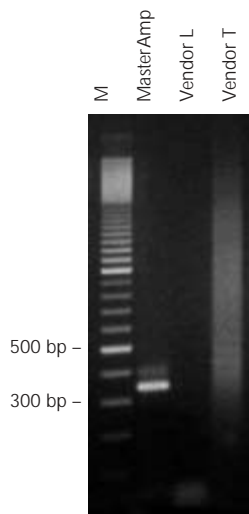
The MasterAmp RT-PCR Kit for High Sensitivity uses a single reaction mix for both reverse transcription and PCR. Each MasterAmp reaction contained: 1X RT-PCR Buffer, 3mM MgCl<sub>2</sub>, 0.5 mM MnSO<sub>4</sub>, 400 μM each dNTP, 12.5 pmoles of each primer, 25-100 pg placental RNA and 2.5 U of RetroAmp™ RT DNA Polymerase. RNA was reverse transcribed at 60°C for 30 minutes, and then immediately PCR amplified using the following parameters: denature at 94°C for 45 seconds, anneal to primers at 55°C for 45 seconds, and primer extend at 72°C for 60 seconds for 40 cycles.

RT-PCR was performed using dilutions of total cellular RNA from human placenta. Primers were designed to amplify a 343 base RNA region of the human glycoprotein hormone alpha subunit. The sequences of the primers were: 5'GTCAACGCGCT-GAACACATCCTGC and 5'GACACTCCCCATTAC-CATGACCCTG.

Reverse transcription and RT-PCR were performed with kits from other vendors using the protocols recommended by the respective manufacturers. Reverse transcription temperatures ranged from



**Figure 1. The MasterAmp RT-PCR Kit for High Sensitivity detects lower levels of RNA than RT-PCR kits from other vendors.** RT-PCR was performed as described in Methods using 25pg of total human placental RNA and primers to the human glycoprotein hormone alpha subunit. M, DNA ladder.



**Figure 2. The MasterAmp RT-PCR Kit for High Sensitivity results in a more specific, high temperature RNA amplification.** RT-PCR was performed as described in Methods using 25-100 pg of total human placental RNA and primers to the human glycoprotein hormone alpha subunit. M, DNA ladder.

37°C to 60°C. PCR cycling conditions were identical to those described above for the MasterAmp Kit with the exception of two kits, which recommended significantly longer extension times. Those recommendations were followed.

## Results

The MasterAmp RT-PCR Kit was more sensitive than any other RNA amplification systems (Figure 1). Prominent, specific RT-PCR products were produced from 25-100 picograms of total cellular RNA from human placenta using the MasterAmp RT-PCR Kit for High Sensitivity, whereas none of the other kits or systems tested resulted in a detectable product from 25 picograms of RNA. The higher sensitivity of the MasterAmp Kit held true, both for systems that used a single reaction mix for reverse transcription and PCR (Vendors P and R) and for more time-consuming systems that used two different reaction mixtures and separate steps for reverse transcription and PCR (Vendors E, T, S and L).

The MasterAmp RT-PCR Kit was also compared to two vendors' high temperature RNA amplification kits (Figure 2). Reverse transcription was performed at 50°C to 60°C for 30 minutes followed by PCR amplification with

identical cycling profiles. As recommended by the supplier, PCR was performed as a second separate reaction with Vendor L's kit. As found previously, the MasterAmp RT-PCR Kit for High Sensitivity detected lower levels of RNA and produced a single, prominent RT-PCR product. Amplifications using the other kits resulted in a non-specific amplification (i.e., a smear) or no amplification when only 25 picograms of RNA was used as template.

## Summary

The MasterAmp RT-PCR Kit for High Sensitivity is the easiest and most sensitive method for RNA detection. The kit uses a single reaction mix for both reverse transcription and PCR. Since reverse transcription is performed at the highest temperature, there is no need for a separate template denaturation step, and problems due to RNA secondary structure are eliminated.

## References

1. Brooks, E.M. *et al.* (1995) *BioTechniques* **19**, 806.
2. Freeman, W.M. *et al.* (1996) *BioTechniques* **20**, 782.

### MasterAmp™ RT-PCR Kit for High Sensitivity

RT71225	25 Reactions
RT712100	100 Reactions

### MasterAmp™ High Fidelity RT-PCR Kit

RF91025	25 Reactions
RF910100	100 Reactions

For more information, please circle reader service number N734 on the reply card found in the center insert or visit our website at [www.epicentre.com/catalog/high\\_sensitivity\\_rt-pcr.htm](http://www.epicentre.com/catalog/high_sensitivity_rt-pcr.htm)

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# High Efficiency Packaging of Methylated DNA for Genomic Library Construction using MaxPlax™ Lambda Packaging Extracts

Mike Fiandt, EPICENTRE

Successful construction of genomic lambda 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 degrade methylated DNA. All commercial suppliers of lambda packaging extracts claim high packaging efficiency when using a control DNA that is in no way representative of the DNA used for genomic library production. Our intent was to demonstrate the packaging efficiency that a user will likely find when preparing genomic libraries from highly methylated DNA using the MaxPlax™ Lambda Packaging Extracts. EPICENTRE's MaxPlax Lambda Packaging Extracts are derived from *E. coli* BHB2688 and a restriction-minus strain, NM759 to facilitate high efficiency packaging of methylated DNA.

**DNA Methylation.** T7 DNA was methylated *in vitro* by Sss I Methylase which specifically methylates the cytosine in the dinucleotide sequence 5'-CG-3'. This methylation pattern closely mimics that found in the genomic DNA of higher eukaryotes. Overnight digestion of methylated and untreated T7 DNA with *Hpa* II - a restriction endonuclease that digests unmethylated DNA at 5'-CCGG-3' but will *not* cut methylated DNA - was

used to assay for completeness of the methylation reaction (Figure).

**Lambda Packaging.** Untreated and methylated T7 DNAs were independently cloned into pWEB™ Cosmid vector (EPICENTRE) using Fast-Link™ DNA Ligase (EPICENTRE). Four microliters of each ligation reaction were individually packaged using MaxPlax Lambda Packaging Extract according to the product protocol. Dilutions of packaged DNA were used to infect *E. coli* EPI305 cells and infected bacteria grown on LB

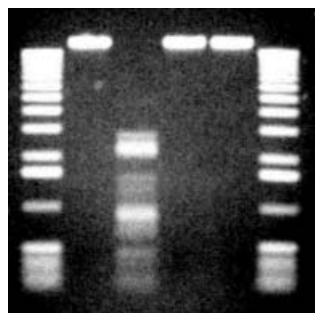
plates at 37°C overnight. Plaques were counted and the packaging efficiencies calculated.

High efficiency lambda packaging of methylated DNA. The Figure demonstrates that methylation of T7 DNA at its 5'-CG-3' dinucleotide sequences by Sss I Methylase went to completion as determined by its resistance to overnight *Hpa* II incubation. Cosmid cloning of both the methylated and untreated T7 DNA and subsequent packaging using MaxPlax Lambda Packaging Extracts each generated libraries of >3 x 10<sup>7</sup> plaques per µg of DNA. Thus, MaxPlax Lambda Packaging Extracts produce cosmid libraries from methylated DNA with the same high efficiency as from unmethylated DNA.

**Table 1. Packaging of methylated and unmethylated T7 DNA using MaxPlax Lambda Packaging Extracts.**

Cosmid Insert	Plaques per plate	Pfu/µg DNA
T7 DNA	140	3.5 x 10 <sup>7</sup>
Methylated T7 DNA	155	3.9 x 10 <sup>7</sup>

M 1 2 3 4 M



**Figure. Complete methylation at 5'-CG-3' dinucleotides in T7 DNA was confirmed by its resistance to overnight incubation with *Hpa* II.** Lane 1, T7 DNA; Lane 2, T7 DNA after *Hpa* II incubation; Lane 3, Sss I Methylase treated T7 DNA; Lane 4, methylated T7 DNA after *Hpa* II incubation. M, DNA ladder.

### MaxPlax™ Lambda Packaging Extracts

The highest efficiency and best value

5 Extracts	MP5105
10 Extracts	MP5110
20 Extracts	MP5120

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

Extracts are available in bulk quantity. Please inquire.

For more information, please visit our website at [www.epicentre.com/catalog/maxplax.htm](http://www.epicentre.com/catalog/maxplax.htm)

## New High Efficiency TransforMax™ EC100 Electrocompetent *E. coli*



With a transformation efficiency of  $>5 \times 10^9$  cfu/ $\mu$ g DNA (pUC19), EPICENTRE's new TransforMax™ EC100 electrocompetent *E. coli* are ideal for all cloning applications. And since TransforMax EC100 cells are restriction minus and lack transformation size bias against large inserts (like DH10B™ cells), they can be used to generate complete and unbiased primary cosmid and BAC libraries. Their high efficiency (Table), lack of size bias and other features also make them ideal for transformation of EZ::TN™ Transposon insertion clones or for generating deletion libraries using the EZ::TN pWEB::TNC™ cosmid and pPDM™ plasmid deletion vectors.

Transformation Efficiency:  $> 5 \times 10^9$  cfu/ $\mu$ g DNA (pUC19)

### Genotype

*F*<sup>-</sup> *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\phi$ 80d/*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *endA1* *araD139*  $\Delta$ (*ara, leu*)7697 *galU* *galK*  $\lambda^-$  *rpsL* *nupG*

### Relevant Phenotype

- Blue/white screening of vectors expressing the LacZ'  $\alpha$ -complementing peptide.
- Restriction minus for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- Accepts large clones for unbiased, primary cosmid and BAC library production.
- Endonuclease minus (*endA1*) to ensure high yields of plasmid clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

**Table. TransforMax™ EC100 Electrocompetent *E. coli* have a higher transformation efficiency than competent cells from other vendors.**

	Transformation Efficiency (cfu/ $\mu$ g DNA)*
TransforMax™ EC100 <i>E. coli</i>	$9.2 \times 10^9$
Competitor S	$5 \times 10^9$
Competitor I	$4 \times 10^9$
Competitor B	$3 \times 10^9$

\*Average of eight independent transformations with a pUC vector. DH10B is a trademark of LTI.

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

EC10005 5 X 100  $\mu$ l (10 Electroporations)  
 EC10010 10 X 100  $\mu$ l (20 Electroporations)

Each includes pUC19 Control DNA.

For more information, please visit our website at [www.epicentre.com/catalog/ec100.htm](http://www.epicentre.com/catalog/ec100.htm)

## DNA Ligation in as little as 5 Minutes at room temperature! Fast-Link™ DNA Ligation Kit

EPICENTRE's Fast-Link™ DNA Ligation Kits are specially formulated to provide fast and efficient DNA ligations for both routine and high throughput cloning.

### Fast and Efficient DNA Ligations

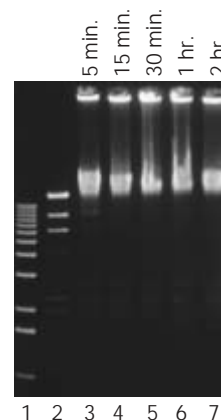
- Ligate cohesive-end DNA in 5 minutes at room temperature!
- Ligate blunt-end DNA in 15 minutes at room temperature!
- Ligate PCR product with A-overhangs in 1 hour or less at 16°C.

### Transform Cells Without Desalting the Ligation Reaction

Desalting the DNA ligation reaction prior to electroporation is not necessary when using the Fast-Link DNA Ligation Kits.

For more information visit our website at [www.epicentre.com/catalog/fastlink.htm](http://www.epicentre.com/catalog/fastlink.htm)

**Figure . Time course for cohesive-end ligation using the Fast-Link 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.**



### Fast-Link™ DNA Ligation Kit

LK6201H 100 ligations

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

## Obtain the Highest Yield of Capped RNA from an *In Vitro* Transcription Reaction

### Use AmpliCap™ High Yield Message Maker Kits

EPICENTRE's new 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 convenient 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, T3 and SP6 High Yield Message Maker Kits feature:

- RNA yields up to 45 µg per reaction using the AmpliCap T7 and T3 Kits and up to 35 µg using an AmpliCap SP6 Kit. (Twenty microliter reactions using Control Template DNA).
- 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 is also included for efficient production of long, 5'-capped RNA.
- AmpliCap Kits utilize concentrations of NTPs that are high enough to inhibit conventional transcription reactions.

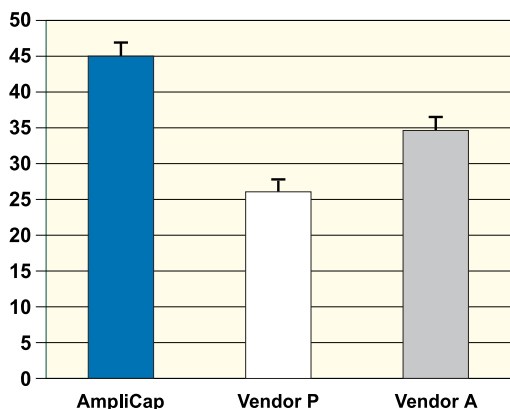


Figure 1. AmpliCap T7 High Yield Message Maker Kits consistently produce the highest yield of 5'-capped RNA transcript. A standard 20 µl reaction produces more 5'-capped RNA than the leading competitor's kits.



<b>T7</b> AC0707	25 Reactions
<b>T3</b> AC0703	25 Reactions
<b>SP6</b> AC0706	25 Reactions

Each kit contains the respective AmpliCap™ Enzyme Solution (includes RNase inhibitor), Cap/NTP PreMix, 20 mM GTP, 10 X AmpliCap™ Transcription Buffer, 100 mM DTT, RNase-free DNase I, Control Template DNA, RNase-Free Water

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