

# EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

## The BuccalAmp™ DNA Extraction Kit: A New Tool for Easier, Faster Sample Collection and Extraction of PCR-Ready Genomic DNA from Large Numbers of Samples

Judith E. Meis, EPICENTRE

### Introduction

In both clinical diagnostics and basic scientific research, fast and reproducible DNA extraction from large numbers of samples is becoming increasingly important. EPICENTRE's one-step BuccalAmp™ DNA Extraction Kit, has become a product of choice for PCR-ready DNA extraction, due to its simplicity, reliability, and high DNA yields.

Genomic DNA extraction of buccal (cheek) cells with the BuccalAmp Kit requires only heating to prepare DNA ready for PCR (Figure 1). Buccal cell sampling with the newly designed foam Catch-All™ Sample Collection Swab is noninvasive and painless, ideal for any segment of the population including children. Moreover, buccal cell samples can be stored and transported for an extended period of time at ambient temperature. These features make BuccalAmp genomic DNA extraction an ideal method for high throughput applications. In this report, we simulate a high throughput environment and demonstrate the efficiency of genomic DNA extraction from a large number of samples for PCR.

### Methods

#### Buccal Cell Sample Collection

Catch-All Sample Collection Swabs were distributed to 96 individuals varying in age. Samples were self-collected, except when young children were assisted by adults. Healthcare professionals were not involved with sample collection.

Each swab containing a buccal cell sample was sent to the laboratory in its

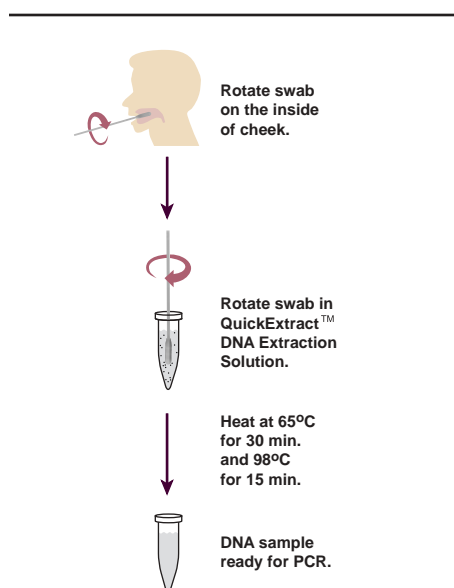


Figure 1. Procedure for obtaining PCR-ready DNA using the BuccalAmp™ DNA Extraction Kit.

original hard plastic collection tube at ambient temperature. Tubes were stored at 4° C for up to 4 weeks before being processed.

#### Buccal Cell DNA Extraction

Buccal cell DNA was extracted from the swab samples from all 96 individuals according to the standard protocol. Specifically, each sample swab was rotated in 500 µl of QuickExtract™ DNA Extraction Solution. Extraction solutions were then heated in separate wells of a 96 well plate (2 ml per well capacity) at 65° C for 30 minutes followed by 98° C for 15 minutes. The solutions containing extracted DNA were cooled and stored at -20° C until used for PCR amplifications.

#### β-Globin PCR Amplification

All samples were amplified simultaneously using the FailSafe™ PCR System. The β-globin PCR amplifications were carried out in 50-µl reactions containing 5 µl of

... continued on page 2

## FailSafe™ PCR System "Never Fail at PCR Again"

### In This Issue

- 1 BuccalAmp™ DNA Extraction Kit
- 3 Consistent PCR of 20 Kb to > 40 Kb Templates
- 4 Prepare Custom EZ::TN™ Transposons by PCR
- NEW! 6 BAC Cloning Vectors and High Efficiency Electrocompetent Cells
- 7 Screen Your BAC Clones in 3 Hours and Plasmid Clones in 1 Hour
- 8 Stop Cloning or Subcloning Artifacts and Improve Your Cloning Efficiency
- 9 Sequence Through GC-Rich and AT-Rich Sequences
- 10 Make Stable Genomic Libraries of Cosmid-Sized Clones
- NEW! 11 Clone a PCR Product into the Vector of Your Choice
- 12 Direct PCR from a Single Bacterial Colony without DNA Extraction
- 13 High Yields of Yeast DNA
- 14 Create Random Gene Knockouts in Living Cells
- NEW! 15 Make Your Own R6K $\gamma$ ori Containing Transposon
- 16 DNA Ligations in 5 Minutes!

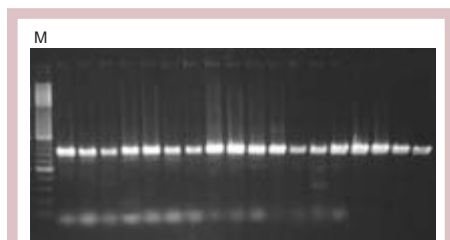
the extraction solution (i.e., 1% of the total volume), 2.5 Units of FailSafe™ PCR Enzyme Mix, and 10 pmoles of each PCR primer in FailSafe™ PCR PreMix A. PCR was performed in a 96-well plate using 30 cycles of 92° C for 40 seconds, 50° C for 40 seconds, and 72° C for 60 seconds. PCR reactions were analyzed by agarose gel electrophoresis.

**Multiplex PCR Amplification**

Multiplex PCR amplifications using Cooperative Human Linkage Center (CHLC) Markers were performed in 50-µl reactions containing 5 µl of extracted buccal cell DNA, 10 pmoles of each of the 10 primers, and 1.25 Units of FailSafe PCR Enzyme Mix in FailSafe PCR PreMix G. The cycling profile for the multiplex amplification was 30 cycles of 92° C for one minute, 50° C for one minute, and 72° C for one minute.

**Results and Discussion**

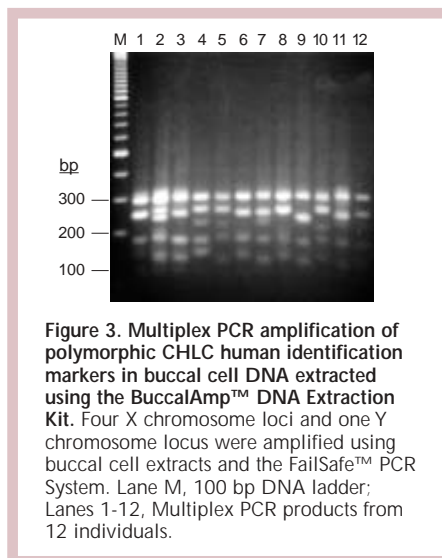
The DNA yield from BuccalAmp DNA Extraction Kit samples typically range from 1-7 µg, which is sufficient for at least 100 PCR amplifications. The human β-globin gene was successfully amplified for all 96 samples as shown by the presence of a 750-bp PCR product on agarose gels (Figure 2). (Note: we have also successfully amplified the β-globin gene using only 1 µl of sample extract.)



**Figure 2. PCR amplification of the β-globin gene using human buccal cell DNA extracted using the BuccalAmp™ DNA Extraction Kit.** Lanes M, 100 bp DNA ladder; Other lanes show representative PCR results obtained using buccal cell extracts from 18 self-collected samples.

In the second set of experiments, genomic DNA obtained using the BuccalAmp DNA Extraction Kit was used for genotyping using CHLC primers. CHLC primers amplify a group of highly polymorphic short tandem repeats consisting of tri- and tetra-nucleotide repeats. Four of the sets of CHLC primers used amplify regions of the X chromosome, and the fifth set amplifies a region on the Y chromosome. Multiplex amplifications were performed with these primers using 12 DNA samples. All twelve samples amplified well

with the X chromosome primers which produce products ranging in size from 118 to 299 bp in length (Figure 3). The Y chromosome primers produce products between 205 and 221 bp in length. Products from these primers were seen in samples 2, 4, 5, 7, 8, and 10-12 (Figure 3). As shown, the high quality of the extract DNA resulted in good multiplex amplification using CHLC primers, enabling the genotypes of all samples to be readily differentiated.



**Figure 3. Multiplex PCR amplification of polymorphic CHLC human identification markers in buccal cell DNA extracted using the BuccalAmp™ DNA Extraction Kit.** Four X chromosome loci and one Y chromosome locus were amplified using buccal cell extracts and the FailSafe™ PCR System. Lane M, 100 bp DNA ladder; Lanes 1-12, Multiplex PCR products from 12 individuals.

Based on the experiments described above, the BuccalAmp DNA Extraction Kit offers many benefits and advantages over other methods. These include:

**Simple and rapid sample processing:** The only step required for the DNA extraction process is heating for 45 minutes. No centrifugation or column step is needed. Due to the simplicity of the protocol, many samples can be processed simultaneously, decreasing the time required per sample. After dispersing the buccal cells into the QuickExtract™ DNA Extraction Solution, sample preparation was less than one hour for a 96-well plate and actual hands-on time was only seconds per sample.

**No risk for sample cross contamination:** The elimination of other steps, such as centrifugation and DNA columns, reduces the possibility of cross contamination and avoids mislabeling of tubes. Reduction in these risks, common for multiple step DNA extraction processes, ensures the accuracy of results.

**Simpler and safer sample collection and handling:** The Catch-All Swab supplied with the BuccalAmp Kit is made from a soft foam and causes no distress even for children. This results in improved

compliance and cooperation from sample donors.

**Self-sampling with reduced sampling cost:** Samples can be self-collected by donors with minimal instruction. The fact that the self-collected samples were amplifiable in multiplex PCR demonstrates that BuccalAmp DNA extraction is an excellent alternative to blood draws.

**Easy and safer sample storage and transportation:** Dry buccal cell samples on the Catch-All Swabs can be stored for an extended period of time and transported at ambient temperature without compromising the genomic DNA. Concerns over sample spillage and deterioration, frequently encountered in blood sample collection, are eliminated.

**No blood born hazards:** Since there is no blood involved, BuccalAmp DNA Extraction eliminates exposure to any blood borne hazards.

**Conclusion**

The one-step BuccalAmp DNA Extraction Kit provides an effective and efficient new method to extract genomic DNA for PCR. The simplicity of buccal cell sampling and collection offers convenience for large-scale genomic research or clinical diagnosis and offers a less hazardous alternative to blood sampling. The simplicity of DNA extraction using the kit also facilitates high throughput applications or automated processes.

**BuccalAmp™ DNA Extraction Kit**

BQ0901S	1 Kit
BQ0908S	8 Kits
BQ0916S	16 Kits

**Contents:**

- 15 tubes (1 extraction/tube) of BuccalAmp™ QuickExtract™ Solution 1.0.
- 15 individually-packaged sterile Catch-All™ Swabs.

**QuickExtract™ DNA Extraction Solution 1.0**

QE09050	50 ml
Bulk solution, sufficient to perform 100 extractions.	

**Catch-All™ Sample Collection Swabs**

QEC091H	100 swabs
100 individually-packaged swabs in sterile hard-pack plastic cylinders.	

Contact EPICENTRE or your local distributor to discuss discounts for larger quantities, special packaging, or standing orders for any of the above items.

# Get Consistent PCR of Templates 20 Kb to >40 Kb Using the MasterAmp™ Extra-Long PCR System

The MasterAmp™ Extra-Long PCR System enables consistent, high fidelity amplification of long DNA sequences of 20 Kb to >40 Kb in length. This increased consistency and fidelity is accomplished by combining the MasterAmp™ Extra-Long DNA Polymerase ... a unique blend of high fidelity thermostable polymerases...with an extensively tested set of MasterAmp™ Extra-Long PCR 2X PreMix solutions. Each PreMix contains dNTPs, buffer, and various amounts of MgCl<sub>2</sub> and MasterAmp™ PCR Enhancer.

Obtaining optimal results using the MasterAmp Extra-Long PCR System is easy. Start with the MasterAmp Extra-Long PCR Kit which contains the MasterAmp™ Extra-Long DNA Polymerase Mix and 9 MasterAmp Extra-Long PCR 2X PreMixes. Then...

- 1 Prepare a PCR cocktail containing your template/primer pair and the MasterAmp Extra-Long DNA Polymerase Mix. Perform your long PCR using each of the 9 MasterAmp Extra-Long PreMixes (Figure 1).
- 2 Run the PCR products on a gel and identify the PreMix that provides the PCR product of the correct size (Figure 2). Use the chosen MasterAmp Extra-Long PCR PreMix every time that you perform this particular PCR to ensure consistent and reliable results.
- 3 Repeat the process with every new template/primer pair or purchase the MasterAmp Extra-Long DNA Polymerase Mix and the MasterAmp Extra-Long PCR 2X PreMixes separately.

With the MasterAmp™ Extra-Long PCR System you get:

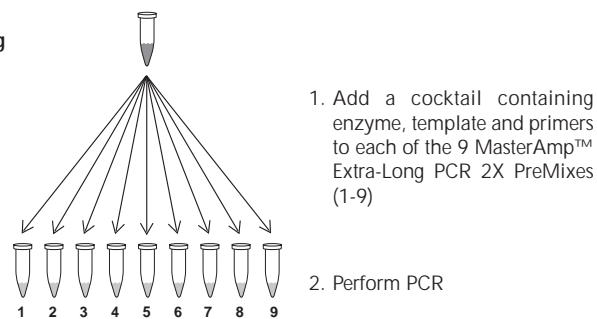
## DNA Amplification up to >40 Kb from Any Template

Using the MasterAmp Extra-Long PCR System, amplifications of 20 Kb up to >40 Kb are obtained rapidly and consistently from any genomic DNA template (Figure 3). Typically, "hot start" PCR is not required.

## Consistent and Reliable Results

The MasterAmp Extra-Long PCR System gives consistent and reliable results due in part to the MasterAmp Extra-Long PCR

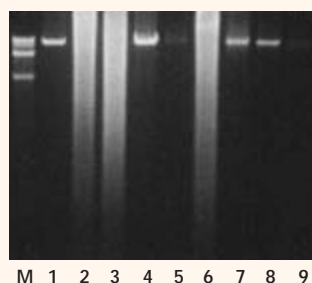
Figure 1. Procedure for rapid optimization of long PCR using the MasterAmp™ Extra-Long PCR Kit.



1. Add a cocktail containing enzyme, template and primers to each of the 9 MasterAmp™ Extra-Long PCR 2X PreMixes (1-9)

2. Perform PCR

Figure 2. Amplification of a 20 Kb region of lambda DNA using each of the 9 MasterAmp Extra-Long PCR 2X PreMixes (1-9) contained in the MasterAmp™ Extra-Long PCR Kit. PreMix 4 produced optimal results. M, DNA size marker.



3. Choose the best MasterAmp Extra-Long PreMix (Figure 2)

2X PreMixes. Select the MasterAmp Extra-Long PreMix that provides the best amplification for your template/primer pair (Figure 2) and use that PreMix every time you perform that PCR for the most consistent and reliable long PCR results.

## High Fidelity PCR of Long DNA for Sequencing and Expression

The MasterAmp Extra-Long DNA Polymerase Mix contains a 3' → 5' proof-reading enzyme that delivers fidelity at least 3-times higher than Taq DNA Polymerase.

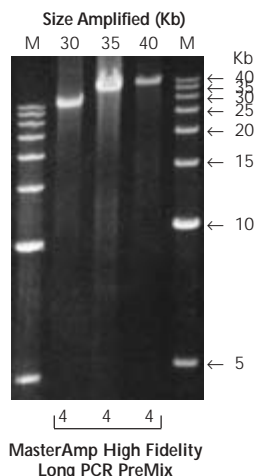


Figure 3. Amplification of 30, 35, and 40 Kb sequences from lambda DNA. One nanogram of lambda DNA was used to amplify 30, 35, and 40 Kb sequences. Lane M, 5 Kb DNA ladder. Results were analyzed on a 0.5% agarose gel.

## MasterAmp™ Extra-Long PCR Kit

MHF9220 50 Reactions

### Contents:

MasterAmp™ Extra-Long PCR 2X PreMixes 1-9  
MasterAmp™ Extra-Long DNA Polymerase Mix  
Control Lambda DNA/Primers

MasterAmp™ Extra-Long DNA Polymerase and individual Extra-Long PCR 2X PreMixes are available separately. Visit [www.epicentre.com/catalog/extra\\_long.htm](http://www.epicentre.com/catalog/extra_long.htm)

# Prepare Custom EZ::TN™ Transposons by PCR Using Primers with Transposase-Specific Mosaic End (ME) Sequences

Haiying Grunenwald and Jim Pease, EPICENTRE

An EZ::TN™ Transposon can be made from any DNA sequence by placing it between two 19-basepair inverted repeat Mosaic End (ME) sequences that are uniquely and specifically recognized by EZ::TN Transposase (Figure 1). EPICENTRE provides a number of pre-made EZ::TN Transposons for a variety of applications (e.g., see [www.epicentre.com/transposomics.htm](http://www.epicentre.com/transposomics.htm)). One way to make custom EZ::TN Transposons is to clone DNA of interest into the multiple cloning site of one of a series of EZ::TN pMOD™ <MCS> Transposon Construction Vectors available from EPICENTRE (see page 15). In this report we present an alternative method, for generating an EZ::TN Transposon called the “ME-Tailed PCR Method”.

The ME-Tailed PCR Method for making custom EZ::TN Transposons is shown in Figure 2. The DNA of interest, which can be anything - a resistance marker, a gene, a control element, etc. - is amplified by PCR using primers that, in addition to having 3'-sequences homologous to the template, also have non-homologous tails with 19-base ME sequences at their 5'-ends. PCR amplification of the template using these ME-Tailed primers produces an EZ::TN Transposon that can be used directly, without further purification, for *in vitro* insertion into any DNA target.

## Materials and Methods

### PCR templates

Three different DNA templates – a plasmid, genomic DNA, and a PCR product - were amplified using the ME-Tailed PCR Method. The three templates were an

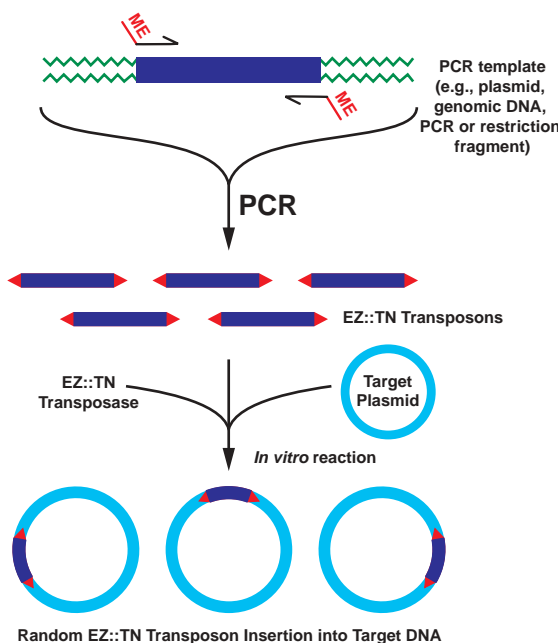


Figure 2. An EZ::TN™ Transposon can be rapidly produced from any DNA template by PCR using primers that contain the 19-base ME sequence at their 5'-ends. The EZ::TN Transposon produced can be used, without purification, for efficient and random insertion into a target DNA, *in vitro*. The ◀▶ designate the ME sequence at the ends of the EZ::TN Transposon.

1108-bp kanamycin resistance marker (Kan<sup>R</sup>) in pJK1; an 887-bp Dihydrofolate Reductase (DHFR) gene in *E. coli* strain BW19851 genomic DNA; and the same 887-bp DHFR gene as a PCR product.

### ME-tailed PCR primers

ME-Tailed PCR primers were designed to contain the 19-base EZ::TN Transposon ME sequence at their 5'-ends in addition to at least a 19-base sequence, homologous to the template at the 3'-ends. For example, the PCR primers used to produce a Kan<sup>R</sup> EZ::TN Transposon from pJK1 were:

5' - **CTGTCTCTTATACACATCTCT** - CAAAATCTCTGATGTTACATTGC-3'

5' - **CTGTCTCTTATACACATCT**GGTTGATGAGAGCTTTGTTGAGGT-3'

The sequence shown in black is homologous to the template while sequence shown in red is the 19-base ME sequence.

### PCR conditions

PCR reactions were performed in 50 μl containing 50 pmoles of each primer, 1 ng of template DNA, 2.5 U of FailSafe™ PCR Enzyme Mix, and either FailSafe PCR 2X PreMix C for amplifying the Kan<sup>R</sup> gene or FailSafe PCR 2X PreMix D for amplifying the DHFR gene. Cycling conditions used for the Kan<sup>R</sup> EZ::TN Transposon production were: 94°C for 2 minutes followed by 30 cycles of 1 minute at 94°C, 1 minute at 63°C, 1.5 minutes at 72°C.

The yield of each EZ::TN Transposon PCR product was determined by fluorometry. The EZ::TN Transposons produced by the ME-Tailed PCR Method were designated EZ::TN <KAN> and EZ::TN <DHFR>. The “< >” denote the 19-base ME sequence.

### *In vitro* insertion of EZ::TN Transposons into target DNA

The EZ::TN <KAN> and EZ::TN <DHFR> Transposons produced by PCR were used

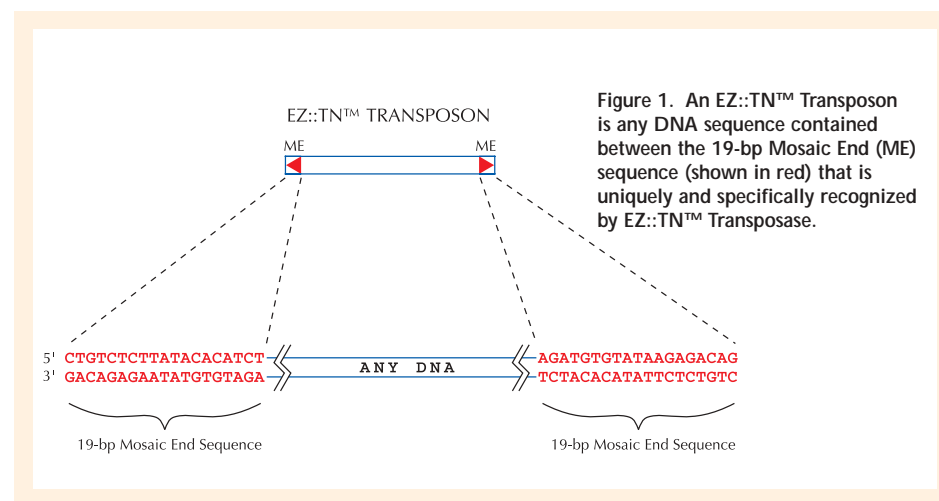


Figure 1. An EZ::TN™ Transposon is any DNA sequence contained between the 19-bp Mosaic End (ME) sequence (shown in red) that is uniquely and specifically recognized by EZ::TN™ Transposase.

for *in vitro* insertion into target DNA without further purification or modification (e.g. end-polishing). The target DNA contained a 3.4-Kb insert in pUC19 (designated as pUC19/3.4). Standard EZ::TN Transposon *in vitro* insertion reactions were:

1 µl	10X Reaction Buffer (0.50 M Tris-acetate, pH 7.5; 1.5 M potassium acetate; 100 mM magnesium acetate; 40 mM spermidine)
2 µl	0.2 µg of pUC19/3.4 target DNA (100 ng/µl)
X µl	Molar equivalent of EZ::TN <KAN> or EZ::TN <DHFR> Transposon
X µl	Sterile water to a final volume of 9 µl
1 µl	EZ::TN Transposase (10 U/µl)
10 µl	Total Volume

Insertion reactions were incubated for 2 hours at 37°C, and then terminated by the addition of 1 µl of 1% SDS and heating for 10 minutes at 70°C.

A 1 µl aliquot of each insertion reaction was used for electroporation of TransforMax™ EC100™ *E. coli* cells. Transposon insertion clones were selected by overnight growth on plates containing 100 µg/ml of ampicillin to select for the vector and either 50 µg/ml of kanamycin to select for EZ::TN <KAN> Transposon insertion clones or 10 µg/ml of trimethoprim to select for EZ::TN <DHFR> Transposon insertion clones.

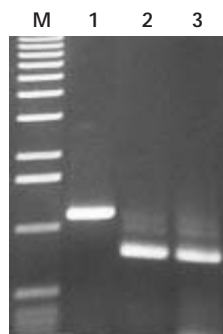
## Results

### Yield and integrity of EZ::TN Transposons made using the ME-Tailed PCR Method

The FailSafe PCR system was used for PCR in order to assure reliable, high fidelity synthesis of functional EZ::TN Transposons. As determined by agarose gel electrophoresis, full-length transposons were produced for both EZ::TN <KAN> and EZ::TN <DHFR> Transposons (Figure 3). Approximately 7.5 µg of EZ::TN Transposon was obtained from each 50 µl PCR reaction – enough for >100 *in vitro* insertion reactions.

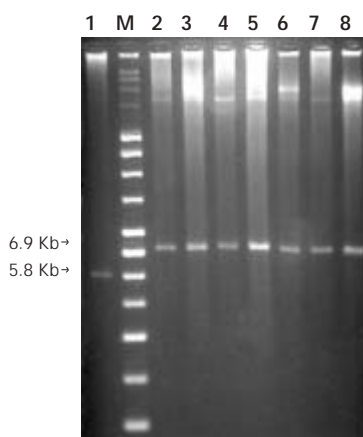
### *In vitro* transposition efficiency of the EZ::TN <KAN> and EZ::TN <DHFR> Transposons

Transposition efficiencies, defined as the ratio of the number of Amp<sup>R</sup>/Kan<sup>R</sup> or Amp<sup>R</sup>/Trimethoprim<sup>R</sup> EZ::TN Transposon



**Figure 3.** The ME-Tailed PCR Method produced full-length 1108 bp EZ::TN <KAN> Transposon from a plasmid DNA template (Lane 1) and 887 bp EZ::TN <DHFR> Transposon from *E. coli* genomic DNA template (Lane 2) and from a PCR product template (Lane 3). Lane M, DNA size markers.

insertion clones to Amp<sup>R</sup> target clones in seven independent *in vitro* insertion reactions, averaged 0.13% for the EZ::TN <KAN> Transposon and 0.68% and 0.30%, respectively, for the genomic- and PCR template-derived EZ::TN <DHFR> Transposons. Although the transposition efficiencies of transposons prepared using the ME-Tailed PCR Method were about 10-fold lower than those of EPICENTRE's commercially available EZ::TN <KAN-2> and EZ::TN <DHFR-1> Transposons, transposons prepared using the ME-Tailed PCR Method still generate sufficient numbers of *in vitro* insertions for many applications. For example, the three transposons prepared using the ME-Tailed PCR Method described here generated >10<sup>5</sup> *in vitro* insertion clones per 0.2 µg of



**Figure 4.** An EZ::TN Transposon produced by the ME-Tailed PCR Method is efficiently inserted into a target DNA, *in vitro*. Seven randomly chosen Amp<sup>R</sup>/Kan<sup>R</sup> clones were individually processed using the Colony Fast-Screen™ Kit (see p. 7) and the size of the clones analyzed by agarose gel electrophoresis in less than 1 hour. Lane M, Supercoiled DNA size marker; Lane 1, 5.8 Kb pUC19/3.4 DNA; Lanes 2-8, randomly chosen EZ::TN <KAN> Transposon insertion clones. The size of these clones (6.9 Kb) reflect the insertion of a single 1.1 Kb EZ::TN <KAN> Transposon into pUC19/3.4.

pUC19/3.4 as a target. These results compare to >10<sup>6</sup> insertion clones per insertion reaction obtained using the comparable commercially available EZ::TN Transposons from EPICENTRE.

### Analysis of the insertion clones

Putative EZ::TN <KAN> Transposon and EZ::TN <DHFR> Transposon insertion clones were analyzed based on size in less than an hour using EPICENTRE's Colony Fast-Screen™ Kit (see p. 7). All clones analyzed had the expected size for insertion of a single 1108-bp EZ::TN <KAN> Transposon or 887-bp EZ::TN <DHFR> Transposon, respectively. Representative gel data are shown in Figure 4.

## Conclusions

Custom EZ::TN Transposons can be prepared by PCR amplification of plasmid-, genomic- or PCR-derived templates using PCR primers with a non-template-homologous 5'-tail, consisting of a 19-base ME sequence that is recognized by the transposase. Transposons generated using this ME-Tailed PCR Method can be used directly, without purification, for random *in vitro* insertion into any target DNA in a simple 2-hour reaction. The *in vitro* transposition efficiencies of unpurified transposons generated using the ME-Tailed PCR Method are about 10-fold less than the corresponding purified commercially available EZ::TN Transposons. These unpurified transposons are more than active enough (e.g., transposition efficiency of about 10<sup>5</sup> insertion clones per 0.2 µg of pUC19/3.4 target) for use in many *in vitro* insertion applications.

At this time, we recommend using EZ::TN Transposons made using the EZ::TN pMOD™ <MCS> series of Transposon Construction Vectors (see p. 15) for making EZ::TN Transposomes - stable complexes of an EZ::TN Transposon and EZ::TN Transposase that can be electroporated directly into living cells.

### EZ::TN™ Transposase

TNP92110 10 Units

### Colony Fast-Screen™ Kit

FS08250 1 Kit  
Reagents sufficient for screening 250 colonies.

### \*FailSafe™ PCR PreMix Selection Kit

FS99060 48 Reactions

\* See center insert for details.

Special Offer good until 8/3/01  
Limit of 3 per order, please.

# Cloning-Ready Bacterial Artificial Chromosome (BAC) Vectors

## pIndigoBAC-5 (*Bam*H I-Cloning Ready) Vector

## pIndigoBAC-5 (*Hind* III-Cloning Ready) Vector

EPICENTRE's Cloning-Ready pIndigoBAC-5 is derived from pBeloBAC11 and pIndigoBAC. pIndigoBAC-5 has been linearized at either its unique *Bam*H I or its unique *Hind* III site. The linearized DNA is then dephosphorylated and purified and is ready for cloning *Bam*H I or *Hind* III restriction cut genomic DNA. Each preparation of Cloning-Ready pIndigoBAC-5 vectors is tested to ensure the completeness of linearization, dephosphorylation and for the integrity of the *Bam*H I and *Hind* III ends.

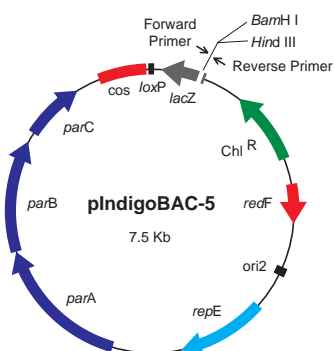


Figure 1. Cloning-Ready pIndigoBAC-5 vectors are supplied linearized at either the unique *Bam*H I or *Hind* III site and then completely dephosphorylated.



Figure 2. A genomic BAC library constructed in pIndigoBAC-5 (*Hind* III-Cloning Ready) vector produced >95% white recombinant clones.

Sequencing primers for BAC end-sequencing of pIndigoBAC-5 clones are available separately.

### Features of the Cloning-Ready pIndigoBAC-5 vectors

- Derived from pBeloBAC11 and pIndigoBAC.
- Linearized and dephosphorylated and ready for cloning.

- Enhanced blue/white screening of recombinants.
- Tested to ensure complete linearization and dephosphorylation and the integrity of the *Bam*H I and *Hind* III ends.

### pIndigoBAC-5 (*Bam*H I-Cloning Ready)

BACB085H 500ng 25 ng/μl  
Supplied linearized at *Bam*H I site and dephosphorylated.

### pIndigoBAC-5 (*Hind* III-Cloning Ready)

BACH095H 500 ng 25 ng/μl  
Supplied linearized at *Hind* III site and dephosphorylated.

### BAC End-Sequencing Primers

#### pIndigoBAC-5 Forward Sequencing Primer

BFP0701 1 nmole

#### pIndigoBAC-5 Reverse Sequencing Primer

BRP0801 1 nmole  
Each Primer is supplied at 50 μM in TE Buffer.

## Get >4X10<sup>6</sup> BAC Clones/μg DNA Using TransforMax™ EC100™ Electrocompetent *E. coli*

TransforMax™ EC100™ Electrocompetent *E. coli* provide the highest transformation efficiency available and are ideal for preparing BAC libraries and for almost every cloning application. TransforMax EC100 cells are restriction minus for efficient cloning of mammalian DNA and readily accept large clones (Table 1, Figure 1).

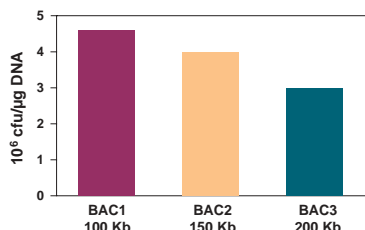


Table 1. Transformation efficiency of TransforMax™ EC100™ Electrocompetent *E. coli* using purified BAC clones containing inserts of 100 Kb, 150 Kb and 200 Kb. Results shown are the averages of nine transformation experiments.

### Genotype

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



Figure 1. TransforMax™ EC100™ Electrocompetent *E. coli* can produce > 4X10<sup>6</sup> BAC clones/μg DNA. 1 μl (77 ng) of a purified BAC clone containing a 150 Kb insert was used to transform 50 μl TransforMax EC100 Electrocompetent *E. coli* and produced >4X10<sup>6</sup> cfu/μg DNA.

### Important Phenotypes & Applications

- Blue/white screening of vectors expressing the *LacZ*' α-complementing peptide.
- Restriction minus for efficient cloning of methylated DNA (e.g. mammalian genomic DNA).
- Accepts large clones for unbiased BAC library production (Table 1).
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

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

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

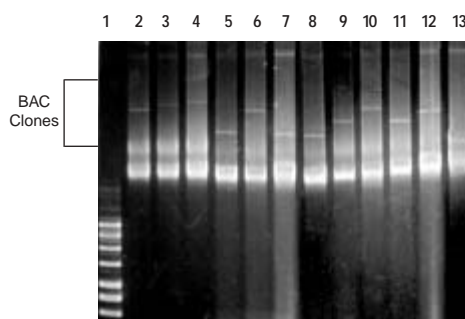
Each includes pUC19 control DNA.

## Screen Your BAC Clones in 3 Hours and Plasmid Clones in 1 Hour Without Minipreps and Restriction Digests Using the Colony Fast-Screen™ Kit

Save days in screening your BAC library without the need to grow cultures, perform minipreps and restriction endonuclease (e.g. *Not*I) digestions using the Colony Fast-Screen™ Kit. The process for using the Colony Fast-Screen Kit for screening BAC clones is shown in Figure 1. Plasmid clones can be screened in 1 hour or less with a slight modification to the BAC screening procedure.

### The Colony Fast-Screen Kit is:

- **Fast.** Screen BAC clones in 3 hours. Screen plasmid clones in 1 hour or less.
- **Sensitive.** Even low copy clones (e.g. BAC and Fosmid clones) are readily detected without the need to grow cultures.
- **High Through-put Capability.** Amenable to 96-well microtiter format.
- **Efficient.** Estimate the size of cloned inserts without the need to grow cultures or restriction endonuclease digests.



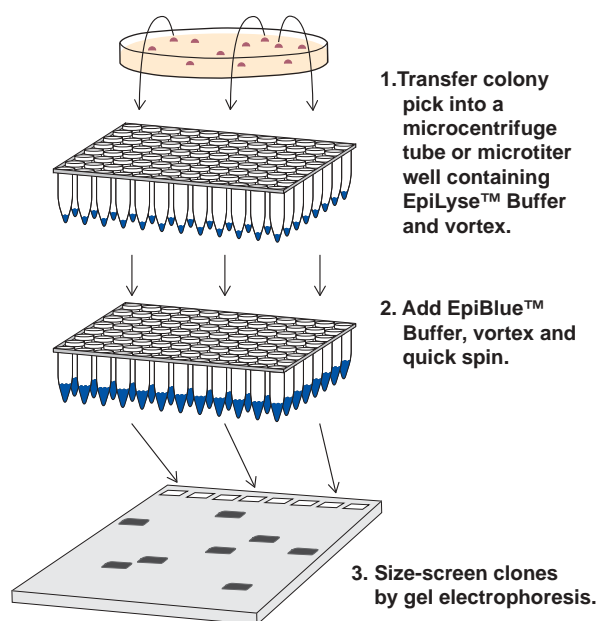
**Figure 2. The size of a BAC clone can be estimated in 3 hours using the Colony Fast-Screen™ Kit.**

Randomly chosen BAC clones were picked from an overnight plate and individually processed using the Colony Fast-Screen Kit. 25 µl aliquots of each processed clone were loaded on a 0.8% agarose gel in 1X TAE. The gel was run at 5V/cm for 3 hours at 4°C and then stained using SYBR® Gold. Lane 1, Supercoiled DNA ladder; Lane 2, 100 Kb BAC clone control; Lane 3, 150 Kb BAC clone control; Lane 4, 200 Kb BAC clone control; Lanes 5-12, randomly chosen BAC clones; Lane 13, vector clone (pIndigoBAC-5).

### Colony Fast-Screen™ Kit

FS08250 1 Kit  
Reagents sufficient for screening 250 colonies.

SYBR® is a registered trademark of Molecular Probes, Inc.



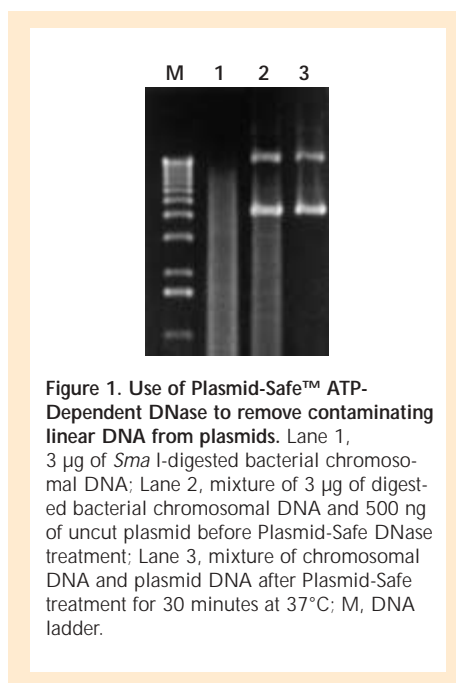
**Figure 1. BAC clones can be screened in 3 hours using the Colony Fast Screen™ Kit. Plasmid clones can be screened in 1 hour or less.**

## Stop Cloning or Subcloning Artifacts!

### Remove All Chromosomal DNA From Your Plasmid, Cosmid or BAC Cloning Vector Preps Using Plasmid-Safe™ DNase

Preparations of plasmid, cosmid, fosmid and BAC cloning vectors are frequently contaminated with fragments of bacterial genomic DNA that are generated during alkaline lysis. Commercial purification columns and even CsCl centrifugation do not effectively remove all these contaminants. Ultimately, the contaminating DNA fragments are ligated into the cloning vector and result in false positives and high backgrounds.

**Plasmid-Safe™ ATP-Dependent DNase** provides a powerful, fast and easy method to completely remove all traces of bacterial chromosomal DNA contamination from cloning vector preparations in one hour. Plasmid-Safe is an ATP-dependent DNase that selectively digests



**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, mixture of 3 µg of digested bacterial chromosomal DNA and 500 ng of uncut plasmid before Plasmid-Safe DNase treatment; Lane 3, mixture of chromosomal DNA and plasmid DNA after Plasmid-Safe treatment for 30 minutes at 37°C; M, DNA ladder.

linear double-stranded DNA but has no activity on nicked, closed-circular dsDNA or supercoiled DNA. Therefore, Plasmid-Safe DNase is recommended as a final purification step for all cloning vector preparations.

Plasmid-Safe™ ATP-Dependent DNase	
E3101K	1,000 U
E3105K	5,000 U
E3110K	10,000 U

## Simplify Dephosphorylation of Your Cloning Vectors Using Heat-Labile HK™ Phosphatase

Derived from an Antarctic bacterium, HK™ (Heat-Killable) Phosphatase greatly simplifies dephosphorylation of cloning vectors prior to ligation of the DNA insert. The enzyme is as effective as calf intestinal alkaline phosphatase (CIP) and

bacterial alkaline phosphatase (BAP) in removing phosphates from protruding 5'-end of dsDNA generated by many restriction endonucleases (e.g., *Bam*H I, *Eco*R I, *Hind* III, etc.). However, unlike CIP and BAP, HK Phosphatase is completely and irreversibly inactivated by incubation at 70°C for 15 minutes (Table 1). Note: we do not recommend using HK Phosphatase for blunt ends and 3'-protruding ends.

**Unit Definition:** One Molecular Biology Unit (MBU) dephosphorylates 1 µg of *Hind* III-digested pUC19 in one hour at 30°C in 1X TA Buffer.

### Reference

- Hoffman, L.M. and Jendrisak, J. (1990) *Gene* **88**: 97.

**Table 1. HK™ Phosphatase is completely inactivated after heat treatment at 70°C for 15 minutes.**

Phosphatase	% Activity After Heating
HK Phosphatase	0
BAP	41
CIP	62

Each phosphatase was incubated at 70°C for 15 minutes. Remaining phosphatase activity was then determined by incubation with 5'-<sup>32</sup>P-labeled RNA.

### HK Phosphatase is:

- Completely and irreversibly heat-inactivated.
- Active in most restriction enzyme buffers so that restriction enzyme digestion, dephosphorylation and DNA ligation can be performed in a single tube, without time-consuming and yield-reducing phenol extraction or ethanol precipitation.
- Active in removing the 5'-phosphate from DNA and RNA.

### HK™ Phosphatase

H92025	25 MBU*
H92050	50 MBU*
H92100	100 MBU*

Supplied at 1 U/µl. Includes 10X TA Buffer and 0.1 M CaCl<sub>2</sub>.

\*MBU = Molecular Biology Unit

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

EPICENTRE's AmpliScribe™ T7, SP6 & T3 High Yield Transcription Kits and AmpliScribe T7, T3 & SP6 RNA Polymerases, are specially formulated to utilize high concentrations of NTPs that are inhibitory to other systems. An AmpliScribe reaction will incorporate up to 90% of input NTPs. The result is an exceptionally high yield of RNA from an AmpliScribe High Yield Transcription reaction.

## The Highest Yield of Full-Length Long RNA

AmpliScribe T7 High Yield Transcription Kits consistently produce up to 150 µg of long RNA from a standard 20 µl, 2-hour reaction. In a direct comparison with competitors' kits, the AmpliScribe T7 Kit produced the highest yields of RNA from three different templates tested.<sup>1</sup> In addition, the AmpliScribe RNA Polymerase Mixes contain an RNase inhibitor to ensure the integrity of the RNA product.

## The Highest Yield of Short RNA

Now, there is no need to buy a specialized kit to make short (<300) RNA transcripts. An AmpliScribe Transcription Kit will also produce exceptionally high yields of short RNA. In fact, the AmpliScribe T7 High Yield Transcription Kit produced more of a short (63 base) RNA than a competitor's kit designed to make short transcripts.

## Produce Milligram Amounts of RNA

Scale up an AmpliScribe T7 reaction to produce milligram amounts of RNA (Figure 2).

## Now! Produce Fluorescent-Labeled RNA Probes

An AmpliScribe High Yield reaction can be readily modified to incorporate Cy<sup>5</sup>-UTP (Figure 3), Cy3, and fluorescein-12-CTP and fluorescein-12-UTP to produce fluorescent-labeled RNA probes<sup>2</sup> for gene expression studies or *in situ* hybridization.

## The Best Value for *In Vitro* Transcription

Compare our yields. Compare our price. You will see that the AmpliScribe High Yield Transcription Kits provide the best value for *in vitro* transcription.

### References

1. Schanke, J. T. (2000) *EPICENTRE Forum* 7(2), 6.
2. DeLong, E. F., et al., (1999) *App. and Environ. Microbiol.* 65, 5554.

**Save up to 25% per reaction  
compared to a competitor!**

AmpliScribe™	Catalog No.	Size
T7 High Yield Kit	AS2607	25 reactions
	AS3107	50 reactions
T3 High Yield Kit	AS2603	25 reactions
	AS3103	50 reactions
SP6 High Yield Kit	AS2606	25 reactions
	AS3106	50 reactions

Kits include AmpliScribe™ RNA Polymerase (with RNase inhibitor), AmpliScribe™ 10X Reaction Buffer, NTPs, RNase-free DNase I, DTT and Control Template. Labeled nucleotides are not included.

AmpliScribe Kits are available in quantity and bulk discount. Please inquire.

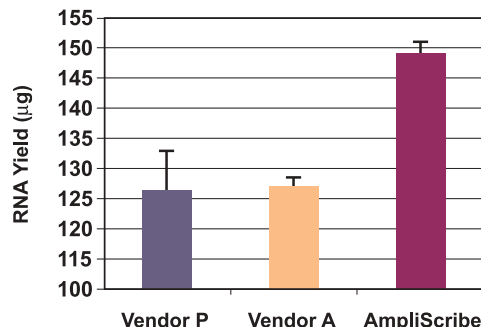


Figure 1. AmpliScribe™ T7 High Yield Transcription Kit consistently produced the highest yield of a 1.8 Kb RNA. Up to 150 µg of RNA was produced using the AmpliScribe™ T7 High Yield Kit in a standard 20 µl, 2-hour reaction.

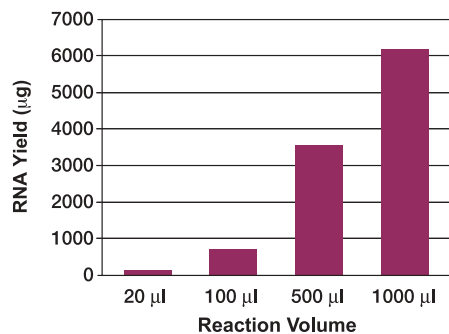


Figure 2. The standard 20 µl AmpliScribe™ T7 High Yield Transcription reaction can be scaled-up to produce milligram amounts of full-length RNA.

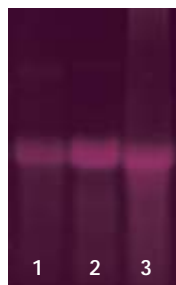


Figure 3. Full-length fluorescent-labeled RNA can be readily produced using AmpliScribe™ High Yield Transcription Kits. A 1.4 Kb Cy5-labeled RNA was produced in a 2-hour, 20 µl AmpliScribe reaction containing 7.5 mM each ATP, CTP, GTP; 5 mM UTP; 1.2 mM Cy5-UTP and 1 µg linearized DNA template. Following gel electrophoresis, the Cy5-labeled RNA was visualized, without staining, on a UV transilluminator. Lane 1, AmpliScribe T7 reaction; Lane 2, AmpliScribe T3 reaction; Lane 3, AmpliScribe SP6 reaction.



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800-284-8474

# New! Get the Highest Yield of 5'-Capped RNA with the AmpliCap™ High Yield Message Maker Kits

## The Highest Yield of 5'-Capped RNA

The new AmpliCap™ T7, SP6 & T3 High Yield Message Maker Kits consistently produce more of a 1.4 Kb 5'-capped RNA than competitors' kits (Figure 1).

## High 5'-Capping Efficiency

An optimized m<sup>7</sup>G[5']ppp[5']G Cap/NTP PreMix is provided to maximize capping efficiency and convenience. Capping efficiencies up to 80% are obtained with all three kits.

## Full-length Capped Transcripts

AmpliCap RNA Polymerase Mixes contain an RNase inhibitor to ensure the integrity of the RNA. A vial of GTP is included for production of long capped RNA.

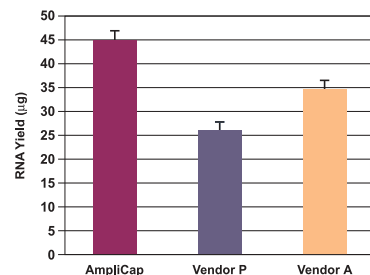


Figure 1. AmpliCap™ T7 High Yield Message Maker Kit consistently produces the highest yield of a 1.4 Kb 5'-capped RNA transcript. A standard 20 µl reaction produces up to 45 µg of 5'-capped RNA from a T7 or T3 reaction, and up to 35 µg from an SP6 reaction.

AmpliCap™	Catalog No.	Size
T7 Message Maker Kit	AC0707	25 reactions
T3 Message Maker Kit	AC0703	25 reactions
SP6 Message Maker Kit	AC0706	25 reactions

*Kits include an AmpliCap™ RNA Polymerase (with RNase inhibitor), AmpliCap™ 10X Reaction Buffer, Cap/NTP PreMix, GTP, RNase-free DNase I, DTT and Control Template*

## The Best Value in RNA Polymerases

Standard formulations of T7, T3 & SP6 RNA Polymerases, which should not be confused with the AmpliScribe™ or AmpliCap™ T7, T3 & SP6 RNA Polymerase formulations, are also available from EPICENTRE. The standard formulations of our RNA Polymerases feature:

### High Purity

Each RNA Polymerase is purified to very high specific activity, and tested to be free of detectable exo- and endonuclease, RNase activities and *E. coli* RNA polymerase activity. In addition each RNA Polymerase is function-tested in an *in vitro* transcription assay.

### Greatest Range of Concentrations Available

Our standard formulation T7, T3 & SP6 RNA Polymerases are available in a range of concentrations from 25 U/µl to 2500 U/µl.

### The Best Value in RNA Polymerases

Compare our quality. Compare our prices. You will find that EPICENTRE's T7, T3 & SP6 RNA Polymerases offer the best value.

Catalog No.	Conc.	Size
<b>T7 RNA Polymerase</b>		
TL910K	25 U/µl	10,000 U
T7905K	50 U/µl	5,000 U
T7910K	50 U/µl	10,000 U
T7925K	50 U/µl	25,000 U
T7950K	50 U/µl	50,000 U
TM905K	200 U/µl	5,000 U
TM910K	200 U/µl	10,000 U
TM925K	200 U/µl	25,000 U
TM950K	200 U/µl	50,000 U
TH925K	1,000 U/µl	25,000 U
TH950K	1,000 U/µl	50,000 U
TU950K	2,500 U/µl	50,000 U

*Enzyme only; Transcription Buffer is not included.*

*T7 RNA Polymerase is also available in bulk. Please inquire.*

Catalog No.	Conc.	Size
<b>SP6 RNA Polymerase</b>		
SL910K	25 U/µl	10,000 U
SL925K	25 U/µl	25,000 U
S7901K	50 U/µl	1,000 U
S7910K	50 U/µl	10,000 U
S7925K	50 U/µl	25,000 U
SM905K	200 U/µl	5,000 U
SM910K	200 U/µl	10,000 U
SM925K	200 U/µl	25,000 U
SH910K	1,000 U/µl	10,000 U
SU925K	2,500 U/µl	25,000 U

*Enzyme only; Transcription Buffer is not included.*

*SP6 RNA Polymerase is also available in bulk. Please inquire.*

Catalog No.	Conc.	Size
<b>T3 RNA Polymerase</b>		
TL010K	25 U/µl	10,000 U
T9001K	50 U/µl	1,000 U
T9010K	50 U/µl	10,000 U
T9025K	50 U/µl	25,000 U
T9050K	50 U/µl	50,000 U
TM005K	200 U/µl	5,000 U
TU050K	2,500 U/µl	50,000 U

*Enzyme only; Transcription Buffer is not included.*

*T3 RNA Polymerase is also available in bulk. Please inquire.*

<b>Transcription Buffer Package</b>		
BP1001		1 Pkg

*Includes 5 ml of 5X Transcription Buffer and 2.5 ml of 100 mM DTT.*

# COMPLETELY SEQUENCE PLASMID, COSMID AND BAC CLONES WITHOUT SUBCLONING OR PRIMER WALKING

The EZ::TN™ Transposon insertion reaction is a simple, one-step enzymatic reaction that randomly inserts an EZ::TN Transposon containing a selectable marker and sequencing primer binding sites into your plasmid, cosmid or BAC clone (Figure 1). Minipreps of plasmid and cosmid clones can be used in the EZ::TN insertion reaction. Transform *E. coli* with an aliquot of the reaction and select for EZ::TN Transposon insertion clones. Prepare sequencing template from randomly chosen transposon insertion clones and sequence each bidirectionally using a single set of sequencing primers (provided in the kits) that are homologous to the ends of the inserted EZ::TN Transposon.

## Reduce Your Sequencing Time by 10-Fold or More

Traditional methods of preparing sequencing template by shotgun subcloning or sequencing large clones by primer walking are labor intensive, time-consuming and costly. In contrast, a single EZ::TN Transposon insertion reaction generates more than one million templates – enough to completely sequence even the largest clone and saving you the time and expense usually spent subcloning or designing and synthesizing sequencing primers.

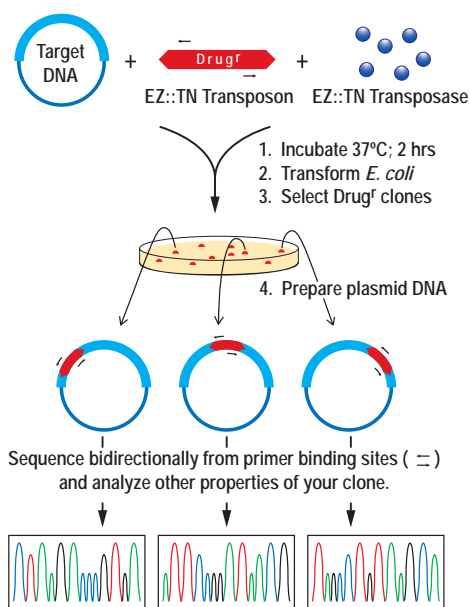


Figure 1. The process for generating insertion clones for sequencing and a myriad of other applications using an EZ::TN™ Insertion Kit.

**“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.

## Complete Sequence Coverage of Your Clone

The EZ::TN Transposon system is based on the hyperactive *in vitro* Tn5 transposition system (Goryshin and Reznikoff (1998) *J. Biol. Chem.*, 273, 7367) and is highly random. This high degree of randomness ensures that the sequencing priming sites are distributed throughout your clone and provides complete sequence coverage of the clone.

**“After evaluating several available methods, including primer walking, concatenated cDNA sequencing and multiple transposon-based systems, we decided to use the Tn5-based EZ::TN Transposon Insertion System because of its high performance in our setting.”** Yuriy Shevchenko, et al., NIH Intramural Sequencing Center, *EPICENTRE Forum* (2001) 8, 2.

Catalog No.	Quantity
<b>EZ::TN™ &lt;KAN-2&gt; Insertion Kit</b>	
EZ1982K	10 Reactions
<b>EZ::TN™ &lt;TET-1&gt; Insertion Kit</b>	
EZ1921T	10 Reactions
<b>EZ::TN™ &lt;DHFR-1&gt; Insertion Kit</b>	
EZ1912D	10 Reactions
Each kit contains the specific EZ::TN™ Transposon, EZ::TN™ Transposase, Buffers and two unlabeled sequencing primers. Contact EPICENTRE or your local distributor to discuss discounts for bulk quantities.	
<b>TransforMax™ EC100™ Electrocompetent <i>E. coli</i></b>	
EC10005	5 x 100 µl (10 electroporations)
EC10010	10 x 100 µl (20 electroporations)
TransforMax™ EC100™ Electrocompetent <i>E. coli</i> have the highest transformation efficiency available and are function tested for optimal EZ::TN Insertion reaction results.	



## The Most Versatile Transposition System

EPICENTRE offers a choice of EZ::TN Transposons because sequencing is often just part of your research project. In addition to primer binding sites and a selectable marker these transposons contain features that can be used in gene analysis, proteomics and RNA research.

### Find Functional Domains or Epitopes of Proteins

The EZ::TN™ In-Frame Linker Insertion Kit was designed to rapidly and easily produce random 19-amino acid (19 codon; 57-nucleotide) in-frame insertions into genes of expressed proteins for protein engineering, functional analysis, and domain or epitope mapping. The kit features the EZ::TN™ <Not I/KAN-3> Transposon, which contains a kanamycin resistance marker flanked by *Not I* restriction sites. Kanamycin-resistant insertion clones are digested with *Not I*, ligated, and re-transformed into *E. coli* (Figure 2). Since each resulting clone contains a random 19-codon insertion that can be read in all three reading frames the protein retains its original amino acid sequence on both sides of the insertion site.

### Synthesize RNA from Any Region of Your Cloned DNA

The EZ::TN™ <T7/KAN-2> Promoter Insertion Kit provides an easy and reliable method to randomly insert a phage T7 RNA polymerase promoter into any target DNA. The transposon does not have a transcription termination sequence so RNA can be produced from chosen insertion clones by *in vitro* transcription using an AmpliScribe™ T7 High Yield Transcription Kit, or *in vivo* after transformation of *E. coli* having an inducible T7 RNA polymerase gene (Figure 3).

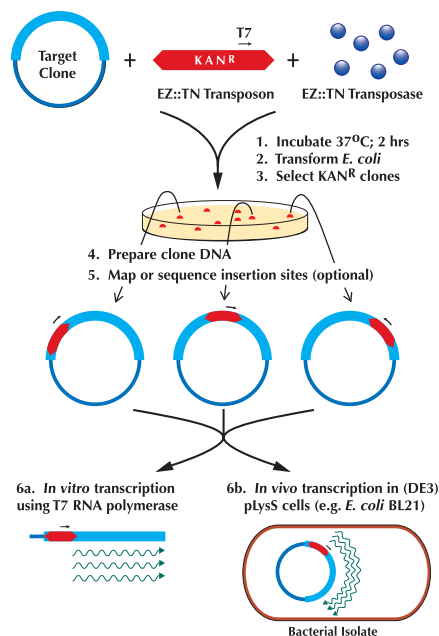


Figure 3. RNA transcripts can be generated *in vitro* or *in vivo* from EZ::TN™ <T7/KAN-2> Transposon insertions in target DNA.

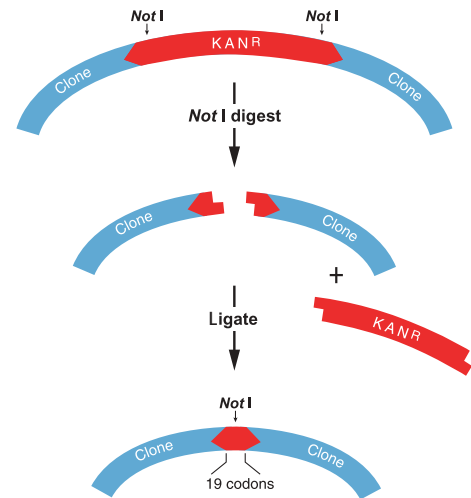


Figure 2. The EZ::TN™ <Not I/KAN-3> Transposon contains a kanamycin resistance gene flanked by *Not I* restriction sites. A 19-codon insertion that can be read in all three reading frames is generated following *Not I* digestion and ligation.

### Insert a Conditional Origin of Replication

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

Catalog No.	Quantity
<b>EZ::TN™ In-Frame Linker Insertion Kit</b>	
EZI04KN	10 Reactions
For sequencing cloned DNA then generating random 19 amino acid in-frame insertions into the encoded protein.	
<b>EZ::TN™ &lt;T7/KAN-2&gt; Insertion Kit</b>	
EZI03T7	10 Reactions
For random insertion of a T7 transcription promoter.	
<b>EZ::TN™ &lt;R6K<math>\gamma</math>ori /KAN-2&gt; Insertion Kit</b>	
EZI011RK	10 Reactions
For random insertion of the <i>E. coli</i> R6K $\gamma$ origin of replication.	
Each of the above kits contains the specific EZ::TN™ Transposon, EZ::TN™ Transposase, Buffers and two unlabeled sequencing primers.	
<b>TransforMax™ EC100D™ <i>pir</i><sup>+</sup> Electrocompetent <i>E. coli</i></b>	
ECP09500	5 X 100 $\mu$ l (10 Electroporations)
Maintains clones at 15 copies per cell. Includes control vector containing an R6K $\gamma$ ori.	
<b>TransforMax™ EC100D™ <i>pir</i>-116 Electrocompetent <i>E. coli</i></b>	
EC6P095H	5 X 100 $\mu$ l (10 Electroporations)
Maintains clones at 250 copies per cell. Includes control vector containing an R6K $\gamma$ ori.	

HIV *E. coli* Yeast

HCV Enterovirus HPV

*B. pertussis* Mammalian

*M. tuberculosis* RSV

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

***We guarantee it.***

Paraffin Tissues Liver

Saliva Serum/Plasma

Whole Blood Mouse Tail

Buccal Cells Kidney Soy

Urine Sputum Maize

Tissue Culture Cell Lines

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

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

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

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

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



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# MasterPure™

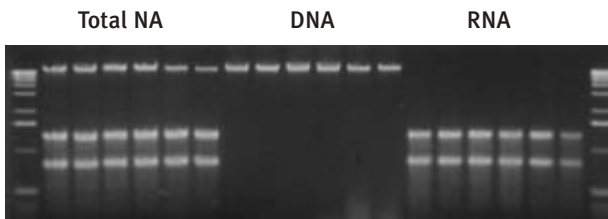
## DNA & RNA Purification Kits

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

TRY OUR  
10 PURIFICATION  
SIZE!

## Consistent Purity

Consistently obtain DNA or RNA free of protein.



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

## Extensive Sample Range

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

### Examples of Targets Analyzed

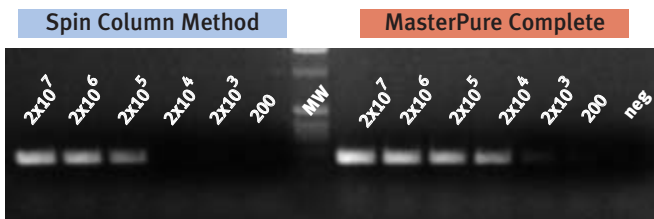
Mammalian Systems  
HIV  
*E. coli*  
HCV  
*B. pertussis*  
RSV  
Yeast  
*M. tuberculosis*  
Enterovirus  
HPV  
Soy  
Maize  
Insect Tissues

### Examples of Samples Extracted

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

## High Sensitivity

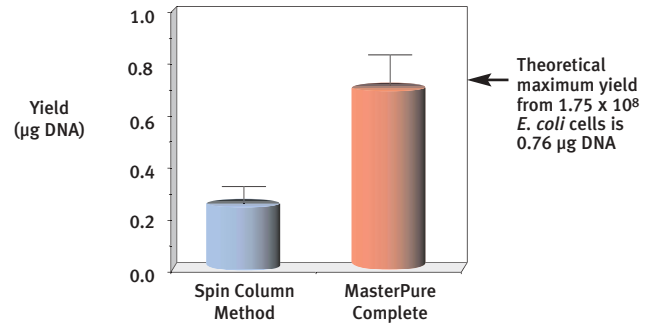
The MasterPure co-precipitant greatly improves sensitivity.



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

## Greater Yields

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



DNA was purified from  $1.75 \times 10^8$  *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

### MasterPure™ DNA Purification Kit

(for isolating TNA or DNA)

MCD85201 200 Purifications

### MasterPure™ RNA Purification Kit

(for isolating RNA only)

MCR85102 100 Purifications

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

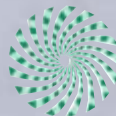
**Whatever the length,  
whatever the sequence,  
the *FailSafe™ PCR System*  
will faithfully amplify  
your template every time.**

At EPICENTRE, we're scientists like you and we usually don't take advertising slogans seriously. But this time we're making an exception. Our new FailSafe PCR System is a very different product; it is truly a much better way to amplify your DNA.

First, it's a **high fidelity system** that makes many fewer mistakes than regular Taq. It's also a **long PCR system** — we've been able to amplify greater than 20 kb and believe we can go much farther. It's a **"tough template" system**. It will amplify the highest GC DNA you can throw at it. And finally, it has our patented PCR Enhancement Technology, which makes it extremely reproducible from reaction to reaction. So you can see why this time we broke our own prohibition against using grandiose marketing claims.

We believe that if you use this enzyme system you will never have a bad PCR reaction, no matter what your template.

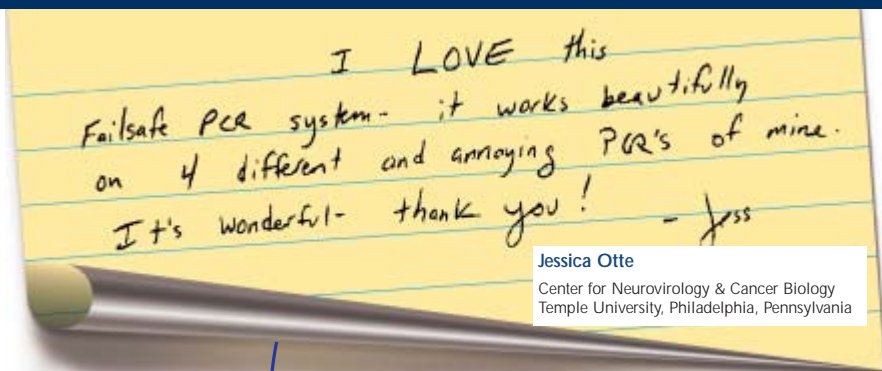
***That's why we signed this ad,  
and that's our promise to you.***



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# Here's what researchers using the FailSafe™ PCR System are telling us...



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



PCR results obtained using the FailSafe™ PCR System to screen for mouse knockout Gene P. PCR reactions with mouse genomic DNA and two forward primers with one shared reverse primer. (Data courtesy of Jessica Otte).

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

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

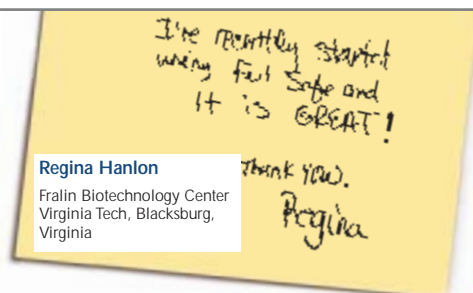


Two regions of *Streptomyces coelicolor* DNA which I had previously found to be impossible to amplify using other DNA polymerases were amplified first time using the FailSafe™ PCR PreMix Selection Kit. The letters A-L denote the PCR PreMixes used from the kit. In each case the J PreMix was optimum. M = marker. (Data courtesy of Karen Jolly).

**From:** Moises Hernandez, CDC  
**To:** Epicentre

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

**Moises Hernandez**  
Centers for Disease Control and Prevention  
Atlanta, Georgia



**Regina Hanlon**  
Fralin Biotechnology Center  
Virginia Tech, Blacksburg,  
Virginia

## FailSafe™ PCR PreMix Selection Kit\*

Cat. No.	Size
FS99060	48 reactions*

\*The FailSafe™ PCR PreMix Selection Kit includes everything you need for PCR amplifications except PCR templates and primers. This kit contains FailSafe™ PCR Enzyme Mix, and 12 different FailSafe™ PCR 2X PreMixes (A to L; 200 µl each). Each PreMix is sufficient for 4 typical PCR amplifications, based on a 50 µl reaction volume.

Limit 3 per order, please.

## FailSafe™ PCR System

Cat. No.	Size	No. of FailSafe PCR 2X PreMixes Included (2.5 ml each)
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 are also available separately.

# Sequencing Through GC-rich and AT-rich Sequences with the SequiTherm™ EXCEL™ II DNA Sequencing Kit

Meena Kumari and Antje Anji, Dept. of Pharmacology, Univ. of Texas Health Science Center

## Introduction

Messenger RNA stability, an important control mechanism of gene expression, is a highly regulated process. The majority of mRNA stability determinants have been located within the 3'-UnTranslated Region (UTR) of an mRNA. To locate stability determinants for a brain-specific receptor protein being studied in our lab, we first generated a full-length cDNA clone. Since the sequence of the UTR of the gene of interest is unknown, we sequenced that part of the cDNA. Here we present a method to sequence a GC-rich double-stranded DNA template using the SequiTherm™ EXCEL™ II DNA Sequencing Kit.

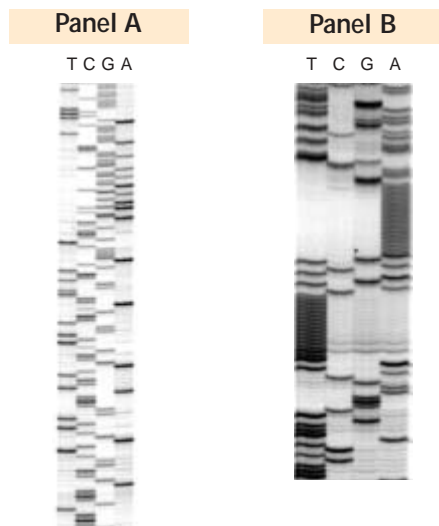


Figure 1. DNA regions from cDNA clones with high GC- (Panel A) or AT- (Panel B) content are readily sequenced by SequiTherm™ EXCEL™ II DNA Polymerase.

## Methods

The UTR of the gene of interest was amplified by RACE and the products were subcloned. Plasmid miniprep DNA was used as sequencing template. Primers, both universal and gene-specific, were 5'-end-labeled with [ $\gamma$ - $^{32}$ P]ATP. Sequencing premix was prepared as follows: 300 fmoles DNA template was vacuum dried, and resuspended in 6  $\mu$ l autoclaved milliQ water. DNA was then mixed with 6  $\mu$ l (12 pmoles) of  $^{32}$ P-labeled primer, 8.5  $\mu$ l of SequiTherm EXCEL II sequencing buffer and stored

on ice for 15 minutes before adding 1  $\mu$ l (5 Units) of SequiTherm EXCEL II DNA polymerase. Five microliters of the sequencing premix were added to each of four tubes containing the respective SequiTherm EXCEL II termination mix (A= 2.4  $\mu$ l; C= 3  $\mu$ l; G= 3  $\mu$ l and T= 2.4  $\mu$ l). The cycle DNA sequencing reaction was performed as follows: Step 1 = 5 minutes at 95°C x 1 cycle; Step 2 = 30 seconds at 95°C, 50 seconds at 68°C, 60 seconds at 72°C x 30 cycles; Step 3 = soak at 6°C. At the end of the sequencing reaction, 3  $\mu$ l of Stop/Loading buffer was added to each of the tubes and each tube was denatured at 85°C for 3 minutes, before loading samples (1.5 – 4.5  $\mu$ l) on a 5% denaturing polyacrylamide gel. Following electrophoresis, the gel was fixed, dried and then exposed to Kodak Biomax X-ray film overnight at room temperature for 24 hours.

## Results and Discussion

Using the SequiTherm EXCEL II Kit and the sequencing conditions described here, we were able to generate sequence data beginning very near the 3'-end of the primers. Typically an electrophoretic run resulted in a separation of a easily readable sequence of at least 300 nucleotides. Analysis of the sequences generated revealed regions of very high (74%) GC-content (Figure 1A). The SequiTherm EXCEL II DNA Polymerase successfully read through these regions without any noticeable "stops" or bands in all four lanes as can occur with some polymerases in high GC regions.

While sequencing other UTR clones we found that some clones were unique and not related to our gene of interest. One such sequence is shown in Figure 1B. This sequence has a high AT-content which again was easily sequenced by the SequiTherm EXCEL II DNA Polymerase. Taken together our sequence data (shown and not shown) indicates that the SequiTherm EXCEL II Kit is an excellent kit for sequencing both GC-rich and AT-rich DNA templates.

## Acknowledgment

This work was supported by NIH-NIAA Grant #AA12070.

## Finish Your DNA Sequencing Project—Complete Difficult Regions with SequiTherm™ EXCEL™ II DNA Sequencing Kits

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 your most difficult regions with SequiTherm EXCEL II DNA Sequencing Kits: 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.

### SequiTherm™ EXCEL™ II DNA Sequencing Kit

For manual cycle and isothermal sequencing

SEM79020	20 Sequences
SEM79050	50 Sequences
SEM79100	100 Sequences

### SequiTherm™ EXCEL™ II DNA Sequencing Kit-LC for 25-41 cm gels

For LI-COR® or NEN® Global IR<sup>2</sup> Sequencers

SE101LC	100 Sequences
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### SequiTherm™ EXCEL™ II DNA Sequencing Kit-LC for 66 cm gels

For LI-COR® or NEN® Global IR<sup>2</sup> Sequencers

SE9202LC	100 Sequences
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### SequiTherm™ EXCEL™ II Long-Read™ DNA Sequencing Kit-ALF

For ALF™ DNA Sequencers or ABI Prism™ Sequencers (dye-primer)

SE8301A	100 Sequences
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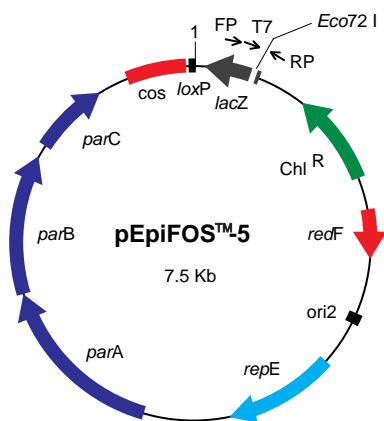
LI-COR and Global IR<sup>2</sup> are trademarks or registered trademarks of LI-COR, Inc. NEN is a registered trademark of NEN Life Products, Inc.

ALF is a registered trademark of Amersham Pharmacia Biotech.

ABI Prism is a registered trademark of PE Biosystems.

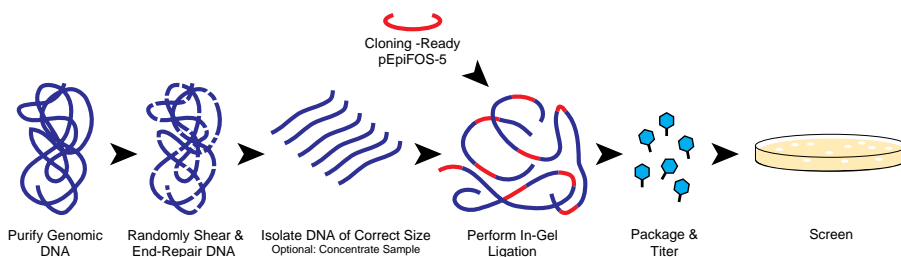
# Produce Unbiased Libraries of Stable, Cosmid-Sized Clones Using the EpiFos™ Fosmid Library Production Kit

Fosmid vectors<sup>1,2</sup> provide an improved method for cloning and stably maintaining libraries of cosmid-sized (about 40 Kb) clones in *E. coli*. The pEpiFOS™-5 Fosmid Vector (Figure 1) provided in the EpiFOS™ Library Production Kit is derived from the single copy F-factor of *E. coli*. Thus, the cosmid-sized clones produced using pEpiFOS-5 are propagated as a single copy. Low copy number has been shown to improve clone stability compared to high copy cosmid clones. In addition, pEpiFOS-5 contains *cos* sites for *cos* site-mediated lambda phage packaging and high efficiency fosmid library production. The pEpiFOS-5 vector is provided linearized and dephosphorylated - ready for use in library production.



**Figure 1. Map of pEpiFOS™-5 Fosmid Vector.** pEpiFOS-5 is provided linearized at the *Eco721* site and dephosphorylated in the EpiFOS Fosmid Library Production Kit.

The EpiFOS Fosmid Library Production Kit provides all reagents needed to construct complete and unbiased fosmid libraries in about 2 days using a novel cloning strategy (Figure 2). Genomic DNA is first sheared by passing it through a syringe needle (not supplied with the kit). Shearing the DNA into approximately 40 Kb fragments leads to the highly random generation of DNA fragments in contrast to more biased libraries that result from fragmenting the DNA by partial restriction endonuclease digestion. The sheared DNA is then end-repaired to generate blunt ends using reagents supplied in the kit and size-selected using low melting point agarose

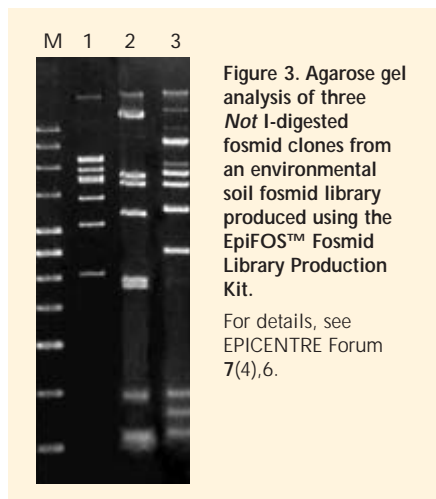


**Figure 2. The process for preparing a complete and unbiased fosmid library using the EpiFOS™ Fosmid Library Production Kit.** Cloning randomly sheared genomic DNA fragments results in a complete and unbiased fosmid library. The EpiFOS Fosmid Library Production Kit provides all necessary reagents (except for genomic DNA).

gel. Finally, the size-selected DNA is ligated into the supplied linearized and dephosphorylated pEpiFOS-5 Fosmid Vector, packaged using ultra-high efficiency packaging extracts (>10<sup>9</sup> pfu/μg for phage lambda), also included in the kit, and plated on the supplied EPI100™ *E. coli* plating cells. The result is a complete and unbiased primary fosmid library.

### EpiFOS Fosmid Library Production Kit provides:

- Unbiased genomic libraries containing clones averaging about 40 Kb in size.
- Stable clones produced in the single copy pEpiFOS-5 vector.
- Reagents for up to 10 cloning and packaging reactions.
- High efficiency packaging extracts to maximize the number of clones produced.



### References

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

### EpiFOS™ Fosmid Library Production Kit

FOS0901 1 Kit  
For up to 10 Fosmid libraries.

#### Contents:

Kit includes pEpiFOS™-5\* Fosmid Vector, End-repair Enzyme Mix, End-repair 10X Buffer, dNTP Mix, Fast-Link™ DNA Ligase, Fast-Link™ 10X Ligation Buffer, ATP Solution, GELase™ Gel-digesting Preparation, GELase™ 50X Reaction Buffer, MaxPlax™ Lambda Packaging Extracts, Ligated Lambda Control DNA, Control DNA, EPI100™ Plating strain, Control Lambda Plating strain.

**pEpiFOS™-5 Forward Sequencing Primer**  
F5FP010 1 nmole 50 μM

**pEpiFOS™-5 Reverse Sequencing Primer**  
F5RP011 1 nmole 50 μM

\* pEpiFOS™-5 is exclusively licensed by EPICENTRE Technologies.

# Efficiently Clone a PCR Product into the Vector of *Your Choice* Using the End-It™ DNA End-Repair Kit

PCR amplification products, produced using Taq Polymerase and other thermostable DNA Polymerases, frequently contain a non-template-coded 3'-A overhang. The End-It™ DNA End-Repair Kit rapidly and efficiently converts PCR product into 5'-phosphorylated, blunt-ended DNA for efficient and economical cloning into any blunt-end cloning vector (Figure 1). The conversion to 5'-phosphorylated, blunt-end DNA is accomplished by exploiting the 5'-3' polymerase and 3'-5' exonuclease activities of T4 DNA Polymerase and T4 Polynucleotide Kinase contained in the End-Repair Enzyme Mix. ATP, dNTPs and 10X Reaction Buffer are also included in the kit. The resulting 5'-phosphorylated, blunt-end DNA is ready for ligation into the blunt-ended cloning vector of your choice, using, for example, EPICENTRE's Fast-Link™ DNA Ligation Kit (see back cover).

The End-It DNA End-Repair Kit is also useful for repairing genomic DNA fragments containing damaged or incompatible 5'- or 3'-ends that result from shearing or restriction endonuclease digestion. The resulting 5'-phosphorylated, blunt-end DNA can be cloned into any blunt-end plasmid, cosmid, fosmid or BAC vector.

For PCR product cloning, the End-It™ DNA End-Repair Kit provides:

## Efficiency

Cloning efficiencies of  $> 10^7$  cfu/ $\mu$ g of PCR product can be obtained (Table 1).

## Versatility

Other PCR cloning methods require cloning into a limited number and type of specialized and costly vectors. Using the End-It DNA End-Repair Kit you can clone your PCR product into the blunt-ended, dephosphorylated vector of your choosing.

## Economy

One kit will end-repair and 5'-phosphorylate up to 20 PCR products for subsequent blunt-end cloning.

	Colonies/ $\mu$ g DNA
PCR product without End-It Kit treatment	0
PCR product made blunt-ended with End-It Kit	$4 \times 10^7$
Blunt-ended DNA control (Pvu II-cut DNA)	$4 \times 10^7$

**Table 1. Cloning efficiency of PCR product treated with the End-It™ DNA End-Repair Kit.** 50ng of a 1.3 Kb PCR product, produced using Taq DNA Polymerase, was ligated into a linearized and dephosphorylated cloning vector before and after end-repair and 5'-phosphorylation using the End-It DNA End-Repair Kit. The positive control was a 1.3 Kb DNA digested with Pvu II. Aliquots of the ligation reactions were used to transform TransforMax™ EC100™ Electrocompetent *E. coli*.

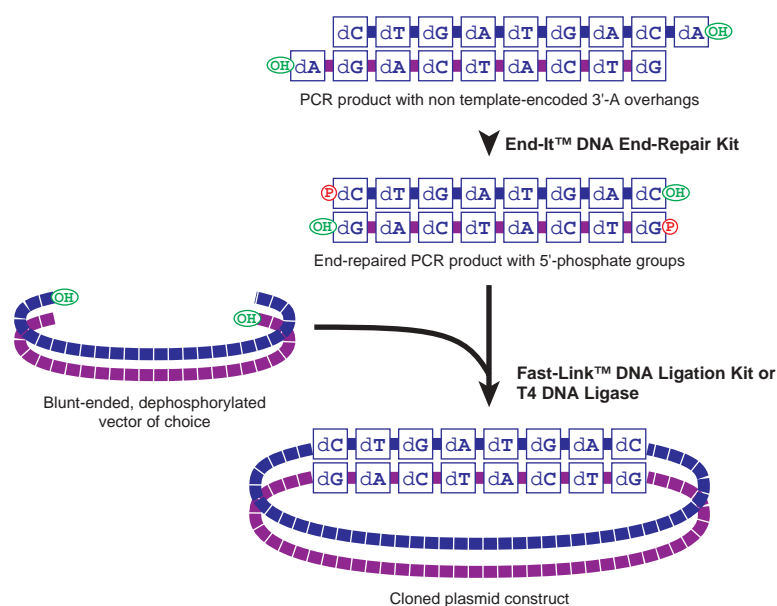
## End-It™ DNA End-Repair Kit

ER0720 20 Reactions

For end-repair and 5'-phosphorylation of up to 100  $\mu$ g of DNA.

### Contents:

End-Repair Enzyme Mix, 10X End-Repair Buffer, dNTP Solution, ATP Solution.



**Figure 1. The End-It™ DNA End-Repair Kit converts PCR product with 3'-A overhangs to blunt-end, 5'-phosphorylated DNA for cloning into a blunt-end site of any vector.**

Make a blunt-end PCR product using the End-It™ DNA End-Repair Kit. Then, ligate the PCR product into the blunt-ended cloning vector of your choice in as little as 15 minutes (!) using the Fast-Link™ DNA Ligation Kit (See back cover for information on the Fast-Link Kit).

# Direct PCR from a Single Bacterial Colony Without DNA Extraction Using the FailSafe™ PCR System

Haiying Grunenwald, EPICENTRE

## Introduction

PCR template preparation that requires DNA purification can be tedious, costly, and time consuming, especially with large numbers of samples. In previous reports,<sup>1,2</sup> we demonstrated that the FailSafe™ PCR System enabled direct PCR amplification using whole blood and dried blood spots collected on Guthrie cards or glass slides, all without prior DNA purification. This article reports reproducible PCR amplification directly from single *E. coli* colonies using the FailSafe PCR System.

## Methods and Results

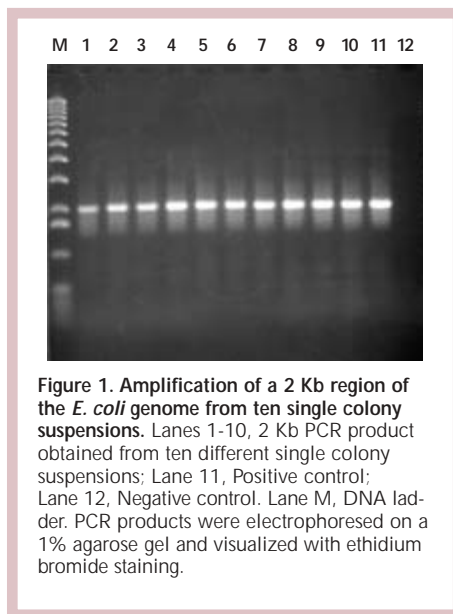
### Direct PCR from bacterial colonies using the FailSafe PCR System

The FailSafe™ PCR PreMix Selection Kit contains the unique FailSafe™ PCR Enzyme blend and 12 PreMixes (A to L), each representing a different PCR condition. A PCR amplification will succeed under at least one condition for any template and primer pair combination.

In order to evaluate the ability of the FailSafe PCR System to amplify directly from bacterial cells, 10 single *E. coli* colonies, 1 mm in diameter, were first picked from the plate and suspended in 10 µl of water. Each suspension was used for PCR in separate reactions with four different sets of PCR primer pairs that amplify different regions of the *E. coli* genome. Primer Pair A generates a PCR product of 2 Kb in size, Primer Pair B 4 Kb, Primer Pair C 6 Kb, and Primer Pair D 8 Kb. PCR was carried out with each primer pair using the FailSafe PCR PreMix Selection Kit in a 50 µl volume that contained 1 µl of the resuspended *E. coli* cells, 50 pmoles of each primer, 25 µl of the optimal FailSafe™ 2X PCR PreMix, and 2.5 Units of FailSafe PCR Enzyme Mix. PCR cycling profile was: 3 minutes at 99°C, followed by 30 cycles of 20 seconds at 98°C and 3 minutes (for Primer Pair A and B) or 5 minutes (for Primer Pair C and D) at 68°C.

The PCR amplifications were successful for all four sets of PCR primers using the *E. coli* cell suspensions from the ten colonies. Direct PCR amplification of *E. coli* with Primer Pair A using PreMix C is shown in Figure 1.

Direct PCR using *E. coli* cell suspensions from an overnight cell culture was also

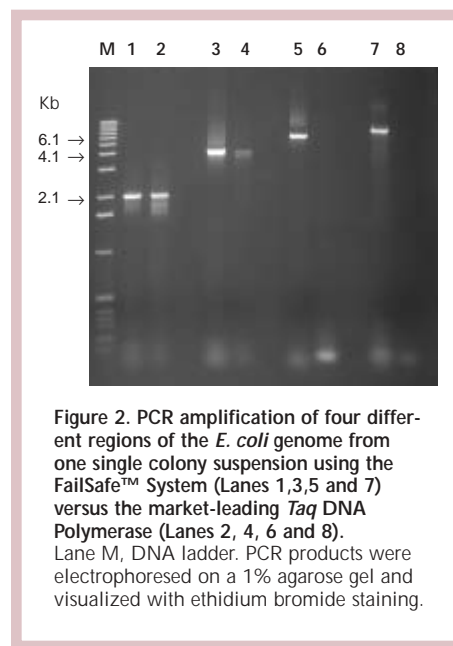


**Figure 1. Amplification of a 2 Kb region of the *E. coli* genome from ten single colony suspensions.** Lanes 1-10, 2 Kb PCR product obtained from ten different single colony suspensions; Lane 11, Positive control; Lane 12, Negative control. Lane M, DNA ladder. PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide staining.

successful (data not shown). The cell suspension was prepared by centrifuging 1 ml of a saturated *E. coli* culture and resuspending the cell pellet in 1 ml of water. One microliter of resuspended cells was used per PCR reaction as indicated above.

### Comparison of direct PCR from an *E. coli* colony using FailSafe and the leading Taq DNA Polymerase

All PCR amplifications using the FailSafe System were performed as indicated



**Figure 2. PCR amplification of four different regions of the *E. coli* genome from one single colony suspension using the FailSafe™ System (Lanes 1,3,5 and 7) versus the market-leading Taq DNA Polymerase (Lanes 2, 4, 6 and 8).** Lane M, DNA ladder. PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide staining.

above. PCR amplifications using the market-leading *Taq* DNA Polymerase were performed as directed using the supplied 10X Buffer provided by the manufacturer. Cycling conditions were identical to those used for FailSafe. The differences in PCR results between the FailSafe System and the market leader are shown in Figure 2. FailSafe consistently amplified *E. coli* DNA using all of the primer pairs tested, while the leading *Taq* DNA Polymerase amplified *E. coli* DNA with only 2 of the 4 primer pairs. Also, of the two positive PCR reactions using the leading *Taq* DNA Polymerase, the yield of the PCR products was visibly lower than that obtained using the FailSafe PCR System.

## Conclusions

The FailSafe PCR System reliably amplified DNA from *E. coli* colonies without a DNA extraction step.

## References

1. Grunenwald, H. (2000) EPICENTRE Forum 7(4), 10.
2. Grunenwald, H. (2001) EPICENTRE Forum 8(2), 4.

### 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\*\*\*  
(4 x 250 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).

See the center insert for more information on the FailSafe™ PCR System.

## Get the Highest Yields of Yeast DNA Using the MasterPure™ Yeast DNA Purification Kit

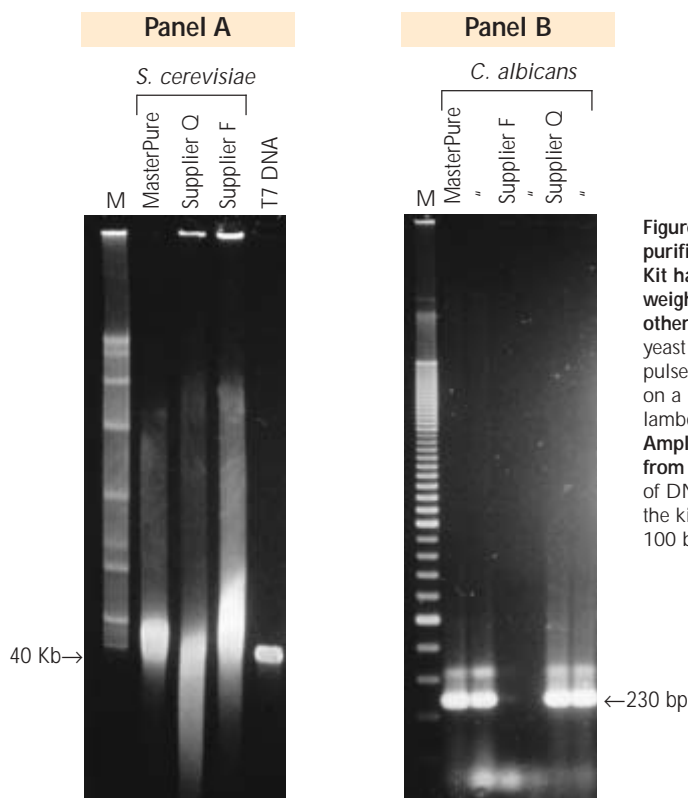
The MasterPure™ Yeast DNA Purification Kit is a simple and nonenzymatic approach to yeast genomic DNA purification. High quality yeast DNA can be obtained in less than 40 minutes without columns, resins or organic extractions. Recover DNA from a wide variety of yeast species including *Candida*, *Saccharomyces*, *Pichia*, and *Schizosaccharomyces*, and filamentous fungi such as *Asperigillus* and *Penicillium*.

### Higher yield than the competition

Using the simple, short protocol (Table 1), DNA yields obtained with the MasterPure Kit were consistently above those of two competing kits (Figure 1). For example, the kit from supplier F averaged 0.25 µg of DNA from 1.5 ml of *S. cerevisiae*, whereas the MasterPure Kit produced an average of 2.94 µg, almost twelve times as much. The supplier Q kit produced 1.8 µg of *S. cerevisiae* DNA from the same culture volume.

**Table 1. Protocol for MasterPure Yeast DNA Purification Kit (for 1.5 ml culture):**

- 1 Lyse cells in the Yeast Cell Lysis Solution.
- 2 Precipitate and remove proteins using MPC Protein Precipitation Reagent.
- 3 Precipitate, wash, and resuspend the nucleic acids.



**Figure 2. Panel A:** Yeast DNA purified using the MasterPure™ Kit has a higher molecular weight than DNA purified using other kits. 500 ng of purified yeast DNA was analyzed by pulsed field gel electrophoresis on a 1% agarose gel. Lane M, lambda DNA ladder. **Panel B:** Amplification of the DUT gene from *C. albicans*. Ten nanograms of DNA purified using each of the kits was amplified. Lane M, 100 bp ladder.

### High molecular weight DNA

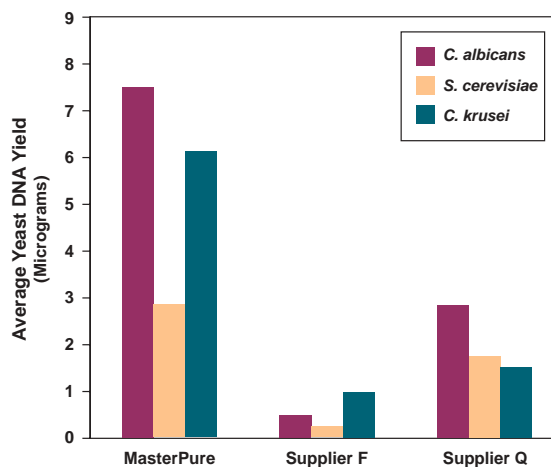
The MasterPure Kit yields high molecular weight yeast DNA. As determined by pulsed field gel electrophoresis, the size of the *S. cerevisiae* DNA isolated with the MasterPure Yeast DNA Purification Kit is approximately 40-50 Kb, while DNA of the same yeast species purified with the kit from supplier Q was mostly degraded to fragments smaller than 40 Kb (Figure 2A). A similar size discrepancy was observed for *C. albicans* DNA (data not shown).

### PCR-Ready DNA

The MasterPure Kit produces high-quality DNA that can be used directly for many applications including PCR amplification, restriction endonuclease digestion, Southern blotting, and genomic library preparation. Figure 2B shows PCR amplification of the dUTP pyrophosphatase (DUT) locus using *C. albicans* DNA as the template.

### DNA purification from single yeast colonies

The protocol can be easily adjusted for larger or smaller samples, including single yeast colonies. For example, the average yield for *S. cerevisiae* colonies was 188 ng and for *C. albicans*, 538 ng. Increasing the volume of the preparation twenty-fold, the DNA yield from a 30 ml culture of *S. cerevisiae* was 77 µg, and for *C. albicans*, 430 µg.



**Figure 1. The MasterPure™ Yeast DNA Purification Kit gives higher yields of DNA than other kits.** DNA yields were from 1.5 ml cultures and quantitated by fluorometry with Hoescht 33258 dye. The data represent the average of duplicate extractions from either one (*C. krusei*) or two (*S. cerevisiae* and *C. albicans*) experiments.

### MasterPure™ Yeast DNA Purification Kit

MPY80010 10 Purifications  
MPY80200 200 Purifications

#### Contents:

Yeast Cell Lysis Solution, MPC Protein Precipitation Reagent, TE Buffer, RNase A

# Create Random Gene Knockouts in Living Cells Using the EZ::TN™ Transposome™

A transposon is a DNA sequence that “hops” or transposes into a DNA molecule in a reaction catalyzed by a transposase enzyme. These genetic elements have been used in applications such as distributing sequencing primer binding sites, creating gene knockouts, and introducing a variety of markers for genetic analysis. EZ::TN™ Transposomes™ provide an efficient and reliable method of randomly inserting transposons into the genomes of many different genera.

## A simple one-step transposition system

An EZ::TN Transposome is the stable complex formed by incubating an EZ::TN™ Transposon with EZ::TN™ Transposase in the absence of Mg<sup>2+</sup>. EZ::TN Transposomes are so stable that they can be electroporated into many living cells that can be transformed by electroporation. Once in the cell, the EZ::TN Transposome is activated by Mg<sup>2+</sup> and the EZ::TN Transposon is randomly inserted into the host's genomic DNA (Figure 1).<sup>1</sup>

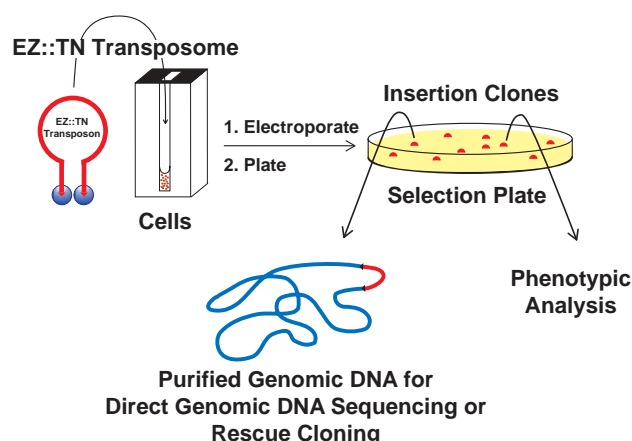
No other transposition system is so simple or versatile. The only requirement is that DNA can be introduced into the host by electroporation. There is no need for cell conjugation, suicide vectors or specific host factors.

## A broad host range system

EZ::TN Transposomes have already been used successfully by scientists working with a variety of different species including *Escherichia*, *Corynebacterium*, *Mycobacterium*, *Pseudomonas*, *Proteus*, *Salmonella*, *Xylella*, *Saccharomyces* and more.<sup>1-4</sup> Various methods (e.g. Southern blot and sequence analysis) have verified that insertion mutants from these studies are both random and stable. Combined with ease of use, these properties make the EZ::TN Transposome ideal for generating libraries of mutants in species that have poorly described genetic systems or lack adequate molecular tools.

**Table 1. Average 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>Salmonella typhimurium</i>	>10 <sup>4</sup>
<i>Pseudomonas sp</i>	>10 <sup>2</sup>
<i>Proteus vulgaris</i>	>10 <sup>3</sup>
<i>Mycobacterium smegmatis</i>	>10 <sup>2</sup>



**Figure 1. An EZ::TN™ Transposome™ can be electroporated into living cells where it randomly inserts the transposon component into the host's genomic DNA. The EZ::TN Transposon insertion site can be analyzed by a variety of methods.**

The number of transposition clones obtained is highly dependent on the transformation efficiency of the host cell (Table 1). The higher the transformation efficiency of the cell, the more clones will be produced. Electroporation of *E. coli* with a transformation efficiency of >10<sup>9</sup> cfu/µg typically results in >10<sup>5</sup> independent insertion clones when 1 µl of EZ::TN Transposome is used.

Pre-formed EZ::TN Transposomes are available containing either a kanamycin selectable marker (<KAN-2> or <R6Kγori /KAN-2>) derived from Tn903 or a dihydrofolate reductase gene (<DHFR-1>) that can be selected on plates containing trimethoprim.

## Create your own Transposome

You can create your own EZ::TN Transposome using one of the EZ::TN™ pMOD™<MCS> Transposon Construction Vectors (see page 15) and EZ::TN Transposase. To prepare your transposon, clone the DNA of interest into the multiple cloning site and then release the transposon by PCR or by digestion with *Pvu* II or *Psh* A I. Nanogram amounts of transposon DNA are then incubated with EZ::TN Transposase in the presence of glycerol to form an EZ::TN Transposome for random insertion into genomic DNA. A custom EZ::TN Transposon might include antibiotic resistant determinants for insertional mutagenesis, a reporter gene to facilitate studies of gene regulation and protein localization, or rare restriction enzyme sites for genome mapping.

## Sequencing your gene knockout is easy

EZ::TN Transposons contain unique primer binding sites at either end for bidirectional sequencing. Hence, once a gene knockout has been selected the affected gene can be sequenced directly using bacterial genomic DNA as template and primers homologous to the ends of the inserted transposon. The transposon insertion can also be “rescued” and the flanking DNA sequenced when mutations are made with an EZ::TN Transposon containing an *E.coli* conditional origin of replication (*R6Kγori*) (see page 15).

## References

- Goryshin, I.Y. *et al.* (2000) *Nature Biotechnol.* **18**, 97.
- Derbyshire, K. M. *et al.* (2000) *EPICENTRE Forum* **7**(2), 1.
- Guillabert, M. R. *et al.* (2000) *EPICENTRE Forum* **8**(2), 1.
- Abstr. Annu. Meet. Am. Soc. Microbiol. (2001) Losada, L. C. *et al.*, B107, p. 64, Marra, D. *et al.*, B99, p.62.

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

TSM99K2 10 Reactions

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

TSM99D1 10 Reactions

### EZ::TN™<R6Kγori /KAN-2>Tnp Transposome™ Kit

TSM08KR 10 Reactions

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

## Rapidly Rescue Clone Transposed Genomic DNA

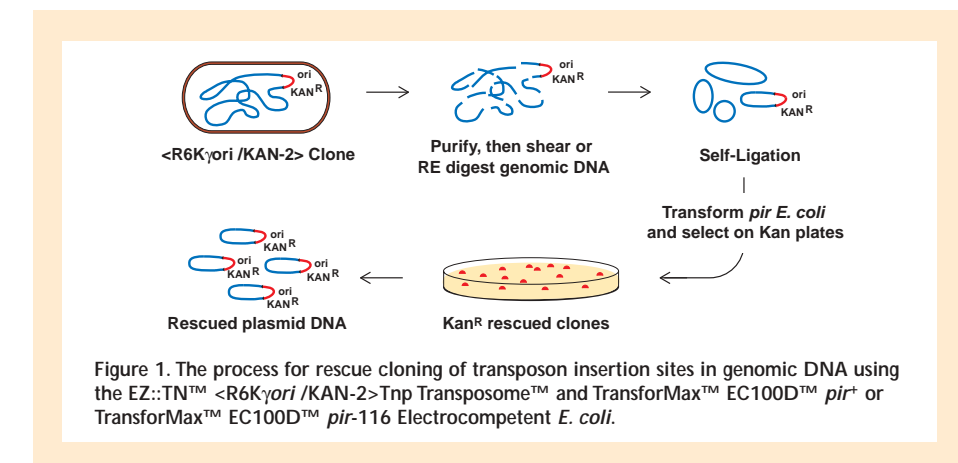
Among the advantages of transposon mutagenesis is that the transposon serves as a marker that can be used to clone and sequence the region of genomic DNA that has been disrupted. Nothing makes this cloning process easier than creating mutations *in vivo* with the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™. In addition to encoding a kanamycin-resistance gene, the transposon contains an *E.coli* conditional origin of replication (R6Kγori). The presence of this origin of replication enables you to propagate or “rescue” the region of genomic DNA into which the transposon has been inserted in a three step process (Figure 1):

- 1 Purify genomic DNA from a single kanamycin resistant insertion clone or a pool of clones, and then fragment the DNA by digestion with restriction endonuclease(s) or by random shearing.
- 2 Self-ligate the genomic DNA fragments using Fast-Link™ DNA Ligase or another suitable ligase. For efficient ligation of randomly sheared DNA, the ends of the DNA can be repaired using the End-It™ DNA End-Repair Kit (see p. 11).
- 3 Transform an *E. coli* host expressing the π protein (*pir* gene product), such as TransforMax™ EC100D *pir*<sup>+</sup> or TransforMax™ EC100D *pir*-116 Electrocompetent *E. coli* and select on kanamycin plates. Only those clones containing the EZ::TN <R6Kγori /KAN-2> Transposon will grow.

Rescue clones can then be sequenced bidirectionally using the provided primers that are homologous to the ends of the transposon.

### Make your own R6Kγori containing transposon

EPICENTRE now offers the EZ::TN™ pMOD™-3<R6Kγori /MCS> Transposon Construction Vector (Figure 2) so that you can quickly and easily prepare a custom EZ::TN Transposon that can also be used for rescue cloning. To prepare your transposon, clone any DNA sequence of interest (e.g. selectable marker, control element, reporter gene) into the multiple cloning site and then prepare the transposon by PCR amplification using the Forward and Reverse PCR Primers provided with the vector, or by restriction enzyme digestion with *Pvu* II or *PshA* I.



The transposon can be incubated with EZ::TN Transposase in the absence of Mg<sup>2+</sup> to form an EZ::TN Transposome for random insertion into the genomic DNA of living cells (see page 14). DNA flanking the transposon insertion site can then be rescued by modifying the three step process outlined above to include a selection process other than kanamycin resistance. Your custom EZ::TN Transposon can also be used for insertion into any target DNA *in vitro*. *In vitro* transposition of R6Kγori containing transposons can be used, for example, to rescue plasmids which ordinarily do not replicate in *E. coli* because they lack a recognizable origin of replication and/or a selectable marker.

Your custom EZ::TN Transposon will also include unique primer binding sites at either end for bidirectional sequencing of the insertion site using the pMOD™ <MCS> Forward and Reverse Sequencing Primers (available separately). No need to design your own primers.

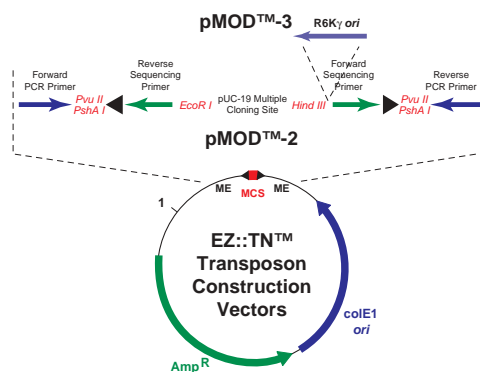


Figure 2. A custom EZ::TN™ Transposon containing any sequence of interest can be prepared using a Transposon Construction Vector. Transposons made with the pMOD™-3 vector can also be used for rescue cloning.

### EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ Kit

TSM08KR 10 Reactions  
Kit contains pre-formed Transposome™ and two unlabeled sequencing primers.

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

MOD0602 20 µg  
Includes: pMOD™-2<MCS> Vector and the Forward and Reverse PCR Primers.

### EZ::TN™ pMOD™-3<R6Kγori /MCS> Transposon Construction Vector

**NEW!**  
MOD1503 20 µg  
Includes: pMOD™-3<R6Kγori /MCS> Vector and the Forward and Reverse PCR Primers.

### EZ::TN™ Transposase

TNP92110 10 Units

### TransforMax™ EC100D™ *pir*<sup>+</sup> Electrocompetent *E. coli*

ECP09500 5 X 100 µl  
(10 Electroporations)  
Maintains clones at 15 copies per cell. Includes control vector containing an R6Kγori.

### TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*

EC6P095H 5 X 100 µl  
(10 Electroporations)  
Maintains clones at 250 copies per cell. Includes control vector containing an R6Kγori.



# DNA Ligation in 5 Minutes!

## At room temperature

Lab Tested ...  
Scientist Approved.

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

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

### Fast and Efficient DNA Ligations.

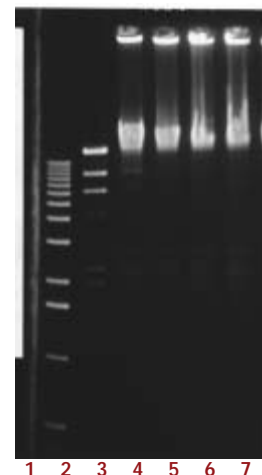
- Ligate cohesive-end DNA in 5 minutes at room temperature.
- Ligate blunt-end DNA in 15 minutes at room temperature.
- Ligate PCR product with A-overhangs in 1 hour or less at 16°C.

### Transform Cells Without Desalting the Ligation Reaction.

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

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

Ligation Time in Minutes  
5 15 30 60 120



### Representative Transformation Results with the Fast-Link DNA Ligation Kit\*

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

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

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

### Fast-Link™ DNA Ligation Kits

LK0750H 50 ligations  
LK6201H 100 ligations

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

### HOW TO CONTACT US AT EPICENTRE

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### EPICENTRE FORUM

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