

Best Wishes for the New Year!

Epicentre Forum

New Tools & Techniques for Molecular Biology

Direct Sequencing of BAC Clones Without Subcloning or Primer Walking Using EZ::TN™ Transposon Tools

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INVITATION TO CONTRIBUTE

THE EPICENTRE FORUM, SENT TO THOUSANDS OF RESEARCHERS WORLDWIDE, INVITES YOU TO CONTRIBUTE ARTICLES AND TECHNICAL TIPS ON THE USE OF OUR PRODUCTS. PLEASE CONTACT US FOR MORE INFORMATION.

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- 16 DNA Ligations in 5 Minutes at Room Temperature!

Volume 7 Number 4 2000

Bacterial Artificial Chromosome (BAC) vectors are frequently used for cloning genomic DNA because they will accommodate large (100 Kb - 300 Kb) inserts with a low frequency of sequence rearrangement. Typically, sequencing of BAC clones is accomplished by the laborious, time consuming and costly process of subcloning and shotgun sequencing or by BAC end-sequencing and primer walking. This report demonstrates faster and easier methods for direct sequencing of BAC clones without subcloning or primer walking.

Introduction

The process for direct sequencing of BAC clones is outlined in Figure 1. The BAC clone of interest is first purified using PSIΨClone™ BAC DNA Kit which removes contaminating *E. coli* chromosomal DNA. Then, an EZ::TN™ Transposon containing a kanamycin resistance marker and sequencing primer binding sites is randomly inserted into each BAC molecule in a two hour, *in vitro* reaction. An aliquot of the transposon insertion reaction is used to transform electrocompetent *E. coli* and independent BAC insertion clones containing an EZ::TN Transposon are isolated on kanamycin plates. Finally, BAC insertion clones are grown up, purified in a miniprep format and

sequenced bidirectionally using a single set of sequencing primers provided in the EZ::TN <KAN-2> Insertion Kit that are homologous to sites within the inserted transposon.

Methods and Results

Purification of BAC clone DNA for the EZ::TN Transposon insertion reaction.

Unlike minipreps of high copy plasmid and cosmid clones, which can be used directly in the EZ::TN Transposon

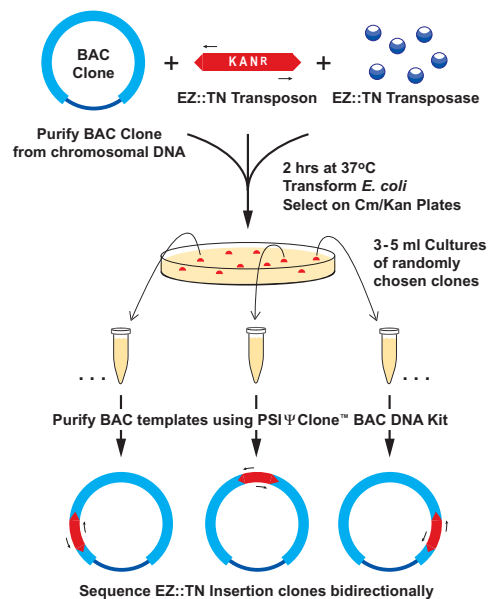


Figure 1. A complete population of BAC sequencing templates is generated in a single EZ::TN Transposon insertion reaction. BAC clones containing a randomly inserted EZ::TN Transposon can then be sequenced directly using primers that are homologous to the ends of the transposon.

insertion reaction, BAC DNA preparations are contaminated with a higher molar proportion of *E. coli* chromosomal DNA due to their low copy number. Contaminating chromosomal DNA prevents an accurate determination of the amount of BAC DNA in the sample and competes with BAC DNA as target for EZ::TN Transposon insertions. Therefore, effective BAC purification prior to the transposon insertion reaction increases the efficiency of *in vitro* transposon insertions into the BAC clone.

Prior to purification, BAC clones chosen for sequencing were grown overnight in 5 ml Terrific Broth¹ (TB) containing 20 µg/ml chloramphenicol. Use of TB and inclusion of chloramphenicol at 20 µg/ml in the medium consistently gave the best yields of BAC clone DNA compared to other combinations of culture medium and chloramphenicol concentrations².

BAC DNA was then purified from 5 ml overnight cultures using a simple mini-prep procedure described in the PSIΨClone BAC DNA Kit (Princeton Separations). The resulting DNA pellet was resuspended in 20 - 25 µl of TE Buffer and BAC DNA yield was estimated using the Hoechst Assay (Molecular Probes). Yields of 0.6 - 1 µg of highly purified BAC DNA were typically found though yields in excess of 1 µg were observed. The resulting DNA was sufficiently pure for use in the EZ::TN Transposon insertion reaction.

The EZ::TN Transposon Insertion reaction and selection of transposon insertion clones

0.2 µg of purified BAC DNA was incubated with an equal molar amount of EZ::TN <KAN> Transposon as described in the EZ::TN <KAN> Insertion Kit literature. It is imperative to use equal molar amounts of BAC clone DNA and EZ::TN Transposon in the reaction so as to maximize the transposon insertion efficiency and minimize multiple transposon insertion events. One µl of the reaction mix was used to transform 40 µl electrocompetent *recA1 E. coli*. Following electroporation, cells were allowed to recover by addition of 1 mL SOC¹ broth and incubation at 37°C for 45-60 minutes then plated directly on LB plates containing kanamycin (Kan; 40 µg/ml) and chloramphenicol (Cm; 12.5 µg/ml). Plates were incubated overnight at 37°C yielding >2 x 10² clones per plate each containing a BAC clone with a single randomly inserted EZ::TN <KAN> Transposon. A single *in vitro* transposon insertion reaction yielded >2 X 10⁴ insertion clones. This number of independent, randomly distributed insertions is more than enough to determine the complete sequence of a 100 Kb BAC clone insert.

Growth of BAC insertion clones and preparation of BAC sequencing templates

Optimal sequencing results were obtained when BAC insertion clones were inoculated into 5 ml cultures and

grown overnight within 3 days following transformation. After 3 days on the plate, yield of BAC DNA was reduced (Table 1), resulting in shorter sequence reads and diminished peak intensities.

Table 1. Yields of BAC DNA from cultures of individual colonies picked from plates following overnight growth exceeded yields resulting from cultures picked from the same plate 1 week later.

Cultures were grown in 5 ml of TB containing 20 µg/ml chloramphenicol for 18 hours prior to purification using the PSIΨClone BAC DNA Kit.

BAC insertion clone designation	DNA yield from colonies on plate	
	overnight	1 week
P1	0.82 µg	0.61 µg
JA	0.90 µg	0.51 µg
K2	0.66 µg	0.59 µg

Each Kan^R/Cm^R colony, representing a separate EZ::TN Transposon insertion event was picked at random and inoculated into 5 ml TB containing 40 µg/ml kanamycin and 20 µg/ml chloramphenicol in a 15 ml conical tube and incubated at 37°C for 18-24 hours with shaking (225 - 250 rpm). Chloramphenicol selection in broth culture appeared to be essential to maintain the BAC molecule. Following the 18-24 hour growth of the BAC cultures, any insertion clones that were not immediately sequenced should be pelleted and stored at -20°C until needed.

BAC DNA was isolated from the individual 5 ml cultures using the PSIΨClone BAC DNA Kit as described by the manufacturer. After the desalting and precipitation step, the recovered BAC DNA was resuspended in 20-25 µl of TE Buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA). Typical yields of EZ::TN transposed BAC clones were 0.6 - 1 µg.

Sequencing of BAC clones containing EZ::TN Transposon insertions.

Purified, BAC clones were sequenced using the single set of sequencing primers that are provided in the EZ::TN <KAN> Insertion Kit. These primers are homologous to the ends of the inserted EZ::TN Transposon. Sequencing was performed using 11 µl (approximately 400 - 600 ng) BAC clone DNA, 1 µl (3.2 pmoles) of unlabeled sequencing primer, and 8 µl Big Dye Terminator Reaction Mix (Applied BioSystems). Following an initial denaturation step for 3 minutes at 98°C, cycle sequencing conditions consisted of 34 cycles of 98°C for 30 seconds, 53°C for 30 seconds, 60°C for 4 minutes, followed by a final 60°C for 5 minutes. Sequencing reactions were cleaned up using Centri•Sep™ purification columns (Princeton Separations), dried and resuspended in 2.5 µl of loading buffer (formamide, EDTA, Blue Dextran). The entire 2.5 µl was loaded per well of an ABI 373 DNA sequencer.

Under these conditions, sequence reads of 450 - 500 bases per run were typically obtained, and reads of >600 bases were frequently seen (Figure 2). Sequence reads of <400

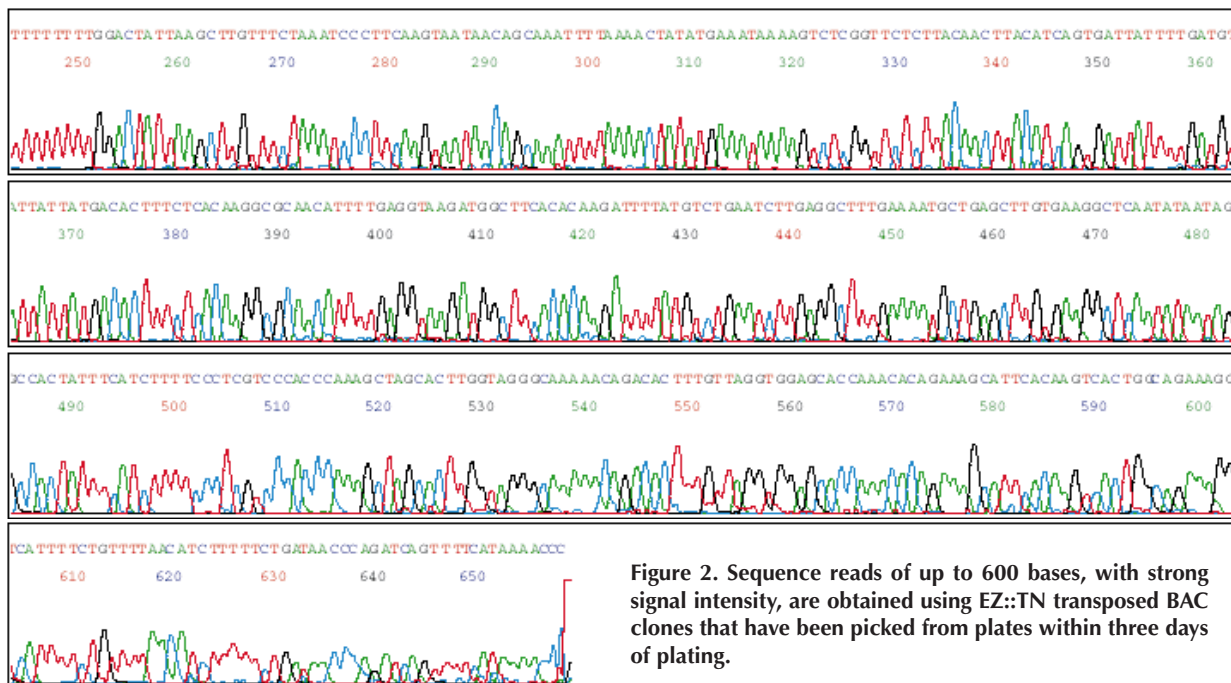


Figure 2. Sequence reads of up to 600 bases, with strong signal intensity, are obtained using EZ::TN transposed BAC clones that have been picked from plates within three days of plating.

bases with an associated drop in signal intensity were observed from clones aged past 5 days on plates. This is probably due to a drop in BAC DNA yield (Table 1).

Summary

This report demonstrates a rapid and simple process for direct sequencing of BAC clones without the need for sub-cloning or primer walking. This process relies on the random nature of the EZ::TN Transposon insertion reaction³ and on the availability of a simple and efficient method for purifying BAC DNA (e.g. the PSIΨClone BAC DNA Kit). A summary of the critical steps for successful BAC clone sequencing is presented in Table 2.

Table 2. The critical steps for direct sequencing of BAC clones:

- 1) Purify the target BAC clone to remove contaminating *E. coli* chromosomal DNA prior to the EZ::TN Transposon insertion reaction to improve transposition efficiency.
- 2) Use equal molar amounts of BAC clone DNA and EZ::TN Transposon in the EZ::TN Transposon insertion reaction, to obtain maximum transposition efficiency and minimize multiple insertions.
- 3) Pick all Kan^R/Cm^R colonies to be sequenced from the plate within 3 days of plating to maximize BAC DNA yield and optimize sequencing results.
- 4) Culture individual BAC insertion clones in TB containing 20 µg/ml chloramphenicol and 40 µg/ml kanamycin to maximize the yield of transposed BAC DNA.
- 5) Purify the EZ::TN Transposon-containing BAC DNA prior to the DNA sequencing for maximum reads.

References

1. Sambrook, J. *et al.* (1989) in *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press v3:A.2
2. O'Mullan, P.J. (2001) Manuscript in preparation.
3. Meis, R. (2000) EPICENTRE *Forum* v.7:4 (p. 5 of this issue of EPICENTRE Forum)

For product information on PSIΨClone BAC DNA Kits and Centri•Sep purification columns, contact Princeton Separations at www.prinsep.com.

EZ::TN™<KAN-2> Insertion Kit

EZI982K 10 Reactions

Contents: EZ::TN™ <KAN-2> Transposon, EZ::TN™ Transposase, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, Forward and Reverse Sequencing Primers, Control Target DNA, Sterile Water

TransforMax™ EC100™ Electrocompetent *E. coli*.

EC10005 5 x 100 µl (10 electroporations)

EC10010 10 X 100 µl (20 electroporations)

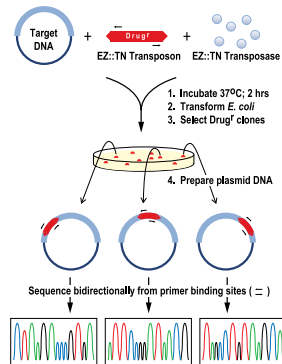
Transformation efficiency of >5X10⁹ cfu/µg. See p. 13 for information on TransforMax EC100 cells.

www.epicentre.com/transposomics.htm

Will you be making a BAC library soon? See p. 8 for information on EPICENTRE's new Cloning Ready linearized and dephosphorylated pIndigoBAC-5 Vectors.

How to Make Sequencing Faster & Easier Using EZ::TN™ Transposon Tools

EPICENTRE offers EZ::TN™ Transposon Tools kits and reagents designed to make almost any DNA sequencing project faster and easier using one of 3 basic strategies.

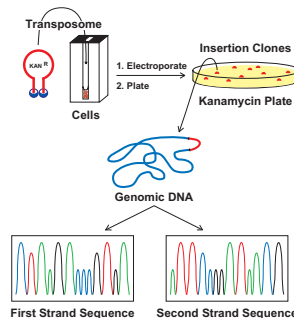


The *In Vitro* Insertion Strategy

The *In Vitro* Insertion Strategy is used if you already have DNA clones that are too big to sequence with a single set of sequencing primers (e.g. clones of >2 Kb). A simple 2-hour *in vitro* reaction randomly inserts an EZ::TN Transposon into your clone. Transform *E. coli* with an aliquot of the reaction mix and select on medium containing the transposon-encoded antibiotic. You obtain >10⁶ of independent clones - enough to completely sequence even the largest clone - each containing a single randomly inserted EZ::TN Transposon. Sequence the clones bidirectionally using a single set of primers (provided in the kits) that are homologous to the ends of the inserted EZ::TN Transposon. See the preceding article for use of the *In Vitro* Insertion Strategy in sequencing BAC clones, without sub-cloning or primer walking.

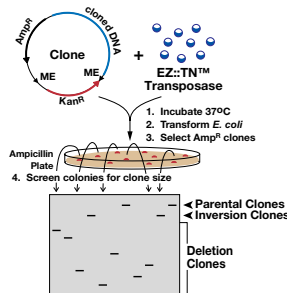
The Transposome™ Strategy

The Transposome™ Strategy is used if you're interested in finding a gene related to a specific phenotype. An EZ::TN Transposome is a stable complex formed between the EZ::TN Transposase and an EZ::TN Transposon. An EZ::TN Transposome is so stable that it can be electroporated into living cells where it is activated by intracellular Mg²⁺ and randomly inserts into the host cell's genomic DNA to create gene knockouts. Identify gene knockouts of interest and then sequence the affected gene directly, without cloning, using total bacterial genomic DNA template and primers that are homologous to the ends of the inserted EZ::TN Transposon. An EZ::TN Transposome containing an R6Kγ origin of replication, enabling rescue cloning of DNA comprising the transposon insertion site, is also available. See p. 14.



The *In Vitro* Deletion Strategy

The *In Vitro* Deletion Strategy should be considered if you need to generate a new plasmid or cosmid library for a sequencing project. First, clone your DNA into one of the specially-constructed plasmid or cosmid vectors. Incubate a single clone with EZ::TN™ Transposase to generate a complete population of random deletion subclones. Transform *E. coli* with an aliquot of the reaction mix and select deletion subclones on antibiotic plates. Size chosen deletion subclones by agarose gel and then generate the complete sequence of the original clone by choosing and sequencing a set of deletion subclones that span the entire size range of the original clone using a single primer that is provided with the kits.



Kits for the *In Vitro* Insertion Strategy

EZ::TN™ <KAN-2> Insertion Kit
EZI982K 10 Reactions

EZ::TN™ <TET-1> Insertion Kit
EZI921T 10 Reactions

EZ::TN™ <DHFR-1> Insertion Kit
EZI912D 10 Reactions

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

Kits for the Transposome™ Strategy

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 Transposome complex and two unlabeled sequencing primers.

Kits for the *In Vitro* Deletion Strategy

EZ::TN™ Plasmid-Based Deletion Machine
DPM9401 10 Reactions

pWEB::TNC™ Cosmid Cloning Kit
WEBC931 10 Reactions

Complete kit for producing 10 unbiased cosmid libraries.

pWEB::TNC™ Deletion Cosmid Transposition Kit
WEBC942 10 Reactions

Complete kit for generating a complete population of random deletion subclones from clones produced using the pWEB::TNC Cosmid Cloning Kit.

www.epicentre.com/transposomics.htm

EZ::TNTM Transposon Insertions Into Target DNA *In Vitro* are Highly Random

Ronald J. Meis, EPICENTRE

Introduction

EZ::TNTM Transposon Tools, based on a hyperactive *in vitro* Tn5 transposition system¹, can be used to speed up and simplify a myriad of genomics and proteomics procedures. For many applications, including complete sequencing of a DNA target that is too large to sequence with a single set of sequencing reactions, it is important that insertion of the EZ::TN Transposon into target DNA is highly random. In order to further evaluate randomness of *in vitro* insertion obtained using EZ::TN Insertion Kits, the transposon insertion sites were sequenced for 55 colonies picked at random from among >10⁶ insertion clones obtained following transformation of *E. coli* cells with an aliquot of the insertion reaction.

Materials and Methods

The DNA target used for the insertion reaction, referred to as “pIndigoBAC-5/269,” was a 7775-bp DNA comprising a 269-bp fragment cloned in the pIndigoBAC-5 vector.

In vitro insertions into the pIndigoBAC-5/269 target were made by incubating the target with an equal molar amount of an EZ::TN <TET-1> Transposon, encoding tetracycline resistance, and EZ::TN Transposase for 2 hours at 37°C as described in the EZ::TN <TET-1> Insertion Kit literature. Following transformation of TransforMaxTM EC100TM Electrocompetent *E. coli* cells with an aliquot of the *in vitro* insertion reaction, insertion clones were selected by growth on tetracycline plates. Greater than >10⁶ tetracycline-resistant pIndigoBAC-5/269 insertion clones were obtained. Fifty-five of these insertion clones, each containing a single EZ::TN <TET-1> Transposon, were randomly chosen from the plate, and used to prepare DNA for use as templates in DNA cycle sequencing reactions. The DNAs were sequenced using the SequiTherm EXCELTM II DNA

Sequencing Kit LC and IRD800-labeled forward and reverse transposon-specific primers. See Figure 1 on page 4 for an overview of the EZ::TN Transposon insertion, selection and sequencing process.

Results

The map position of each of the 55 EZ::TN Transposon insertions into pIndigoBAC-5/269 is shown in Figure 1A. An analysis of the localized G+C content of pIndigoBAC-5/269, based on a 50-bp window, is shown in Figure 1B. Further data and a detailed analysis of the results will be published separately at a later date. However, from the insertion data presented in Figure 1, the following conclusions can be surmised:

1. There was no transposon insertion bias either for or against high G+C or high A+T regions in the target DNA - see for example, insertion clones 679, 683, 752, 705, 727, 740, 688, 718, and 724 in Figure 1B.
2. The complete sequence of the target was easily obtained. The longest regions of pIndigoBAC-5/269 without an insertion were 510 bp between clones 683 and 752 and 488 bp between clones 745 and 678. Both of these spans were easily sequenced from clones 683/752 and 745/678, respectively.

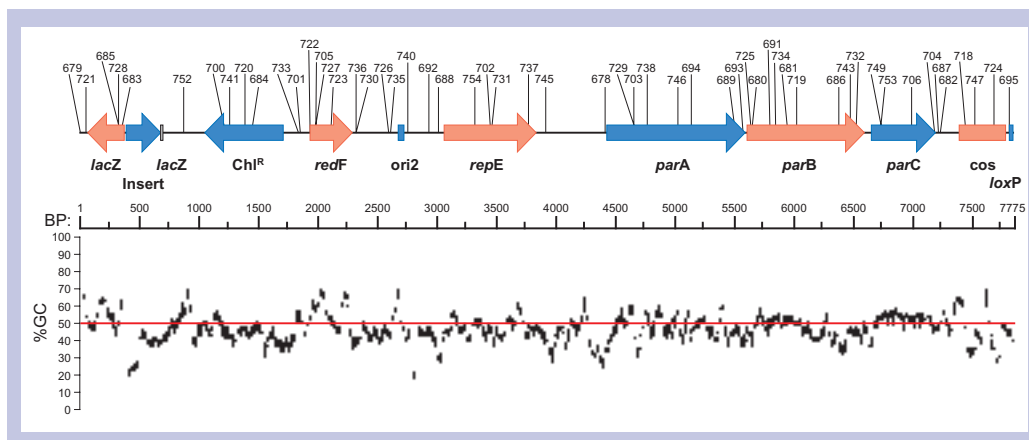
Conclusion

Although the sample size of 55 insertion clones was small relative to the >10⁶ transposon insertion clones generated in the *in vitro* EZ::TN Transposon reaction, the data strongly supported the conclusion that EZ::TN Transposon insertions into target DNA are highly random.

Reference

1. Goryshin, I.Y. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**: 7367-7374.

Figure 1. Insertion data for 55 EZ::TNTM <TET-1> Transposon insertions into pIndigo BAC-5/269: (1A) Map position of insertions; (1B) G+C content of target sequence determined using a 50-bp window.



Construction of an Environmental Genomic DNA Library from Soil using the EpiFOS™ Fosmid Library Production Kit

Mike Fiandt, EPICENTRE

Fosmid vectors^{1,2} provide an improved method for cloning and stably maintaining cosmid-sized (35 - 45 kb) libraries in *E. coli*. The pEpiFOS™-5 Fosmid Vector, provided linearized and dephosphorylated in the EpiFOS™ Fosmid Library Production Kit, is derived from the single copy F-factor of *E. coli* to insure that the clones produced are propagated as single copies in the cell to improve their stability compared to conventional cosmid libraries.

The EpiFOS Fosmid Library Production Kit utilizes a novel strategy (Figure 1) for cloning randomly sheared and end-repaired genomic DNA. Shearing of DNA into approximately 40-kb fragments leads to highly random generation of fragments in contrast to the conventional approach of generating DNA fragments by partial restriction endonuclease digestion. Like cosmids, fosmids are introduced into *E. coli* by high efficiency lambda packaging. The result is a complete and unbiased fosmid library.

Here we describe the production and preliminary analysis of an environmental genomic DNA fosmid library from soil using the EpiFOS Fosmid Library Production Kit.

DNA preparation

17.5 g of soil (taken from the grounds of EPICENTRE, Madison, WI) was suspended in buffer containing 1% SDS, Proteinase K, RiboShredder™ RNase Blend and RNase A and incubated at 70°C for 1 hour. The “slurry” was filtered and the DNA was precipitated from the filtrate. Following centrifugation, the DNA pellet was dried and resuspended in TE.

Fosmid cloning and library production

The crude soil DNA was further purified and size selected by agarose gel electrophoresis. The purified DNA was made blunt and 5'-phosphorylated, and then ligated into the provided linearized and dephosphorylated pEpiFOS-5 Fosmid vector. The EpiFOS Fosmid Library Production Kit provides all buffers and enzymes for the end repair of the DNA as well as for ligation into the pEpiFOS-5 vector. pEpiFOS clones were packaged using the provided high efficiency MaxPlax™ Lambda Packaging Extracts and plated on the supplied EPI100™ *E. coli* strain.

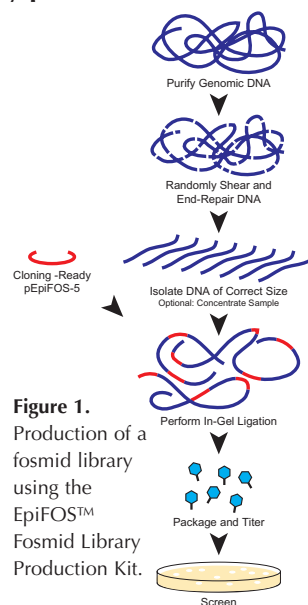


Figure 1. Production of a fosmid library using the EpiFOS™ Fosmid Library Production Kit.

Analysis of the Fosmid soil library

The EpiFOS Fosmid Library Production Kit produced a library of 6×10^5 colonies/ μg of soil DNA. Analysis of the fosmid clones was initially done by restriction endonuclease “fingerprinting” and fosmid end-sequencing using the pEpiFOS-5 Forward and Reverse Sequencing Primers.

Three fosmid clones were randomly chosen from the library and analyzed by *Not* I (GCGGCCGC) restriction



Figure 2. Gel analysis of 3 *Not* I-digested Fosmid clones.

Lane 1, size marker;
Lane 2-3, Clone F1 undigested and *Not* I-digested
Lane 4-5, Clone F2 undigested and *Not* I-digested
Lane 6-7, Clone F3 undigested and *Not* I-digested

digestion. These clones were found to contain multiple *Not* I sites (figure 2) - a surprising find considering the approximately 40kb size of the clones - providing our first evidence of high G+C content. The DNA sequences generated from end-sequencing of each of three other clones revealed 63% - 70% G+C content. BLAST homology search against GenBank revealed no significant homology between any of the 3 fosmid clones and any sequence in GenBank. Thus, these clones likely contain unique and previously uncharacterized sequences.

Analysis of these and other fosmid clones including one that demonstrates a phosphatase-like activity, is ongoing.

References

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

EpiFOS™-Fosmid Library Production Kit

FOS0901 1 Kit

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

*Exclusively licensed by EPICENTRE Technologies.

pEpiFOS™-5 Forward Sequencing Primer

F5FP010 1 nmole 50 μM

pEpiFOS™-5 Reverse Sequencing Primer

F5RP011 1 nmole 50 μM

www.epicentre.com/catalog/fos5.htm

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

Just swab the inner cheek, rotate in QuickExtract™ Solution, and heat to obtain PCR-ready genomic DNA for >100 amplifications.

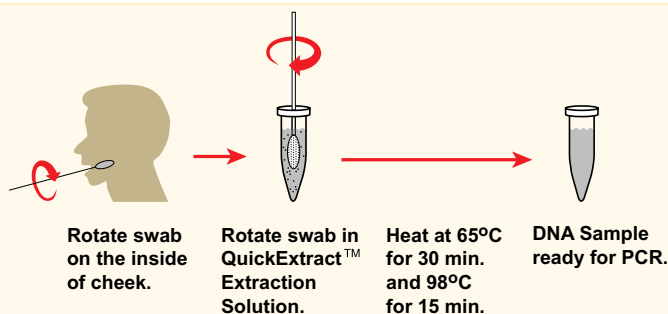


Figure 1

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

The QuickExtract Solution is pre-aliquoted into individual sample tubes. To obtain PCR-ready DNA, just rotate the buccal sample swab in one of these tubes, mix and heat. No centrifugation step is needed, making it easy to process one to hundreds of samples in less than an hour - without phenol, chloroform, or other toxic organic solvents. DNA yields range from 1-7 µg – more than other methods and enough to perform at least 100 PCR assays. Figure 2 shows typical PCR results from DNA extracted using the BuccalAmp Kit.

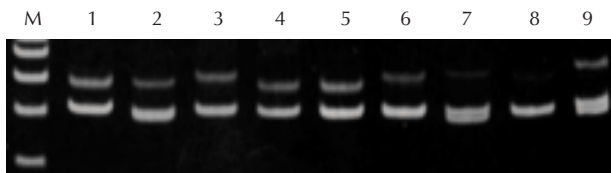


Figure 2. PCR of polymorphic repeat sequences in DNA from buccal samples from 9 individuals obtained using the BuccalAmp™ DNA Extraction Kit. DNA was amplified using the FailSafe™ PCR System (FS99060). (Lane M = 100 bp marker).

BuccalAmp Kits include Catch-All™ Sample Collection Swabs – a soft foam swab on a soft, flexible plastic handle (Figure 3). Catch-All Swabs provide gentle, safe buccal sample collection, even for infants, and the porous foam on these swabs catches more of the sample than buccal brushes. Catch-All Swabs are provided individually packaged in sterile hard-pack plastic cylinders. After collecting the sample, return the sample swab to the cylinder package, for safe, secure storage and transport from the collection site to the analysis site.

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

QuickExtract DNA Extraction Solution and Catch-All Sample Collection Swabs are available separately and in bulk for high throughput applications.

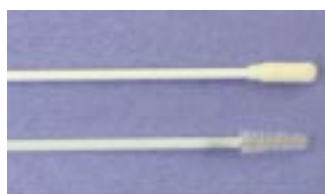


Figure 3. Catch-All™ Sample Collection Swabs (upper) improve DNA yields by adsorbing more sample than is possible with buccal brushes (lower)

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

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.	

Cloning Ready BAC Vectors

pIndigoBAC-5 (*Bam*H I-Cloning Ready) & pIndigoBAC-5 (*Hind* III-Cloning Ready)

pIndigoBAC-5* is the first commercially available Bacterial Artificial Chromosome (BAC) vector for cloning and preparation of primary BAC libraries. The vector is derived from pBeloBAC11 and pIndigoBAC¹ and will accommodate and stably maintain DNA inserts of >100 Kb. pIndigoBAC-5 has been linearized at either its unique *Bam*H I or its unique *Hind* III site. The linearized DNA was then dephosphorylated and is ready for cloning the respective *Bam*H I or *Hind* III restriction cut genomic DNA. The linearized and dephosphorylated vectors are highly purified and tested for low vector background and dephosphorylation.

BAC libraries can be produced using the high efficiency TransforMaxTM EC100TM Electrocompetent *E. coli* (available separately; see p. 13). In addition, BAC clones produced in pIndigoBAC-5 can be sequenced directly, without sub-cloning, using the Forward and Reverse BAC end-sequencing primers² (available separately) and the EZ::TN<KAN-2> Insertion Kit (see p. 1 for details).

Features of pIndigoBAC-5 Cloning Ready vectors

- Derived from pBeloBAC11 and pIndigoBAC.
- Already linearized and dephosphorylated and ready for cloning.
- Tested for low background and for complete dephosphorylation.
- Enhanced blue/white screening of recombinants.

Quality control

Greater than 10⁶ white colonies/μg of pIndigoBAC-5 DNA are observed when pIndigoBAC-5 (*Bam*H I-Cloning Ready or *Hind* III - Cloning Ready) DNA is ligated with control DNA (*Bam*H I-cut or *Hind* III-cut *E. coli* chromosomal DNA) and transformed into TransforMaxTM EC100TM Electrocompetent *E. coli*. Greater than 95% of white colonies contain an insert and less than 10% background (colonies due to non-linearized pIndigoBAC-5 DNA) is observed.

References

1. Birren, B., Mancino, V. and Shizuya, H., (1999) Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual* v.3, 241-295, Cold Spring Harbor Press.
2. Hurowitz, E.H. *et al.*, (2000) *DNA Research* 7:2,1

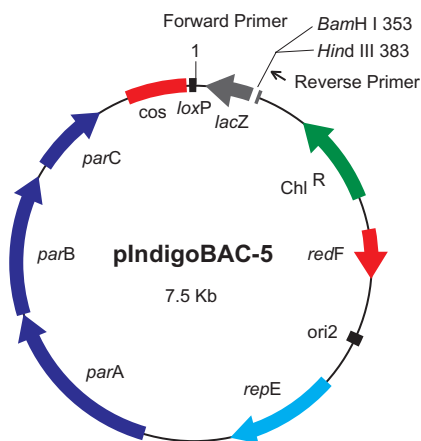


Figure 1. Cloning-Ready pIndigoBAC-5 Vectors are supplied linearized at either the unique *Bam*H I or *Hind* III site and completely dephosphorylated.

pIndigoBAC-5 (<i>Bam</i>H I-Cloning Ready)		
BACB085H	500ng	25 ng/μl
Supplied linearized at <i>Bam</i> HI site and dephosphorylated.		
pIndigoBAC-5 (<i>Hind</i> III-Cloning Ready)		
BACH095H	500 ng	25 ng/μl
Supplied linearized at <i>Hind</i> III site and dephosphorylated.		
pIndigoBAC-5 Forward Sequencing Primer		
BFP0701	1 nmole	50 μM
pIndigoBAC-5 Reverse Sequencing Primer		
BRP0801	1 nmole	50 μM
TransforMaxTM EC100TM Electrocompetent <i>E. coli</i>		
EC10005	5 X 100 μl	(10 Electroporations)
EC10010	10 X 100 μl	(20 Electroporations)
Each includes pUC19 control DNA.		
See p. 13 for information about TransforMax cells.		

*pIndigoBAC-5 is exclusively licensed by EPICENTRE Technologies.

Get the Highest Possible Yield of Full Length RNA

AmpliScribe™ High Yield Transcription Kits

EPICENTRE's AmpliScribe™ T7, T3 & SP6 High Yield Transcription Kits are specially formulated to utilize high concentrations of NTPs that are inhibitory to other kits and "homemade" *in vitro* transcription systems. The result is the highest possible yield of RNA from an *in vitro* transcription reaction.

The Highest Yield of Large RNA

AmpliScribe T7 High Yield Kits consistently produce the highest yield of large RNA...up to 150 µg of 1.8 Kb RNA per µg of DNA in a standard 20 µl, 2-hour reaction. AmpliScribe Kits incorporate up to 90% of input NTPs into RNA product and will produce up to 20 times more full-length RNA than conventional *in vitro* transcription reactions.

Full-length Transcripts

AmpliScribe High Yield Kits contain RNase inhibitor to ensure the integrity of the RNA produced.

The Highest Yield of Short RNA

AmpliScribe T7 Kits produce more short (<300 nt) RNA than a competitor's kit that was designed for short transcripts (Figure 3). Generate up to mg amounts of short RNA from a 100 µl AmpliScribe reaction.

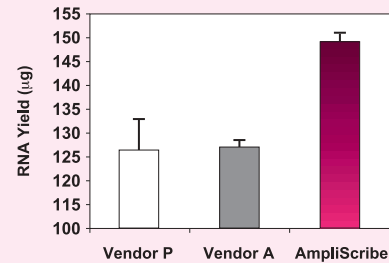


Figure 1

Highest yield of large RNA. AmpliScribe T7 High Yield Transcription Kit consistently produced more full-length 1.8 Kb RNA transcript than kits from two competitors.

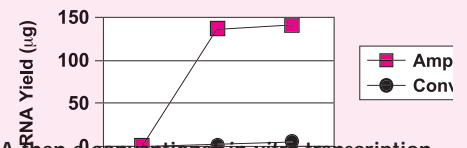


Figure 2

More RNA than a conventional *in vitro* transcription reaction. An AmpliScribe T7 High Yield Transcription reaction produces >20 times more RNA than a conventional reaction.

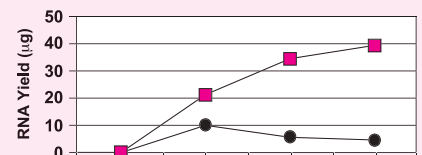


Figure 3

Highest yield of short RNA. A 20 µl AmpliScribe T7 reaction produces more of a 63-base RNA than a competitor's kit specifically designed for short transcripts.

AmpliScribe™ High Yield Transcription Kits

	<u>Catalog No.</u>	<u>Kit Size</u>
AmpliScribe™ T7 High Yield Transcription Kit	AS2607	25 Reactions
	AS3107	50 Reactions
AmpliScribe™ T3 High Yield Transcription Kit	AS2603	25 Reactions
	AS3103	50 Reactions
AmpliScribe™ SP6 High Yield Transcription Kit	AS2606	25 Reactions
	AS3106	50 Reactions

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

www.epicentre.com/catalog/ascribe.htm



EPICENTRE®

www.epicentre.com

Get the Highest Yield of 5'-capped RNA

AmpliCap™ T7, T3 & SP6 High Yield Message Maker Kits

EPICENTRE's new AmpliCap™ T7, T3 & SP6 High Yield Message Maker Kits are specially formulated to produce the highest yield of m⁷G[5']ppp[5']G capped RNA from an *in vitro* transcription reaction. With the AmpliCap Message Maker Kits you get:

The Highest Yield of 5'-Capped RNA

AmpliCap High Yield Message Maker Kits consistently produce more 5'-capped RNA than kits from the leading competitors (Figure 1)...up to 45 µg of m⁷G[5']ppp[5']G- capped RNA from a standard 20 µl T7 or T3 reaction and up to 35 µg of capped RNA from an SP6 reaction.

High 5'-Capping Efficiency

An optimized m⁷G[5']ppp[5']G Cap/NTP PreMix solution is provided in the kits to maximize capping efficiency, RNA yield and convenience. Capping efficiencies up to 80% are obtained with all three kits.

Full-length Capped Transcripts

AmpliCap Message Maker Kits contain RNase inhibitor to ensure the integrity of the RNA produced. In addition, a vial of 20 mM GTP is included for efficient production of long capped RNA transcripts.

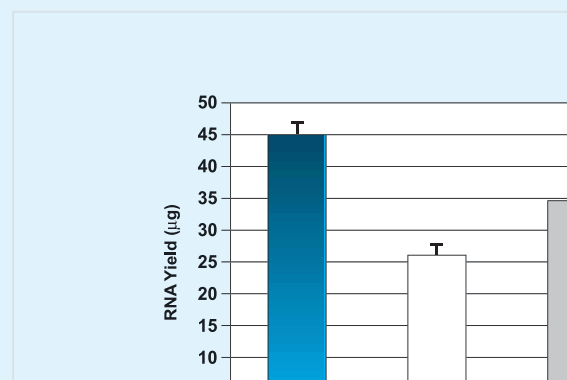


Figure 1. Yield of capped RNA using the AmpliCap™ T7 Message Maker Kit compared to other kits. The AmpliCap T7 High Yield Message Maker Kit consistently produced more full-length 5'-capped RNA than kits from two competitors. All transcription reactions were performed for two hours using the AmpliCap T7 control template.

AmpliCap™ High Yield T7, T3 & SP6 Message Maker Kits

<u>Catalog No.</u>	<u>Kit Size</u>
T7 AC0707	25 Reactions
T3 AC0703	25 Reactions
SP6 AC0706	25 Reactions

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

www.epicentre.com/catalog/acap.htm



EPICENTRE®
www.epicentre.com



Get Superior
RT-PCR Fidelity & Sensitivity
with EPICENTRE's

MasterAmp™ RT-PCR Kits

Now you can get the best sensitivity and fidelity with EPICENTRE's MasterAmp™ RT-PCR Kits.

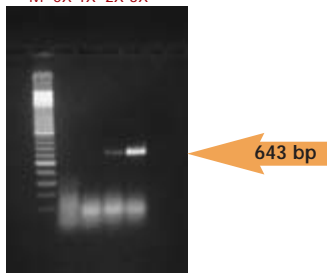
EPICENTRE's MasterAmp™ PCR Enhancement Technology (with betaine)* can substantially improve the yield and specificity of amplification reactions, and is especially useful where the template has a high G+C content and secondary structure.

1. The MasterAmp™ RT-PCR Kit for High Sensitivity: Excellent sensitivity and yield.

- Highest Sensitivity:**
 Detect specific target RNA from as little as 1 picogram of total cellular RNA. (better than all other kits on the market.)
- High Temperature Reverse Transcription:**
 RetroAmp™ RT reduces problems associated with secondary structure.
- Amplify Difficult Templates Easily:**
 MasterAmp™ PCR Enhancement Technology improves amplification of templates with high G+C content.
- High Specificity:**
 The MasterAmp PCR Enhancement Technology improves specificity and yield, and greatly reduces spurious, non-specific amplification.
- Convenient:**
 The one-step, one-tube continuous reaction protocol greatly reduces the likelihood of contamination.
- Complete:**
 Kit contains everything you need for RT-PCR.

The MasterAmp™ PCR Enhancer improves the yield of your PCR product.

MasterAmp PCR Enhancer Concentration
M 0X 1X 2X 3X

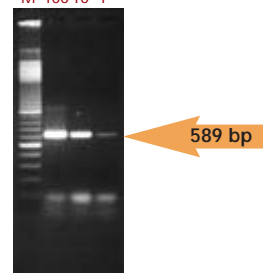


A rare bovine PRLR transcript was amplified from 100 ng of total bovine liver RNA. The MasterAmp PCR Enhancer improved the yield of the RT-PCR product.

To learn more about these experiments, please see *Epicentre Forum* 5:1, pages 1-4.

You can amplify specific RNA species from as little as 1 picogram of total cellular RNA.

Picograms of Total RNA
M 100 10 1

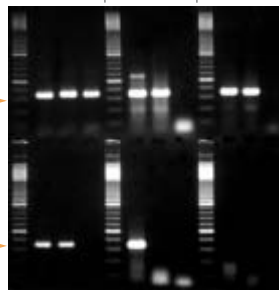


High temperature reverse transcription using RetroAmp RT DNA Polymerase permitted amplification of a 589 base fragment of Human Chorionic gonadotropin alpha (hCG α). 1 pg RNA corresponds to approximately 50 copies of hCG α RNA.

To learn more about these experiments, please see *Epicentre Forum* 5:1, pages 8-10 and the EPICENTRE 2001 Catalog, page 4-4.

The MasterAmp RT-PCR Kit for High Sensitivity detects lower levels of RNA than RT-PCR Kits from other manufacturers.

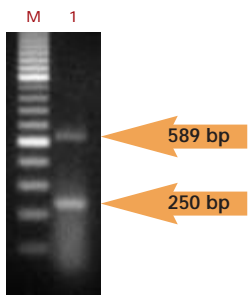
Picograms of Total RNA
MasterAmp Vendor P Vendor R
M 100 50 25 | M 100 50 25 | M 100 50 25



M 100 50 25 | M 100 50 25 | M 100 50 25
Vendor E | Vendor S | Vendor L

To learn more about these experiments, please see *Epicentre Forum* 7:3, page 11.

You can perform multiplex RT-PCR using either of the MasterAmp RT-PCR Kits.



M, 100 bp ladder
Lane 1, co-amplification of 589 bp CG α and 250 bp β -actin from 100 ng human placental RNA

To learn more about these experiments, please see *Epicentre Forum* 6:3, page 10.

* Covered by German Patent No. DE4411588C1 and other pending and issued patents in the United States and other countries assigned or exclusively licensed to EPICENTRE.

2. The MasterAmp™ High Fidelity RT-PCR Kit: Excellent fidelity and reliability.

- **High Fidelity:**
Makes far fewer errors than other RT-PCR kits.
- **Longer Amplification:**
The MMLV-RT Plus and MasterAmp™ TAQurate™ DNA Polymerase Mix yield longer, full-length RT-PCR products.
- **Flexible:**
Amplify tough templates and multiple templates in a single reaction.
- **Reliable and Efficient:**
The MasterAmp PCR Enhancement Technology improves the yield and efficiency of first strand synthesis and PCR, especially on tough templates.
- **Convenient:**
The unique PreMix system makes RT-PCR set-up easy.
- **Complete:**
Kit contains everything you need for RT-PCR.

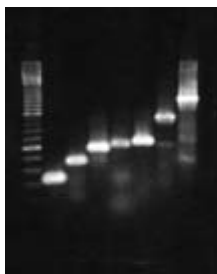
The MasterAmp High Fidelity RT-PCR Kit permits generation of error-free RT-PCR products for cloning and gene expression studies.



Representative electropherogram of sequence analysis of an RT-PCR product generated using the MasterAmp High-Fidelity RT-PCR Kit. The sequence of a full-length (850 bp) rabbit tissue factor cDNA clone generated from rabbit brain mRNA was found to be error-free.

Amplify templates from many different sources using the MasterAmp High Fidelity RT-PCR Kit.

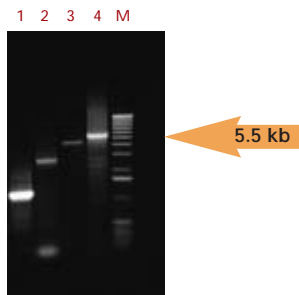
M 1 2 3 4 5 6 7



M, 100 bp ladder
Lane 1, 250 bp human lung β -actin
Lane 2, 350 bp human lung GAPDH
Lane 3, 463 bp region of TMV
Lane 4, 479 bp *E. coli* 16s rRNA
Lane 5, 589 bp human placental CG α
Lane 6, 850 bp human heart β -actin
Lane 7, 1248 bp region of TMV

To learn more about these experiments, please see *Epicentre Forum* 6:3, page 10.

With the MasterAmp High Fidelity RT-PCR Kit, you can amplify templates \geq 5.5 kb in length.



RT-PCR amplification of TMV RNA.
Lane 1, 1,248 bp fragment from TMV RNA
Lane 2, 2,795 bp fragment from TMV RNA
Lane 3, 4,517 bp fragment from TMV RNA
Lane 4, 5,503 bp fragment from TMV RNA
M, Kb ladder

To learn more about these experiments, please see *Epicentre Forum* 6:3, page 10.

The EPICENTRE website contains information on the MasterAmp™ RT-PCR Kit for High Sensitivity and MasterAmp™ High Fidelity RT-PCR Kit. View articles from the Epicentre Forum newsletter, and obtain detailed product protocols.

www.epicentre.com

How to Select the best MasterAmp™ RT-PCR Kit for your application

	MasterAmp™ High-Fidelity RT-PCR Kit	MasterAmp™ RT-PCR Kit for High Sensitivity
Highest fidelity	+	
Highest sensitivity		+
Amplification system	MMLV-RT Plus and MasterAmp TAQurate™ DNA Polymerase Mix (High Fidelity)	RetroAmp™ RT DNA Polymerase (High Sensitivity)
One-Step and Two-Step Protocols	+	+
Includes MasterAmp™ PCR Enhancement Technology* for difficult templates	+	+
Includes a Single Premix containing dNTPs, MgCl ₂ & Buffer	+	
Includes Random/Oligo dT Primers	+	
Longest Amplifications	+	
Best for cDNA cloning	+	
Best for detecting rare RNA species, in small samples		+
Best for cloning and sequencing RT-PCR products	+	
High Temperature cDNA Synthesis		+

* Covered by German Patent No. DE4411588C1 and other pending and issued patents in the United States and other countries assigned or exclusively licensed to EPICENTRE.

Ordering Information

Catalog Number	Description	Size
RT71225	MasterAmp™ RT-PCR Kit for High Sensitivity (Trial size)	25 reactions
RT712100	MasterAmp™ RT-PCR Kit for High Sensitivity	100 reactions
RT91025	MasterAmp™ High Fidelity RT-PCR Kit (Trial Size)	25 reactions
RT910100	MasterAmp™ High Fidelity RT-PCR Kit	100 reactions

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EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector Frequently Asked Questions

The EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector enables researchers to easily construct custom EZ::TN Transposons containing virtually any DNA sequence of interest (e.g. species-specific selectable markers, genetic control elements, cDNA, genes) for use in *in vitro* insertion reactions or for preparation of EZ::TN Transposomes™. Here, we provide answers to the questions regarding preparation of EZ::TN Transposons using pMOD-2<MCS> Vector.

Q1 What is the general protocol for preparing a custom EZ::TN Transposon using pMOD-2<MCS>?

A1 Preparation of an EZ::TN Transposon using pMOD-2<MCS> is a three step process:

1. First, clone your DNA of interest into the multiple cloning site of pMOD-2<MCS>.
2. Then, isolate the custom EZ::TN Transposon either by digestion with *Pvu* II or *Psh* AI (*Box* I) or by PCR amplification of the transposon region.
3. Finally, purify (see Q6) the newly constructed EZ::TN Transposon.

Q2 How can I make an EZ::TN Transposome from the custom EZ::TN Transposon?

A2 It's easy!. Just incubate the EZ::TN Transposon with EZ::TN Transposase in the absence of Mg²⁺.

Q3 What is the largest EZ::TN Transposon that I can make?

A3 To date, the largest custom EZ::TN Transposon reported by our customers is about 7 Kb. In theory, larger EZ::TN Transposons can be prepared. However, in general, the larger the transposon, the less efficiently it is inserted into the target DNA either *in vitro* or *in vivo* as an EZ::TN Transposome. One theory as to why large transposons are inserted into target DNA less efficiently is that there is a higher probability of a large transposon undergoing *intramolecular* transposition into itself rather *intermolecular* insertion into the target DNA.

Q4 What is the smallest EZ::TN Transposon that I can make?

A4 To date, the smallest EZ::TN Transposon that has been used successfully is about 290 bp.

Q5 Does the pMOD-2<MCS> vector contain genetic control elements?

A5 No. The DNA of interest (e.g. a selectable marker, gene or cDNA) must contain all necessary genetic control elements when cloned into pMOD-2<MCS> if it is to be expressed in a cell. Also, when an EZ::TN Transposon is used to make an EZ::TN Transposome for *in vivo* insertion into the cell's genome, it will be replicated as a single copy. Therefore, the level of expression of the gene or selectable marker is likely

to be diminished compared to expression in a high copy plasmid. If the transposon contains an antibiotic selection marker, it may be necessary to plate transposened cells on two or more plates containing different levels of antibiotic in order to detect transpositions into different cellular targets.

Q6 Does the newly prepared EZ::TN Transposon need to be purified prior to use?

A6 Whether the custom EZ::TN Transposon is released from pMOD-2<MCS> by *Pvu* II or *Psh* AI (*Box* I) digestion or by PCR amplification, it should be purified away from the vector and any trace of uncut pMOD-2 <MCS>. Agarose gel electrophoresis is one of the most effective methods of purification. Avoid or limit exposure of the EZ::TN Transposon to maintain its activity and integrity.

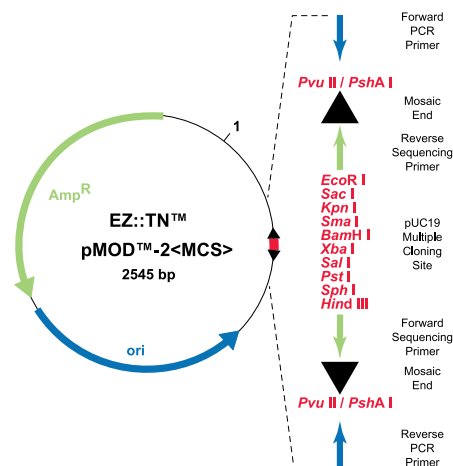


Figure 1. EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector.

EZ::TN™ pMOD-2™<MCS> Transposon Construction Vector	
MOD0602	20 µg
Includes: pMOD-2™<MCS> Vector and the Forward and Reverse PCR Primers	
EZ::TN™ Transposase	
TNP92110	10 Units
pMOD™<MCS> Forward Sequencing Primer	
MODFSP201	1 nmole
pMOD™<MCS> Reverse Sequencing Primer	
MODRSP202	1 nmole

www.epicentre.com/catalog/pmod2.htm

Direct PCR from Whole Blood

Haiying Grunenwald, EPICENTRE

Abstract

Reliable PCR assay results were obtained for the human hemochromatosis gene, for the human apo E gene, which has a G+C content of 75%, and for multiplex assays for the cystic fibrosis transmembrane regulator (CFTR) using the FailSafe™ PCR System to amplify genomic DNA in one microliter of heparin-treated whole blood that was stored for up to six months.

Introduction

Researchers often purify DNA from blood samples prior to performing PCR because it is believed that blood constituents and the reagents commonly used to preserve blood samples (e.g., anticoagulants) interfere with PCR.¹ In order to save the time and expense required for template purification, several methods have been reported for direct PCR of blood samples. These include microwave irradiation², hydrogen peroxide treatment³, and boiling in NaOH.⁴ Here, we report PCR amplification results using the FailSafe PCR System to amplify several human genes from unpurified DNA in untreated whole human blood and in whole blood stored for up to six months at +4°C, -20°C, or -70°C in the presence of the commonly-used anticoagulants sodium or lithium heparin, sodium citrate, and EDTA.

Methods and Results

Blood collection

Blood samples were collected using standard blood draw procedures and placed in various types of collection tubes corresponding to the respective method tested. In a limited number of cases, a portion of a collected sample was used for PCR within 5 minutes of collection without any treatment. The remaining blood of each sample was stored in a Vacutainer™ tube with Hemogard™ closure (Becton Dickinson, NJ) containing one of four different commonly-

used anticoagulants to prevent clotting: sodium heparin; lithium heparin; 3.2% sodium citrate; or 7.5% K₃EDTA. The different preserved blood samples were then stored at +4°C, -20°C, or -70°C for various times before use.

PCR Conditions

PCR primers and PCR temperature cycling conditions were as described elsewhere by researchers working on the respective templates. The optimal FailSafe PCR PreMix (which contains PCR buffer, MgCl₂, dNTPs, and FailSafe™ PCR Enhancer), was determined for each template and primer pair as described in the FailSafe PCR PreMix Selection Kit protocol using purified human genomic DNA as a template; the same optimal FailSafe PCR PreMix was then used to amplify the respective gene from whole untreated or anticoagulant-treated blood.

Analysis of the effect of different blood anticoagulants on PCR of the human hemochromatosis and apo E genes

Blood samples are generally stored in anticoagulants until use. In order to test whether PCR amplifications would be successful using whole blood samples stored in various anticoagulants without DNA extraction, the hemochromatosis gene was amplified from blood samples stored at +4°C in four common anticoagulants - sodium heparin, lithium heparin, sodium citrate, and EDTA. Each 50- μ l reaction included 1 X FailSafe™ PCR PreMix D, 50 pmoles of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 μ l of the whole blood. Cycling conditions were 5 minutes at 95°C, followed by 38 cycles of 30 seconds at 96°C, 30 seconds at 55°C, and 1 minute at 72°C, then 7 minutes at 72°C. One nanogram of purified human genomic DNA was also amplified under the same conditions as a positive control, while the negative control reaction included everything except for the template.



Never fail at PCR again.
We Promise.

“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 kit worked first time and the J mix gave a superbly clear band at precisely the correct molecular weight.”

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

The ability to amplify difficult sequences, such as sequences with high G+C content, can be crucial for some studies. To test whether the FailSafe™ PCR systems can be used to successfully amplify a G+C-rich sequence directly from blood samples stored in various anticoagulants, PCR was carried out for the apo E gene, which has a G+C content of 75%. Each 50- μ l reaction included 1 X FailSafe™ PCR PreMix J, 25 pmoles of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 μ l of an anticoagulant-treated whole blood sample. Cycling conditions were 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 1 minute at 72°C. One nanogram of purified human genomic DNA was also amplified under the same conditions as a positive control, while the negative control reaction included everything except the template.

Multiplex PCR of CFTR in heparin-treated whole blood

Multiplex PCR is useful for many assay situations, such as for genetic linkage studies using microsatellite markers or for detection of multiple potential pathogens. In order to determine whether unpurified whole blood samples could be used for multiplex PCR, blood samples stored at -20°C in sodium heparin were used to amplify a five-band multiplex PCR of the cystic fibrosis transmembrane regulator (CFTR). Five exons (4, 10, 11, 20, and 21) of CFTR were amplified using the FailSafe PCR System. Each 50 μ l reaction included 1 X FailSafe™ PCR PreMix C, 25 pmoles of each of all 5 sets of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 μ l of whole blood sample stored in sodium heparin at -20°C. Cycling conditions were 2 minutes at 94°C, followed by 30 cycles of 10 seconds at 94°C, 10 seconds at 53°C, and 10 seconds at 74°C, then 5 minutes at 74°C.

Effect of storage temperature and time of storage on the ability of heparin-treated whole blood to be used for PCR

Whole blood samples treated with sodium heparin were assayed as described above for their ability to be used for PCR amplification following storage for up to six months at +4°C, -20°C, or -70°C.

Analysis of PCR products

Approximately 10% of each reaction was electrophoresed on a 2% agarose gel and visualized by staining with SyberGold™ (Molecular Probes, OR), unless otherwise stated.

Results and Discussion

Satisfactory PCR amplifications of the hemochromatosis and apo E genes were obtained using freshly drawn untreated blood samples tested (i.e., without any anticoagulants) (data not shown). However, due to the difficulty of

working with untreated blood, samples are typically treated with an anticoagulant prior to use or storage.

Our studies indicated that treatment of whole blood with heparin is preferable to treatment with sodium citrate or EDTA if the sample is to be used for PCR amplification. PCR assays for the hemochromatosis gene, the apoE gene, and the CFTR multiplex were always successful for all of the heparin-treated samples tested (Figure 1, Panel A). On the other hand, although amplification was sometimes successful using sodium citrate- or EDTA-treated blood (data not shown), PCR consistency was poor, with an estimated PCR failure rate of about 50%. The consistency of PCR was 100% in sodium heparin-treated blood for the target templates and samples tested. However, it should be noted that this result was based on using only 1 μ l of blood per 50- μ l PCR reaction. It is possible that use of greater amounts of blood would result in inhibition of PCR, but this was not tested. If PCR of a heparin-treated blood sample fails, the author recommends first increasing the number of PCR cycles in preference to increasing the amount of blood used.

Our data (Figure 1, Panel B) also showed that sodium heparin-treated whole blood can be used successfully to amplify target DNA templates with high G+C content using the FailSafe™ PCR System, such as the apoE gene, which has a G+C content of 75%.

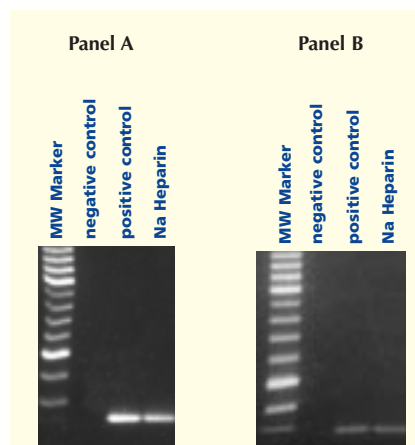


Figure 1. Amplification of hemochromatosis and apoE gene using blood samples stored in sodium heparin.

Panel A. A 200 bp region of hemochromatosis gene was amplified with blood samples stored at 4°C in sodium heparin.

Panel B. A 268 bp region of apo E gene was amplified with blood samples stored at 4°C in sodium heparin.

The positive and negative controls were also included.

Further, the 5 bands representing 5 exons of the CFTR gene were all amplified from DNA in heparin-treated whole blood (Figure 2), indicating that even difficult multiplex PCR targets can be amplified in blood using the FailSafe™ PCR System.

Blood samples are generally stored at +4°C, -20°C, or -70°C. Successful direct PCR amplifications were obtained from heparin-treated blood samples stored at all of these temperatures for up to 6 months. The amount of PCR product appeared to be the same from sodium heparin-treated blood samples stored at all three temperatures for the hemochromatosis and apo E genes. For example, Figure 3, Panel B illustrates successful PCR amplifications of hemochromatosis and apo E genes from blood samples stored in sodium heparin for 6 months at -70°C. However, since the intensity of the PCR product bands appeared to decrease somewhat for multiplex PCR of CFTR in sodium heparin-treated blood samples stored for 6 months at +4°C, the author recommends keeping heparin-treated blood samples in a freezer for long-term storage whenever possible. No decrease in the amount of PCR product was observed following multiplex PCR of sodium heparin-treated blood stored for 6 months at -20°C (Figure 3, Panel A).

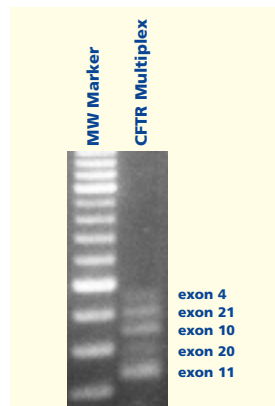


Figure 2. Five-band CFTR multiplex PCR with blood. Blood samples stored in sodium heparin at -20°C were used to amplify CFTR exons, 4, 10, 11, 20, and 21.

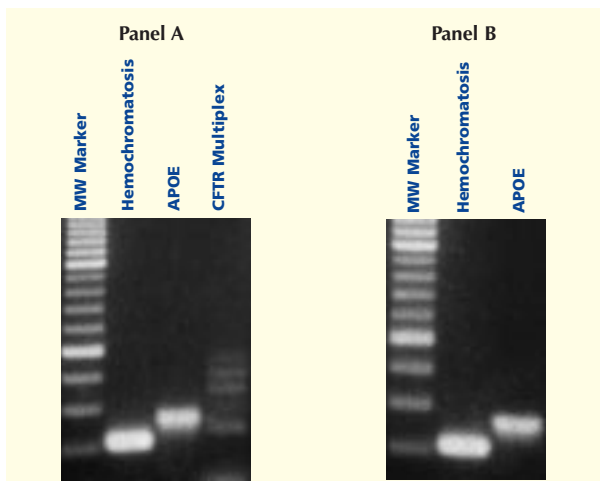


Figure 3. PCR of blood samples stored at -20°C or -70°C. Blood samples in sodium heparin stored at either -20°C (Panel A) or -70°C (Panel B) were used to PCR amplify hemochromatosis and apo E genes. CFTR multiplex PCR was also done with samples stored at -20°C. Approximately 25% of each reaction was electrophoresed on a 2% agarose gel.

Tips for Successful PCR of Whole Blood

1. Use sodium heparin as the anticoagulant. Lithium heparin was just as successful for the assays tested, but fewer assays were performed with this anticoagulant.
2. Use only 1 µl of mixed whole blood per 50-µl PCR assay if possible.
3. If necessary, use more PCR cycles rather than more blood sample to improve amplification efficiency.
4. For short-term storage (i.e., up to about 2 weeks), store blood samples at +4°C. However, for long-term storage, store at -20°C or -70°C since some degradation of DNA, which led to reduced PCR efficiency, was seen with blood samples stored at +4°C compared to those stored at the freezer temperatures.
5. The FailSafe PCR System provides a simple and fast way to find the optimal PCR conditions for any template and primer pair. With every new set of primers tested, obtain the most optimal PCR conditions by using the FailSafe™ PCR PreMix Selection Kit with purified DNA. After the optimal FailSafe™ PCR PreMix is chosen, then use this PreMix for direct PCR from whole blood.
6. While the data presented in this report were obtained for PCR assays using 1 µl of well-mixed whole blood, using 1ml of the buffy coat in the PCR reactions should provide similar results.
7. Use of whole blood in PCR reactions will result in a reddish color in the final PCR products. This does not interfere with the detection of PCR products via electrophoresis.

Conclusion

Consistent successful PCR amplifications were achieved from untreated or heparin-treated unpurified whole blood using the FailSafe™ PCR System. Reliable PCR results were obtained for difficult templates, including multiplex templates and templates with high G+C content. Consistent PCR results were obtained for all targets tested using heparin-treated whole blood samples that were stored for at least 2 weeks at +4°C, or for at least 6 months at -20°C or -70°C.

References

1. Wang, J-T, *et al.* (1992) *J. Clin. Microbiol.* **30**, 750.
2. Ihara, M, *et al.* (1994) *BioTechniques* **17** (4), 726.
3. Rudbeck, L. and Dissing, J. (1998) *BioTechniques* **25** (4), 588.
4. M. I. Queipo-Ortuna, M. A., *et al.* (1999) *BioTechniques* **27** (2), 248.

FailSafe™ PCR PreMix Selection Kit

FS99060 60 Units

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

FailSafe™ PCR System

FS99100 100 Units

Includes FailSafe™ PCR Enzyme Mix and choice of one FailSafe™ PCR 2X PreMix.

FS99250 250 Units

Includes FailSafe™ PCR Enzyme Mix and choice of two FailSafe™ PCR 2X PreMixes.

FS9901K 1,000 Units

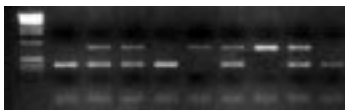
Includes FailSafe™ PCR Enzyme Mix and choice of eight FailSafe™ PCR 2X PreMixes.

Never fail at PCR again.
We Promise.

“The FailSafe System worked beautifully! ... I’m responsible for providing PCR data trust-

worthy enough to make breeding decisions for each line. Our experiments require screening of the animals’ genotypes. We are now using the FailSafe System to screen two knockout lines (multiplex) and four transgenic (single PCR product) lines of mice. Soon we will be using the FailSafe PreMix Selection Kit again when we start working with a new line of knockout mice.”

— Jessica Otte,

Center of Neurovirology & Cancer Biology,
Temple University, Philadelphia, PA

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 (0.1 µg each) were incubated for 30 cycles of 90°C for 15 sec., 55°C for 15 sec., and 72°C for 1 min., followed by 72°C for 10 minutes. (Data courtesy of Jessica Otte).



Obtain the Highest Transformation Efficiency Possible Using TransforMax™ EC100™ Electrocompetent *E. coli*

With a transformation efficiency of $>5 \times 10^9$ cfu/µg DNA (pUC19) EPICENTRE's new TransforMax™ EC100™ Electrocompetent *E. coli* have the highest transformation efficiency of any electrocompetent cells. And since TransforMax EC100 cells are restriction minus and lack transformation size bias against large clones, they are ideal for almost every cloning application. For example, use of TransforMax EC100 cells results in complete and unbiased BAC libraries, ensuring the presence of every clone in the library. Their high efficiency, lack of size bias and other features also make them ideal for generating EZ::TN Transposon insertion clones or deletion subclones from DNA cloned in the pWEB::TNC™ cosmid or pPDM™ plasmid deletion vectors.

Transformation Efficiency $> 5 \times 10^9$ cfu/µg DNA (pUC19)**Genotype**F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) *ø80dlacZ*ΔM15 Δ*lacX74* *recA1 endA1 araD139* Δ(*ara, leu*)7697 *galU galK λ- rpsL nupG***Relevant Phenotype**

- Blue/white screening of vectors expressing the LacZ' α-complementing peptide.
- Restriction minus for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- Accepts large clones for complete and unbiased BAC library production.

- Endonuclease minus (*endA1*) to ensure high yields of plasmid clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

Table 1. The average transformation efficiency of eight independent transformations of TransforMax™ EC100™ Electrocompetent *E. coli* with pUC vector was 9.2×10^9 . All values are in cfu/µg DNA.

	Transformation efficiency
TransforMax EC100 <i>E. coli</i>	9.2×10^9
Competitor S	5×10^9
Competitor I	4×10^9
Competitor B	3×10^9

TransforMax™ EC100™ Electrocompetent *E. coli*

EC10005 5 X 100 µl (10 Electroporations)

EC10010 10 X 100 µl (20 Electroporations)

Each includes pUC19 control DNA. TransforMax cells are available in bulk. Please inquire.

www.epicentre.com/catalog/ec100.htm

Rescue Cloning of Bacterial Genomic DNA using the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™

A «Transposome™» is a stable complex between an EZ::TN™ Transposon and EZ::TN™ Transposase that forms spontaneously in the absence of magnesium cations. The complex is so stable that it can be used to transform competent *E. coli* and other microorganisms. Once in the cell, the EZ::TN Transposase is activated by intracellular magnesium cations and randomly inserts a single EZ::TN Transposon into the chromosome or extrachromosomal DNA of each cell *in vivo*. EPICENTRE offers EZ::TN Transposomes with transposons that encode kanamycin-, tetracycline-, or trimethoprim-resistance genes. Insertion clones are selected by plating on medium containing the respective antibiotic. Also, insertions into genes can create functional gene knockouts, which can often be screened for or selected based on a change in phenotype.

In addition to encoding a kanamycin-resistance gene, the EZ::TN Transposon in the new EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ contains a conditional origin of replication (R6Kγori). The presence of this origin of replication enables easy rescue cloning of the gene or region of DNA containing the transposon for each insertion clone (Figure 1).

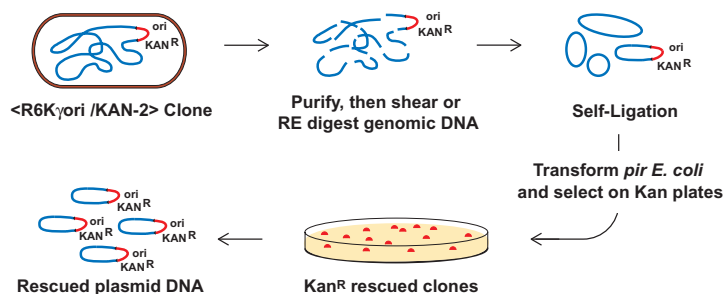


Figure 1. The process for rescue cloning of transposon insertion sites in genomic DNA using the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ and TransformMax™ EC100D™ *pir*⁺ or TransformMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*.

Rescue of insertion clones is extremely powerful for functional and genetic analysis. With *E. coli*, greater than 10⁴ transposon insertion clones are obtained following electrotransformation with only 1 µl of EZ::TN <R6Kγori /KAN-2>Tnp Transposome. A gel with examples of typical rescue clones is shown in Figure 2.



Figure 2. Gel analysis of 11 rescue clones.

Lane 1, size markers; Lanes 2-12, rescue clones of *E. coli* chromosomal DNA produced using EZ::TN <R6Kγori /KAN-2>Tnp Transposome.

Rescue cloning of the region of host genomic DNA containing the inserted transposon is a three step process:

1. Purify approximately 1 µg of genomic DNA from a single chosen insertion clone or from a pooled population of clones using the MasterPure™ DNA Purification Kit. Fragment the genomic DNA by digestion with restriction endonuclease(s) or by random shearing.
2. Self-ligate the genomic DNA fragments with a DNA Ligase (e.g. Fast-Link™ DNA Ligase). Genomic DNA fragments produced by random shearing or by digestion with multiple restriction endonucleases must first be end-repaired (made blunt-ended) and then 5'-phosphorylated using, for example, the End-It™ DNA End-Repair Kit (available separately).
3. Transform an aliquot of the ligation reaction into an *E. coli* cell line that expresses the Π protein (*pir* gene product), such as TransformMax™ EC100D™ *pir*⁺ or TransformMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* (see p. 15) and grow on plates containing kanamycin. Only those clones containing the EZ::TN <R6Kγori /KAN-2> Transposome will grow. Yields of >10⁴ rescue clones per µg of genomic DNA are typically seen.

Two unlabeled sequencing primers that are homologous to the ends of the inserted transposon are provided for bidirectional sequencing of the rescue clones.

Note: To randomly insert an R6Kγori into a cloning vector or other DNA *in vitro*, use the new EZ::TN™ <R6Kγori /KAN-2> Insertion Kit described on p. 15.

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

TSM08KR 10 Reactions

Includes two unlabeled sequencing primers.

MasterPure™ Complete DNA & RNA Purification Kit

MC89010 10 Purifications

End-It™ DNA End-Repair Kit

ER0720 20 Reactions

For end-repair of up to 100 µg of DNA.

www.epicentre.com/catalog/r6ktnp.htm

EZ::TN™ <R6Kγori /KAN-2> Insertion Kit

The new EZ::TN™ <R6Kγori /KAN-2> Insertion Kit can be used to randomly insert the *E. coli* R6Kγ conditional origin of replication (R6Kγori) into target DNA *in vitro*. The EZ::TN <R6Kγori /KAN-2> Transposon contains the R6Kγori and a kanamycin selection marker. A single 2-hour *in vitro* reaction randomly inserts the <R6Kγori /KAN-2> Transposon into the target DNA. Use an aliquot of the reaction to transform *E. coli* hosts expressing the *pir* gene product (Π protein) such as EPICENTRE's TransforMax™ EC100D™ *pir*⁺ or TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* and select on kanamycin plates. Only those clones harboring DNA containing the EZ::TN <R6Kγori /KAN-2> Transposon will grow.

Use the EZ::TN <R6Kγori /KAN-2> Insertion Kit to:

- Introduce the R6Kγori into any cloning vector.
- Propagate vectors from non-*E. coli* species in *E. coli*.
- Propagate genomic DNA fragments from any species as independently replicating DNA in *E. coli*.

Two unlabeled Sequencing Primers, homologous to the ends of the inserted transposon, are provided in the kit for bidirectional sequencing of the transposon insertion clones.

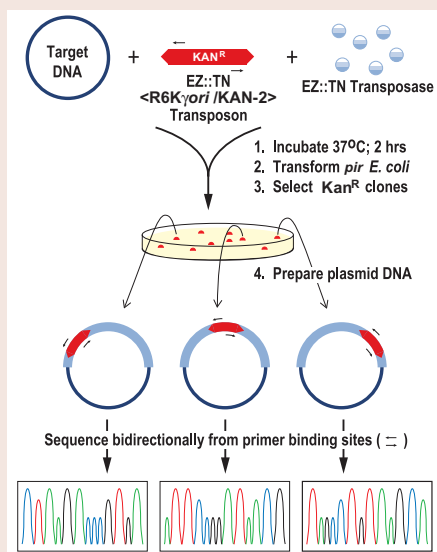


Figure 1. The process for generating and sequencing R6γori - containing clones.

EZ::TN™ <R6Kγori /KAN-2> Insertion Kit

EZ1011RK

10 Reactions

Contains: EZ::TN™ <R6Kγori /KAN-2> Transposon, EZ::TN™ Transposase, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, KAN-2 FP-1 Forward Sequencing Primer, R6KAN-2 Reverse Sequencing Primer, Control Target DNA, Sterile Water

Electrocompetent *E. coli* for Rescue Cloning

TransforMax™ EC100D™ *pir*⁺ Electrocompetent *E. coli* and TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* each express the Π protein (*pir* gene product) for replication of vectors containing the R6Kγ conditional origin of replication (R6Kγori). The cells are derived from EPICENTRE's TransforMax™ EC100™ Electrocompetent *E. coli* by P1 phage transduction with a strain containing the *pir*⁺ or *pir*-116 gene linked to a dihydrofolate reductase (DHFR) marker. Both cell strains can be used for propagation of vectors and rescue clones transposed by the EZ::TN <R6Kγori /KAN-2> Transposon.

Transformation Efficiency

Greater than 1 X 10⁹ cfu/μg supercoiled DNA

Genotypes

TransforMax EC100D *pir*⁺ Electrocompetent *E. coli*

Maintains plasmids at approximately 15 copies per cell¹. F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d*lacZ*ΔM15 Δ*lacX74* *recA1* *endA1* *araD139* Δ(*ara, leu*)7697 *galU* *galK* λ⁻ *rpsL* *nupG* *pir*⁺(DHFR).

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

Maintains plasmids at approximately 250 copies per cell¹. F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d*lacZ*ΔM15 Δ*lacX74* *recA1* *endA1* *araD139* Δ(*ara, leu*)7697 *galU* *galK* λ⁻ *rpsL* *nupG* *pir*-116(DHFR).

Applications and Important Phenotypes

- Expresses the Π protein for propagation of vectors containing the R6Kγori.
- Blue/white screening of vectors expressing the LacZ' α-complementing peptide.
- Restriction minus for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

Reference

1. Metcalf, W.M. *et al.* (1994) *Gene* **138**,1

TransforMax™ EC100D™ *pir*⁺ Electrocompetent *E. coli*

ECP09500

5 X 100 μl

(10 Electroporations)

Includes control vector containing an R6Kγori.

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

EC6P095H

5 X 100 μl

(10 Electroporations)

Includes control vector containing an R6Kγori.

www.epicentre.com/catalog/ecpir.htm

P R O D U C T R E V I E W

DNA Ligation in 5 Minutes at Room Temperature!

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

Fast and Efficient DNA Ligations.

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

Transform Cells Without Desalting the Ligation Reaction.

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

Lab Tested...Scientist-approved.

Visit www.biowire.com and search for "Fast-Link" to view unsolicited comments from users of EPICENTRE's Fast-Link DNA Ligation Kit.

Table 1. Fast-Link Representative Results.

	Ligation Time	% White Colonies	Recombinants per µg DNA
Overhang	5 min.	93	2.0 x 10 ⁶
Blunt	5 min.	71	4.4 x 10 ⁵
PCR product	1 hr.	68	1.2 x 10 ⁴

Fast-Link™ DNA Ligation Kits

NEW! LK0750H 50 ligations
LK6201H 100 ligations
Includes Fast-Link™ DNA Ligase, Fast-Link™ 10X Ligation Buffer, ATP

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Do you need high efficiency electrocompetent *E. coli* for your cloning work? See p. 13 for information on EPICENTRE's new TransforMax™ EC100™ Electrocompetent *E. coli*.

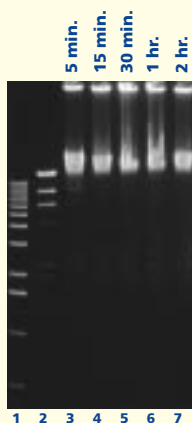


Figure 1. Time course for cohesive-end ligation using the Fast-Link™ Kit. Lambda *Hind* III markers were ligated in a standard Fast-Link reaction using 2 U of Fast-Link DNA Ligase (Lanes 3-7). Lane 1, 1 kb ladder; Lane 2, no enzyme.

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