# EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research


# Extract PCR-Ready Soil DNA in Less Than an Hour with the New SoilMaster™ DNA Extraction Kit

*Judith Meis and FengLing Chen, EPICENTRE*

## Introduction

The analysis of DNA from microbial populations in soil and sediment samples has been fraught with difficulties. The direct lysis of cells within the soil matrix, often results in the coextraction of other soil components, including potent organic inhibitors such as humic and fulvic acids. These components can prevent the amplification of DNA by the polymerase chain reaction (PCR).[1, 2]

The SoilMaster™ DNA Extraction Kit provides a reliable, simple method for producing PCR-ready DNA from soil and sediment samples. This method is based on hot-detergent lysis methods [3, 4] and incorporates an inhibitor removal chromatography step (Figure 1). Genomic DNA was extracted from different soil and sediment samples varying in composition and origin. The integrity of the purified DNA is demonstrated by agarose gel electrophoresis. The diversity of the extracted PCR-ready genomic DNA is demonstrated by amplification of 1 µl of the extracted DNA (<1% of the total), using the FailSafe™ PCR System, with a series of primers with different target specificities.

The bacterial DNA was also amplified with consensus bacterial 16S ribosomal PCR primers and cloned using the CopyControl™ PCR Cloning Kit. The diversity of the cloned DNA was examined by restriction fragment length polymorphism (RFLP) analysis of the 16S clones.

## Methods

### Soil samples

Soil samples included forest soil and marsh soil from Madison, WI. Cave soil was obtained from Dr. Tina Robach, Department of Biology, at the University of Louisville.

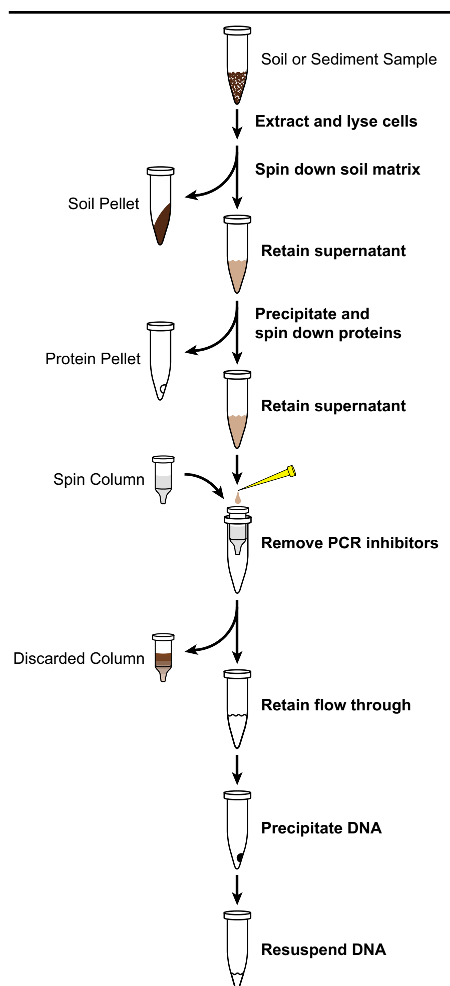


**Figure 1. SoilMaster™ DNA Extraction Kit protocol.**

### DNA extraction

Genomic DNA was purified from soil samples using the SoilMaster™ DNA Extraction Kit. The protocol is illustrated in Figure 1. Briefly, 250 µl of Soil DNA Extraction Buffer and 100 µg of Proteinase K were added to 100 mg of soil, followed by the addition of 50 µl of Soil Lysis Buffer. The sample was vortexed, heated to 70°C for 10 minutes, vortexed again, and the soil matrix was spun down. Protein Precipitation Reagent (60 µl) was added and the sample was held on ice. The proteins were spun down and the supernatant was spun through an Inhibitor Removal Spin Column to remove enzymatic inhibitors from the soil DNA. The genomic soil

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In This Issue


*New!* 1 Extract PCR-Ready Soil DNA in Less Than an Hour with the New SoilMaster™ DNA Extraction Kit

3 How to Never Fail at PCR

*New!* 4 Rapid and Efficient Cloning of Large PCR Products, Up to 15 Kb, Using the New CopyControl™ PCR Cloning Kits

6 Rapid Identification of EZ::TN™ Transposon Insertion Sites in the Genome of *Neisseria gonorrhoeae*

*New!* 8 TypeOne™ Inhibitor Improves Transformation Efficiencies by Blocking Type I Restriction and Modification Systems *In Vivo*

*New!* 9 An Improved Approach for Construction and Analysis of BAC Libraries Using the CopyControl™ pCC1BAC™ Vector Containing an Inducible High-Copy Origin

*New!* 12 Size Your BAC Clones in 4 Hours without Growing Cultures or Performing Restriction Digests

13 Isolation of Bovine and Mouse DNA from Buccal and Vaginal Samples Using the BuccalAmp™ DNA Extraction Kit

15 Get Consistently High Yields of Full-Length RNA From Both Long and Short DNA Templates Using AmpliScribe™ T7 High Yield Transcription Kits




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DNA present in the column flow-through was precipitated with DNA Precipitation Solution, and washed twice with Pellet Wash Solution. The DNA was resuspended in 300 µl of TE Buffer.

### *PCR amplification*

The primers used for PCR amplification are listed in Table 1. Purified soil DNA was amplified with the FailSafe™ PCR System. Briefly, 50 µl reactions contained 25 µl of the appropriate FailSafe™ 2X PreMix, 2.5 U FailSafe™ Enzyme Mix, 10 pmoles of each primer and 1 µl of purified soil DNA. The thermocycling parameters were: 92°C for 2 minutes, followed by 30 cycles of 92°C for 45 seconds, a variable annealing temperature for 45 seconds and 72°C for 60 seconds. Ten to twenty percent of the amplification reaction product was analyzed by agarose gel electrophoresis.

### *PCR product cloning*

PCR products were cloned using the CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Electrocompetent *E.coli*. Briefly, 1.3-Kb PCR products amplified with 16S bacterial consensus primers (Table 1) were precipitated with the PCR Precipitation Solution. The purified PCR product was then treated with the PCR End-Repair Enzyme Mix to generate blunt-ended and 5′-phosphorylated PCR products for cloning. The PCR product was ligated into the CopyControl™ pCC1™ (Blunt-Cloning Ready) Vector using Fast-Link™ DNA Ligase. The ligation reaction was then transformed into TransforMax™ EPI300™ Electrocompetent *E.coli* by electroporation. The resulting clones were quick screened for inserts by the direct lysis of colonies and the size selection of the DNA using EpiLyse™ Solution and EpiBlue™ Solution.

Restriction Fragment Length Polymorphism (RFLP) was performed on a series of 16S pCC1™ clones restricted with *Rsa* I (6). The fragments were separated by agarose gel electrophoresis.

## Results

### *Larger size and more intact DNA*

The DNA isolated with the SoilMaster DNA Extraction Kit was compared to the DNA purified with two other soil DNA kits incorporating bead beating or vortex mixing in the presence of beads. Extracted DNA was examined by agarose gel electrophoresis (Figure 2). The DNA extracted with the SoilMaster Kit was of larger size and contained more intact DNA than DNA purified by other methods.

**Table 1. PCR Amplification Primers**

| Organism Group | Primer Pair (reference) | DNA Sequence | Product Size (bp) |
|---|---|---|---|
| Bacteria consensus | 63f/1387r (5) | CAGGCCTAACACATGCAAGTC<br>GGGCGGWGTGTACAAGGC | 1325 |
| Plants | NS3/NS4 (6) | GCAAGTCTGGTGCCAGCAGCC<br>CTTCCGTCAATTCCTTTAAG | 597 |
| Fungi, Protists, and Green Algae | NS1/NS2 (7) | GTAGTCATATGCTTGTCTC<br>GGCTGCTGGCACCAGACTTGC | 555 |
| High G+C Gram Positive Bacteria | Actino F/R (8) | GGCCTTCGGGTTGTAAACC<br>CTTTGAGTTTTAGCCTTGCGGC | 542 |
| Bacteria consensus | P4/P5 (9) | AACGCGAAGAACCTTAC<br>CGGTGTGTACAAGGCCCGGGAACG | 450 |
| *Bacillus* and relatives | BacF/BacR (8) | AGGGTCATTGGAAACTGGG<br>CGTGTTGTAGCCCAGGTCATA | 600 |

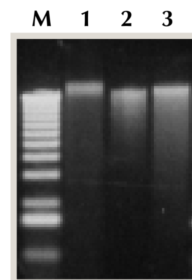


**Figure 2. The SoilMaster™ DNA Extraction Kit extracts high molecular weight intact DNA from compost soil sample.** Lane M, Kb DNA ladder; Lane 1, soil DNA extracted with the SoilMaster Kit; Lanes 2 and 3, DNA purified using other soil kits.

### *Difficult DNA extractions*

The SoilMaster Kit extracts DNA from difficult-to-extract soil and sediment samples. Cave sediment DNA was successfully extracted using the SoilMaster Kit, but no visible DNA was purified in attempts with two other kits, as shown when examining proportional amounts of DNA preparations by agarose gel electrophoresis (Figure 3).

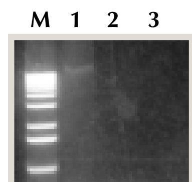


**Figure 3. The SoilMaster™ DNA Extraction Kit extracts DNA from difficult-to-extract soil and sediment samples.** Lane M, Kb DNA ladder; Lane 1, cave sediment DNA extracted with the SoilMaster Kit; Lanes 2 and 3, purification attempts of cave sediment DNA using other soil kits.

### *Amplification of diverse organisms*

PCR amplification results illustrate the diverse set of organisms represented in the extracted DNA. DNA from cave sediment, forest soil, and marsh soil was extracted and specific targets were subsequently amplified with the FailSafe PCR System. The extracted genomic DNA was amplified by a series of DNA primers with different specificities, including 1) two sets of consensus bacterial primers, 2) fungi, protists, and green algae primers, 3) plant primers, 4) primers to high G+C, gram positive bacteria, and 5) *Bacillus* primers (Table 1). Amplification products were obtained from all five primer sets

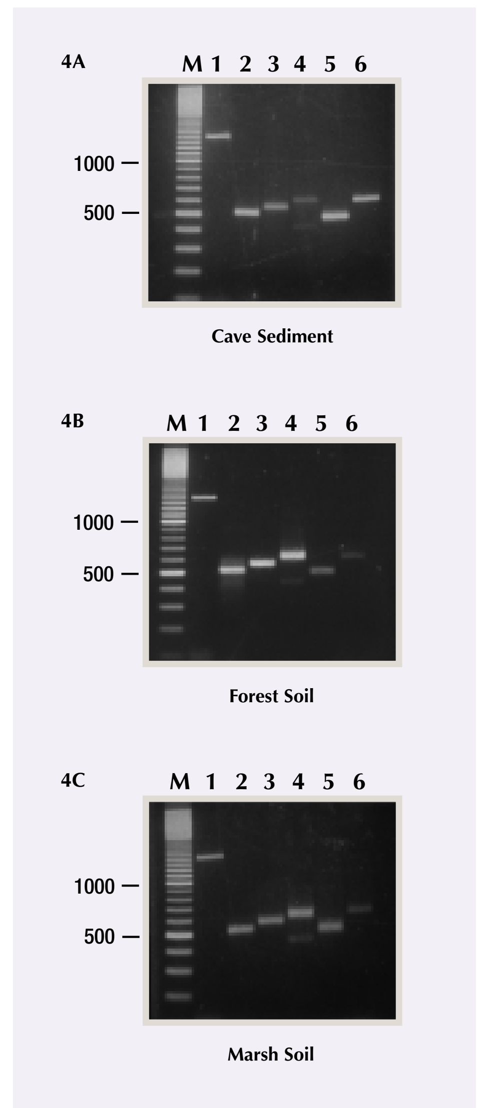


**Figure 4. FailSafe™ PCR amplification of extracted soil DNA.** DNA was extracted from 3 distinct soil types including cave sediment (Panel A), forest soil (Panel B), marsh soil (Panel C). The extracted soil was amplified using the following primers: Lanes 1 and 2, consensus bacterial primers to the 16S ribosomal RNA gene; Lane 3, fungi, protists and green algae primers; Lane 4, plant primers NS3/NS4; Lane 5, high G+C gram positive bacterial primers; Lane 6, *Bacillus* primers.

using the extracted DNA from all samples tested (Figure 4).

### *Successful cloning and RFLP analysis*

The DNA amplified with 16S bacterial consensus primers was cloned into pCC1™ with the CopyControl™ PCR Cloning Kit (see p.4 and center insert). Clones containing the 1.3-Kb PCR product were examined by RFLP with *Rsa* I to examine sequence variations in the cloned fragments. The RFLP analysis of clones demonstrates the diversity of 16S sequences amplified from the extracted soil DNA (Figure 5). This indicates that a wide variety of organisms and species are represented in the extracted soil DNA.

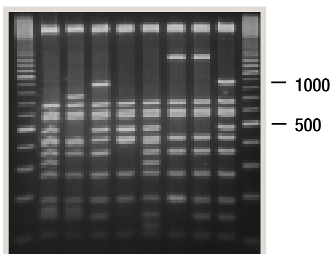


**Figure 5. RFLP analysis of clones containing 1.3 Kb 16S ribosomal RNA gene PCR products.** Eight clones were restricted with *Rsa* I, and the resulting fragments were separated by agarose gel electrophoresis. The varied banding patterns demonstrate the diversity of the 16S ribosomal gene sequences that were amplified and cloned into pCC1™.

## Discussion

The SoilMaster™ DNA Extraction Kit efficiently extracts PCR-ready DNA from a wide variety of organisms from soil including difficult-to-extract sediments. DNA from soil and sediments can be effectively amplified by FailSafe PCR amplification and subsequently cloned for further characterization.

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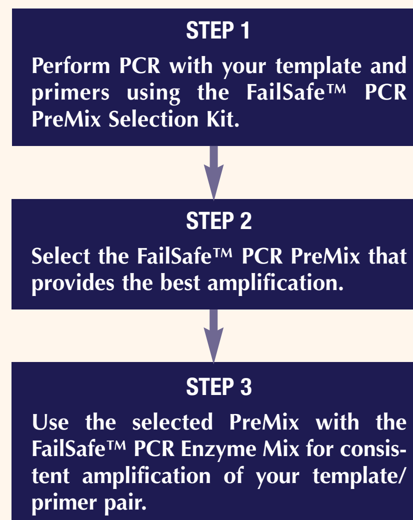
**SoilMaster™ DNA Extraction Kit**

SM02050 50 Reactions

# Here is How to Never Fail at PCR

The FailSafe™ PCR System combines a unique blend of high fidelity thermostable enzymes with an extensively tested set of FailSafe PCR PreMixes to provide a new standard for PCR performance and reliability. The FailSafe System gives consistent amplification of any template up to about 20 Kb in length, even difficult templates, such as those with high GC content or secondary structure, and multiplex PCR reactions.

### FailSafe PCR is an easy 3-step process

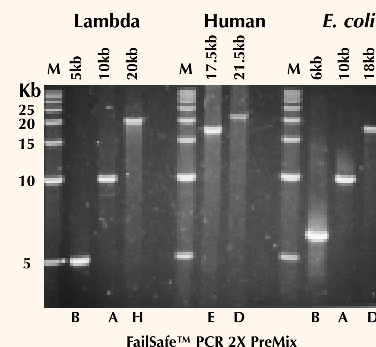



Use these three steps for each template/primer pair you wish to amplify.

### Obtain high fidelity PCR with no loss in sensitivity

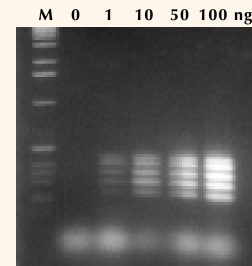
The FailSafe™ PCR Enzyme Mix contains a 3′-5′ proofreading enzyme that delivers fidelity at least three times higher than *Taq* DNA polymerase.

### Amplify templates up to 20 Kb long




**Amplification of a wide range of sequence sizes from different sources using the FailSafe™ PCR System.**

### Get successful multiplex amplification

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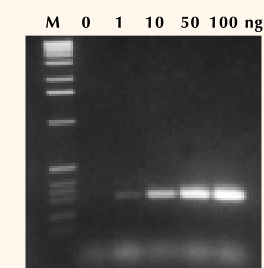
### Easily amplify GC-rich templates




**Amplification of a segment of human DNA with 85% GC content: Fragile X FMRI was amplified using 1 ng of human genomic DNA by FailSafe™ PCR.**

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# Rapid and Efficient Cloning of Large PCR Products, Up to 15 Kb, Using the New CopyControl™ PCR Cloning Kits

*Haiying Grunenwald, EPICENTRE*

## Introduction

Cloning large PCR products can be a laborious and time consuming process. For example, most methods require gel purification of PCR products >3 Kb to remove non-specific or incomplete amplification products that would be preferentially cloned. EPICENTRE's new CopyControl™ PCR Cloning Kits significantly reduce the time and labor required to clone PCR products up to 15 Kb (Table 1). The CopyControl PCR Cloning Kits utilize a blunt-end cloning process (Figure 1) that facilitates the use of any thermostable polymerase and a rapid colony screening process that identifies full-length clones without the need to grow overnight cultures or perform restriction digests. The high cloning efficiency of these kits and the fast colony screening process eliminates the need for gel purification of large PCR products prior to cloning.

The CopyControl PCR Cloning Kits incorporate the revolutionary CopyControl technology that allows the user to grow the clones at single copy to ensure insert stability, reduce the likelihood of segment deletions (due to high AT or repetitive sequences) and permit cloning of PCR products encoding expressed toxic peptides or proteins. Once the CopyControl clones have been screened, they can then be induced to high copy number for high yields of DNA for sequencing or other applications. For additional information on the advantages of the CopyControl PCR Cloning Kits, see the insert section of this *Forum*.

| | CopyControl Procedure | | Other Method | |
|---|---|---|---|---|
| | PCR Reaction | | PCR Reaction | |
| Preparation | • Precipitate PCR product<br>• Convert to 5′-phosphorylated, blunt-ended DNA | 1 hour | • Load and run agarose gel<br>• Excise the DNA<br>• Purify the DNA | 2-3 hour |
| Cloning | • Ligate into pCC1 Vector<br>• Transform EPI300™ cells<br>• Plate and select overnight | 18-20 hours | • Ligate into vector<br>• Transform cells<br>• Plate and select overnight | 18-20 hours |
| Screening | • Pick clones and process<br>• Load, run and stain gel | 1-2 hours | • Pick clones<br>• Culture overnight<br>• Isolate DNA<br>• Perform RE digests or PCR<br>• Load, run and stain gel | 28-36 hours |
| | Elasped Time 24 hours or less | | Elasped Time > 48 hours | |

**Table 1. Cloning and screening of large PCR products is faster and easier using the CopyControl™ PCR Cloning Kit than by other commonly used methods.**

In this report, we demonstrate rapid and efficient cloning of large PCR products using the CopyControl PCR Cloning Kit with TransforMax™ EPI300™ Electrocompetent *E. coli*.

## Methods and Results

Note: the complete CopyControl PCR cloning procedure can be viewed on the EPICENTRE web site at: www.epicentre.com/ccpcr.asp

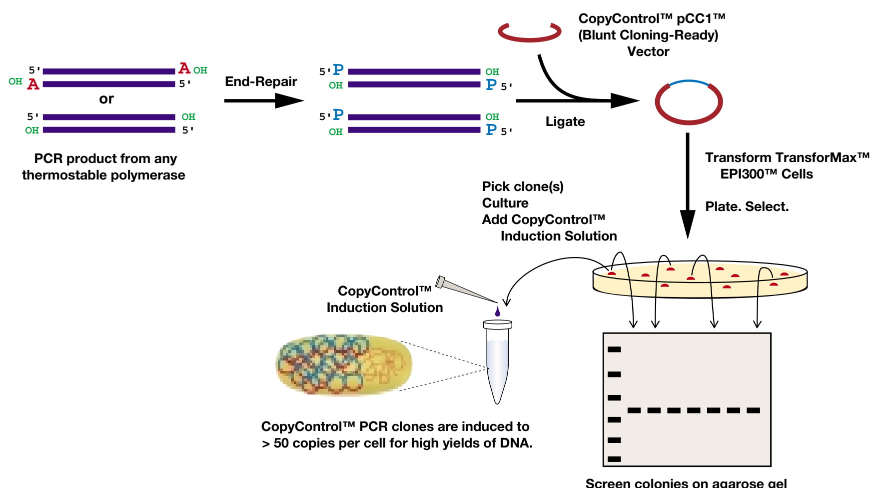


**Figure 1. Efficient, blunt-end cloning of a PCR product up to 15 Kb and screening of clones can be completed in 24 hours using the CopyControl™ PCR Cloning Kit.** Once clones are screened, they can be induced to high copy number for high yields of DNA for sequencing, *in vitro* transcription or other applications.

### *Cloning of large PCR products*

PCR amplification of a 5-Kb, a 10-Kb and a 15-Kb region of Lambda DNA was performed using EPICENTRE's FailSafe™ PCR System. The FailSafe™ PCR Enzyme Mix produces PCR products with a mix of blunt-end and 3′-A overhanging ends. PCR products were recovered from the PCR reactions by addition of an equal volume of the PCR Precipitation Solution (provided in the CopyControl PCR Cloning Kits) followed by centrifugation. Using the CopyControl PCR cloning process, there is no need to gel purify large PCR products prior to cloning as is required using other methods.

The PCR products were converted to 5′-phosphorylated, blunt-ended DNA in a 30 minute reaction with the PCR End-Repair Enzyme mix. Following a brief heat inactivation step, Fast-Link™ DNA Ligase and 75 ng of the CopyControl pCC1™ (Blunt Cloning-Ready) Vector were added and the ligation reaction carried out for 2 hours at room temperature. One microliter of the ligation reaction was used to transform TransforMax™ EPI300™ Electrocompetent *E. coli*. (TransforMax™ EPI300™ Chemically competent *E. coli* can also be used but generally result in a 10 to 100-fold reduction in the number of clones obtained). Following cell out-

growth, 100 µl of a 1:10 dilution of the transformed cells were plated on LB plates supplemented with chloramphenicol (12.5 µg/ml). Plates were incubated overnight at 37ºC.

### *Screening the CopyControl PCR clones*

Screening the size of the CopyControl PCR clones was completed in 2 hours by a unique colony screening process that does not require overnight cultures or restriction digests. Typically, 10 – 20 clones were randomly picked from the plates and processed using the EpiBlue™ Solution and EpiLyse™ Solution supplied with the kit. Fifteen microliters of each processed clone were loaded into the wells of a 1% agarose gel. An 8.1-Kb supercoiled DNA size marker, equal in size to the CopyControl pCC1 Vector and provided in the kit, was used as a size standard. Following electrophoresis, gels were stained with SYBR® Gold and full-length clones identified. Figure 2 shows colony screening results for 10-Kb PCR clones.

As summarized in Table 2, all three of the large PCR products were efficiently cloned using the CopyControl PCR Cloning Kit with TransforMax EPI300 Electrocompetent *E. coli*. Though the cloning efficiency and percentage of full-length PCR clones decreased as the size of the PCR product increased, full-length 15-Kb clones were readily identified using the rapid colony screening process.

Though not necessary for most users, the insert size of full-length clones was confirmed by *Not* I digestion. Clones determined to be full length by the colony screening procedure were digested with *Not* I, which releases the cloned insert from the vector. The *Not* I digestion products were sized by agarose gel electrophoresis. In all cases tested, clones determined to be full-length by the colony screening method of the kit were confirmed to be full-length by *Not* I digestion.

| Size of the PCR product cloned | cfu/µg of PCR product | cfu/reaction | Percentage of full length clones |
|---|---|---|---|
| 5 Kb | ≈ 1 x $10^7$ | ≈ 5 x $10^5$ | ≈ 90% |
| 10 Kb | ≈ 3.5 x $10^6$ | ≈ 2.8 x $10^5$ | ≈ 80% |
| 15 Kb | ≈ 9.2 x $10^5$ | ≈ 1.1 x $10^5$ | ≈ 30% |

**Table 2. Large PCR products of 5 Kb, 10 Kb and 15 Kb were efficiently cloned, without the need for gel purification, using the CopyControl™ PCR Cloning Kit.** The percentage of full-length clones was determined by the colony screening procedure described in the article.

### *CopyControl PCR clones can be induced to high copy number for high yields of DNA*

CopyControl PCR clones can be grown at single-copy number to ensure the stability of the cloned insert and the clonability of sequences which would express levels of a toxic protein that would be detrimental to the host cell if grown at high copy number. A single colony from each of three PCR clones was induced from single-copy to high-copy number according to the procedure described in the product literature. Briefly, the clones were grown in LB + chloramphenicol liquid medium overnight at 37ºC. Following the overnight growth, the cells were diluted into fresh media in duplicate. CopyControl™ Induction Solution was added to one of the duplicate cultures (the "induced culture") resulting in expression of the *trf*A gene of the TransforMax EPI300 host cells and subsequent amplification of the clone from single-copy to high-copy number. CopyControl Induction Solution was not added to the other culture (the "uninduced control"). Both cultures were incubated for 5 hours at 37ºC with shaking.

DNA isolated from an equal number of cells from induced and uninduced cultures was analyzed by 1% agarose gel electrophoresis (Figure 3). Typically, the induced cultures yielded > 50-fold more DNA than uninduced cultures.

## Conclusion and Discussion

We have demonstrated that the CopyControl PCR Cloning Kit with TransforMax EPI300 *E. coli* provides an efficient and time-saving method to clone PCR products. The blunt-end cloning process utilized by the kit enables the use of any thermostable polymerase or polymerase blend in the PCR reaction. PCR products up to 15 Kb can be readily cloned without the need for gel purification. The colony screening procedure featured in the kits is a rapid and reliable method to identify full-length clones without the need for overnight cultures and restriction digests. Finally, the CopyControl PCR clones can be grown at single copy to ensure insert stability and cloning of sequences encoding an expressed toxic protein and then induced to up to >50 copies per cell for high yields of DNA.

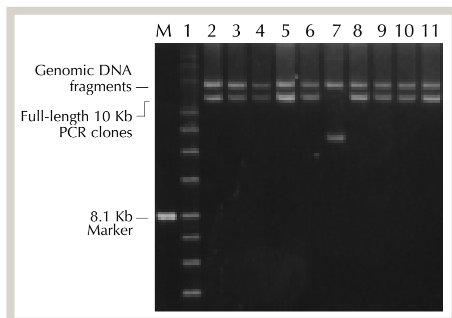


**Figure 2. Full-length 10-Kb CopyControl™ PCR clones were readily identified in 2 hours without the need for overnight cultures or restriction digests.** CopyControl PCR clones were randomly picked from a plate and processed using the EpiLyse™ and EpiBlue™ Solutions by the method described in the product literature. Supercoiled DNAs were sized by 1% agarose gel electrophoresis and the gel stained with SYBR® Gold. Lane M, supercoiled 8.1-Kb size marker; Lane 1, supercoiled DNA size standards; Lanes 2-11, randomly picked CopyControl PCR clones. Full length clones are 18.1 Kb (10-Kb PCR product + 8.1-Kb pCC1™ vector).

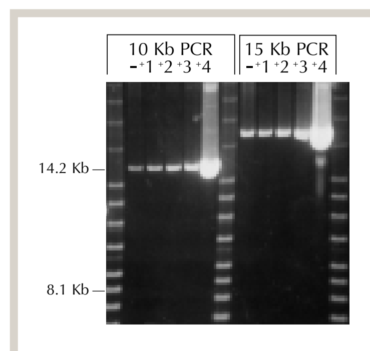


**Figure 3. CopyControl™ PCR clones can be induced from single copy to > 50 copies per cell.** DNA was extracted from an equal number of cells of uninduced (-) cultures and induced (+) cultures of CopyControl™ PCR clones. DNA from the induced cultures was further diluted 1:200 (Lanes +1), 1:100 (Lanes +2) and 1:50 (Lanes +3) and all samples, including undiluted, induced DNA (Lanes +4) were run side-by-side on a 1% agarose gel. The level of clone induction was estimated by visually comparing the staining intensity of the diluted, induced samples with the uninduced sample.


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**CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Electrocompetent *E. coli*.**

CCECPCR1 20 Reactions

**CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Chemically Competent *E. coli*.**

CCPCR1CC 20 Reactions

***Contents:***

CopyControl™ pCC1™ (Blunt Cloning-Ready) Vector, PCR Precipitation Solution, 10X Reaction Buffer, PCR End-Repair Enzyme Mix, Fast-Link™ DNA Ligase, EpiLyse™ Solution, EpiBlue™ Solution, CopyControl™ Induction Solution, Control PCR Product, Supercoiled DNA Size Marker, Water, and either TransforMax™ EPI300™ Electrocompetent *E. coli* or TransforMax™ EPI300™ Chemically Competent *E. coli*.

# Rapid Identification of EZ::TN™ Transposon Insertion Sites in the Genome of *Neisseria gonorrhoeae*

*Thomas F. Ducey and David W. Dyer*
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## Introduction

*Neisseria gonorrhoeae* is a Gram-negative, diplococcal, sexually transmitted bacterium responsible for over 300,000 infections in the United States (US) every year. Thus, gonococcal infections are a serious public health problem in the US. In males this infection is often self-limiting and symptomatic, in females the infection often goes undetected and can cause a multitude of secondary problems. Since we have recently determined the genome sequence of the gonococcus, we are now exploring methods for functional analysis of uncharacterized genes.

Gene inactivation and examination of the resulting phenotypes is a mainstay of genetics. Although this strategy can yield a greater understanding of the biological function of a particular gene or a set of genes, this process can be long and tedious, especially when one attempts to target large groups of genes simultaneously. However, using the EZ::TN™ <KAN-2> Transposon in a mutagenesis protocol that exploits the natural competency of *N. gonorrhoeae*, we are making random, easily identifiable mutations in a majority of the genes in the gonococcal genome (Figure 1). We then employ a two-step process to analyze these mutants. The first step is a polymerase chain reaction (PCR) protocol called random amplification of transposon ends (RATE).[1] The second step involves the sequencing of these RATE products. By analyzing the DNA sequence, we can then map these transposon mutants to the exact basepair of insertion in the gonococcal genome (Figure 2).

## Materials and Methods

### *Insertional mutagenesis and selection*

Gonococcal genomic DNA was sheared into blunt-ended products by nebulization, performed as follows: 50 µg of DNA was mixed into 2 ml of a buffered solution (final concentrations: 50% glycerol, 50 mM Tris-HCl (pH 8.0), and 15 mM $MgCl_2$) and then placed into a nebulizer cup (IPI Medical Products). The cup was then placed into an ice-water bath and the solution was nebulized using nitrogen gas. DNA fragments of a given size were reproducibly generated depending on the amount and duration of pressure that was placed on the solution in the cup. These conditions were dictated by DNA concentration and the % G+C content. In the case of the gonococcus (52% G+C) the following times and pressures were used to produce variously sized DNA fragments: 4-6 Kb (45 seconds @ 4 psi), 2-4 Kb (90 seconds @ 6 psi), 1-3 Kb (150 seconds @ 10 psi). After nebulization, the DNA was salt-precipitated overnight at -20ºC, washed once with 70% EtOH, and then resuspended in TE buffer. The DNA was then run on a 1% agarose TAE gel and properly sized DNA fragments were gel purified.

Two hundred nanograms of genomic DNA (1-6 Kb) was used in a standard EZ::TN <KAN-2> Transposon insertion reaction, the reaction was stopped and the DNA stored at -20ºC. Tranformation of the gonococcus with transposon-laden gonococcal DNA was performed as described by Bernstein *et al*.[2] We did not find it necessary to repair the short single-stranded gaps introduced at the ends of the transposon during the insertion reaction. A library of 25,000 to 50,000 kanamycin-resistant gonococci was routinely obtained from a 10-µl reaction.

### *RATE protocol*

RATE is a three-step, single-primer PCR protocol, as diagrammed in Figure 2A.

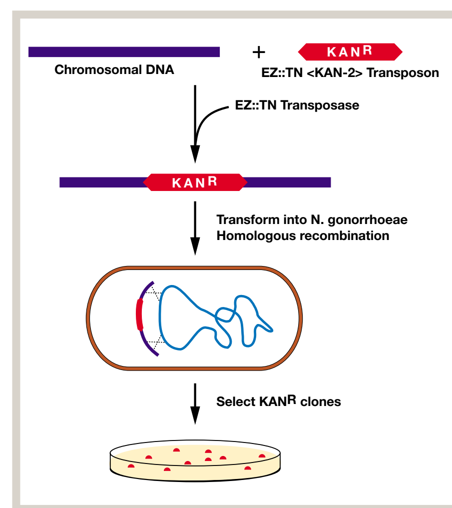


**Figure 1. Strategy for generating EZ::TN™ <KAN-2> Transposon insertions in the genome of *N. gonorrhoeae*.** Chromosomal DNA was mutagenized *in vitro* with the EZ::TN <KAN-2> Transposon and transformed into naturally competent gonococcus. Cells which integrated the transposon insertions via double homologous recombination were then selected on kanamycin plates.

The first and third steps of the protocol are performed at a stringent temperature, no more than 5ºC lower than the $T_m$ of the primer selected. The second step is done at 30ºC, which allows nonspecific amplification of the single-stranded product generated in the first step, while the third step will amplify both the specific and non-specific products generated in the second step.

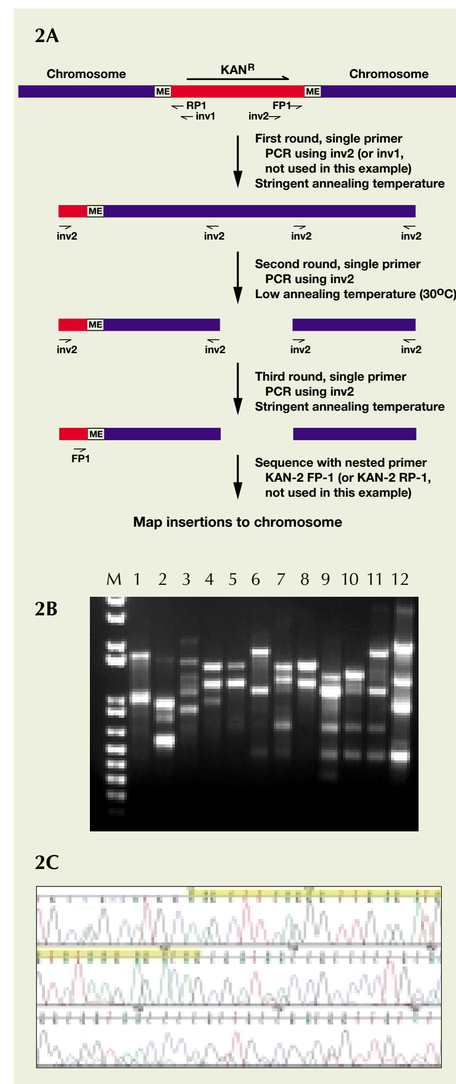


**Figure 2. Genomic EZ::TN™ <KAN-2> Transposon insertion sites were determined by sequencing PCR products which comprise one end of the transposon and a region of flanking chromosomal DNA. Panel A:** Overview of the single-primer RATE PCR protocol used to amplify transposon-target junctions. **Panel B:** PCR products generated after amplification of DNA from 12 randomly chosen *N. gonorrhoeae* insertion clones (Lanes 1-12). M, size ladder. **Panel C:** Clear sequence reads are obtained using nested primers (KAN-2 FP-1 or KAN-2 RP-1) that bind only transposon-specific products.

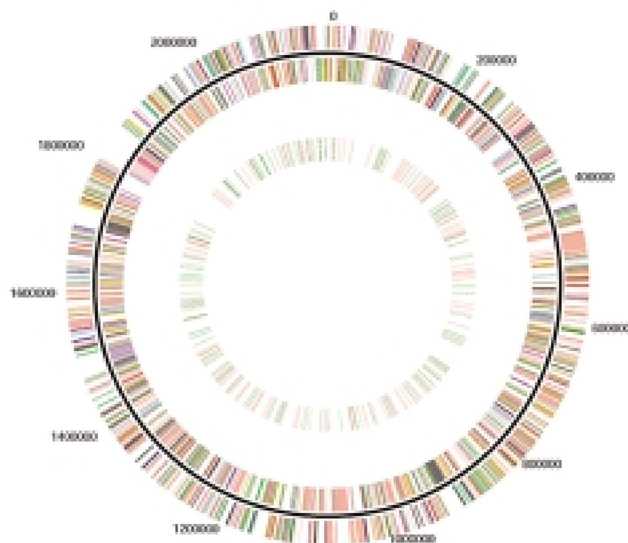


**Figure 3. Location of EZ::TN™ <KAN-2> Transposon insertions on a circular map of the *N. gonorrhoeae* chromosome.** The three rings of colored hash marks (outermost to innermost) indicate positions for the following: open reading frames (ORFs) in the plus strand; ORFs in the minus strand; and transposon insertions in the plus (green) or reverse (salmon) strands. Colors in the two largest rings represent different categories of genes.

Specifically, a small sample of each kanamycin-resistant gonococcal colony was placed into 50 µl of water and boiled for 10 minutes. One microliter of the crude cell extract was then used as template in a standard PCR reaction with the Inv-1 (ATGGCTCATAACACCCCTTGTATTA) or Inv-2 (GAACTTTTGCTGAGTTGAAGGATCA) primer. The first 30 cycles of the PCR reaction are done at 55ºC, with a 30- second extension. The next 30 cycles are done at 30ºC, with a similar extension time, while the last 30 cycles are performed at 55ºC with a 2 minute extension time. The RATE reactions were then analyzed on a 1% agarose TAE gel.

### *Sequencing of RATE products*

RATE PCR products were sequenced with BigDye™ terminators according to Applied Biosystems specifications using the forward (KAN-2 FP-1) or reverse (KAN-2 RP-1) primers supplied with the EZ::TN <KAN-2> Insertion Kit. After sequencing, the locations of the transposon inserts were determined by a blastn comparison with the sequence of *Neisseria gonorrhoeae* (http://seqgen.ouhsc.edu/blast.html).

## Results and Discussion

We have demonstrated a new approach to locating transposon insertions found within a genomic sequence. Prior attempts to locate transposons within a genomic sequence were restricted to either Southern hybridization analysis, which does not pinpoint the transposon location, or by sequencing from genomic DNA, which requires large amounts of template DNA and can be a difficult process. By employing the RATE protocol and coupling it with a sequencing reaction (Figure 2), we have easily identified over 300 EZ::TN <KAN-2> Transposon insertion sites to the exact basepair. Our approach keeps DNA preparation to a minimum so that large numbers of samples can be analyzed quickly and efficiently at relatively low cost.

One of the primary purposes for creating and identifying so many transposon insertions is to generate a bank of mutants for down-stream analysis. This requires that transposon insertions be random and stable within the genome of the organism. To assess randomness, we compared our sequencing results to the published sequence for *N. gonorrhoeae*. As shown in Figure 3, each insertion occurred at a different site on the gonococcal genome and there was no clustering at any particular region of the chromosome. We also did not detect any local DNA rearrangements such as deletions or inversions.

Stability was of considerable concern in the case of the gonococcus since the organism has several copies of a putative transposase gene within the genome (unpublished observations). Therefore, we cultured gonococci containing the EZ::TN <KAN-2> Transposon every day for 30 days to check the stability of the transposon. We also performed these studies without antibiotic selection, testing to see if the gonococcus "kicked out" the transposon over the 30-day period. Southern blot analyses showed that, with or without antibiotic selection, the transposon is stable and non-mobile within the genome of the gonococcus (data not shown). This means that the EZ::TN <KAN-2> Transposon is an ideal vehicle for creating transposon mutants within the gonococcus.

## References

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2. Burnstein, K.L. *et al*. (1988) *J. Gen. Microbiol*. **134**, 547.

**www.epicentre.com/transposomics.asp**

**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, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.

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

**Editor's note:** Drs. Virginia Clark and Janice Spence at the University of Rochester Medical Center have used the EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ to make random transposon insertions in *N. gonorrhoeae in vivo*. Gonococci were washed, resuspended in 0.3 M sucrose at 2 X $10^{10}$ cfu/ml, and 1 µl of the EZ::TN Transposome was electroporated into the cells under standard conditions. Immediately after electroporation the solution was diluted 10-fold and plated onto GC Medium Base agar containing Kellogg's supplements (GCK). Gonococci are quite sensitive to electroporation, with about 80% killed during electroporation and a 99% loss of viability if cells are outgrown in broth. Outgrowth on plates without selection is necessary to prevent this loss in viability. After 6 hours, the gonococci were removed from the plates, diluted, and plated onto GCK containing 40 µg kanamycin/ml. $Kan^R$ mutants were obtained at a frequency of 2.7 X $10^{-5}$, for a total of ~1 X $10^5$ $Kan^R$ colonies per microliter of EZ::TN Transposome.

*See the center insert for more information on EZ::TN™ Transposomes™.*

# TypeOne™ Inhibitor Improves Transformation Efficiencies by Blocking Type I Restriction and Modification Systems *In Vivo*

*Les M. Hoffman, Darin J. Haskins, and Jerry Jendrisak, EPICENTRE*

## Introduction

DNA transformation can be difficult to achieve in many bacterial strains due to the presence of one or more restriction and modification (R-M) systems which cleave unmodified DNA. Here we demonstrate that EPICENTRE's new TypeOne™ Restriction Inhibitor* significantly increases transformation efficiencies of unmodified DNA in bacterial strains with type I R-M systems. Developed as a unique preparation of a phage protein called "ocr",[1] TypeOne Inhibitor can be electroporated into cells along with transforming DNA. *In vivo* this protein acts as a molecular decoy that blocks the DNA binding site of type I R-M enzymes and inhibits cleavage of unmodified DNA.

## Methods

Fifty microliters of electrocompetent cells, prepared according to standard methods, were electroporated with the amount of unmodified DNA indicated in Table 1, with or without 5 μg (1 μl) of TypeOne Inhibitor. Transformed cells were plated on media containing ampicillin (pUC19), chloramphenicol (48 Kb fosmid), or kanamycin (EZ::TN™ Transposon).

## Results

Type I R-M systems are widespread in Eubacteria and Archaebacteria.[2] For example, *Salmonella typhimurium* strain LT2 has a type I R-M enzyme called *Sty*L TIII, that cleaves pUC19 DNA at three sites. When unmodified pUC19 plasmid DNA was electroporated into *S. typhimurium* strain LT2 in the presence of TypeOne Restriction Inhibitor, 100-fold more ampicillin-resistant colonies were obtained than when the inhibitor was not included in the transformation mixture (Table 1). Addition of TypeOne Inhibitor made transformation efficiencies much more comparable to those of the non-restricting *S. typhimurium* strain LB5000.

TypeOne Inhibitor blocks type I R-M enzymes that recognize different DNA target sequences and therefore can be used to increase transformation efficiencies in a variety of host cells.[1] *E. coli* strain MG1655, for example, contains the *Eco*KI type I R-M enzyme that recognizes a different target sequence than the R-M enzyme found in *S. typhimurium* strain LT2. As shown in Table 1, the addition of TypeOne Inhibitor to electroporations of *E. coli* strain MG1655 with an uncharacterized 48 Kb fosmid clone resulted in a nearly 500-fold increase in transformation efficiency.

| Strain (Type I R-M system) | TypeOne™ Inhibitor | Type of DNA or Transposome™ | Recombinants per μg DNA |
|---|---|---|---|
| *S. typhimurium* LT2 (*Sty*L TIII) | - | pUC19 (100 pg) | 3.0 X $10^{6}$ |
| *S. typhimurium* LT2 (*Sty*L TIII) | + | pUC19 (100 pg) | 3.0 X $10^{8}$ |
| *S. typhimurium* LB5000 (none) | - | pUC19 (100 pg) | 2.0 X $10^{10}$ |
| *E. coli* MG1655 (*Eco*K1) | - | 48 Kb fosmid (50 ng) | 3.0 X$10^{3}$ |
| *E. coli* MG1655 (*Eco*K1) | + | 48 Kb fosmid (50 ng) | 1.4 X $10^{6}$ |
| *S. typhimurium* LT2 (*Sty*L TIII) | - | EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ (1 μl) | 1.3 X $10^{4}$ |
| *S. typhimurium* LT2 (*Sty*L TIII) | + | EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ (1 μl) | 1.0 X $10^{6}$ |
| *A. tumefaciens* (none) | - | EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ (1 μl) | 2.2 X $10^{5}$ |
| *A. tumefaciens* (none) | + | EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ (1 μl) | 1.3 X $10^{5}$ |

**Table 1. Effect of TypeOne™ Restriction Inhibitor on transformation efficiencies.**

EZ::TN™ Transposomes™ - the stable complex between EZ::TN™ Transposase and an EZ::TN™ Transposon – have been used in a variety of microorganisms to create gene knockouts and facilitate sequencing of genomic DNA (see the center insert for more information on this product).[3] The EZ::TN™ <R6Kγ*ori* /KAN-2>Tnp Transposome™ contains six recognition sites for the type I *S. typhimurium Sty*L TIII nuclease. When this Transposome was electroporated into *S. typhimurium* LT2 together with TypeOne Inhibitor, the number of insertion clones was increased by 75-fold (Table 1). The addition of TypeOne Inhibitor did not cause a significant change (neither an increase nor a decrease) in insertion efficiencies in other bacterial species when there was either no restriction activity in the cell (e.g., *Agrobacterium tumefaciens,* Table 1) or no recognition sites for the host type I R-M enzyme in the transposon (data not shown).

## Conclusion

Electroporation of TypeOne Restriction Inhibitor provides a new and powerful method for increasing DNA transformation efficiencies in bacterial strains with type I R-M systems. Use of TypeOne Inhibitor does not require prior knowledge of the restriction sites on the transforming DNA or the restriction specificity of the type I R-M system.

## References

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3. Hoffman, L.M. *et al.* (2000) *Genetica* **108**, 19.

**www.epicentre.com/typeone.asp**

**TypeOne™ Restriction Inhibitor***
TY0261H 100 μg

**Patent pending.*

# An Improved Approach for Construction and Analysis of BAC Libraries Using the CopyControl™ pCC1BAC™ Vector Containing an Inducible High-Copy Origin

*Dilara Begum, Mike Fiandt, and Jerry Jendrisak, EPICENTRE*

## Introduction

The low yield of DNA from single copy BACs is a bottleneck to high throughput processes such as BAC end sequencing, fingerprinting and shotgun library construction. EPICENTRE's new CopyControl™ BAC Cloning Kits, based on an important new cloning technology developed in the laboratory of Dr. Waclaw Szybalski,[1-3] allows the user to induce the BAC plasmid from single copy to 10–20 copies per cell, resulting in high yield of purer DNA, enough for several high-throughput processes from a single 1-ml DNA preparation.

The CopyControl BAC Cloning Kit utilizes the CopyControl™ pCC1BAC™ Vector and the TransforMax™ EPI300™ *E. coli*. pCC1BAC (Figure 1), a derivative of pBeloBAC11 and EPICENTRE's pIndigo-BAC-5, contains the *E. coli* F-factor single-copy origin of replication and *ori*V-a high-copy origin of replication. The TransforMax EPI300 cells have been engineered to contain a mutant *trf*A gene, whose gene product is required for initiation of replication of *ori*V, under tight control of an inducible promoter. BAC clones constructed in pCC1BAC and grown in TransforMax EPI300 cells are maintained as single copy when grown on LB-plates containing chloramphenicol. The CopyControl BAC clones can then be induced to multiple copies whenever desired by addition of CopyControl™ Induction Solution to a subculture of a clone.

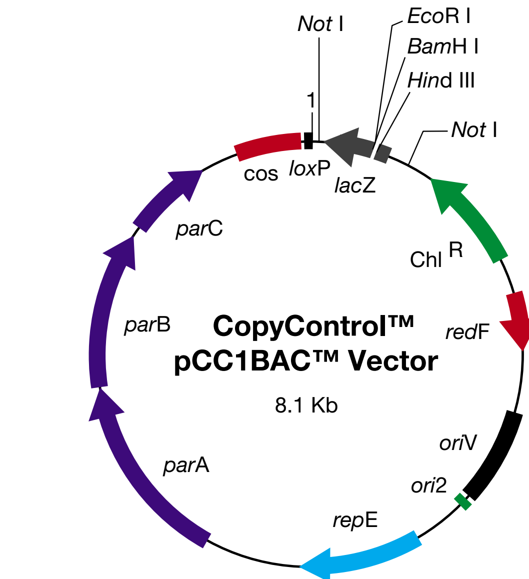



**Figure 1. The CopyControl™ pCC1BAC™ Vector contains both the single-copy *E. coli* F-factor and the *ori*V high-copy origins of replication.** pCC1BAC is supplied linearized (at its *Bam*H I, *Eco*R I, or *Hin*d III site), dephosphorylarted, and highly purified in the CopyControl™ BAC Cloning Kits.

Here we report an improved approach for construction of BAC libraries using the CopyControl BAC Cloning Kit and demonstrate the significant benefit of inducing the BAC clones from single copy to high copy number.

## BAC library construction

A library of the *E. coli* genome was constructed using the CopyControl BAC Cloning Kit (*Hin*d III). Complete protocols for CopyControl BAC Cloning Kits can be viewed on the EPICENTRE web site at www.epicentre.com/ccbac.asp. Briefly, high molecular weight (HMW) DNA plugs were prepared from *E. coli* genomic DNA and subjected to a pre-run followed by a partial digestion with *Hin*d III restriction enzyme and finally size selection of the partially digested DNA by pulse field gel electrophoresis.[4,5] Ligation reactions were performed using 100 ng of electro-eluted DNA from size selected 100-Kb to 150-Kb DNA fragments, 25 ng of the cloning-ready pCC1BAC (*Hin*d III-cut) vector and 4 units of Fast-Link™ DNA Ligase at 16°C for 4 hours. The ligation reaction was desalted for 1 hour on ice and then 2 µl was electroporated into 50 µl of TransforMax EPI300 Electrocompetent *E. coli*. Following cell outgrowth, 100 µl of transformation reaction was plated on LB + chloramphenicol (12.5 µg/ml) + X-Gal + IPTG and the plate was incubated overnight at 37°C.

## Sizing the BAC inserts without growing cultures and *Not* I digestions

The size of the inserts was determined by a rapid colony screening process without the need to grow cultures or perform restriction digests. Briefly, white colonies were randomly picked from the plate and processed, according the product literature, using the EpiLyse™ Solution and EpiBlue™ Solution (included in the kits). An aliquot of each processed clone was individually loaded into a well of a 0.8% agarose gel and the BAC-Tracker™ Supercoiled DNA Ladder (included with the kit and available separately; see page 12) was loaded as size markers. The gel was run for 3 hours at 4°C and stained with SYBR® Gold and the sizes of the BACs were estimated by comparing their mobility to that of the DNAs contained in the BAC-Tracker DNA Ladder. The size estimates obtained by this rapid colony screening process were confirmed by *Not* I digestion of each of the clones followed by CHEF gel electrophoresis.

Using the CopyControl BAC Cloning Kit (*Hin*d III), we constructed an *E. coli* BAC library of 122,000 clones (3000X genome coverage) from a single 4-hour ligation reaction. The library contained >97% white colonies with an average insert size of 130 Kb as determined by both the 4-hour colony screening procedure (Figure 2A) and by *Not* I digest analysis (Figure 2B). The ability to screen a BAC library in 4 hours is a significant improvement because it eliminates the drop in transformation efficiency of the stored ligation reaction that occurs when BAC clones are sized by standard procedures that require overnight cultures and restriction digestions.

## Induction of the CopyControl BAC clones to high-copy number

The most important feature of the CopyControl cloning technology is the

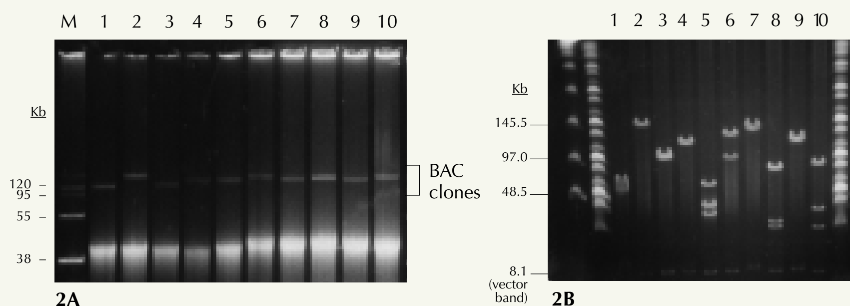



**Figure 2. An average insert size of >120 Kb was determined in 4 hours. 2A:** Ten randomly picked CopyControl™ BAC clones were sized by the rapid colony screening procedure provided in the CopyControl™ BAC Cloning Kits and as described in the text. Total time including processing the clones, running and staining the gel was 4 hours. Lane M, BAC-Tracker™ Supercoiled DNA Ladder; Lanes 1-10, CopyControl BAC clones. **2B.** The same 10 clones were sized by standard methods which included overnight culture, DNA purification, *Not* I restriction digestion and CHEF gel electrophoresis. The two procedures gave comparable results thus validating the colony screening procedure provided in the kits.

ability to induce the CopyControl BAC clones from single-copy to high-copy number. Four CopyControl BAC clones, with insert sizes ranging from 128 Kb to 145 Kb were chosen to evaluate this process. An isolated colony of each of these clones was grown in 1 ml of culture medium and then induced to high-copy number by adding the CopyControl Induction Solution. These clones were designated as the "induced" clones. A duplicate sample of each clone was grown at single copy (designated "uninduced") as control. DNA was isolated from an equal number of cells of both induced and uninduced cultures and digested with *Hin*d III and the digests were analyzed by 1% agarose gel electrophoresis. The restriction analyses of the BAC DNAs from induced and uninduced cultures of the four CopyControl BAC clones is shown in Figure 3.

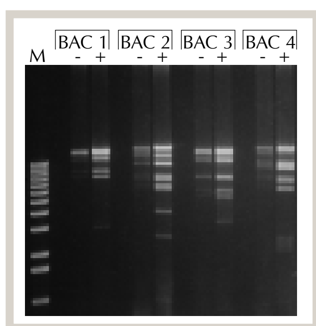



**Figure 3. The copy number of CopyControl™ BAC clones induced to high copy number greatly improve analyses of the cloned inserts.** One microliter of DNA, isolated from an equal number of cells from induced (+) and uninduced (-) cultures of four CopyControl BAC clones, was digested with *Hin*d III and the digestion products analyzed by agarose gel electrophoresis.

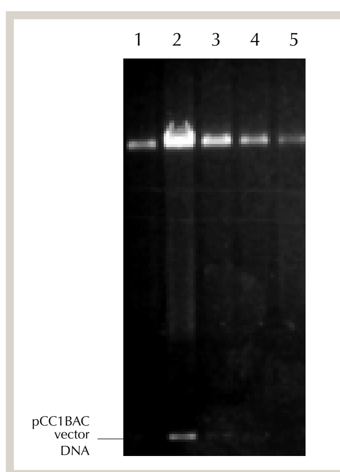



**Figure 4. CopyControl™ BAC clones can be induced to 10 to 20 copies per cell.** DNA was isolated from an equal number of cells of an induced and an uninduced culture of a 128-Kb CopyControl BAC clone. The DNA from the induced culture was diluted and then 1 µl of each sample was digested with *Not* I and digestion products resolved by PFGE followed by staining with SYBR®Gold. Visual comparison of the band intensity of DNA from the uninduced culture with the DNA dilutions from the induced culture indicate that induction resulted in 10 to 20 fold more BAC DNA from the same number of cells. Lane 1, DNA from uninduced 128-Kb clone; Lane 2, DNA from induced 128-Kb clone; Lanes 3-5, 1:5, 1:10 and 1:15 dilutions of DNA from the induced clone.

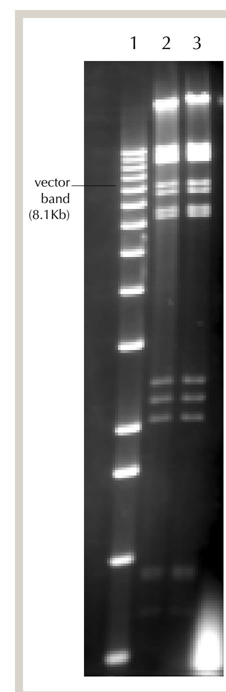
The clone copy number of the induced cultures was estimated by comparing the band intensity of the single-copy *Not* I-digested BAC DNA from the uninduced cultures with the band intensity of *Not* I digestion of 1:5, 1:10: and 1:15 serial dilutions of DNA from the induced cultures. As shown in Figure 4, the induced culture of the 128-Kb clone yielded ~15-fold more BAC DNA than the uninduced culture. Additionally, it was demonstrated that inserts are stably maintained at single copy in the pCC1BAC Vector (Figure 5). A more complete investigation of the stability of CopyControl BAC clones at single copy and after induction to high-copy number will be presented in an upcoming issue of the *EPICENTRE Forum*.

## Conclusion

The CopyControl BAC Cloning Kits provide an improved protocol for construction of BAC libraries that include a 4 hour ligation reaction and, significantly, the ability to screen the size of the clones in 4 hours.

The CopyControl pCC1BAC Vector, with its single-copy and *ori*V high-copy origins of replication, is the vector of choice for construction of large insert BAC libraries.

**Figure 5. CopyControl™ BAC clones are stable at single copy.** A 145-Kb CopyControl BAC clone was digested with *Hin*d III before and after growth for 100 generations at single copy in TransforMax™ EPI300™ *E. coli*. The DNAs produced identical *Hin*d III fingerprints demonstrating that the CopyControl BAC did not undergo deletions or rearrangements after 100 generations of growth. Lane 1, linear DNA size marker; Lane 2, *Hin*d III fingerprint of the CopyControl BAC clone from generation 0; Lane 3, *Hin*d III fingerprint of the CopyControl BAC clone after 100 generations.




Large inserts cloned into pCC1BAC Vectors and grown in TransforMax™ EPI300™ *E. coli* are stable when maintained at single copy and, when desired can be induced to 10 to 20 copies per cell for higher yields of purer DNA. Thus, the pCC1BAC Vector offers significant cost and time-saving advantages over all other low-copy BAC vectors for applications such as fingerprinting and BAC end-sequencing.

## Construction of CopyControl™ BAC libraries from plant genomes

Drs. P. Piffanelli, C. Lanaud and J.C. Glaszmann BAC-TROP, CIRAD-AMIS, France have recently reported success in constructing BAC libraries from tropical

plant species using the CopyControl BAC Cloning Kits. Libraries constructed in the pCC1BAC Vector will be used as a resource for genome sequencing and map-based cloning of agronomically important genes. The ability of the pCC1BAC clones to induce to >15 fold makes the use of pCC1BAC Vector as an "ideal choice of vector" for high throughput sequencing and DNA fingerprinting.

Look for a complete article in an upcoming *Epicentre Forum*.

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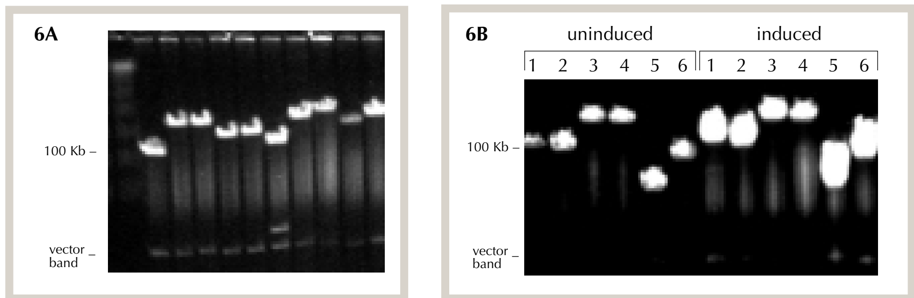



**Figure 6. A cocoa plant genomic library was constructed at CIRAD-AMIS, France using the CopyControl™ BAC Cloning Kit (*Hind* III). 6A:** *Not* I-digest analysis of 10 CopyControl™ BAC clones. **6B:** Comparison of BAC DNA yield from an equal number of cells of uninduced and induced cultures of six clones.


www.epicentre.com/ccbac.asp

**CopyControl™ BAC Cloning Kit (*Bam*H I)**

CCBAC1B 1 Kit

**CopyControl™ BAC Cloning Kit (*Eco*R I)**

CCBAC1E 1 Kit

**CopyControl™ BAC Cloning Kit (*Hind* III)**

CCBAC1H 1 Kit

***Contents:***

pCC1BAC™ (*Bam*H I) or pCC1BAC™ (*Eco*R I) or pCC1BAC™ (*Hind* III) Cloning-Ready Vector, Fast-Link™ DNA Ligase and Buffer, ATP, BAC-Tracker™ Supercoiled DNA Ladder, EpiBlue™ Solution, EpiLyse™ Solution, Control DNA Insert, and Control BAC Clone (145 Kb).

TransforMax™ EPI300™ Electrocompetent *E. coli*, required to induce CopyControl BAC clones to high copy number, are available separately.

**TransforMax™ EPI300™ Electrocompetent *E. coli***

EC300105 5 X 100 µl

EC300110 10 X 100 µl

EC300150 50 X 100 µl

***Contents:***

Electrocompetent *E. coli* of >5 X $10^9$ cfu/µg, pUC19 Control DNA, and CopyControl™ Induction Solution.


# Integrate CopyControl™ Capability into Existing BAC and Fosmid Clones

EPICENTRE's new EZ::TN™ <*ori*V /KAN-2> Insertion Kit enables researchers to integrate CopyControl™ capability into existing single-copy BAC and fosmid clones. The kit features the EZ::TN™ <*ori*V /KAN-2> Transposon, which contains the *ori*V high-copy origin of replication and a kanamycin selectable marker. A short, one-step *in vitro* reaction catalyzed by EZ::TN™ Transposase randomly inserts the transposon into existing BAC or fosmid clones. An aliquot of the transposition reaction is then used to transform TransforMax™ EPI300™ Electrocompetent *E. coli* (available separately) and insertion clones are selected by growth on kanamycin (Figure 1).

## Obtain high yields of BAC and fosmid DNA for sequencing and fingerprinting

A single, reaction generates up to thousands of random transposon insertion clones. Like the CopyControl™ pCC1™ Vectors, BAC and fosmid clones containing the EZ::TN <*ori*V /KAN-2> Transposon can be maintained at single copy to ensure insert stability but can then be induced to 10 to 50 copies per cell whenever desired, to maximize the yield and purity of DNA for sequencing, fingerprinting and other applications.

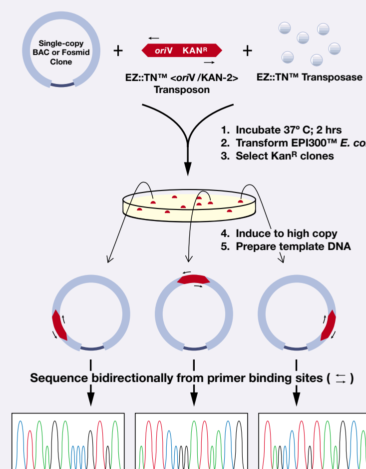



**Figure 1. The process for generating EZ::TN™ <*ori*V /KAN-2> Transposon insertion clones for high yields of DNA and bidirectional sequencing.**

## Sequence bidirectionally from randomly distributed primer binding sites

Each insertion clone not only contains *ori*V and a kanamycin marker, but unique primer binding sites near the ends of the transposon. DNA flanking the transposon can be sequenced bidirectionally from these unique sites using the primers provided in the kit. Thus, insertion of the *ori*V-containing transposon accomplishes two functions; it converts a single-copy clone to one which has CopyControl capability and it generates a library of sequencing templates with random transposon insertions permitting complete sequencing of the clone with only two sequencing primers. The need for subcloning or primer walking strategies has been eliminated.


www.epicentre.com/transposomics.asp

**EZ::TN™ <*ori*V /KAN-2> Insertion Kit**

EZI02VK 10 Reactions

***Contents:***

EZ::TN™ <*ori*V /KAN-2> Transposon, EZ::TN™ Transposase, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.

TransforMax™ EPI300™ Electrocompetent *E. coli*, required to induce EZ::TN™ <*ori*V/KAN-2> transposed clones to high copy number, are available seperately.

# Size Your BAC Clones in 4 Hours without Growing Cultures or Performing Restriction Digests

Until now sizing BAC clones and assessing the quality of a BAC library was a time-consuming and labor-intensive process requiring growth of overnight cultures, isolation of DNA, digestion with restriction enzymes and Pulse Field Gel Electrophoresis (PFGE). EPICENTRE's Colony Fast-Screen™ Kit and new BAC-Tracker™ Supercoiled DNA Ladder eliminate these steps and reduce the time required to size BAC clones from days to 4 hours.

**Benefits of the Colony Fast-Screen™ Kit**

- **Fast.** Screen BAC clones in 4 hours. Screen smaller clones in as little as 1 hour.
- **Efficient.** No need to grow cultures or perform restriction digests.
- **Amenable** to both high throughput and routine cloning applications.

The Colony Fast-Screen Kit contains the EpiLyse™ Solution and EpiBlue™ Solution. The EpiLyse Solution lyses the cells releasing the supercoiled BAC DNA as well as genomic DNA fragments. The EpiBlue™ Solution is added in preparation for loading the gel. Standard agarose gels can be used. Load 10 µl of the BAC-Tracker™ Supercoiled DNA Ladder into lanes adjacent to the BACs and run the gel. Following electrophoresis, stain with SYBR® Gold (or other highly sensitive stain) and size the BAC clones (Figure 2). Total processing time, including running and staining an agarose gel, is about 4 hours.

The BAC-Tracker™ Supercoiled DNA Ladder (Figure 3) is the first commercially available size marker that is suitable for estimating the size of large supercoiled DNAs such as BAC clones. The Ladder contains supercoiled DNAs of 38 Kb to 120 Kb in a ready-to-load solution. The BAC-Tracker Ladder has been formulated so that each band gives approximately equal staining intensity.

The Colony Fast-Screen Kit and the BAC-Tracker Supercoiled DNA Ladder are also supplied in EPICENTRE's new Copy-Control™ BAC Cloning Kits (see pages 9 – 11 and the center insert).

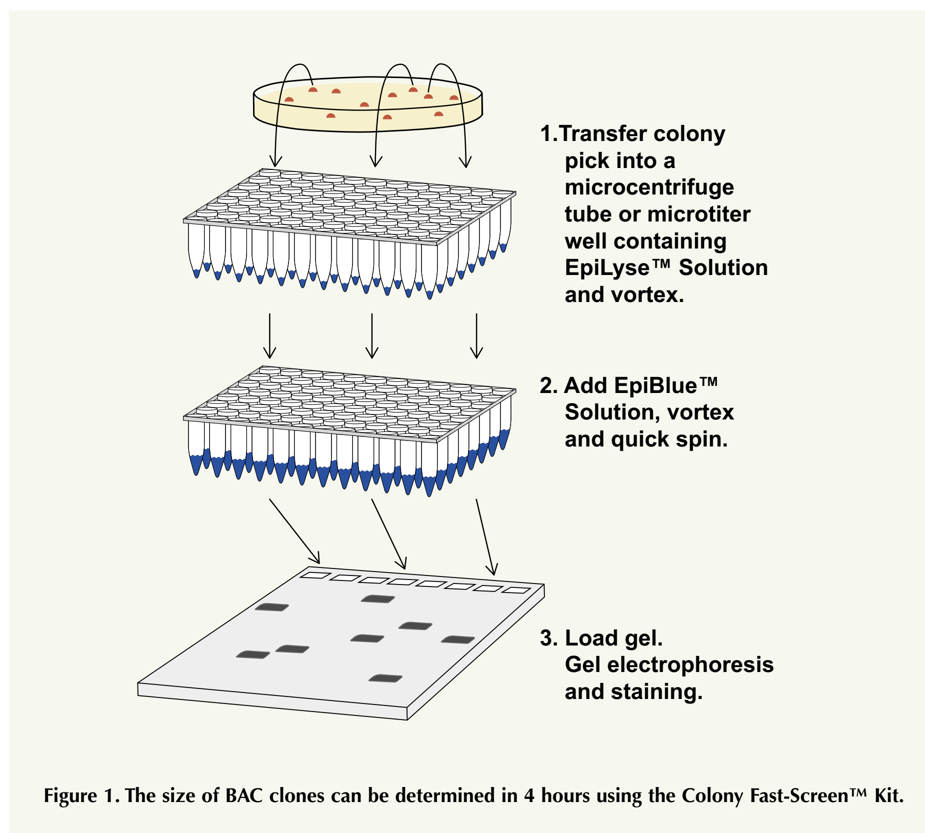



**Figure 1. The size of BAC clones can be determined in 4 hours using the Colony Fast-Screen™ Kit.**

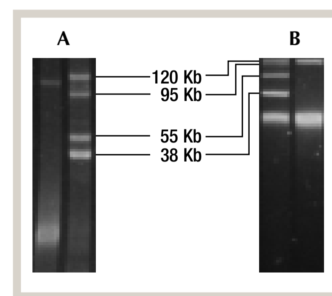



**Figure 3. A purified 115-Kb BAC clone sized using the BAC-Tracker™ Supercoiled DNA Ladder by PFGE (Panel A) and by 0.8% agarose minigel (Panel B).** Gels were stained using SYBR® Gold.

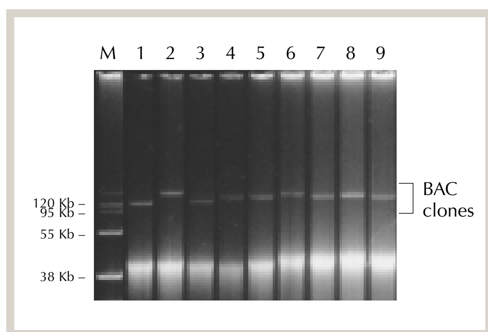



**Figure 2. The size of 9 randomly picked BAC clones was determined using the Colony Fast-Screen™ Kit and the BAC-Tracker™ Supercoiled DNA Ladder as size markers.** The BAC clones were processed using the EpiLyse™ Solution and the EpiBlue™ Solution as described in the Colony Fast-Screen Kit literature. Aliquots of the processed clones were loaded into the wells of a 0.8% agarose gel. Ten microliters of the BAC-Tracker Supercoiled DNA Ladder was loaded and electrophoresis was performed for 3 hours (4.5 V/cm) at 4°C. The gel was stained with SYBR® Gold to visualize the single-copy BAC DNAs.

**www.epicentre.com/fastscreen.asp**
**www.epicentre.com/bactracker.asp**

**Colony Fast-Screen™ Kit**

FS08250 250 Screenings

***Contents:***

EpiBlue™ Solution and EpiLyse™ Solution.

**BAC-Tracker™ Supercoiled DNA Ladder**

BT010950 50 Gel Lanes

***Contains:***

Supercoiled DNAs of 38 Kb, 55 Kb, 95 Kb and 120 Kb in a ready-to-load solution.

# Isolation of Bovine and Mouse DNA from Buccal and Vaginal Samples Using the BuccalAmp™ DNA Extraction Kit

## Isolation of Bovine DNA from Buccal and Vaginal Samples

*R. S. Sadler, M. A. Anderson, and M. S. Ashwell*
*USDA, ARS, Gene Evaluation and Mapping Laboratory*
*Beltsville, MD*

Our research focuses on identification of quantitative trait loci affecting economically important traits like milk production and reproduction in commercial dairy cattle. This research requires large numbers of animals from complex pedigrees that are genotyped at microsatellite markers located throughout the genome. We study both males and females in our projects, isolating DNA from semen for males and normally using blood as a reliable source for females. However, collection of blood from cows located on the farm is expensive because a veterinarian usually collects the blood and then the samples must be quickly shipped to the laboratory for processing. Upon arrival, samples would be extracted using either an inexpensive, labor-intensive phenol-chloroform method or quicker, more expensive, solid-phase or spin-column methods.

We elected to try the BuccalAmp™ DNA Extraction Kit to collect samples from cows due to the ease of sample collection, economical method of storage and shipping, and the rapid processing of the samples. We collected buccal and vaginal samples from cows using the Catch-All™ Sample Collection Swabs provided in the kit. Swabs were processed on the same day of collection, while others were kept at room temperature for one week and –20ºC for one month.

The kit was easy to use, requiring only a vortex mixer and a hot water bath. After quantitating the extractions through DNA fluorometry, we found no discrepancy in the quality or quantity of DNA obtained from swabs that were processed the same day and those that were stored and processed at a later time. Furthermore, the actual DNA yield surpassed our expectations, with an average DNA concentration of 200 µg/µl. Most importantly, all of these samples repeatedly PCR-amplified using fluorescently-tagged primers (Figure 1). In conclusion, the BuccalAmp Kit was ideal for acquiring vaginal and buccal samples using noninvasive methods, providing high quality DNA in a timely fashion.

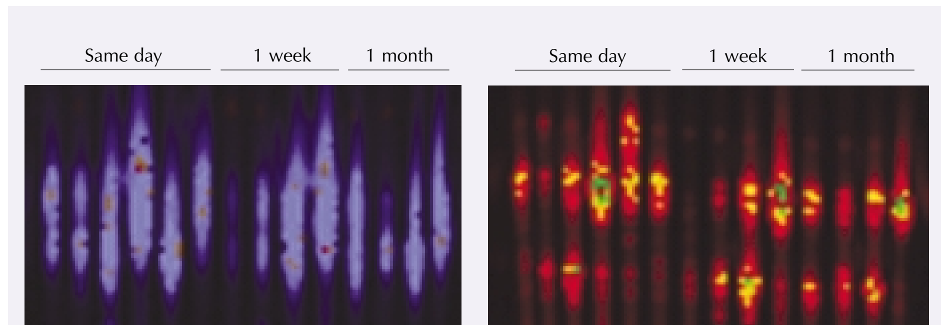



**Figure 1. PCR amplification of buccal and vaginal samples.** Each 12 µl reaction included 50 ng of template, 1X buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM of each primer, and 0.3 U *Taq* polymerase. PCR products were produced with the following protocol: 95ºC for 3 minutes, 94ºC for 15 seconds, 56ºC for 15 seconds, 72ºC for 15 seconds, for 35 cycles, and then 60ºC for 30 minutes. An aliquot (1 µl) of each reaction was analyzed on an ABI 377 Automated DNA Sequencer.

(Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.)

## Isolation of Mouse DNA from Buccal Samples

*Jonathon Golas and Janet Grod*
*Wyeth Research*
*Pearl River, NY*

Multiple intestinal neoplasia (*Min*) is an autosomal dominant trait that involves a nonsense mutation in codon 850 of the murine *Apc* gene [murine *Apc* is a homologue to the human *APC* (adenomatous polyposis coli) gene ].[1] Because the condition of the *Min* mouse mocks human FAP, the C57BL/6J-*Min*/+ strain is a very important model for finding therapies for the prevention of colon cancer. C57BL/6J females can be crossed with C57BL/6J-*Min*/+ males to produce pups that are either heterozygous for the *Apc* mutation, or normal C57BL/6J. One hundred percent of the C57BL/6J-*Min*/+ mice raised on a high fat diet (AIN) develop adenomas throughout the intestinal tract and most die by the age of 120 days.[2]

Normal C57BL/6J females (usually 2) are placed in a cage with one C57BL/6J-*Min*/+ male. Plugs will be present up to 12 hours post-copulation. Plugged females are separated and after approximately 19-21 days, parturation occurs. The pups are weaned at 21-28 days post parturation.

Traditionally, tail snips have been used to genotype for the *Apc* mutation. A 1-2 mm piece of tail is cut with a razor blade and placed into 30 µl of DNA extraction buffer. Then, proteinase K is added and a series of vortexes and water bath incubations occur until finally, PCR-ready DNA is prepared. An allele-specific PCR assay using the primers MAPC MT, MAPC 9, and MAPC 15 is used to genotype the mice. A PCR product of 327 bp is generated in the presence of *Min* alleles, but no product is evident in its absence. MAPC 9 and MAPC 15 together, generate a 618 bp product surrounding the *Min* nonsense mutation. This band is generated in +/+ mice and *Min*/+ mice.[3]

Recently, DNA extractions were attempted using EPICENTRE's BuccalAmp™ DNA Extraction Kit as an alternative to the use of tail snips. Swabs of the cheek cells of the mice were rotated in the tubes containing the QuickExtract™ DNA Extraction Solution, and DNA extraction was performed following the kit's protocol. The kit provided PCR-ready DNA that was incorporated into the PCR protocol mentioned above. The DNA yield was sufficient, and the samples were run against tail digested samples, on a 4% agarose gel with ethidium bromide (Figure 1).

The BuccalAmp Kit proves more beneficial than tail-snip digestion in two ways. First it saves time in the elimination of tail-tissue digestion. It also provides a less invasive means of sampling DNA from mice, without sacrificing DNA yield.

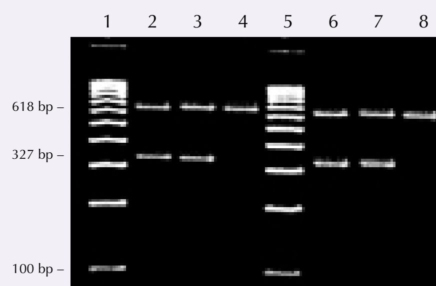



**Figure 1. PCR products obtained using the FailSafe™ PCR System and run on a 4% gel with ethidium bromide.** Lanes 1 and 5 contain the 100 bp marker. Lanes 2, 3, and 4 are DNA samples collected from the cheek cells of mice. The DNA was extracted using the BuccalAmp™ DNA Extraction Kit. Lanes 6, 7, and 8 are DNA samples collected from tail snips of the same mice. Samples in lanes 2 and 3 and lanes 6 and 7 were positive for the *Apc* gene mutation (*Min*/+). Samples in lanes 4 and 8 did not have the *Apc* gene mutation (+/+).

## References


1. Su, L.K. *et al.* (1992) *Science* **256**, 668.
2. Moser, A.R. *et al.* (1990) *Science* **247**, 322.
3. Dietrich, W.F. *et al.* (1993) *Cell* **75**, 631.



**www.epicentre.com/buccalamp.asp**

**BuccalAmp™ DNA Extraction Kits**

| | |
|---|---|
| BQ0901S | 1 Kit |
| BQ0908S | 8 Kits |
| BQ0916S | 16 Kits |

***Contents:***

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

**www.epicentre.com/failsafe.asp**

**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.


# Science Students Amplify Human Globin Gene DNA from Buccal Samples


*Students Kathryn Karalis, Emily Leaner, Justin Liu and Dr. Jack Sanford*
*The Derryfield School, Manchester, NH*


The Derryfield School offers a 10-week biology independent science course for high school seniors as an opportunity to get hands-on scientific experience. Students are expected to write a proposal outlining their interests and expectations for the course. Under the direction of the science teacher, students discuss research plans and specific experiments that can be done in the classroom laboratory. They then design their experiments and proceed through the classical steps of observation, hypothesis, data collection and analysis of results.

Last year, three students, Emily Learner (now at Vasser College), Justin Liu (now at Virginia Military Institute) and Kathryn Karalis (now at Northeastern University) [Figure 1], performed a variety of experiments including the extraction and amplification of human globin gene DNA from buccal samples using the BuccalAmp™ DNA Extraction Kit and the FailSafe™ PCR System.

## DNA Extraction

DNA was obtained with the BuccalAmp™ DNA Extraction Kit from EPICENTRE. First, the oral cavity was thoroughly rinsed with water, the inside surface of the cheek was gently brushed with a toothbrush and the oral cavity was rinsed again with water. Samples of buccal epithelial cells were then collected using the sterile Catch-All™ sample collection swab according to directions on the package. The swab was placed directly into a tube containing the QuickExtract™ DNA Extraction Solution and rotated clockwise and counterclockwise 5 times in each direction. Sample tubes were hand vortexed and heated for 30 minutes at 65ºC. Samples were hand vortexed again for 15 seconds and heated for 8 minutes at 98ºC. This step was repeated and the extracted DNA solutions were stored at –20ºC.

## DNA Amplification

Amplification primers were purchased from and purified by Integrated DNA Technologies, Inc. Primers were diluted to 1 µM final concentration in the amplification reaction. Five microliters of extracted DNA were amplified in the presence of the FailSafe™ PCR Enzyme Mix, the FailSafe PCR 2X PreMixes A through K and globin DNA primers in a total volume of 50 µl. PCR cycling conditions were 30 cycles of a three-step cycling program with a denaturing temperature of 98ºC for 1 minute, annealing at 55ºC for 0.5 minute, and extension at 73ºC for 1 minute. Amplified DNA products were examined via electrophoresis using 0.8% agarose in a Tris/Borate/EDTA buffer. Gels were stained with SYBR® Gold and viewed with a Dark Reader System. Digital images were recorded with a Nikon Coolpix 880 (Figure 2).

## Conclusion

Emily, Justin, and Kathryn were thrilled with the process of doing science, particularly the detailed work required to accurately perform PCR. This technique lends itself to a number of tasks that students can do and then connect to the classroom discussions. For instance, collecting their own cells and extracting the DNA, preparing PCR tubes, pouring agarose gels and using a thermalcycler are all exciting tasks done in order to visualize DNA that belongs to you, the experimenter.

**Figure 1. Student participants (from left) Kathryn Karalis, Emily Leaner, Justin Liu and their science teacher Dr. Jack Sanford.**

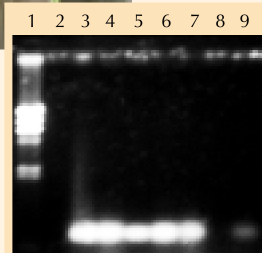



**Figure 2. PCR amplification results using the FailSafe™ PCR System.** Agarose gels were loaded with 10 µl aliquots from PreMix reactions D through J. Lane 1, *Hind* III lambda digest (1 µg); Lane 2, negative control; Lanes 3-9, PCR products generated in the presence of PreMixes D through J.
(Images courtesy of Claudette Knieriem)

# Get Consistently High Yields of Full-Length RNA From Both Long and Short DNA Templates Using AmpliScribe™ T7 High Yield Transcription Kits

Getting consistent *in vitro* transcription of full-length RNA transcripts from a variety of DNA templates is critical to the success of many gene expression experiments. For this reason, EPICENTRE's AmpliScribe™ High Yield Transcription Kits have become the preferred *in vitro* transcription kits for many labs. Recently, a competitor reported their "inability" to produce full-length transcripts >1 Kb using EPICENTRE's AmpliScribe T7 High Yield Transcription Kits. We find this result quite remarkable since a number of researchers have reported to us that, in side-by-side comparisons, AmpliScribe Kits have consistently produced better results with their templates than kits from competitors.

Here, as reported previously (see *EPICENTRE Forum* 7:2) we demonstrate the yield and integrity of RNA produced using the AmpliScribe T7 High Yield Transcription Kits. In this study, we report the results of transcribing ten different DNA templates, producing transcripts ranging in size from 46 bases to 8 Kb using AmpliScribe T7 High Yield Transcription Kits.

## High yields of full-length, long RNA transcripts from an AmpliScribe™ T7 High Yield Transcription reaction

Five linearized DNA templates, producing RNA transcripts of 2.8 Kb, 3.8 Kb, 4.8 Kb, 7.3 Kb, and 8.0 Kb, were individually transcribed in standard 20 µl AmpliScribe T7 High Yield Transcription reactions for 2 hours at 37ºC. As shown in Figure 1, each reaction produced a full-length RNA transcript. RNA yields ranged from 154 µg to 160 µg of RNA.

**"The main thing I like about the AmpliScribe Kits is their consistently higher yields and lower price than the competition."**
*– Dr. JLE Dean, Imperial College School of Medicine, London*

**"I just finished a side-by-side comparison of the AmpliScribe Kit to the (competitor's kit). I wanted to let you know that the AmpliScribe Kit by far and away blows (the competitor's kit) away. In terms of both quality and yield"**
*– Aaron Nagel, Genome Solutions*

## High yields of full-length, short RNA transcripts from an AmpliScribe™ T7 High Yield Transcription reaction

Five linearized DNA templates, producing RNA transcripts of 46 bases, 69 bases, 88 bases, 95 bases, and 242 bases, were individually transcribed in standard 20 µl AmpliScribe T7 High Yield Transcription reactions for 2 hours at 37ºC. Figure 2 shows that each reaction produced the expected full-length transcript. RNA yield ranged from 41 to 78 µg of RNA.

## AmpliScribe™ T7 High Yield Transcription reactions consistently produce high yields of RNA transcripts

Each new batch of AmpliScribe T7 High Yield Transcription Kits must meet stringent quality testing parameters for RNA yield prior to release for sales. The RNA yield from five recent batches of the AmpliScribe T7 High Yield Transcription Kits is shown in Figure 3. Each lot of kits produced >150 µg of a 1.4 Kb RNA transcript.

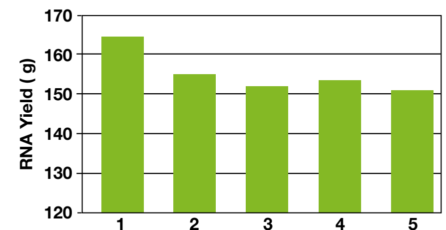



**Figure 3. AmpliScribe™ T7 High Yield Transcription Kits consistently produce high yields of RNA.** AmpliScribe T7 transcription reactions from five recent lots of kits each generated >150 µg RNA as determined by EPICENTRE's Quality Control Lab using 1 µg of linearized control DNA template, producing a 1.4-Kb RNA transcript, in a 2 hour reaction.

## AmpliScribe™ High Yield Transcription Kits are the best value for *in vitro* transcription

EPICENTRE's competitively-priced AmpliScribe High Yield Transcription Kits consistently produce high yields of RNA transcripts from both long and short DNA templates.

**Figure 1. An AmpliScribe™ T7 High Yield Transcription reaction produces full-length, long RNA transcripts.** Aliquots from standard 20 µl AmpliScribe T7 High Yield Transcription reactions were loaded onto a 1% agarose-formaldehyde gel and stained with ethidium bromide. The size of the RNA transcripts and RNA yields (in parentheses) were: Lane 1, 2.8 Kb (155 µg); Lane 2, 3.8 Kb (160 µg); Lane 3, 4.8 Kb (154 µg); Lane 4, 7.3 Kb (156 µg); Lane 5, 8.0 Kb (156 µg).

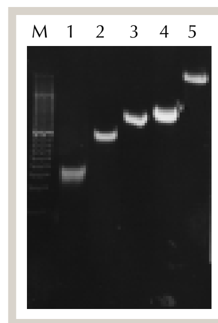



**Figure 2. An AmpliScribe™ T7 High Yield Transcription reaction produces full-length, short RNA transcripts.** Aliquots from standard 20 µl AmpliScribe T7 High Yield Transcription reactions were loaded onto a 12% polyacrylamide gel and stained with ethidium bromide. The size of the RNA transcripts and RNA yields (in parentheses) were: Lane 1, 46 bases (41 µg); Lane 2, 69 bases (55 µg); Lane 3, 88 bases (61 µg); Lane 4, 95 bases (65 µg); Lane 5, 242 bases (78 µg).

www.epicentre.com/ampliscribe.asp

**AmpliScribe™ High Yield Transcription Kits**

**T7**

| | |
|---|---|
| AS2607 | 25 Reactions |
| AS3107 | 50 Reactions |

**SP6**

| | |
|---|---|
| AS2606 | 25 Reactions |
| AS3106 | 50 Reactions |

**T3**

| | |
|---|---|
| AS2603 | 25 Reactions |
| AS3103 | 50 Reactions |

***Contents:***

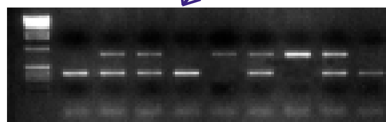
AmpliScribe™ T7, SP6 or T3 Enzyme Mix (with added RNase inhibitor), 100 mM ATP, CTP, GTP and UTP Solutions, AmpliScribe™ 10X Reaction Buffer, RNase-free DNase I, RNase-free Water, DTT, and Linearized Control DNA Template.

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

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

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

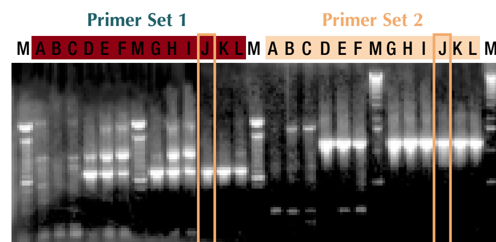

**Jessica Otte**
Center for Neurovirology & Cancer Biology
Temple University, Philadelphia, Pennsylvania

**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 leters 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


I've recently started using FailSafe and it is GREAT! Thank You, Regina


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


**Visit www.epicentre.com/failsafe.asp for product and ordering information.**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