

EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research



The FailSafe™ Real-Time PCR System Provides Highly Sensitive and Specific Real-Time PCR Analysis

Haiying Grunenwald, EPICENTRE

The FailSafe™ Real-Time PCR System extends the unsurpassed specificity, sensitivity, and consistency of the FailSafe™ PCR System to quantitative PCR applications. The FailSafe™ Real-Time PCR System incorporates SYBR® Green I dye for the detection and quantitation of PCR products without the expense of labeled PCR primers, molecular beacons, or other labeled probes. Like the standard Fail-Safe™ PCR System, this new real-time PCR kit ensures successful quantitative PCR the first time and every time.

The FailSafe Real-Time PCR System uses the FailSafe PCR Enzyme Mix, a unique blend of thermostable enzymes that is capable of amplifying the most difficult DNA templates with extremely high sensitivity and high fidelity. In addition, the patented FailSafe PCR Enhancer (with betaine)* greatly improves the specificity and consistency of PCR.

The final key components of the system are 12 unique FailSafe PCR PreMixes. Each FailSafe PCR PreMix contains everything else you need for a successful quantitative PCR except your own template and primers: SYBR Green I dye, dNTPs, buffer, and varying amounts of MgCl₂ and EPICENTRE's patented FailSafe PCR Enhancer. A separate container of ROX, a fluorescent passive reference dye that is required for signal normalization in SYBR Green dye reactions assayed using ABI real-time PCR instruments, is also provided.

In this report, we compare the specificity, sensitivity, and dynamic range of the FailSafe Real-Time PCR System with real-time PCR kits of leading suppliers.

Methods and Results

Higher specificity with the FailSafe™ Real-Time PCR System

To test the specificity of real-time PCR reactions, a 357-bp fragment of human

First Time

Perform PCR with your template and primers using the **FailSafe™ Real-Time PCR PreMix Selection Kit** and choose the PreMix that provides the best quantitative amplification.



Every Time

Get the selected PreMix with the **FailSafe™ Real-Time PCR System** and use it for consistent amplification of your template/primer pair.

Duchenne Muscular Dystrophy (DMD) exon 43 was amplified using 100 ng of human genomic DNA, 500 nmol each of the forward and reverse primers, and either the FailSafe Real-Time PCR System or another supplier's kit according to the manufacturer's directions. FailSafe PreMix E was found to be optimal in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions with this template and primer set. All reactions were set up at room temperature. Prior to thermocycling, hot-start enzymes of other suppliers were reactivated according to each of the other manufacturer's instructions. The FailSafe System does not use a hot-start enzyme and no reactivation step was required. The PCR cycling program

consisted of 50 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C.

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Figure 1. Higher specificity with the FailSafe™ Real-Time PCR System. Real-time PCR amplification of a 357-bp fragment from human DMD exon 43 was performed with FailSafe Real-Time PCR System and 5 major hot-start real-time PCR suppliers' kits. **Panel A**, PCR quantification graph. **Panel B**, Melt curve analysis. **Panel C**, Agarose gel electrophoresis. Real-time PCR amplification was carried out on MJ Research's Opticon® 2 Real-Time PCR Detection System.

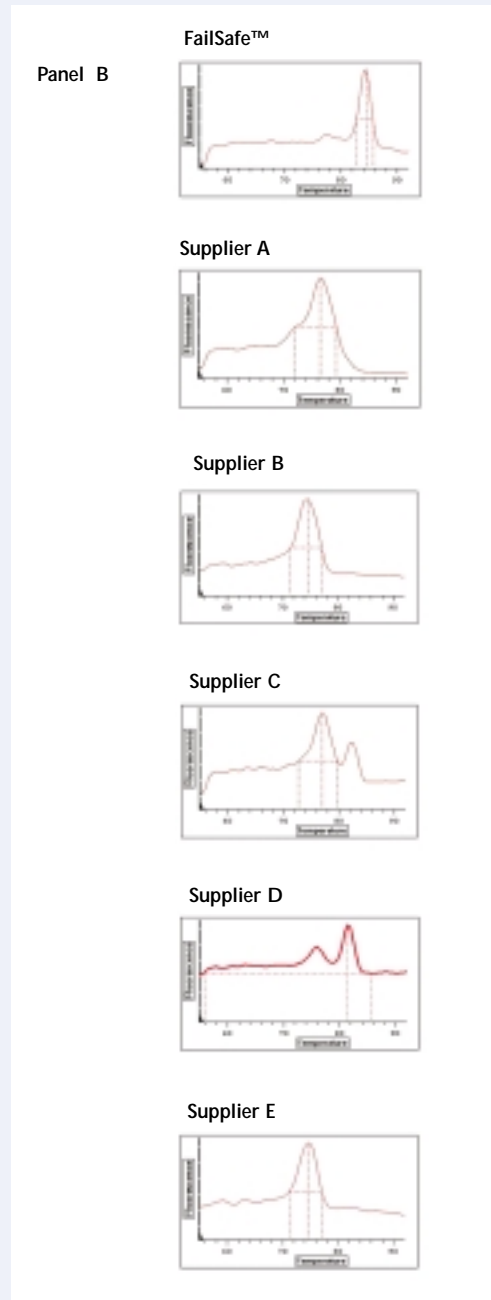
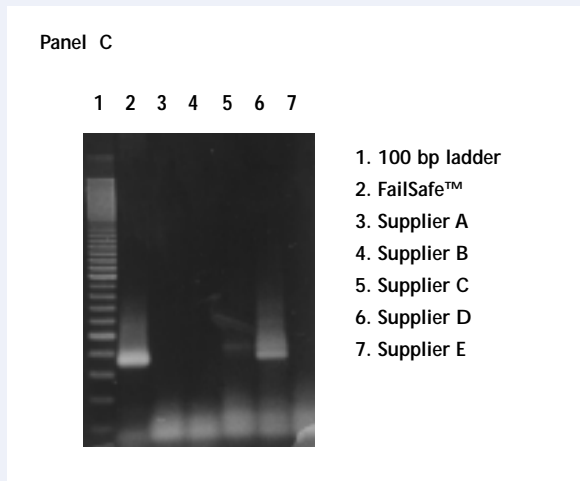
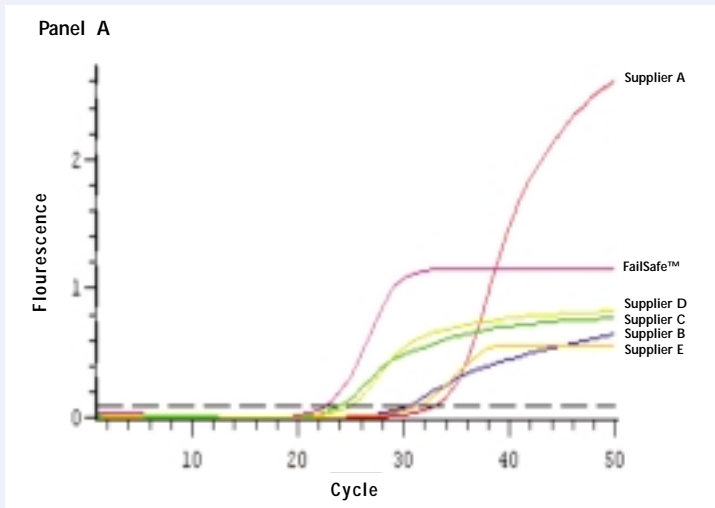


Figure 1, Panel A shows real-time PCR results obtained using the various kits. A significantly faster cycle threshold (C_T) was obtained using the FailSafe Real-Time PCR System compared to all of the other kits tested. Figure 1, Panel B illustrates melt curve analysis. Although some kits resulted in higher overall fluorescence than the FailSafe System, the melt curve analysis demonstrated that this higher fluorescence was due to non-specific or primer-dimer amplification rather than specific primer-dependent amplification. The fact that the FailSafe Real-Time PCR System provided the best true

amplification was also apparent by agarose gel electrophoresis of the PCR products, as shown in Figure 1, Panel C.

Higher sensitivity with the FailSafe™ Real-Time PCR System

To compare the sensitivity of quantitative PCR, a 181-bp fragment of human DMD exon 47 was amplified using the FailSafe Real-Time PCR System and five other suppliers' kits using 10 ng of human genomic DNA and 500 nmol each of the forward and reverse primers according to the manufacturer's directions. FailSafe™ Real-Time PreMix B was found to be opti-

mal with this template and primer set in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions. All reactions were set up at room temperature. Again, prior to thermocycling, the hot-start enzymes of each of the other suppliers were reactivated according to the other manufacturer's instructions, while no reactivation step was required or used for the FailSafe System. The PCR cycling program consisted of 50 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C. As seen in Table 1, the FailSafe Real-Time PCR System provided the fastest C_T value.

Supplier	C _T Value (Average of Triplicate Reactions)
FailSafe™ Real-Time PCR System	24.5
Supplier A	26.1
Supplier B	30.1
Supplier C	28.0
Supplier D	27.4
Supplier E	29.8

Table 1. Higher sensitivity and faster C_T value with the FailSafe™ Real-Time PCR System. Real-time PCR amplification of a 181-bp fragment from human DMD exon 47 was performed with 10 ng of human genomic DNA using the FailSafe Real-Time PCR System and 5 major hot-start real-time PCR suppliers' kits. C_T values obtained from triplicate reactions of each PCR kit were averaged. Real-time PCR amplification was carried out on Bio-Rad's iCycler iQ™ Real-Time PCR Detection System.

Broader dynamic range with the FailSafe™ Real-Time PCR System

In order to evaluate the FailSafe Real-Time PCR System and five other suppliers' kits for dynamic range in quantitative real-time PCR, a 460-bp sequence was amplified using from as little as 1 molecule to 10⁶ molecules of bacteriophage lambda DNA as a template. Each 50- μ l real-time PCR was carried out using lambda DNA ranging from 1 molecule to

10⁶ molecules and 500 nmol each of the forward and reverse primers according to the manufacturer's directions. FailSafe™ Real-Time PreMix E was found to be optimal in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions with this template and primer set. All reactions were set up at room temperature. Each manufacturer's instructions were followed on reactivating their hot-start PCR enzyme, a step not required with the FailSafe Real-Time PCR

System. The PCR cycling program consisted of 45 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C. As demonstrated in Figure 2, the FailSafe Real-Time PCR System provided more sensitive quantitative PCR amplification, as well as a broader dynamic range.

Conclusions

The FailSafe Real-Time PCR System provides highly specific and sensitive quantitative PCR data with broad dynamic range. The reported quantitative PCR analysis was performed on Bio-Rad's iCycler iQ™ and MJ Research's Opticon® 2. The FailSafe™ Real-Time PCR System has also been tested on ABI Prism 7700.

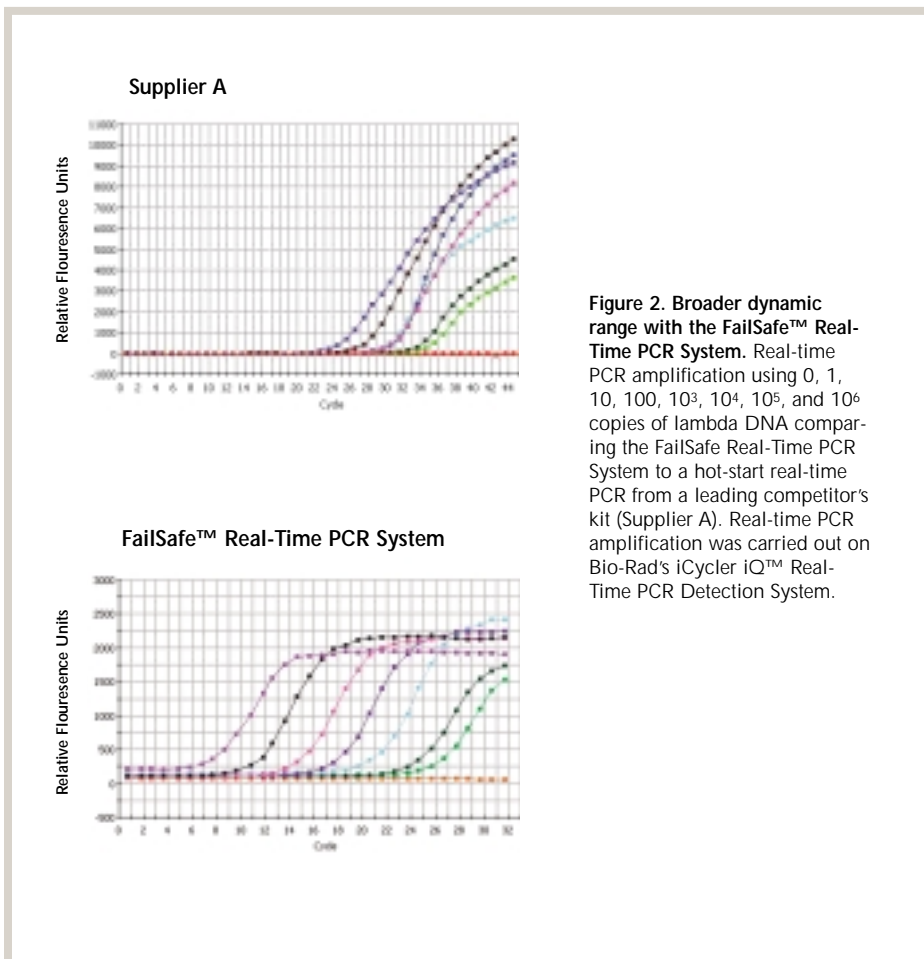


Figure 2. Broader dynamic range with the FailSafe™ Real-Time PCR System. Real-time PCR amplification using 0, 1, 10, 100, 10³, 10⁴, 10⁵, and 10⁶ copies of lambda DNA comparing the FailSafe Real-Time PCR System to a hot-start real-time PCR from a leading competitor's kit (Supplier A). Real-time PCR amplification was carried out on Bio-Rad's iCycler iQ™ Real-Time PCR Detection System.

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FailSafe™ Real-Time PCR PreMix Selection Kit

FSR0360 48 Reactions

Contents:

FailSafe™ PCR Enzyme Mix, 12 FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

FailSafe™ Real-Time PCR System

FSR03200 200 Reactions

Contents:

FailSafe™ PCR Enzyme Mix, choice of two FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

* The use of betaine in DNA or RNA polymerase reactions is covered by patent rights exclusively licensed to EPICENTRE Technologies. Purchase of EPICENTRE's products for use under these rights is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for non-commercial life science research.

SYBR is a registered trademark of Molecular Probes, Inc. SYBR® Green I Dye is covered by patents.

This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or purchased, i.e., an authorized thermal cycler.



The MasterAmp™ Real-Time RT-PCR Kit Provides Superior Sensitivity and Consistent Quantitation

Judith E. Meis, EPICENTRE

Introduction

The MasterAmp™ Real-Time RT-PCR Kit provides all the necessary components to perform high sensitivity one-step quantitative RT-PCR. The kit includes a ready-to-use reaction mix containing SYBR® Green I dye, buffer, dNTPs, MgCl₂, and MasterAmp™ PCR Enhancer (with betaine)*. In addition, the kit uses RetroAmp™ RT DNA Polymerase, which is both a thermostable DNA polymerase and has reverse transcription activity. RetroAmp™ Polymerase allows for higher RT temperatures (up to 70°C), significantly increasing the specificity and sensitivity of the RT reactions while decreasing secondary structure of RNA templates.

Another factor that improves the specificity and sensitivity of RT-PCR is the use of the MasterAmp™ PCR Enhancer, which is incorporated in the MasterAmp™ Real-Time RT-PCR Kit. The Enhancer reduces DNA and RNA secondary structure, eliminates sequence composition dependence of nucleic acid melting, and reduces pauses during DNA synthesis, thus improving the yield and specificity of many template amplifications.¹⁻³

Here we demonstrate consistent real-time RT-PCR amplification of gene sequences from as little as 1-5 pg of total cellular RNA from *E. coli* or human cells.

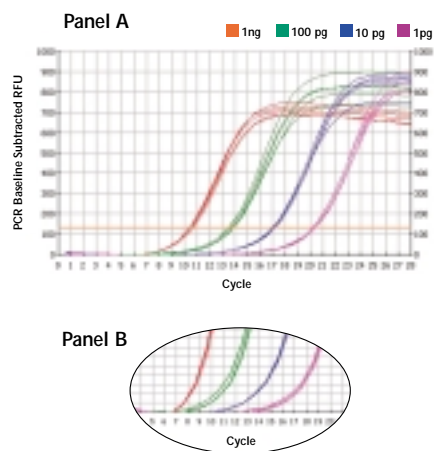


Figure 1. High sensitivity and C_T uniformity of RT-PCR using the MasterAmp™ Real-Time RT-PCR Kit. Panel A, Real-time RT-PCR using 16S rRNA consensus primers and 1 ng, 100 pg, 10 pg, or 1 pg of total *E. coli* RNA. Panel B, Expanded view showing cycle threshold uniformity.

Methods

Real-time RT-PCR was performed using total *E. coli* RNA and consensus primers to the *E. coli* 16S rRNA gene or total cellular RNA from HeLa human cells and gene-specific primers to the human β -actin gene. Total cellular RNA was purified using the MasterPure™ RNA Purification Kit as described in the product protocol. Real-time RT-PCR was carried out with 1 pg to 50 ng of the respective total cellular RNA using the MasterAmp Real-Time RT-PCR Kit according to the one-step protocol supplied with the kit. Real-time RT-PCR reactions were monitored using Bio-Rad's iCycler iQ™. Amplification products were also analyzed by agarose gel electrophoresis.

Results

Figure 1 presents the results obtained using the MasterAmp Real-Time RT-PCR Kit to amplify from 1 pg to 1 ng of *E. coli* cellular RNA using 16S rRNA consensus primers. The data show high sensitivity and cycle threshold (C_T) uniformity over the complete range of template.

We next examined RT-PCR amplification of the β -actin gene sequence using total RNA from human HeLa cells. Under standard RT-PCR conditions, little or no RT-PCR product was detected using the β -actin primers (Figure 2). However, 1X or 2X MasterAmp PCR Enhancer in the reaction resulted in an abundant specific amplification of the β -actin gene sequence from total HeLa cell RNA.

One-step real-time RT-PCR of the β -actin gene sequence was then performed using 5 pg to 50 ng of total HeLa cell RNA and either the MasterAmp™ Kit or a leading competitor's kit according to the manufacturer's protocol. As seen in Figure 3, amplification using the MasterAmp Real-Time RT-PCR Kit resulted in consistently higher sensitivity based on lower C_T values compared to results obtained with the competitor's kit.

Conclusions

The MasterAmp Real-Time RT-PCR Kit provides superior sensitivity and C_T uniformity in a ready-to-use pre-mix format. The patented MasterAmp PCR Enhancer significantly increases the specificity and consistency of real-time RT-PCR and eases the burden of primer design for both standard and difficult templates.

References

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2. Mytelka, D.S. and Chamberlin, M.J. (1996) *Nucl. Acids Res.* 24, 2774.
3. Weissensteiner, T. and Lanchbury, J.S. (1996) *BioTechniques* 21, 1102.

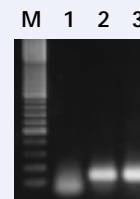


Figure 2. Improved RT-PCR due to the incorporated MasterAmp™ PCR Enhancer. A 132-bp region of β -actin was amplified using the MasterAmp™ Real-Time RT-PCR Kit from 100 ng of human placental cellular RNA. Lane M, 100-bp ladder; Lane 2, 1X Enhancer; Lane 3, 2X Enhancer.

Lane 1, no added enhancer; Lane 2, 1X Enhancer; Lane 3, 2X Enhancer.

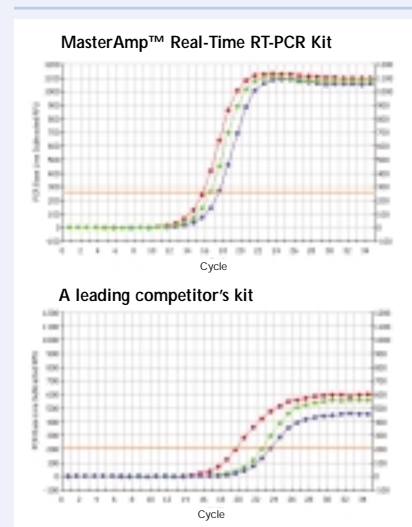


Figure 3. Increased sensitivity using the MasterAmp™ Real-Time RT-PCR Kit. Real-time amplification of β -actin RNA was performed using 50 ng (red), 500 pg (green) and 5 pg (blue) of total cellular RNA comparing the MasterAmp Real-Time RT-PCR Kit to a leading competitor's kit.

www.epicentre.com/realtimertpcr.asp

MasterAmp™ Real-Time RT-PCR Kit

MAR03100 100 Reactions

Contents:

RetroAmp™ RT DNA Polymerase, 2X Green RT-PCR PreMix, MasterAmp™ 10X PCR Enhancer, 25 mM MgCl₂, 25 mM MnSO₄, and Sterile Water.

* Covered by issued and pending patents as described on page 3.

See license and trademark information on page 3.

6 Reasons Why DuraScript™ RNA Is Better for RNAi

EPICENTRE's DuraScript™ T7 Transcription Kit* produces 2'-Fluorine-CMP and 2'-Fluorine-UMP modified RNA transcripts—called DuraScript™ RNA—that are completely resistant to RNase A digestion.[†] Recent research indicates that double-stranded DuraScript RNA provides many benefits for use in RNA interference (RNAi)¹.

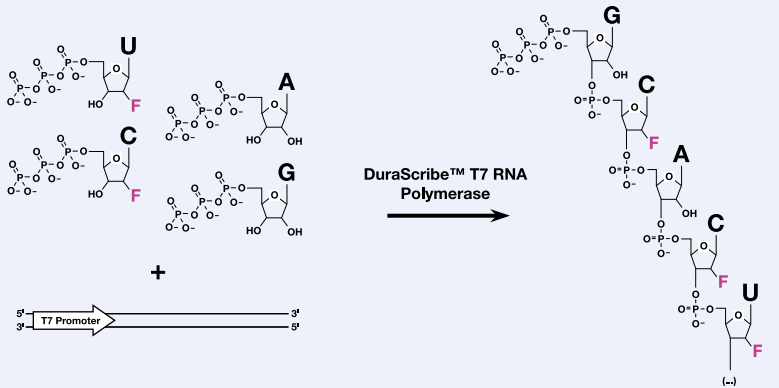


Figure 1. DuraScript™ T7 RNA Polymerase efficiently incorporates 2'-F-CTP and 2'-F-UTP into full length DuraScript™ RNA. The presence of the fluorine at the 2'-position of the 2'-F-C and 2'-F-U nucleotides prevents RNase A digestion. The result is DuraScript™ RNA that is completely resistant to RNase A and related ribonucleases.

1. Produce up to 50 µg of DuraScript RNA from a DuraScribe reaction.

A standard DuraScribe reaction produces approximately 50 µg (2.5 mg/ml) of DuraScript RNA.

2. Make DuraScript RNA from DNA templates with a standard T7 promoter.

The DuraScript™ T7 RNA Polymerase, provided in the kit, recognizes the same T7 transcription promoters as standard T7 RNA Polymerase but is about 100-fold more active in incorporation of 2'-fluorine-pyrimidines.

3. DuraScript RNA is completely resistant to RNase A and the nucleases found on human hands.

There is no need for gloves, DEPC-treatment of reagents, or RNase inhibitors when making or working with DuraScript RNA.

4. DuraScript RNA is stable in cell culture media containing 10% fetal calf serum (Figure 2).

5. Double-stranded DuraScript RNA is effective for RNAi-mediated gene silencing (see sidebar).

6. Double-stranded DuraScript RNA has been delivered into cultured cells in the presence of serum and without the need for transfection reagents.²

References

1. Meis, J.E. and Chen, F. (2002) *EPICENTRE Forum* 9 (1), 10.
2. Capodici, J. *et al.* (2002) *J. Immunol.* **169**, 5196.

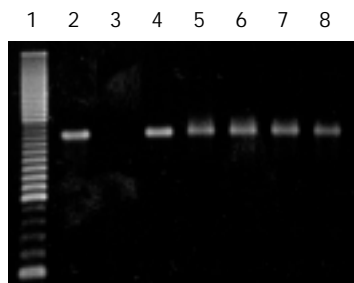


Figure 2. DuraScript™ RNA is stable in tissue culture media for at least 2 hours. Five micrograms of a 1.4-kb standard RNA transcript and a DuraScript RNA transcript were incubated in 100 µl of tissue culture media (D-MEM + 10% fetal calf serum) at 37°C. Lane 1, RNA ladder; Lane 2, standard RNA; Lane 3, standard RNA after 15 minutes in tissue culture media; Lane 4, DuraScript RNA; Lanes 5-8, DuraScript RNA after 15, 30, 60, and 120 minutes, respectively, in tissue culture media.

Double-Stranded DuraScript™ RNA Inhibits HIV-1 Infection and Can Be Delivered into Cultured Cells without the Use of Transfection Reagents

In ground-breaking work a recent publication by Capodici *et al.* demonstrated that double-stranded DuraScript™ RNA, produced using the DuraScript™ T7 Transcription Kit and targeted to the HIV-1 *gag* gene effectively inhibited HIV replication in primary CD4⁺ cells. Significantly, the double-stranded DuraScript RNA was delivered into the cells in the presence of serum and without the need for transfection reagents. Canonical dsRNAs produced by *in vitro* transcription or by chemical synthesis yielded similar results but only when complexed to transfectin.

Capodici, J. *et al.* (2002) *J. Immunol.* **169**, 5196.

www.epicentre.com/durascribe.asp

DuraScript™ T7 Transcription Kit

DS010910	10 Reactions
DS010925	25 Reactions

Contents:

DuraScript™ T7 Enzyme Mix, DuraScript™ T7 10X Reaction Buffer, ATP, GTP, 2'-F-CTP, 2'-F-UTP, DNase I, DTT, Control Template, and Water.

* The use of DuraScript T7 Transcription Kit to synthesize nucleic acids with non-canonical bases or for partial ribo-substitution is covered by U.S. patents 5,849,546; 6,107,037 and other patents issued or pending. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for life science research. Contact EPICENTRE concerning licenses for other uses.

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Production of High-Activity Digoxigenin-Labeled Riboprobes for *In Situ* Hybridization Using the AmpliScribe™ T7 High Yield Transcription Kit

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Introduction

Our laboratory uses non-radioactive *in situ* hybridization (ISH) to study switching of collagen gene expression in skeletal tissues during normal and abnormal growth and development.^{1,2} Digoxigenin-labeled, antisense riboprobes are well-suited for this purpose because they can be stored frozen for long periods before use, they bind tightly to their cognate mRNA, and they give superior histological resolution compared to radioactive labels. Despite the fact, however, that collagen genes are generally highly expressed, we found some commercial *in vitro* transcription systems were yielding low levels of probe activity with too much background. We therefore tested EPICENTRE's AmpliScribe™ T7 High Yield Transcription Kit to prepare digoxigenin-labeled riboprobes according to a modified procedure described by Kaplan *et al.*³

Methods and Results

Preparation of the T7 transcription template

Transcription templates were generated by PCR amplification of regions of rat collagen cDNA clones (Figure 1). By using PCR-generated transcription templates we effectively eliminate backgrounds caused by uncut plasmid or other DNA. Briefly, a reverse PCR primer (5' TTTC-CATTGCCATTTCAG 3') was designed to bind 169 bases "downstream" from the T7 transcription promoter contained on the cloning vector. This 169-base "leader" sequence helps ensure that the T7 RNA polymerase can initiate at its promoter site efficiently. We then designed a different forward PCR primer for each of the different collagen cDNAs we wanted to detect. The forward primers were selected so that the final PCR product would be about 700 to 1000 bp long—a good length for ISH probes.

Typically 1 ng of cDNA clone DNA per 500- μ l PCR reaction volume was used as PCR template. We performed 5 X 100- μ l PCR reactions under standard conditions for 38 cycles. Following PCR, the reaction product was cleaned up on a spin column, the OD measured, and then sodium acetate/ethanol precipitated and washed. The PCR product was then resuspended to approximately 1 μ g/ μ l and used as template for the AmpliScribe T7 *in vitro* transcription reaction.

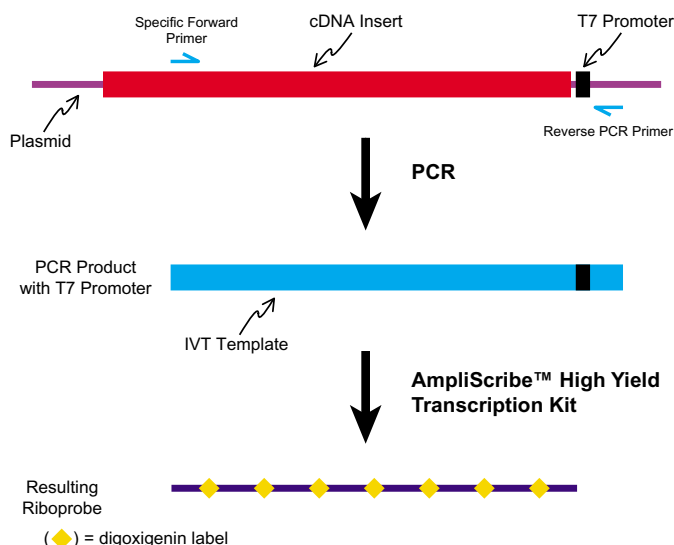


Figure 1. Schematic overview of how digoxigenin-labeled riboprobes were prepared. PCR was carried out on plasmid containing a collagen cDNA. This yields a dsDNA template for *in vitro* transcription (IVT) that includes 700-1000 bases of cDNA plus 169 bases of vector that includes the T7 transcription initiation site. Having the short downstream vector sequence increased the efficiency of the IVT reaction.

Transcription of digoxigenin-labeled riboprobes using the AmpliScribe T7 High Yield Transcription Kit

The AmpliScribe T7 *in vitro* transcription reaction was set up at room temperature in the following order:

PCR product template.	1 μ g (about 1 μ l)
AmpliScribe™	
10X Reaction Buffer.	2 μ l
ATP, CTP, GTP (100 mM each).	1.5 μ l each
100 mM UTP	1 μ l
10 mM Digoxigenin-11UTP*	6 μ l
100 mM DTT	2 μ l
Water	to 18 μ l
AmpliScribe™ T7 Enzyme Solution	2 μ l

TOTAL VOLUME 20 μ l

*Roche Applied Science, Cat. No. 1209256

The reaction was mixed and incubated 2 hours at 37°C and then treated with RNase-Free DNase to remove the template DNA. The digoxigenin-labeled riboprobe was precipitated with ammonium acetate, washed with ethanol, dried, and resuspended in 52 μ l of water. Yields measured by A₂₆₀ were up to 200 times the amount of input DNA template.

Quantifying the activity of the digoxigenin-labeled riboprobe

One microliter dilutions of the riboprobe were used to titrate the probe to measure incorporation of digoxigenin by chemiluminescent immuno-dot blots probed with alkaline phosphatase-conjugated anti-digoxigenin (Roche Applied Science) and developed with CSPD chemiluminescent substrate. A parallel dilution series was made with a positive control digoxigenin-labeled RNA that is supplied with Roche Applied Science's digoxigenin labeling kit (which represents "maximum" labeling, according to the manufacturer) and digoxigenin-labeled riboprobe produced by a competitor's *in vitro* transcription kit. As shown in Figure 2, the AmpliScribe T7 Kit yielded 3-fold higher incorporation than Roche's "maximum" control and 10-

to 30-fold higher probe activity than the competitor's kit. Following quantitation, the riboprobes were divided into 5- μ l aliquots, flash frozen in liquid nitrogen, and stored at -70°C.

In situ hybridization

Optimum dilutions for probing tissue samples were determined empirically. Preparation of tissues and hybridization conditions have been described in detail.² Briefly, tissues are dissected and immediately placed in cold, neutral 4% para-formaldehyde, fixed overnight, then decalcified by storing in 12.5% EDTA.

Tissues were embedded in paraffin, sectioned (5-7 μ m), and deparaffinized by standard techniques. After prehybridization for one hour, probe is incubated with the tissue overnight at 42°C, and any non-hybridized probe was removed by RNase A digestion. After washes of increasing stringency, anti-digoxigenin-alkaline phosphatase conjugate was incubated with the sections for an hour, followed by PBS washes. Finally, NBT-BCIP chromogenic substrate was added and incubated at room temperature until signal develops, the reaction was stopped with water, and the slides dried and cover slipped (Figure 3).

Conclusion

The AmpliScribe T7 High Yield Transcription Kit proved to be the most effective product for preparing the high-activity, digoxigenin-labeled riboprobes needed for our projects. With typically a 30-fold higher yield, we spend substantially less time, effort, and expense in reagent preparation and optimization. This provides us a long-lasting supply of consistent probe to process samples in large experimental series.

Acknowledgment

This work was supported in part by grant DE 07444 to P.O. from the US-NIDCR. Its contents are solely the responsibility of the authors and do not necessarily reflect official views of the NIDCR or the NIH.

References

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2. Marks, Jr., S.C. *et al.* (2000) *Int. J. Dev. Biol.* 44 (3), 309.
3. Kaplan, E.D. *et al.* (1996) *EPICENTRE Forum* 3 (2), 1.

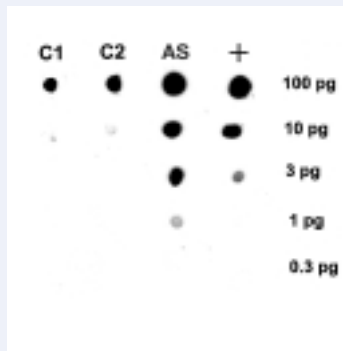


Figure 2. An AmpliScribe™ T7 High Yield Transcription reaction yielded 10- to 30-fold higher digoxigenin-labeled riboprobe activity than the competitor's reaction. Each column is a dilution series of one sample. C1, digoxigenin-labeled rat collagen type II (α 1) riboprobe produced in a competitor's *in vitro* transcription reaction; C2, a repeat of the competitor's reaction; AS, digoxigenin-labeled rat collagen type II (α 1) riboprobe produced in an AmpliScribe T7 transcription reaction; +, digoxigenin-labeled positive control RNA (Roche Applied Science).

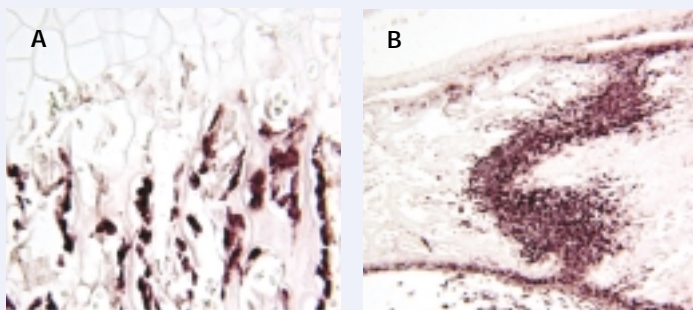


Figure 3. *In situ* hybridization results using digoxigenin-labeled riboprobes produced using the AmpliScribe™ T7 High Yield Transcription Kit. Panel A, type I collagen in the rat tibia. Type I-positive osteoblasts are intensely stained along the bone trabeculae of the metaphysis in the lower portion of the panel, while the cartilage at the top is completely negative, as expected. Panel B, type III collagen in the rat premaxillary-maxillary suture. The sigmoidal shape of the suture is seen clearly in the right panel, with type III-expressing, undifferentiated precursor cells filling the space between the bony surfaces. Original magnifications, Panel A 160X and Panel B 43X.

www.epicentre.com/ampliscribe.asp

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

Contents:

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

AmpliScribe™ T7-Flash™ Transcription Kit

Now Available

- Fast 30-minute *in vitro* transcription reaction
- Higher yields of RNA – up to 180 μ g from a 20- μ l reaction

See page 8 for more information.

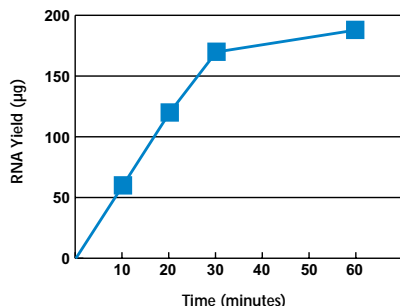


Introducing EPICENTRE's New AmpliScribe™ T7-Flash™ Transcription Kit ...

Transcribe More RNA in 30 Minutes Than the Leading Competitor's "High Yield" Transcription Kit Can Do in 2 Hours

EPICENTRE's new AmpliScribe™ T7-Flash™ Transcription Kit has been developed to produce the highest yields of RNA in the shortest time. Using 1 µg of standard 1.4-kb T7 promoter-containing DNA template in a 20-µl reaction, an AmpliScribe T7-Flash Transcription reaction yields up to 180 µg of RNA (9 mg/ml) in 30 minutes (Figure 1). The leading competitor's "high yield" transcription kit produces less RNA using a 2-hour reaction.

Figure 1. An AmpliScribe™ T7-Flash™ reaction produces up to 180 µg of RNA in 30 minutes.



Make More Long or Short RNA Transcripts

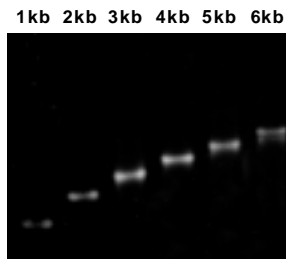
The AmpliScribe T7-Flash Transcription Kit produces good yields of RNA from templates ranging in size from <50 bp to several kbp (e.g. Figure 2) in 30 minute reactions.

Try an AmpliScribe™ T7-Flash™ Transcription Kit At No Risk

EPICENTRE's new AmpliScribe T7-Flash Transcription Kit produces the highest

yields of full-length RNA in the shortest time compared to conventional *in vitro* transcription reactions and the leading competitor's *in vitro* transcription kit. But don't take our word for it. Compare the AmpliScribe T7-Flash Transcription Kit with the *in vitro* transcription system that you are currently using.

Figure 2. Full length RNA transcripts from <50 bases to several kb are produced using the AmpliScribe™ T7-Flash™ Transcription Kit.



www.epicentre.com/t7-flash.asp

AmpliScribe™ T7-Flash™ Transcription Kit

ASF3257	25 Reactions
ASF3507	50 Reactions

Contents:

AmpliScribe™ T7-Flash™ Enzyme Solution (with added RNase inhibitor), AmpliScribe™ T7-Flash™ 10X Reaction Buffer, ATP, CTP, GTP, UTP, RNase-Free Water, RNase-Free DNase I, DTT, and Control DNA Template (linearized).

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

EPICENTRE's AmpliCap™ T7, T3, and 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.

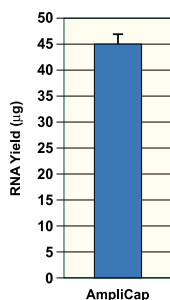


Figure 1. AmpliCap™ T7 High Yield Message Maker Kits consistently produce the highest yield of 5'-capped RNA transcript.

The AmpliCap T7, T3, and SP6 High Yield Message Maker Kit features:

- RNA yields up to 45 µg per reaction using the AmpliCap T7 and T3 Kits and up to 35 µg using an AmpliCap SP6 Kit. (Twenty microliter reactions using Control Template DNA).
- Up to 80% of the RNA is capped using all three kits.
- An optimized m⁷G[5']ppp[5']G Cap/NTP PreMix solution is provided.
- A separate vial of GTP is also included for efficient production of long, 5'-capped RNA.
- AmpliCap Kits utilize concentrations of NTPs that are high enough to inhibit conventional transcription reactions.

www.epicentre.com/amplicap.asp

AmpliCap™ High Yield Message Maker Kits

AC0707	25 Reactions
AC0703	25 Reactions
AC0706	25 Reactions

Contents:

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

The SoilMaster™ DNA Extraction Kit Provides PCR-Ready Soil DNA in Less Than an Hour

Judith Meis and Feng Ling 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.

Larger size and more intact DNA

Genomic DNA was purified from soil samples that included forest, marsh, and cave soil using the SoilMaster™ DNA Extraction Kit following the kit's protocol. The DNA isolated with the SoilMaster 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 1). The DNA extracted with the SoilMaster Kit was of larger size and contained more intact DNA than DNA purified by other methods.

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 2).

Amplification of diverse organisms

Purified soil DNA was amplified and the PCR 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, pro-

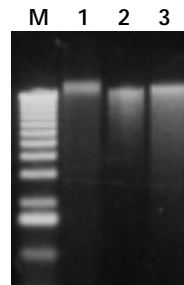


Figure 1. The SoilMaster™ DNA Extraction Kit extracts high molecular weight intact DNA from compost soil sample. Lane M, kb DNA ladder; Lane 1, soil DNA extracted with the SoilMaster Kit; Lanes 2 and 3, DNA purified using other soil kits.

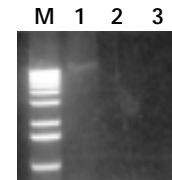


Figure 2. The SoilMaster™ DNA Extraction Kit extracts DNA from difficult-to-extract soil and sediment samples. Lane M, kb DNA ladder; Lane 1, cave sediment DNA extracted with the SoilMaster Kit; Lanes 2 and 3, purification attempts of cave sediment DNA using other soil kits.

tists, and green algae primers, 3) plant primers, 4) primers to high G+C, gram positive bacteria, and 5) *Bacillus* primers. Amplification products were obtained from all 5 primer sets using the extracted DNA from all samples tested (Figure 3).

PCR product cloning and RFLP analysis

The DNA amplified with 16S bacterial consensus primers was cloned into pCC1™ with the CopyControl™ PCR Cloning Kit. 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 demonstrated the diversity of 16S sequences amplified from the extracted soil DNA (data not shown). This indicates that a wide variety of organisms and species are represented in the extracted soil DNA.

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.

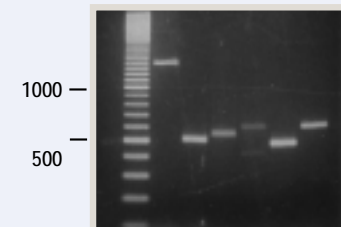
References

1. Tsai, Y.-L and Olson, B.H. (1992) *Appl. Environ. Microbiol.* **58**, 754.
2. Tebbe, C.C. and Vahjen, W. (1993) *Appl. Environ. Microbiol.* **59**, 2657.
3. Selenska, S. and Klingmuller, W. (1991) *Letters in Appl. Microbiol.* **13**, 21.
4. Zhou, J. et al. (1996) *Appl. Environ. Microbiol.* **62**, 316.

www.epicentre.com/soilmaster.asp

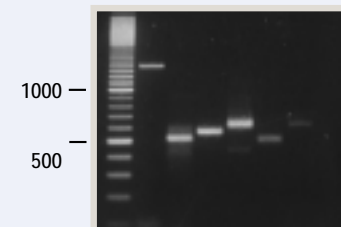
SoilMaster™ DNA Extraction Kit
SM02050 50 Reactions

3A M 1 2 3 4 5 6



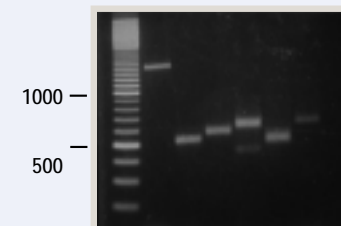
Cave Sediment

3B M 1 2 3 4 5 6



Forest Soil

3C M 1 2 3 4 5 6



Marsh Soil

Figure 3. FailSafe™ PCR amplification of extracted soil DNA. DNA was extracted from 3 distinct soil types including cave sediment (Panel A), forest soil (Panel B), and 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.

Which Kit is Best for Your Genomic DNA Cloning Project— the CopyControl™ Fosmid Library Production Kit or the CopyControl™ BAC Cloning Kit?

EPICENTRE's new CopyControl™ Fosmid Library Production Kit and CopyControl™ BAC Cloning Kits are based on cloning technology developed in the laboratory of Dr. Waclaw Szybalski.¹ CopyControl cloning technology enables the user to grow CopyControl Fosmid or CopyControl BAC clones at single-copy number to ensure insert stability and successful cloning of sequences encoding expressed toxic protein. Then, whenever desired, the CopyControl clones can be induced from single copy to high copy for higher yields of higher purity DNA (Figures 1 and 2). Higher yields of higher purity DNA means more applications (e.g., fingerprinting, end-sequencing) from a single DNA prep, more DNA for sub-cloning and often longer DNA sequence reads. For more information on the CopyControl Cloning process, see the side bar on p. 11 or visit www.epicentre.com/cc_tutorial.asp.

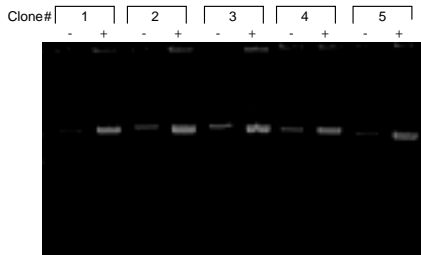


Figure 1. CopyControl™ Fosmid clones can be induced up to 50 copies per cell to greatly increase DNA yield. Five randomly chosen CopyControl Fosmid clones were grown in culture in duplicate. One sample of each was induced (+) to multiple-copy number by addition of CopyControl™ Induction Solution. The other sample was an uninduced control (-). DNA was isolated from an equal number of cells of each and analyzed by agarose gel electrophoresis.

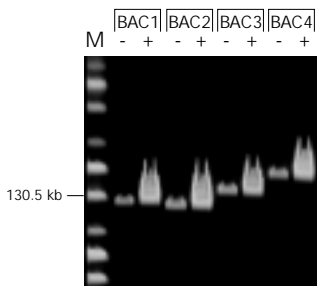


Figure 2. *Not I* digestion of DNA from an equal number of induced (+) and uninduced (-) cultures of 4 CopyControl™ BAC clones. The induced cultures yielded 10- to 20-fold more DNA, M = DNA marker.

Table 1. A comparison of the CopyControl™ Fosmid and the CopyControl™ BAC cloning processes.

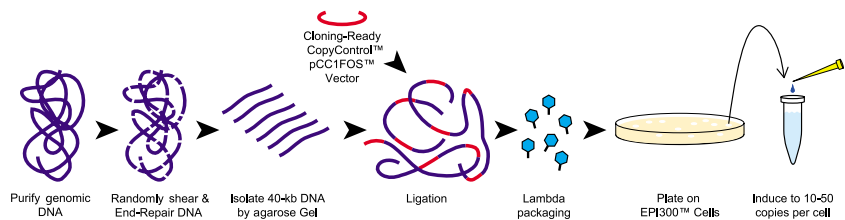
Library Construction Parameter	CopyControl™ Fosmid Library Production Kit (Figure 3A)	CopyControl™ BAC Cloning Kits (Figure 3B)
Vector	CopyControl™ pCC1FOS™ provided linearized at <i>Eco</i> 72 I (blunt) site, dephosphorylated, and purified.	CopyControl™ pCC1BAC™ vector provided linearized at either the <i>Bam</i> H I-, <i>Eco</i> R I-, or <i>Hind</i> III-site, dephosphorylated, and highly purified.
Preparation of genomic DNA	Random shearing followed by end-repair to 5'-phosphorylated, blunt-ended DNA. End-repaired DNA is size-selected by standard agarose gel electrophoresis.	Partial restriction endonuclease digestion. DNA fragments are size-selected by pulse field gel electrophoresis (PFGE).
Introduction into host	High efficiency lambda phage packaging followed by infection of TransforMax™ EPI300™ cells.	Electroporation of high efficiency TransforMax™ EPI300™ Electrocompetent <i>E. coli</i> .
Background	Virtually 100% white colonies.	Greater than 95% white colonies.
Insert size	All inserts are approximately 40 kb.	Typically an average size of 80 kb to 200 kb.
Cloning efficiency	Approximately 10-fold greater than BAC cloning.	Approximately 10-fold lower than fosmid cloning.
Induction of the CopyControl clones to high-copy number	Inducible from single-copy to 10-50 copies per cell.	Inducible from single-copy to 10-20 copies per cell.

Here we present a brief summary and comparison (Table 1 and Figure 3) of the processes and advantages of constructing genomic libraries using the CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits in order to aid the user in selecting the best kit for their need.

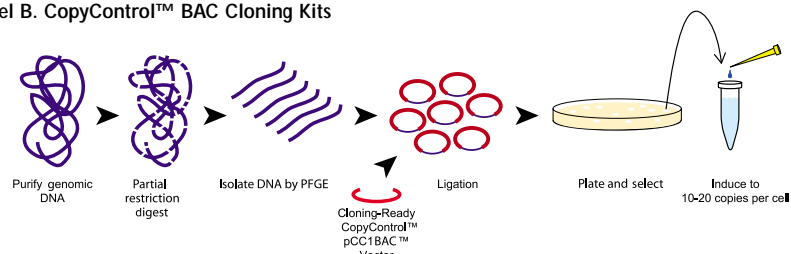
Additionally, CopyControl capability can be incorporated into existing single-copy BAC and fosmid clones using a simple transposon-based *in vitro* reaction. For more information, visit www.epicentre.com/transposomics.asp.

Figure 3. Overview of the process for preparing a genomic library using the CopyControl™ Fosmid Library Production Kit (Panel A) or the CopyControl™ BAC Cloning Kits (Panel B).

Panel A. CopyControl™ Fosmid Library Production Kit



Panel B. CopyControl™ BAC Cloning Kits



Discussion

The CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits have been used successfully by many researchers to construct libraries from both large and small genomes. As a general rule, fosmid cloning can save the user time and labor constructing a genomic library while BAC cloning can save the user time and labor in analyzing the genomic clones.

For example, constructing a CopyControl Fosmid library does not require costly equipment (e.g., Pulse Field Gel Electrophoresis and electroporator) or labor (preparing and isolating large genomic fragments and assessing the quality of the library) that is needed for constructing a

BAC library. The CopyControl Fosmid Library Production Kit utilizes a process of random shearing and blunt-end cloning of the DNA which eliminates the need to optimize and then perform partial restriction endonuclease digestions, as required for BAC cloning. In addition, a fosmid library constructed from randomly sheared DNA will likely be a more complete and unbiased library than a BAC library that was constructed from partial restriction endonuclease digested DNA.

In contrast, BAC libraries typically produce clones with inserts 3- to 5-fold larger than a fosmid clone so that there are fewer clones required for complete genome coverage. The larger insert sizes of BAC clones can mean reduced time

and labor needed for fingerprinting and end-sequencing and ultimately, assembly of contigs—compared to the same library constructed in a fosmid vector.

Reference

1. Wild, J. *et al.* (2002) *Genome Research* **12**, 1434.

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

Each kit contains sufficient reagents for constructing the equivalent of one 10X human genomic library.

Note: TransforMax™ EPI300™ Electrocompetent *E. coli* or Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli*, required for inducing CopyControl BAC clones to high-copy number, are available separately.

www.epicentre.com/epi300.asp

TransforMax™ EPI300™ Electrocompetent *E. coli*

EC300105 5 X 100 µl

EC300110 10 X 100 µl

Transformation efficiency > 1 x 10¹⁰ cfu/µg.

Includes CopyControl™ Induction Solution and pUC19 control DNA.

Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli*

EC02T15 5 X 100 µl

EC02T110 10 X 100 µl

Transformation efficiency > 1 x 10¹⁰ cfu/µg.

Includes CopyControl™ Induction Solution and pUC19 control DNA.

www.epicentre.com/ccfos.asp

CopyControl™ Fosmid Library Production Kit

CCFOS110 1 Kit

Kit contains sufficient reagents to produce up to 10 CopyControl Fosmid libraries.

Phage T1-Resistant EPI300™-T1^R *E. coli* cells, required for inducing CopyControl Fosmid clones to high-copy number, are supplied with the kit.

Incorporate CopyControl™ capability into existing single-copy BAC and fosmid clones.

www.epicentre.com/transposomics.asp

EZ::TN™ <*ori*V/KAN-2> Insertion Kit

EZ102VK 10 Reactions

How the CopyControl Cloning and Clone Induction Process Works

CopyControl™ cloning combines the advantages of both single-copy vectors and high-copy vectors without the disadvantages of either. The CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits enable users to make and maintain fosmid or BAC libraries at single-copy number to ensure insert stability and cloning of potentially toxic expressed DNA segments, and then, whenever desired, to induce the clones to high-copy number for high yields of higher purity DNA for downstream processes such as fingerprinting and DNA sequencing (Figure 1).

The pCC1FOS™ Vector and the CopyControl pCC1BAC™ Vectors, provided in the respective kits contain two origins of replication—the single copy *E. coli* F-factor replicon and a high-copy origin of replication, *ori*V.

Initiation of replication from *ori*V requires the *trfA* gene product supplied by the TransforMax EPI300 *E. coli*—a host strain engineered by scientists at EPICENTRE to contain the *trfA* gene under tight control of an inducible promoter.

In the absence of the *trfA* gene induction, replication of pCC1FOS clones and CopyControl pCC1BAC is controlled by the F-factor replicon and the vector is present at one copy per cell. Addition of the CopyControl Induction Solution to CopyControl Fosmid and CopyControl BAC clones grown in individual cultures or in a 96-deep well format induces expression of the *trfA* gene resulting in initiation of replication from *ori*V and subsequent amplification of the clone to 10-50 copies per cell for the CopyControl Fosmid clones and 10-20 copies per cell for the CopyControl BAC clones.

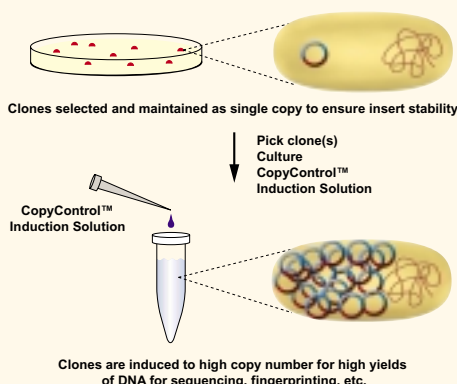


Figure 1. Overview of the CopyControl™ Cloning Systems.

CopyControl Fosmid or BAC clones are grown at single copy in TransforMax™ EPI300™ *E. coli*. Individual clones are chosen, grown in small volume culture, and then induced to high-copy number by addition of the CopyControl™ Induction Solution.



Now, There Are Two Ways to Rapidly Screen Your Clones without Cultures, DNA Purifications, or Restriction Digests

EPICENTRE's Colony Fast-Screen™ Kit (Size Screen) and new Colony Fast-Screen™ Kit (PCR Screen) provide two rapid, sensitive, and easy methods for screening clones and libraries without the need to grow cultures, isolate DNA, or perform restriction endonuclease digestions.

1. Colony Fast-Screen™ Kit (Size Screen)

Rapidly screen the size of cloned inserts

The Colony Fast-Screen™ Kit (Size Screen), EPICENTRE's original Colony Fast-Screen™ Kit, facilitates screening the size of cloned inserts (Figure 1A). The size of most inserts can be determined in 1 hour or less (Figure 2) and the size of Bacterial Artificial Chromosome (BAC) clones can be estimated in as little as 4 hours.

Benefits

- Rapid—determine the size of PCR, cDNA, and other cloned DNAs in 1 hour and of BAC clones in as little as 4 hours without the need to grow cultures, isolate DNA, or perform restriction digests.
- Sensitive—the size of single-copy BAC clones is readily estimated.
- High-throughput capability—the kit is amenable to both high-throughput and routine cloning applications.
- Flexible—can be used with all standard *E. coli* host strains and any plasmid vector.

Figure 1. The Colony Fast-Screen™ processes are rapid and simple

2. Colony Fast-Screen™ Kit (PCR Screen)

Rapidly prepare clones for screening by PCR

The new Colony Fast-Screen™ Kit (PCR Screen) provides a rapid method for preparing clones—in about 10 minutes—for screening by PCR without the need to grow cultures or isolate DNA (Figure 1B). The kit can be used with all standard *E. coli* hosts and all plasmid vectors, including single-copy vectors. Thermostable polymerase and PCR primers are not provided.

Benefits

- Rapid—prepare PCR-ready DNA in <10 minutes without the need to grow cultures or purify DNA.
- Sensitive—enables screening of single-copy and high-copy clones by PCR amplifications up to 10 kb.
- High-throughput capability—the kit is amenable to both high-throughput and routine cloning applications.
- Reproducible PCR—the PCR-Lyse™ Solution provided does not interfere with pre-established PCR reaction conditions.
- Flexible—can be used with any plasmid vector and all standard *E. coli* host strains.

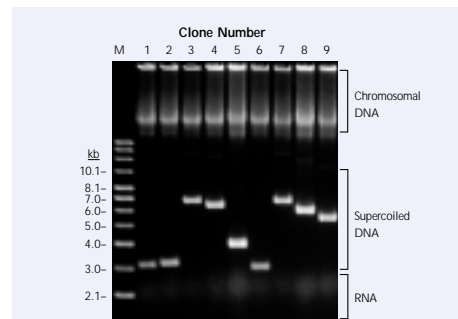
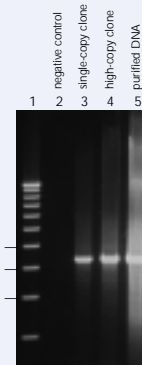


Figure 2. Agarose gel size analysis of plasmid clones using the Colony Fast-Screen Kit (Size Screen). Very small portions of nine randomly chosen clones were picked from an overnight plate, processed using the Colony Fast-Screen Kit (Size Screen), and the size of the cloned insert of each determined by agarose gel electrophoresis. Total time including running the gel was 45 minutes. M, Supercoiled DNA Ladder.

Figure 3. The Colony Fast-Screen™ Kit (PCR Screen) enables screening of both high-copy and single-copy clones by PCR without the need for cultures and DNA purifications.

Colonies containing a 5-kb lambda fragment cloned into the CopyControl™ pCC1™ vector and grown on plates at either single-copy or high-copy number were picked and processed using the Colony Fast-Screen Kit (PCR Screen) as described in Figure 1. PCR was performed using primers homologous to the ends of the cloning vector. Lane 1, DNA size marker; Lane 2, *E. coli* DNA (negative control); Lane 3, single-copy clone; Lane 4, high-copy clone; Lane 5, PCR of 1 ng of purified DNA.

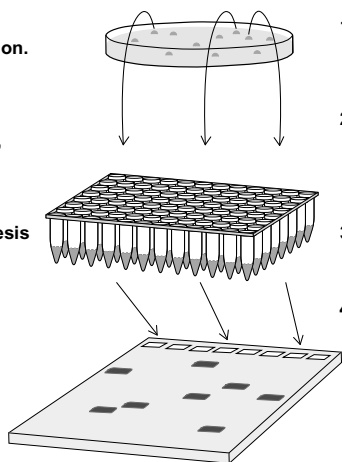


A. Colony Fast-Screen™ Kit (Size Screen)

1. Pick colony into EpiBlue™ Solution.
2. Add EpiLyse™ Solution, vortex, and quick spin.
3. Gel electrophoresis and staining.

B. Colony Fast-Screen™ Kit (PCR Screen)

1. Pick colony into tube or microtiter well.
2. Add PCR-Lyse™ Solution, vortex, and heat 5'.
3. Perform PCR.
4. Gel electrophoresis and staining.



www.epicentre.com/fastscreen.asp

Colony Fast-Screen™ Kit (Size Screen)

Rapidly screen the size of cloned inserts.
FS08250 1 Kit
Sufficient reagents to screen 250 colonies.

Contents:

EpiLyse™ Solution
EpiBlue™ Solution

Colony Fast-Screen™ Kit (PCR Screen)

Rapidly prepare clones for screening by PCR.
FS0322H 1 Kit
Sufficient reagents to screen 200 colonies.
Thermostable Polymerase and PCR primers are not included.

Contents:

PCR-Lyse™ Solution
Gel Loading Solution

New

Direct Selection of Genes Encoding Non-Cytoplasmic Proteins

The EZ::TN™ β -Lactamase Fusion Kit was developed for the direct selection of genes encoding membrane and secreted proteins. The kit features the EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon, which contains a β -lactamase gene (*blaM*) that lacks both promoter and secretory signal sequences.

Screen a clone or library of clones with a simple, one-step *in vitro* reaction that randomly inserts a single EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon into the target DNA. Then, transform *E. coli* cells with an aliquot of the reaction and plate on media containing ampicillin (Figure 1, A). Only insertion clones with transcriptional fusions to genes encoding extracytoplasmic proteins will grow. These fusions generate hybrid proteins that can transport the *blaM* moiety through the inner membrane and confer resistance to ampicillin (Figure 1, B).

Once Amp^R clones are selected, use the primer binding sites at the ends of the EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon to map or bidirectionally sequence the

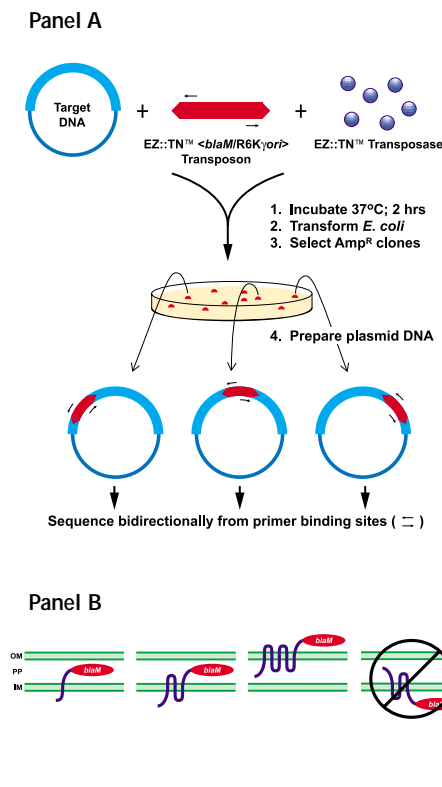


Figure 1. The process for selecting cloned genes encoding membrane and secreted proteins with the EZ::TN™ β -Lactamase Fusion Kit (Panel A). Only fusions that transport the *blaM* moiety through the inner membrane will grow on ampicillin (Panel B).

insertion site with primers provided in the kit. When large genomic clones (e.g., fosmids, cosmids, or BACs) are screened, DNA flanking the insertion site can be subcloned or “rescued” as an Amp^R plasmid that replicates from the R6K γ origin of replication also contained on this EZ::TN Transposon (see center insert).

www.epicentre.com/transposomics.asp

EZ::TN™ β -Lactamase Fusion Kit

EZ131BL 10 Reactions

Contents:

EZ::TN™ Transposase, EZ::TN™ $\langle blaM/R6\gamma ori \rangle$ Transposon, Reaction Buffer, Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.

Efficient Mapping of Functional Domains or Epitopes of Proteins

The EZ::TN™ In-Frame Linker Insertion Kit was designed to rapidly and easily produce random 19-amino acid (19 codon) in-frame insertions into genes of expressed proteins to facilitate mapping of functional domains or epitopes.

The kit features the EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon, which contains a kanamycin resistance marker flanked by *Not I* restriction sites. A simple *in vitro* reaction catalyzed by EZ::TN™ Transposase randomly inserts this transposon into target DNA. Following transformation of *E. coli*, a library of $>10^6$ independent Kan^R insertion clones is obtained. Insertion clones can be identified for further analysis by altered activity, restriction mapping, or sequencing from the ends of the transposon.

Once clones are chosen, the kanamycin-resistance gene is excised from the EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon by *Not I* digestion. Each *Not I*-digested clone is then ligated and re-transformed into

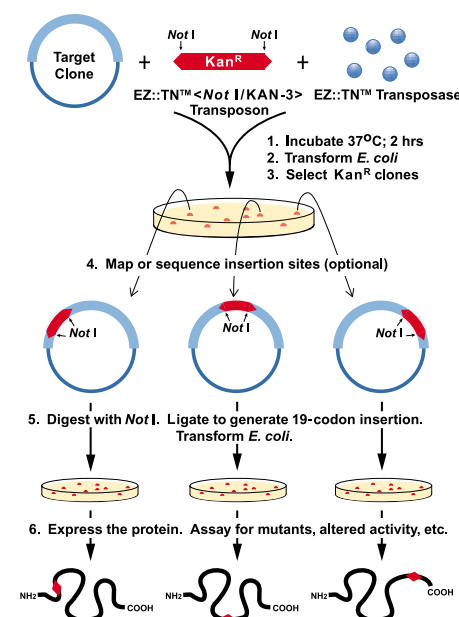


Figure 1. The EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon contains a kanamycin resistance gene flanked by *Not I* restriction sites. A 19-codon insertion that can be read in all three reading frames is generated following *Not I* digestion and ligation.

E. coli (Figure 1). Since the ends of the transposon have been modified to eliminate translational stops, the resulting clones each contain a random 19-codon insertion that can be read in all three reading frames. Thus, the protein is unchanged except for the random insertion of 19 amino acids.

www.epicentre.com/transposomics.asp

EZ::TN™ In-Frame Linker Insertion Kit

EZ104KN 10 Reactions

Contents:

EZ::TN™ Transposase, EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon, Reaction Buffer, Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.



A Mu-Based Transposition System that Is At Least 50-Fold More Efficient than the Competition

The first *in vitro* transposition system was developed by Dr. Kiyoshi Mizuuchi using the well characterized temperate bacteriophage Mu.^{1,2} However, until now, commercially-available Mu-based systems have used a MuA transposase that has a transposition efficiency 50-100 times lower than EPICENTRE's Tn5-based EZ::TN™ Transposon Tools. High transposition efficiencies are critical for obtaining a sufficient number of transposon insertions to completely sequence a clone, especially those with large inserts, as well as other applications.

Now, EPICENTRE is pleased to introduce HyperMu™ Transposon Tools that use HyperMu™ Transposase, a hyperactive enzyme that retains the highly random insertion characteristics of MuA transposase³ but is at least 50-times more active *in vitro* than the enzyme available from other suppliers. Thus, the high quality and superior performance of EZ::TN Transposon Tools are now available in these new Mu-based Transposomics™ products.

HyperMu Transposon Tools are now available for strategies that simplify and speed up DNA sequencing and analysis of gene function. HyperMu Transposase, which recognizes the same R1 and R2 end sequences as MuA Transposase, is also sold separately so that these strategies can be used with EPICENTRE's HyperMu Transposons as well as other artificial Mu Transposons.

The *In Vitro* Insertion Strategy for Sequencing Cloned DNA

A primary application of the *In Vitro* Insertion Strategy and the HyperMu™ <KAN-1> Insertion Kit is for sequencing any clone that is too large to sequence with a single set of sequencing reactions. A simple, one-step reaction catalyzed by HyperMu Transposase randomly inserts the HyperMu <KAN-1> Transposon containing sequencing primer binding sites and a kanamycin-selectable marker into any DNA molecule *in vitro*. Then, transform *E. coli* and select on kanamycin plates (Figure 1). Up to millions of independent insertion clones are obtained, each of which can be sequenced bidirectionally using only the sequencing primers provided in the kit that anneal to each end of the HyperMu Transposon.

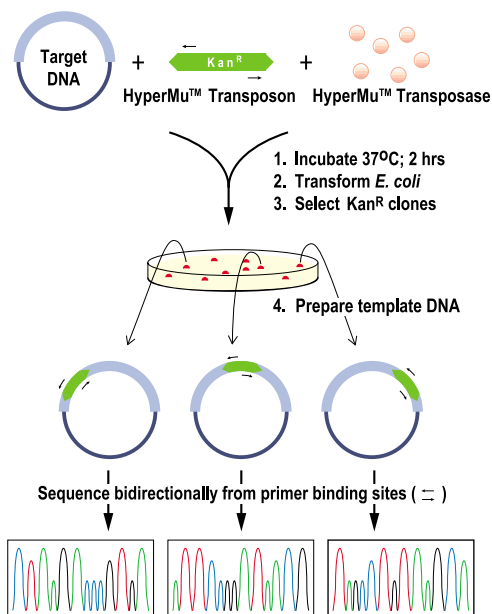


Figure 1. The process for complete sequencing of a target DNA using the HyperMu™ <KAN-1> Insertion Kit. Sequence even the largest BAC clone without the time and expense of subcloning or primer walking.

Consistently high transposition efficiencies are required for complete, overlapping sequencing of target DNA. A 150-kb BAC, for example, would require at least 700 insertion clones to approximate 100% coverage (assuming 1 kb of DNA sequenced per transposon; 500 bases in each direction). As shown in Table 1, only EPICENTRE's HyperMu <KAN-1> Insertion Kit produces enough templates to reliably sequence a large BAC clone. Similar efficiencies for both the 6-kb and 150-kb target DNAs were also obtained using Competitor A's artificial Mu transposon and EPICENTRE's HyperMu Transposase (data not shown).

Table 1. The HyperMu™ <KAN-1> Insertion Kit generates more transposon insertion clones per µg of target DNA than a leading competitor's kit.

Target DNA	EPICENTRE* (cfu/µg)	Competitor A* (cfu/µg)
6-kb plasmid (300 ng)	> 5X10 ⁶	< 10 ⁵
150-kb BAC (1µg)	> 10 ⁵	< 10 ²

*Transposition reactions (20µl) were performed using the manufacturer's protocol and electroporated into TransforMax™ EC100™ Electrocompetent *E. coli*.

The Transposome™ Strategy for Making Random Insertions into Living Cells

HyperMu Transposase and an artificial Mu transposon can also be used to make a HyperMu™ Transposome™ complex that can be electroporated into living cells to generate random transposon insertion clones *in vivo* (Figure 2).^{*} There is no need for cell conjugation, suicide vectors, or specific host factors, thus HyperMu Transposomes can be used to create insertion mutants (e.g., "gene knockouts") in species that have poorly described genetic systems or lack adequate molecular tools. Gene knockouts created with the ready-to-use HyperMu <R6K_γori/KAN>Tnp Transposome can be sequenced directly or "rescued" as a plasmid propagated from the R6K_γ origin of replication (see the center insert).

Although the increased efficiency of the HyperMu Transposase relative to MuA transposase enables practical use of HyperMu Transposome systems, researchers should consider using an EZ::TN™ Transposome™ system in lieu of or in addition to a HyperMu Transposome system. EZ::TN Transposomes typically generate 10-100 times more *in vivo* insertions using *E. coli* than a HyperMu Transposome.

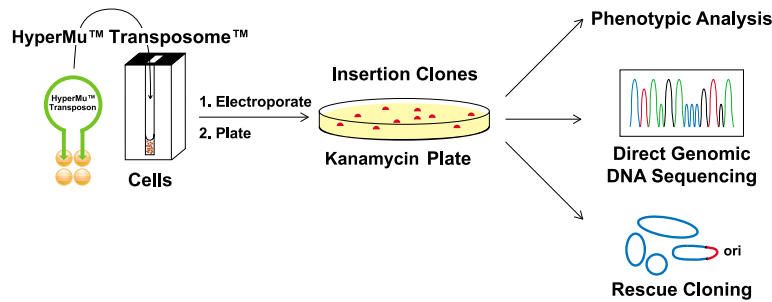


Figure 2. The HyperMu™ <R6K γ ori/KAN-1>Tnp Transposome™ can be electroporated into living cells where it randomly inserts the transposon component into the host's genomic DNA. The HyperMu Transposon insertion site can be analyzed by a variety of methods.

Strategies That Use Both EZ::TN and HyperMu Transposon Systems

Since EZ::TN and HyperMu Transposases do not recognize the same end sequences for transposition, they can also be used in strategies in which it is desirable to use more than one transposon system. For example, EPICENTRE scien-

tists have "rescued" plasmids and other episomes from heterologous bacterial systems that are not capable of replicating in *E. coli* by inserting into them an *E. coli* ori-containing EZ::TN Transposon. Then, the plasmid or episome can be completely sequenced following *in vitro* insertion of a HyperMu Transposon.

References

1. Mizuuchi, K. (1983) *Cell* **35**, 785.
2. Chaconas, G. and Harshey, R.M. (2002) In: *Mobile DNA II*, Ed. by N.L. Craig *et al.*, ASM Press, Washington, D.C., Chapter 17, pp. 385-402.
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www.epicentre.com/transposomics.asp

HyperMu™ <KAN-1> Insertion Kit

HMI032K 10 Reactions

HyperMu™ <R6K γ ori/KAN-1>Tnp Transposome™ Kit

MTS32RK 10 Reactions

HyperMu™ Transposase

THM03210 10 U

* The use of Transposome™ complexes for *in vivo* insertion of a transposon, including, but not limited to HyperMu™ and EZ::TN™ Transposome™ complexes, is covered by U.S. Patent No. 6,159,736 and related patent applications, exclusively licensed to EPICENTRE.



Phage T1-Resistant Electrocompetent *E. coli* with a Transformation Efficiency of $>1 \times 10^{10}$

Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli*

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- Genomic library construction
- cDNA library construction
- Cloning rare or limiting DNA
- Shotgun library construction

Once introduced into the lab environment, bacteriophage T1 rapidly lyses *E. coli* strains that are commonly used in cloning applications. The result can be significant lab downtime and the loss of valuable clones and entire libraries. Bacteriophage T1 is particularly difficult to eliminate from the lab and can lay dormant for many years. The *tonA* genotype protects the Phage T1-Resistant TransforMax EC100-T1^R cells, and your clones, from attack by phage T1 (and phage T5).

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Important Benefits (compare to DH10B*)

- Greater than 10^{10} cfu/ μ g DNA.
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- Readily accepts large DNAs for construction of large-insert genomic libraries.
- Restriction minus (*mcrA*, Δ (*mrr-hsdRMS-mcrBC*)) enables efficient cloning of methylated DNA for more complete genomic libraries.
- Endonuclease minus (*endA1*) to ensure high yields of DNA.
- Restriction minus (*recA1*) for greater stability of large cloned inserts.
- *lacZ* Δ M15 for blue/white screening of recombinants.

Genotype

F⁻ *mcrA* Δ (*mrr-hsdRMS-mcrBC*)
 ϕ 80*dlacZ* Δ M15 Δ *lacX74* *recA1* *endA1*
araD139 Δ (*ara, leu*)7697 *galU* *galk* λ -
rpsL *nupG* *tonA*

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Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli*

ECO205T1 5 X 100 μ l

ECO210T1 10 X 100 μ l

Transformation efficiency $>1 \times 10^{10}$ cfu/ μ g.
 Includes pUC19 control DNA.

Phage T1-Resistant TransforMax™ EC100™-T1^R Chemically Competent *E. coli*

CCT10210 10 X 50 μ l

Transformation efficiency $>5 \times 10^7$ cfu/ μ g.
 Includes pUC19 control DNA.

* DH10B is a trademark of Invitrogen Corporation.

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2. microarray strategies & probe synthesis
3. new nucleic acid amplification methods
4. high-throughput SNP screening
5. RNA interference

Please tell us your expertise in these areas in your application.



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