

# EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

Volume 11-6

*Featuring:*

*EPICENTRE's Nucleic  
Acid Purification Products*

**Purify RNA from 10 to  
10,000 Eukaryotic Cells**

**Purify Microbial DNA  
from Water Samples**

**Transposon Kits  
Generate Unidirectional  
Protein Deletions**

**Novel *In Vitro* Transcription  
Products Use ssDNA**

Cover Photo: Canada Life Building, Toronto, Canada  
Photograph by: Adam Richardson  
InterScience, Markham, ON, Canada

# Now your cloning nightmares can have a happy ending

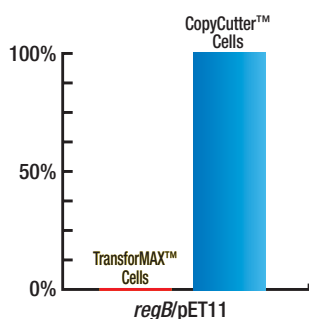
Many cloning nightmares start with DNA inserts that can not be stably maintained in high-copy number vectors and you end up with clones that are “empty” or contain mutations.



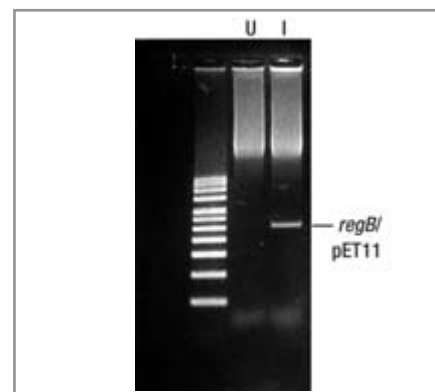
EPICENTRE's new **CopyCutter™ EPI400™ *E. coli* cells\*** significantly lower the copy number of a wide variety of vectors so that you can more readily clone “toxic” genes or other unstable DNA sequences. Engineered from our high-transformation efficiency **TransforMAX™ EC100™ cells**, CopyCutter EPI400 cells contain a modified *pcnB* gene linked to an inducible promoter. The *pcnB* gene controls the copy number of vectors containing ColE1 and pMB1 origins, like derivatives of pUC, pBluescript®, pGEM®, pET and many others. Hence, transforming our CopyCutter cells with vectors like pUC19 reduces the copy number by 25-fold compared to the parental strain.

As an example, consider the *regB* gene. This gene encodes a restriction endoribonuclease that cleaves vital bacterial messages and is therefore highly toxic to *E. coli*, even in very small quantities. We successfully cloned *regB* into a pET11 vector only when recombinants were transformed into CopyCutter cells (FIG 1). A brief induction to higher-copy number was then used to improve plasmid yields (FIG 2).

## Plasmids with Correct Insert Size



**FIG 1.** Successful cloning of the *regB* gene. Of the 19 **TransforMAX™ EC100™** clones screened, 17 contained no insert and 2 had large deletions. All 29 **CopyCutter™ EPI400™** clones screened contained a full-length insert and the sequenced insert was free of mutations.



**FIG 2.** Uninduced **CopyCutter™ EPI400™** cells containing a *regB* clone (Lane U) were induced to higher copy-number for 4 hours using the **CopyCutter™ Induction Solution** (Lane I).



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### On the Cover:

EPICENTRE thanks Adam Richardson for the use of his photograph of the Canada Life Building and the CN tower in Toronto. At 1,815 feet, the CN tower still holds the record for the world's tallest free-standing structure. As the Technical Marketing Manager for InterScience, Adam coordinates sales representatives across Canada, making sure that they have the latest information on the wide variety of scientific equipment and products offered by InterScience. He also provides technical support for InterScience's products and gets great satisfaction from helping customers solve their research problems. InterScience has proudly distributed EPICENTRE products to Canadian researchers for the past 8 years.

### Hands on the Cover:

Mike Fiandt, EPICENTRE Research Scientist

New!

## Purify Microbial DNA from Water Samples Using the WaterMaster™ DNA Purification Kit

Bruce W. Jarvis, EPICENTRE

The WaterMaster™ DNA Purification Kit provides all of the reagents needed to purify high-molecular-weight microbial DNA from water samples, including DNA from most bacteria and many eukaryotes, such as protozoa. The purified DNA can be used for restriction digests, PCR, sub-cloning, sequencing, or microarray analysis. The simple, gentle procedure requires no toxic organic solvents or mechanical cell disruption. The final volume of the purified DNA solution is 60 µl, 50-fold less than other commercial kits, and does not need to be concentrated.

### Environmental sample

To test the WaterMaster DNA Purification Kit on an environmental sample, spring-fed pond water (100 ml) was filtered, first through Miracloth (Calbiochem, EMD Biosciences) to remove large particles,

and then through a 0.45-micron filter, the maximum pore size used to trap bacteria. The filter unit used in this study was a pre-sterilized Millipore 0.45 micron Microfil® V, 47 mm diameter filter available from Fisher Scientific. Because different applications require different filter sizes, the kit does not include filters.

DNA was purified from the collected bacteria using the WaterMaster Kit and assayed on an agarose gel. Figure 1 shows the high molecular weight DNA purified from the pond water sample. The DNA sample was further tested by PCR and shown to contain *Bacillus sp.*, as indicated by amplification of the expected 600-bp product from the 16S ribosomal RNA gene (rDNA)<sup>1</sup> (Figure 2). Some bacteria are more difficult to lyse<sup>2</sup> and many require an additional step for

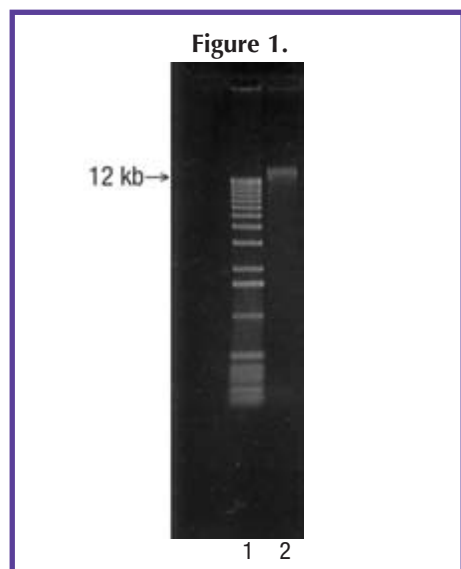
lysis, as outlined in the WaterMaster Kit protocol (available on EPICENTRE's website at [www.epicentre.com](http://www.epicentre.com)).

### Limits of detection

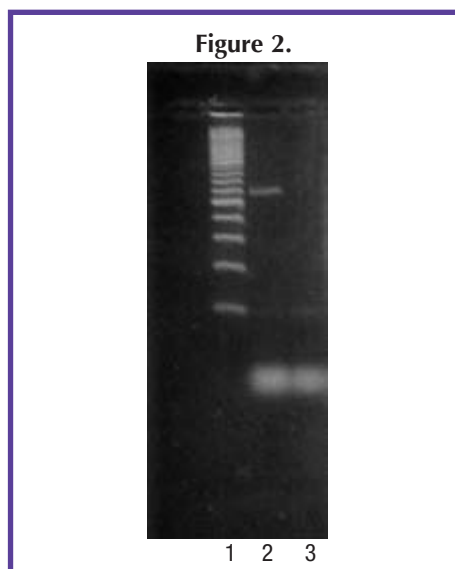
To test the detection limit for bacteria in water samples, varying amounts of *E. coli* ( $10^5$  to 10 cells) were added to distilled water (100 ml) and filtered through 0.45 micron filters. DNA was purified using the WaterMaster Kit and detected by PCR using the FailSafe™ PCR System with PreMix B and universal primers for eubacterial rDNA.<sup>3</sup> Figure 3 shows that DNA from as few as 10 bacteria per 100 ml of water can be purified with the WaterMaster Kit and detected by PCR.

If eubacterial 16S rDNA primers are used to amplify DNA from an environmental

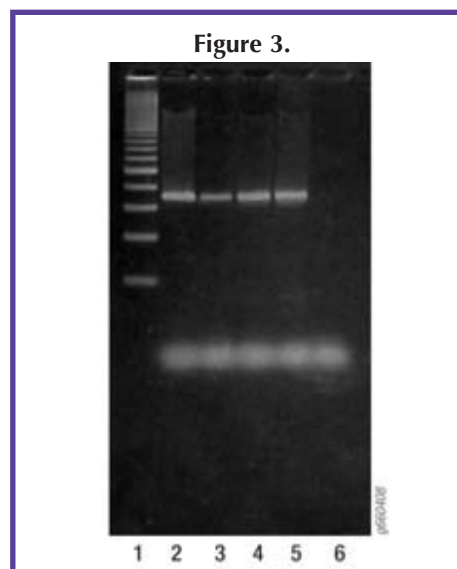
*Continued on page 6*



**Figure 1.** High molecular weight DNA was purified from microbes in a water sample from a spring-fed pond using the WaterMaster™ DNA Purification Kit. The 1% agarose gel was stained with SYBR® Gold. **Lane 1**, kb DNA ladder; **Lane 2**, 5 ng DNA purified with the WaterMaster Kit.



**Figure 2.** PCR Amplification of *Bacillus* DNA purified from an environmental water sample using the WaterMaster™ DNA Purification Kit. Pond water (100 ml) was filtered through a 0.45 micron filter and DNA was purified with the WaterMaster Kit. Using 1 µl (1 ng) of the purified DNA, the 16S rDNA of a *Bacillus sp.* was amplified by PCR using forward primer 5'-AGG GTC ATT GGA AAC TGG G and reverse primer 5'-CGT GTT GTA GCC CAG GTC ATA.<sup>1</sup> Cycle conditions were 95°C (2 minutes) and 30 cycles of 95°C (45 seconds), 55°C (45 seconds), 72°C (45 seconds) followed by 72°C (2 minutes). **Lane 1**, 100-bp ladder; **Lane 2**, PCR product from pond DNA; **Lane 3**, no-template control PCR.



**Figure 3.** The detection limit of the WaterMaster™ DNA Purification Kit for *E. coli* in a water sample was assayed using PCR. Ten-fold serial dilutions ( $10^5$  to 10 cells) of *E. coli* K12 were added to distilled water (100 ml) and filtered through 0.45 micron filters. DNA was purified using the WaterMaster Kit and 1 µl was used in PCR with universal primers for eubacterial rDNA<sup>3</sup>: 5'-CTG CTG CCT CCC GTA GGA GT and 5'-AGA GTT TGA TCC TGG CTC AG. Cycling conditions were 96°C (3 minutes) and 28 cycles of 96°C (30 seconds), 57°C (30 seconds), 72°C (45 seconds). **Lane 1**, 100-bp ladder; PCR products from DNA purified from varying numbers of *E. coli* cells: **Lane 2**,  $10^5$  cells; **Lane 3**,  $10^3$  cells; **Lane 4**,  $10^2$  cells; **Lane 5**, 10 cells; **Lane 6**, no template, negative control. The size of the expected amplicon is 350 bp.



## Purify RNA from 10 to 10,000 Eukaryotic Cells Using the ArrayPure™ Nano-scale RNA Purification Kit

Bruce W. Jarvis and Judith E. Meis, EPICENTRE

The ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from a few hundred eukaryotic cells, a quantity typically obtained with Laser Capture Microdissection procedures.<sup>1,2</sup> The kit contains only aqueous solutions and requires no toxic organic solvents.<sup>3</sup> This nano-scale protocol has been specifically designed for and tested on 10 to 10,000 eukaryotic cells. To purify RNA from a greater number of cells, the original MasterPure™ RNA Purification Kit is recommended.

### Purify RNA from 10 cells

The ArrayPure Kit was tested and compared to two other commercially available kits. Those two kits gave results comparable to each other, so the data from only one kit is shown here. Ten-fold serial dilutions of intact HeLa cells were dispensed into tubes at 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, 10 or 0 (medium control) cells per tube. These were dilutions of living cells, not dilutions

of a cell lysate, which other vendors have used to claim low cell numbers. The RNA at each cell dilution was purified using the ArrayPure Nano-scale RNA Purification Kit or the other supplier's kit, in duplicate.

The ArrayPure method initially uses RNase-free DNase I. In subsequent steps the DNase is removed from the purified RNA by precipitation. Tests showed no detectable double-stranded DNA and no residual DNase activity in the final purified RNA.

For 10<sup>4</sup> and 10<sup>3</sup> cells, RNA was quantitated using RiboGreen® fluorescence. The average total RNA obtained from 10<sup>4</sup> and 10<sup>3</sup> cells with the ArrayPure Kit was 213 and 21 ng, respectively, and with the other supplier's kit was 197 and 15 ng, respectively. The amount of RNA purified from 100 cells and 10 cells was below the RiboGreen® assay detection limit; so RNA from all samples was compared by quantitative RT-PCR.

### Prepare cDNA for real-time PCR

To compare the amount of RNA purified from each cell dilution, cDNA was prepared from the RNA and used in real-time PCR reactions. Figure 1 shows the amplification plots resulting from the ArrayPure samples (1A) and from the other supplier's samples (1B). The ArrayPure samples have lower threshold cycles (C<sub>T</sub>) at lower cell concentrations, indicating that the reactions contain more template. Data for the standard curves derived from the amplification plots are shown in Table 1, on page 6. An acceptable range for PCR efficiency is 90 to 110%. The low PCR efficiency (83.5%) for the samples prepared with the other supplier's kit indicates that the standard curve is too inaccurate to use for template quantification.

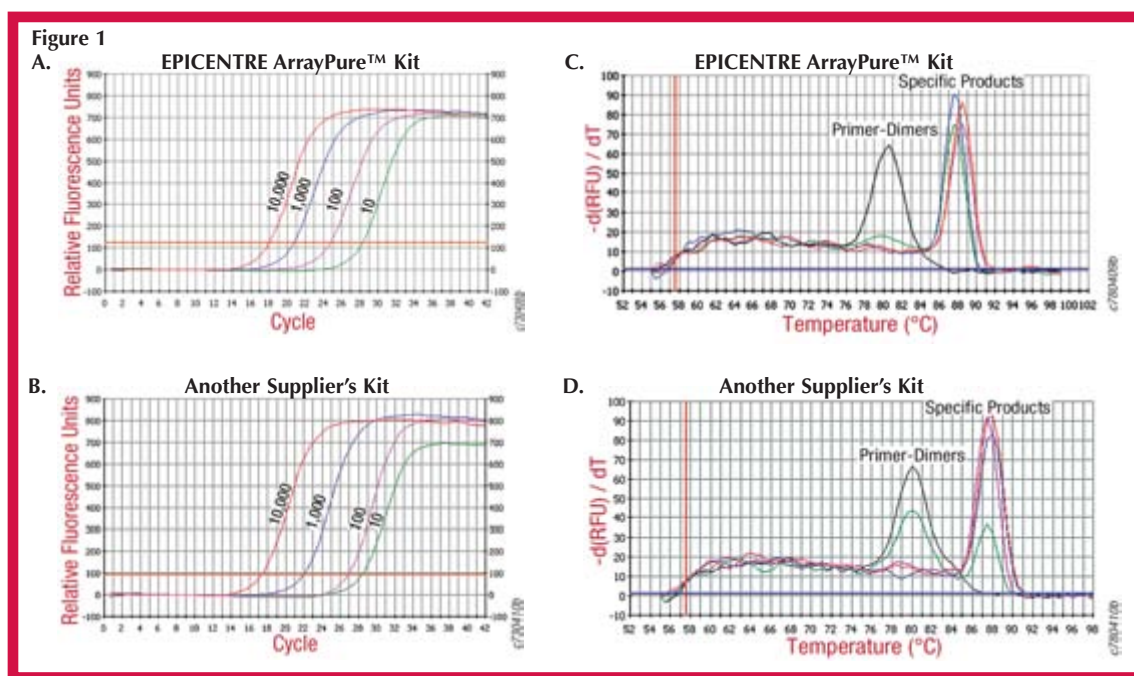
Melt curve analyses (1C and 1D) indicate that the medium control (0 cells) purified by ArrayPure (1C) had no RNA and

*Continued on page 6*

**Figure 1. Quantitative RT-PCR amplification plots were generated using cDNA prepared from RNA purified with the ArrayPure™ Nano-scale RNA Purification Kit or with another supplier's kit. A.** RNA purified with the ArrayPure Kit. **B.** RNA purified with another supplier's kit. Intact HeLa cells were serially diluted ten-fold in growth medium, washed with PBS, and dispensed at 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, 10 or 0 (medium control) cells per tube. RNA was purified using either the ArrayPure Kit or the other supplier's kit, in duplicate, for each cell concentration. Purified HeLa RNA was converted to cDNA using EPICENTRE's MMLV reverse transcriptase. The corresponding cDNAs were amplified using the FailSafe™ Real-Time PCR System with SYBR® Green I dye on a Bio-Rad iCycler

iQ™ Real-Time PCR Detection System. Cycling conditions were 95°C (2 minutes) and 45 cycles of 95°C (20 seconds), 53°C (30 seconds), and 72°C (30 seconds). Primers were for human cyclophilin A (peptidylprolyl isomerase A) 5'- CAT ACG GGT CCT GGC ATC TTG and 5'- GCC ATT CCT GGA CCC AAA GC.

**Melt Curve Analysis of the Quantitative RT-PCR. C.** RNA purified with ArrayPure. PCR amplification of cDNA corresponding to RNA purified from 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup> and 10 HeLa cells all yielded specific PCR products (peaks at 88°C). The 0-cell (medium control) sample yielded only primer-dimers (peak at 80°C to 81°C) indicating the absence of detectable RNA, as expected. **D.** RNA purified with another supplier's kit. The cDNA corresponding to RNA purified from 10 HeLa cells produced primarily primer-dimers and the 0-cell (medium control) sample produced only primer-dimers.



## Purify Microbial DNA from Water Samples. . . (continued from page 4)

source, PCR may yield additional, slower migrating amplicons. These heteroduplex amplicons are created when rDNA from different species anneal together, and can be prevented by a method known as "PCR reconditioning."<sup>4</sup> In this method the amplification reaction is diluted 1:10 in fresh reaction mix before the last 3 PCR cycles.

### Conclusion

The EPICENTRE WaterMaster DNA Purification Kit effectively purifies microbial DNA from environmental water sources using a simple procedure, with no toxic solvents or bead beating. DNA is recovered in a low, 60-µl

volume and is ready for PCR or other techniques, as described in the poster presented at the 12<sup>th</sup> International Meeting on Microbial Genomes (2004) <http://www.epicentre.com/posters/enviroDNAposterweb.pdf>.

### References

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2. Jarvis, B.W. and Hoffman, L.M. (2004) *EPICENTRE Forum* **11**(3), 5.
3. Brow, M.A.D. et al. (1996) *J. Clin. Microbiol.* **34**, 3129.
4. Thompson, J. R. et al. (2002) *Nucleic Acids Res.* **30**, 2083.

[www.epicentre.com/watermaster.asp](http://www.epicentre.com/watermaster.asp)

### WaterMaster™ DNA Purification Kit

WM04005	5 Purifications
WM04020	20 Purifications

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SEE PRODUCT DATA SHEET  
ON PAGE 9

FOR MORE INFORMATION ON  
THE WATERMASTER™ DNA  
PURIFICATION KIT

info

## Purify RNA from 10 to 10,000 Eukaryotic Cells. . . (continued from page 5)

produced only primer-dimers, as expected. ArrayPure RNA from as little as 10 HeLa cells produced cDNA that gave a specific real-time PCR product. RNA purified from 10 HeLa cells with the other supplier's kit did not produce a significant amount of cDNA as indicated by the substantial primer-dimer peak in the 10-cell PCR reaction (1D).

### RNA amplification

The ArrayPure Nano-scale RNA Kit is designed to prepare RNA that can be amplified, which is often necessary for

microarray work. From 2.5 x 10<sup>4</sup> cells, 1 round of RNA amplification was sufficient to amplify 220 ng of purified RNA to 40 µg of RNA. With 20 HeLa cells (400 pg RNA), 2 rounds of amplification produced 32 µg of amplified RNA. After both 1 and 2 rounds of amplification, the RNA could be used for real-time PCR.

### Acknowledgments

We would like to thank Anupama Khanna and Ramesh Vaidyanathan for RNA amplification results and technical discussions.

### References

1. Emmert-Buck, M.R. et al. (1996) *Science* **274**, 998.
2. Bonner, R.F. et al. (1997) *Science* **278**, 1481.
3. Miller, S.A. et al. (1988) *Nucleic Acids Res.* **16**, 1215.

[www.epicentre.com/arraypure.asp](http://www.epicentre.com/arraypure.asp)

### ArrayPure™ Nano-scale RNA Purification Kit

MPS04050	50 Purifications
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ON PAGE 10

FOR MORE INFORMATION ON  
THE ARRAYPURE™ NANO-SCALE  
RNA PURIFICATION KIT

info

Table 1. Standard curve data generated from quantitative RT-PCR amplification plots.

RNA Purification Kit	Correlation Coefficient	Slope	PCR Efficiency (%)
ArrayPure™ Kit	0.998	-3.412	96.4
Other supplier's kit	0.980	-3.793	83.5

# DNA Isolation from a Filamentous Fungus Using the MasterPure™ Yeast DNA Purification Kit

Brian Wickes, University of Texas Health Science Center



## Dr. Brian Wickes

Associate Professor and Director, Advanced DNA Technologies Core Facility Ph.D., Catholic University

E-mail address: wickes@uthscsa.edu  
Phone: (210) 567-3938

*We have found that it works equally well on both yeast and filamentous fungal species.*

## Introduction

Methods for isolation of fungal DNA generally can be grouped into enzymatic, chemical, and physical. Large yields of high molecular weight DNA are most frequently recovered using cell wall-degrading enzymes to spheroplast cells, followed by gentle lysis and additional purification steps. Filamentous fungi offer much more diversity in their cell walls, as well as different morphologies (i.e., conidia vs. hyphae), all of which can make choosing and optimizing a method for a spheroplasting enzyme laborious. Consequently DNA preps for filamentous fungi typically employ bead beating or grinding in liquid nitrogen. Unfortunately, these methods can lead to sheared DNA (bead beating) or can be hazardous and laborious (grinding pathogenic fungi in an open mortar and pestle, or prepping multiple isolates). In an effort to circumvent these issues, we used EPICENTRE's MasterPure™ Yeast DNA Purification Kit to recover DNA from *Aspergillus fumigatus*, a filamentous fungus that is an opportunistic pathogen of humans and animals.<sup>1</sup>

## Methods and Results

Multiple isolates of *A. fumigatus*, from a variety of backgrounds (laboratory strains, clinical isolates, environmental isolates), were tested under variable conditions, that included preparing DNA from hyphae or conidia, using broth or agar cultures, and growing isolates for varying lengths of time prior to preparing DNA.<sup>2</sup> The standard preparation consisted of 200 mg of hyphae harvested from an overnight broth culture, although 72-hour conidia harvested from a single plate also worked well. The protocol accompanying the kit was followed with some modifications. The amount of Yeast Cell Lysis

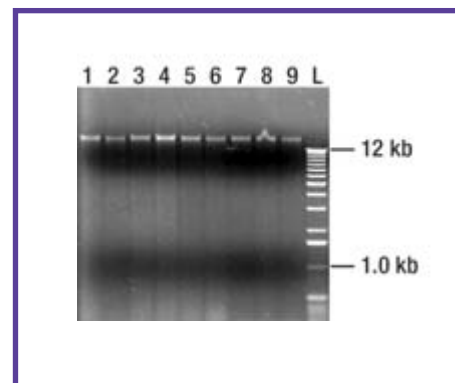
Solution was increased to 450 µl and successive steps were scaled accordingly. Isolates were heated at 65°C for 1 hour, and in some cases, a phenol-chloroform extraction was included, however, grinding was not needed under any circumstance. The yield from these conditions was typically 500-700 µg, by OD<sub>260</sub> (Figure 1) and the DNA was easily digested with restriction enzymes (Figure 2).

## Summary

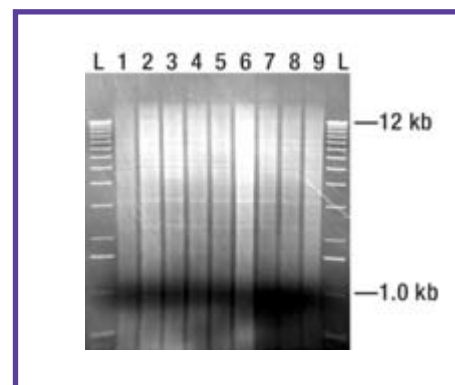
The MasterPure Yeast DNA Purification Kit has proven to be extremely robust and versatile in our laboratory. We have found that it works equally well on both yeast and filamentous fungal species. In addition to the ease of use, the amount of DNA recovered in a single preparation was enough to perform numerous Southern blots. We also found the DNA is of high molecular weight and suitable as a template for PCR reactions. In addition to working for *A. fumigatus*, we have found that the kit is suitable for other members of the aspergilli. Periodically we have also used the kit for DNA recovery from non-aspergilli and observed comparable results. The MasterPure Kit has proven particularly valuable for working with biohazardous fungi and for preparing DNA from multiple species in microtiter tube formats.

## References

- Marr K.A. et al. (2002) *Infect. Dis. Clin. N. Am.* **16**, 875.
- Jin, J. et al. (2004) *J. Clin. Microbiol.* **42** 4293.



**Figure 1. Quality of genomic DNA prepared from nine different strains of *Aspergillus fumigatus*.** Lane 1, AF293 (used for genomic sequence); Lane 2, ATCC 64746; Lane 3, ATCC 14110; Lane 4, Clinical isolate; Lane 5, Environmental isolate; Lanes 6-9, Clinical isolates.



**Figure 2. Eco RI digestion of genomic *Aspergillus fumigatus* DNA from Figure 1.**

[www.epicentre.com/masterpure\\_yeast.asp](http://www.epicentre.com/masterpure_yeast.asp)

### MasterPure™ Yeast DNA Purification Kit

MPY80010 10 Purifications  
MPY80200 200 Purifications

#### Contents:

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

## SoilMaster™ DNA Extraction Kit

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 and incorporates an inhibitor removal spin column. The SoilMaster™ Kit isolates more intact DNA than other soil DNA kits incorporating bead-beating or vortex mixing in the presence of beads.

### Applications

- \* Extraction of PCR-ready DNA from soil and sediment for PCR amplification of diverse organisms
- \* Humic acid and protein removal

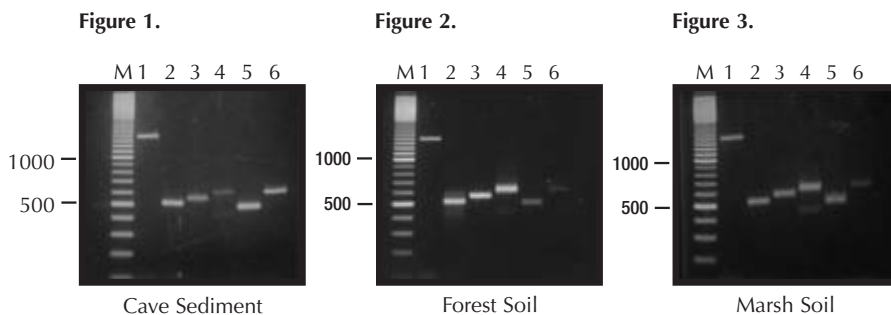
### Benefits

- \* Extract PCR-ready microbial DNA from soil in less than 45 minutes
- \* No need for extra equipment or bead-beating
- \* Recover higher molecular weight DNA



Technical Support  
techhelp@epicentre.com

**Extract**  
**PCR-Ready Soil DNA**  
in less than **45** minutes



**Figures 1-3.** One microliter of extracted soil DNA (less than 1% of the total purified) was amplified using the FailSafe™ PCR System and primers to the following templates; **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; and **Lane 6**, *Bacillus* primers.

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#### SoilMaster™ DNA Extraction Kit

SM02050 50 Reactions

#### Contents:

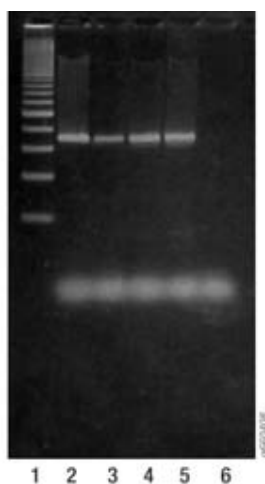
Soil DNA Extraction Buffer  
Proteinase K  
Soil Lysis Buffer  
Protein Precipitation Reagent  
Inhibitor Removal Resin  
Spin Columns  
DNA Precipitation Solution  
Pellet Wash Solution  
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MasterPure™ Gram Positive DNA Purification Kit  
MasterPure™ Yeast DNA Purification Kit  
MasterPure™ Yeast RNA Purification Kit



**Figure 1. The detection limit of *E. coli* in a water sample was tested by PCR.** Varying amounts ( $10^5$  to 10 cells) of *E. coli* K12 were diluted in 100-ml distilled water samples and filtered through 0.45 micron filters. DNA was purified using the WaterMaster™ DNA Purification Kit and detected by PCR using the FailSafe™ PCR System with PreMix B and universal primers for eubacterial rRNA genes.<sup>1</sup> **Lane 1**, 100 base pair ladder; Amplification of DNA purified from varying numbers of *E. coli* cells: **Lane 2**,  $10^5$  cells; **Lane 3**,  $10^3$  cells, **Lane 4**,  $10^2$  cells, **Lane 5**, 10 cells, **Lane 6**, no template. The size of the amplicon is 350 bp. DNA from as few as 10 cells per 100 ml water can be purified with the WaterMaster Kit and detected by PCR.



#### Reference

1. Brow, M.A.D. et al. (1996) *J. Clin. Microbiol.* **34**, 3129.

#### NEW! Easy Shortcut to Product Information

Go to our website at [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **WMD01**

## WaterMaster™ DNA Purification Kit

The WaterMaster™ DNA Purification Kit provides all of the liquid reagents needed to purify microbial DNA from any water source after passage through a filter of your choice. This capability includes DNA from bacteria and eukaryotes. The DNA is suitable for restriction digests, PCR, subcloning, sequencing, or microarray analysis. The kit uses a simple, gentle procedure with no toxic organic solvents or mechanical cell disruption. The final volume of the purified DNA solution is 60  $\mu$ l, 50-fold less than other commercial kits. Thus, there is no need to concentrate the DNA.

### Applications

- \* PCR
- \* Cloning
- \* Sequencing
- \* DNA microarrays

### Benefits

- \* Simple, gentle procedure
- \* No toxic organic solvents
- \* No mechanical cell disruption
- \* Small final volume of DNA

#### WaterMaster™ DNA Purification Kit

WM04005      5 Purifications  
WM04020      20 Purifications

#### Related Products

SoilMaster™ DNA Extraction Kit



Technical Support  
techhelp@epicentre.com

# ArrayPure™ Nano-scale RNA Purification Kit

The ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from a few hundred eukaryotic cells, a quantity typically obtained with Laser Capture Microdissection procedures. The kit contains only aqueous reagents, requires no toxic organic solvents, and has been specifically designed for and qPCR-tested on 10 to 10,000 eukaryotic cells. These assays used dilutions of living cells, not dilutions of a cell lysate, which other vendors have used to claim low cell numbers. ArrayPure™ RNA from 20 HeLa cells has been used to produce µg amounts of RNA using RNA 2-round amplification techniques.

## Applications

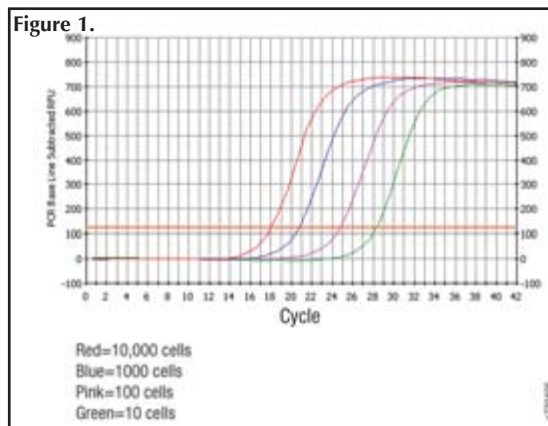
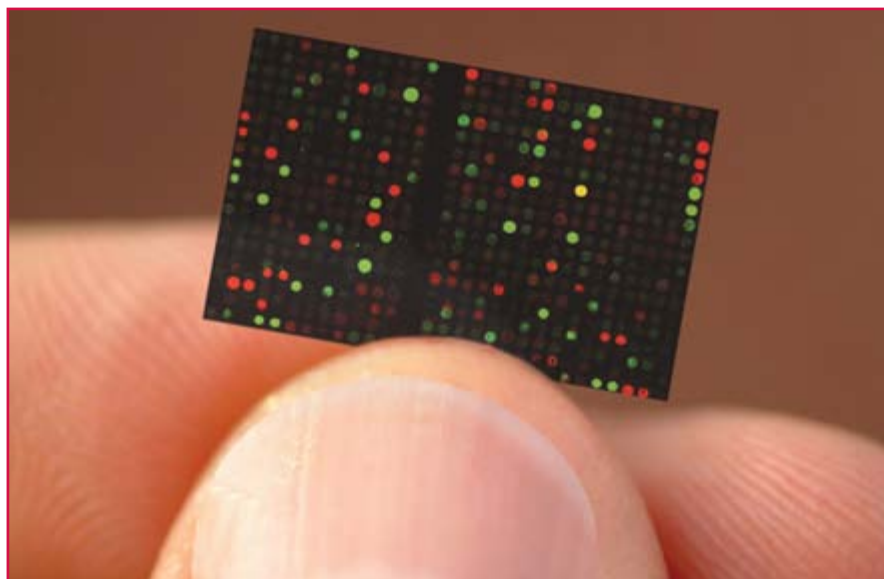
- \* RT-PCR
- \* Amplified RNA
- \* LCM

## Benefits

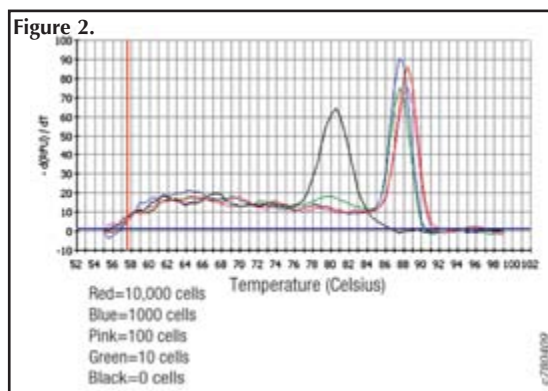
- \* Quantitatively isolate RNA from as few as 10 cells
- \* Purify RNA for RNA amplification
- \* Avoid carrier RNA



Technical Support  
techhelp@epicentre.com



**Figure 1. Quantitative RT-PCR.** Intact HeLa cells were serially diluted ten-fold in growth medium to dispense 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, 10 or 0 (medium control) cells per tube. RNA was purified at each cell concentration with the ArrayPure™ Nano-scale RNA Purification Kit. Purified HeLa RNA was converted to cDNA using EPICENTRE's MMLV reverse transcriptase. Corresponding cDNAs were amplified using the FailSafe™ Real-Time PCR System.



**Figure 2. Melt Curve Analysis.** The cDNA corresponding to RNA purified from 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, and 10 HeLa cells yielded PCR products (peaks at 87°C to 88°C). The 0 cell (medium control, black line) sample yielded only primer-dimers (peak at 80°C to 81°C) indicating the absence of detectable RNA, as expected.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **ARD01**

#### ArrayPure™ Nano-scale RNA Purification Kit

MPS04050      50 Purifications

#### Related Products

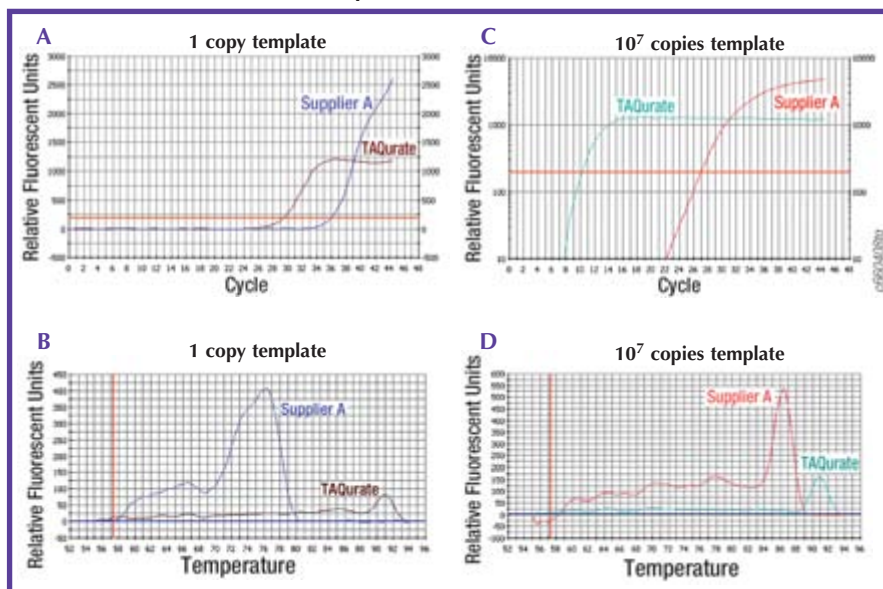
MasterPure™ RNA Purification Kit

# Accurate Results

## TAQurate™ Real-Time PCR Master Mix

The TAQurate™ Real-Time PCR Master Mix with SYBR® Green I dye is formulated for reliable, consistent real-time PCR results with routine template and primer sets. It contains everything needed for successful PCR. Simply add your primers and template to the Master Mix, mix thoroughly, and begin your PCR thermal cycler. The high-performance TAQurate™ Enzyme Blend consistently amplifies most templates, and the PCR Enhancer (with betaine\*) ensures high amplification efficiencies and fewer non-specific real-time PCR products.

### Higher Sensitivity and Faster C<sub>T</sub> Values



**Figure 1. Higher sensitivity and faster threshold cycle (C<sub>T</sub>) values with the TAQurate™ Real-Time PCR Master Mix.** Real-time PCR amplification of one copy and 10<sup>7</sup> copies of lambda DNA was performed with the TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** and **C** are amplification plots for one and 10<sup>7</sup> copies, respectively. **B** and **D** are melt curves for one and 10<sup>7</sup> copies, respectively.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **TQD01**

#### TAQurate Real-Time PCR Master Mix

TM046200 200 Reactions  
TM049048 48 Reactions

#### Related Products

FailSafe™ Real-Time PCR  
PreMix Selection Kit

\* 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 Invitrogen Detection Technologies. 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.

### Benefits

- \* Convenient, ready-to-use Master Mix reduces set-up time and liquid handling steps. Just add templates and primers.
- \* Broad dynamic range and more accurate standard curves.
- \* Highly sensitive and specific amplifications for reliable, consistent results.

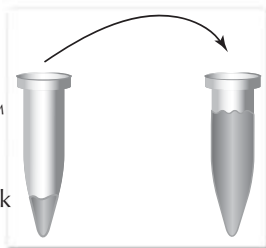


Technical Support  
techhelp@epicentre.com

# TAQurate™ Real-Time PCR Master Mix

## General Overview

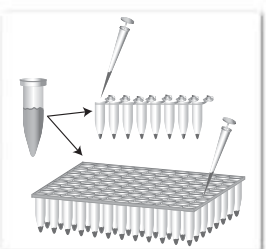
Add 50 µl of TAQurate™ Enzyme Blend to 2x TAQurate™ Master Mix to prepare the Master Mix Stock Solution



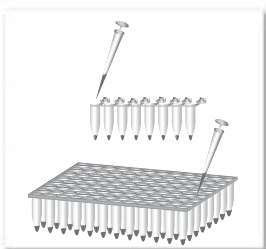
Vortex



Aliquot Master Mix Stock Solution into each reaction tube or well



Add template and primers



Run PCR



## Dynamic Range and Standard Curve Comparison

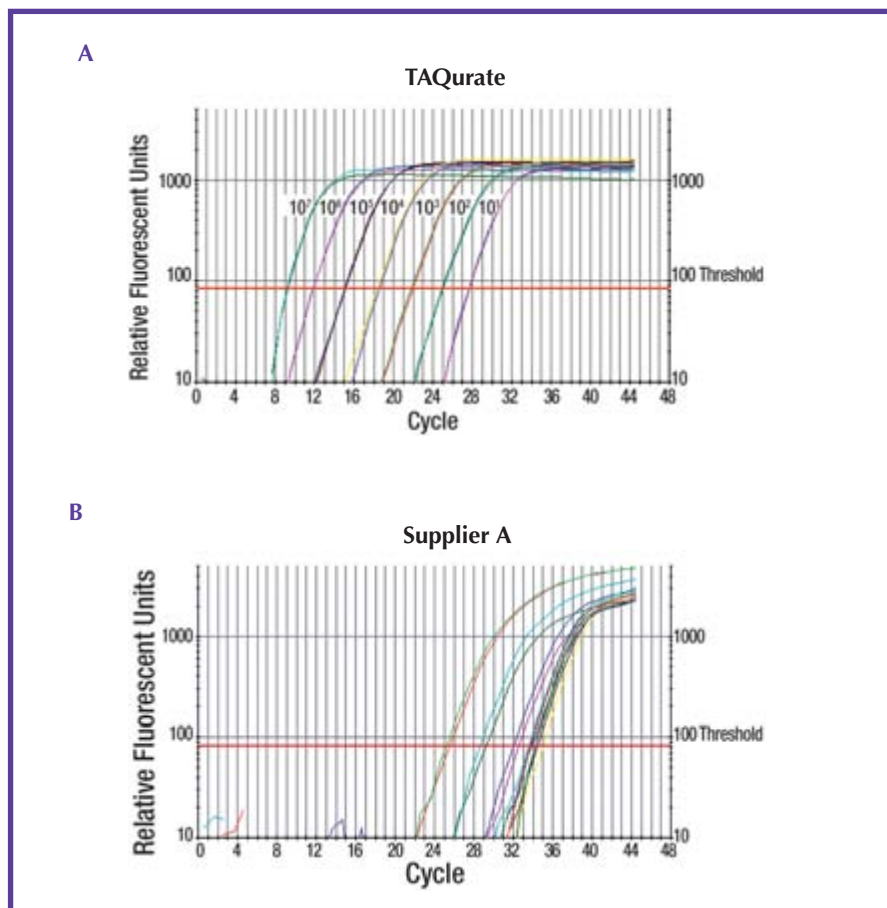
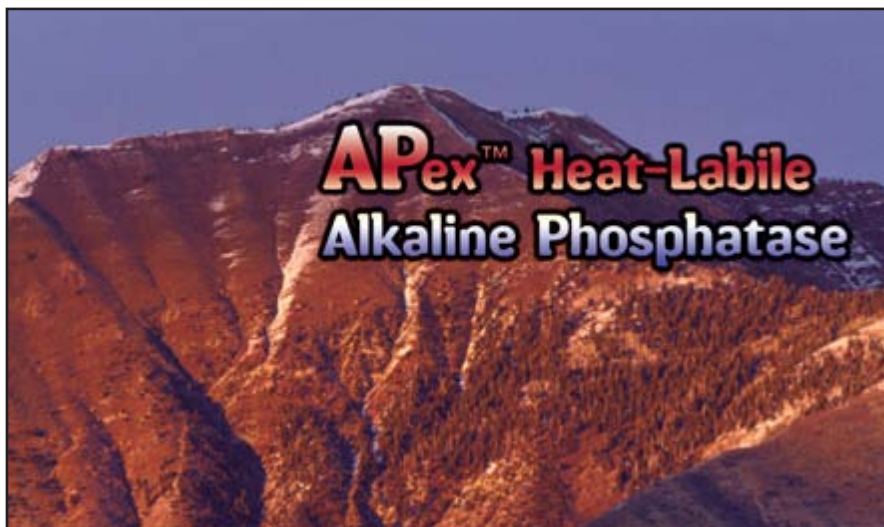


Figure 2. Wider dynamic range generates a more accurate standard curve with the TAQurate™ Real-Time PCR Master Mix. Real-time PCR of lambda DNA ( $10^7$ ,  $10^6$ ,  $10^5$ ,  $10^4$ ,  $10^3$ ,  $10^2$ , and 10 copies) was performed with TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** (EPICENTRE) and **B** (Supplier A) PCR amplification plots.

Table 1. Standard curve data derived from amplification plots in Figure 2.

Master Mix	Correlation Coefficient	Slope	PCR Efficiency (%)
TAQurate	0.999	-3.19	105.8
Supplier A	0.831	-1.29	493.9



## APex™ Heat-Labile Alkaline Phosphatase

APex™ Heat-Labile Alkaline Phosphatase is a novel alkaline phosphatase that dephosphorylates a broad range of substrates using a single, quick, and convenient protocol. APex™ Phosphatase removes the 5'-phosphate from all types of DNA ends in a wide variety of buffer conditions, including most restriction enzyme buffers. The enzyme is irreversibly heat-inactivated by incubation at 70°C for 5 minutes.

5'-overhang	
5'-(4-base) overhang:	<i>Hind</i> III, <i>Eco</i> R I
5'-(3-base) overhang:	<i>Eco</i> O109 I
5'-(2-base) overhang:	<i>Nde</i> I
Blunt-end	
no overhang:	<i>Sma</i> I, <i>Ssp</i> I
5'-recessed	
3'-(4-base) overhang:	<i>Sph</i> I, <i>Sac</i> I, <i>Pst</i> I
3'-(3-base) overhang:	<i>Alw</i> N I
3'-(1-base) overhang:	<i>Ahd</i> I

Table 1 Restriction enzyme overhangs tested.

**Activity:** 1 µl of APex Phosphatase dephosphorylates 1 µg of pUC19 vector DNA digested with *Hind* III (5'-protruding), *Hinc* II (blunt) or *Pst* I (5'-recessed) in 10 minutes at 37°C.

**Quality Control:** APex Phosphatase is function-tested to meet our Cloning Quality Standard of >99% inhibition of *Pst* I-cut vector self-ligation as assayed by *E. coli* transformation, when treating 1 µg of pUC 19 DNA with 1 µl of enzyme for 10 minutes at 37°C.

**Contaminating Activity Assays:** APex Phosphatase is free of detectable DNA exo- and endonuclease, and RNase activities.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **APD01**

APex™ Heat-Labile Alkaline Phosphatase		Related Products
AP49010	10 Reactions	Fast-Link™ DNA Ligation Kit
AP49050	50 Reactions	
AP49100	100 Reactions	

### Applications

- \* Dephosphorylate DNA vectors for cloning.
- \* Prepare 5'-nucleic acid termini for end labeling.
- \* Dephosphorylate DNA and RNA substrates.

### Benefits

- \* Highly purified from a recombinant source.
- \* Fast, complete, and irreversible heat-inactivation.
- \* Active in a wide range of temperatures, pH, salt concentrations, and buffers.
- \* Active on blunt, 5'-overhang and 5'-recessed DNA ends.
- \* Simple 10-minute protocol for most applications.



Technical Support  
techhelp@epicentre.com

# NTPhos™ Thermolabile Phosphatase

NTPhos™ Thermolabile Phosphatase is a phosphatase formulation designed to hydrolyze and remove NTPs, dNTPs, and other nucleotides from *in vitro* reactions, to prevent nucleotide interference in downstream reactions. The enzyme is irreversibly inactivated by incubation at 65°C for 15 minutes.

## Applications

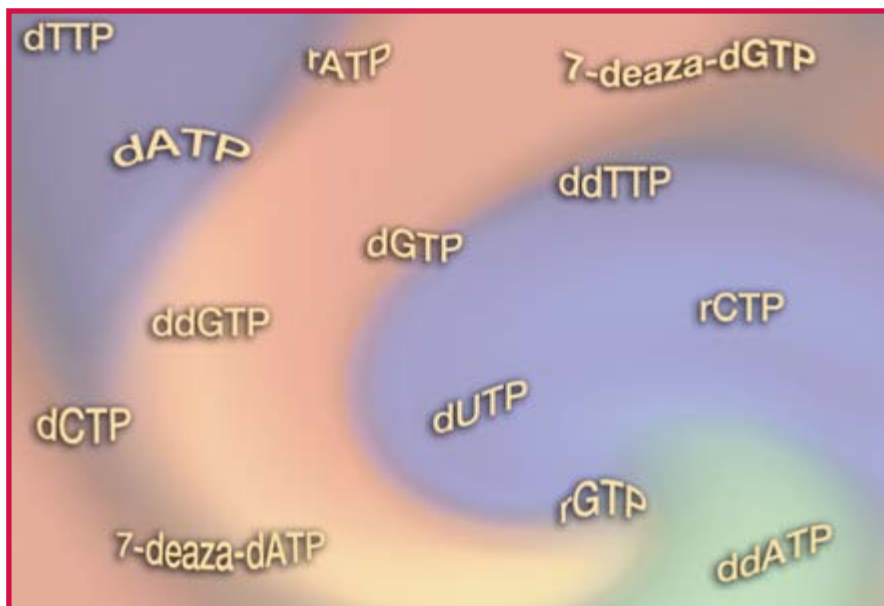
- \* Remove 5'-phosphates from modified or unmodified NTPs, NDPs, NMPs, dNTPs, dNDPs, dNMPs and inorganic pyrophosphate from *in vitro* reactions.
- \* Remove ATP from enzymatic reactions.

## Benefits

- \* High activity.
- \* Highly purified from a recombinant source.
- \* Active in a wide variety of reaction buffers.
- \* Quick and irreversibly heat-inactivated at 65°C in 15 minutes.



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dATP	ddATP	7-deaza-dATP	3'-dATP	2'-fluoro-dATP	rATP	rADP
dGTP	ddGTP	7-deaza-dGTP			rGTP	rGMP
dCTP	ddCTP			2'-fluoro-dCTP	rCTP	rCDP
dTTP	ddTTP					
dUTP				2'-fluoro-dUTP	rUTP	
dNTP mix					rNTP mix	

### Nucleotides tested and hydrolyzed by NTPhos™ Thermolabile Phosphatase

**Unit Definition:** One unit results in the release of one nanomole of inorganic phosphate from ATP or dATP in 1 minute at 37°C under standard assay conditions. NTPhos Phosphatase is supplied at a concentration of 20 units per microliter.

**Storage Buffer:** 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 mM zinc acetate, 10 mM MgCl<sub>2</sub>, 0.1 M NaCl, and 0.1% Triton X-100.

**Quality Control:** NTPhos Phosphatase is free of detectable DNA exo- and endonuclease and RNase activities.

### NEW! Easy Shortcut to Product Information

Go to our website at [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **NTD01**

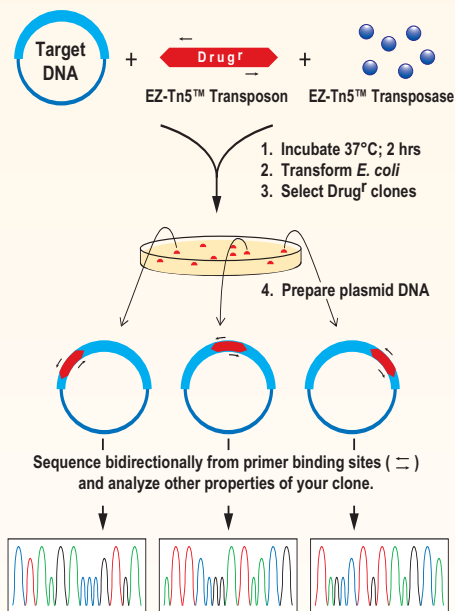
### NTPhos™ Thermolabile Phosphatase

NT4905H	20 Units/μl	500 Units
NT4910K	20 Units/μl	10,000 Units
NT4920K	20 Units/μl	20,000 Units

# EPICENTRE EZ-Tn5™ Insertion Kits

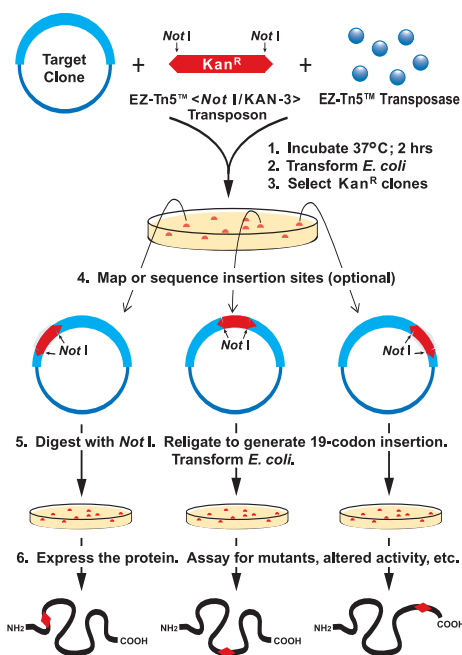
## Overview

EZ-Tn5™ Insertion Kits are based on a hyperactive Tn5 transposition system that can be used for sequencing and a myriad of other applications. Each kit relies on a simple, one-step enzymatic reaction catalyzed by EZ-Tn5™ Transposase to randomly insert an EZ-Tn5™ Transposon into any DNA molecule *in vitro*. Following transformation of *E. coli* and selection for a marker encoded by the transposon, up to millions of independent insertion clones are obtained, each with a single EZ-Tn5 Transposon at a different site. Insertion clones are sequenced bidirectionally from primer binding sites near the ends of the EZ-Tn5 Transposon and further analyzed using other unique features of the randomly inserted DNA.



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## Find Functional Domains or Epitopes of Proteins



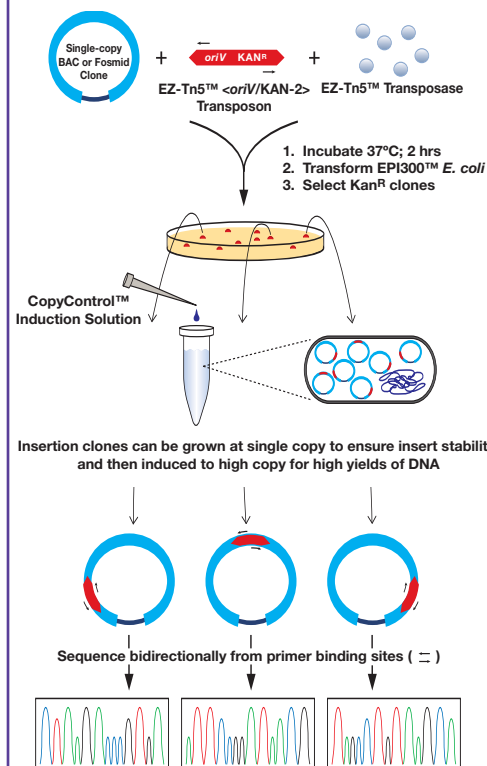
The EZ-Tn5™ In-Frame Linker Insertion Kit was designed to rapidly and easily produce random 19-amino acid (19 codon; 57-nucleotide) in-frame insertions into genes of expressed proteins for protein engineering, functional analysis, and domain or epitope mapping. The kit features the EZ-Tn5™ <Not I/KAN-3> Transposon, which contains a kanamycin resistance marker flanked by Not I restriction sites. Kanamycin-resistant insertion clones are digested with Not I, ligated, and re-transformed into *E. coli*. Since each resulting clone contains a random 19-codon insertion that can be read in all three reading frames the protein retains its original amino acid sequence on both sides of the insertion site. Relying on random transposon insertions rather than pre-existing restriction enzyme sites makes this approach more versatile than traditional linker scanning mutagenesis.

[www.epicentre.com/in\\_frame\\_linker.asp](http://www.epicentre.com/in_frame_linker.asp)

EZ-Tn5™ In-Frame Linker Insertion Kit  
EZI04KN 10 Reactions

## Integrate CopyControl™ Capability into BAC and Fosmid Clones

The EZ-Tn5™ <oriV/KAN-2> Insertion Kit enables researchers to integrate CopyControl™ capability into existing single-copy BAC and fosmid clones. Like the CopyControl™ pCC1™ Vectors, BAC and fosmid clones containing the EZ-Tn5™ <oriV/KAN-2> Transposon can be maintained in TransforMax™ EPI300™ Electrocompetent *E. coli* at single copy to ensure insert stability. Whenever desired, clones can be induced to 10 to 50 copies per cell for purification of microgram quantities of DNA from less than 2 ml of culture. Then sequence even the largest BAC clone with only two sequencing primers and avoid making a shotgun library or primer walking.



Insertion clones can be grown at single copy to ensure insert stability and then induced to high copy for high yields of DNA

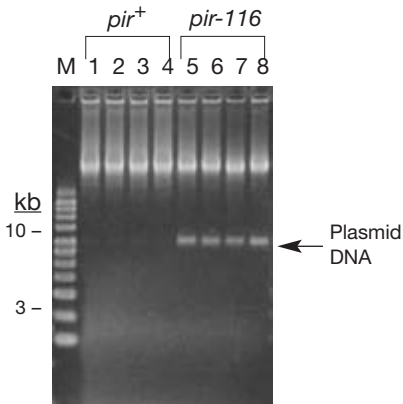
[www.epicentre.com/kan2\\_insert.asp](http://www.epicentre.com/kan2_insert.asp)

EZ-Tn5™ <oriV/KAN-2> Insertion Kit  
EZI02VK 10 Reactions

# EPICENTRE EZ-Tn5™ Insertion Kits

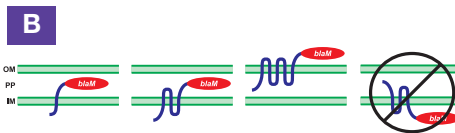
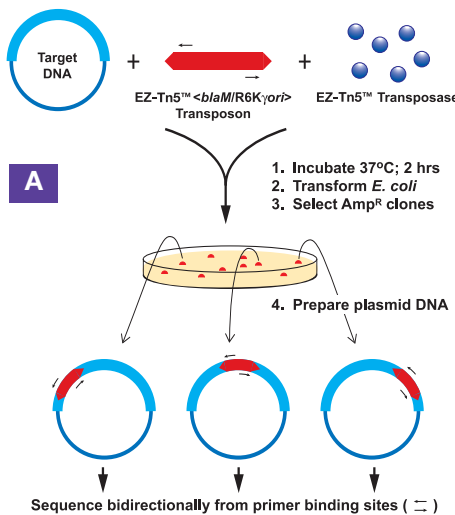
## “Rescue” Plasmid and Mitochondrial DNA

The EZ-Tn5™ <R6Kγori/KAN-2> Insertion Kit facilitates propagation and sequencing of circular DNA molecules, such as plasmids and mitochondrial DNAs, that would not otherwise replicate in *E. coli*. The EZ-Tn5 Transposon containing an *E. coli* R6Kγ origin of replication and a kanamycin resistance marker is randomly inserted into target DNA. An aliquot of the reaction is then transformed into TransforMax™ EC100D™ *pir*<sup>+</sup> or TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* which express the *pir* gene product required for replication from R6Kγori, and insertion clones are selected on kanamycin plates. The resulting library of random gene knockouts can also be used to facilitate genetic analysis of plasmid-encoded genes.



A plasmid containing the EZ-Tn5™ <R6Kγori/KAN-2> Transposon can be maintained in TransforMax™ EC100D™ *pir*<sup>+</sup> cells at approximately 15 copies per cell (Lanes 1-4) or TransforMax™ EC100D™ *pir*-116 cells at approximately 250 copies per cell (Lanes 5-8).

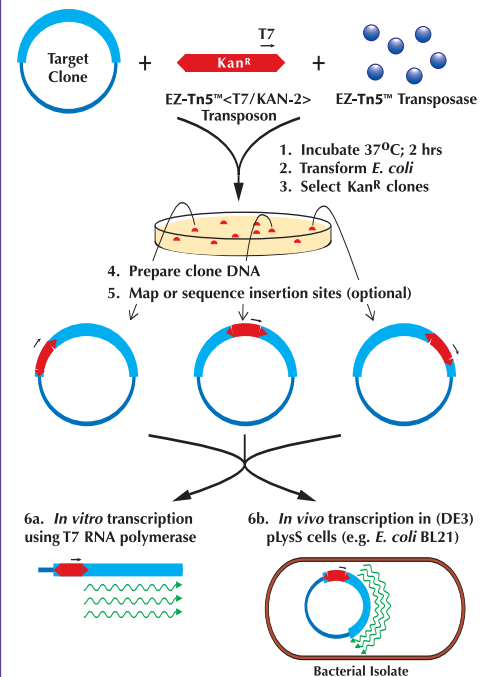
## Direct Selection of Genes Encoding Non-Cytoplasmic Proteins



The EZ-Tn5™ β-Lactamase Fusion Kit was developed for the direct selection of genes encoding membrane and secreted proteins. The kit features the EZ-Tn5™ <blaM/R6Kγori> Transposon which contains a β-lactamase gene (*blaM*) that lacks both promoter and secretory signal sequences. The transposon is randomly inserted into target DNA which is then transformed into *E. coli* and selected on ampicillin (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 (B).

## Synthesize RNA from Any Region of Cloned DNA

The EZ-Tn5™ <T7/KAN-2> Promoter Insertion Kit provides an easy and reliable method to randomly insert a phage T7 RNA polymerase promoter into any target DNA. The transposon does not have a transcription termination sequence so RNA can be produced from chosen insertion clones by *in vitro* transcription from the T7 promoter using, for example, AmpliScribe™ T7 High Yield Transcription Kit, AmpliCap™ T7 High Yield Message Maker Kit, DuraScribe® T7 Transcription Kit or T7 RNA Polymerase. RNA can also be generated for *in vivo* expression studies after transformation of cells having a T7 RNA polymerase gene.



[www.epicentre.com/r6gori\\_kan2\\_insert.asp](http://www.epicentre.com/r6gori_kan2_insert.asp)

### EZ-Tn5™ <R6Kγori/KAN-2> Insertion Kit

EZI011RK 10 Reactions

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

ECP09500 5 X 100 µl

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

EC6P095H 5 X 100 µl

[www.epicentre.com/fusion.asp](http://www.epicentre.com/fusion.asp)

### EZ-Tn5™ β-Lactamase Fusion Kit

EZI31BL 10 Reactions

[www.epicentre.com/t7\\_kan2\\_promoter.asp](http://www.epicentre.com/t7_kan2_promoter.asp)

### EZ-Tn5™ <T7/KAN-2> Promoter Insertion Kit

EZI03T7 10 Reactions

Based on user suggestions, we will gradually replace the “EZ::TN™” name by “EZ-Tn5™” so it will be easier to distinguish our hyperactive Tn5-based transposon products from our “HyperMu™” products.

# Environmental Genomics Studies Use EPICENTRE's Fosmid Kits to Generate Libraries

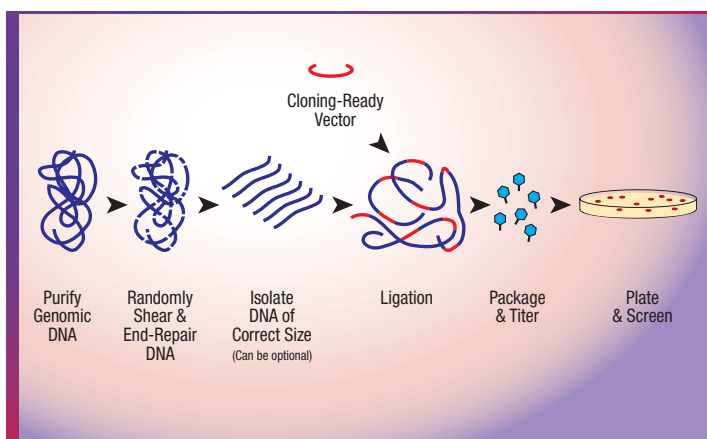
Merriann Carey, EPICENTRE

EPICENTRE's first kit for genomic library production was the pWEB™ Cosmid Cloning Kit. The kit's novel cloning strategy generates 40-kb fragments by random shearing, rather than partial restriction enzyme digestion (Figure 1). This approach eliminates the need for very high molecular weight genomic DNA, required for partial digestion, and results in a truly random library with better sequence coverage.

The pWEB™ Vector, however, contains a ColE1 origin of replication, which maintains clones at a relatively high-copy number. Continuous propagation of some clones at high-copy number results in deletions, inversions, and rearrangements of the insert DNA. Having observed this loss in library fidelity, Kim *et al.* developed a new type of cosmid, the fosmid.<sup>1</sup>

Fosmids contain an F factor replication origin and are stably maintained in *E. coli* at one copy per cell. To document the benefits of working at single-copy, Kim *et al.* prepared human genomic libraries in fosmid and cosmid vectors, grew each serially for multiple generations, and analyzed the restriction patterns of randomly chosen clones. Nearly 50% of the cosmid clones showed altered restriction patterns, primarily due to large deletions, within 20 to 40 generations after primary culture. When DNA that proved to be unstable in a cosmid was re-cloned into a fosmid vector, the authors detected no changes in restriction patterns, even after 80 generations of growth.<sup>1</sup>

Convinced of the merits of working at single-copy, EPICENTRE developed the EpiFOS™ Fosmid Library Production Kit for generating cosmid-sized clones that are maintained at one copy per cell. The EpiFOS Kit is being used in the new field of "metagenomics," or environmental genomics. Scientists wanting to tap into the complexities of uncultivated microbial communities are cloning large genomic fragments directly from the environment rather than from a flask.



**Figure 1. Overview of the production process for a library of 40-kb inserts using an EPICENTRE cosmid or fosmid vector.**

Edward DeLong, a leader in this field, used the EpiFOS Kit to create genomic libraries of methane-oxidizing archaeal populations found in marine sediments.<sup>2,3</sup> His lab recognized the "high degree of fidelity" fosmids offer, reasoning that the fosmid library "would likely reflect the composition of the sample and that the individual clones would accurately preserve the arrangement of cloned genes."<sup>4</sup>

Christa Schleper and co-workers used the EpiFOS Kit to archive 3 Gbp of DNA from soil and discovered "a huge variety of genomic DNA." They also obtained a very respectable cloning efficiency – around 51,000 clones from a single lambda packaging reaction.<sup>5</sup>

Fosmids have one negative "side-effect" – low DNA yields. EPICENTRE's remedy was an inducible, high-copy origin, "oriV". The CopyControl™ Fosmid Library Production Kit includes a cloning-ready fosmid vector that contains both *oriV* and F factor replication origins. In the absence of an inducer, replication proceeds from the F factor replicon, which maintains the vector at one copy per cell. Addition of an inducer to the growth medium drives replication from the *oriV*, producing up to 50 copies of a clone per cell. Unlike constitutively high-copy cosmids, induced CopyControl fosmid clones are held at high copy for 4 hours or less, and the insert instability associated with cosmids has not been observed.

The CopyControl Fosmid Kit has also played a part in some very exciting science. The Joint Genome Institute, which sequences both microbial communities and individual microbes (over 60 genomes), uses the CopyControl Fosmid Kit as standard operating procedure for production sequencing.<sup>6</sup>

Oak Ridge National Labs, also interested in high-throughput techniques, is developing fosmid microarrays to identify novel gene sequences in different environmental samples<sup>7</sup> using the CopyControl Fosmid Kit.

CopyControl Fosmid Kit.

Muller *et al.* are screening CopyControl fosmids to determine how bioactive metabolites associated with the symbiotic relationship between sponges and bacteria are produced. These authors obtained  $4 \times 10^7$  colony forming units per microgram of bacterial DNA and averaged 5  $\mu$ g of fosmid DNA from 1-ml cultures induced to higher copy-number.<sup>8</sup>

As genomic library technology progresses, EPICENTRE continues to provide innovative library production tools and techniques.

## References

1. Kim, U.J. *et al.* (1992) *Nucleic Acids Res.* **20**, 1083.
2. Hallam, S.J. *et al.* (2003) *Appl. Environ. Microb.* **69**, 5483.
3. Hallam, S.J. *et al.* (2004) *Science* **305**, 1457.
4. Stein, J.L. *et al.* (1996) *J. Bacteriol.* **178**, 591.
5. Treusch, A.H. *et al.* (2004) *Environ. Microbiol.* **6**, 970.
6. [www.jgi.doe.gov/sequencing/protocols/prots\\_production.html](http://www.jgi.doe.gov/sequencing/protocols/prots_production.html)
7. Fields, *et al.* (2003) Oak Ridge National Lab, Directed Research and Development, Annual Report, Project Number: 3211-2029
8. Muller, W.E.G. *et al.* (2004) *Appl. Environ. Microb.* **70**, 2332.

[www.epicentre.com/fosmid.asp](http://www.epicentre.com/fosmid.asp)

### CopyControl™ Fosmid Library Production Kit

CCFOS110 1 Kit  
For producing up to 10 complete and unbiased fosmid libraries.

# Quickly Extract DNA from Tissue in 8 Minutes

By Bruce W. Jarvis, EPICENTRE

QuickExtract™ DNA Extraction Solution 1.0 prepares PCR-ready DNA from animal tissue, hair follicles, feather quills, and mouse tail snips.<sup>1</sup> The classic protocol uses incubations at two different temperatures, for a total of 46 minutes. This report shows the results from a rapid 8-minute procedure (Table 1) to process solid tissue, such as mouse liver and mouse tail snips.

Table 1.

Protocol	Minutes at 65°C	Minutes at 98°C	Total Minutes
Classic	30	16	46
Rapid	6	2	8

The rapid protocol saves time and is convenient for multiple samples and high throughput techniques, but does not extract the maximum DNA from tissues (Figure 1). Depending upon which parameter is more important, one can optimize the DNA yield with the classic protocol or save time with the rapid protocol. In either case, the QuickExtract Solution provides DNA that can be efficiently amplified by PCR.

For DNA in QuickExtract Solution, the recommended storage temperature is -20°C. In a stringent test of DNA stability,

DNA was extracted from mouse liver with QuickExtract Solution, using both the classic and rapid protocol, and subsequently stored at 37°C for 4 weeks. After 4 weeks, QuickExtract DNA samples were tested for stability by PCR using EPICENTRE's FailSafe™ PCR System as described in Figure 2. Band intensities are comparable for PCR products amplified from DNA extracted by either protocol, indicating that subjecting the DNA to extreme conditions (37°C for 4 weeks) did not affect template fidelity for PCR (Figure 2).

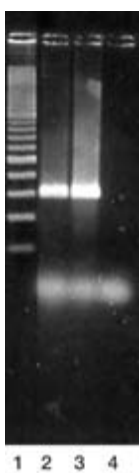
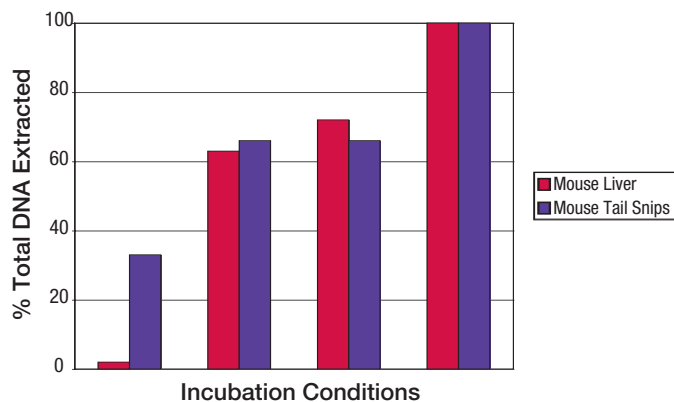


Figure 2. PCR results using mouse liver DNA stored in QuickExtract™ DNA Extraction Solution at 37°C for 4 weeks. DNA in QuickExtract Solution (1 µl) was amplified using the FailSafe™ PCR System with forward primer 5'-GCC CGG ACA CTT TCT CTG ATG and reverse primer 5'-AGA CTG CTG CTG ACG ACA CAC G for the IRF-1 gene, amplifying a 300-bp DNA fragment. Cycle conditions were 95°C (2 minutes) and 30 cycles of 95°C (30 seconds), 55°C (30 seconds), and 74°C (30 seconds). Lane 1, 100-bp ladder; Lane 2, PCR product from mouse liver DNA extracted by the classic protocol; Lane 3, PCR product from mouse liver DNA extracted by the rapid protocol; Lane 4, no template PCR.

Figure 1. Relative yields of DNA extracted by different incubation regimens using QuickExtract™ DNA Extraction Solution 1.0. Mouse liver and tail snips were incubated in QuickExtract Solution for the indicated times. After each incubation regimen the supernatant was assayed for DNA by fluorimetry with Hoechst dye 33258. Extracted DNA values are expressed as a percentage of the maximum amount extracted.



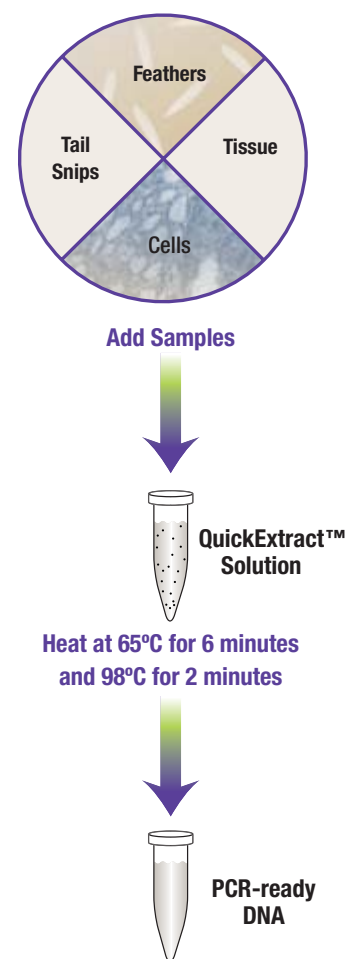
Minutes at 65°C	1	6	20	30
Minutes at 98°C	2	2	10	16
		Rapid Protocol		Classic Protocol

## Conclusion

For tissue, such as mouse liver and tail snips, the 8-minute QuickExtract DNA Extraction Solution protocol prepares PCR-ready DNA that is stable during long-term storage. However, if maximum DNA yield is more important than speed, the classic 46-minute protocol is still recommended.

## References

- Meis, J.E. and F. Chen (2003) EPICENTRE Forum 10 (2), 8.



[www.epicentre.com/buccalamp.asp](http://www.epicentre.com/buccalamp.asp)

QuickExtract™ DNA Extraction Solution 1.0  
QE09050 50 ml

Bulk solution, sufficient to perform 100 extractions.

# Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

## Questions about QuickExtract™ DNA Extraction Solution

**Q.** Can I restriction digest, clone, or directly sequence the extracted DNA prepared with QuickExtract™ DNA Extraction Solution?

**A.** No. The extracted DNA amplifies efficiently by PCR, but the extraction procedure does not remove proteins and other cell debris and the DNA cannot be used for restriction digests, cloning, or direct sequencing. The resulting PCR product, amplified from a specific genomic DNA target, can be purified and restriction digested, cloned, or sequenced.

**Q.** What is the best way to quantify DNA extracted from samples using the QuickExtract Solution?

**A.** Because there is residual degraded RNA in the sample, using OD<sub>260</sub> to quantify the DNA will give an artificially high estimate of the DNA concentration. The best method to quantify the DNA is by fluorimetry using a DNA-specific dye, such as Hoechst 33258<sup>1</sup> (bisbenzimidazole), or PicoGreen®. These dyes bind specifically to double-stranded DNA and not to nucleotides, single-stranded DNA, or RNA.

**Q.** Can I use the new 3-minute QuickExtract protocol<sup>2</sup> with any sample?

**A.** For individual cells, such as buccal, HeLa, or bacterial (gram-positive or gram-negative), we recommend the 3-minute protocol. We have found no difference in the resulting PCR amplifications between the 3-minute protocol and the standard 46-minute procedure. However, not all samples behave the same and may require different incubation times. For a more rapid protocol with samples such as animal tissue, mouse tails, hair follicles, or feather quills, we recommend an 8-minute protocol (please see article on page 18). If you are uncertain about

which procedure to use, please contact us about extraction information for your specific sample type.

**Q.** What kinds of samples have been successfully extracted with QuickExtract DNA Extraction Solution?

**A.** So far, we and our customers have successfully extracted and amplified DNA from buccal cells (human, bovine, and murine), gram-positive and gram-negative bacteria, hair follicles, chicken feather quills, blood (liquid or dried on either Guthrie cards or filter paper), and organ and tissue samples, such as liver.

**Q.** Can the QuickExtract DNA Extraction Solution be used with samples in forensic analysis?

**A.** Yes, QuickExtract DNA Extraction Solution has been tested and used by the California Department of Justice to obtain DNA for different forensic analyses. Please contact us for more information.

**Q.** Can DNA prepared with QuickExtract Solution be used in whole genome amplification procedures, such as GenomiPhi™, REPLI-g™ or TempliPhi™ Multiple Displacement Amplifications (MDA)?

**A.** Yes. A recent paper<sup>3</sup> cites the use of DNA extracted using EPICENTRE's QuickExtract Solution with Amersham's TempliPhi kit and Molecular Staging's REPLI-g kit for Multiple Displacement Amplification of the DNA. The authors state that under certain conditions MDA enhances downstream PCR, genotyping, and fingerprinting results.

**Q.** What is the difference between the QuickExtract DNA Extraction Solution and EPICENTRE's MasterAmp™ DNA Extraction Solution?

**A.** The MasterAmp DNA Extraction Solution has a different formulation, which includes a proprietary bead matrix that was used to remove PCR inhibitors from the DNA extract. This matrix was later found to be unnecessary, especially in non-buccal cell sample types, and is not used in the QuickExtract Solution.

### References

1. Hoffman, L. and Moan, E. (1998) *EPICENTRE Forum* 5(4), 1.
2. Jarvis, B.W. (2004) *EPICENTRE Forum* 11(4), 4.
3. Sorensen, K.J. et al. (2004) *Anal. Biochem.* 324, 312.

*PicoGreen® is a registered trademark of Molecular Probes Invitrogen Detection Technology*

Thanks, Hank.

I was just reading the EPICENTRE Forum and read how good the customer service is (well you could be biased). Now this just proves to me that you are wonderful and excellent and comments in the Forum are true.

Keep up the good work. Thanks again.

Regards,

Dilip Dias  
Transgenic Plant Analysis Group  
Garst Seed Company  
Slater, IA



# Transposon-Based Method to Create and Express N-Terminal and C-Terminal Protein Deletions

Mike Fandt, EPICENTRE

## Introduction

Characterizing a protein, whether to understand the relationship between structure and function, map a domain or epitope, or ultimately create a chimeric enzyme, often requires a set of nested deletion mutants. Creating deletions using pre-existing restriction enzyme sites limits the depth of such a collection. Timed nuclease digestion methods are tedious and require careful monitoring at each reaction step.

EPICENTRE offers two new transposon-based kits, the EZ-Tn5™ Protein Truncation Kit and the Mu-End™ Protein Truncation Kit, as simple and reliable alternatives. Used consecutively, these kits can generate a library of unidirectional deletions from the N-terminal and C-terminal ends of any protein coding sequence. The transposon-derived deletions are propagated in *E. coli* as kanamycin-resistant “rescue” clones that can be expressed from a T7 promoter.

## Methods and Results

### Creating transposon insertions

The EZ-Tn5 Protein Truncation Kit features the EZ-Tn5™ <p15Aori / KAN-2 / T7Exp> Transposon, which contains the low-copy p15A origin of replication, a kanamycin resistance marker, and a T7-promoter region (Figure 1). First, a simple, *in vitro* reaction catalyzed by EZ-Tn5™ Transposase is used to randomly insert this EZ-Tn5 Transposon into any target DNA. In this work and in a similar study,<sup>1</sup> the *E. coli* DNA polymerase I gene (*polA*), which contains three well-characterized domains, was chosen as a model target. A *polA* PCR product was used in the 2-hour transposition reaction, but the target DNA can be almost any DNA containing the desired sequence (e.g., an existing plasmid, a fosmid or cosmid clone, or a restriction fragment).

### PCR amplification and cloning

Next, the transposition reaction is amplified by PCR (Figure 2) using one primer to the transposon end and the other primer to a fixed point in the target sequence. In this example, the transposition reaction was amplified with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, P15 FP-1, and the 3'-end *polA* primer, POL RP. Since the transposon is randomly inserted along

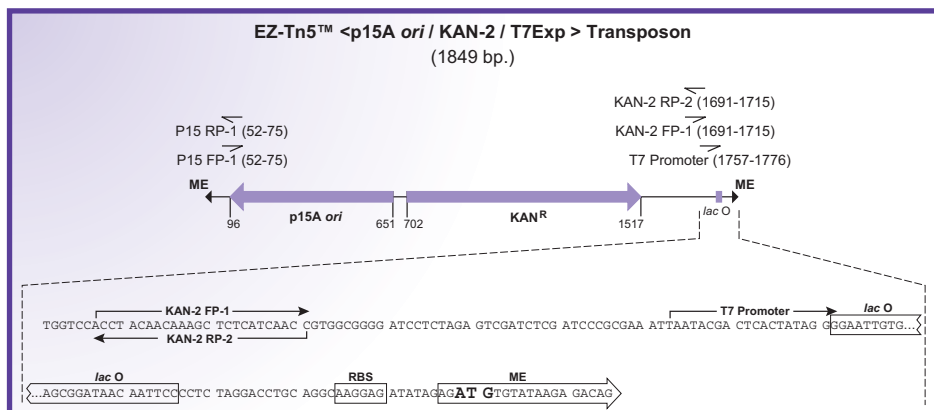


Figure 1. A schematic representation of the EZ-Tn5™ <p15Aori / KAN-2 / T7Exp> Transposon. ME refers to the 19-bp “mosaic end” sequences required for transposition.

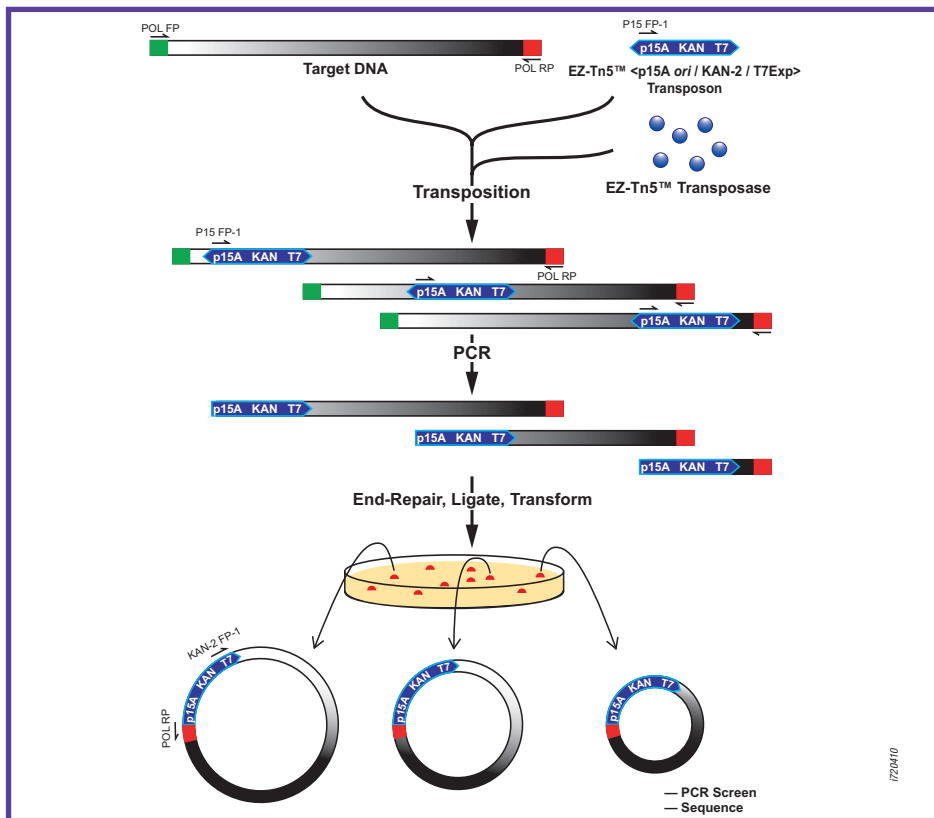
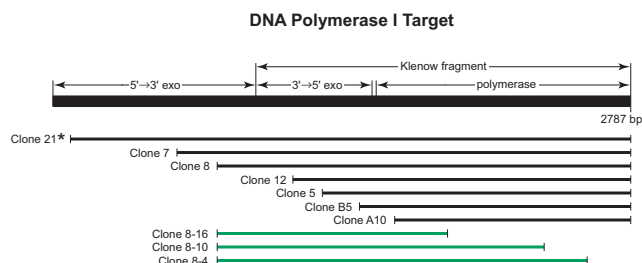


Figure 2. The EZ-Tn5™ Protein Truncation Kit can be used to create random, unidirectional deletions from the 5'-end (shown here) or 3'-end of a target sequence.

the length of the *polA* gene, amplification with this primer pair generates a library of N-terminal deletions. Likewise, amplification with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, KAN-2 RP-2, and the 5'-end *polA* primer, POL FP, generates a library of C-terminal deletions (data not shown).

PCR products will contain a portion of the sequence of interest, the p15A origin of replication, and a kanamycin-resistance marker. Using the End-Repair Mix and Fast-Link™ DNA Ligase provided in the kit, PCR products are blunt-ended and self-ligated to create a library of “rescue” clones that can replicate after transformation into standard *E. coli* strains.



**Figure 3. Unidirectional deletions in *polA* were generated using the EZ-Tn5™ Protein Truncation Kit (black lines) and the Mu-End™ Protein Truncation Kit (green lines).** Asterisk indicates an insertion that is not in frame, and cannot be expressed using the T7 promoter.

**Screening**

Colonies can be easily screened, and the size of the target DNA deletion estimated, using the Colony Fast-Screen™ Kit (PCR Screen) and the appropriate primers. For example, the *polA* clones containing N-terminal deletions were screened with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, KAN-2 FP-1, and the 3'-end *polA* primer, POL RP. PCR products in the size range of interest were then sequenced with the same terminal transposon primer, KAN-2 FP-1, to determine precisely where the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon has inserted.

**Expression of N-terminal deletions**

Each rescue clone with an N-terminal deletion will also contain a transposon-derived T7-promoter region, which includes the lac operator (lacO), a ribosome-binding site, and an ATG start site. At least one-third of these clones will generate in-frame protein fusions that can be expressed in cells containing an inducible T7 RNA polymerase gene (e.g., *E. coli* BL21). The EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon fusion protein contains only 6 transposon-encoded amino acids.

Clone 8, for example, results in a 264-amino acid, N-terminal deletion that creates an in-frame fusion with *polA* (Figure 3). Expression in a BL21 host generates the expected 74-kD protein which is slightly larger than the Klenow fragment. An in-gel assay for DNA polymerase activity shows that the fusion is capable of DNA synthesis (Figure 4).<sup>2</sup> Clones 12 and 5 result in 387- and 435-amino acid deletions, respectively. They do not retain polymerase activity, even though both contain the entire polymerase domain (Figure 3). Presumably, more of the 3'→5' exonuclease domain is required for proper folding of the active polymerase.

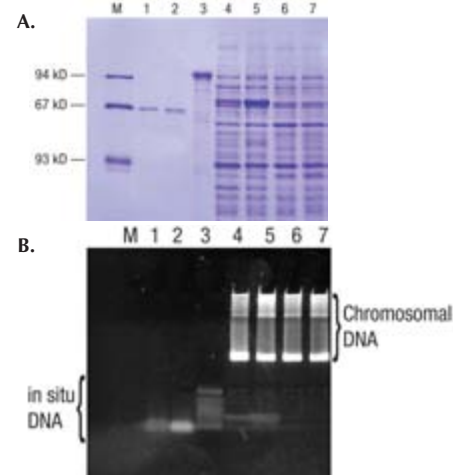
**Making C-terminal deletions**

The Mu-End Protein Truncation Kit was developed to make unidirectional deletions from the carboxyl end of proteins generated with the EZ-Tn5 Protein

Truncation Kit. This kit features a 56-bp fragment that contains the R1 and R2 end sequence of a bacteriophage Mu transposon. An *in vitro* transposition reaction, catalyzed by EPICENTRE's HyperMu™ Transposase, randomly inserts the Mu-end into a selected clone.

PCR amplification of the resulting insertion reaction, using Mu-specific and EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon-specific primers, generates a library of deletions, which retain the transposon-derived origin, kanamycin marker, and T7 promoter region. These products can be "rescued" as plasmids following the end-repair and ligation steps described above. The Mu-specific primer used in the amplification contains stop codons in all three reading frames to avoid adding unwanted amino acids.

The Mu-End Protein Truncation Kit was used to create C-terminal deletions in



**Figure 4. Analysis of *polA* deletion clones by SDS-PAGE and an in-gel DNA polymerase activity assay.** **A.** SDS-PAGE analysis of *polA* truncations. Samples in lanes 1 to 3 are purified protein. Samples in lanes 4 to 7 are crude extracts. **Lane 1**, Klenow fragment; **Lane 2**, Klenow fragment with a point mutation in the 3'→5' exo domain; **Lane 3**, *E. coli* DNA polymerase I; **Lane 4**, Clone 8, uninduced; **Lane 5**, Clone 8, induced; **Lane 6**, Clone 8-10, uninduced; **Lane 7**, Clone 8-10, induced. **M**, MW marker. **B.** An SDS-PAGE gel containing a poly(dA-dT) substrate was run with the same samples as in A. After being subjected to renaturation conditions, the gel was incubated with dATP and dTTP, stained with SYBR® Gold, and bands with polymerase activity were visualized with UV light.

Clone 8. Clones were PCR screened with the Colony Fast-Screen Kit for deletions in the desired size range. Selected clones were sequenced with the transposon primer, P15 RP-1, to precisely define the Mu-end insertion site. Clone 8-10, for example, has a 139-amino acid deletion from the C-terminal end of Clone 8 (Figure 3). When the resulting 58-kD protein was expressed, DNA polymerase I activity was not detected in an in-gel assay (Figure 4).

**Summary**

The transposon-based EZ-Tn5 Protein Truncation Kit and the Mu-End Protein Truncation Kit provide convenient methods for generating libraries of N-terminal and/or C-terminal protein deletions that can be expressed in *E. coli*. Although not emphasized here, these kits are also useful for the functional characterization and sequencing of genes and regulatory elements, by allowing the generation of unidirectional libraries from either end of a target DNA.

**References**

1. Fiantdt, M. (2004) 30th Steenbock Sympos.; May 20-23; Madison, WI. Poster 15.
2. Spanos, A. and Hübscher, U. (1993) In: Hirs, C. Methods in Enzymology Vol. 91. San Diego; Academic Press. 263.

[www.epicentre.com/truncation.asp](http://www.epicentre.com/truncation.asp)

**EZ-Tn5™ Protein Truncation Kit**  
EZI41110      10 Reactions  
**Contents:**  
EZ-Tn5™ Transposase  
EZ-Tn5™ <p15Aori /KAN-2 /T7Exp> Transposon  
EZ-Tn5™ 10X Reaction Buffer  
EZ-Tn5™ 10X Stop Solution  
Control Target DNA  
Sterile Water  
End-Repair Enzyme Mix  
End-Repair 10X Buffer  
ATP  
dNTPs  
Fast-Link™ DNA Ligase  
10X Fast-Link™ Buffer  
PCR Precipitation Solution  
P15 RP-1  
P15 FP-1  
KAN-2 RP-2  
KAN-2 FP-1

**Mu-End™ Protein Truncation Kit**  
HMI41110      10 Reactions  
**Contents:**  
HyperMu™ Transposase  
Mu-End™ Transposon  
HyperMu™ 10X Reaction Buffer  
HyperMu™ 10X Stop Solution  
Sterile Water  
End-Repair Enzyme Mix  
End-Repair 10X Buffer  
ATP  
dNTPs  
Fast-Link™ DNA Ligase  
10X Fast-Link™ Buffer  
PCR Precipitation Solution  
MU-1 RP-1



# Rapidly Screen Bacterial RNA Polymerase Inhibitors Using Innovative Kool™ NC-45™ Technology

Dr. Eric Kool observed that certain small (28 to 150 nucleotides) circular, single-stranded DNAs (ssDNA nanocircles) are very efficiently transcribed *in vitro*, without requiring promoter sequences or primers, by a variety of DNA-dependent RNA Polymerases. EPICENTRE offers the first commercially available products based on Dr. Kool's patented Rolling Circle Transcription (RCT) technology,<sup>1,2,3</sup> the **Kool™ NC-45™ Universal RNA Polymerase Template** and the **Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit**. These products enable the

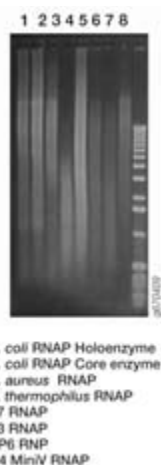
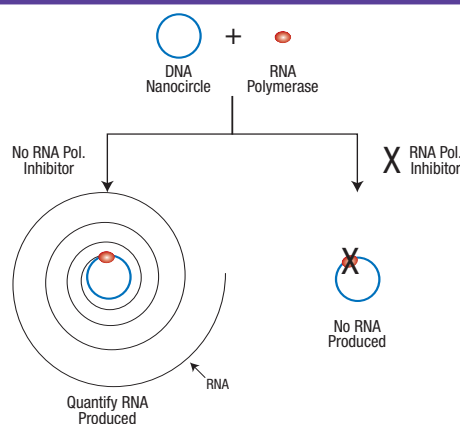
user to rapidly assay the activity of any prokaryotic RNA polymerase or screen for inhibitors of *E. coli* or other bacterial RNA polymerases (Figure 1).

The Kool NC-45 Template, which forms the basis of these unique products, is a circular 45-base ssDNA that functions as a template for phage and bacterial RNA polymerases in *in vitro* transcription reactions. The Kool NC-45 Template has been tested with a number of bacterial and phage RNA polymerases as shown in Figure 2.

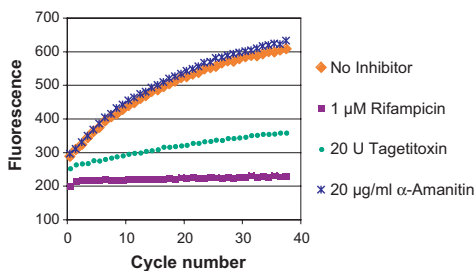
The Kool NC-45 RNAP Activity & Inhibitor Screening Kit contains the Kool NC-45 Template to assay activity and screen compounds for inhibition of *E. coli* RNA polymerase. The kit provides *E. coli* RNA Polymerase (Core Enzyme) and SYBR® Green I dye for real-time detection of RNA polymerase activity in the presence and absence of potential inhibitors (Figure 3).

Real-time detection methods using SYBR® Green, SYBR® Gold, RiboGreen® and Molecular Beacons,<sup>4</sup> as well as end-point detection methods (e.g., gel electrophoresis or radioactivity) can be used to monitor RNA polymerase activity with both the Kool NC-45 Universal RNA Polymerase Template and the Kool NC-45 RNAP Activity & Inhibitor Screening Kit.

**Figure 1. The Kool™ NC-45™ Universal RNA Polymerase Template and the Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit provide a rapid and easy method to screen compounds for inhibition of *E. coli* and other bacterial RNA Polymerases.** In the absence of an RNA Polymerase inhibitor, large amounts of RNA are produced from the Kool NC-45 Template (provided in the screening kit and sold separately). The RNA can be detected by real-time fluorescent methods or end-point detection methods. In the presence of a functional inhibitor, no RNA is produced.



**Figure 2. Rolling circle transcription of Kool™ NC-45™ Universal RNA Polymerase Template.** Kool NC-45 Template (50 ng) was incubated for 1 hour at 37°C in 1X reaction buffer containing the following RNA polymerases: **Lane 1**, *E. coli* RNA polymerase Holoenzyme; **Lane 2**, *E. coli* RNA polymerase core enzyme; **Lane 3**, *S. aureus* RNA polymerase; **Lane 4**, *T. thermophilus* RNA polymerase; **Lane 5**, T7 RNA polymerase; **Lane 6**, T3 RNA polymerase; **Lane 7**, SP6 RNA polymerase; **Lane 8**, MiniV™ RNA polymerase. Reaction products were analyzed by agarose gel electrophoresis.



**Figure 3. The Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit using real-time detection of rolling circle transcription by *E. coli* RNA Polymerase in the presence and absence of inhibitors.** Transcription is strongly inhibited by rifampicin, a known inhibitor of bacterial RNA Polymerase,<sup>5</sup> and partially inhibited by tagetitoxin,<sup>6</sup> while α-amanitin, an inhibitor of eukaryotic RNA polymerase II, has no effect.

## References

1. Daubendiek, S.L. et al. (1995) *J. Am. Chem. Soc.* **117**, 7818.
2. Diegelman, A.M. et al. (1998) *Nucleic Acids Res.* **26**, 3235.
3. Daubendiek, S.L. et al. (1997) *Nat. Biotechnol.* **15**, 273.
4. Marras, S.A. et al. (2004) *Nucleic Acids Res.* **32**, e72.
5. Hartmann, G. et al. (1967) *Biochim. Biophys. Acta* **145**, 843.
6. Mathews, D.E. and Durbin, R.D. (1990) *J. Biol. Chem.* **265**, 493.

[www.epicentre.com/kool.asp](http://www.epicentre.com/kool.asp)

**Kool™ NC-45™ Universal RNA Polymerase Template**  
(for Bacterial RNA Polymerases)  
KN411100 100 pmoles  
(100 Reactions)

**Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit**  
(for Bacterial RNA Polymerases)  
KNK49025 25 Reactions

### Contents:

- Kool™ NC-45™ Template
- E. coli* RNA Polymerase (Core enzyme)
- Reaction Buffer
- NTPs
- SYBR® Green I dye

*SYBR® and RiboGreen® are registered trademarks of Molecular Probes Invitrogen Detection Technologies.*

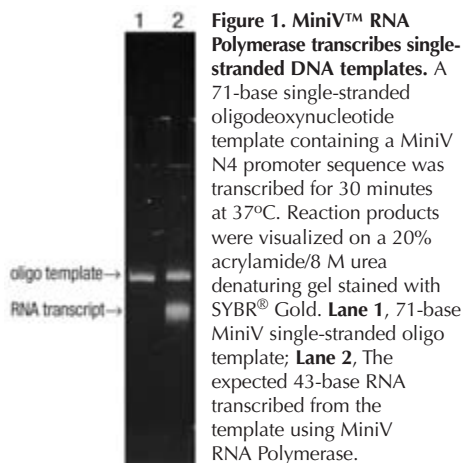
New!

## Transcribe ssDNA Templates with Single-Stranded Promoters Using the MiniV™ *In Vitro* Transcription Kit

EPICENTRE, the leader in *in vitro* transcription technology, is pleased to introduce the new MiniV™ *In Vitro* Transcription Kit. The MiniV *In Vitro* Transcription Kit enables users to produce RNA transcripts *in vitro* from single-stranded DNA (ssDNA) templates that are joined to a single-stranded N4 bacteriophage promoter.

MiniV™ RNA Polymerase\* is the transcriptionally-active 1,106-amino acid domain of the N4 virion RNA polymerase. The enzyme lacks RNA strand displacement and unwinding activity on RNA:DNA hybrids and requires *E. coli* Single-Stranded DNA Binding Protein (EcoSSB) to displace the RNA strand from the DNA template for efficient *in vitro* transcription.

Linear templates of any length, including oligodeoxynucleotides and single-stranded cDNAs, that contain an N4 promoter can be transcribed. Functional N4 transcription promoter sequences (19 to 21 bases in length) are provided in the product



**Figure 1. MiniV™ RNA Polymerase transcribes single-stranded DNA templates.** A 71-base single-stranded oligodeoxynucleotide template containing a MiniV N4 promoter sequence was transcribed for 30 minutes at 37°C. Reaction products were visualized on a 20% acrylamide/8 M urea denaturing gel stained with SYBR® Gold. **Lane 1**, 71-base MiniV single-stranded oligo template; **Lane 2**, The expected 43-base RNA transcribed from the template using MiniV RNA Polymerase.

literature and on EPICENTRE's website at [www.epicentre.com](http://www.epicentre.com). The yield of RNA from a MiniV transcription reaction varies depending on the sequence of the DNA template. Typically 1 to 5 moles of RNA are produced per mole of ssDNA template.

[www.epicentre.com/miniv.asp](http://www.epicentre.com/miniv.asp)

### MiniV™ *In Vitro* Transcription Kit

MV41025 25 Reactions

#### Contents:

MiniV™ RNA Polymerase (with RNase Inhibitor)

MiniV™ 5X Transcription Buffer

ATP, CTP, GTP, UTP

DTT

*E. coli* SSB Protein

RNase-free DNase I

MiniV™ ssDNA Control Template

RNase-free Water

\*Covered by U.S. patent Application No. 2003/0096349 and related patent applications in other countries.

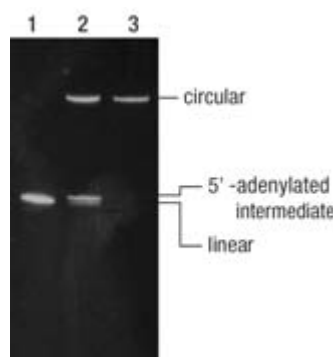
SYBR® is a registered trademark of Molecular Probes Invitrogen Detection Technologies.

New!

## Produce Templates for Rolling Circle Replication and Transcription in 1 Hour Using CirLigase™ ssDNA Ligase

CirLigase™ ssDNA Ligase is a thermo-stable, ATP-dependent ligase that catalyzes intramolecular ligation (circularization) of single-stranded DNA (ssDNA) with 5'-phosphate and 3'-hydroxy termini. Linear ssDNA >30 bases can be circularized by CirLigase ssDNA Ligase.

Traditional methods for preparing circular ssDNA involve the use of oligo "splints" spanning the 5'- and 3'- termini of the oligonucleotide to be circularized. Even under the best of conditions, both linear and circular concatamers are produced and extensive purification is necessary to remove them. In contrast, the standard 60-minute CirLigase reaction, produces virtually no linear or circular concatamers, making the enzyme ideal for easily and rapidly preparing closed-circular ssDNA templates for rolling circle replication or rolling circle transcription.



**Figure 1. Under standard reaction conditions, CirLigase™ ssDNA Ligase converts linear single-stranded DNA (ssDNA) into circular ssDNA.**

A 71-base ssDNA oligo was converted to circular DNA using CirLigase ssDNA Ligase. **Lane 1**, 71-base ssDNA oligo; **Lane 2**, Circularization proceeds through an adenylated intermediate; **Lane 3**, To confirm that the reaction product was a closed circle, the reaction was treated with exonuclease I, a DNase that specifically digests linear ssDNA.

### Applications

Produce circular ssDNA templates for:

- Rolling circle replication experiments.
- Rolling circle transcription experiments.
- RNA Polymerase activity and inhibitor screening assays. (See Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit on p. 22)

[www.epicentre.com/circligase.asp](http://www.epicentre.com/circligase.asp)

### CirLigase™ ssDNA Ligase

CL4111K 1000 Units

CL4115K 5000 Units

#### Contents:

CirLigase™ ssDNA Ligase

CirLigase™ 10X Reaction Buffer

ATP

50 mM MnCl<sub>2</sub>

CirLigase™ Linear ssDNA Control Substrate Water

# Screen *E. coli* Colonies by Restriction Enzyme Analysis without Overnight Cultures

## Colony Fast-Screen™ Kit (Restriction Screen) Determine size and orientation of clones

### Rapid—

Go from colonies to gel loading in 25 minutes or less.

### Flexible—

Use with any endA<sup>-</sup> *E. coli* host strain and for DNA fragments as low as 100 bp.

### Convenient—

Just lyse the cells, add restriction enzyme, and run a gel. No need for overnight cultures, plasmid preps, or lengthy restriction digests.

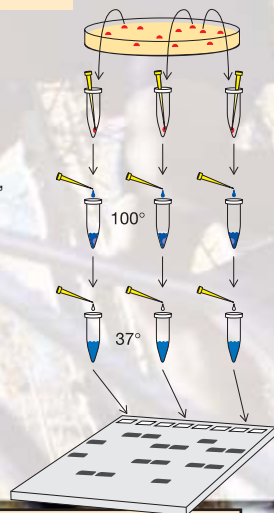
### 4 Quick Steps:

1. Pick colony into a microcentrifuge tube or microplate well.
2. Add Restricti-Lyso™ Solution, vortex, heat for 60 seconds at 100°C.
3. Perform restriction digestion on the entire tube of lysed cells for 15 minutes at 37°C.
4. Load gel, electrophorese, and stain.

### Colony Fast-Screen™ Kit (Restriction Screen)

FS0472H

Sufficient reagents to screen 200 colonies.



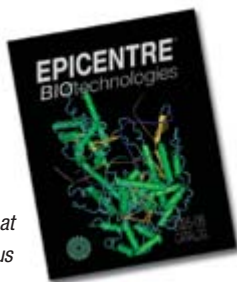
For more information, go to [www.epicentre.com](http://www.epicentre.com) and enter this QuickInfo code: **COA01**

## EPI Announcements, etc.

**New!**

**2005-2006 Catalog  
Mailing Soon**

Be sure that you are on our mailing list for the new catalog. You can go to our website at [www.epicentre.com/reply\\_card.asp](http://www.epicentre.com/reply_card.asp) or call us toll free (in the U.S.) at 800 284-8474.



## Meet DeAnna Mabis

If you e-mail [customerservice@epicentre.com](mailto:customerservice@epicentre.com), DeAnna Mabis is usually the person responding to your request. DeAnna also has primary responsibility for EPICENTRE's international orders, which includes working with our distributors and other international customers, providing pricing, order processing, customs documents, shipping, and order follow-up. DeAnna enjoys her work, and apparently her smile shines through the phone because the distributors and customers have had nothing but praise for DeAnna in the 9 1/2 years that she has been with EPICENTRE.



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