EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

Volume 11-5

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CopyCutter™ *E. coli* Strain Lowers Copy Number of Common Vectors

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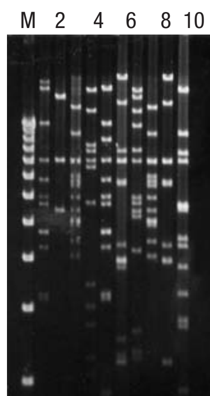

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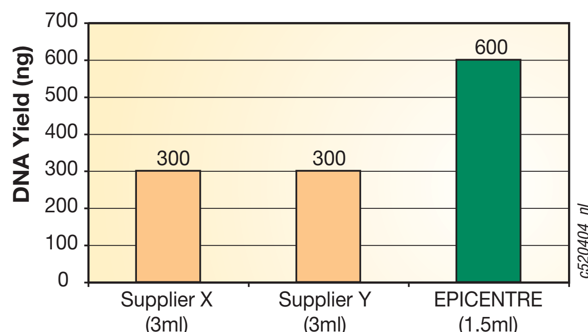

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EPICENTRE thanks Alexander Karasin for the use of his photograph taken in Wisconsin's Governor Dodge State Park. Alexander is an Associate Scientist at the University of Wisconsin-Madison in the Department of Pathobiological Sciences, School of Veterinary Medicine. He researches the molecular epidemiology and pathogenesis of influenza viruses, studying structural-functional relationships in influenza using reverse genetics.

Hands on the Cover:
Marlena Skarbek, EPICENTRE Product Quality Manager

APex™ Heat-Labile Alkaline Phosphatase Dephosphorylates Any DNA Ends in Just 15 Minutes

Ron Meis, EPICENTRE

A variety of alkaline phosphatases (APs) (Enzyme Commission number 3.1.3.1) have been used in common molecular biology techniques for decades. Applications include preventing cloning vector recircularization during ligations, preparing 5′-nucleic acid termini for 5′-end labeling with polynucleotide kinase, dephosphorylating DNA and RNA substrates, and nucleotide degradation in polymerase reactions, including PCR.[1] Each alkaline phosphatase used to date offers advantages, with some disadvantages. Now EPICENTRE introduces APex™ Heat-Labile Alkaline Phosphatase, with all of the benefits of previous alkaline phosphatases, without their drawbacks.

BAP, CIAP

The classic enzymes, Bacterial Alkaline Phosphatase (BAP) and Calf Intestinal Alkaline Phosphatase (CIAP), are inefficiently heat-inactivated, requiring substrate purification to remove the enzymes prior to subsequent manipulations.[1]

HK™ Phosphatase

In 1988, EPICENTRE introduced HK™ (**H**eat-**K**illable) Thermolabile Phosphatase, the first irreversibly heat-inactivated alkaline phosphatase.[2] Because HK Phosphatase is completely inactivated by heating at 65°C for 15 minutes, the enzyme doesn't need to be removed from the reaction, which makes it much more convenient to use than BAP or CIAP. The enzyme efficiently removes phosphates from RNA and from 5′-overhang DNA ends, but has reduced activity on blunt and 5′-recessed DNA ends, does not hydrolyze nucleotides, and requires addition of Ca^{++} to the buffer.

SAP

In the early 1990's, Northern shrimp was identified as a source for alkaline phosphatase.[3] Shrimp Alkaline Phosphatase (SAP) is irreversibly heat-inactivated (65°C for 15 minutes); active on blunt, 5′-overhang and 5′-recessed DNA ends; and hydrolyzes nucleotides. These properties have made SAP a popular choice for alkaline phosphatase applications. However, SAP is isolated from a non-recombinant source (shrimp-processing waste water),[4] requires extended reaction incubation times (up to 60 minutes for blunt-end DNA),[5] and shows reduced activity in many common restriction enzyme (RE) buffers, unless the buffers are supplemented.[5]

TAB5-AP

More recently TAB5 Antarctic Bacterial Alkaline Phosphatase (TAB5-AP) has been introduced.[6] TAB5-AP is isolated from a recombinant source, can be heat-inactivated, is active on all three types of DNA ends, and can hydrolyze nucleotides. However, the enzyme requires extended reaction incubation time (up to 60 minutes for 5′-recessed DNA ends)[5] and requires addition of a supplemental buffer to common RE buffers.[5]

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. Isolated from a recombinant source, this proprietary new enzyme is highly purified, with no nuclease contamination. Most applications require treatment with just 1 µl of APex Alkaline Phosphatase for 15 minutes at 37°C (>100 nmol P_i hydrolyzed). The enzyme is then quickly and irreversibly inactivated by a 5-minute incubation at 70°C.

Reaction and inactivation temperatures

While APex Heat-Labile Alkaline Phosphatase is optimally active at 37°C (typically 15 minutes), and is easily and irreversibly inactivated at 70°C (5 minutes), the enzyme is surprisingly tolerant of moderately elevated reaction temperatures and lengthened reaction times. For example, virtually 100% activity remained following overnight (18-hour) incubations at both room temperature and 37°C, and after a 3-hour incubation at 42°C. In fact, APex Alkaline Phos-phatase maintained ≥90% activity after 90-minute incubations at both 47°C and 50°C. Above 50°C, the half-life of the enzyme rapidly decreases to <12 minutes at 55°C, <5 minutes at 60°C, and <3 minutes at 65°C.

Dephosphorylates any DNA ends

The enzyme is fully active on blunt, 5′-overhang and 5′-recessed DNA ends, regardless of the length of the overhang. Activity was assayed by comparing the religation of restricted vectors that were either treated with APex Alkaline Phosphatase or untreated. After incubation with ligase and transformation of *E. coli*, the APex Alkaline Phosphatase-treated vectors showed >99% reduction in colonies compared to the untreated vectors, which indicates an inability to religate after dephosphorylation. To verify that the inability of the vectors to religate wasn't due to damaged DNA ends, restricted and dephosphorylated vectors were kinased and religated. Colony counts of *E. coli* transformed with these kinased and religated vectors were comparable to the counts of religated vectors that had not been dephosphorylated. See Table 1 for list of the restriction enzymes tested.

Table 1 Restriction enzyme overhangs tested.

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

Active in most buffers from pH 5.5 to 12

APex Heat-Labile Alkaline Phosphatase can be added directly to most RE buffers, without supplementation. Of the 21 RE buffers tested, APex Alkaline Phosphatase had full activity in all of them. Further

analysis of buffer conditions showed that the enzyme had ≥95% activity under the following conditions:

- pH range of 5.5 to 12.
- Concentration range of 0 to 1000 mM Na^+, NH_4^+, K^+, Cl^-, and Acetate$^-$.
- Concentration range of 0 to 10% Triton™ X-100.

Hydrolyzes nucleotides

APex Heat-Labile Alkaline Phosphatase hydrolyzed phosphate groups from solutions of the nucleotides listed in Table 2, but did not hydrolyze the RNA Cap Analogs GpppG or 7-methyl-GpppG.

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	

Table 2. Nucleotides tested and hydrolyzed by APex™ Heat-Labile Alkaline Phosphatase.

Conclusion

EPICENTRE introduces APex Heat-Labile Alkaline Phosphatase, a novel alkaline phosphatase which offers improved flexibility and convenience over other commercially available heat-labile alkaline phosphatases. APex Alkaline Phosphatase can be directly added to most enzymatic buffers without requiring supplementation. The enzyme functions over a wide range of pH, ionic strength, and temperature conditions and is irreversibly heat-inactivated at 70°C in 5 minutes. APex Alkaline Phosphatase is active on all types of DNA ends and hydrolyzes a broad range of nucleotide substrates. All of this with a simple treatment with 1 µl of APex Heat-Labile Alkaline Phosphatase for 15 minutes at 37°C.

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LK0750H	50 Reactions
LK6201H	100 Reactions

Clone Unstable DNA by Lowering the Copy Number of Common Vectors Using CopyCutter™ EPI400™ *E. coli* Cells*

Darin Haskins, EPICENTRE

Introduction

If you clone DNA on a regular basis, chances are good that you've been caught in the lab, gel image in hand, mumbling to yourself "Where's the insert?" Your controls look great and you've optimized everything you can think of, but your clones are "empty" or they contain an insert that's too small or that doesn't map correctly. Often this problem arises because the insert can not be stably maintained in a high-copy number vector.

The insert may code for a protein that interferes with normal cellular functions and inhibits cell growth. As a result, the clone either dies or is overtaken by "empty" or mutated recombinants that can grow faster. Similarly, although for reasons less well understood, AT- and GC-rich sequences or sequences with strong secondary structure, can also be unstable at high-copy number and are often selected against.

One of the easiest solutions to such cloning impasses is to use a lower-copy number vector. But you probably chose the vector you did for a reason. Besides the high-copy number that makes plasmid purification easier, the vector's multiple cloning site works well with your cloning strategy and/or the vector contains other sequences that you need for downstream applications. Keeping this in mind, EPICENTRE engineered our new CopyCutter™ EPI400™ *E. coli* cells.

Here we demonstrate how the CopyCutter EPI400 cells can significantly lower the copy number of a wide variety of popular, high-copy number vectors so that you can clone "toxic" genes or unstable DNA sequences into your favorite vectors. Moreover, following a short incubation in the presence of the CopyCutter™ Induction Solution, you can increase the copy number of the vector to improve plasmid yields.

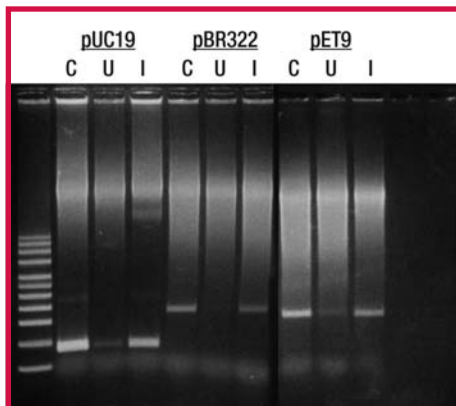

Figure 1. The copy number of ColE1-type plasmids is lowered 4- to 25-fold in CopyCutter™ EPI400™ *E. coli* cells. Lane C, TransforMAX™ EC100™ cells; Lanes U and I, uninduced and induced CopyCutter EPI400 cells, respectively. Crude extracts of plasmid DNA were prepared from cells grown in selective media and analyzed by agarose gel electrophoresis. Approximately the same number of lysed cells (based on OD_{600}) were loaded per lane.

Methods and Results

The CopyCutter EPI400 cell line was derived from our high-transformation efficiency phage T1-resistant TransforMAX™ EC100™ *E. coli* strain by manipulating a gene that controls the copy number of ColE1-type plasmids. This constitutively expressed gene, *pcnB* (plasmid copy number), was deleted from the TransforMAX EC100 strain and replaced with a modified *pcnB* gene linked to an inducible promoter, creating the CopyCutter EPI400 strain.

Lower copy number

ColE1-type plasmids include the naturally occurring ColE1, pMB1, and p15A, as well as pBR322, the pUC plasmids, the pET series, the pBluescript series, and many others. In standard *E. coli* strains the copy number of pUC19 can reach well over 100 copies per cell.[1] As shown in Table 1, the copy number of this vector is reduced by approximately 25-fold in the CopyCutter EPI400 strain compared to the parental TransforMAX EC100 strain, grown under the same conditions. Since lower-copy number plasmids, like pBR322 and many pET-derivatives contain an additional control element, their copy number is kept at 30 to 70 copies per cell in standard strains.[2] In the CopyCutter EPI400 strain the copy number of these vectors is reduced approximately 4-fold compared to the parental TransforMAX EC100 strain (Table 1).

Raise copy number

To induce plasmids in CopyCutter cells to higher copy number, a single clone was grown overnight in selective medium. Following overnight growth, cells were diluted into fresh medium to an OD_{600} of 0.2 and CopyCutter Induction Solution was added. Cultures were incubated for 4 hours at 37°C with shaking. As shown in Table 1 and Figure 1, raising the copy number of pUC19, pBR322 or pET9

Table 1. Comparing Plasmid Load in CopyCutter™ EPI400™ and TransforMAX™ EC100™ *E. coli* Cells

E. coli Host Cells	Growth Condition	Approximate Number of Vector Copies Per Cell*		
		pUC19 (Amp)	pBR322 (Amp)	pET9 (Kan)
TransforMAX™ EC100™ cells	Normal	~216	~71	~33
CopyCutter™ EPI400™ cells	Uninduced	~9	~17	~9
CopyCutter™ EPI400™ cells	Induced	~200	~66	~19

* *Based on the molar amount of plasmid DNA obtained from at least 10^{10} ampicillin or kanamycin resistant cells. Cultures were grown overnight in selective media (EC100 and EPI400-uninduced) or induced for 4 hours with the CopyCutter™ Induction Solution as described in the text.*

increased the plasmid load per ampicillin or kanamycin-resistant cell by approximately 22-, 4-, or 2-fold, respectively.

Random plasmid distribution

Because ColE1-type plasmids are randomly distributed to daughter cells during cell division, some daughter cells will receive plasmids and others will not.[1] After overnight growth in the presence of ampicillin, CopyCutter EPI400 cells containing pBR322 or pUC18 were plated on LB plates and on LB-ampicillin plates. The number of colonies obtained on LB plates was 40 to 60% higher than the number of colonies on LB-ampicillin plates, indicating the percentage of daughter cells that did not receive a plasmid. After a 4-hour induction in the presence of ampicillin, similar percentages of plasmid-free cells were observed with these vectors in CopyCutter EPI400 cells.

Kanamycin selection is more stringent in this application and fewer daughter cells were found to be plasmid-free. Comparisons were made between uninduced and induced cultures of the CopyCutter EPI400 strain containing a pET-derivative with a kanamycin marker. The number of colonies on LB plates was no more than 10% higher than the number of colonies on LB-kanamycin plates.

Clone toxic genes

To demonstrate the utility of the CopyCutter EPI400 strain we used two "potent" gene sequences that others have been unable to maintain in ColE1-type vectors. The first gene, *acpP*, encodes *E. coli* acyl carrier protein (ACP). When ACP is overproduced from multicopy plasmids, an unmodified form of the protein accumulates and inhibits cell growth.[3] A 423-bp PCR product containing *acpP* and its native promoter was ligated into pUC18 and aliquots of the reaction were electroporated into TransforMAX EC100 and CopyCutter EPI400 cells. Although 3 of the 36 TransforMAX EC100 clones screened contained a full-length insert, sequencing showed that each contained multiple point mutations, resulting in non-conserved amino acid substitutions. Quite remarkably, 27 of the 29 *acpP* CopyCutter EPI400 clones screened contained inserts of the expected size (Figure 2). Plasmid DNA purified from 3 clones induced to a higher copy number was also of the expected size and the sequenced inserts contained no mutations.

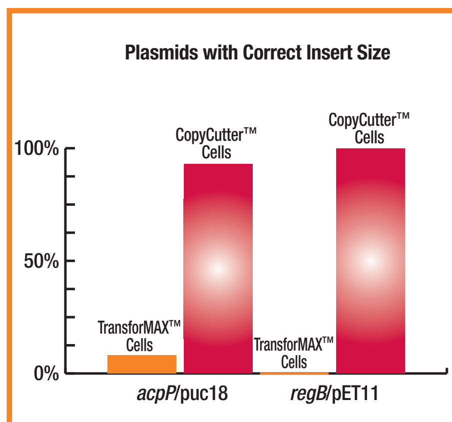

Figure 2. DNA inserts encoding toxic gene products were successfully cloned into high-copy number vectors using CopyCutter™ EPI400™ *E. coli* cells. After sequencing, the full-length *acpP* clones in TransforMAX™ EC100™ cells were found to contain multiple point mutations.

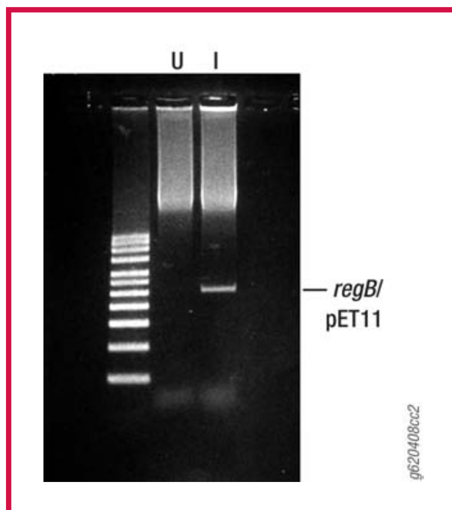

Figure 3. Uninduced CopyCutter™ EPI400™ *E. coli* cells containing a *regB* clone (Lane U) are induced to higher-copy number (Lane I) using the CopyCutter™ Induction Solution. Crude extracts of plasmid DNA were prepared from cells grown in selective media and analyzed by agarose gel electrophoresis. Approximately the same number of lysed cells (based on OD_{600}) were loaded per lane.

Cloning the *regB* gene from phage T4 provided a second set of dramatic results. This gene encodes a restriction endoribonuclease that cleaves vital bacterial messages and is therefore highly toxic to *E. coli* even in very small quantities.[4] A 461-bp PCR fragment containing a promoterless *regB* gene was ligated into the T7 expression vector, pET11a, and aliquots of the reaction were electroporated into TransforMAX EC100 and CopyCutter EPI400 cells. Although neither strain contains a T7 RNA polymerase gene, basal expression of regB in pET-derivatives has been observed in similar hosts, presumably from cryptic *E. coli* promoters on the plasmid.[4] Of the 19 TransforMAX EC100 clones screened, 17 contained no insert, and 2 had large deletions. In contrast, all 29 CopyCutter EPI400 clones screened contained a full-length insert (Figure 2). Inducing a regB clone to higher copy number severely retarded cell growth but plasmid DNA purified from the induced culture was the correct size and the sequenced insert was free of mutations (Figure 3).

Summary

EPICENTRE's new CopyCutter EPI400 *E. coli* cells significantly lower the copy number of many common vectors so that you can more readily clone unstable DNA sequences. Since you often can't predict whether the gene or chromosomal region you are cloning will be unstable, we supply these cells in a convenient, single-use format so you can easily incorporate them into your cloning regimen.

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* Patent pending.

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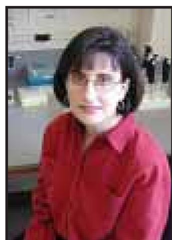
Undergraduates Study Gene Expression in *S. cerevisiae* Using a Student-Optimized Protocol

Karen K. Bernd and Victoria Statler
Davidson College, Davidson, NC



Karen Bernd is an Associate Professor of Biology at Davidson College. Her laboratory studies protein targeting and localization. Her Cell Biology course extends this interest into student-designed studies of signaling cascades and changes of protein localization during the mating reaction of *Saccharomyces cerevisiae*. Davidson College, Biology Department, Box 7118, Davidson, NC 28035. Tel (704) 894-2889, Fax (704) 892- 2512, E-mail kabernd@davidson.edu Web page http://www.bio.davidson.edu/Biology/kabernd/BerndCV/Lab/Berndhm.htm

Victoria Statler graduated from Davidson College in 2003 and presently attends the University of Louisville Medical School.

Courses in Davidson College's Biology Department rely on investigative laboratories to teach biology through research experiences. My Cell Biology lab studies extracellular and intracellular signaling using *S. cerevisiae* mating as a model. Students characterize mating mutants using a variety of procedures, including expression of genes involved in the mating reaction cascade. Since this is a teaching lab and the students have varying research experience, an RNA isolation method that did not use caustic chemicals (phenol) was preferred. Previous RNA isolation methods did not produce reliably high yields for the students and did not fit conveniently into the lab session.

Last year Victoria Statler (now at University of Louisville Medical School) worked to optimize an RNA isolation that was safe and reliable for undergraduates, gave high RNA yields, and fit into a standard three-hour laboratory session. To determine the protocol that would work best in student hands, she used EPICENTRE's MasterPure™ RNA Purification Kit with several combinations of cell-wall disruption steps (lysis with lyticase, bead beating, and freeze/thaw cycles). In the fall of 2003 my Cell Biology students used the optimized protocol, including bead beating and the MasterPure Kit, to study a collection of novel *S. cerevisiae* mating mutants.

EPICENTRE now offers the MasterPure™ Yeast RNA Purification Kit that releases RNA, which is largely free of DNA and protein, in about 40 minutes, without mechanical force or caustic reagents.

I have a collection of MATa *S. cerevisiae* mating mutants, isolated through UV mutagenesis. Working in seven groups, the nineteen Cell Biology students characterized the mating defect strains from this collection using morphology, complementation and gene expression studies. The gene expression studies relied on total RNA isolated from the mating mutant strains using Victoria's optimized protocol. Spectrophotometry was used to evaluate the purity and quantity of RNA. These experiments were done before the release of the MasterPure Yeast RNA Purification Kit and demonstrate the versatility of EPICENTRE products, a must when teaching budgets require that you get as much out of every reagent as possible.

Conclusion

On their first attempt, all seven lab groups isolated high purity samples (by OD 260/280 ratios). Samples were also evaluated by running 1 µg on 0.8% agarose, TAE gels containing EtBr and visualized with a Bio-Rad Fluor-S® MultiImager. This group of students, who had never used a UV spectrophotometer before, let alone isolate RNA, isolated fourteen samples of high quality RNA with yields of 79.75 µg to 239.8 µg per prep. As a bonus, the entire isolation, including getting settled, the prep, RNA quantification, and cleanup were completed within a standard three-hour laboratory session. The students and I were proud of their yields. We found they had plenty of RNA to perform any of the additional experiments that they designed. In fact, I still have RNA to use for follow-up experiments in my research lab.

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Individual components for all MasterPure™ DNA & RNA Purification Kits are available separately.

Introducing TAQurate™ Real Time PCR Master Mix, A Single High-Performance Mix

Haiying Grunenwald and Gordon S. Hunter, EPICENTRE

Introduction

Real-Time PCR (quantitative PCR, qPCR) measures the amount of PCR product generated over time and is relative to the amount of template in the reaction. The technique is used to compare gene expression levels in different tissues, determine viral and bacterial loads, and validate microarray results for gene expression studies. Many of these applications are being done in high throughput environments, amplifying similar templates with routine sets of primers.

EPICENTRE's new TAQurate™ Real-Time PCR Master Mix provides successful real-time PCR with most routine templates and primer pairs. TAQurate Master Mix contains SYBR® Green I Dye, the TAQurate™ Real-Time PCR Enzyme Blend, and our patented PCR Enhancer (with betaine*), in addition to optimized buffer, salts, and dNTP concentrations.

The TAQurate enzyme blend provides high sensitivity and specificity, and reliable amplification of even difficult templates. The PCR Enhancer (with betaine*) ensures high amplification efficiencies and fewer nonspecific PCR products by stabilizing the DNA polymerase and reducing pauses and stops, even in troublesome GC-rich template regions.[1]

To use the TAQurate Master Mix, simply add the primers and template, mix thoroughly, and begin PCR. The ready-to-use TAQurate Master Mix saves setup time and reduces liquid-handling steps. This report demonstrates the dynamic range, sensitivity, and specificity of real-time PCR performed with TAQurate Real-Time PCR Master Mix, and compares the results with a PCR master mix from another leading supplier (Supplier A).

Broad dynamic range, more accurate standard curve

To allow a more equitable comparison between the TAQurate Master Mix and Supplier A's master mix, lambda DNA, a relatively easy template to amplify,[2] was used to evaluate the dynamic range. Duplicate reactions containing 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 copies of lambda DNA were set up at room temperature. To ensure a uniform distribution and fair comparison, distilled water, primers for the lambda *cII* gene (460-bp amplicon) and template DNA were mixed together and then aliquoted into the master mixes from both EPICENTRE and Supplier A. Supplier A's reactions were hot-start activated according to the manufacturer's recommendations. TAQurate Master Mix does not require a hot start. Reactions were amplified with an initial denaturation at 95°C (2 minutes), followed by 45 cycles of 94°C (30 seconds), 55°C (30 seconds) and 72°C (30 seconds), and analyzed using the BioRad iCycler iQ™ Real-Time PCR Detection System.

Standard curve results

As indicated in Figure 1, the TAQurate Master Mix generated good quantification graphs over a dynamic range of 7 orders of magnitude. Plotting the log of the template concentration versus the threshold cycle (C_T) generates a standard curve, which for the TAQurate Master Mix gave a 0.999 correlation coefficient. Amplification efficiency is derived from the slope of the standard curve ($[10^{(-1/slope)}]-1 \times 100$), which for TAQurate Master Mix gave a 105.8% amplification efficiency. The accepted amplification efficiency range is 90% to 110%. Over the dynamic range used here, PCR results with Supplier A's master mix gave a 0.831 correlation coefficient and 493.9% amplification efficiency, values which are not acceptable for a useful standard curve.

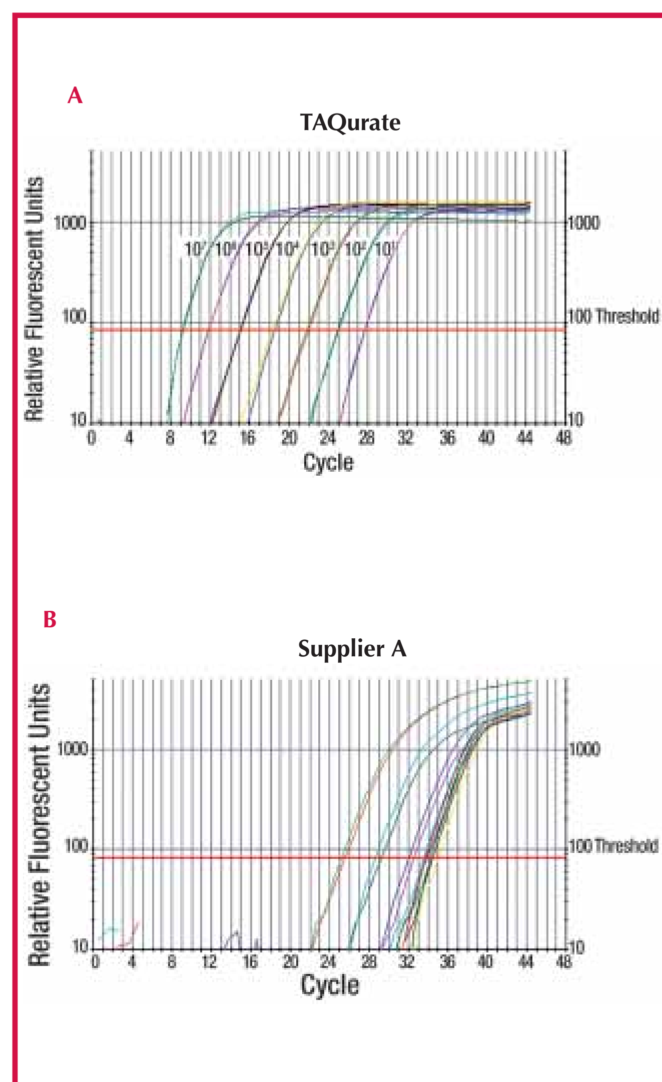


Figure 1. Wider dynamic range generates a more accurate standard curve with the TAQurate™ Real-Time PCR Master Mix. Real-time PCR of lambda DNA (10, 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 copies) was performed with TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** (EPICENTRE) and **B** (Supplier A) PCR quantification graphs.

High sensitivity, faster C_T values

Faster (lower) C_T values indicate greater PCR sensitivity. To compare the sensitivity of TAQurate Master Mix to Supplier A's master mix, reactions containing one copy or 10^7 copies of lambda DNA were amplified using the same reaction condi-

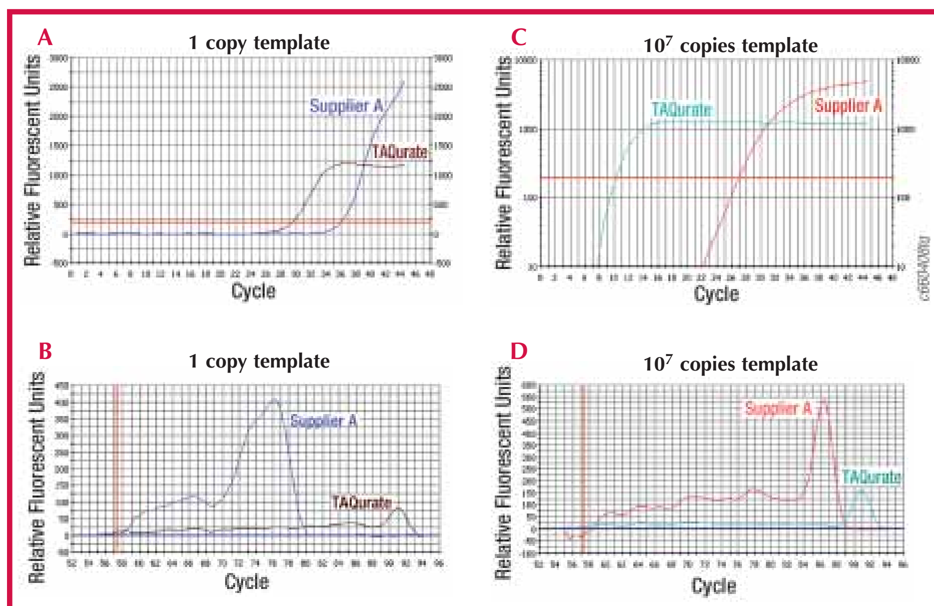


Figure 2. Higher sensitivity and faster threshold cycle (C_T) values with the TAQurate™ Real-Time PCR Master Mix. Real-time PCR amplification of one copy and 10^7 copies of lambda DNA was performed with the TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** and **C** are quantification graphs for one and 10^7 copies, respectively. **B** and **D** are melt curves for one and 10^7 copies, respectively.

tions described above. With one copy of template DNA, the TAQurate reaction had a C_T of 29.5 cycles and the Supplier A reaction had a significantly slower C_T of 36 cycles (Figure 2A). Higher relative concentrations of SYBR® Green I dye in Supplier A's master mix result in a higher fluorescence signal, but can actually inhibit the reaction and contribute to the slower C_T value.[3] The melt curves in Figure 2B show formation of a specific PCR product in the TAQurate reaction, but only primer-dimers and non-specific PCR products in the Supplier A reaction.

With 10^7 copies of template DNA, the TAQurate reaction had a C_T of 11 cycles compared to the Supplier A reaction with a C_T of 27 cycles (Figure 2C). At this higher template concentration, both master mixes gave good melt curves (Figure 2D). The significantly faster C_T values for the TAQurate reactions, at both low and high template concentrations, indicate greater PCR sensitivity.

Effective two-step real-time RT-PCR

To determine how well the TAQurate Master Mix works with cDNA, as used in gene expression studies, human cDNA was prepared from 1 ng of HeLa cell total RNA using EPICENTRE's MMLV reverse transcriptase in a 100-µl reaction. The human ß-actin message was then amplified by real-time PCR, in triplicate 50-µl reactions, using 1 µl of the above HeLa cell cDNA, 25 pmole of the forward and reverse primers, and either TAQurate or Supplier A's master mix. Supplier A reactions were hot-start activated; TAQurate reactions were not. Reactions were amplified with an initial denaturation at 95ºC (2 minutes), and 40 cycles of 95ºC (10 seconds), 60ºC (10 seconds), and 72ºC (30 seconds).

As indicated in Figure 3A, the TAQurate Master Mix reactions had an average C_T value of 19 compared to Supplier A's reactions with an average C_T value of 35. Both master mixes gave satisfactory melt curves, as shown in Figures 3B (EPICENTRE) and 3C (Supplier A). Using cDNA as the template, TAQurate Master Mix provides a more sensitive real-time PCR amplification.

Conclusion

EPICENTRE's new TAQurate Real-Time PCR Master Mix provides highly sensitive and specific real-time PCR amplifications for routine applications over a broad dynamic range. For more difficult templates, or to optimize real-time PCR conditions, EPICENTRE offers the FailSafe™ Real-Time PCR PreMix Selection Kit.[2]

References

1. Mytelka, D.S. and Chamberlin, M.J. (1996) *Nucleic Acids Res.* **24**(14), 2774.
2. Grunenwald, H. and Hunter, G.S. (2003) *EPICENTRE Forum* **10**(2), 14.
3. Nath, K. *et al.* (2000) J. *Biochem. Biophys. Methods* **42**(1-2), 15.

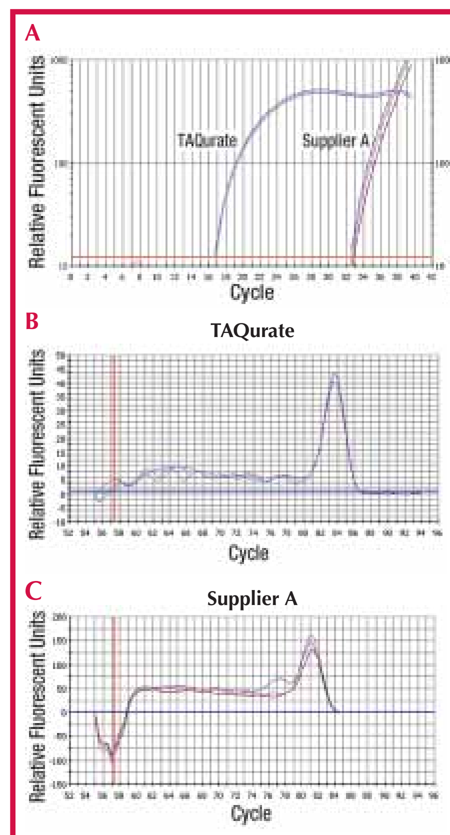


Figure 3. Two-step real-time RT-PCR of human cDNA derived from HeLa cell total RNA was amplified with the TAQurate™ Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). A, quantification graphs; **B**, TAQurate melt curves; **C**, Supplier A melt curves.

www.epicentre.com/taqurate.asp

TAQurate™ Real-Time PCR Master Mix

TM046200 200 Reactions

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.

* 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.

Mesophilic DNA Polymerases

Product Name	Activity				
	5'→ 3' exonuclease	3'→ 5' exonuclease	Nick translation	Heat Inactivation[a]	Strand displacement
DNA Polymerase I, *E. coli*	+	++	+	75°C 20 minutes	–
Klenow DNA Polymerase	–	++	–	75°C 20 minutes	+
Klenow Fragment Exo- DNA Polymerase	–	-	–	75°C 20 minutes	+
RepliPHI™ Phi29 DNA Polymerase	–	++	–	65°C 10 minutes	++++
T4 DNA Polymerase	–	+++	–	75°C 20 minutes	–
T7 DNA Polymerase, Unmodified	–	+++	–	75°C 20 minutes	–

[a] *Indicated treatment results in complete inactivation under standard reaction composition conditions.*

EPICENTRE offers a variety of high-quality, exceptionally pure, mesophilic DNA polymerases for common molecular biology techniques. We hope this table provides an easy comparison of DNA polymerase properties to help you select the best enzyme for your work. For additional information or questions about a specific enzyme or technique, please contact EPICENTRE's Technical Consultants at techhelp@epicentre.com.

www.epicentre.com

DNA Polymerase I, E. coli

DP021K 10 U/µl 1,000 U
DP0415K 10 U/µl 5,000 U

Klenow DNA Polymerase

KP04061K 1,000 U

Klenow Fragment Exo– DNA Polymerase

KL04011K 20 U/µl 1,000 U

RepliPHI™ Phi29 DNA Polymerase 1 µg/µl

RepliPHI™ Phi29 DNA Polymerase (Enzyme only)
PP031010 10 µg 1 µg/µl
(10,000 U) (1,000 U/µl)

RepliPHI™ Phi29 Reagent Set (Enzyme, dNTPs, Buffer, DTT)
RH031110 10 µg 1 µg/µl
(10,000 U) (1,000 U/µl)

RepliPHI™ Phi29 DNA Polymerase 0.1 µg/µl

RepliPHI™ Phi29 DNA Polymerase (Enzyme only)
PP040110 10 µg 0.1 µg/µl
(10,000 U) (100 U/µl)

RepliPHI™ Phi29 Reagent Set (Enzyme, dNTPs, Buffer, DTT)
RH040210 10 µg 1 µg/µl
(10,000 U) (100 U/µl)

RepliPHI™ Phi29 Polymerase Dilution Buffer
RPB04041 1 ml

T4 DNA Polymerase

D0602H 200 U
D0605H 500 U
Includes 10X Reaction Buffer.

T4 DNA Polymerase is also available in bulk. Please inquire.

T7 DNA Polymerase, Unmodified

D07250 10 U/µl 250 U
D07500 10 U/µl 500 U
D0701K 10 U/µl 1,000 U
Includes 10X Reaction Buffer.

T7 DNA Polymerase, Unmodified is also available in bulk. Please inquire.

Technical Support
techhelp@epicentre.com

Thermophilic DNA Polymerases

Product Name	Activity					
	Reverse transcriptase	5'→ 3' exonuclease	3'→ 5' exonuclease	Thermostability[b]	Fidelity[c]	Strand displacement
rBst DNA Polymerase[a]	+	+	–	<15 min at 75°C <1.5 min at 95°C	N/A	–
rBst DNA Polymerase Large Fragment[a] (IsoTherm™ DNA Polymerase)	+	–	–	<15 min at 75°C <1.5 min at 95°C	N/A	+++
MasterAmp™ AmpliTherm™ DNA Polymerase	–	–	–	N/A	N/A	N/A
MasterAmp™ *Taq* DNA Polymerase	Very weak. Requires Mn^{2+}	+	–	9 min at 97.5°C	0.38-1.82 x 10-4	N/A
MasterAmp™ *Tfl* DNA Polymerase	–	+	–	40 min at 95°C	8.3-9.0 x 10-5	N/A
MasterAmp™ *Tth* DNA Polymerase	++ Requires Mn^{2+}	+	–	20 min at 95°C	2.2 x 10-4	N/A

[a] rBst DNA polymerase and rBst DNA Large Fragment (IsoTherm™ DNA Polymerase) are for DNA replication at ≤ 65°C. Not suitable for typical PCR. The other thermostable enzymes listed in this chart are suitable for PCR cycling applications.

[b] Values represent half-lives of a given enzyme. 50% of the activity is retained after a given time at a given temperature.

[c] Defined as the average number of correct nucleotides a polymerase incorporates before making an error.

N/A - Not available.

In addition to optimized PCR reagents and kits, EPICENTRE offers a selection of high-quality, exceptionally pure, thermophilic DNA polymerases for DNA amplification techniques. We hope this table provides an easy comparison of DNA polymerase properties to help you select the best enzyme for your work. For additional information or questions about a specific enzyme or technique, please contact EPICENTRE's Technical Consultants at techhelp@epicentre.com.

rBst DNA Polymerase

BH1100	5 U/µl	100 U
BH1500	5 U/µl	500 U
BH101K	5 U/µl	1,000 U

rBst DNA Polymerase Large Fragment (IsoTherm™ DNA Polymerase)

BL901K	5 U/µl	1,000 U

Includes 10X Reaction Buffer.

BL1805K	50 U/µl	5,000 U
BL1950K	50 U/µl	50,000 U

Enzyme only.

Technical Support
techhelp@epicentre.com

MasterAmp™ AmpliTherm™ DNA Polymerase

AT72100	5 U/µl	100 U
AT72250	5 U/µl	250 U
AT72500	5 U/µl	500 U
AT7201K	5 U/µl	1,000 U
AT7205K	5 U/µl	5,000 U (5 x 1,000 U)

Includes 10X PCR Buffer, 25 mM $MgCl_2$, and MasterAmp™ 10X PCR Enhancer.

MasterAmp™ Taq DNA Polymerase

Q82100	5 U/µl	100 U
Q82250	5 U/µl	250 U
Q82500	5 U/µl	500 U
Q8201K	5 U/µl	1,000 U
Q8205K	5 U/µl	5,000 U (5 x 1,000 U)

Includes 10X PCR Buffer, 25 mM $MgCl_2$, and MasterAmp™ 10X PCR Enhancer.

MasterAmp™ Taq PCR Core Kit

MCQ74200 200 Reactions

Contents:

MasterAmp™ *Taq* DNA Polymerase
10X PCR Buffer
MasterAmp™ 10X PCR Enhancer
dNTP Mix, 2.5 mM each
25 mM $MgCl_2$
Enzyme Dilution Buffer
Control Template and Primers Mix

MasterAmp™ Tfl DNA Polymerase

F72100	1 U/µl	100 U
F72250	1 U/µl	250 U
F72500	1 U/µl	500 U
F7201K	1 U/µl	1,000 U
F7205K	1 U/µl	5,000 U (5 x 1,000 U)

Includes 20X PCR Buffer, 25 mM $MgCl_2$, and MasterAmp™ 10X PCR Enhancer.

MasterAmp™ Tth DNA Polymerase

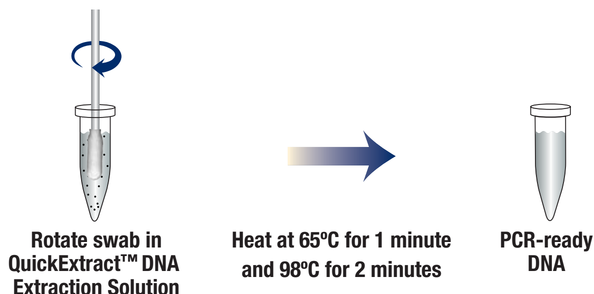
TTH72100	5 U/µl	100 U
TTH72250	5 U/µl	250 U
TTH72500	5 U/µl	500 U
TTH7201K	5 U/µl	1,000 U
TTH7205K	5 U/µl	5,000 U (5 x 1,000 U)

Includes a 20X PCR Buffer (without Mg^{2+} or Mn^{2+}) plus separate 25 mM solutions of $MgCl_2$ and $MnSO_4$, and MasterAmp™ 10X PCR Enhancer.

Product Data Sheet

BuccalAmp™ DNA Extraction Kit

The BuccalAmp™ DNA Extraction Kit is a single-tube system for rapid preparation of DNA from buccal (cheek) swabs for use in PCR amplification assays. To obtain PCR-ready DNA, just rotate the buccal sample swab in one of these tubes, mix, and heat for 3 minutes. No centrifugation step is needed.



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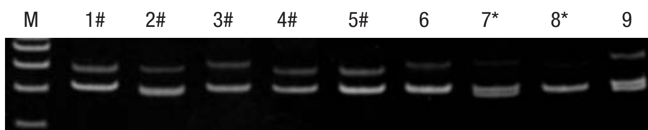


PCR of polymorphic repeat sequences in DNA from buccal samples from 9 individuals obtained using the BuccalAmp™ DNA Extraction Kit. DNA was amplified using the FailSafe™ PCR System. Lane M, 100 bp marker.

remote-site collected sample * pediatric sample

Applications

- Rapid, easy extraction of DNA from buccal swabs
- Human or animal identity testing
- SNP analysis
- Assays for viruses, bacteria or other microorganisms

Benefits

- Simple and rapid sample collection
- Soft, gentle foam swab
- 3 minute DNA extraction protocol
- Safe - no toxic solvents

NEW! ***Easy Shortcut to Product Information***

Go to our website at **www.epicentre.com** and enter this QuickInfo code: **BAD01**

BuccalAmp™ DNA Extraction Kits

BQ0901S	1 Kit (15 Tubes & Swabs)
BQ0908S	8 Kits (120 Tubes & Swabs)
BQ0916S	16 Kits (240 Tubes & Swabs)

Contents per kit:

15 tubes (1 extraction per tube) of QuickExtract™ DNA Extraction Solution 1.0

15 individually-packaged sterile Catch-All™ Swabs

QuickExtract™ DNA Extraction Solution 1.0

QE09050	50 ml

Bulk solution, sufficient to perform 100 extractions.

Catch-All™ Sample Collection Swabs

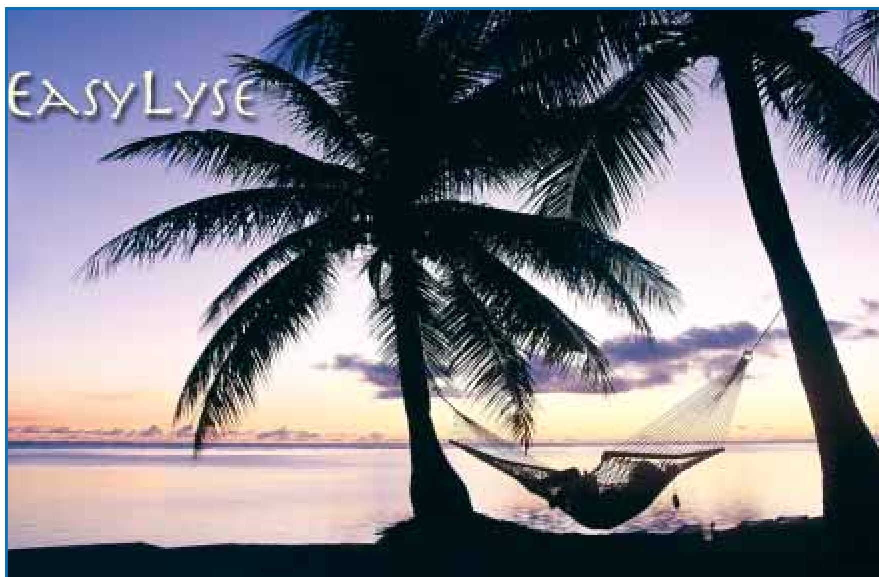
QEC0925	25 Swabs
QEC091H	100 Swabs

Technical Support
techhelp@epicentre.com

Product Data Sheet

EasyLyse™ Bacterial Protein Extraction Solution

The EasyLyse™ Bacterial Protein Extraction Solution is designed for lysing bacterial cells for the isolation of proteins, especially recombinant gene products expressed in E. coli, without significant loss of enzymatic activity. EasyLyse is formulated for ease of use as a homogeneous reagent in high-throughput applications.

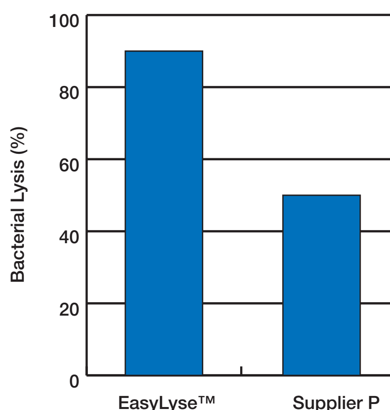


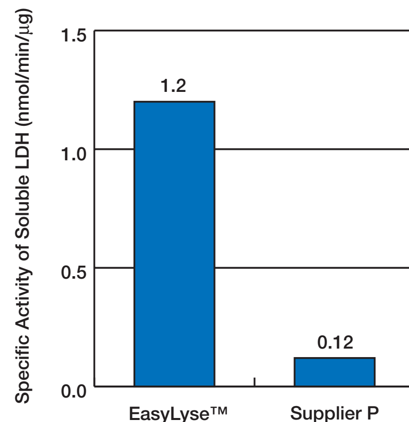
Applications

- Gently lyses bacteria
- Digests nucleic acids
- Single, 1-step addition
- Ideal for high throughput applications

Benefits

- Obtain high yield of soluble protein in one step
- Retain more enzyme activity





NEW! ***Easy Shortcut to Product Information***

Go to our website at **www.epicentre.com** and enter this QuickInfo code: **ELD01**

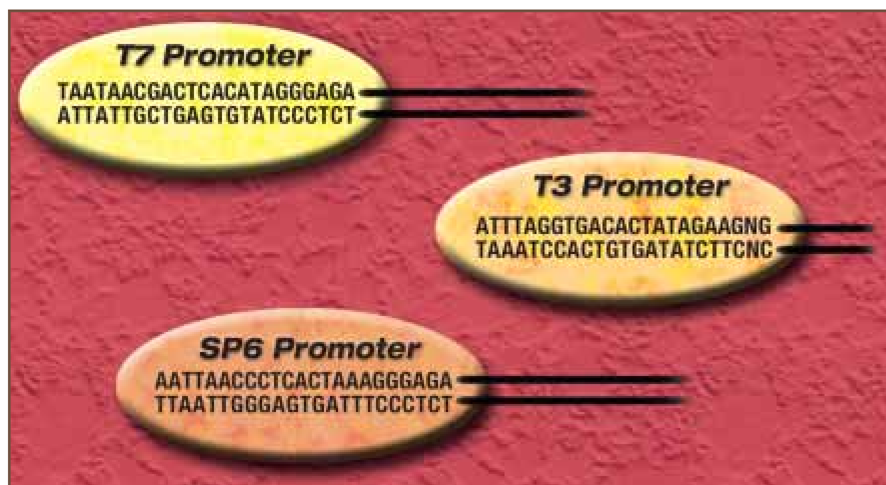
EasyLyse™ Bacterial Protein Extraction Solution		***Related Products***
RP03750	500 x 1 ml or 48 x 96 wells	ReadyLyse™ Lysozyme Solution
Contents: Lysis Buffer, Enzyme Mix, $MgCl_2$ Solution		

Technical Support
techhelp@epicentre.com

Product Data Sheet

T7, T3, & SP6 RNA Polymerases

T7, T3, and SP6 RNA Polymerases produce defined RNA by in vitro transcription of DNA cloned into a plasmid or other vector downstream from the respective RNA polymerase promoter.



Unit Definition: One unit of phage RNA polymerase catalyzes the incorporation of 1 nmole of a labeled ribonucleoside triphosphate into RNA in 1 hour at 37°C in 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, and 0.5 mM each NTP using a DNA template with the appropriate T7, T3, or SP6 promoter.

Storage Buffer: 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1.0 mM DTT, 0.1 mM EDTA, and 0.1% Triton™ X-100.

5X Transcription Buffer (available separately): 0.2 M Tris-HCl (pH 7.5), 50 mM NaCl, 30 mM $MgCl_2$, and 10 mM spermidine.

Quality Control: The phage RNA polymerases are tested in *in vitro* transcription reactions, and are free of detectable exo- and endonuclease and RNase activities, and *E. coli* RNA polymerase activity.

Applications

- RNA synthesized can be used as a probe, anti-sense RNA, a ribozyme, a template for *in vitro* translation, or as a precursor mRNA for splicing or other processing studies.
- Synthesis of RNA for nucleic acid amplification methods or gene expression studies.

Benefits

- High purity, specificity, and activity.
- Greatest range of enzyme concentrations available, from 25 U/µl to 2,500 U/µl.
- Extremely high promoter specificity.
- The best value in phage RNA Polymerases.

www.epicentre.com/rnapol.asp

T7 RNA Polymerase

TL910K	25 U/µl	10,000 U
T7905K	50 U/µl	5,000 U
T7910K	50 U/µl	10,000 U
T7925K	50 U/µl	25,000 U
T7950K	50 U/µl	50,000 U
TM905K	200 U/µl	5,000 U
TM910K	200 U/µl	10,000 U
TM925K	200 U/µl	25,000 U
TM950K	200 U/µl	50,000 U
TH925K	1,000 U/µl	25,000 U
TH950K	1,000 U/µl	50,000 U
TU950K	2,500 U/µl	50,000 U

Enzyme only; Transcription Buffer is not included.

T3 RNA Polymerase

TL010K	25 U/µl	10,000 U
T9001K	50 U/µl	1,000 U
T9010K	50 U/µl	10,000 U
T9025K	50 U/µl	25,000 U
T9050K	50 U/µl	50,000 U
TM005K	200 U/µl	5,000 U
TU050K	2,500 U/µl	50,000 U

Enzyme only; Transcription Buffer is not included.

SP6 RNA Polymerase

SL910K	25 U/µl	10,000 U
SL925K	25 U/µl	25,000 U
S7901K	50 U/µl	1,000 U
S7910K	50 U/µl	10,000 U
S7925K	50 U/µl	25,000 U
SM905K	200 U/µl	5,000 U
SM910K	200 U/µl	10,000 U
SM925K	200 U/µl	25,000 U
SH910K	1,000 U/µl	10,000 U
SU925K	2,500 U/µl	25,000 U

Enzyme only; Transcription Buffer is not included.

Transcription Buffer Package

BP1001 1 Pkg

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

RNA polymerases are also available in bulk. Please inquire.

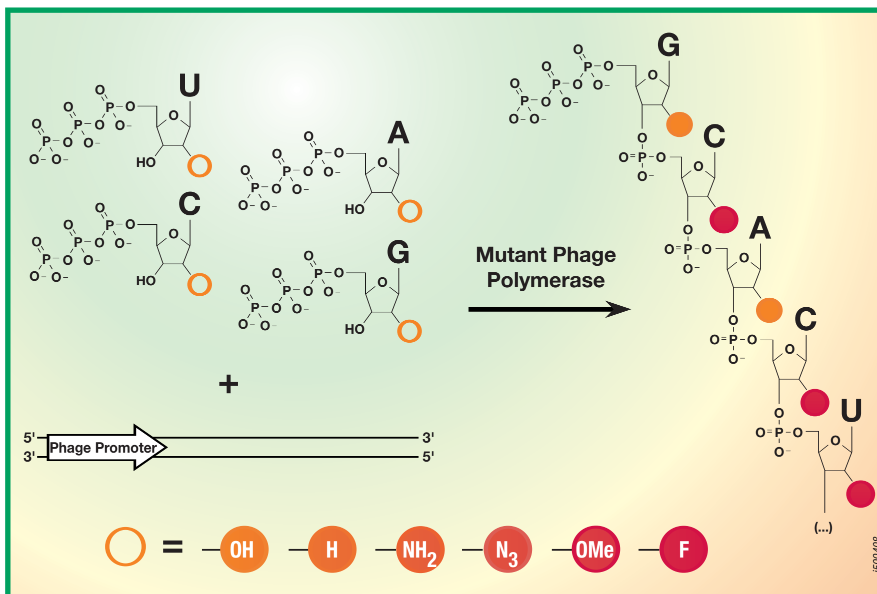
Related Products

AmpliScribe™ High Yield Transcription Kits
AmpliCap™ High Yield Message Maker Kits
DuraScribe™ T7 Transcription Kit
T7 and SP6 R&DNA™ Polymerases
NTPs
RiboScribe™ RNA Probe Synthesis Kits
Cap Analogs

Technical Support
techhelp@epicentre.com

T7 R&DNA™ Polymerase and SP6 R&DNA™ Polymerase

The mutant T7 and SP6 R&DNA™ Polymerases, like the corresponding wild-type T7 and SP6 RNA Polymerases are useful for in vitro transcription of RNA from a DNA template containing a T7 or SP6 transcription promoter. However, unlike the wild-type T7 and SP6 RNA Polymerase, the T7 and SP6 R&DNA Polymerases will efficiently incorporate 2′-deoxy-NTPs and 2′-modified-NTPs such as 2′-Fluorine-dNTP (2′-F-dNTPs) in addition to the canonical NTPs into full length transcripts.*



Applications

- Synthesize "RNA" transcripts of mixed rNMP/2′-dNMP or rNMP/2′-modified-dNMP composition.
- Synthesize "RNA" transcripts with altered sensitivity to specific RNases (e.g. RNase A) for greater stability in most RNA applications.

Benefits

- The mutant T7 and SP6 R&DNA Polymerases utilize the same T7 or SP6 promoters as the wild-type T7 and SP6 RNA polymerases.
- Readily incorporate 2′-dNTPs and 2′-modified-dNTPs into transcripts.

The ability of these mutant enzymes to incorporate 2′-deoxy-NTPs and 2′-modified-dNTPs permits either primed or unprimed *in vitro* synthesis of transcripts composed of mixed rNMP/2′-dNMP or rNMP/2′-modified-dNMP composition for a variety of applications.

Solutions of 2′-Fluorine-dCTP (2′-F-dCTP) and 2′-Fluorine-dUTP (2′-F-dUTP), which are readily incorporated into transcripts by T7 and SP6 R&DNA Polymerase, are available separately.

* The use of T7 and SP6 R&DNA™ 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.

www.epicentre.com/rdna.asp

T7 R&DNA™ Polymerase

D7P9201K	50 U/µl	1,000 U
D7P9205K	50 U/µl	5,000 U

Contents:
T7 R&DNA™ Polymerase
5X Reaction Buffer
100 mM DTT

SP6 R&DNA™ Polymerase

D6P9301K	50 U/µl	1,000 U
D6P9305K	50 U/µl	5,000 U

Contents:
SP6 R&DNA™ Polymerase
5X Reaction Buffer
100 mM DTT

2′-Fluorine-dCTP (2′-F-dCTP)

R2F110C	50 mM	1 µmole

2′-Fluorine-dUTP (2′-F-dUTP)

R2F110U	50 mM	1 µmole

Related Products

DuraScribe™ T7 Transcription Kit
NTPs
dNTPs
EZ::TN™ <T7/KAN-2> Promoter Insertion Kit

Technical Support
techhelp@epicentre.com

Product Data Sheet

Ask Frank

by Fred and Hank

Fred Hyde

Hank Daum

Questions about some of EPICENTRE's Enzymes

Q: Why are EPICENTRE's enzymes of such high quality?

A: EPICENTRE's enzymes are of extremely high quality because of our dedication to developing optimized purification methods and rigorous quality control testing. These objectives result in outstanding enzyme activity and long-term stability. EPICENTRE uses stringent, proprietary processes, which greatly improve enzyme purity and thus ensure reproducible performance.

Q: Will Tobacco Acid Pyrophosphatase (TAP) cut an ApppG mRNA cap?

A: Yes, Tobacco Acid Pyrophosphatase will digest this, and other capped RNAs with modified cap analogs. TAP cleaves the capped RNA transcript to a 5′-phosphate (α-pG-RNA), a free phosphate (β-p), and the residual cap nucleotide (γ-Ap).

Q: What is a good dilution buffer for TAP?

A: For long-term storage purposes, use TAP storage buffer (50% glycerol, 10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.01% Triton™ X-100). For same-day use, dilute the enzyme in 1X TAP reaction buffer (10X reaction buffer is included with the enzyme). For additional information on TAP, please see page 23.

Q: What non-standard nucleotides can be incorporated into RNA using EPICENTRE's mutant T7 and SP6 R&DNA Polymerases?

A: T7 and SP6 R&DNA Polymerases have been used to incorporate 2′-deoxy, 2′-NH_2, 2′-F, 2′-OMe and 2′-N_3 dNTPs into nascent RNA strands. EPICENTRE offers the DuraScribe™ T7 Transcription Kit to prepare 2′ Fluorine-modified RNA transcripts, which are RNase A resistant. (For more information please go to www.epicentre.com/durascribe.asp).

Q: Can I completely substitute a specific rNTP in an RNA molecule using the mutant T7 or SP6 R&DNA Polymerase?

A: Sometimes. For example, modified RNAs can be made using 100% 2′-F-dUTP or 2′-F-dCTP, but the corresponding 2′-F-dATP and 2′-F-dGTP are incorporated somewhat less efficiently. The T7 and SP6 R&DNA Polymerases incorporate different modified NTPs at different rates.[1] Processivity and transcription yield are further compromised when two or more different dNTPs or modified NTPs are used. Increasing the reaction time and increasing the reaction temperature to 42°C can improve the yield of the resulting transcript. For more information on T7 and SP6 R&DNA Polymerase, please see Product Data Sheet, page 16.

Q: Do any of your thermophilic DNA polymerases have proofreading activity?

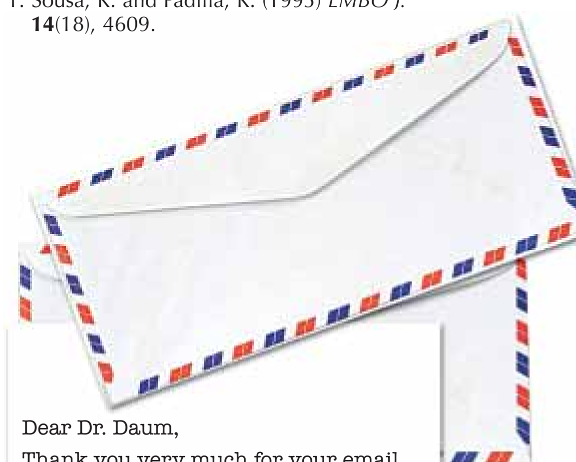
A: FailSafe™ PCR Enzyme Mix and MasterAmp™ Extra-Long DNA Polymerase Mix are enzyme blends that contain proofreading activity. The fidelities of EPICENTRE's polymerase blends are greater than three times that of standard *Taq* Polymerase.

Q: Which of EPICENTRE's thermophilic DNA Polymerases add non-template A's on the 3′-end of the PCR product?

A: EPICENTRE's *Taq*, *Tfl*, *Tth* and AmpliTherm™ DNA Polymerases incorporate a non-template encoded A to the 3′-end of PCR products. The FailSafe PCR Enzyme Mix and MasterAmp Extra-Long DNA Polymerase Mix, result in a mixture of PCR products – some with an A and others without. Thus, PCR products made using FailSafe or MasterAmp Extra-Long Kits can be efficiently cloned in either a blunt-end or T/A cloning vector. For more information on EPICENTRE's DNA polymerases, please see Product Data Sheets, pages 11 and 12.

Reference

1. Sousa, R. and Padilla, R. (1995) *EMBO J.* **14**(18), 4609.



Dear Dr. Daum,

Thank you very much for your email. It has always been a pleasure communicating with you. Your help and directions and in depth knowledge has opened new avenues for me. Research is always exciting but it is more so when you feel like a community.

I am really grateful for all the time and effort you took to extend your helpful hand. It only proves your generosity and the company culture.

Because of the nature of our research, I am sure we would be dealing more with your company in the future.

With many thanks and best regards,

Nusrat Sharmeen
Advanomics Corporation
Montreal, Quebec, Canada

Restriction-Enzyme Screen Clones from Colony to Gel in 25 Minutes or Less

Ron Meis, EPICENTRE

Because ligation reactions can generate different insert orientations, multiple inserts, and empty vectors, cloning recombinant DNA in *E. coli* usually requires a screen of the resulting colonies for the correct clone. Potential clones can be size-screened by electrophoresis of the uncut plasmid, or screened for insert size and orientation by PCR or restriction enzyme digestion. Each method has benefits and drawbacks.

The Colony Fast-Screen™ Kit (Restriction Screen) provides a significantly faster and more convenient way to screen E. coli colonies for clones by restriction analysis than ever before.

Uncut plasmid analysis

Uncut plasmid analysis is quick (just lyse the cells and run a gel) and convenient (can be performed directly on colonies, no additional overnight cultures and plasmid preps). EPICENTRE offers the Colony Fast-Screen™ Kit (Size Screen) for uncut plasmid analysis. If the orientation of the clone is not a concern, this method is very efficient.

PCR analysis

PCR analysis is convenient (can be performed directly on colonies, no additional overnight cultures and plasmid preps) and can determine the orientation of the clone. EPICENTRE offers the Colony Fast-Screen™ Kit (PCR Screen) for PCR analysis. If the necessary PCR primers are available and the extra time required for cycling is not a problem, this method provides more specific information about the clone than uncut plasmid analysis.

Restriction analysis

Restriction enzyme digest analysis uses a common tool found in most molecular biology labs (restriction enzymes) and can determine insert orientation. However, traditionally this method also required overnight cultures and plasmid preps. Now EPICENTRE offers the Colony Fast-Screen™ Kit (Restriction Screen), which allows restriction analysis directly from individual colonies.

The new Colony Fast-Screen Kit (Restriction Screen) allows restriction analysis of clones, from colony to gel-loading, in only 25 minutes with the standard protocol, or in as little as 10 minutes with the accelerated protocol. Briefly, the procedure consists of colony resuspension in the kit's proprietary solution, heat treatment, restriction enzyme digestion and agarose gel electrophoresis. The kit's Restricti-Lyse™ Solution lyses cells in an environment that is not inhibitory to restriction enzyme activity.

To demonstrate the use of the kit, an insert (1.2 kb) was blunt-end ligated into a vector (2.4 kb). Resulting colonies were screened using *Sca* I in both the Restriction Screen standard protocol and the accelerated protocol. *Sca* I cuts in both the insert and the vector. In one insert orientation (A) a *Sca* I digest produces 2.5- and 1.1-kb fragments. In the other orientation (B), the digest produces two 1.8-kb fragments. Digestion of the recircularized vector, without an insert, gives a single 2.4-kb fragment.

Standard protocol

For the standard protocol, each colony was vortexed in 10 µl of Restricti-Lyse Solution to resuspend the cells and incubated at 100°C for 90 seconds. After the tubes were briefly cooled at room temperature, 1 µl of 10X restriction enzyme buffer and 10 Units (1µl) of *Sca* I were added. Reactions were incubated at 37°C for 15 minutes, 2 µl of 6X gel loading buffer were added and the entire reaction

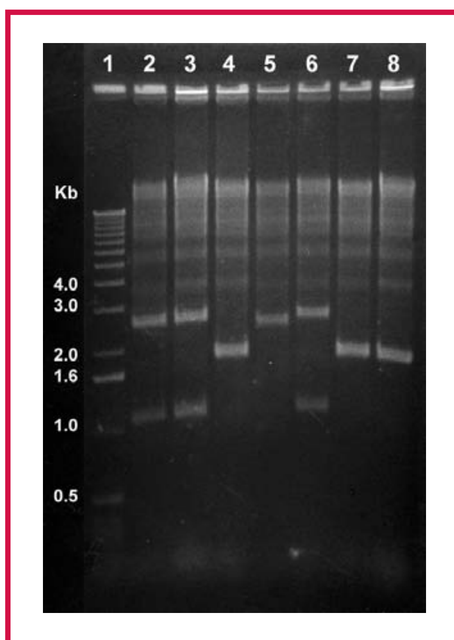


Figure 1. Results of the standard protocol with the Colony Fast-Screen™ Kit (Restriction Screen), which goes from colony to gel loading in 25 minutes. Lane 1, molecular size marker; Lanes 2 to 8, *Sca* I digests of DNA from 7 colonies prepared according to the kit's standard protocol. Lanes 2, 3, and 6 show clones in orientation A; Lanes 4, 7, and 8 show clones in orientation B; and Lane 5 is a recircularized vector.

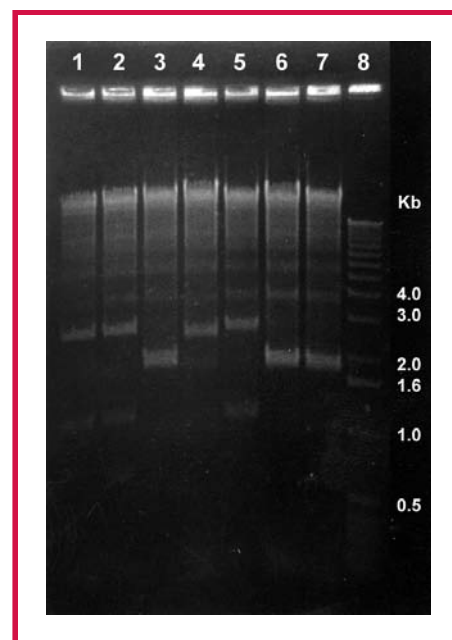


Figure 2. Results of the accelerated protocol with the Colony Fast-Screen™ Kit (Restriction Screen), which goes from colony to gel loading in 10 minutes. Lanes 1 to 7, *Sca* I digests of DNA from 7 colonies prepared according to the kit's accelerated protocol; Lane 8, molecular size marker. Lanes 1, 2, and 5 show clones in orientation A. Lanes 3, 6, and 7 show clones in orientation B. Lane 4 is a recircularized vector.

was subjected to electrophoresis on a 1% agarose gel. Figure 1 shows the results of the standard protocol.

Accelerated protocol

With the accelerated protocol, each colony was resuspended by pipetting in 10 µl of Restricti-Lyse Solution and incubated at 100°C for only 10 seconds. The *Sca* I digest was set up as in the standard protocol, but incubated at 37°C for only 5 minutes. The reaction was prepared and electrophoresed as for the standard protocol. Figure 2 shows the restriction digest results using the accelerated protocol. The resulting DNA bands from the accelerated protocol are generally discernable, but have less DNA and stain less intensely than the standard protocol.

Conclusion

The Colony Fast-Screen Kit (Restriction Screen) provides a significantly faster and more convenient way to screen *E. coli* colonies for clones by restriction analysis than ever before.

www.epicentre.com/cfs_res.asp

Colony Fast-Screen™ Kit (Restriction Screen)

Screen the size and orientation of clones.

FS0472H 1 Kit

Sufficient reagents to screen 200 colonies.

Colony Fast-Screen™ Kit (Restriction Screen) Identifies Subclones with 124-bp Fragment

Judith Meis, EPICENTRE

The Colony Fast-Screen™ Kit (Restriction Screen) was used to screen colonies directly for the presence of a small restriction enzyme fragment, quickly and easily, without growing overnight cultures or purifying plasmid DNA. Here pUC19-based clones with a 97-bp insert were screened for the presence of a 124-bp restriction fragment by a double restriction enzyme digest.

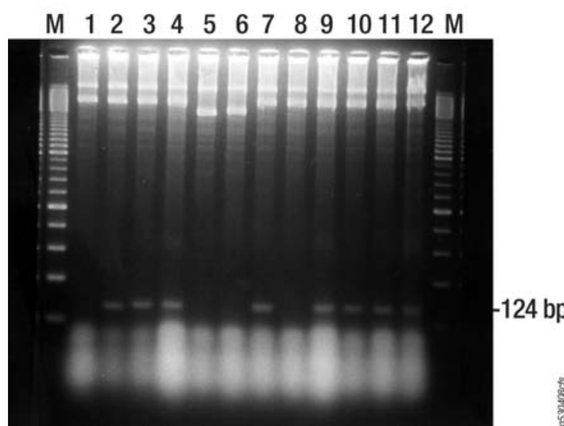


Figure 1. Electrophoresis of restriction digests of 12 plasmid DNA samples prepared with the Colony Fast-Screen™ Kit (Restriction Screen). DNA prepared from 12 large colonies was double-digested with *Eco*R I and *Xba* I. Samples were run on a 3% agarose gel to identify clones containing the desired 124-bp fragment. M, 100 bp DNA Ladder. Lanes 2, 3, 4, 7, 9, 10, 11, and 12 show positive clones.

Methods

Ligation and transformation

A blunt 97-bp fragment was ligated into the *Sma* I site of pUC19 using the Fast-Link™ DNA Ligation Kit. One microliter of the ligation reaction was electroporated into TransforMax™ EC100™ Electrocompetent *E. coli* and the cells were plated and grown on LB-ampicillin overnight.

DNA preparation and restriction screening

DNA was prepared for restriction screening by transferring most of a large colony (about 2 mm) into 10 µl of the kit's Restricti-Lyse™ Solution. Part of the colony was also touched to a new LB-ampicillin grid plate for future propagation. Cells were resuspended by vortexing and lysed by incubating at 100°C for 1 minute. Restriction buffer (1 µl of 10X buffer), *Eco*R I (1 Unit), and *Xba* I (1 Unit) were added directly to the prepared DNA samples. To ensure a complete double digest with only 1 Unit of each enzyme, the digestion reactions were incubated at 37°C for 1 hour, rather than the typical 15 minutes needed with 5 to 10 Units of enzyme. Loading buffer was added directly to the samples, and they were assayed on a 3% agarose gel and stained with SYBR® Gold.

Results

Twelve large colonies were screened for the expected 124-bp *Eco*R I/*Xba* I fragment containing the desired 97-bp insert. Large colonies were used to increase the amount of DNA and more readily detect the small fragments. Figure 1 shows that 8 of the 12 colonies screened contain the insert. The Colony Fast-Screen Kit (Restriction Screen) prepares enough DNA to quickly and directly screen plasmids for even small restriction fragments, without overnight cultures and mini-plasmid preps.

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

www.epicentre.com/fastlink.asp

Fast-Link™ DNA Ligation Kit

LK11025 25 Ligations
LK0750H 50 Ligations
LK6201H 100 Ligations

Contents:
Fast-Link™ DNA Ligase
Fast-Link™ 10X Ligation Buffer
10 mM ATP

TransforMax™ EC100™ Electrocompetent *E. coli*

EC10005 5 X 100 µl
EC10010 10 X 100 µl
Includes pUC19 control DNA.

TransforMax™ EC100™ Chemically Competent *E. coli*

CC02810 10 X 50 µl
Includes pUC19 control DNA.

The Oyster Bacterial Biome: Who Else Is Inside the Shell?

Les Hoffman, EPICENTRE

Introduction

A recent approach to the analysis of bacterial communities inhabiting various environments uses ribosomal RNA gene sequences (rDNA) to identify the various species present.[1] DNA is extracted, the rDNA genes are amplified by PCR, and the ribosomal amplicons are sequenced.[2] We applied this technique to a specialty food, the oyster, which contains heterogeneous populations of bacteria, some of which can be harmful to humans. *Crassostrea virginica*, the Eastern oyster, is the species most consumed in the United States (www.agmrc.org/aquaculture/profiles/oysterprofiles.pdf). The ecology of its microbial symbionts is not well studied, and published reports differ widely in the bacterial species found in oysters.[3-5]

EPICENTRE's MasterPure™ Plant Leaf DNA Purification Kit was used to extract DNA from *C. virginica*. The isolated DNA contains both oyster genomic DNA (shown by amplification of metallothionein genes) and DNA from a number of bacterial species in the oyster. Bacterial identification was made by amplifying 16S rDNA, cloning the PCR products using EPICENTRE's CopyControl™ PCR Cloning Kit, and sequencing the cloned genes. Eight species of gram-negative bacteria, some unidentified, were found. No known human pathogens were represented by the amplified rDNAs. However, an ampicillin-resistant *Pseudomonas* species was found.

Materials and Methods

DNA isolation

Mucopolysaccharides and glycogen in shellfish often make the isolation of nucleic acids difficult. The MasterPure Plant Leaf DNA Purification Kit effectively removes polysaccharides and isolates DNA from glycogen and carbohydrate-rich organisms, such as mollusks. *C. virginica* was harvested in New England and purchased in Madison, WI. Samples of 120 to 180 mg of freshly shucked oyster were macerated in 1.5-ml tubes using a plastic pestle and 300 µl of the kit's DNA Extraction Reagent. The remainder of the isolation was done as described in the kit literature, without organic solvents or enzymatic treatments. Yields were approximately 1 to 1.5 µg of DNA for each of four oyster tissue samples. Figure 1 shows DNA purified from the four samples. The lower part of the lanes shows eukaryotic, nucleosomal DNA bands.

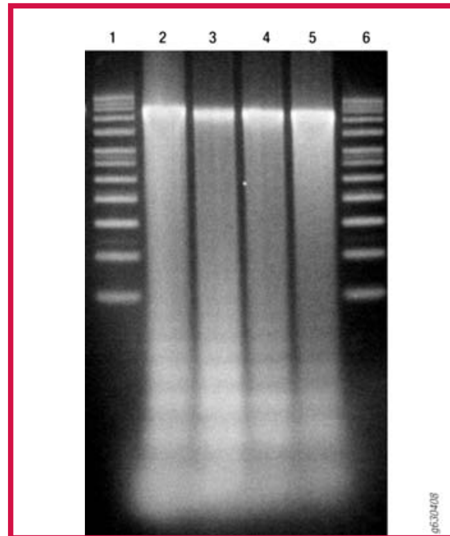


Figure 1. Oyster DNA was purified using the MasterPure™ Plant Leaf DNA Purification Kit. Aliquots from four preparations of oyster DNA were electrophoresed in a 1% agarose gel and stained with SYBR® Gold. Lanes 1 and 6, DNA ladder; Lanes 2 to 5, oyster DNA.

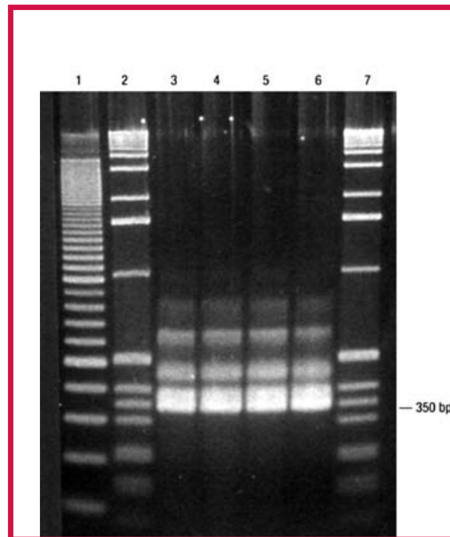


Figure 2. The 16S ribosomal DNA sequences from four oyster samples were amplified using the FailSafe™ PCR System and universal eubacterial primers. After amplification, 5 µl of each 50-µl reaction was assayed in a 2% agarose gel and stained with SYBR® Gold. Lane 1, 100 bp ladder; Lanes 2 and 7, kb ladder; Lanes 3 to 6, oyster DNA PCR products. The bands above the approximately 350-bp rDNA amplicons are probably heteroduplexes, as described in the text.

PCR amplification

16S rDNA sequences were amplified using 1 µl of oyster DNA, universal eubacterial primers[6] and the FailSafe™ PCR 2X PreMix B, which previously had been determined to be optimal with these primers and similar templates. Primers used were EB-L, 5′-CTG CTG CCT CCC GTA GGA GT-3′ and EB-R, 5′-AGA GTT TGA TCC TGG CTC AG-3′. Cycling conditions were: 96°C (2 minutes), followed by 28 cycles of 96°C (30 seconds), 55°C (30 seconds), and 72°C (45 seconds).

Figure 2 shows the 16S rDNA PCR products (approximately 350 bp) from the four DNA samples. Each lane contains additional, slower migrating amplicons, which are probably heteroduplexes arising from the annealing of rDNAs from different species. These heteroduplexes can be removed by a procedure called "PCR reconditioning".[7] If cloned, heteroduplexes can be repaired in *E. coli* after transformation, producing mosaic rDNA sequences.[7]

For oyster-specific genes, PCR primers were designed to amplify oyster metallothionein genes, which code for a family of detoxifying proteins.[8] Primers used were 5′-ATG TCT GAT CCA TGT AAC TGC ACT-3′ and 5′-TCA TTC AAG AAT TAG ATA TCG AGC T-3′. To quickly determine the optimum PCR reaction conditions for oyster genomic DNA and the metallothionein primers, reactions were set up with PreMixes from the FailSafe™ PCR PreMix Selection Kit, as directed. Cycling conditions were: 95°C (3 minutes), followed by 28 cycles of 95°C (30 seconds), 55°C (30 seconds), and 72°C (30 seconds), then 72°C (2 minutes).

Figure 3 shows PCR results from reactions set up with Premixes A through I from the FailSafe PCR PreMix Selection Kit. These primers amplified the oyster metallothionein genes from the purified genomic

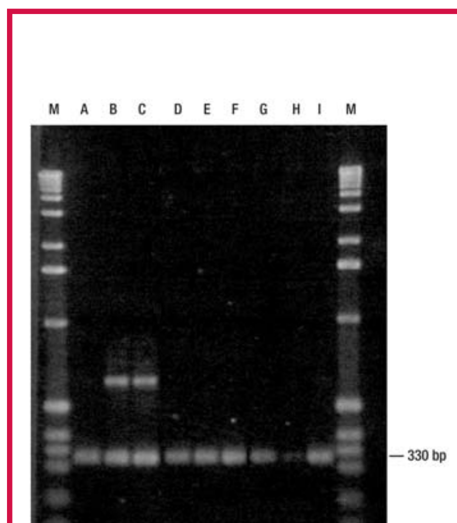


Figure 3. Oyster metallothionein genes were amplified using the FailSafe™ PCR PreMix Selection Kit. After amplification, PCR products were electrophoresed in a 2% agarose gel and stained with SYBR® Gold. Lanes A to I correspond to the FailSafe PreMixes used; Lanes M, kb marker.

DNA under all of the reaction conditions. PreMixes B and C gave additional products and PreMix H did not give optimal results, but any of the other PreMixes could be used with this template and these primers in future reactions.

PCR cloning

Amplified rDNAs were end-repaired and cloned into the CopyControl™ pCC1™ (Blunt Cloning-Ready) Vector as directed in the CopyControl PCR Cloning Kit. Ligations were electroporated into the kit's TransforMAX™ EPI300™ Electrocompetent *E. coli* and plated on LB plates containing 12.5 µg/ml of chloramphenicol and 1X CopyControl™ Induction Solution. Clones were grown under inducing conditions to increase the plasmid copy number and facilitate screening and plasmid purification. (For more information on the advantages of using the inducible CopyControl™ System to stably maintain clones at single copy, please see www.epicentre.com/ccpcr.asp.) Clones containing one to three copies each of the rDNA amplicons were selected using the Colony Fast-Screen™ (Size Screen) reagents included in the kit.

Sequencing and analysis of rDNAs

Selected clones were grown as described in the CopyControl PCR Cloning Kit. DNA was isolated, using either EPICENTRE's new FosmidMAX™ DNA Purification Kit or a spin-column plasmid isolation kit from another supplier, and sequenced. Sequencing results were consistently better with plasmids isolated using the FosmidMAX DNA Purification Kit than with the spin-column plasmid isolation kit (data not shown). Results were compared by BLAST analysis to known bacterial 16S rDNA sequences.

Results

Table 1 shows that of nine sequenced clones, eight are unique. Three clones represent *Pseudomonas sp.*, which are principal agents in the spoilage of fresh fish and shellfish, where they may inhibit the growth of other spoilage organisms.[9] The abundance of *Pseudomonas sp.* in oysters purchased in Wisconsin may reflect the length of time between their harvest and molecular analysis. *Shewanella sp.* have the interesting ability to reduce Fe III in the absence of oxygen, and have been found in many environments around the world. *S. baltica*, previously named *S. putrifaciens*, produces odorous compounds, such as trimethylamine, during fish spoilage.

Notably, 16S sequences of several clones (clones OB7, 14, 19, and 21) match the closest GenBank species by 94 to 98%, and may represent previously unidentified bacteria. Also, only gram-negative bacteria were found. Because the bacterial DNA was isolated without lysozyme treatment, gram-positive bacteria may have been present in the oyster, but not detected here. EPICENTRE offers the MasterPure™ Gram Positive DNA Purification Kit specifically for isolating DNA from hard-to-lyse gram-positive bacteria.

There are no pathogenic bacterial sequences in this set of rDNA genes. However, plating the surface bacteria from an oyster on antibiotic media revealed an ampicillin-resistant, culturable strain. No organisms resistant to 10 µg/ml tetracycline were found. The rDNA sequences were amplified from single colonies of the ampicillin-resistant strain and sequenced directly. The rDNA sequence is a 100% match with *Pseudomonas sp.* NZ101, isolated in New Zealand, which causes a disease in cultured mushrooms (Godfrey, S.A.C., Harrow, S.A., Marshall, J.W. and Klena, J.D., unpublished). Ampicillin-resistant bacteria are common in many rivers in the United States[10] and by inference might be found in river estuaries that support oysters.

Conclusion

EPICENTRE offers a wide variety of molecular tools for environmental DNA analysis, including the MasterPure Plant Leaf DNA Purification Kit, the

Table 1. Bacteria identified by rDNA sequences.

Clone	Closest database match*	% similarity	Putative phylum
OB1	*Flavobacterium sp.* A43	100	gram-negative
OB4	*Pseudomonas sp.* MSB2046	100	γ-proteobacteria, gram-negative
OB7	trout intestinal bacterium B76	97	gram-negative
OB12	*Shewanella baltica* OS155	100	γ-proteobacteria, gram-negative
OB14	*Pseudomonas Sp.* AU4899	94	γ-proteobacteria, gram-negative
OB17	*Shewanella baltica* CSQ1	100	γ-proteobacteria, gram-negative
OB19	trout intestinal bacterium B76	98	gram-negative
OB21	*Pseudomonas tolaasii* NZ032	98	γ-proteobacteria, gram-negative
OB26	eubacterium dtb25	99	gram-negative

*Gene assignment by BLAST searching the NCBI GenBank collection of bacterial 16S rDNA sequences.

CopyControl PCR Cloning Kit, FailSafe PCR PreMix Selection Kit, FosmidMAX DNA Purification Kit, and the MasterPure Gram Positive DNA Purification Kit.

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SYBR® Gold is a registered trademark of Molecular Probes.

www.epicentre.com/masterpure_plant.asp

MasterPure™ Plant Leaf DNA Purification Kit

MPP92010 10 Purifications
MPP92100 100 Purifications

Contents:

Plant DNA Extraction Solution
Cleanup Solution
TE Buffer

www.epicentre.com/ccpcr.asp

CopyControl™ PCR Cloning Kit (with electrocompetent cells)

CCECPCR1 20 Reactions

Contents:

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

www.epicentre.com/ccpcr.asp

CopyControl™ PCR Cloning Kit (with chemically competent cells)

CCPCR1CC 20 Reactions

CopyControl™ Induction Solution

CCIS125 25 ml
1000X concentrated solution. Filter sterilized.

www.epicentre.com/failsafe.asp

FailSafe™ PCR PreMix Selection Kit

FS99060 60 Units

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

FailSafe™ PCR System

FS99100 100 Units

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

FS99250 250 Units

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

FS9901K 1,000 Units

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

www.epicentre.com/fosmidmax.asp

FosmidMAX™ DNA Purification Kit

FMAX046 1 Kit
Reagents sufficient for 150 X 1.5-ml; 10 X 40-ml; or 5 X 100-ml purifications.

Contents:

FosmidMAX™ Solutions 1 to 4
RiboShredder™ RNase Blend
TE Buffer

www.epicentre.com/masterpure_gpdna.asp

MasterPure™ Gram Positive DNA Purification Kit

MGP04020 20 reactions
MGP04100 100 reactions

Contents:

Gram Positive Cell Lysis Solution
MPC Protein Precipitation Reagent
Ready-Lyse™ Lysozyme
Proteinase K
TE Buffer
RNase A

New! Transposon Construction Vectors

A custom EZ::TN™ Transposon containing any DNA sequence of interest can be easily prepared using an EZ::TN™ pMOD™ Transposon Construction Vector. The Transposon can be used for *in vitro* insertion into any target DNA, or it can be incubated with EZ::TN™ Transposase to form an EZ::TN™ Transposome™ complex, for making transposon insertion libraries in microorganisms.

Replication of these two new vectors is dependent on the *pir*-gene product found in our TransforMAX™ EC100D™ *pir*-strains. Both vectors were developed to optimize results obtained with EZ::TN Transposons that are prepared by restriction enzyme digestion of a pMOD-derivative rather than by PCR amplification.

www.epicentre.com/pmod.asp

EZ::TN™ pMOD™-4 <MCS> Transposon Construction Vector

MOD4804 20 µg

EZ::TN™ pMOD™-5 <R6Kγ*ori*/MCS> Transposon Construction Vector

MOD4805 20 µg

Tobacco Acid Pyrophosphatase (TAP) Functional Assay Ensures Decapped mRNA

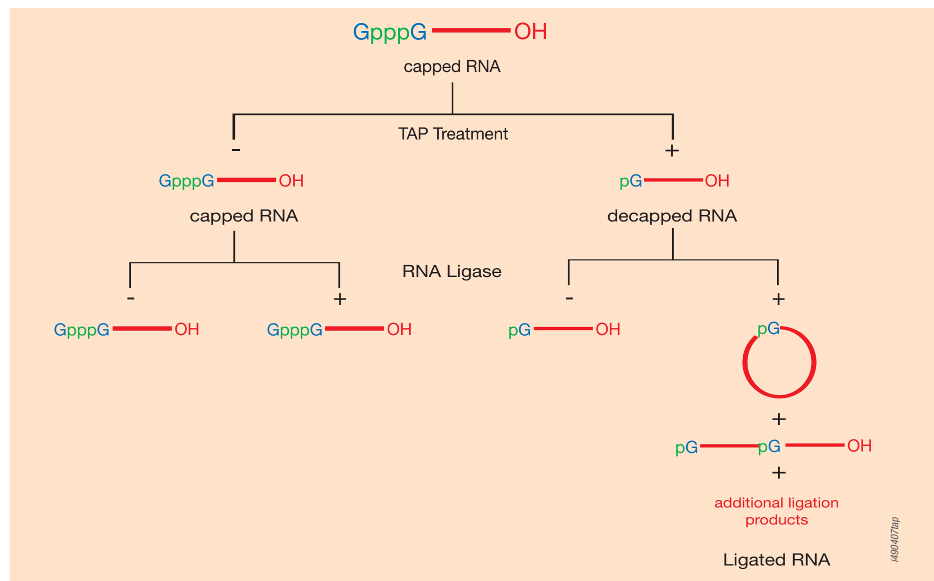
Tobacco acid pyrophosphatase (TAP) removes the "cap" structure from the 5′-end of eukaryotic mRNAs,[1] or the 5′-triphosphate from unprocessed prokaryotic transcripts,[2] leaving a 5′-monophosphate. This prepares the RNA molecule for ligation to other acceptor molecules. For example, T4 RNA ligase has been used to ligate oligoribonucleotides to TAP-treated cellular RNAs, allowing construction of full-length cDNA libraries[3] and accurate mapping of transcription initiation sites, for both eukaryotic[4] and prokaryotic[2] transcripts.

TAP activity is routinely assayed, using ATP[1,5] or *p*-nitrophenyl phosphate[5] as the substrate, by measuring the liberation of phosphate or *p*-nitrophenol. Assays are also performed to ensure that TAP preparations are not contaminated with phosphatases, which could remove the monophosphate remaining on the 5′ end of the decapped RNA, or with RNases, which could degrade the RNA substrate. However, none of these assays directly determine that the enzyme specifically decaps an RNA substrate.

Functional TAP assay

Each lot of EPICENTRE's TAP is tested in a functional assay, as outlined in Figure 1. A short, capped RNA transcript is synthesized using EPICENTRE's AmpliCap™ SP6 High Yield Message Maker Kit and incubated in the presence or absence of TAP. Each reaction is divided into two and incubated either in the presence or absence of T4 RNA ligase.

Figure 1. Schematic outline of EPICENTRE's functional TAP assay.



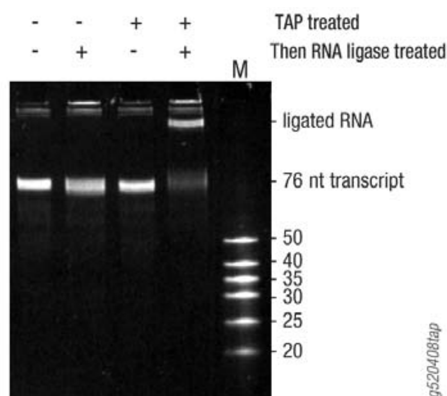


Figure 2. Lot # 750-30850 TAP treatment of capped RNA allows efficient self-ligation.

The ligation reaction is assayed on an 8 M urea, 20% polyacrylamide gel in a Tris-borate-EDTA buffer and stained with SYBR® Gold. Figure 2 shows that only capped RNA that was treated with TAP and RNA Ligase produced ligation products, as indicated by a shift in the RNA band from the non-ligated position to the slower migrating ligated position.

Conclusion

EPICENTRE functionally assays our TAP to ensure the expected specificity on capped RNA molecules. Specific ligation products would not be observed if the TAP was inactive, contaminated with a phosphatase, which would remove the 5′ phosphate from the decapped RNA, or contaminated with ribonuclease, which would produce smaller non-ligatable degradation products containing 3′ phosphates.

References

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www.epicentre.com/tap.asp

Tobacco Acid Pyrophosphatase (TAP)

T19050	50 U
T19100	100 U
T19250	250 U
T19500	500 U

Includes 10X Reaction Buffer.

AmpliCap™ SP6 High Yield Message Maker Kit

AC0706 25 Reactions

T4 RNA Ligase

LR5010	5 U/µl	1,000 U
LR5025	5 U/µl	2,500 U
LR5050	5 U/µl	5,000 U

Includes 10X Reaction Buffer and a 10 mM ATP Solution.

T4 RNA Ligase is also available in bulk. Please inquire.

SYBR® Gold is a registered trademark of Molecular Probes.

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

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

FailSafe™ PCR PreMix Selection Kit FS99060

Contains 60 units of the FailSafe™ PCR Enzyme Mix and all 12 FailSafe™ PCR 2X PreMixes. The FailSafe PCR System is also available with 100, 250, or 1,000 units of FailSafe PCR Enzyme Mix and your choice of FailSafe PCR 2X PreMixes.

*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.

EPI Announcements, etc.

COVER PHOTOS FOR EPICENTRE FORUM

Have your lab recognized and your photo on the cover of the EPICENTRE Forum. Please send us your original photos of local scenes. We will select 6 photos to appear on EPICENTRE Forum covers in 2005.

Send original photographs and a paragraph about your lab to:

EPICENTRE Forum Cover Photo
726 Post Road
Madison, WI 53713

Or submit high resolution digital photos to: julie.capadona@epicentre.com

Meet Your Customer Service Manager

Sandy Larson joined EPICENTRE 12 years ago as a customer service representative; now she manages that department as well as the company database. Each day she calmly and methodically responds to a variety of internal requests to fix problems and provide data support. Sandy may also answer your call to EPICENTRE when she's pitching in on the phones in order to keep things running smoothly in customer service.

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