

## Articles Citing Promega Products in Novel and Interesting Applications



This new section continues to present brief citations of peer-review journal articles featuring Promega products in interesting or novel applications. We welcome suggestions for similar citations of general interest to our readers.

### ***in vitro* RNA-dependent RNA polymerase assay/T-vector cloning**

**Expression of enzymatically active rabbit hemorrhagic disease virus RNA-dependent RNA polymerase in *Escherichia coli*.** López Vázquez, A., Martín Alonso, J.M., Casais, R., Boga, J.A. and Parra, F. (1998) *J. Virol.* **72**, 2999.

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In this work, the coding region of the rabbit hemorrhagic disease virus (RHDV) RNA-dependent RNA polymerase 3D (3D<sup>pol</sup>) was cloned as a *Bam*H I cassette by PCR amplification, and the resulting fragment was ligated into the pGEM<sup>®</sup>-T Vector<sup>(a,b)</sup>. The pGEM<sup>®</sup>-T Vector contains a single 3'-thymidine (T) overhang at the insertion site, which greatly improves the ligation efficiency of PCR products because of the nontemplate-dependent addition of a single deoxyadenosine (A) to the 3'-ends of PCR products by many thermostable enzymes. The fragment containing the 3D<sup>pol</sup> was purified from the pGEM<sup>®</sup>-T Vector and inserted into an expression vector, which was transformed into *E. coli* cells. The expression vector directed synthesis of a fusion protein composed of glutathione *S*-transferase (GST) and RHDV 3D<sup>pol</sup>. The 3D<sup>pol</sup> moiety of this fusion protein was released from the carrier protein by proteolysis.

In a poly(A)-dependent, oligo(U)-primed poly(U) polymerase assay, the recombinant 3D<sup>pol</sup> demonstrated poly(U) polymerase activity, which was dependent on oligo(U) primer and poly(A) template but was not inhibited by either rifampicin or actinomycin D.

The recombinant 3D<sup>pol</sup> enzyme was assayed for RNA-dependent RNA polymerase activity in a reaction with RNasin<sup>®</sup> Ribonuclease Inhibitor using a synthetic RHDV subgenomic RNA as template. In the presence of full-length template, the recombinant enzyme synthesized template-sized products from an oligo(U) primer. However, in the absence of the oligo(U) primer, the enzyme produced RNA molecules up to twice the size of the template (2L). The results were consistent with this activity being template-dependent RNA polymerase activity and not due to a terminal transferase reaction. The enzyme activity was dependent on ATP, CTP and GTP, as well as Mg<sup>2+</sup>. Treatment with RNase A revealed that the 2L product from this assay was double-stranded, and Northern blot analysis suggested that this 2L RNA was composed of covalently linked sense and antisense strand template.

### Ordering Information

Product	Size	Cat.#
pGEM <sup>®</sup> -T Vector System I <sup>(a,b)</sup>	20 reactions	A3600
pGEM <sup>®</sup> -T Easy Vector System I <sup>(a,b)</sup>	20 reactions	A1360
Recombinant RNasin <sup>®</sup> Ribonuclease Inhibitor <sup>(c)</sup>	2,500 units	N2511
	10,000 units	N2515

*pGEM and RNasin are trademarks of Promega Corporation and are registered with the U.S. Patent and trademark Office.*

### **primer extension/gel mobility shift assay/PCR product purification/DNase I footprinting**

**QacR is a repressor protein that regulates expression of the *Staphylococcus aureus* multidrug efflux pump QacA.** Grkovic, S., Brown, M.H., Roberts, N.J., Paulsen, I.T. and Skurray, R.A. (1998) *J. Biol. Chem.* **273**, 18665.

*School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006 Australia.*

The QacA protein of *Staphylococcus aureus* is a multidrug transporter, and QacR is a repressor protein that regulates the expression of *qacA*. Primer extension analysis was performed using **M-MLV Reverse Transcriptase** and 50µg of total RNA from *S. aureus* to map transcription start points (tsp) for the divergently transcribed *qacA* and *qacR* mRNAs. The location of promoter elements and regulation of *qacA* by QacR were further investigated with chloramphenicol acetyltransferase (CAT) assays. Gel mobility shift assays were performed using PCR-generated radiolabeled probes that were purified using a **Wizard® DNA Purification System**. These assays demonstrated that QacR binds to a DNA fragment in the *qacA* promoter that contains an inverted repeat (IR1), which is the putative operator site for QacR binding. DNase footprinting experiments using **RQ1 RNase-Free DNase** confirmed the gel shift results and showed that QacR bound specifically to IR1, overlapping the *qacA* tsp. Further, deletion of half of the IR1 dyad symmetry resulted in constitutive expression of a *qacA*-CAT fusion.

#### Ordering Information

Product	Size	Cat.#
M-MLV Reverse Transcriptase	10,000 units	M1701
	50,000 units	M1705
Wizard® DNA Clean-Up System <sup>(d)</sup>	100 preps	A7280
Wizard® PCR Preps DNA Purification System <sup>(e)</sup>	50 preps	A7170
RQ1 RNase-Free DNase	1,000 units	M6101

*Wizard* is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office.

### isolation of apoptotic DNA

**The role of CED-3-related cysteine proteases in apoptosis of cerebellar granule cells.** Eldadah, B.A.<sup>1,2</sup>, Yakovlev, A.G.<sup>1,3</sup> and Faden, A.I.<sup>1,3</sup> (1997) *J. Neurosci.* **17**, 6105, and

**The activation of CPP32-like caspases contributes to neuronal apoptosis and neurological dysfunction after traumatic brain injury.** Yakovlev, A.G.<sup>1,3</sup>, Knoblich, S.M., Fan, L., Fox, G.B., Goodnight, R. and Faden, A.I.<sup>1,3</sup> (1997) *J. Neurosci.* **17**, 7415.

<sup>1</sup>Georgetown Institute for Cognitive and Computational Sciences, <sup>2</sup>Interdisciplinary Program in Neuroscience, and <sup>3</sup>Department of Neurology, Georgetown University Medical Center, Washington, DC 20007 USA

Apoptosis is characterized by morphological changes, such as nuclear and cytoplasmic condensation, decrease in cell volume, plasma membrane bleb formation and fragmentation into apoptotic bodies. These changes are often accompanied by internucleosomal cleavage of genomic DNA. In these two studies, apoptotic cell death was assessed qualitatively by DNA fragmentation analysis.

In the work of Eldadah and colleagues, genomic DNA was extracted from cells that were incubated for 12 hours in control, serum-deprived, K<sup>+</sup>-deprived or serum/K<sup>+</sup>-deprived media. Yakovlev and colleagues examined induction of apoptosis in the cortex and hippocampus of rats subjected to traumatic brain injury (TBI). In both studies, cells were lysed with guanidine hydrochloride, and DNA was isolated using the **Wizard® DNA Purification Resin** and **Wizard® Minicolumns** or **Midicolumns**. Isolated DNA was analyzed for fragmentation, which is seen as DNA ladders, by ethidium bromide fluorescence or by labeling with [alpha-<sup>32</sup>P]dATP. Serum deprivation had little effect, but K<sup>+</sup> deprivation and serum/K<sup>+</sup> deprivation substantially increased the degree of cell death. In the TBI study, DNA fragmentation was observed in the injured cortex and hippocampus, beginning four hours after TBI and continuing for 72 hours.

#### Ordering Information

Product	Size	Cat.#
Wizard® Maxipreps DNA Purification Resin <sup>(d)</sup>	500ml	A7401
Wizard® Midicolumns	100	A7651
Wizard® Minipreps DNA Purification Resin <sup>(d)</sup>	250ml	A7141
Wizard® Minicolumns	250	A7211

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## PCR product purification/mutagenesis/sequencing/*in vitro* transcription

**Multiple virulence determinants of foot-and-mouth disease virus in cell culture.** Baranowski, E.<sup>1</sup>, Sevilla, N.<sup>1,2</sup>, Verdager, N.<sup>3</sup>, Ruiz-Jarabo, C.M.<sup>1</sup>, Beck, E.<sup>4</sup> and Domingo, E.<sup>1</sup> (1998) *J. Virol.* **72**, 6362.

<sup>1</sup>Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid 28049 Madrid, Spain; <sup>2</sup>Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037 USA; <sup>3</sup>Centre de Investigació i Desenvolupament (C.S.I.C.) 08028 Barcelona, Spain; and <sup>4</sup>Institut für Biochemie, University of Giessen, D-35392 Giessen, Germany.

This study investigated the genetic alterations of a hypervirulent foot-and-mouth disease virus (FMDV) variant. Amino acids of the G-H loop of the capsid protein VP1 enhance the binding of the virus to heparin and contribute to increased virulence in BHK-21 cells.

A number of derivatives of plasmid pFMDV-YEP-poly(C), containing FMDV strain 01K cDNA under the control of the SP6 promoter, were constructed. Plasmid p3,242/01K was constructed by subcloning a 3,242bp fragment of the original pFMDV-YEP-poly(C) plasmid into a derivative of **pGEM<sup>®</sup>-5Zf(+)** Vector<sup>(a)</sup>. Full-length, chimeric cDNAs were produced by substituting the coding region for two other capsid proteins for the 01K sequence in the plasmid. Chimeric plasmids were linearized and transcribed using **SP6 RNA Polymerase** in the presence of **RNasin<sup>®</sup> Ribonuclease Inhibitor**. To assess virulence of these chimeras, BHK-21 cells were transfected with these RNAs.

Confluent cell monolayers were inoculated with FMDV. Viral RNA extraction, cDNA synthesis and reverse transcription-PCR amplification were performed on infected cells. PCR products were purified using the **Wizard<sup>®</sup> PCR Preps DNA Purification System** <sup>(e)</sup>. Consensus nucleotide sequences were determined from the PCR-amplified DNA using the **fmol<sup>®</sup> DNA Cycle Sequencing System**.

For mutagenesis of the FMDV sequences, FMDV cDNA fragments were cloned into the **pALTER<sup>®</sup>-1 Vector** <sup>(a)</sup>. Single-stranded DNA template was obtained from *E. coli* DH5alpha<sup>®</sup> F' cultures containing the pALTER<sup>®</sup>-derived constructs. Site-directed mutagenesis was performed on these templates using the **Altered Sites<sup>®</sup> II *in vitro* Mutagenesis System**.

FMDV mutants that were generated and selected for inability to bind heparin demonstrated reduced virulence for some cell types.

### Ordering Information

Product	Size	Cat.#
Wizard <sup>®</sup> Minicolumns	250	A7211
Wizard <sup>®</sup> PCR Preps DNA Purification System	50 preps	A7170
pALTER <sup>®</sup> -1 Vector	20µg	Q6301
pGEM <sup>®</sup> -5Zf(+) <sup>(a)</sup> Vector	20µg	P2241
Altered Sites <sup>®</sup> II <i>in vitro</i> Mutagenesis System	20 reactions	Q6210
fmol <sup>®</sup> DNA Cycle Sequencing System	100 reactions	Q4100
fmol <sup>®</sup> DNA Cycle Sequencing System Sample	20 reactions	Q4110
SP6 RNA Polymerase	1,000 units	P1085
SP6 RNA Polymerase (HC)	2,500 units	P4084
Recombinant RNasin <sup>®</sup> Ribonuclease Inhibitor <sup>(c)</sup>	2,500 units	N2511
	10,000 units	N2515

Altered Sites, fmol, pALTER, pGEM, RNasin and Wizard are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office.

DH5alpha is a registered trademark of Life Technologies, Inc.

<sup>(a)</sup>*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.*

<sup>(b)</sup>*Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.*

<sup>(c)</sup>*U.S. Pat. No. 5,552,302 has been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor (PRI). Inhibitors of Angiogenin, which comprises a segment of human PRI, is the subject of U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687 assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.*

<sup>(d)</sup>*U.S. Pat. No. 5,658,548 has been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures.*

<sup>(e)</sup>*Licensed under U.S. Pat. No. 5,075,430.*

<sup>(f)</sup>*U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.*

<sup>(g)</sup>*The method of recombinant expression of Coleoptera luciferase is covered under U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.*

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