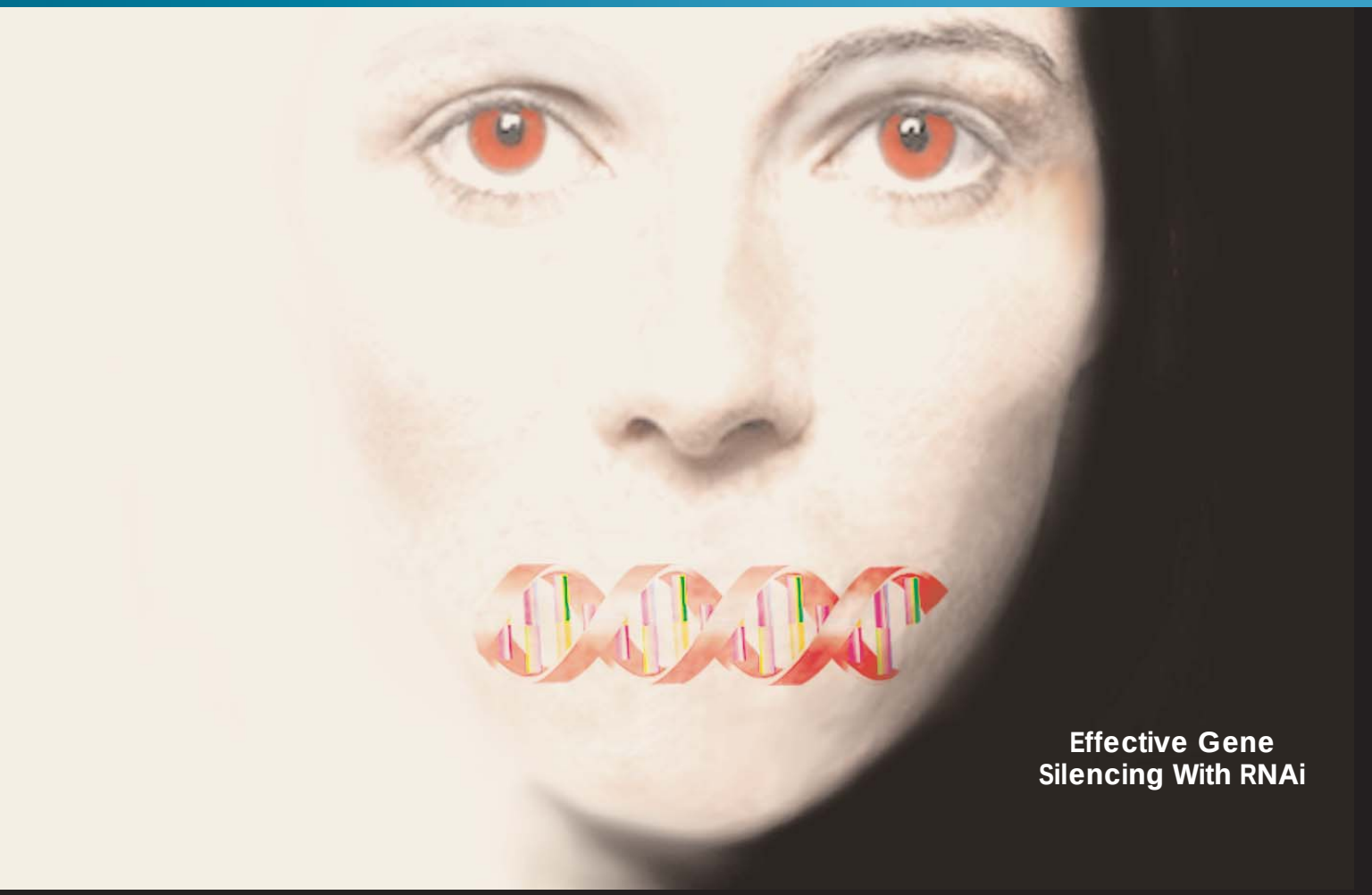
GTS Delivery



Silence of the Genes

A Newsletter for Advanced Molecular Delivery Tools

Volume 2 Issue 2

New Product

New Transfection Reagent for Effective Gene Silencing

GeneSilencer™ siRNA Transfection Reagent

Small interfering RNA (siRNA) is emerging as an important tool for inhibiting gene expression in mammalian cells. In order to help researchers fully take advantage of this powerful technique, a new reagent, GeneSilencer™, is now available for efficient transfection of functional siRNAs into diverse cell lines.

Why Transfect siRNA?

RNA interference (RNAi) is characterized by targeted mRNA degradation after introduction of sequence-specific double stranded RNA (dsRNA) into cells. Although cellular uptake of long dsRNA by organisms such as *C. elegans* and *Drosophila* has proven to be an effective method to induce RNAi, it tends to result in non-specific gene suppression in vetebrate cells due in part to interferon response. Recently, it has been discovered that short (less than 30 nucleotides) dsRNAs, referred to as small interfering RNAs (siRNA), can cause gene-specific silencing in mammalian cells (1,2,3). In addition, the RNAi effect caused by siRNA can be detectable even after many cell divisions. These properties make siRNA transfection a useful tool for gene silencing in mammalian cells.

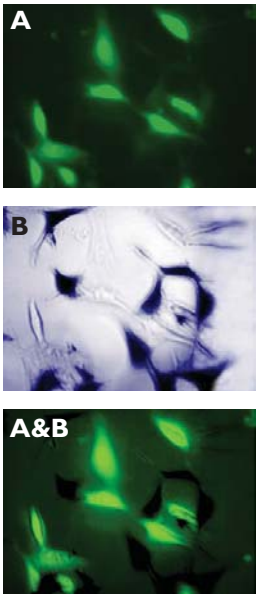
Efficient siRNA Transfection.

Because most commercially available wide-spectrum transfection reagents deliver poor results with siRNA, we formulated GeneSilencer, a cationic lipid based reagent that has been extensively screened in many mammalian cell lines in order to achieve:

- Efficient delivery of siRNA
- Functional gene silencing post siRNA delivery
- Compatibility with diverse growth conditions (with and without serum)
- Low cytotoxicity

These qualities combined with the easy-to-use protocol make GeneSilencer the ideal choice for siRNA transfection.

Figure 1. β-galactosidase Gene Silencing by siRNA Transfection into NIH 3T3 Cells



NIH 3T3 cells stably expressing ß-galactosidase were transfected with the fluorescent anti-*lacZ* siRNA oligos using the GeneSilencer™ Transfection Reagent. Approximately 50% of the cells were transfected with siRNA (green fluorescence). When stained with X-Gal, only cells that were not transfected with siRNAs stain positive for ß-galactosidase (blue color). Cells that took up siRNA (green fluorescence) did not show any visible X-Gal staining, demonstrating that anti-*lacZ* siRNA oligos delivered with GeneSilencer efficiently suppress ß-galactosidase expression.

New Product

Superior Gene Silencing.

To ensure efficient and functional siRNA delivery we have tested the GeneSilencer™ reagent with several siRNA fragments in various cell lines. Figure 1 shows that anti-*lacZ* siRNAi oligos, when transfected with the GeneSilencer™ reagent, effectively suppress ß-galactosidase expression in NIH 3T3 cells. In addition, when compared with other commercially available transfection reagents, GeneSilencer™ consistently offers superior transfection efficiencies and more effective gene silencing (Figures 2, 3).

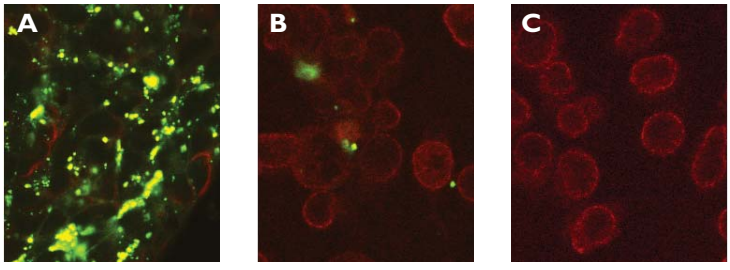
Silence Your Gene Today.

Get superior transfection results and functional RNA interference with the GeneSilencer reagent. Call GTS for more information or order today.

References

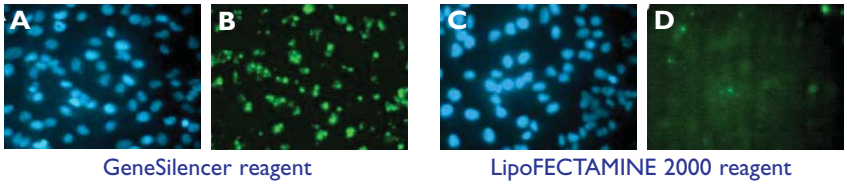
1. Elbashir, S.M. *et al.* (2001) *Nature* **411**: 494-498.
2. Caplen, N.J. *et al.* (2001) *Proc Natl Acad Sci* USA **98**: 9742-9747.
3. Sharp, P.A. (2001) *Genes and Development* **15**: 485-490.

Figure 2. Lamin Gene Silencing by siRNA Transfection into HeLa Cells



Logarithmically growing HeLa cells were transfected with anti-human lamin siRNA oligomers that have been fluorescently labeled with FITC (green). Two commercially available transfection reagents were used: (A) GeneSilencer (GTS), (B) Oligofectamine (Invitrogen), and (C) saline solution. The experiment was performed following the manufacturers' protocols. The images were taken 36 hours post transfection and all siRNAs that have not been taken up were washed away. Fluorescently-labeled anti-lamin antibody (red) was used to examine lamin protein levels inside the transfected cells. The highest amounts of lamin were found in the saline control (C), whereas the lowest amounts were detected in the experiment where GeneSilencer reagent was used (A).

Figure 3. Transfection of FITC-labeled anti-Lamin siRNA into HeLa Cells



HeLa cells were transfected with fluorescent siRNA that target the human lamin transcript. Two different transfection reagents were used and nuclei were stained with DAPI (A and C). In B, the uptake of fluorescent siRNA oligomers was shown using the GeneSilencer siRNA Transfection Reagent (DAPI control was shown under A). In D, Lipofectamine 2000 from Invitrogen was used as the transfection reagent for the uptake of fluorescent siRNA (DAPI control was shown under C).

Product	Quantity	Catalog no.	Price
GeneSilencer™ siRNA	**Transfection Reagent**		
	0.75 ml (200 rxn.)	T500750	$275
	5 x 0.75 ml (5 x 200 rxn.)	T505750	$1165

*Each transfection is for delivering 200 ng of siRNA.

Click to Learn More

New Product

Easy and Efficient Transfection of Neuronal Cells

NeuroPORTER™ Transfection reagent

Transfecting neuronal cells, especially dissociated cultures of primary neurons, can be a difficult task. That's why we developed the NeuroPORTER™ Transfection Reagent. It is the first transfection reagent specifically screened and optimized for efficient transfection of neuronal cells including primary neurons, neuronal cell lines, and glial cells.

Innovative Formulation.

NeuroPORTER is a new cationic lipid reagent that has been selected from a variety of large lipid libraries based on the following criteria:

- Superior transfection efficiency in primary neurons, cultured neuronal cell lines, and glial cell lines
- Minimized cytotoxicity

The formulation of NeuroPORTER was further optimized to allow a simplified protocol, serum compatibility and stability. This reagent has been rigorously tested in multiple cell types to ensure the highest transfection efficiency (percentage of cells expressing transgene) and activity (transgene product activity in cell extracts).

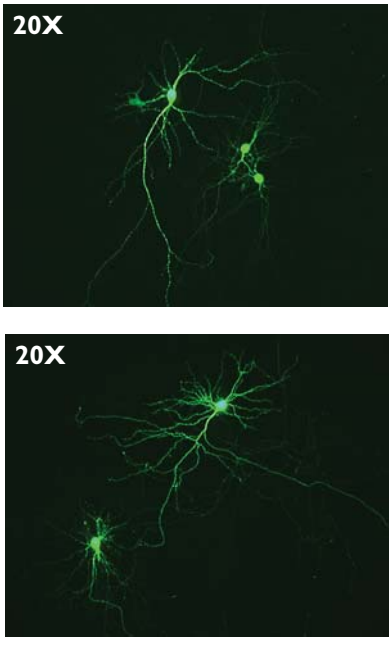
Neuron Transfection Made Easy

NeuroPORTER Transfection Reagent eliminates the hassles associated with viral transfection. Simply mix DNA with the NeuroPORTER reagent and you are ready to transfect neurons. NeuroPORTER reagent is compatible with serum so there is no need to change medium during transfection and there is no need to remove NeuroPORTER after transfection. For your convenience, three cell-type specific protocols designed for primary neurons, neuronal cell lines, and glial cell lines are provided in a comprehensive manual. In addition, detailed optimization examples are outlined. With NeuroPORTER reagent, neuronal transfection can not be easier.

Superior Results

Cultured primary neurons are difficult to transfect because they are post-mitotic and very sensitive to culture conditions. Figure 1 demonstrates the efficient transfection of a plasmid expressing GFP (green fluorescent protein) into a primary culture of rat cortical neurons. After the cells are transfected, there are no signs of neurodegeneration or withdrawal of neurites that are frequently observed when using calcium phosphate and other commercially available transfection reagents. In addition, we have compared NeuroPORTER reagent with other transfection reagents using human NT2 neuron precursor and C6 glioma cell lines. Figure 2 shows the superior transfection and gene

Figure 1. Transfection of plasmid expressing GFP into rat primary cortical neurons



Rat primary cortical neuron cells were grown on cover slip in 24 well plates. 0.5 µg of gWIZ/GFP vector (GTS Cat. No. P040400) were transfected into these cells with 2.5 µl of NeuroPORTER reagent. Images were taken 48 hours post transfection.

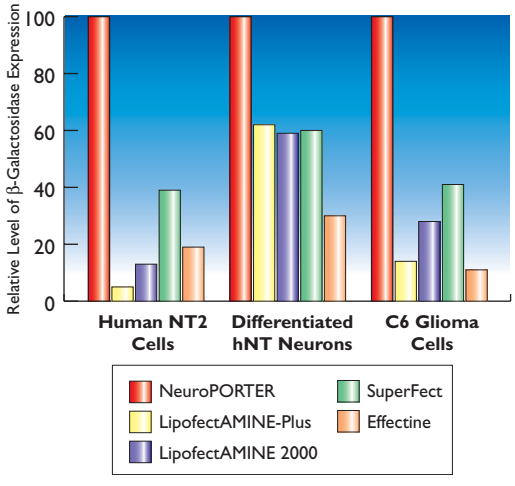
New Product

expression results obtained with the NeuroPORTER reagent.

Experience Efficient Neuronal Transfection.

No more hassles with viruses. No more frustrations with mediocre transfection results. Experience easy and efficient transfection with NeuroPORTER reagent. Order NeuroPORTER reagent today and start getting great neuronal transfection results.

Figure 2. β-galactosidase expression comparison in NT-2 and C6 cell lines



Product	Quantity	Catalog no.	Price
NeuroPORTER™ Transfection Reagent			
	1.5 ml (75-300 rxn.)	T400150	$275
	5x1.5 ml (375-1500 rxn.)	T400750	$1165

*Each transfection is for delivering 2 µg of DNA in 6-well plates.

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Application note

A Simple Method for Delivery and Antigen Presentation of a Malaria Protein in Human Dendritic Cells

Denise L. Doolan and Norma L. Graber
Naval Medical Research Center, Silver Spring, MD 20910 USA

BioPORTER™ Transfection reagent

Introduction

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play critical roles in the initiation and modulation of immune responses. Because of their potent antigen presenting ability, dendritic cells offer a promising strategy for eliciting immunity against tumors or pathogens of interest when they are loaded with target antigens. Historically, transfecting dendritic cells with DNA or RNA has proved to be problematic and most researchers can achieve only very low transfection efficiencies using retroviral delivery methods. This article demonstrates successful delivery and subsequent presentation of a malaria antigen in human dendritic cells using BioPORTER®, a novel cationic lipid-based protein delivery reagent.[1]

Materials & Methods

Dendritic Cells and T Cells

Peripheral blood mononuclear cells (PBMC's) were isolated from a human volunteer immunized with radiation-attenuated sporozoites from *P. falciparum*, the infectious agent responsible for malaria. The PBMC's were cultured with GM-CSF/IL-4 for 6 days to generate dendritic cells, which were used as APCs in an IFN-γ ELIspot assay. Autologous PBMC populations were used as a source of responder cells.

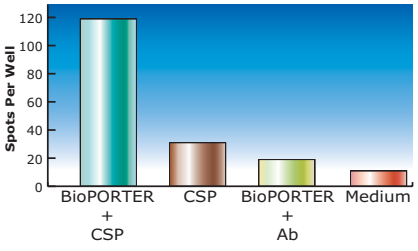
Antigen Delivery.

A total of 10 µg of recombinant *P. falciparum* circumsporozoite protein (PfCSP) expressed in *Saccharomyces cerevisiae* was diluted to a final volume of 40 µl in serum-free medium (OptiMEM). The protein solution was added to one pre-coated vial containing BioPORTER reagent (BioPORTER QuikEase™ Kit, GTS) and incubated at room temperature for 5 min. The BioPORTER /protein mixture was adjusted to a final volume of 0.5 ml in serum-free medium (OptiMEM). Then, 125 µl of the BioPORTER /protein mixture was added to 1 x 10^5 cultured dendritic cells, in a 12 ml snap-cap polypropylene tube, and incubated for 4 hrs at 37°C. Cells were washed twice with PBS and resuspended in standard cell culture medium (RPMI 1640 supplemented with 10% FCS).

IFN-γ ELIspot assay

PfCSP protein transduced dendritic cells were transferred to standard ELIspot plates precoated with anti-IFN-γ mAb. Autologous PBMCs were added to each well, at a target cell:effector cell (DC:PBMC) ratio of 1:10 (50,0000 DC/500,000 PBMCs and 25,000 DC/250,000 PBMCs). Cells were cultured for 36 hours at a temperature of 37°C in an atmosphere of 5% CO_2. Plates were then processed as per a

Figure 1. Cellular Immunity Assay



Dendritic cells were generated from PBMCs of a volunteer immunized with radiation-attenuated *P. falciparum* sporozoites, for use as target cells. Recombinant *P. falciparum* CSP protein was delivered to the dendritic cells using BioPORTER. Autologous PBMC populations were added to the protein-transduced dendritic cells and cultures processed as per a standard ELIspot assay. A total of 120 PfCSP antigen-specific T-cells out of 250,000 input PBMCs were detected, translating to 480 SFCs per million PBMCs.

Application note

standard ELIspot assay for evaluation of the number of PfCSP-specific IFN-γ secreting cells. The number of spots corresponding to IFN-γ producing cells was determined visually using a stereomicroscope (KS ELIspot, Zeiss). Results were expressed as the number of IFN-γ-secreting cells per 10^6 PBMCs.

Result and Discussion.

ELIspot assay results are presented in Figure 1. A total of 120 antigen specific T-cells were detected in the 250,000 input PBMCs, translating to 480 spot forming colonies (SFCs) per million PBMC's, when antigen specific target cells were generated by delivering PfCSP to the cells in the presence of BioPORTER reagent. In contrast, the signal was barely above background when the dendritic cells were treated with either recombinant CSP alone, BioPORTER + control mouse IgG, or culture medium alone. These data confirm results from additional studies demonstrating efficient delivery of fluorescent goat IgG into dendritic cells using BioPORTER reagent (Figure 2). Based on these results, we conclude that BioPORTER is an effective reagent for efficiently delivering proteins into dendritic cells, and that BioPORTER-mediated antigen delivery to dendritic cells results in functional antigen presentation. These properties of BioPORTER reagent make it a valuable tool for studying antigen presentation in difficult-to-transfect dendritic cells.

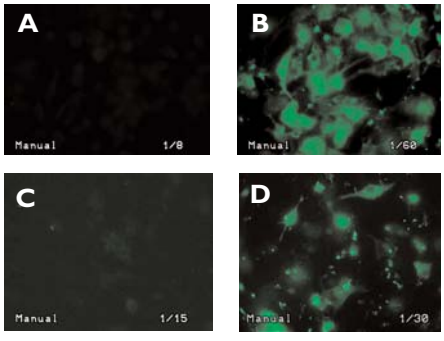
Reference

1. Zelphati, O. *et al.* (2001) *J. Biol. Chem.* **276**: 35103-35110.

Acknowledgments

This work was supported by funds allocated to the Naval Medical Research Center work unit 61102A.S13.F.A0009. The studies reported herein were conducted in accordance with US Navy regulations governing the protection of human subjects in medical research, and the protocols employing human subjects were reviewed and approved by the Naval Medical Research Center's Committee for the Protection of Human Subjects. The opinions and assertions herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the U.S. Navy or the Department of Defense.

Figure 2. Delivery of fluorescent antibody to human and mouse dendritic cells



A fluorescein labeled goat IgG antibody was delivered to human dendrtitic cells (A&B), or mouse bone marrow dendritic cells (C&D) either with BioPORTER (B&D) or without BioPORTER (A&C). For both cell types the results show that BioPORTER can very efficiently deliver antibodies to most of the cells in the culture. (Courtesy Gene Therapy Systems)

New Product

Speed Up Your *E. coli* Transformation with TurboCells™

TurboCells™ Competent *E. coli*

It has been a long day. You are tired and hungry. You just want to go home. But you still have to spend an hour or two to transform competent cells for overnight incubation….. To many of you this is a familiar scenario. That's why we developed TurboCells™ Chemically Competent E. coli. They allow efficient transformation in just 3 minutes so you can save hours and still get the colonies you need.

3-Minute Transformation

TurboCells Chemically Comptent *E. coli* are specially optimized to achieve excellent transformation efficiency with a novel 3-minute protocol (Figure 1). Together with the TurboCells Transformation Buffer, TurboCells allow efficient transformation without the need for a lengthy transformation reaction or hour-long recovery step. Compared to other competent cells, which require a 1-2 hour transformation protocol, TurboCells Competent E. coli can save you hours of time. In addition, with TurboCells you can perform the heat shock step at 37ºC, eliminating the inconvenience of setting up a 42ºC water bath.

Robust Results

With TurboCells, you do not have to sacrifice performance for speed. You can achieve transformation efficiencies of up to 5 x 10^7 – 1 x 10^8 cfu/µg of supercoiled DNA using the rapid 3-minute protocol, which is more than sufficient for most cloning experiments (Figure 2). Additionally, when using the traditional 1.5 – 2 hour transformation protocol with TurboCells, efficiencies of > 1 x 10^9 cfu/µg can be achieved.

Powerful Strain

TurboCells Competent *E. coli* are prepared using a unique procedure to allow consistent performance under diverse conditions. For example, 10 µl of undiluted ligation mix can be added directly to 50 µl of competent cells without significantly compromising transformation results. The fast and easy protocol, useful genotype (Table 1), and consistent performance make TurboCells Competent *E. coli* a great choice for both routine cloning experiments and demanding high-throughput transformations.

Convenient, Easy-to-Use Format

For optimal transformation results and for your convenience, TurboCells are supplied in single-use 50 µl aliquots. This avoids efficiency-robbing freeze-

Figure 1. Comparison of Transformation Protocols

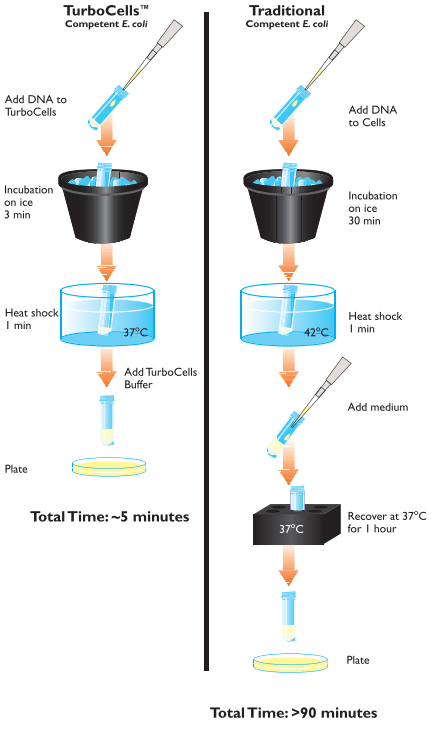
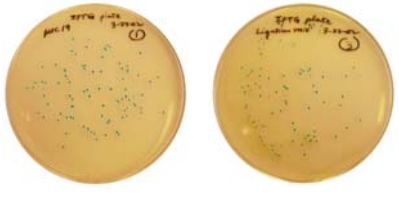


Figure 2. Transformation Results with TurboCells



pUC19 + TurboCells on IPTG/Amp plates

pUC19 self ligation+ TurboCells on IPTG/Amp plates

New Product

thaw cycles and wasted cells. Turbo charge your next cloning experiment with TurboCells Competent *E. coli* and get fast and efficient transformation results. Call GTS and order TurboCells today.

Table 1. Genotypes of TurboCells

TurboCells™ *F⁻*: *recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ80lacZΔM15 Δ(lacZYA-argF)U169*

TurboCells™ *F´*: *F recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ80lacZΔM15 Δ(lacZYA-argF)U169*

Genotype	Advantage
recA1	Reduced homologous DNA recombination
endA1	Low non-specific endonuclease activity and improved plasmid yield
hsd	Improved cloning efficiencies and representations of methylated DNA
lacZΔM15	Allow blue/white screening through b-galactosidase complementation

Product	Quantity	Catalog no.	Price
TurboCells™ Competent *E. coli*	20 x 50 µl	C300020	$170
TurboCells™ F´ Competent *E. coli*	20 x 50 µl	C301020	$170

Click to Learn More

20% Off Zeus Cuvettes With Any Newsletter Product Purchase

Zeus™ Electroporation Cuvettes are manufactured to strict quality standards to ensure consistent delivery results. They are ideal for electroporation of bacteria, yeast, mammalian and other cells. Cuvettes are available in three sizes for different cell types and protocols. Zeus Electroportation Cuvettes offer the following benefits:

- Compatible with all common electorporation devices (*e.g.* Bio-Rad, BTX, Eppendorf)
- Individually wrapped and gamma-irradiated to ensure sterility
- Gap tolerance of 0.02 mm to ensure reproducible results
- Color-coded, leak-resistance caps for easy identification of gap width
- Economical price

Product	Size	Quantity/Bag	Cat. No.	Price	Discount
Zeus™ Electroporation Cuvette					
	0.1 cm (white cap)	50	C901050	$99	$79
	0.2 cm (blue cap)	50	C902050	$99	$79
	0.4 cm (red cap)	50	C904050	$99	$79

Q & A

A Revolution in Cloning Technology

Xi-Clone™ Rapid PCR Cloning System: Questions & Answers

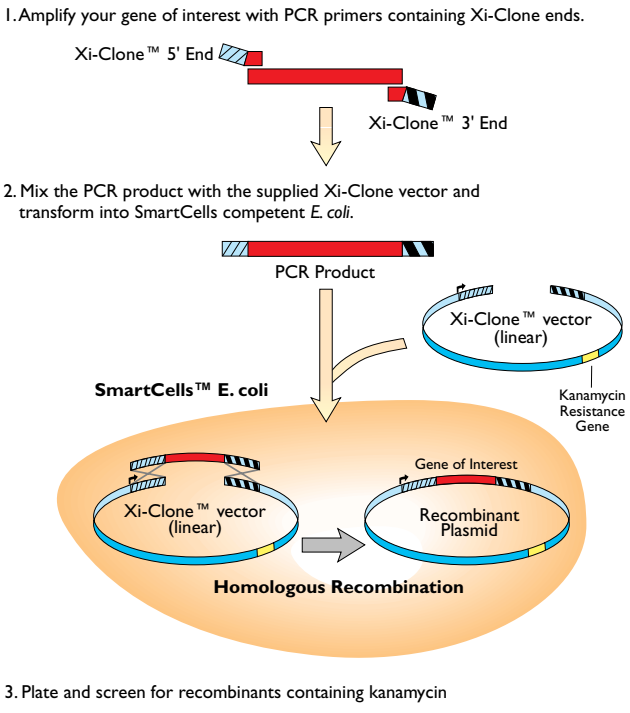
1. What is Xi-Clone™?

Xi-Clone™ is a powerful new PCR cloning system allowing researchers to save tremendous time and resources by cloning their genes-of-interest into high expression plasmid vectors without using any restriction or ligation enzymes. By simply mixing the PCR amplified gene with the linearized Xi-Clone vector, and then adding this mix to the SmartCells™ competent *E. coli* (included with the Xi-Clone kit), researchers can insert their gene of interest into a powerful mammalian or *in vitro* expression vector after only a few minutes of labor.

2. How does Xi-Clone work?

The Xi-Clone system uses homologous recombination in *E. coli* to insert the gene of interest into a linearized Xi-Clone expression vector (Figure 1). Initially, the gene is amplified by PCR using primers that contain both gene-specific and Xi-Clone end sequences. Next, the PCR product is mixed with the supplied linearized Xi-Clone vector, and the mixture is added to the SmartCells competent *E.coli*. After transformation, endogenous bacterial recombinases incorporate the gene of interest into the Xi-Clone vector, and > 85% of the transformants contain the insert in the correct orientation

Figure 1. How Xi-Clone Works



3. What are the available Xi-Clone expression vectors?

At present, Xi-Clone expression vectors are available for CMV promoter based constitutive expression in mammalian cells (phCMV/Xi vectors) and in T7 promoter based *in vitro* expression systems (pIX/Xi vectors). For your convenience, each of these vectors is available for expression of recombinant proteins in native or HA epitope-tagged formats.

Q & A

4. I use traditional restriction enzyme / ligase cloning. Why should I switch to Xi-Clone PCR Cloning?

The Xi-Clone system will save you days of labor compared to traditional restriction enzyme / ligase cloning. With the Xi-Clone system, there are no restriction digestions and no overnight ligations. In addition, screening will be significantly reduced because >85% of the transformants are the desired clones. More importantly, no extra sequences (i.e., restriction site nucleotides) are incorporated into your expression cassette. This means your expression product will not contain any unwanted amino acids.

5. I use TOPO/TA cloning, which is simple and takes 5 minutes. Why should I switch to Xi-Clone PCR Cloning?

If you are currently using TOPO/TA cloning system, you would benefit from using the Xi-Clone system because:

a. There are no topoisomerase recognition sequences necessary with Xi-Clone. So, no junk sequences are incorporated into your expression element, and no unwanted amino acids are incorporated into your expression product.

b. With Xi-Clone, your gene is always cloned in the correct orientation, whereas depending on which TOPO kit you use, you may get recombinants with your gene in both orientations.

c. The phCMV/Xi mammalian expression vectors utilize a highly optimized CMV promoter to give expression levels up to 5 times higher than those obtained with TOPO/TA counterparts.

d. For high-throughput users, no incubation steps are necessary.

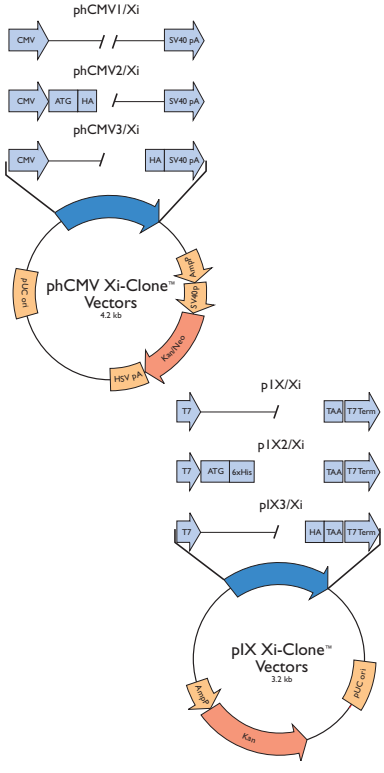
6. What is the largest gene I can insert into the Xi-Clone vector?

An upper size limit for the gene insert has not been determined. However, the largest gene we have successfully cloned into the Xi-Clone vector is 3. 7 kb.

7. Can I use my own vector for the Xi-Clone process?

GTS provides a custom vector service in which we can convert your vector into a Xi-Clone compatible vector. Contact our Technical Support Department at 888-428-0558, Ext. 3 for details.

Figure 2. Xi-Clone phCMV and pIX Vectors



Product	Quantity	Catalog no.	Price
phCMV1 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003120	$360
phCMV2 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003220	$360
phCMV3 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003320	$360
pIX Xi-Clone Kit for in vitro Translation	20 rxn.	XC004120	$345
pIX2 Xi-Clone Kit for in vitro Translation	20 rxn.	XC004220	$345
pIX3 Xi-Clone Kit for in vitro Translation	20 rxn.	XC004320	$345

*Each kit also contains sufficient SmartCells™ Chemically Competent *E. coli* for 20 transformations.

Click to Learn More

Product Review

The Key to High-Level Expression in Mammalian Cells

phCMV Plasmids

The new phCMV series of vectors offers significantly improved expression levels when compared to other CMV promoter-based constitutive expression vectors (e.g. pcDNA3.1). Available for expression of native proteins or HA-tagged fusion proteins, the phCMV vectors provide the ultimate flexibility and highest yield.

Built to Express

If you are working with difficult-to-transfect cells, such as neurons or macrophages, the higher the expression level of your vector, the easier it will be to identify your gene product. The phCMV vectors simply provide the highest expression levels of any mammalian expression plasmid available today. Not all CMV promoter-based plasmids are created equal. The CMV IE promoter/enhancer sequences contained in the phCMV vectors have been systemically analyzed and modified. The modifications include removing the sequences that are redundant and deleterious to high levels of expression while retaining those sequences with high transcriptional activity. In comparison to the most commonly used expression vectors, such as pcDNA3.1, phCMV vectors give up to 5x higher expression levels (Figure 1).

User-Friendly Vector Design

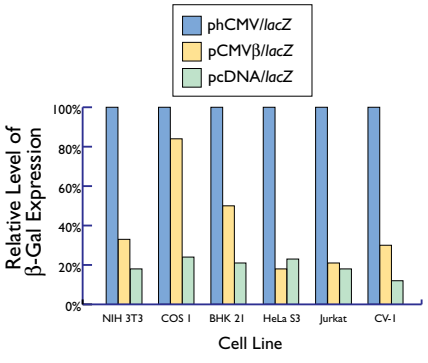
The phCMV series of plasmids offer tremendous flexibility in protein expression. Versions are available for expression of native proteins (phCMV1), as well as N-terminal HA-tagged proteins (phCMV2) or C-terminal HA tagged proteins (phCMV3), allowing for easy detection and purification (Figure 2). Additionally, the multiple cloning site of the phCMV vectors contains an extensive selection of common restriction sites that make your cloning task easier. Finally, the rigorously engineered vector backbone ensures high copy numbers in *E. coli* to allow easier plasmid preparation.

Optimized for Easy Stable Selection

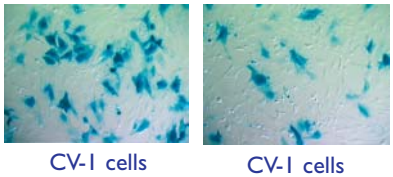
The neomycin resistance gene of the phCMV vectors provides for efficient selection of stable transformants using G418. Additionally, the combined

Figure 1. Expression Level Comparison

A. β-gal expression level comparison in different cell lines



B. β-gal expression in CV-1 cells



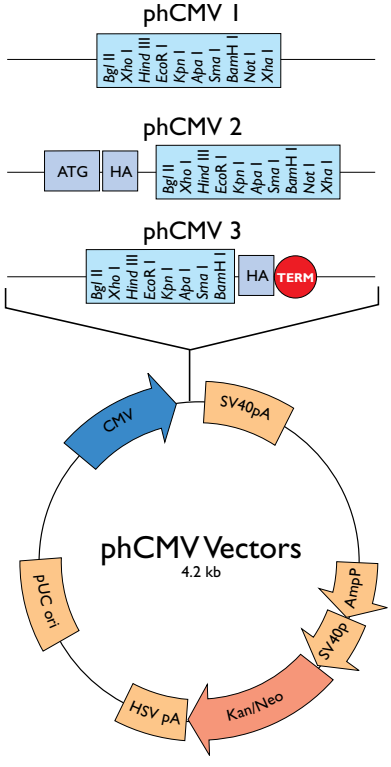
CV-1 cells transfected with phCMV/LacZ

CV-1 cells transfected with pcDNA/LacZ

CV-1 cells were transfected with 2 µg of plasmid DNA and 10 µl of GenePORTER reagent. After the initial 4 hours of incubation in serum free media 10% FBS-containing medium was added to the chambered slides and cells were cultured for 48 hours. Cells were then stained with X-gal.

Kanamycin/Neomycin resistance gene allows for a smaller plasmid size (approximately 4.2 kb) to ensure easy transfection. Taken together, the features of the phCMV vectors provide for easy, flexible and efficient cloning with the highest expression levels available. Unlock the door to high-level gene expression – call GTS and order your phCMV vector today.

Figure 2. phCMV Vector Maps



Product	Quantity	Catalog no.	Price
phCMV1 vector	25 µg	P003100	$250
phCMV2 vector	25 µg	P003200	$250
phCMV3 vector	25 µg	P003300	$250

Each kit includes 25 µg of expression vector and 10 µg of positive control expression vector.
* The phCMV vectors are also Xi-Clone™ adapted to allow enzyme-free PCR cloning. For more information see page 10.

Click to Learn More

Application note

Transfection of a 231 kb Bacterial Artificial Chromosome Vector

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GenePORTER™ Transfection Reagent

Introduction

Transfection of bacterial artificial chromosome (BAC) clones, which are large vectors commonly in excess of 100 kb, remains very difficult compared to transfection of common plasmid vectors, which are typically < 15 kb. In this article, we demonstrate the efficient transfection of Chinese hamster mutant V-C8 cells with a bacterial artificial chromosome (BAC) containing the breast cancer susceptibility gene, *Brca2*, using the GenePORTER™ transfection reagent. This experiment was performed to verify if V-C8 cells are defective in *Brca2* and to determine if hypersensitivity in response to various DNA damaging agents is caused by Brca2 deficiency (1).

Materials and Methods

Cell line.

V-C8 is a Chinese hamster mutant cell that represents the XRCC11 complementation group among X-ray-sensitive rodent cell mutants. It is extremely sensitive to various DNA-damaging agents (2, 3, 4). The high level of spontaneous and cross-linked induced chromosomal aberrations manifested by V-C8 cells indicates a possible defect in DNA repair. Indeed, V-C8 cells have an impaired capacity to repair double-stranded breaks after irradiation (3). A key player in double-stranded break repair through homologous recombination is the Rad 51 protein (5), a homolog of the E. coli RecA protein. Impaired formation of Rad51 foci, in response to DNA damage has been demonstrated in mammalian Brca1 or Brca2 defective cells, as well as in hamster cells defective in Rad51 paralogs.

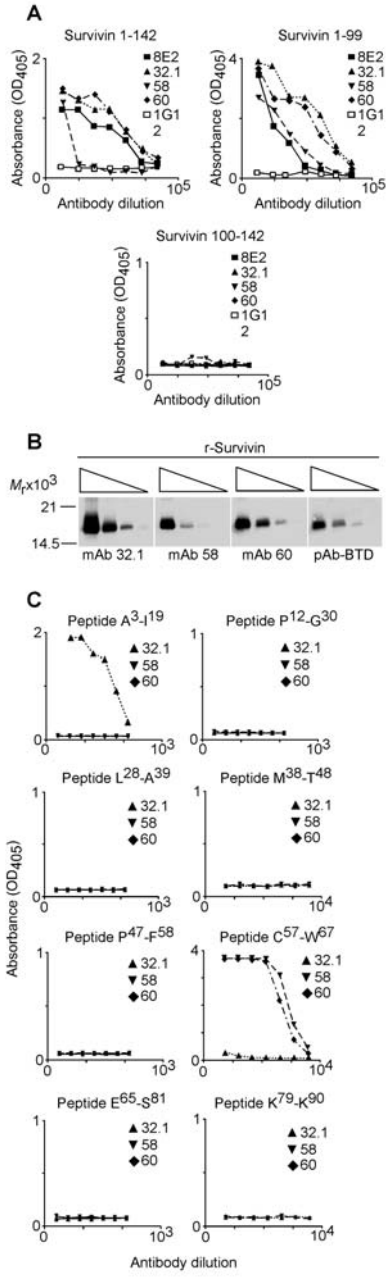
BAC vector

pBAC421-Neo is a bacterial artificial chromosome vector based on the 11.5 kb pBACe3.6 vector. pBAC421-Neo contains a 220-kb genomic sequence consisting of the full-length 60 kb murine *Brca2* gene, 70 kb of upstream sequences, and 90 kb of downstream sequences. This BAC has been used to completely rescue the embryonic lethality associated with the Brca2 mutation in mice (S. Swaminathan and S. K. Sharan, unpublished data).

Transfection

Transfection of pBAC421-Neo was performed using the GenePORTER transfection reagent according to the manufacturer's protocol. Briefly, V-C8 cells were incubated overnight so they were 60-90% confluent on the day of transfection. pBAC421-Neo DNA was mixed with GenePORTER transfection reagent in serum-free medium and allowed to incubate for 10-45 minutes. The DNA/GenePORTER complexes were added to the cells and allowed to incubate in serum-free medium at 37˚C. After 3-5 hours, one volume of medium containing

Figure 1. Cell Survival After Exposure



20% FCS was added to the transfection, and the mix was allowed to incubate overnight under 5-10% CO_2 at 37˚C. Cells were assayed for clonogenic survival after 24-72 hours.

Clonogenic Survival Assays

To determine clonogenic survival of V-C8 cells after transfection of pBAC421-Neo, cell cultures were transferred to 10 cm dishes and exposed either to X rays, or to the DNA damaging agents mitomycin C (MMC) and methanesulfonate (MMS). After treatment, the cells were rinsed with 0.9% NaCl, stained with 0.25% methylene blue, and visible colonies were counted.

Results

Using the clonogenic survival assay described, we observed rescue of clonogenic survival in the pBAC421-Neo transfected V-C8 cells compared to non-transfected cells (Figure 1). This confirmed the results of a separate experiment in which a single human chromosome 13 providing the *Brca2* gene (1) was transferred into V-C8 cells by microcell-mediated chromosome transfer (1). The sensitivities of V-C8 to X rays, MMC, MMS, and UV light (data not shown) were largely complemented by human chromosome 13 (Figure 1).

Discussion

In conclusion, the Chinese hamster cell mutant, V-C8, is defective in the breast cancer susceptibility gene *Brca2*. Transfection of a BAC containing the *Brca2* gene into V-C8 cells allows for rescue of resistance to DNA damaging agents and clonogenic survival. GenePORTER transfection reagent effectively delivers BAC's into these cells, allowing the gene of interest to be expressed and the effects of the expressed protein to be studied.

References

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