PROTEIN FUNCTION & ANALYSIS

Transfection

+ How do you study and control gene expression?

+ How do you detect mycoplasma contamination?

+ How do you ensure the success of your RNA or protein transfection?

STRATAGENE®

Introduction to Transfection

Transfection is the delivery of DNA, RNA, proteins, and macromolecules into eukaryotic cells. Goals for transfection include the study of gene regulation as well as protein expression and function. The success of transfection depends on transfection efficiency, low cytotoxicity, and reproducibility.

To ensure high efficiency of your transfection, you need reliable transfection technology and reagents to optimize your cell culture conditions. We provide you with a variety of reagents for your preferred transfection method and *mycoplasma* detection kits to ensure early detection of cell culture contamination.

For more information on our transfection products, please visit **www.stratagene.com/transfection.**

CONTENTS:

Table 1: Selection Guide for Transfection Reagents

	RECOMMENDED APPLICATION	TRANSFECTION METHOD	FEATURES	INCUBATION TIME
Mammalian Transfection Kit	DNA transfection	$CaPO_4$, DEAE	+ Works for many cell types	+ 12 - 24 hrs
MBS Mammalian Transfection Kit	DNA transfection	Modified $CaPO_4$	+ Works with low DNA concentration + Recommended for CHO and HEK293 cells	+ 3 hrs
LipoTaxi® Transfection Reagent	DNA transfection	Liposome based	+ Works for many cell types + Requires small amounts of DNA + Gentle on cells + Room temperature storage	+ 4 - 6 hrs
GeneJammer® Transfection Reagent	DNA transfection	Polyamine based	+ Good for hard to transfect (e.g. primary cells) + Works for many cell types + Effective in presence of serum (no media changes required) + Less cytotoxic than lipids	+ 3 hrs
GeneEraser™ siRNA Transfection Reagent	siRNA transfection	Proprietary formulation	+ Optimized for siRNA transfection into many cell types + Knocks down target gene even at low siRNA concentration + Low cell cytotoxicity + Works in presence of serum	+ 20 - 72 hrs
BioTrek™ Protein Delivery Reagent	Protein, peptide, macromolecule delivery	Lipid formulation	+ Works for many cell types + Conveniently packaged in single use vials + Includes control proteins	+ 4 hrs
ViraPack™ Transfection Kit	Viral packaging	$CaPO_4$	+ Optimized for viral packaging cell lines	+ 3 hrs

Transient and Stable Transfections

Classical transfection techniques include the DEAE-dextran method, calcium phosphate method, and liposome-mediated transfection (lipofection). Your choice of transfection technology will influence your results. We offer a variety of transfection reagents and kits to suit your specific needs.

DEAE-dextran and Calcium Phosphate Methods

We offer several mammalian transfection kits based on the original methods used to introduce DNA into cultured mammalian cells: DEAE-dextran and calcium phosphate. Our original Mammalian Transfection Kit utilizes the modified calcium phosphate method, which yields up to 100-fold efficiency over traditional methods, depending on the cell line to be transfected (Figure 1). Both are relatively simple techniques, with DEAE-dextran better suited for transient transfections and the calcium phosphate method for stable transfections.

To increase transfection efficiency over these standard methods, we utilize a specially modified bovine serum in our MBS Mammalian Transfection Kit[1] for use during transfection. This results in significantly higher efficiencies as well as reduced assay time (Figure 2). This kit exhibits superior performance with CHO (Chinese Hamster Ovary) and HEK293 (Human Embryonic Kidney) cells.

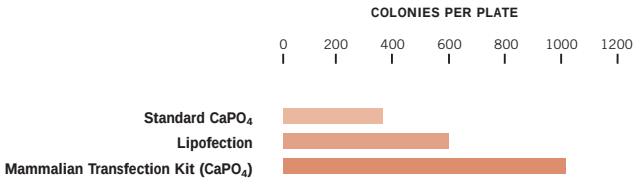


Figure 1
INCREASED YIELD WITH MAMMALIAN TRANSFECTION KIT

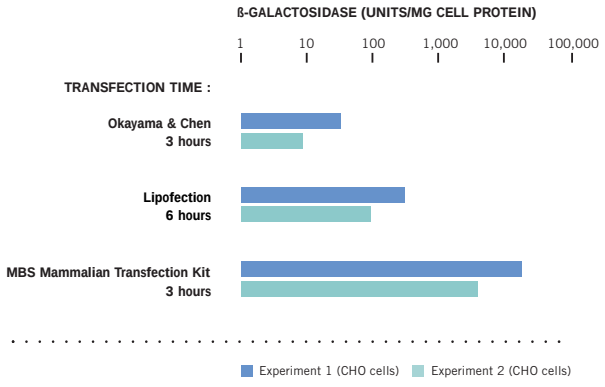


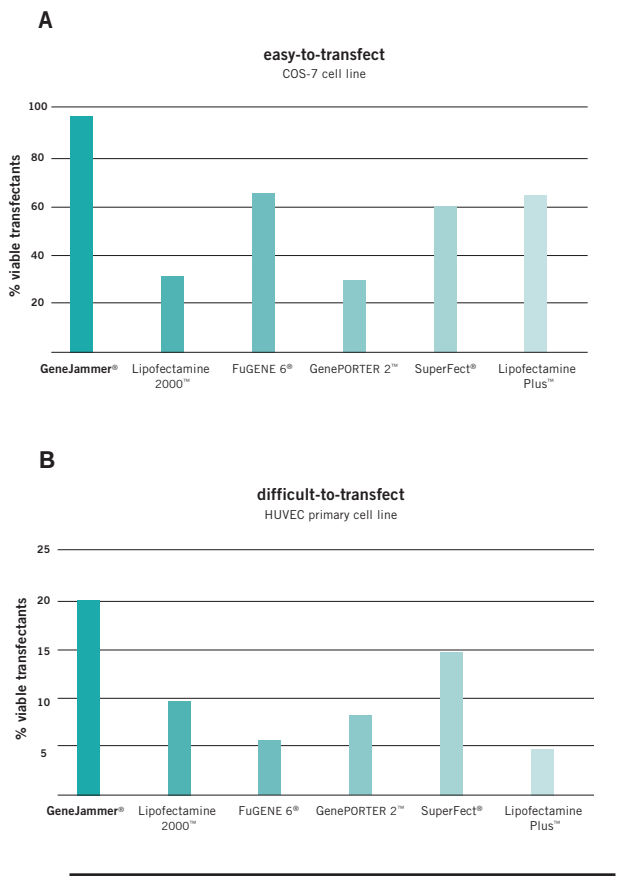
Figure 2
INCREASED EFFICIENCY WITH MBS MAMMALIAN TRANSFECTION KIT

Lipofection

Compared to the DEAE-dextran and calcium phosphate methods, liposomes often offer higher transfection efficiency and better reproducibility. Using our LipoTAXI® Transfection Reagent results in stable and transient transfections with low cytotoxicity and consistent high transfection efficiencies (a 10-fold increase over calcium phosphate/DEAE mediated transfection in many cell lines). Furthermore, our LipoTAXI reagent can be conveniently stored at room temperature.

Polyamines

Our GeneJammer® Transfection Reagent provides the high transfection efficiencies that you need, even in the most problematic conditions such as in the presence of serum or in transfection of primary cells (Figure 3). Furthermore, this easy-to-use reagent results in significantly reduced levels of cell damage as compared to the traditional liposomal method (Figure 4).



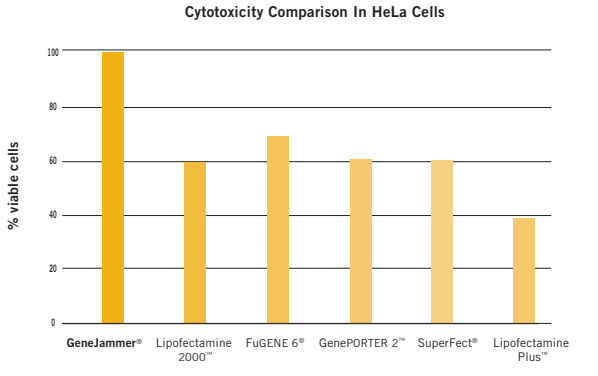


Figure 3

GENEJAMMER® REAGENT ENSURES HIGH TRANSFECTION EFFICIENCY IN BOTH EASY- AND DIFFICULT-TO-TRANSFECT CELLS

We used pCMV-beta-galactosidase to transfect COS-7 cells (A) and human umbilical vein endothelial cells (B), using GeneJammer® reagent and competitor transfection reagents.

Figure 4

GENEJAMMER® REAGENT DEMONSTRATES LOWEST TOXICITY, HIGHEST NUMBER OF VIABLE CELLS

To compare viability, we transfected HeLa cells with pCMV-beta-galactosidase and GeneJammer® reagent or competitive reagents. 24 hours after transfection, cell viability was evaluated by counting cells stained with trypan blue.

Special Transfection Needs

We also offer a variety of specialty transfection reagents and kits to meet your experimental needs such as transfecting RNA, proteins, or for virus packaging.

Protein Delivery

Our BioTrek™ Protein Delivery Reagent[2] allows you to directly translocate your protein into a living cell. Conveniently packaged in single use vials, this provides an easy method to effectively deliver proteins, peptides, and other macromolecules directly into a broad range of cell types at efficiencies of 50 – 95% in as little as 3 – 4 hours (Figure 5). This enables you to rapidly and accurately study cellular and protein function.

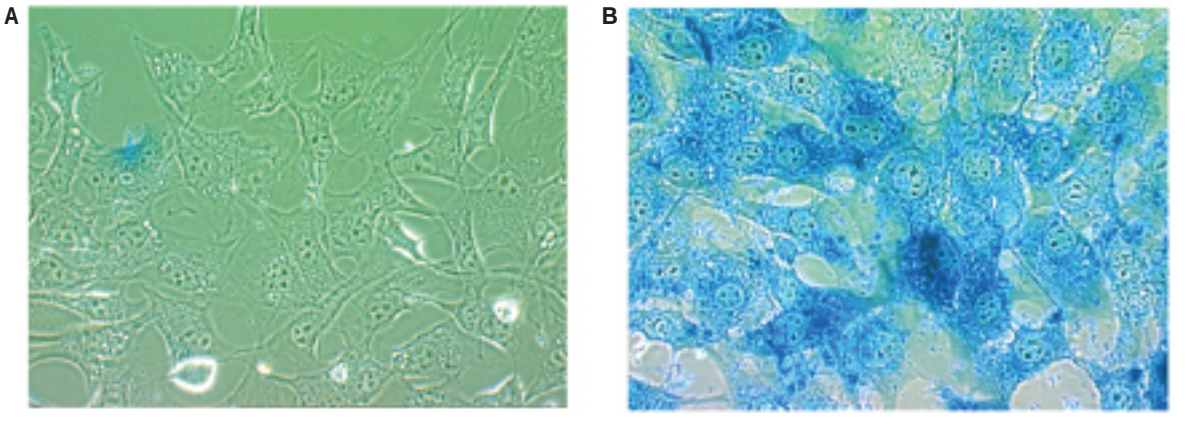


Figure 5

PROTEIN TRANSFECTION OF BETA-GALACTOSIDASE WITH BIOTREK™ REAGENT

BioTrek™ reagent mediated intracellular delivery of beta-galactosidase in NIH-3T3 cells (A). After 4 hours, cells were fixed, stained for activity with the X-gal reagent, then compared to standard transfection (B).

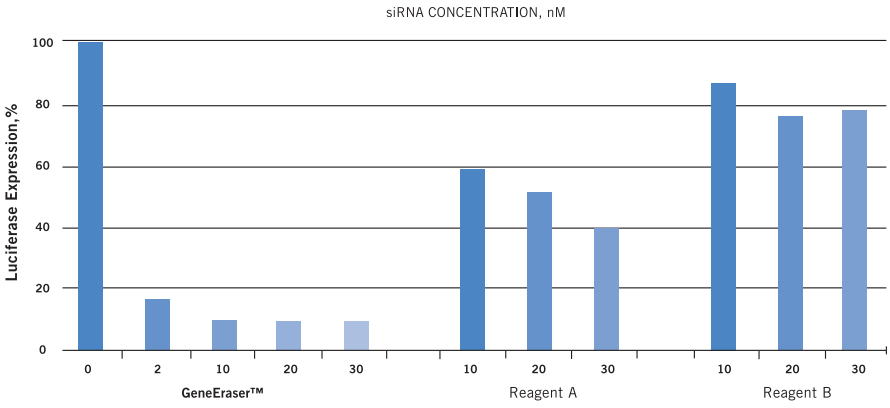
siRNA Transfection

RNA interference (RNAi) is a powerful method for inhibiting the expression of a particular gene. RNAi experiments require a transfection reagent that is specially optimized for siRNA delivery into mammalian cells.

Our GeneEraser™ siRNA Transfection Reagent[3] enables the use of very low levels of siRNA to knock out stable or transient gene expression in a wide variety of cell lines and has a high transfection efficiency (Figure 6). Provided in a ready-to-use format, the GeneEraser reagent and your siRNA can be added directly to cells in media, even in the presence of serum.

High Titer Virus Production

The transfection efficiency of the packaging cell line in any viral delivery experiment is critical. Our ViraPack™ Transfection Kit[1] transfects the HEK293 packaging cell line with exceptional efficiency, so you can increase the number of infectious virus particles produced per transfection.



CELL TYPES

Monkey Kidney SV40-Transformed
CHO-K1, Hamster Ovary
NIH/3T3, Mouse Embryo Fibroblast
HT-29 Human Colon Adenocarcinoma
A549, Human Lung Carcinoma
293, Human Kidney Transformed
HeLa, Human Cervical Carcinoma

Figure 6
SILENCING EFFECT OF LUCIFERASE siRNA BY GENEERASER™ siRNA TRANSFECTION REAGENT
Hela cells were transfected with 10 µg luciferase reporter plasmid pGL2 (Promega) using our GeneJammer™ siRNA Transfection Reagent. Cells were then transfected with luciferase GL2 siRNA duplex (Dharmacon Research) using different transfection reagents (2.5 µl). 83 to 90% knock out of luciferase expression was observed when 2 to 30 nM concentration of siRNA was complexed with the GeneEraser reagent, compared to 40 to 60% knock out with Reagent A and 13 to 22% knock out with Reagent B.

Table 2
The GeneEraser™ siRNA Transfection Reagent has been used to successfully deliver siRNA into the cell types listed above.

Mycoplasma Detection

Present in 5 - 35% of cell cultures, *mycoplasma* contamination can alter growth characteristics, enzyme patterns, cell membrane composition, chromosomal structure, and transfection efficiency. These lead to unreliable experimental results as well as loss of time and precious cell lines. Therefore, routine screening for *mycoplasma* contamination is essential. Unlike bacteria and fungi, *mycoplasma* contamination cannot be detected by visual inspection. Their small size allows them to pass through most sterilizat sion filters and they are resistant to common antibiotics.

Table 3: Mycoplasma Selection Guide

	METHOD	FEATURES	RECOMMENDED APPLICATION
Mycoplasma Plus™ PCR Primer Set	PCR	+ Includes positive and internal controls	+ Detection, speciation
MycoSensor™ PCR Assay Kit	PCR	+ Primer design prevents detection of *E. coli* DNA that often contaminates recombinant *Taq* DNA polymerase preparations + Short cycle time (50% faster) + Protocol ensures decontamination of carry-over PCR product + Detects as few as 10 copies of *mycoplasma* genomic DNA, depending on species	+ Detection
MycoSensor™ QPCR Assay Kit	Real-Time QPCR	+ Quick results (2 hours) + Convenient master mix format + Detects as few as 10 copies of *mycoplasma* genomic DNA, depending on species + Includes positive and internal amplification controls + Includes PCR purification kit + Tested on multiple real-time QPCR platforms	+ Detection

Mycoplasma Detection

Traditional mycoplasma detection methods, such as culture testing, staining, and immunofluorescence detection, are very time-consuming, not sensitive, and difficult to interpret. PCR amplification provides a quick and easy method for mycoplasma detection.

The Mycoplasma Plus™ PCR Primer Set provides you reliable PCR detection and speciation of mycoplasma. This kit provides an internal control to confirm PCR amplification was successful and a noninfectious mycoplasma genomic DNA template that serves as a positive control.

Our next generation PCR amplification kit, the MycoSensor™ PCR Assay Kit[4], features enhanced primer design to improve detection of the eight most common contaminating species (Table 4) and to prevent the detection of E. coli DNA, which often contaminates recombinant Taq DNA polymerase preparations. The MycoSensor kit features shorter run time (50% faster) and detects as few as 10 copies of Mycoplasma genomic DNA, depending on species (Figure 7) .

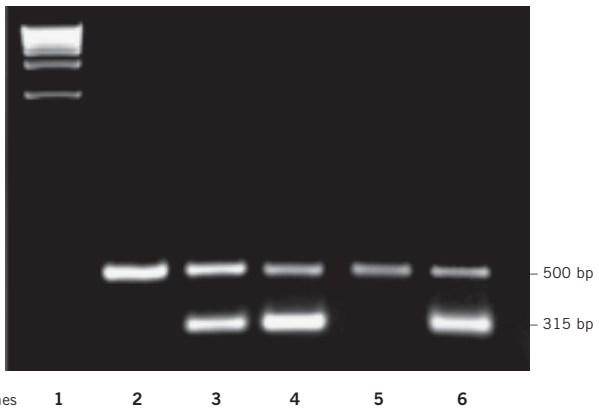


Figure 7
ELIMINATING FALSE NEGATIVES IN YOUR MYCOPLASMA DETECTION

The internal control in the MycoSensor™ PCR Assay Kit reduces false negatives by verifying that PCR amplification was successful, indicated by a 500-bp band that is distinguishable from the mycoplasma target band. **Lane 1**: kb ladder; **Lane 2**: Water + internal control (no template); **Lane 3**: Internal control + M. orale (Positive control); **Lane 4**: Internal control + A. laidlawii (Positive control); **Lane 5**: Internal control + CHO cell supernatant (Myco -); **Lane 6**: Internal control + HeLa cell supernatant (Myco +).

EIGHT MOST COMMON SPECIES OF MYCOPLASMA AND ACHOLEPLASMA

1	*A. laidlawii*
2	*M. arginini*
3	*M. fermentans*
4	*M. hominis*
5	*M. hyorhinis*
6	*M. orale*
7	*M. pirum*
8	*M. salivarium*

Table 4

Our MycoSensor™ PCR Assay Kit detects the eight most common contaminant species of mycoplasma and acholeplasma.

Mycoplasma Detection by Real-Time PCR

Our new MycoSensor™ QPCR Assay Kit[5] detects mycoplasma infection of cell cultures by real-time quantitative PCR, using SYBR® Green dye detection, in less than 2 hours. The convenient master mix format provides all the reagents needed for amplification and fluorescence detection, and the kit detects the eight most common cell culture contaminating species of mycoplasma (Table 4). Customers can detect as few as 10 copies of contaminating genomic DNA from 100 µl of cell culture supernatant, or test for weak mycoplasma infection in extracts made from cell pellets (Figure 8).

The kit includes two positive control templates to validate the detection of successful PCR amplification of mycoplasma template and confirm the SYBR® Green dissociation profile for your test samples. If your test cell line is infected, the SYBR Green dissociation profile results in a melting peak at 82°C. As seen by a melting peak around 85°C, the amplification control confirms the absence of PCR inhibitors, thus reducing false negative results (Figure 9). To minimize false positives, the closed tube real-time detection format minimizes the potential for cross-contamination with PCR amplicon. We include a passive reference dye in the kit to allow you the flexibility of running the assay on most real-time QPCR platforms. The kit also contains DNA purification reagents for removal of potential PCR inhibitors in cell culture supernatants and cell pellets.

The MycoSensor QPCR assay kit provides a fast, convenient method for the routine testing of cell cultures for mycoplasma and acholeplasma contamination. The kit delivers consistent results and includes controls designed to eliminate false negatives and false positives. We've optimized the MycoSensor QPCR assay kit for maximum performance on multiple real-time QPCR platforms, including our Mx3000P™ and Mx4000® Quantitative PCR instruments, the Cephied Smart Cycler®, and the ABI PRISM® 7700.

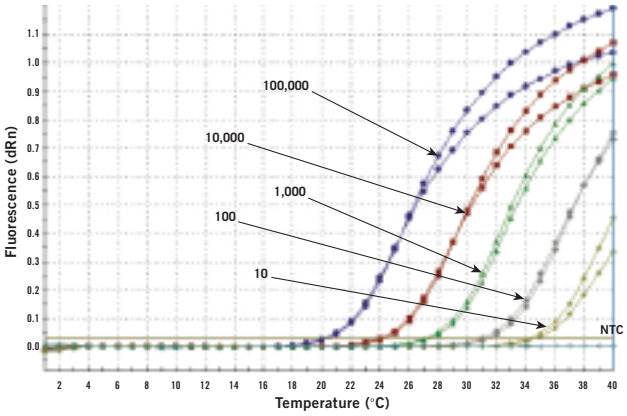


Figure 8
DETECTION OF M.ORALE GENOMIC DNA USING MYCOSENSOR™ QPCR ASSAY KIT

The MycoSensor™ QPCR Assay Kit detects a range from 100,000 copies to 10 copies of *M.orale* genomic DNA in the presence of HeLa cell culture supernatant. The reactions were done in duplicate on the Mx3000P™ system and the amplification plots are shown here.

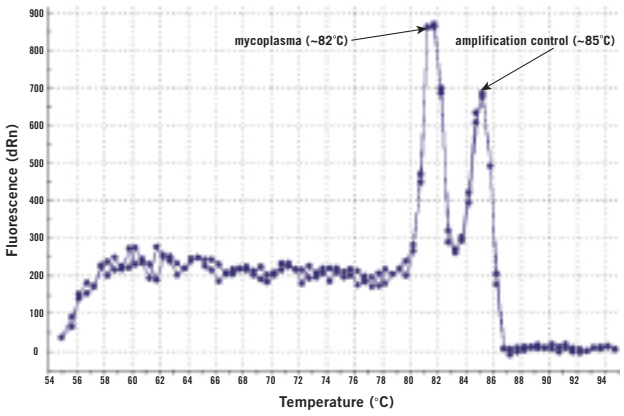


Figure 9
SPECIFIC DETECTION OF MYCOPLASMA CONTAMINATION AND AMPLIFICATION CONTROL

The SYBR® Green dissociation curve generated on the Mx3000P™ system for the *mycoplasma* primers and the amplification control primers indicates distinct melting temperatures of -82°C for mycoplasma and -85°C for the amplification control, respectively.

Ordering Information

Description	Catalog No.	Size	Price ($)
Mycoplasma Detection Kits			
MYCOPLASMA PLUS™ PCR PRIMER SET	1 kit (100 rxn)	302008	335
MYCOSENSOR™ PCR ASSAY KIT	1 kit (100 rxn)	302108	335
MYCOSENSOR™ QPCR ASSAY KIT	1 kit (100 rxn)	302106	395
Transfection Reagents & Kits			
GENEJAMMER® TRANSFECTION REAGENT	0.3 ml	204132	102
	1.0 ml	204130	202
	4 x 1.0 ml	204131	728
LIPOTAXI® TRANSFECTION REAGENT	3 x 1 ml	204110	211
MAMMALIAN TRANSFECTION KITS			
CAPO4 AND DEAE	1 kit (30 transfections each)	200285	237
CAPO4	1 kit (30 transfections)	200385	113
DEAE	1 kit (30 transfections)	200386	113
MBS MAMMALIAN TRANSFECTION KIT	1 kit	200388	330
Protein Delivery			
BIOTREK™ PROTEIN DELIVERY REAGENT	1 kit (24 rxn)	204140	270
siRNA Transfection			
GENEERASER™ SIRNA TRANSFECTION REAGENT	0.4 ml	204152	177
	1.0 ml	204150	358
Viral Delivery			
VIRAPACK™ TRANSFECTION KIT	1 kit	200488	190
Other Related Products			
BETA-GALACTOSIDASE ASSAY KIT	1 kit (100 rxn)	200383	149
BETA-GALACTOSIDASE ASSAY KIT (HIGH SENSITIVITY)	1 kit (100 rxn)	200710	180
BETA-GALACTOSIDASE ASSAY KIT (IN SITU)	1 kit	200384	154
G418 SULFATE	100 mg	200397	27
	500 mg	200398	52
	1 mg	200399	93
	10 ml	200049	62
HYGROMYCIN B	1 MU (1 x 10^6 U)	200715	129
LUCIFERASE ASSAY KIT	1 kit (100 rxn)	219020	90
QUANTOS CELL PROLIFERATION ASSAY KIT	1 kit	302011	221

LEGAL INFORMATION

1 U.S. Patent No. 5,330,904

2 BioTrek™ Protein Delivery Reagent is manufactured for Stratagene by Gene Therapy Systems, Inc.

3 GeneEraser™ siRNA Transfection Reagent is manufactured for Stratagene by Mirus

4 Patent pending
Purchase of this PCR-related product does not convey any rights under the PCR patents owned by Hoffmann-La Roche. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

5 Patent pending
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