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A Newsletter for Advanced Molecular Delivery Tools

Volume 3 Issue 1

Feature Article

Efficient and Economical Generation of Functional siRNAs

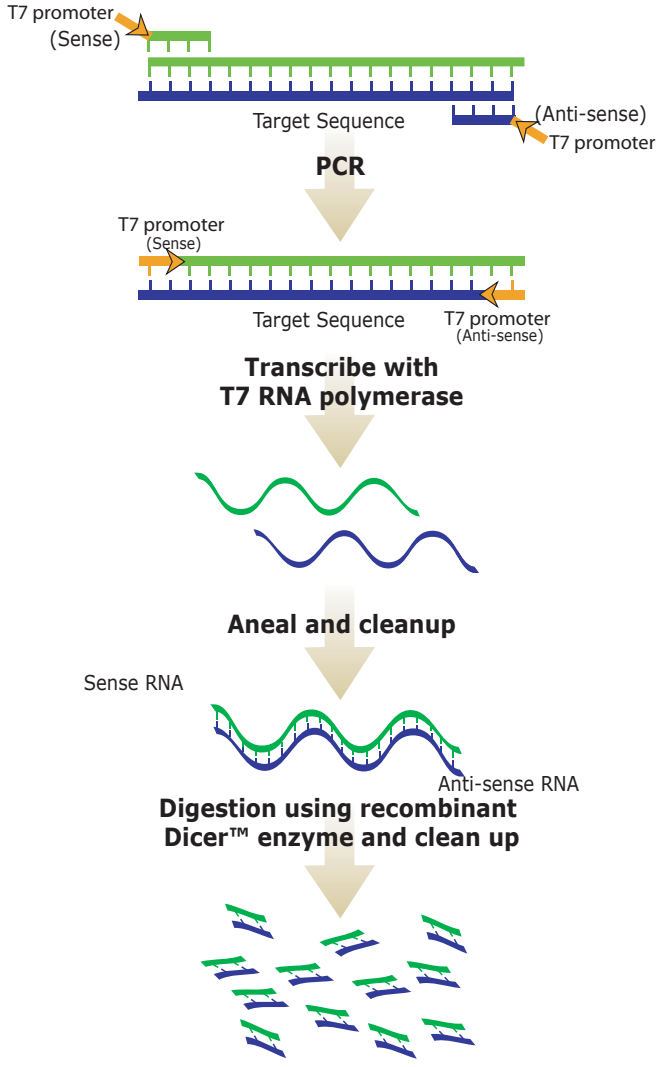
Dicer siRNA Generation Kit

Small Interfering RNA (siRNA)-mediated gene silencing is an exciting and valuable tool for studying gene function. However, most researchers are limited by their ability to test only a handful of chemically synthetic siRNAs due to high cost and lack of design guidance. Dicer siRNA Generation Kit allows cost-effective production of siRNAs targeted against entire gene sequences for efficient gene silencing.

Harness the Natural Process.

Most researchers purchase 3 to 8 chemically synthesized double-stranded siRNAs for each gene of interest to test whether they can get any strong gene silencing effect. This means synthesizing and processing 6 to 16 RNA oligonucleotides at a minimum cost of $1500. Because the rules for effective siRNA design are not well established, all the time and money spent could be potentially wasted. The Dicer siRNA Generation Kit mimics the natural RNA interference process by using the recombinant human dicer enzyme to cleave *in vitro* transcribed dsRNA into a pool of 20 to 21-bp siRNAs (Figure 1). Compared to conventional siRNA construction methods such as chemical synthesis and hairpin siRNA expression

Figure 1. How Dicer siRNA Generation Kit Works



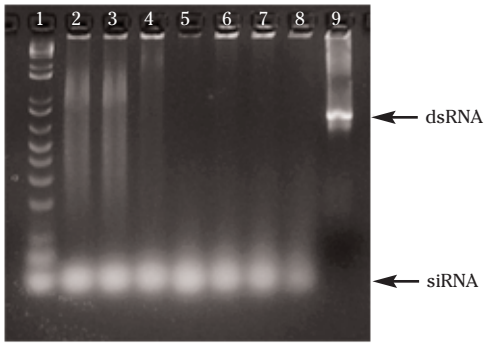
vectors, the Dicer siRNA Generation Kit offers the following advantages:

- No guesswork – A mixture of siRNAs against the full-length gene sequence has a better chance of success than a single siRNA design
- Cost effective – Less trial-and error on untested synthetic siRNA sequences
- High efficiency – the same or greater effectiveness as well-designed, chemically synthesized siRNA
- Optimized siRNA transfection conditions with GeneSilencer™ siRNA Transfection Reagent

Efficient siRNA Generation.

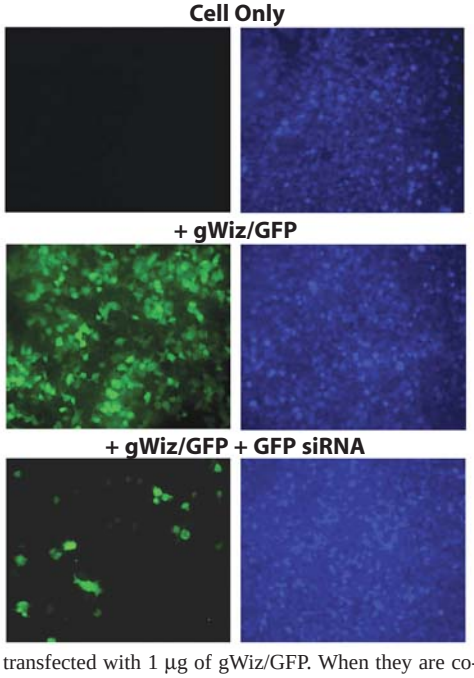
The Dicer siRNA Generation Kit relies on two powerful technologies for efficient siRNA production. First, the novel TurboScript™ T7 Transcription Kit allows rapid synthesis of 10 to 50 times the amount of RNA produced by conventional *in vitro* transcription reactions. The secret behind this high yield is that each DNA template is copied hundreds of times. This ensures that you will have sufficient dsRNA after annealing the transcribed sense and antisense RNA strands. The second key technology is an ultra-active form of human recombinant dicer enzyme which can cleave more than 90-95% of dsRNA template into 20 to 21-bp siRNAs within 12 hours under optimized reaction conditions (Figure 2). With an abundant

Figure 2. Generating siRNA with Recombinant Dicer Enzyme



1 µg of a 700 bp dsRNA after 12 hour of digestion by the dicer enzyme. Lanes: 1. marker; 2. 1 µl dicer, 3. 1.5 µl dicer, 4. 2 µl dicer, 5. 2.5 µl dicer, 6. 3 µl dicer, 7. 3.5 µl dicer, 8. 4 µl dicer, 9. 1 µg dsRNA (control)

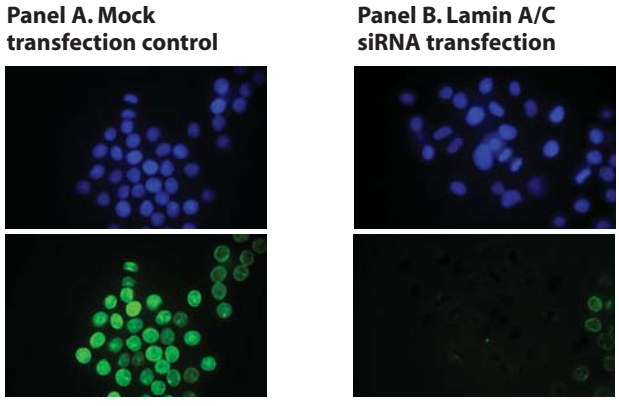
Figure 3. lac Z Gene Silencing in HeLa Cells



293 cells transiently transfected with 1 µg of gWiz/GFP. When they are co-transfected with 500 ng of diced GFP siRNA, GFP expression was efficiently suppressed. Cells were imaged 48 hours post-transfection. (GFP: green, left panels. DAPI: blue, right panels).

New Product

Figure 4. Endogenous lamin A/C Gene Silencing in HeLa Cells



HeLa cells were transfected with GeneSilencer and immunostained for Lamin A/C (green). A mock transfected control was compared to 100nM of either Dicer-processed lamin A/C siRNAs. Nucleii were counterstained (blue) with DAPI. In panel B more than 50% of the cells showed gene silencing.

supply of dsRNA templates and subsequent efficient dsRNA cleavage you are sure to have sufficient siRNAs in every single species to achieve highly efficient gene silencing.

Powerful Gene Silencing.

Figure 3 demonstrates the efficient silencing of the green fluorescent protein (GFP) gene expression in 293 cells stably expressing GFP. While successful gene silencing was achieved in one experiment with the Dicer siRNA Generation Kit, it was necessary to employ several chemically synthesized siRNAs using generally-accepted design guidelines before an siRNA sequence was found to exhibit any GFP suppression. In another experiment, we showed that siRNAs generated using the Dicer siRNA Generation Kit are at least as effective in silencing endogenous lamin A/C gene expression as published synthetic siRNA (Figure 4).

Easy-to-Use Kit.

The Dicer siRNA Generation Kit includes all the necessary reagents to generate sufficient siRNAs for 50 gene silencing experiments with up to 5 genes. The entire procedure takes about 2 days, which represents significant time savings compared to the 1-2 weeks required for ordering and testing chemically synthesized siRNAs. In addition, each kit comes with optimized GeneSilencer™ siRNA Transfection Reagent to ensure efficient delivery of your precious siRNA sample. The comprehensive product manual includes easy-to-follow protocols and recommended transfection conditions to guide you step by step. Expand your research by testing more than just a few siRNAs. Call GTS today to order your Dicer siRNA Generation Kit.

Product	Quantity	Catalog no.
Dicer siRNA Generation Kit		
	1 Kit	T510001
Recombinant Dicer Enzyme Kit		
	50 Units	T510002
TurboScript™ T7 Transcription Kit		
	20 reactions	T510003
GeneSilencer™ siRNA Transfection Reagent		
	0.75 ml (200 rxn.)	T500750
	5 x 0.75 ml (5 x 200 rxn.)	T505750

*Each transfection is for delivering 200 ng of siRNA.

Application note

Gene Silencing of 12/15-lipoxygenase in Mouse Primary Peritoneal Macrophages

Jinjin Fan, Lei Zhao, and Colin D. Funk
Univ. of Pennsylvania School of Medicine

GeneSilencer™ siRNA Transfection reagent

Introduction

12/15-lipoxygenases (12/15-LO) have been implicated in the maturation process of red blood cells and the oxidative modification of low-density lipoproteins related to atherogenesis. To study the interactions between lipoxygenase pathways and to determine the role of 12/15-LO-catalyzed oxidation of LDL in atherogenesis, expression of 12/15-LO was silenced in primary murine peritoneal macrophages using anti-12/15-LO siRNA transfected with the GeneSilencer™ siRNA Transfection Reagent.

Methods and Materials

Transfections

Transfection of primary murine peritoneal macrophages was performed using 3 µg of anti-12/15-LO siRNA or 3 µg of control anti-GL2-FITC siRNA (Dharmacon). The cells were subcultured in 6-well plates at 3-5 X 10^6/well. The siRNA was diluted with 25 µl of siRNA diluent and 15 µl of DMEM. This was added to 20-30 µl of GeneSilencer™ Reagent and allowed to incubate for 5 minutes before adding to the cells.

Fluorescent and Light Microscopy

To estimate transfection efficiency, the cells transfected with control GL2-FITC siRNA were examined under fluorescent and light microscope 24 hours post-transfection.

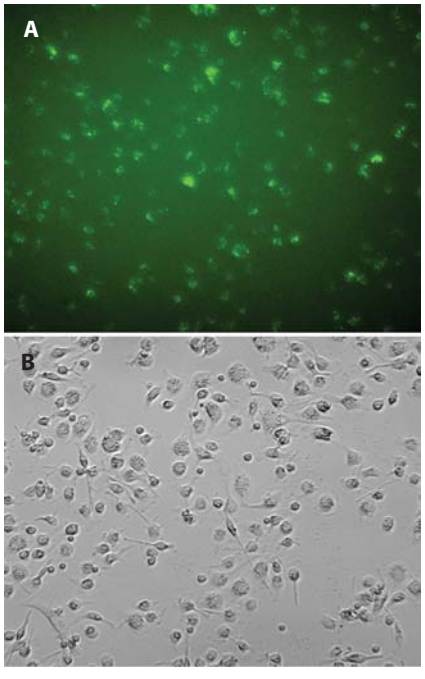
Western Blot Analysis

Cells transfected with anti-12/15-LO siRNA were analyzed for silencing of 12/15-LO expression using Western blot analysis 48 and 96-hrs post-transfection. Cell lysate was run on a 10% SDS-PAGE denaturing/reducing gel. Western Blot analysis was performed using ECL Hybond nitrocellulose membrane (Amersham) and an in-house rabbit anti mouse 12/15-LO.

Results

Figure 1A shows transfection of primary peritoneal macrophages using GeneSilencer Reagent and GL2-FITC siRNA dulpex. The transfection efficiency was approximately 60%. Figure 1B shows the same field under light microscopy. Figure 2 shows knockdown of 12/15-LO expression at 48 and 96 hr post-transfection. After normalizing the

Figure 1. Transfection of anti-GL2-FITC siRNA into Primary Murine Peritoneal Macrophages



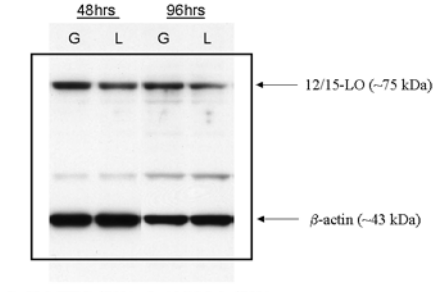
A. Fluorescent Microscopy
B. Light Microscopy

12/15-LO signal to β-actin, the "knockdown" percentage achieved with the individual siRNA species used was approximately 30% at 48hrs post-transfection and 40% at 96 hrs post-transfection.

Discussion

In conclusion, the GeneSilencer siRNA Transfection reagent efficiently delivers siRNA into primary murine peritoneal macrophages and provides effective siRNA-mediated gene silencing. The gene knockdown achieved allows for successful study of the role of 12/15-LO in lipoxygenase pathways and 12/15-LO-catalyzed oxidation of LDL in atherogenesis.

Figure 2: Silencing of 12/15-LO Expression with an Anti-12/15-LO siRNA



G: GL2-FITC siRNA L: 12/15-LO siRNA

Product	Quantity	Catalog no.
GeneSilencer™ siRNA Transfection Reagent		
	0.75 ml (200 rxn.)	T500750
	5 x 0.75 ml (5 x 200 rxn.)	T505750
GeneSilencer™ 96 Titration Plate		
	96-well plate	T500960
GeneSilencer™ 96 Standard Plate (High)		
	96-well plate	T500961
GeneSilencer™ 96 Standard Plate (Low)		
	96-well plate	T500962

New Product

Transfection of Antisense Oligonucleotides with Enhanced Potency

Cytofectin™ Transfection Reagent

Cytofectin™ Transfection Reagent provides efficient transfection of antisense oligonucleotides with high potency, minimal toxicity, and an easy-to-use protocol. Cytofectin has become established as the most reliable reagent for anti-sense oligonucleotide transfection by researchers whose numerous publications attest to its efficacy and quality.

Powerful Formulation

Transfection of functional antisense DNA oligonucleotides into cells using cationic lipids is a valuable method for the elucidation of gene function. However, the use of transfection reagents optimized for plasmid DNA limits researchers from fully capitalizing on the effectiveness of anti-sense oligonucleotide-mediated gene silencing. In contrast, Cytofectin Transfection Reagent is an optimized formulation, which has been specifically screened with antisense oligos for high transfection efficiency, potent antisense activity, broad specificity, and minimal toxicity.[1-3] This convenient combination of benefits makes Cytofectin a powerful tool for antisense oligonucleotide studies.

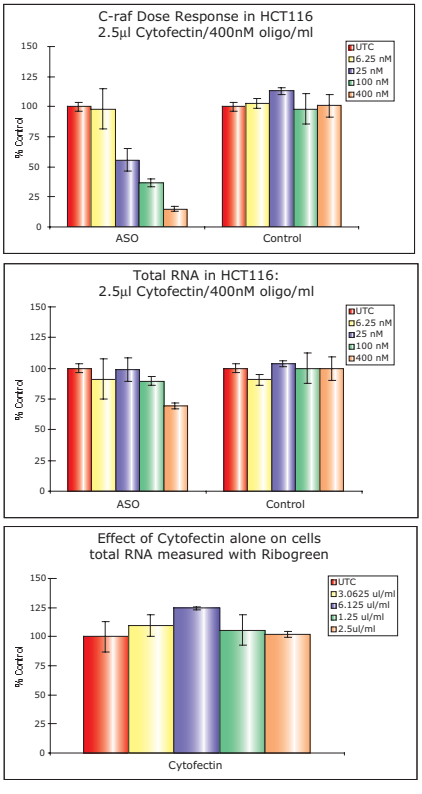
Time-Tested Results

Efficient delivery of antisense oligonucleotides is of no use unless the delivered oligonucleotide DNA can provide effective and target-specific gene suppression. Antisense oligonucleotides delivered with Cytofectin Reagent have been shown to be more effective at reducing target gene expression compared to transfection of the same oligos using other available transfection reagents.[3] Please visit our website at www.genetherapysystems.com to view a complete list of published Cytofectin references.

Easy-to-use Protocol

The Cytofectin protocol is simple and straightforward, making your oligonucleotide transfections fast, easy, and stress-free. Simply mix the Cytofectin reagent and the oligonucleotide DNA, each diluted with serum-free media, and add to your cells growing in serum-containing medium. There are no special diluents required for your DNA and no need to change medium after an initial serum-free incubation period. Also, in contrast to other transfection reagents which work across a narrow range of DNA/lipid ratios, the Cytofectin Reagent works across a much broader range of

Figure 1. C-raf Dose Response in HCT116 Cells



Transfection of antisense oligonucleotide (ASO) into HCT116, a human colon adenocarcinoma cell line. The target gene is *c-raf*, a well characterized Ser/Thr kinase.

Panel A. Dose response of c-raf in HCR116.
Panel B. Total RNA Measured with Ribogree for corresponding treatments from Panel A.
Panel C. Effect of Cytofectin Reagent along on cell viability/toxicity.

Table 1. Cell Lines Successfully Transfected with Cytofectin™ Transfection Reagent

Cell Line	Species	Type
COS-7	Monkey	Kidney
CV-1	Monkey	Kidney
BalbC-3T3	Mouse	Embryo fibroblast
Rat-2	Rat	Embryo
HeLa	Human	Cervical carcinoma
NHDF	Human	Dermal fibroblast
MCF-7	Human	Breast carcinoma
A549	Human	Lung carcinoma
T24	Human	Bladder carcinoma
HCT116	Human	Colon carcinoma
H460	Human	Lung carcinoma
WiDR	Human	Colon carcinoma
HT-29	Human	Colon carcinoma
CasKi	Human	Cervical carcinoma
SiHa	Human	Cervical carcinoma

DNA/lipid ratios, eliminating the need for extensive optimization to find the one ratio that works efficiently.

Smart Solution for Anti-Sense Oligonucleotide Transfection

Now, there is no need to waste hours of time trying to optimize transfection of anti-sense oligonucleotides with transfection reagents optimized for plasmid DNA. Call GTS and start effective and functional transfection of your antisense oligonucleotide today.

References:
1. Wagner, RW *et al.* (1994) *Nature* **372**: 333-335.
2. Wagner, RW *et al.* (1993) *Science* **260**: 1510-1513.
3. Lewis, JG *et al.* (1996) *Proc. Natl. Acad.* U.S.A. **93**: 3176-3181.

Product	Quantity	Catalog no.
Cytofectin™ Transfection Reagent		
	1 ml	T610001
	5 x 1 ml	T610005

Product Review

Efficient Neuronal Cell Transfection

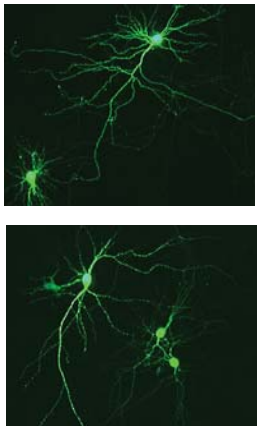
NeuroPORTER™ Transfection Reagent

Transfection of neuronal cells is notoriously difficult, with the percentage of transfected cells commonly <1% for primary neurons and <20% for neuronal cell lines. To improve gene delivery in neuronal cells, we developed the NeuroPORTER™ Transfection Reagent, an innovative cationic lipid specifically designed to achieve the highest transfection efficiencies in a variety of primary neurons, neuronal cell lines, and glial cells.

Superior Neuronal Transfection Efficiencies

Obtaining a high percentage of transfected neurons is a difficult challenge frequently faced by neuroscience researchers. With NeuroPORTER Transfection Reagent, efficient transfection of neuronal cells is now possible. Figure 1 shows dissociated rat primary cortical neurons transfected with a GFP expression vector. The transfection efficiency achieved was 5-10%. Also, note how these cells exhibit none of the cytotoxicity or withdrawal of neuronal dendrites commonly associated with the use of calcium phosphate and other transfection methods. Figure 2 shows a comparison of expression levels obtained using NeuroPORTER Transfection Reagent and other popular transfection reagents in a variety of neuronal cell lines. The NeuroPORTER reagent is clearly the most effective at facilitating high level gene expression. Transfection efficiencies achieved with these cells ranged from 35% to 40%. In addition, customers have obtained transfection efficiencies as high as 80% in certain glial cell lines.

Figure 1: Transfection of 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.

Optimized Neuron Transfection Reagent

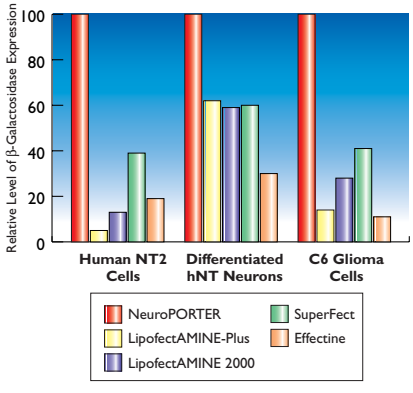
The NeuroPORTER Transfection Reagent is a unique lipid formulation identified by screening hundreds of candidate lipids using a proprietary high-throughput transfection assay. The

NeuroPORTER reagent was chosen because it provides the highest transfection efficiencies in the widest range of neuronal cells. Also, the NeuroPORTER reagent minimizes cytotoxicity, enabling easier post-transfection analysis.

Fast & Easy Protocol

NeuroPORTER Transfection Reagent allows you to avoid excessive time and difficulties associated with viral transfection vectors. Simply combine your plasmid DNA with the NeuroPORTER reagent, incubate at room temperature for 5-10 minutes, and add to your cells. The NeuroPORTER reagent has minimal interaction with serum, so there is no need to switch media after starting your transfection. Also, because it exhibits extremely low cytotoxicity, in most cases there is no need to remove NeuroPORTER from the transfected cells. Finally, the NeuroPORTER reagent is supplied with optimized protocols for primary neurons, neuronal cell lines, and glial or post-miotic cell lines, so you can achieve the highest transfection efficiencies with each neuronal cell type.

Figure 2: β-Galactosidase Expression Comparison in NT2, hNT, and C6 cells



Get the Best Neuronal Transfection

Because of its unprecedented efficiency, low toxicity, broad applicability, and ease-of-use, NeuroPORTER reagent is your best choice for transfecting neurons. Stop stressing over time-consuming viral vectors and low transfection efficiencies: call GTS and order the NeuroPORTER Transfection Reagent today.

Product	Quantity	Catalog no.
NeuroPORTER™ Transfection Reagent		
	75-300 reactions (1.5 ml)	T400150
	375-1500 reactions (5 x 1.5 ml)	T400750

Q & A

Transformation in 3 Minutes: TurboCells™ Chemically Competent *E. coli*

TurboCell™ Chemically Competent *E. coli*

Questions & Answers

1. What is the primary difference between the TurboCells™ Chemically Competent *E. coli* and standard competent *E. coli*?

The TurboCells Chemically Competent *E. coli* are specially optimized to achieve high transformation efficiency with a novel 3-minute transformation protocol. In contrast, transformation using standard competent cells typically requires a 1.5 – 2 hour protocol. Figure 1 demonstrates the efficiency of the TurboCells protocol compared to a standard transformation protocol.

2. What transformation efficiencies can be achieved using TurboCells Chemically Competent *E. coli*?

When using the 3-minute TurboCells transformation protocol, you can achieve efficiencies of 5 x 10^7 - 1 x 10^8 cfu/µg of supercoiled DNA. Also, when using a traditional 1.5 - 2 hour protocol with the TurboCells, greater than 1 x 10^9 cfu/µg of supercoiled DNA can be achieved.

3. What is the difference between TurboCells™ and TurboCells F´?

TurboCells F´ contain a self-transmissible, low-copy plasmid, called F´, that can be used for the generation of single strand DNA (e.g., using M13 bacteriophage techniques).

4. In addition to time-savings, what other advantages do TurboCells Chemically Competent E. coli offer?

TurboCells Chemically Competent *E. coli* are prepared according to a unique procedure that allows robust performance under normally deleterious transformation conditions. For example, it is not required to dilute or purify your ligation mix before transformation. If needed, over 10 µl of full strength ligation mix can be added to 50 µl of competent cells without significantly compromising transformation results. The genotype of TurboCells is suitable for most cloning needs, such as blue/white screening, generation of plasmid based libraries, and the ability to efficiently transform large plasmids.

5. What are the genotypes of the TurboCells Competent *E. coli*?

The genotypes of the TurboCells are summarized in the table 1.

Figure 1: Comparison of TurboCells™ Competent *E. coli* Protocol and Standard Competent *E. coli* Protocol

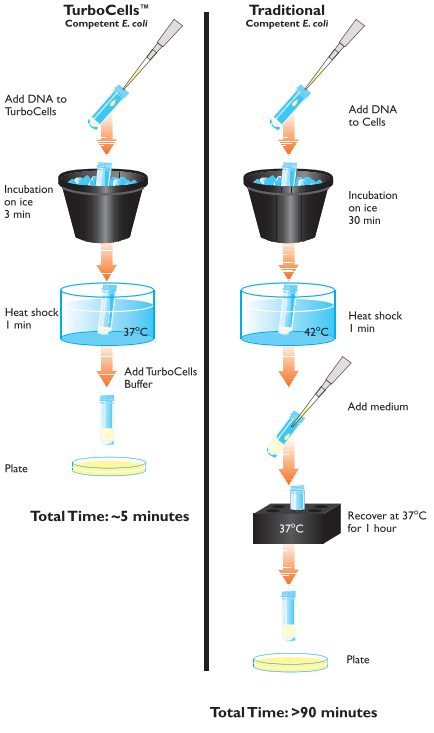
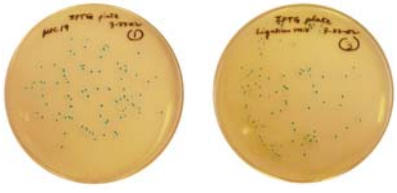


Figure 2. Transformation Results with TurboCells



pUC19 + TurboCells on IPTG/Amp plates

pUC19 self ligation+ TurboCells on IPTG/Amp plates

6. Why are TurboCells Competent *E. coli* packaged in single-use aliquots?

TurboCells are packaged in single-use aliquots to avoid efficiency-robbing freeze/thaws and wasted cells.

7. Are TurboCells available for protein expression?

Yes. TurboCells BL21(DE3) and BL21(DE3) pLysS offers 3-minute transformation of T7-promoter based expression vectors for protein expression.

8. What concentration of kanamycin and ampicillin should I use with/TurboCells?

We use 50 µg/ml and 100 µg/ml, respectively.

Table 1. Genotype of TurboCells™ Chemically Competent E. coli

TurboCells™ : *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*

TurboCells™ BL21(DE3): *recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ80lacZΔM15 Δ(lacZYA-argF)U169 (DE3)*

TurboCells™ BL21(DE3)pLysS: *recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ80lacZΔM15 Δ(lacZYA-argF)U169 (DE3)pLysS(Cam^R)*

Genotype	Advantage
recA1	Mutation in gene(s) responsible for recombination of DNA. This genotype is particularly desirable when cloning genes with direct repeats.
endA	Mutation in the nonspecific endonuclease Endonuclease I. Eliminates non-specific endonuclease activity resulting in improved plasmid preps.
hsd	Mutations in the system of methylation and restriction which allows E. coli to recognize DNA as foreign. The hsd genotype allows efficient transformation of DNA generated from PCR reactions.
lacZΔM15	Element required for b-galactosidase complementation when plated on X-Gal. Used in blue/white screening of recombinants. Usually carried on the lambdoid prophage 80 or F´.
DE3	Lysogen that encodes T7 RNA polymerase. Used to induce expression in T7-driven expression systems.
pLysS	Plasmid that encodes T7 lysozyme. Used to reduce basal expression in T7-driven expression systems by inhibiting basal levels of T7 RNA polymerase.

Product	Quantity	Catalog no.
TurboCells™ Chemically Competent *E. coli*		
	20 x 50 µl	C300020
TurboCells™ F´ Chemically Competent *E. coli*		
	20 x 50 µl	C301020
TurboCells™ BL21 (DE3) Chemically Competent *E. coli*		
	20 x 50 µl	C302020
TurboCells™ BL21 (DE3) pLysS Chemically Competent *E. coli*		
	20 x 50 µl	C303020

New Product

Easily Upgrade Your Favorite Vector for Rapid PCR Cloning

Xi-Clone™ Conversion Kit*

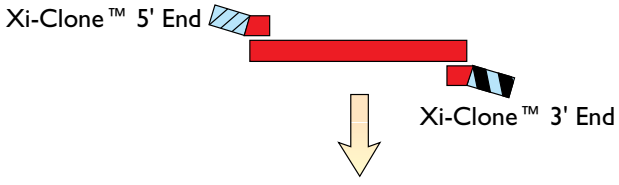
The Xi-Clone™ Vector Conversion Kit allows simple modification of your favorite vectors for rapid directional PCR cloning. By making your existing vectors Xi-Clone-compatible, you will save hours in each cloning experiment by eliminating the need for restriction or ligation enzymes.

How Xi-Clone Works.

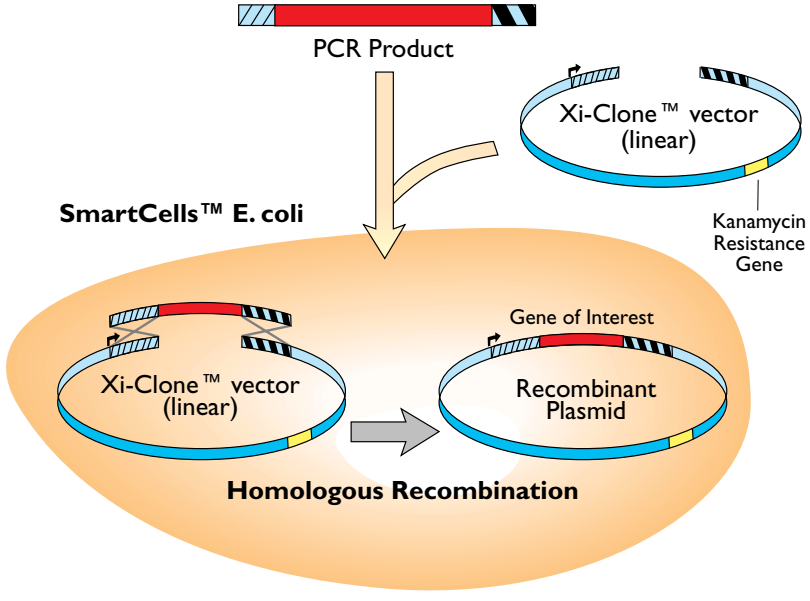
The Xi-Clone PCR Cloning Technology relies on the principle that certain *E. coli* cells are able to recombine homologous sequences with a high rate of specificity. With Xi-Clone, linear DNA fragments are generated through the PCR process to include sequences on the 5´ and 3´ ends that are homologous with end sequences on a linear Xi-Clone-ready vector (Fig. 1). When the PCR fragments and the linear Xi-Clone vector are mixed and transformed into competent *E. coli*, bacterial recombinase activity is able to join the two DNA fragments resulting in a circular plasmid. The proprietary strain, SmartCells™ Chemically Competent *E. coli*, facilitates efficient homologous recombination but it also features the recA genotype which minimizes unwanted recombination in cloning.

Figure 1. How Xi-Clone™ Technology Works

1. Amplify your gene of interest with PCR primers containing Xi-Clone ends.



2. Mix the PCR product with the supplied Xi-Clone vector and transform into SmartCells competent *E. coli*.



3. Plate and screen for recombinants containing kanamycin

Powerful Vector Plug-in.

If multiple cloning experiments need to be performed using the same vector, Xi-Clone-adapted vectors can provide tremendous time savings. With Xi-Clone-ready vectors, you will no longer be limited by restriction sites, and you can clone only the desired sequences into your vector. In addition, because Xi-Clone works with any vector, you will no longer be limited by vector formats dictated by commercial vector suppliers (e.g. TOPO Cloning or Gateway). The benefits of Xi-Clone include:

- Time savings – No restriction digests or overnight ligations in cloning experiments
- Robust cloning efficiency – Greater than 50% of the transformants are recombinant
- Flexibility – Compatible with most vectors and DNA polymerases
- Fast and easy vector conversion protocol

All these features make Xi-Clone vectors ideal for high-throughput cloning projects.

Simple Vector Conversion.

Making a vector Xi-Clone compatible is easy: simply linearize the plasmid, treat it with the enzyme mix and buffer supplied in the Xi-Clone Vector Conversion Kit, and the plasmid is ready to accept properly designed PCR fragments containing the gene of interest. Each Xi-Clone Conversion Kit contains all the required reagents (except for restriction enzyme and DNA polymerase) to generate enough Xi-Clone vectors for 40 PCR cloning reactions as well as SmartCells Competent *E. coli* for recombinant screening. Choose your favorite cloning or expression vector and start saving time with the Xi-Clone Conversion Kit today.

* Patent pending

Product	Quantity	Catalog no.
Xi-Clone™ Vector Conversion Kit		
	40 Reactions	XC005040
SmartCells™ Chemically Competent E. coli		
	20 x 50 µl	C101020
SmartCells™ F´ Chemically Competent E. coli		
	20 x 50 µl	C101120

Delivery

A Newsletter for Advanced Molecular Delivery Tools Volume 3 Issue 1

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