epiTAP Express® Kit

Instruction Manual

Catalog Number
TAP010220

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Purchaser Notification

Limited License

The purchase price paid for the epiTAP Express® kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended **for internal research only** by the purchaser. Such use is limited to the amplification, transfection, and expression of nucleic acids as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Gene Therapy Systems, Inc ("GTS").

Separate licenses are available from GTS for the express purpose of non-research use or applications of the epiTAP Express kit or TAP Express® technology. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, contact the Director of Licensing at GTS.

There is a Patent Pending on the epiTAP Express kit and all of its components. Purchasers may terminate this License at any time by returning all epiTAP Express kit material and documentation to GTS, or by destroying all kit components. Purchasers are advised to contact GTS with the notification that an epiTAP Express kit is being returned in order to obtain a refund and/or to expressly terminate a research only license granted through the purchase of the kit(s).

This document covers in full the terms of the epiTAP Express research only license, and does not grant any other express or implied license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The epiTAP Express kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Licensing, Gene Therapy Systems, Inc.
10190 Telesis Court, San Diego, CA 92121.
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Kit Contents

The epiTAP Express® rapid gene expression kit contains sufficient nucleotide fragments and buffers to perform 20 PCR amplifications or for generating epiTAP Express® fragments from 20 different genes-of-interest. Each PCR amplification produces, on average, 5 µg of DNA, which is enough for five transfections. For animal injections, 25 µg (or 5 PCR amplifications) of the epiTAP Express fragments are required per injection.

Tube Number	Contents	Amount
1	**epiTAP Express® promoter mix:** Contains epiTAP Express promoter fragment and the corresponding epiTAP promoter primer for the Second-Step PCR* amplification.	40 µl
2A	**epiTAP Express® terminator mix A:** Contains TAP Express primer for the Second-Step PCR amplification.	40 µl
2B	**epiTAP Express® terminator mix B:** Contains TAP Express terminator fragment and the corresponding TAP terminator primer for the Second-Step PCR amplification.	40 µl
3	**epiTAP Express® control DNA template:** Contains plasmid DNA with a GFP gene as a positive-control template for amplification using the 5' & 3' control primers	10 µl (10 ng)
4	**epiTAP control 5' primer:** Contains control 5' primer for use with the TAP control DNA template in the First-Step PCR amplification.	10 µl (300 pmol)
5	**epiTAP control 3' primer:** Contains control 3' primer for use with the TAP control DNA template in the First-Step PCR amplification.	10 µl (300 pmol)
6	**GenePORTER® transfection reagent:** Contains proprietary cationic lipid-based transfection mix for transfection of the epiTAP Express fragments into mammalian cells (**see Ordering Information below**).	100 µl (10 transfections)

Stability and Storage

The epiTAP Express® kit is shipped frozen. For maximum stability and long-term use, store all reagent tubes at –20°C upon receipt.

Ordering Information

Product Name	Catalog Number	Number of Reactions
TAP Express® kit	TAP010210	20 PCR amplifications
epiTAP Express® kit	TAP010120	100 PCR amplifications
epiTAP Express® (HA) kit	TAP010220	20 PCR amplifications
TAP Express® kit	TAP010110	100 PCR amplifications
TAP Express® Cloning kit	TAPC2010	10 cloning reactions
GenePORTER® reagent	T201007	75 reactions (0.75 ml)
GenePORTER® 2 reagent	T202007	75 reactions (0.75 ml)
BoosterExpress® reagent	T20100B	3 boosters (4.5 ml)
See GTS Catalog or web site for larger GenePORTER® and GenePORTER® 2 sizes		

You can order the products above by using any of the following contact information.

Toll-Free Number: 888-428-0558	**Fax**: 858-623-9494
E-mail: Orders@genetherapysystems.com	**Web Site**: http://www.genetherapysystems.com

*PCR: The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license. No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of any Gene Therapy Systems PCR-related products. For more information, contact the Director of Licensing at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California, 94501.

Introduction

We congratulate you on your purchase of this exclusive, high-performance product. With this kit, you will be able to transform any gene-of-interest into a transcriptionally active PCR (TAP) fragment that is ready for direct introduction into cells or animals. The epiTAP Express® kit is the latest addition to our TAP Express®line of products that simplify and speed up the rate of discovery in functional genomics and basic research. The TAP Express technology and epiTAP Express kit allows large numbers of gene products to be screened systematically by functional activity while saving you time and money. Until now, the only way to create transcriptionally active genes was to clone PCR fragments into an expression vector, transform bacteria, and purify the plasmid. Although this process can yield large quantities of plasmid, it is time- and labor-intensive especially as more and more genes are simultaneously expressed. The TAP Express system is the first and only tool that considerably speeds up the process from gene to protein expression by eliminating previously required steps and materials. This system makes it possible to rapidly express a large number of different proteins from sequenced genomes even when the proteins' identities and functions are not known. Furthermore, to help identify and purify novel proteins, we have created the epiTAP Express system that effortlessly allows the expression of your genes of interest along with a hemagglutinin (HA) epitope tag fusion. The HA epitope tag (YPYDVPDYA) is well characterized and highly immunoreactive[1]. After transfection of the HA-tagged epiTAP Express fragments into cells, the resulting proteins can be identified with commercially available anti-HA antibodies. The epitope tag is also useful for facilitating purification of the protein, identifying associated proteins, characterizing new proteins by immunoprecipitation, and determining subcellular localization.

Summary of the epiTAP Express® Process

The TAP Express technology is based on the principles of recombinant PCR, in which two or more DNA fragments can be joined in a desired orientation. The proprietary mix of overlapping sequences that is included in the epiTAP Express kit makes this process fast, easy, and reliable. The entire process consists of only two PCR steps (Figure 1). The epiTAP Express system adds more versatility to the TAP Express technology by incorporating a 5' HA tag into the expressed gene product.

Preparation Step

For amplifying your gene-of-interest, we recommend that you order your 5'- and 3'-custom oligos from our licensed vendor GENSET OLIGOS (Methods and Procedures, Section 1.1).

Purpose: To design and construct the 5'- and 3'-custom oligos that contain the 5'- and 3'-epiTAP Ends sequences that are used to amplify the gene-of-interest.

First-Step PCR

The Custom Oligos, provided by GENSET OLIGOS, are used in the First-Step PCR amplification to produce the epiTAP Primary® fragment.

***Purpose*:** To add the 5' HA epitope Tag and 3'-epiTAP End sequences to the gene-of-interest, which is a necessary step for performing the Second-Step PCR amplification.

[1] **Niman, H.L., et al., *Anti-peptide antibodies detect oncogene-related proteins in urine.* Proc Natl Acad Sci U S A, 1985. 82(23): p. 7924-8.)**

Second-Step PCR

epiTAP Express promoter and TAP Express® terminator mixes are added to the epiTAP Primary fragment in the Second-Step PCR.

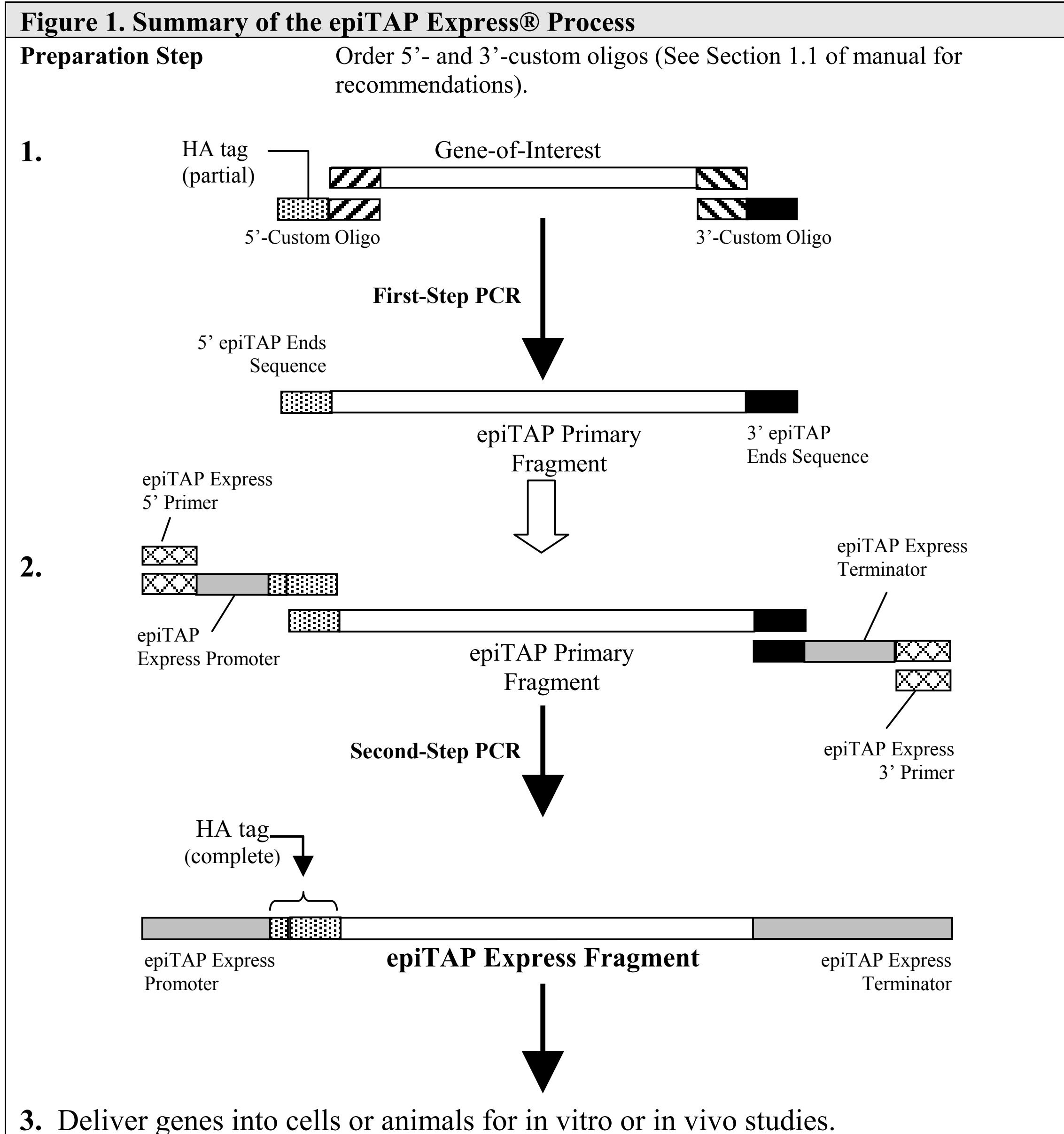
Purpose: To add mammalian promoter (fused with an HA epitope tag) and terminator sequences to your genes-of-interest for their direct expression in cells or animals.

Expression Step

epiTAP Express® fragments, which contain your genes-of-interest plus epiTAP Express promoter and terminator sequences, are ready to transfect into cells or inject into animals.

Figure 1. Summary of the epiTAP Express® Process

Preparation Step Order 5'- and 3'-custom oligos (See Section 1.1 of manual for recommendations).



3. Deliver genes into cells or animals for in vitro or in vivo studies.

METHODS AND PROCEDURES

1. Preparation Step

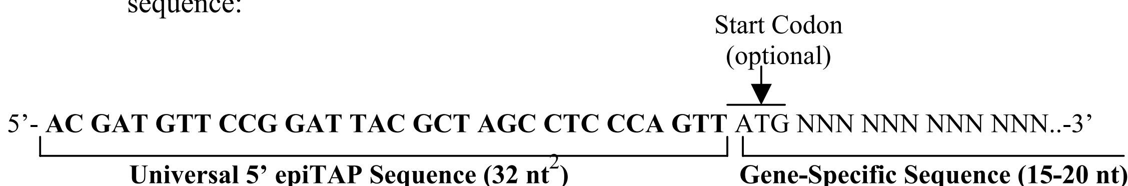
1.1. Designing Gene-Specific PCR Primers and Adding epiTAP Ends Sequences to the Genes-of-Interest

IMPORTANT *The first step of the epiTAP Express® process is to amplify the gene-of-interest with custom primers that add universal epiTAP Ends sequences. Before this preparatory step, you must order high-quality, custom oligos from a reputable vendor.*

We have devoted considerable effort in testing different kit components and suppliers. To offer you the best possible product performance, GTS has selected GENSET OLIGOS as the authorized exclusive vendor of TAP Custom Oligos. GENSET OLIGOS delivers high-quality oligos and consistent service. For you to obtain the best results for your epiTAP Express experiments, we highly recommend GENSET OLIGOS as the source of your Custom Oligos. We have worked with GENSET OLIGOS to provide a dedicated Web site that makes the process of ordering Custom Oligos convenient for you. All epiTAP Express customers can obtain design tips, oligo requirements, and pricing information and can be assured of secure online ordering and technical support. For more information Please visit www.gensetoligos.com/Products/TAP/tapexpress.html or www.genetherapysystems.com

1.2. Designing the 5'-Custom Oligo

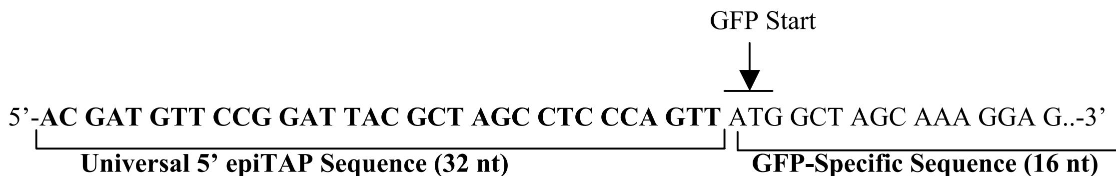
The 5'-custom oligo must contain between 47 and 52 nucleotides; of these, 32 nucleotides comprise the 5'-epiTAP Ends universal sequence and the other 15 to 20 nucleotides make up your gene-specific sequence ("N" = any nucleotide). When designing and ordering the 5'-custom oligo, make sure that it contains the following sequence:



IMPORTANT *Please note that the start codon "ATG" for your gene of interest does not have to be included in the custom oligo sequence, as long as the same open reading frame is used (as shown) to ensure correct fusion between the HA tag and your gene of interest. The start codon for the HA tag is upstream of the sequence shown above.*

[2] Nt = Nucleotides

EXAMPLE The green fluorescent protein (GFP) gene sequence is used below to illustrate the design of the epiTAP control 5' primer for use with the TAP control GFP template:

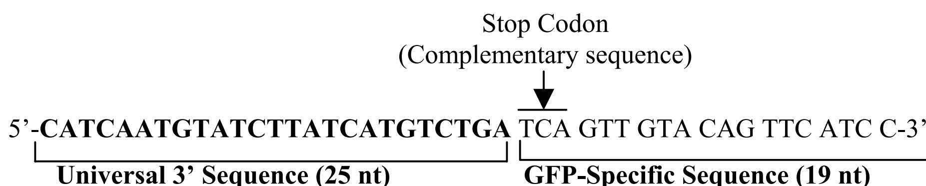


1.3. Designing the 3'-Custom Oligo

The 3'-custom oligo must contain between 40 and 45 nucleotides; of these, 25 comprise the 3'-epiTAP Ends universal sequence and the other 15 to 20 nucleotides are specific to your gene-of-interest. When designing and ordering the 3'-custom oligo, make sure that it contains the following sequence:

We strongly recommend that a complementary stop codon sequence, such as "TCA," be added to the end of the gene sequence to achieve proper translational termination of the expressed gene. Other complementary stop codon sequences, such as "TTA" or "CTA," can also be used.

EXAMPLE The GFP gene sequence is used below to illustrate the design of the epiTAP control 3' primer for use with the TAP control GFP template (note that the complementary sequence is used below):



2. PCR Protocols

2.1. First-Step PCR: Amplifying the Coding Region of Your Gene with Custom Primers

This step generates the epiTAP Primary® fragment, a DNA fragment that contains the gene-of-interest with the added 5'- and 3'-epiTAP Ends. The 5'- and 3'-epiTAP Ends are necessary for adding the epiTAP Express® promoter and terminator fragments in the second PCR step.

The conditions recommended below are optimized for amplifying the GFP positive-control reaction, and have been successfully used to amplify several other genes. To amplify your gene-of-interest, start by following our recommendations below or by optimizing PCR conditions appropriately. For tips on optimizing PCR conditions, please refer to the Troubleshooting Guide in the Appendix (page 14).

2.1.1. Prepare a 50-µl reaction mix as follows[3]:

40 µl of dH_2O
5 µl of 10x PCR buffer
1 µl of 10 mM dNTP mix
1 µl of 5' custom oligo **or** epiTAP control 5' primer (30 pmol or 0.4 µg)
1 µl of 3' custom oligo **or** epiTAP control 3' primer (30 pmol or 0.4 µg)
1 µl of DNA template **or** epiTAP control DNA template (10-1000 pg)
1 µl of PCR DNA polymerase (check with supplier for concentration)

2.1.2. Mix reaction well and start thermal cycling using the following parameters[4]:

94°C for 1 minute

94°C for 30 seconds
58°C for 45 seconds } 32 cycles
68°C for 2 minutes

68°C for 10 minutes
15°C storage (optional)

2.1.3. Transfer 5 µl of the PCR amplification to a fresh tube and add 1 µl of 6x loading buffer for agarose gel electrophoresis. Analyze sample(s) on a 1% agarose gel along with suitable DNA size markers. Stain gel with ethidium bromide and visualize PCR bands.

NOTE *For the kit-provided GFP control, PCR amplification produces a major, 710-bp band.*

[3]These conditions were successfully used with CLONTECH's Advantage cDNA PCR and Perkin Elmer's DNA Thermal Cycler polymerases. Your protocol may vary depending on the source of your PCR enzyme or kit.
[4]We used these conditions successfully with the GFP positive control and other template genes. These conditions might not apply to your reaction conditions depending on the size of the PCR product and the particular primer sequences used.

IMPORTANT *Successful completion of the First-Step PCR amplification is critical for the next step, which is to generate full-length and active epiTAP Express® fragments. Some optimization may be necessary if you observe background bands, low yields, or unexpected sizes of the epiTAP Primary™ fragments.*

2.2. Second-Step PCR: Amplifying the epiTAP Primary®Fragment to Generate a Transcriptionally Active PCR Fragment

This final step adds the epiTAP Express promoter and terminator sequences to your genes-of-interest, via the 5'- and 3' TAP Ends sequences introduced during the First-Step PCR. Two terminator sequences have been included in the kit for you to choose from (see notes on next page for details). The final epiTAP Express fragment is transcriptionally active DNA that can be used directly for in vitro or in vivo expression studies.

2.2.1. Prepare the following 50 µl mix:

38 µl of dH_2O
5 µl of 10x PCR buffer
1 µl of 10 mM dNTP mix
2 µl of epiTAP Express promoter mix
2 µl of epiTAP Express terminator mix A or terminator mix B*
1 µl of DNA template (product from First-Step PCR that is diluted 20-fold in dH_2O)[5]
1 µl of PCR DNA polymerase

2.2.2. Mix reaction well and start thermal cycling using the following parameters:

94°C for 3 minute

94°C for 30 seconds
58°C for 30 seconds } 32 cycles
68°C for 3 minutes

68°C for 10 minutes
15°C storage (optional)

2.2.3. Remove 5 µl from each PCR tube and analyze by agarose gel electrophoresis as in step 2.1.3. The addition of the epiTAP Express® promoter and terminator sequences should increase the size of the gene-of-interest by 0.8 kb using terminator mix A and 1 kb using terminator mix B.

[5] If the First-Step PCR yields are low or if you wish to increase the yield of the final TAP Express®fragment you may use lower dilutions (10- or 5-fold).

NOTES

** Terminator mix A is generally recommended since it is more robust with the second PCR reaction, resulting in higher yield and cleaner product. However, the TAP fragments generated using mix A are not compatible with TAP Express Cloning Kit (Cat. No. TAPC2010). In addition, in our hands, the TAP fragments generated using mix A were less active in vivo (e.g. after direct intramuscular injection) compared with fragments made using terminator mix B.*

Terminator Mix	PCR yield	*In vitro* expression	*In vivo* expression	Use with TAP-Cloning Kit
A	+++	+++	++	no
B	++	+++	+++	yes

For the kit-provided GFP positive control, The Second-Step PCR should produce a major, 1.5-kb band using terminator mix A, or a 1.7-kb band using terminator mix B.

Typical epiTAP Express DNA yields (50-μl reactions) range between 2 and 10 μg.

For certain applications (e.g., in vivo injection), you may wish to remove excess nucleotides and buffers from the epiTAP Express fragments by using commercially available PCR purification kits or by traditional ethanol precipitation and resuspension steps.

3. Delivery of epiTAP Express® Fragments

3.1. Transfecting epiTAP Express® DNA Fragments into Cells

For optimal transfection of the epiTAP Express fragments into cells, the kit includes the GenePORTER® transfection reagent. Please follow the protocol below to perform your transfections. Because the GenePORTER reagent is highly efficient in a wide range of host cells, we recommend that you use the GenePORTER® reagent for all of your transfection studies (for more information on the GenePORTER reagent, please visit our Web site: http://www.genetherapysystems.com). To reorder the GenePORTER® reagent or one of our other products for lipid-based transfections, please refer to the Ordering Information section on page 4.

Using the GenePORTER transfection reagent, follow these steps to verify the expression of your epiTAP Express® fragment or GFP positive control:

3.1.1. The day before transfection, plate cells to achieve 60% to 90% confluency on the day of transfection[6].

3.1.2. Dilute the DNA with serum-free medium using one-half of the final transfection volume (see Table 1 below for suggested DNA amounts and transfection volumes).

3.1.3. Dilute the GenePORTER®reagent with serum-free medium using one-half of the transfection volume (see Table 1 below).

3.1.4. Add the diluted DNA to the diluted GenePORTER® reagent. Pipette this mixture up and down rapidly and incubate at room temperature for 10 to 45 minutes.

3.1.5. Aspirate the culture medium from cells and carefully add the DNA/GenePORTER® mixture to the cells; incubate at 37°C for 3 to 5 hours in a 5% to10% CO_2 incubator.

3.1.6. Following the 3- to 5-hour incubation, add one volume (i.e., total transfection volume) of medium containing 20% fetal calf serum (FCS). Incubate the transfected cells overnight in a 5% to 10% CO_2 incubator set at 37°C.

After 24 hours, add more fresh growth medium as needed[7]. Depending on cell type, expression assays can be performed 24 to 72 hours after the start of the transfection procedure.

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[6]Omitting antibiotics from the medium during transfection can increase expression levels. This effect is cell-type dependent and usually small. We recommend CHO-K1 cells for positive control.

[7] For some cell types, old medium can be replaced with fresh medium at this step.

Table 1. Suggested Volumes for Transfection of Adherent Cells

Format	DNA (μg)	GenePORTER® Reagent (μl)	Transfection Volume (ml)
96-well dish	0.2–1.0	0.5–2.5	0.1
24-well dish	1.0–4.0	2.5–10.0	0.25
6-well dish	4.0–12.0	10.0–30.0	1.0
60-mm culture dish	12.0–16.0	30.0–40.0	2.0
100-mm culture dish	16.0–24.0	40.0–60.0	5.0[8]

NOTE *Although the GenePORTER reagent consistently delivers high transfection efficiencies in a wide range of cell types, some optimization of transfection conditions may be necessary with some cells. The two critical variables are the DNA/GenePORTER reagent ratio and the DNA amount. These two variables may be optimized by first determining the best DNA/GenePORTER reagent ratio by using 3 to 9 μl of reagent for each 1 μg of DNA (use a low amount of DNA when optimizing this ratio). Once the optimal ratio is determined, vary the DNA amount over the suggested range. At this point, cell number can also be optimized.*

3.2. Detecting Green Fluorescent Protein

When GFP expression is measured by fluorescent or confocal microscopy, GFP has an excitation peak at 470 to 490 nm and an emission peak at 510 nm. In addition, the level of GFP expression can be monitored by fluorescence-activated cell sorter analysis[9].

3.3. Detecting epiTAP Express fusion Proteins using anti-HA antibodies

There is a variety of antibody-based HA epitope detection kits available commercially. Since the positive control used by the epiTAP Express kit is a GFP fusion, it is recommended that one should not use any fluorescein-based system to detect the HA-GFP fusion.

[8] For 100-mm or larger dishes, we recommend that you prepare your DNA and GenePORTER® reagent in 1 ml of serum-free medium as for the 6-well dish; mix well and further dilute to the desired volume by adding additional serum-free medium before transfection.

[9] Cheng, L. et al. *Use of Green Fluorescent Protein Variants to Monitor Gene Transfer and Expression in Mammalian Cells*. Nature Biotechnology **14**, 606-609 (1996).

APPENDIX

Quality Control

GENSET OLIGOS manufactures custom oligos using the highest possible standards and manufacturing practices. For information about quality assurance procedures, please contact the local GENSET OLIGOS office from which you obtained your custom oligos (you can also refer to a list of GENSET OLIGOS offices on page 17).

To assure the performance of each lot of the TAP Express® kit, we qualify each component with rigorous standards. The final kit is also qualified for its ability to produce transcriptionally active PCR fragments in vitro.

Quality Control Standards for Kit Components

Kit Component	Quality Control Standard
Primers	Every epiTAP Express primer is analyzed by mass spectrometry
Promoter and terminator fragments	Both epiTAP Express promoter and terminator fragments are isolated and purified through proprietary techniques and qualified by agarose gel electrophoresis for their expected fragment size.
GenePORTER®transfection reagent	GenePORTER® reagent is qualified in a 96-well plate in an in vitro transfection assay in COS-7, NIH 3T3 and Jurkat cells.
Positive-control plasmid DNA template	The epiTAP control DNA template containing the gene sequence for the green fluorescent protein is qualified by restriction digest and analyzed by agarose gel electrophoresis for the correct supercoiled and restriction fragment sizes.

Quality Control Standards for Kit

Kit Function	Quality Control Standard
Amplifying positive-control GFP gene	For the provided positive controls (GFP DNA template and epiTAP control 5' and 3' primers), PCR amplification must produce a major 710-bp band that corresponds to the amplified GFP gene.
Adding epiTAP Express promoter and terminator sequences.	This PCR step adds the epiTAP Express promoter and terminator sequences to the amplified GFP gene. The final epiTAP Express fragment must have a corresponding 1.5-kb size using terminator mix A, or a 1.7-kb size using terminator mix B.
Transfection, gene expression, and immununocyto-chemistry	Transfect CHO-K1 cells with the final epiTAP Express fragment containing the GFP gene using the GenePORTER® reagent provided. The fragment produced is evaluated for GFP expression by fluorescence microscopy and compared to a reference GFP expression profile from a control plasmid. Presence of HA epitope tag is detected by immunocytochemistry using commercial anti-HA-Rhodamine antibody

Satisfaction Guarantee

We want you to be completely satisfied with the quality and performance of the epiTAP Express kit. All kits must conform to all of the above assay tests before release. Please contact our Technical Support department if you have any questions or comments about the contents or performance of your epiTAP Express kit.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
1. High background in First-Step PCR reaction	Too many cycles	• Reduce number of cycles.
	Annealing temperature too low	• Increase annealing temperature in 2°C increments.
	Suboptimal primer design	• Redesign primers by adding or deleting 1 to 3 nucleotides until nonspecific bands are eliminated. • Use primer design software to optimize primer nucleotide content.
	Contamination	• Include a control reaction without DNA template. • Carry out PCR reaction in a separate area. Use clean and sterile components and equipment such as pipettes and pipette tips, whenever possible. • Switch to previously unused diluents such as buffer and water.
2. Second-Step PCR reaction failed	PCR component missing	• Repeat Second-Step PCR with fresh reagents. • Include TAP positive control and any other appropriate positive-control reagents provided by PCR reagent manufacturers—like enzymes, buffers, and dNTPs.
	Error in primer design	• Check sequence of custom oligos ordered for First-Step PCR. Make sure that the universal epiTAP Ends sequences are ALL included correctly.
	Short extension time	• If amplifying longer templates (>2 kb), increase the extension time in 30-second increments. In general, to determine extension time, use 0.5 minutes per kb plus 2 minutes. • Use a DNA polymerase that is optimized for long range PCR.
	Quality of TAP Express Primary fragment	• Check the epiTAP Primary® fragment for expected size and purity. If contaminants appear, refer to section 1 in this table.
3. High background in Second-Step PCR reaction	Quality of TAP Primary® fragment	• Clean up the epiTAP Primary fragment if background problems persist. Use any appropriate general laboratory protocol or PCR purification kit at your disposal.
	Too many cycles or not enough DNA template	• Decrease cycle numbers in 2-cycle increments. • Increase the amount of epiTAP Primary fragment used per PCR reaction by diluting it 5- or 10-fold instead of 20-fold.
	Annealing temperature too low	• The annealing temperature recommended has been optimized for the control reaction. Optimize reaction conditions for your genes-of-interest by increasing or decreasing the annealing temperatures by 1°C increments.
	Contamination	• See Section 1 in Problem column above.
4. Low or no protein expression	Incorrect design of custom oligo	• Confirm accuracy of custom oligo sequence. Make sure that the sequences for the codons are in the correct open reading frame (ORF) with the gene-of-interest.
	Suboptimal transfection	• Try different DNA/GenePORTER® reagent ratios by increasing or decreasing the ratio in 0.5 increments. • Use higher amounts of DNA relative to the number of cells transfected. • Some cell lines, such as Jurkat, are inherently difficult to transfect. Try different cell types (e.g., CHO or COS-7) to test for cell-specific transfection efficiencies.

For additional troubleshooting assistance, please contact our Technical Support Department at 888-428-0558 extension 1 or by E-mailing us at tech1@genetherapysystems.com

epiTAP Express®, epiTAP Primary®, TAP Ends®, and GenePORTER® are trademarks of Gene Therapy Systems, Inc.

Quick Reference Protocol for Experienced Users

<table>
<tr><td rowspan="2">Preparation Step
Designing gene-specific primers</td><td>5'-custom oligo design

5'- AC GAT GTT CCG GAT TAC GCT AGC CTC CCA GTT ATG NNN NNN NNN NNN N...-3'
Universal 5' epiTAP Express Sequence (32 nt) Gene-Specific Sequence (15-20 nt)</td></tr>
<tr><td>3'-custom oligo design

5'-C ATC AAT GTA TCT TAT CAT GTC TGA TCA NNN NNN NNN NNN.N….-3'
Universal 3' epiTAP Express Sequence (25 nt) Gene-Specific Sequence (15-20 nt)</td></tr>
</table>

<table>
<tr><td rowspan="2">First-Step PCR
Amplifying gene-of-interest sequence.

Control Cycling Parameters
94 C, 1 min
94 C, 30 sec
58 C, 45 sec } x 32
68 C, 2 min
68 C, 10 min
15 C, storage (opt)</td><td>Prepare a 50-µl reaction mix as follows:
40 µl of dH_2O
5 µl of 10x PCR buffer
1 µl of 10 mM dNTP mix
1 µl of 5'-custom oligo or epiTAP control 5' primer (30 pmol or 0.4 µg)
1 µl of 3'-custom oligo or epiTAP control 3' primer (30 pmol or 0.4 µg)
1 µl of DNA template or epiTAP control DNA template (10-1000 pg)
1 µl of PCR DNA polymerase (check with supplier for concentration)</td></tr>
<tr><td>Check the result of this PCR amplification on agarose gel for proper size and purity of the epiTAP Primary® fragment.</td></tr>
</table>

<table>
<tr><td rowspan="2">Second-Step PCR
Amplifying the epiTAP Primary fragment

Control Cycling Parameters
94 C, 3 min
94 C, 30 sec
58 C, 30 sec } x 32
68 C, 3 min
68 C, 10 min
15 C, storage (opt)</td><td>Prepare a 50-µl reaction mix as follows:
38 µl of dH_2O
5 µl of 10x PCR buffer
1 µl of 10 mM dNTP mix
2 µl of epiTAP Express Promoter mix
2 µl of epiTAP Express Terminator mix A or Terminator mix B
1 µl of epiTAP Primary fragment, diluted 20-fold in dH_2O[10]
1 µl of PCR DNA polymerase</td></tr>
<tr><td>Check the result of this PCR amplification on agarose gel for proper size and purity of the epiTAP Express fragment.</td></tr>
</table>

<table>
<tr><td rowspan="2">Delivery of TAP Express® Fragments
Introducing epiTAP Express fragment into cells</td><td>1. Start with cells that are 60% to 90% confluent.
2. Dilute DNA in serum-free medium (in one-half the final volume of transfection).
3. Dilute GenePORTER® reagent in serum-free medium (in one-half the final volume of transfection).
4. Mix diluted DNA and GenePORTER® reagent vigorously; incubate at room temperature for 10 to 45 minutes.
5. Aspirate culture medium from cells.
6. Add DNA/GenePORTER® reagent mix to cells; incubate at 37°C for 3 to 5 hours.
7. Add one transfection volume of medium containing 20% FCS to cells; incubate overnight at 37°C in a 5% to 10% CO_2 incubator.
8. Change or add more medium to cells as needed.</td></tr>
<tr><td>Assay cells for protein expression. For the control GFP reaction, use confocal microscopy to detect GFP fluorescence.</td></tr>
</table>

[10] If the First-Step PCR yields are low or if you need to increase the yield of the final TAP Express® fragment you may use lower dilutions (5- or 10-fold).

GENSET OLIGOS Contact and Address Information

After ordering the epiTAP Express® kit, visit either of the following web sites to order your epiTAP custom oligos.
http://www.genetherapysystems.com or
http://www.gensetoligos.com/Products/TAP/tapexpress.html.
Click on the TAP Express button or hyperlink and follow instructions.

Alternatively, you can contact GENSET OLIGOS directly at:

Office Contact	Mailing Address
NORTH AMERICA Tel: 1-800-995-0308 or 1-858-551-3050 Fax: 1-800-551-5291 or 1-858-551-3041 marketing@gensetlj.com techmail@gensetlj.com	GENSET Corp. 875 Prospect Street, Suite 206 La Jolla, CA 92037, USA
EUROPE Tel: 33 (0) 1 43 56 59 00 Fax: 33 (0) 1 43 56 59 48 marketing@gensetoligos.com techmail@gensetoligos.com	GENSET SA 1 rue Robert et Sonia Delaunay 75011 Paris - FRANCE
ASIA AND NEW ZEALAND Tel: 65 873 22 71 Fax: 65 873 10 77 marketing@genset.com.sg techmail@genset.com.sg	GENSET Singapore Biotechnology Pte. Ltd. The Alpha Bldg. # 04-28 10 Science Park Road Singapore Science Park II Singapore 117684
JAPAN Tel: 81 75 313 19 74 Fax: 81 75 313 21 79 marketing@genset.co.jp techmail@genset.co.jp	GENSET KK SCB #3, Kyoto Research Park 1, Awata-cho, Chudoji Shimogyo-ku KYOTO 600-8815 JAPAN
AUSTRALIA Tel: 1 800 730 070 Fax: 1 800 731 170 (free call/fax within Australia) Tel: +61 2 6620 3987 Fax: +61 2 6622 3879 (from outside Australia) marketing@genset.com.au techmail@genset.com.au	GENSET PACIFIC Pty. Ltd. Level 5 T-Block Southern Cross University P.O. Box 3088 Lismore NSW 2480 Australia

GENSET/GTS epiTAP-CUSTOM OLIGOS ORDER FORM

For the fastest service, order your epiTAP oligos on the web at http://www.gensetoligos.com

Use this form to fax your order to one of the following numbers. Please choose the office nearest you.

USA, Canada, and South America	+1-800-551-5291
Europe	+33-1-43 56 68 18
Japan	+81-75 313 2179
Singapore and Asia	+65-873 10 77
Australia	+1-800 731 170

Ship To: Principal Investigator:		Date:	Total number of oligos:
		PO#:	Quotation#:
Ship To:		Bill To:	
Tel:	Fax:	Tel:	Fax:
For confirmation by email, please provide Email address		Shipment: Standard Overnight Delivery	

SEQUENCES (OLIGOS ARE FOR RESEARCH USE ONLY)

To avoid mistakes please write **A**, **C**, **T**, in upper case and **g** in lower case.

Oligo Name:	Extra Name: GTS 5' epiTAP Custom Oligo
Type of Oligo**:** DNA 2 OD (min. 150 reactions) 5 OD (min. 375 reactions)	Comments: FINCO = 0.4 µg/µl
Please fill in your sequence, including the start codon. 5' Oligo must be 47 to 52 nucleotides in length.	

5'- AC gAT GTT CCg gAT TAC gCT AgC CTC CCA gTT _ _ _ (35) _ _ _ (38) _ _ _ (41) _ _ _ (44) _ _ _ (47) _ _ _ (50) _ _ (52) **-3'**

Universal 5' epiTAP Sequence (32 nt)

START CODON

Oligo Name:	Extra Name: GTS 3' epiTAP Custom Oligo
Type of Oligo**:** DNA 2 OD (min. 150 reactions) 5 OD (min. 375 reactions)	Comments: FINCO = 0.4 µg/µl
Please fill in your sequence, including the stop codon. 3' Oligo must be 40 to 45 nucleotides in length.	

5'-C ATC AAT gTA TCT TAT CAT gTC TgA _ _ _ (28) _ _ _ (31) _ _ _ (34) _ _ _ (37) _ _ _ (40) _ _ _ (43) _ _ (45) **-3'**

Universal 3' epiTAP Sequence (25 nt)

STOP CODON

VERSION 041801GTS