

TAP Express® Kit Instruction Manual

**Catalog Number
TAP010210**



Genlantis

A Division of Gene Therapy Systems, Inc.
10190 Telesis Court
San Diego, CA 92121
Phone: 888-428-0558 (US. Toll-Free) • 858-457-1919
Fax: 858-623-9494 • 858-558-3617
E-mail: orders@genlantis.com
Web Site: <http://www.genlantis.com>

Purchaser Notification

Limited License

The purchase price paid for the TAP 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 Genlantis, a division of Gene Therapy Systems, Inc (“GTS”).

Separate licenses are available from GTS for the express purpose of non-research use or applications of the TAP Express kits or 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 TAP Express kit and all of its components. Purchasers may terminate this License at any time by returning all TAP Express kit material and documentation to GTS, or by destroying all TAP Express kit components. Purchasers are advised to contact GTS with the notification that a TAP 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 TAP 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 TAP 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
Genlantis, a division of Gene Therapy Systems, Inc.
10190 Telesis Court, San Diego, CA 92121.
Toll free: 888-428-0558.
Fax: 858-623-9494.
Email: licensing@genlantis.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.

TABLE OF CONTENTS

	Page
OVERVIEW	
Purchaser Notification	2
Kit Contents	4
Stability and Storage	4
Ordering Information	4
Introduction	5
METHODS AND PROCEDURES	
Preparation Step: Designing Gene-Specific PCR Primers	7
PCR Protocols	8
First-Step PCR: Amplifying the Coding Region of Your Gene	8
Second-Step PCR: Generating TAP Express® Fragments	9
Delivery of TAP Express® Fragments	10
Detecting Green Fluorescent Protein	11
APPENDIX	
Quality Control	12
Troubleshooting Guide	13
Quick Reference Protocol for Experienced Users	14

Kit Contents

The TAP Express® rapid gene expression kit contains sufficient reagents to perform 20 PCR amplifications or for generating TAP 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) is required per injection.

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

Stability and Storage

The TAP Express® rapid gene expression 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
TAP Express® kit	TAP010110	100 PCR amplifications
epiTAP Express® (HA) kit	TAP010220	20 PCR amplifications
epiTAP Express® (HA) kit	TAP010120	100 PCR amplifications
TAP Express® Cloning kit	TAPC2010	10 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 Genlantis 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 888-428-0558	Fax 858-623-9494	E-mail orders@genlantis.com
Web Site http://www.genlantis.com		

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. Because of its simplicity and speed, the TAP Express® technology is a powerful tool for functional genomics and basic research. Besides offering time- and cost-savings benefits, the TAP Express kit allows large numbers of gene products to be screened systematically by functional activity. 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 as more and more genes are simultaneously expressed. The TAP Express kit is the first and only tool that considerably speeds up the process from gene cloning to protein expression by eliminating previously required steps and materials. With TAP Express technology, your gene-of-interest is ready for expression in one day.

Summary of the TAP 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 TAP Express kit makes this process fast, easy, and reliable. The entire process consists of only two PCR steps (Figure 1).

Preparation Step

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

Purpose: To design and construct the 5'- and 3'-custom oligos containing the 5'- and 3'-TAP Ends™ sequences that will be used to amplify the gene-of-interest.

First-Step PCR

The custom oligos are used in the First-Step PCR amplification to produce the TAP Primary® fragment.

Purpose: To add the 5'- and 3'-TAP Ends sequences to the gene-of-interest, which is a necessary step for performing the Second-Step PCR amplification.

Second-Step PCR

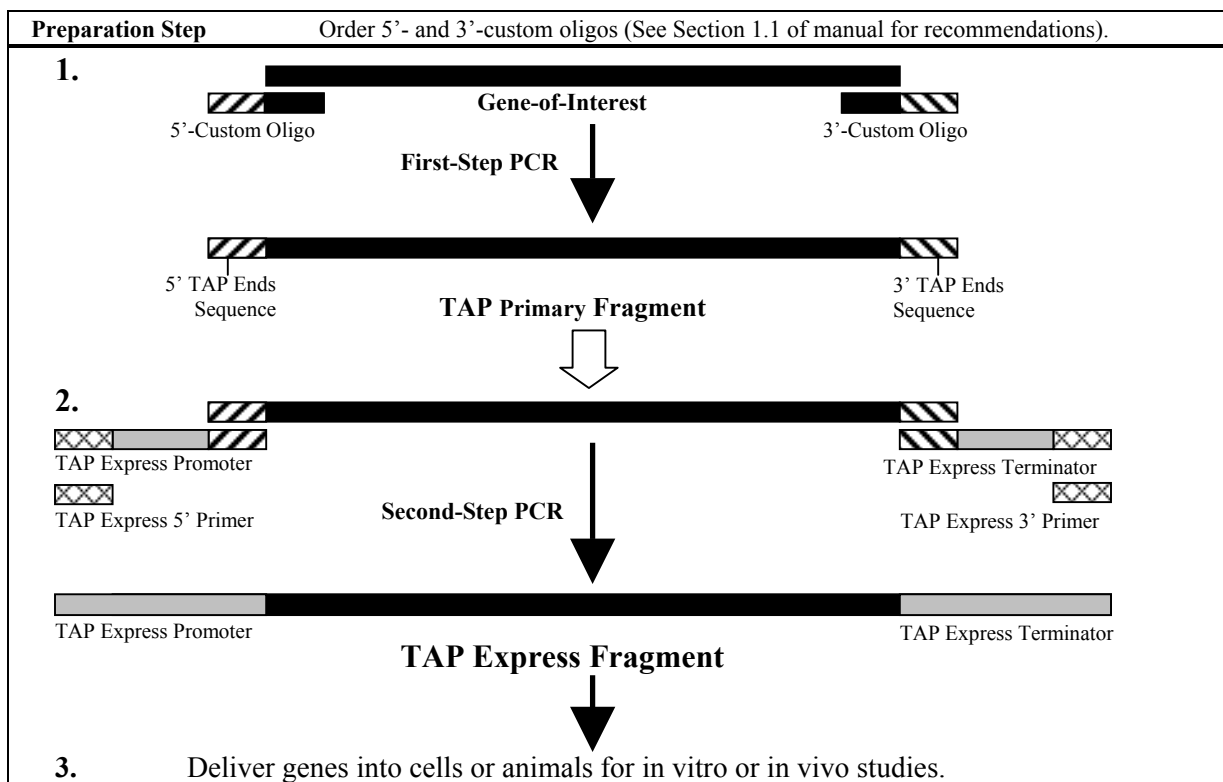
TAP Express promoter and terminator mixes are added to the TAP Primary fragment in the Second-Step PCR.

Purpose: To add mammalian promoter and terminator sequences to your gene-of-interest for its direct expression in cells or animals.

Expression Step

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

Figure 1. Summary of the TAP Express® Process



METHODS AND PROCEDURES

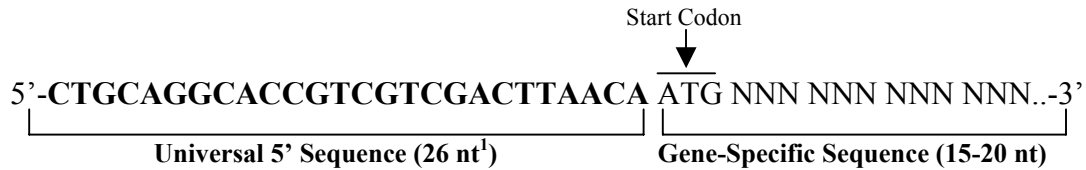
1. Preparation Step

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

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

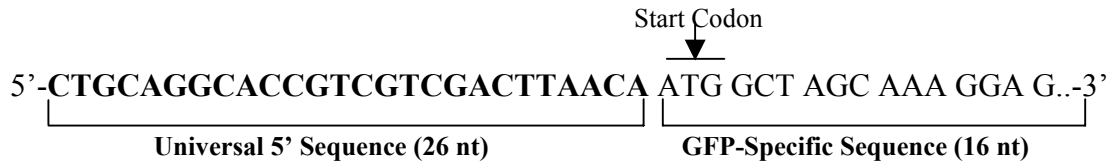
Designing the 5'-Custom Oligo

The 5'-custom oligo must contain between 41 and 46 nucleotides; of these, 26 nucleotides comprise the 5'-TAP 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:



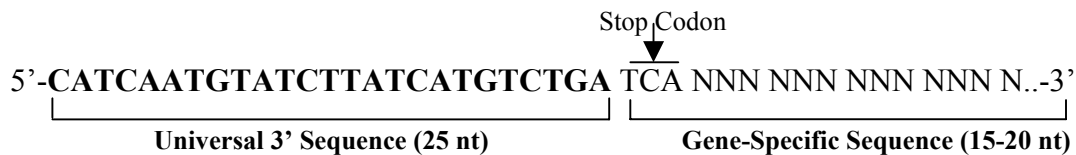
IMPORTANT Please note that the start codon "ATG" should always be included in the custom oligo, especially when the sequence of the gene-of-interest does not have an initiation methionine codon on its 5' end.

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



1.2. Designing the 3'-Custom Oligo

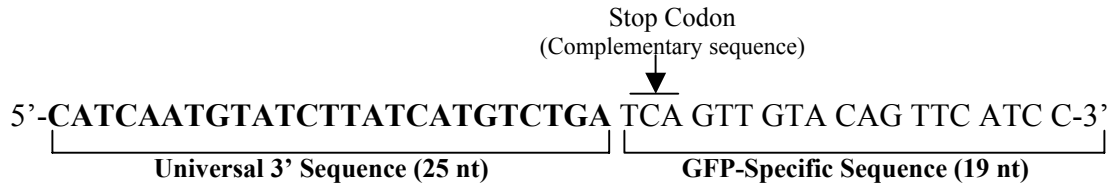
The 3'-custom oligo must contain a minimum of 40 nucleotides; of these, 20 comprise the 3'-TAP Ends® universal sequence and the other 20 nucleotides are specific to your gene-of-interest. When designing and ordering the 3'-custom oligo, make sure that it contains the following sequence:



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.

¹ Nt = Nucleotides

EXAMPLE The GFP gene sequence is used below to illustrate the design of the TAP control 3' primer for use with the TAP control GFP template:



2. PCR Protocols

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

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

The conditions recommended below are optimized for amplifying the GFP positive-control reaction, and we have successfully used these conditions 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 13).

2.1.1. Prepare a 50- μ l reaction mix as follows²:

- 40 μ l of dH₂O
- 5 μ l of 10x PCR buffer
- 1 μ l of 10 mM dNTP mix
- 1 μ l of 5' custom oligo **or** TAP control 5' primer (30 pmol or 0.4 μ g)
- 1 μ l of 3' custom oligo **or** TAP control 3' primer (30 pmol or 0.4 μ g)
- 1 μ l of DNA template **or** TAP control DNA template (10-1000 pg)
- 1 μ l of PCR DNA polymerase (check with supplier for concentration)

²These 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.

Mix reaction well and start thermal cycling using the following parameters³:

94°C for 1 minute

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

68°C for 10 minutes

15°C storage (optional)

2.1.2. 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 positive control, PCR amplification should produce a major, 710-bp band.

IMPORTANT

Successful completion of the First-Step PCR amplification is critical for the next step, which is generating full-length and active TAP Express® fragments. Some optimization may be necessary if you observe background bands, low yields, or unexpected size of the TAP Primary® fragment.

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

This final step adds the TAP Express promoter and terminator sequences to your gene-of-interest, via the TAP Ends® sequences that were introduced during 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 end product, the TAP 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 dH₂O

5 µl of 10x PCR buffer

1 µl of 10 mM dNTP mix

2 µl of TAP Express promoter mix

2 µl of TAP Express terminator mix A or terminator mix B*

1 µl of DNA template (product from First-Step PCR that is diluted 20-fold in dH₂O)⁴

1 µl of PCR DNA polymerase

³We 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 (e.g., certain primers may need an intermediate annealing step, such as 60°C for 30 seconds).

⁴If 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 (5- or 10-fold).

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

94°C for 1 minute
94°C for 30 seconds
60°C for 30 seconds
68°C for 3 minutes } 32 cycles
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 2.1.2. The addition of the TAP 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.

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	<i>In vitro</i> expression	<i>In vivo</i> expression	Use with TAP-Cloning Kit
A	+++	+++	++	no
B	++	+++	+++	yes

For the kit-provided GFP positive control, 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 TAP Express DNA yields (50-µl reactions) range between 2 and 10 µg.
For certain applications (e.g., in vivo injection), you may wish to use either commercially available PCR purification kits or traditional ethanol precipitation and resuspension steps to purify TAP Express fragments by removing excess nucleotides and buffers.

3. Delivery of TAP Express® Fragments

3.1. Transfecting TAP Express® DNA Fragments into Cells

For optimal transfection of the TAP Express fragments into cells, the TAP Express kit includes GenePORTER® transfection reagent. Please follow the protocol below to perform your transfection. Because GenePORTER reagent is highly efficient in a wide range of host cells, we recommend that you try GenePORTER® reagent for all of your transfection studies (for more information, please visit our Web site: <http://www.genlantis.com>). To reorder GenePORTER® reagent or our other products for lipid-based transfections, please refer to Ordering Information, page 4. Using GenePORTER® Transfection Reagent, follow these steps to verify your TAP Express® fragment expression and that of the GFP positive control:

- 3.1.1. The day before transfection, plate cells to achieve 60% to 90% confluency on the day of transfection⁵.
- 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% to 10% CO₂ 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% CO₂ incubator set at 37°C.

After 24 hours, add more fresh growth medium as needed⁶. Depending on cell type, expression assays can be performed 24 to 72 hours after the start of the transfection procedure.

Table 1. Suggested Volumes for Transfection of Adherent Cells

Format	DNA (µg)	GenePORTER® Reagent (µl)	Transfection Volume (ml)
96-well dish	0.1–0.5	0.5–2.5	0.1
24-well dish	0.5–2.0	2.5–10.0	0.25
6-well dish	2.0–6.0	10.0–30.0	1.0
60-mm culture dish	6.0–8.0	30.0–40.0	2.0
100-mm culture dish	8.0–12.0	40.0–60.0	5.0 ⁷

NOTE

Although 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 as described in this reference:

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).

⁵Omitting 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.

⁶For some cell types, old medium can be replaced with fresh medium at this step.

⁷For 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.

APPENDIX

Quality Control

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 TAP Express primer is analyzed by mass spectrometry.
Promoter and terminator fragments	Both TAP 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 the following three cell types: COS-7, NIH 3T3 and Jurkat.
Positive-control plasmid DNA template	The TAP 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 size and restriction fragment size.

Quality Control Standards for Kit

Kit Function	Quality Control Standard
Amplifying the positive-control GFP gene	For the provided positive controls (GFP DNA template and TAP control 5' and 3' primers), PCR amplification must produce a major, 710-bp band that corresponds to the amplified GFP gene.
Adding TAP promoter and terminator sequences	This PCR step adds the TAP Express promoter and terminator sequences to the amplified GFP gene. The final TAP Express control fragment must have a corresponding 1.5-kb size using terminator mix A, or a 1.7-kb size using terminator mix B.
Transfection and gene expression	Transfect CHO-K1 cells with the final GFP TAP Express fragment 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 GFP plasmid.

Satisfaction Guarantee

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

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
1. High background in First-Step PCR reaction	Too many cycles	<ul style="list-style-type: none"> Reduce number of cycles.
	Annealing temperature too low	<ul style="list-style-type: none"> Increase annealing temperature in 2°C increments.
	Suboptimal primer design	<ul style="list-style-type: none"> Redesign primers by adding or deleting 1 to 3 nucleotides until nonspecific bands are eliminated.
	Contamination	<ul style="list-style-type: none"> 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, whenever possible.
2. Second-Step PCR reaction failed	PCR component missing	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Check sequence of custom oligos ordered for First-Step PCR. Make sure that the universal TAP Ends® sequences are ALL included correctly.
	Short extension time	<ul style="list-style-type: none"> If amplifying longer templates (>3 kb), increase the extension time in 30-second increments. In general, to determine extension time, use 0.5 minutes per kb plus 2 minutes.
	Quality of TAP Express Primary fragment	<ul style="list-style-type: none"> Check the TAP Primary® fragment for expected size and purity. If contaminants or fragments of the wrong size appear, refer to section 1 in this table.
3. High background in Second-Step PCR reaction	Quality of TAP Primary® fragment	<ul style="list-style-type: none"> Clean up the TAP 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	<ul style="list-style-type: none"> Decrease cycle numbers in 2-cycle increments. Increase the amount of TAP Primary fragment used per PCR reaction by diluting it 5- or 10-fold instead of 20-fold.
	Annealing temperature too low	<ul style="list-style-type: none"> The annealing temperature recommendation has been optimized for the control reaction. Optimize reaction conditions for your gene-of-interest by increasing or decreasing the annealing temperatures by 1°C increments.
	Contamination	<ul style="list-style-type: none"> See Section 1 in Problem column.
4. Low or no protein expression	Incorrect design of custom oligo	<ul style="list-style-type: none"> Confirm accuracy of custom oligo sequence. Make sure that the sequences for the ATG start codon and the stop codon (if included) are in the correct open reading frame (ORF) of the gene-of-interest.
	Suboptimal transfection	<ul style="list-style-type: none"> 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:

Toll-free number: 888-428-0558, Ext. 3	E-mail: tech1@genlantis.com
---	---

TAP Express®, TAP Ends®, and GenePORTER® are trademarks of Genlantis / Gene Therapy Systems, Inc.

Quick Reference Protocol for Experienced Users

<p>Preparation Step Designing gene-specific primers</p>	<p>5'-custom oligo design</p> <p>5'-CTGCAGGCACCGTCGTCGACTTAACA ATG NNN NNN NNN NNN N...-3'</p> <p style="text-align: center;"> </p> <p style="text-align: center;"> Universal 5' Sequence (26 nt) Gene-Specific Sequence (15-20 nt) </p> <hr/> <p>3'-custom oligo design</p> <p>5'-CATCAATGTATCTTATCATGTCTGA TCA NNN NNN NNN NNN.N....-3'</p> <p style="text-align: center;"> </p> <p style="text-align: center;"> Universal 3' Sequence (25 nt) Gene-Specific Sequence (15-20 nt) </p>
<p>First-Step PCR Amplifying gene-of-interest sequence.</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <p>Control Cycling Parameters</p> <p>94 C, 1 min 94 C, 30 sec 68 C, 2 min } x 32 68 C, 10 min 15 C, storage (opt)</p> </div>	<p>Prepare a 50-μl reaction mix as follows:</p> <p>40 μl of dH₂O 5 μl of 10x PCR buffer 1 μl of 10 mM dNTP mix 1 μl of 5'-custom oligo or TAP control 5' primer (30 pmol or 0.4 μg) 1 μl of 3'-custom oligo or TAP control 3' primer (30 pmol or 0.4 μg) 1 μl of DNA template or TAP control DNA template (10-1000 pg) 1 μl of PCR DNA polymerase (check with supplier for concentration)</p> <p>Check the result of this PCR amplification on agarose gel for proper size and purity of TAP Primary™ fragment.</p>
<p>Second-Step PCR Amplifying the TAP Primary fragment</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <p>Control Cycling Parameters</p> <p>94 C, 1 min 94 C, 30 sec 60 C, 30 sec } x 32 68 C, 3 min 68 C, 10 min 15 C, storage (opt)</p> </div>	<p>Prepare a 50-μl reaction mix as follows:</p> <p>38 μl of dH₂O 5 μl of 10x PCR buffer 1 μl of 10 mM dNTP mix 2 μl of TAP Express promoter mix 2 μl of TAP Express terminator mix A or terminator mix B 1 μl of TAP Primary fragment, diluted 20-fold in dH₂O⁸ 1 μl of PCR DNA polymerase</p> <p>Check the result of this PCR amplification on agarose gel for proper size and purity of TAP Express fragment.</p>
<p>Delivery of TAP Express® Fragments Introducing TAP Express fragment into cells</p>	<p>Start with cells that are 60% to 90% confluent. Dilute DNA in serum-free medium (in one-half the final volume of transfection). Dilute GenePORTER® reagent in serum-free medium (in one-half the final volume of transfection). Mix diluted DNA and GenePORTER® reagent vigorously; incubate at room temperature for 10 to 45 minutes. Aspirate culture medium from cells. Add DNA/GenePORTER™ reagent mix to cells; incubate at 37°C for 3 to 5 hours. Add one transfection volume of medium containing 20% FCS to cells; incubate overnight at 37°C in a 5% to 10% CO₂ incubator; incubate for 24 hours. Change or add more medium to cells as needed.</p> <p>Assay cells for protein expression. For the control GFP reaction, use fluorescence microscopy to detect GFP.</p>

⁸ 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).