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I. DESCRIPTION

By using Genscript proprietary gene synthesis technology, Genscript can deliver to you any human genes you desire. All the genes are cloned into Genscript proprietary pDream2.1 vector (Figure 1), which allows the genes to be expressed in any one of the three major protein expression systems: bacteria, insect cells and mammalian cells.

Most ORF collections available in the market are established by traditional cDNA cloning methods. However, the traditional cDNA cloning methods have the following limitations:

- The collection is not complete. cDNA clones for rare mRNA or long mRNA clones are very difficult to be obtained.
- Clones obtained using RT-PCR method will need mutation correction.
- The cloning vector is usually not an expression vector, or can only be used to express the gene in one of the three major protein expression systems.

Key Features of GenScript GenPool™ ORF Clones:

- All the genes are cloned into pDream2.1 vector, which allows the gene to be directly expressed in bacteria, insect cells and mammalian cells.
- The vector is compatible with Novagen Ek/LIC Cloning Kits, which allows you to move the gene into the LIC vectors from Novagen if needed.
- The vector is compatible with Invitrogen Gateway® system, which allows you to move the gene into other expression vectors from Invitrogen if needed.
- All the genes have a FLAG tag sequence fused to them at the 5'-ends, which allows you to use anti-FLAG antibodies to detect, purify the proteins, and study the gene functions. The tag can also be cleaved with Enterokinase (EK) to make authentic proteins starting with Methionine.

II. VECTOR DESCRIPTION

GenScript pDream2.1 vector (Figure 1) is a protein expression vector for both efficient cloning and high-level expression of any target gene. The gene of interest can be efficiently cloned into the vector using Ligation Independent Cloning (LIC) strategy, and can be expressed directly without any further cloning work in any one of the three major protein expression systems: bacteria, Insect cells and Mammalian cells.

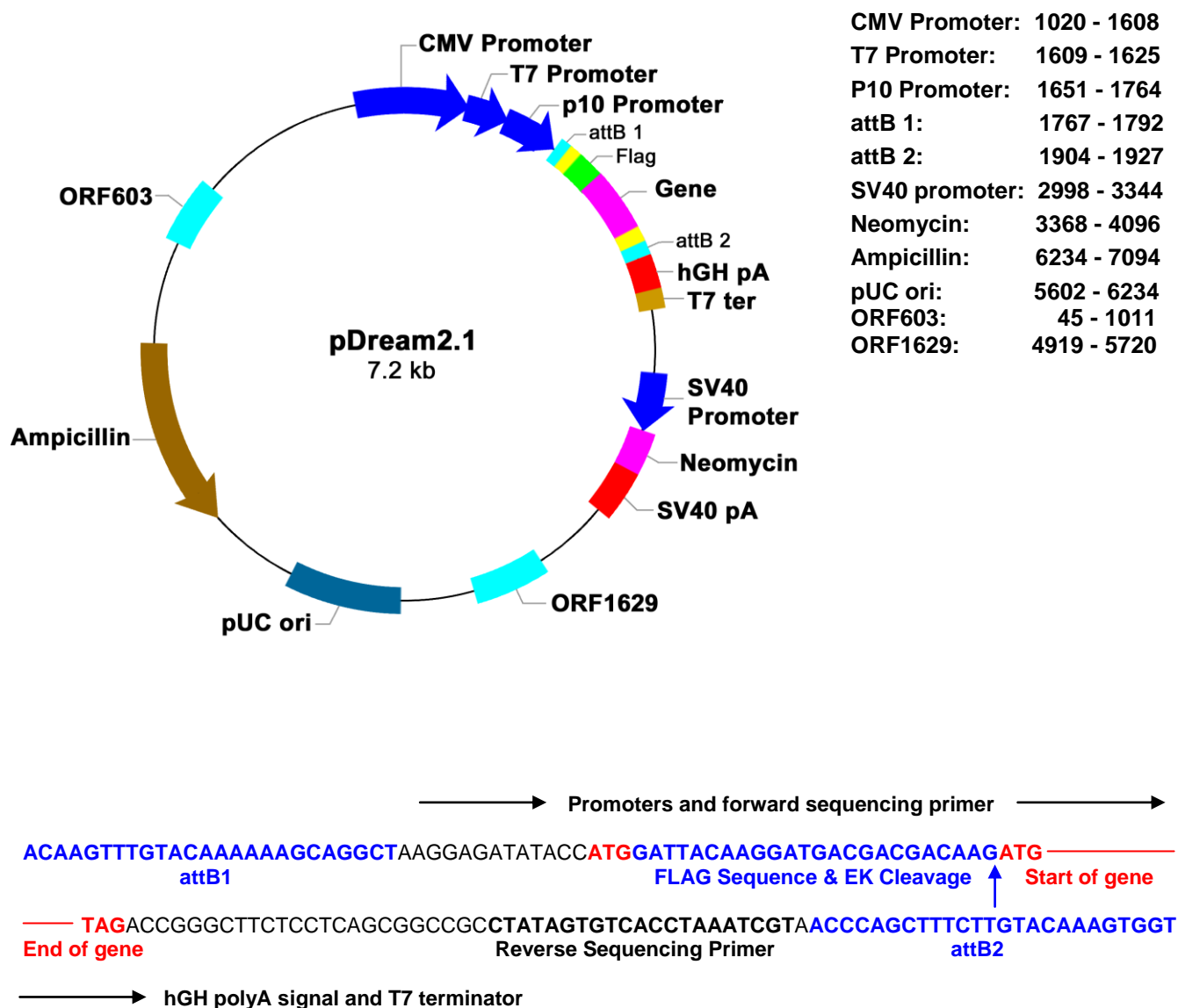


Figure 1. The circle map, the positions of key features and the cloning region of pDream vector

Sequencing Primers:

Forward primer: T7 sequencing primer, [DA0009](#), at T7 promoter

Reverse primer: SP6 sequencing primer, [DA0008](#).

III. RELATED PRODUCTS.

Product	Catalog No.	Price
pDream2.1-cGFP	SD0220	\$350.00
pDream2.1/LIC Kit	SD0221	\$350.00



IV. EXPRESSION IN MAMMALIAN CELLS.

Generate large amount of DNA

Products of the human genes in GenScript pDream2.1 vector are delivered as lyophilized powder, and it can be stored forever at -20°C . You can handle the products in the same way as you handle DNA plasmid. Before use, add 20 μl water to dissolve it. Please vortex diligently and make sure the DNA is dissolved. If needed, incubate at 50°C for 10 min. You can use it directly if you only need less than a few μg of DNA. For large amount of DNA, take 1 or 2 μl of the solution and transform competent DH5a or TOP10 cells for a MaxiPrep using Qiagen MaxiPrep kit.

General considerations before transfection

A variety of protocols such as lipofection and electroporation have been used successfully to transfect human gene constructs into mammalian cells. The transfection procedures are identical to those used for DNA plasmid transfection. The choice of transfection procedures will depend on the mammalian cell line used. In general, we recommend using Lipofectamine™ 2000 or Lipofectamine™ Plus from Invitrogen. The information and protocol for Lipofectamine™ 2000 can be found using this link: <http://www.lifetech.com/content.cfm?pageid=93>. The information and protocol for Lipofectamine™ Plus can be found using this link: <http://www.invitrogen.com/content/sfs/manuals/18324.pdf>.

The following are important issues to be considered before performing the transfection:

- Cell density:** The recommended cell density for transfection using Lipofectamine™ 2000 is 90-95%. If the cell density is less than 90%, the Lipofectamine™ 2000 may have some toxicity on the cells.
- Human gene construct amount:** For 12-well plates, it is recommended to use 1.6 μg as a starting point. For other plate size, the DNA amount can be adjusted proportionally.
- Cell Proliferation:** Maintaining healthy cell culture is critical for cell transfection. It is essential to minimize decreased cell growth associated with nonspecific transfection effects.
- Time:** The optimal time after transfection for analyzing gene expression has to be determined empirically by testing a range of incubation time. The time can vary from 24 to 96 hrs depending on the cells used and the human genes tested.

A protocol based on Lipofectamine™ 2000 from Invitrogen for 12-well plates

1. Purchase Lipofectamine™ 2000 reagent (Cat#11668-027) from Invitrogen
2. Plate the cells the day before transfection so that they are 90-95% confluent on the day of transfection. At the time of plating cells and diluting transfection reagents, avoid antibiotics - this helps cell growth and allows transfection without rinsing the cells. The cell density of 90-95% is very important. If the cell density is less than 90%, transfection may have toxicity on the cells.
3. Dilute the human gene construct with 100 μl of Opti-MEM (Cat# 31985062) from Invitrogen and mix gently.
4. Dilute Lipofectamine™ 2000 Reagent into 100 μl of Opti-MEM medium in a second tube; mix gently and incubate at room temperature for 5 min.
5. Combine diluted DNA (from Step 3) and diluted Lipofectamine™ 2000 Reagent (from step 4); mix and incubate at RT for 20 min.
6. Add 200 μl of DNA-Lipofectamine™ 2000 Reagent complexes to each well. Mix gently by rocking the plate back and forth.
7. Perform assays on the cells 24-48 h after the start of transfection. It is not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.
8. Optimizing transfection: The suggested starting points are listed in Table 1. To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipofectamine™ 2000 concentrations, and cell density. Make sure that cells are greater than 90% confluent and vary DNA (μg): Lipofectamine™ 2000 (μl) ratios from 1:0.5 to 1:5.

**Table I.** Suggested starting amounts of reagents for transfection in different culture vessels

Culture vessel	Surface Area per Well (cm ²)	Relative Surface Area (vs. 24-well)	Volume of Plating Medium	DNA (µg) and Dilution Volume (µl)	Lipofectamine™ 2000 (µl)
96 well	0.3	0.2	100 µl	0.2 µg in 25 µl	0.5 µl in 25 µl
24 well	2	1	500 µl	0.8 µg in 50 µl	2.0 µl in 50 µl
12 well	4	2	1 ml	1.6 µg in 100 µl	4.0 µl in 100 µl
6 well	10	5	2 ml	4.0 µg in 250 µl	10 µl in 250 µl
35 mm	10	5	2 ml	4.0 µg in 250 µl	10 µl in 250 µl
60 mm	20	10	5 ml	8.0 µg in 0.5 ml	20 µl in 0.5 ml
100 mm	60	30	15 ml	24 µg in 1.5 ml	60 µl in 1.5 ml

Selecting antibiotic-resistant transfected cells

There are two major benefits for selecting antibiotic-resistant transfected cells:

1. For cells that are very difficult to be transfected or have very low transfection efficiency, using antibiotic selection will kill the cells that were not transfected with the construct. This will be able to reduce the background when analyzing the expression.
2. By using the antibiotic selection, a stable cell line can be established. The stable cell line can be maintained and assessed for the target gene expression for a long-term period.

The following are general procedures for selecting antibiotic-resistant cells.

1. Before doing antibiotic-resistant selection, perform a transient assay to check the protein expression of the construct by Western or ELISA.
2. Following the transfection procedures as outlined in Section V to perform the cell transfection.
3. After 24 hours of transfection, lift the cells from plates using Trypsin-EDTA. Then add G-418 to the medium for selection.
4. The optimal concentration of G-418 is in the range of 50-1500 µg/ml. 100 µg/ml will be a good start point for optimization.
5. Examine the dishes for viable cells every 2 days. Identify the lowest G-418 concentration that begins to give massive cell death for wild-type cells in approximately 7-9 days, and kills all wild-type cells within 2 weeks. Using this concentration to select cells containing the human gene construct.
6. You can select a mixture of resistant cells or single colony depending on your need.

V. BACTERIA EXPRESSION PROTOCOL

Transformation

1. Thaw *E. coli* BL21(DE3) competent cells on ice. Pipet 50 µl of DE3 competent cells into 1.5 ml microcentrifuge tube.
2. Add 20 ng of plasmid DNA into the 50 µl of BL21DE3 competent cells, and gently mix well. Incubate on ice for 30 minutes.
3. Heat shock at 42°C for 90 seconds. Incubate on ice again for two minutes.
4. Add 1 ml LB. Incubate at 37°C with shaking at 250 rpm for one hour.
5. Spin at 13,000 rpm for 30 seconds in a bench-top microcentrifuge. Remove and discard supernatant. Add 100 µl of room temperature LB, and resuspend pellet by pipeting up and down.
6. Plate 90 µl of the resuspended cells on an LB/Ampicillin (100 µg/ml) agar plate, and the remaining cells on another plate.
7. Incubate plates overnight at 37°C.



Minipreps

1. Pick single colonies and make six 2 ml mini cultures with Ampicillin at 100 µg/ml.
2. Incubate at 37°C with shaking at 250 rpm for 8 – 12 hours.
3. Transfer 1.5 ml of culture into each of six 1.5 ml microcentrifuge tubes, and spin at 13,000 rpm in a bench-top microcentrifuge for 30 seconds. Save the rest of the cultures at 4°C for future use.
4. Remove and discard supernatant, and use GenScript miniprep kits to extract plasmid DNA.
5. Analyze and confirm the plasmid by restriction enzyme digestion. Choose one mini culture for next step, induction and expression.

Induction and Expression

1. Inoculate 1 L LB containing 100 µg/ml of Ampicillin with 250 µl of mini culture.
2. Cover the top of the flask with aluminum foil. Incubate at 37°C with shaking at 250 rpm.
3. Periodically check the OD₆₀₀ of the culture until the OD₆₀₀ reaches 0.5 – 0.6.
4. Set aside 1 ml of the culture as the un-induced control. Induce the culture with 1 ml of 0.4 M IPTG.
5. Grow the culture at 37°C for 3 hours.

Detection and Purification

1. Remove 1 ml of the induced culture for expression analysis. Pellet the cells by spinning at 13,000 rpm in a bench-top microcentrifuge for 30 seconds.
2. Remove and discard the supernatants and resuspend the cell pellets in 50 µl of ddH₂O. Add 1 µl of PMSF to final concentration of 1 mM to each suspension and then add 50 µl 2 X SDS Sample Buffer. Mix the sample rapidly and boil for 3 to 5 min.
3. Centrifuge in a microcentrifuge for 30 sec. Load 5 µl of the supernatant on a normal SDS-PAGE gel.
4. Run the gel for proper time and stain the gel with Coomassie Blue.
5. Purify the protein using Sigma Anti-FLAG®M1 Agarose Affinity Gel ([A4596](#)).

VI. BACULOVIRUS EXPRESSION

pDream is also a baculovirus transfer plasmid that is compatible with [Novagen BacVector®](#) designed for efficient and reliable construction of baculovirus recombinants for protein expression and functional analysis. For the expression of the gene in insect cells, follow the protocols of [Novagen BacVector®](#) (<http://www.emdbiosciences.com/Products/BrowseProductsByCategory.asp?catid=199>) Transfection Kits for optimal performance of the transfer plasmid and the kits.

Order BacVector®-1000 Transfection Kit (Catalog No.: 70059) from Novagen. You can also use BacVector®-2000 Transfection Kit (Catalog No.: 70030) or BacVector®-3000 Transfection Kit (Catalog No.: 70077)

Brief Outlines of Baculovirus Expression Procedures:

1. Use the product as a transfer vector and co-transfect insect cells (SF9 cells) with the BacVector Triple Cut Virus DNA (from Novagen kits).
2. Collect the SF9 cells culture media as recombinant viral stock.
3. Infecting insect cells (SF9 cells) with the recombinant viral stock to produce high level of gene expression.



VII. MOVING GENES TO OTHER VECTORS

The vector, with the gene of interest flanked by *attB*1 and *attB*2 sequences, is compatible with Invitrogen Gateway™ system

(<https://catalog.invitrogen.com/index.cfm?fuseaction=viewCatalog.viewCategories&pc=110&npc=92&nc=109&>).

This allows you to move the gene into other expression vectors from Invitrogen if needed. To do so, you need to order the following kits:

1. PCR Cloning System with Gateway® Technology with pDONR™221 or pDONR™/Zeo (Cat. No. 12535-019 or 12535-027)
2. Expression System with Gateway® Technology.
Cat. No. 11824-026 for *E. coli* expression
Cat. No. 11827-011 for Baculovirus expression
Cat. No. 11826-021 for Mammalian expression

A Brief Procedure to Move the Gene of Interest to Invitrogen Expression Vectors:

1. Create an *attL*-containing entry clone by recombination (BP Reaction) of GenScript product (containing *attB* sites) with a donor vector (containing *attP* sites), pDONR™221 or pDONR™/Zeo.
2. Transform appropriate competent *E. coli* to select for and amplify entry clones.
3. Create an *attB*-containing expression clone by recombination (LR Reaction) of entry clone (containing *attL* sites) with a destination vector (containing *attR* sites), from kit with Cat. No. 11824-026 for *E. coli* expression, or kit with Cat. No. 11827-011 for Baculovirus expression, or kit with Cat. No. 11826-021 for Mammalian expression.
4. Transform appropriate competent *E. coli* to select for and amplify expression clones.
5. Express the gene of interest in the appropriate system using the expression clone.

VIII. cGFP EXPRESSION AS A POSITIVE CONTROL

As a positive control, cGFP gene was cloned into pDream2.1 using Ligation Independent Cloning (LIC) method. cGFP was expressed in all three major protein expression systems: bacteria, insect cells and mammalian cells. The green fluorescence can be directly observed under Fluorescence Microscope. Figure 2, Figure 3 are the cGFP expression in 293H cells and *E. coli*, respectively.

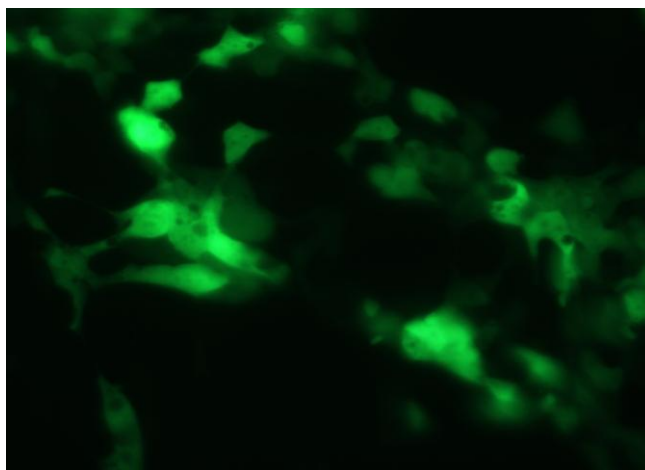


Figure 2. cGFP Expressed in 293H cells

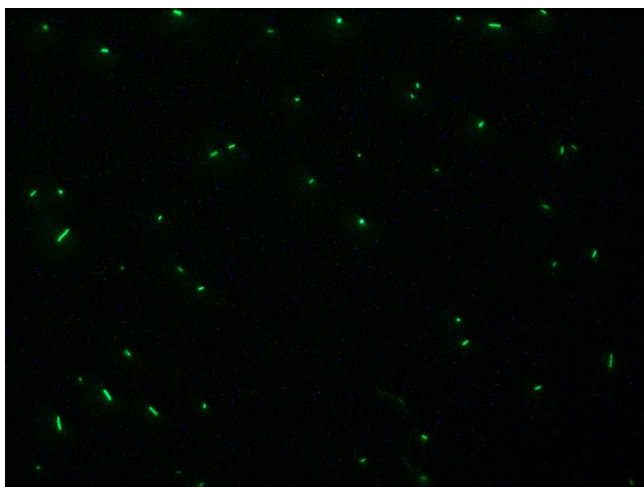


Figure 3. cGFP Expressed in *E. coli*

For Research Use Only.

*** Patent Pending.**

*** Limited License Agreement For Genscript Clones.**

*** Limited Use Label License:** The use of CMV promoter is covered under U. S. Patent No. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation and is sold for research use only. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation (UIRF), 214 Technology Innovation Center, Iowa City, Iowa 52242. For further information, please contact the Associate Director of UIRF, at 319-335-4546.

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