TrueORF Gold is OriGene's premium cDNA clone that has passed the ultimate tests: sequence verification and protein expression validation. Why settle for clones of little validation or wait for gene-synthesis? Get a TrueORF Gold clone and start your experiment immediately.

- Tested individually by Western
- Sequence verified
- Easy-shuttle into over 60 vectors
- Transfection ready
- Next day shipping

TrueORF™ Gold cDNA Clones and PrecisionShuttle™ Vector System

Application Guide

Table of Contents

Revision 03.10.OP

Package Contents and Related Products

The following components are included:

- One (1) vial containing the cDNA clone as 10 ug lyophilized plasmid DNA*.
- Forward (VP1.5) and reverse (XL39) DNA vector sequencing primers; dried onto the bottom of screw cap tubes.
- Certificate of Analysis
- Application Guide

** OriGene plasmids are purified using ion-exchange columns for high-yield, low endotoxin preparations (PowerPrep® HP Midiprep Kit* www.origene.com/other/Plasmid_Purification.)
The cDNA clone is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Related Optional Reagents

Restriction enzymes and buffers
Sgf I or ASIS I from Fermentas
Mlu I from New England Biolabs (NEB)
Nuclease free water
T4 DNA ligase and buffer
Competent *E. coli* cells
LB agar plates with kanamycin, 25 μg/ml (Entry vector)
LB agar plates with ampicillin, 100 μg/ml (Destination vectors)
LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)
DNA purification reagents
Anti-FLAG Antibody – 4C5-AntiDDK (OriGene TA50011)

Related OriGene Products

RapidShuttling Kit	http://www.origene.com/cdna/rapid-shuttling-kit
TrueClone™ FL cDNA clones	http://www.origene.com/cdna/
HuSH™ shRNA Plasmids	http://www.origene.com/rnai/
VERIFY™ Tagged Antigens	http://www.origene.com/lysate/
Validated Antibodies	http://www.origene.com/antibody/
Functional Proteins	http://www.origene.com/protein/
Transfection Reagents	http://www.origene.com/cdna/transfection.mspx
4C5-AntiDDK Antibody	http://www.origene.com/4C5-AntiDDK

Notice to purchaser

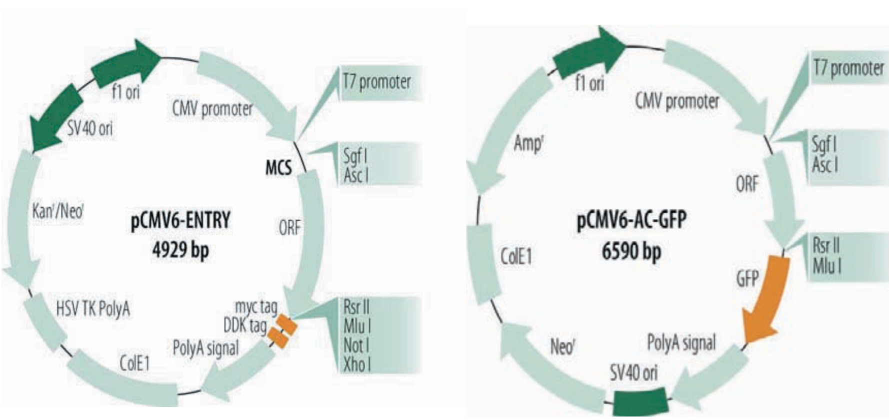
This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Vector Information

RC123456: TrueORFs with a C-terminal fusion of MYC/DDK tag. The cloning expression vector is pCMV6-Entry. The antibiotic selection marker in E. coli is Kanamycin at 25 ug/ml. Unlike other shuttling systems, the entry vector is a functional mammalian expression vector.

All TrueORF clone vectors are described in detail on OriGene's website at http://www.origene.com/cdna/trueorf/destinationvector.mspx

Figure 1. The Vector Maps of pCMV6-Entry and pCMV6-AC-GFP.



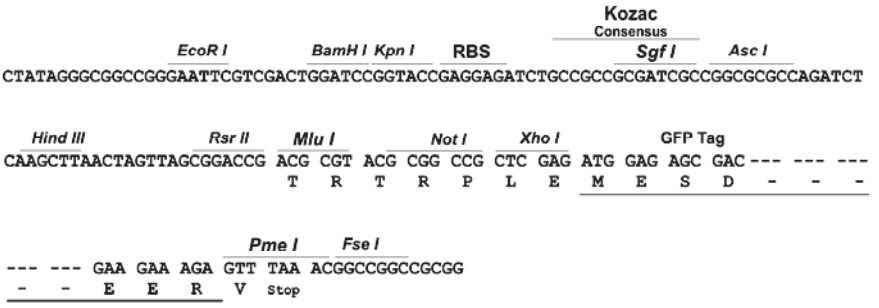
Multiple cloning sites of the PrecisionShuttle pCMV6-Entry Vector

```
                                                                                   Kozac
                                                                                 Consensus
                  EcoR I            BamH I Kpn I   RBS                             Sgf I     Asc I    Bgl II
CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

 Hind III                Rsr II     Mlu I          Not I       Xho I              Myc.Tag
CAAGCTTAACTAGCTAGCGGACCG ACG CGT ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG
                          T   R   T   R   P   L   E   Q   K   L   I   S   E   E

                          EcoR V              Flag.Tag                     Pme I     Fse I
GAT CTG GCA GCA AAT GAT ATC CTG GAT TAC AAG GAT GAC GAC GAT AAG GTT TAA ACGGCCGGCC
 D   L   A   A   N   D   I   L   D   Y   K   D   D   D   D   K   V  Stop
```

Multiple cloning sites in the PrecisionShuttle pCMV6-AC-GFP Vector



The PrecisionShuttle Vector System

The TrueORF vector system is designed to express the ORF of a gene with one or more epitope tags or with a fluorescent marker. There are more than twenty destination vectors in the PrecisionShuttle vector system. These vectors are either N-terminally tagged (pCMV6-AN) or C-terminally tagged (pCMV6-AC). Available tags include Myc, DDK, His, HA, and t-GFP. One untagged PrecisionShuttle vector is also available to express the native (untagged) protein in mammalian cells, or using an *in vitro* protein expression system. Due to the requirement for the Mlu I restriction site, the untagged vector does still append two amino acids (TR) to the C-terminus of the encoded protein.

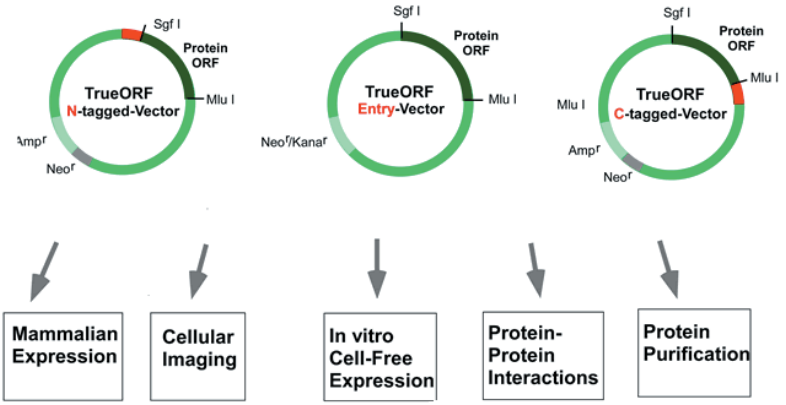


Figure 2. Potential applications of the PrecisionShuttle system for protein analysis. The TrueORF cDNA clones in the pCMV6-Entry vector and other N- or C-tagged-vectors can be directly used in your experiments of protein functional analysis.

All of the plasmids in the PrecisionShuttle™ vector system are designed for high-level target gene expression in mammalian cells or *in vitro* translation in a cell-free system. The plasmids contain the promoter and enhancers of the human cytomegalovirus (CMV) immediate-early gene to drive mammalian gene expression, and the T7 promoter for *in vitro* transcription/translation. A Kozak consensus sequence is included in the plasmid to enhance mammalian expression.

The PrecisionShuttle vector system employs a basic “cut and ligate” molecular cloning method (Figure 3). It is faster, cheaper, more reliable and flexible than a recombination strategy, and no intellectual license is required for either academic or commercial users. The transfer of the ORF from the Entry clone to any destination vector is a rapid process. Digestion, ligation and transformation take as little as 3 hrs (Figure 3) since the Entry vector and destination vectors use different antibiotic selection markers. Unlike recombination-based systems in which the Entry clone is only a preliminary product, OriGene’s Entry Vector contains C-terminal Myc and DDK tags, and can be used directly for many applications including 1) tagged protein expression (C-terminal Myc/DDK in mammalian cells and 2) tagged protein expression in a cell-free system using the T7 promoter.

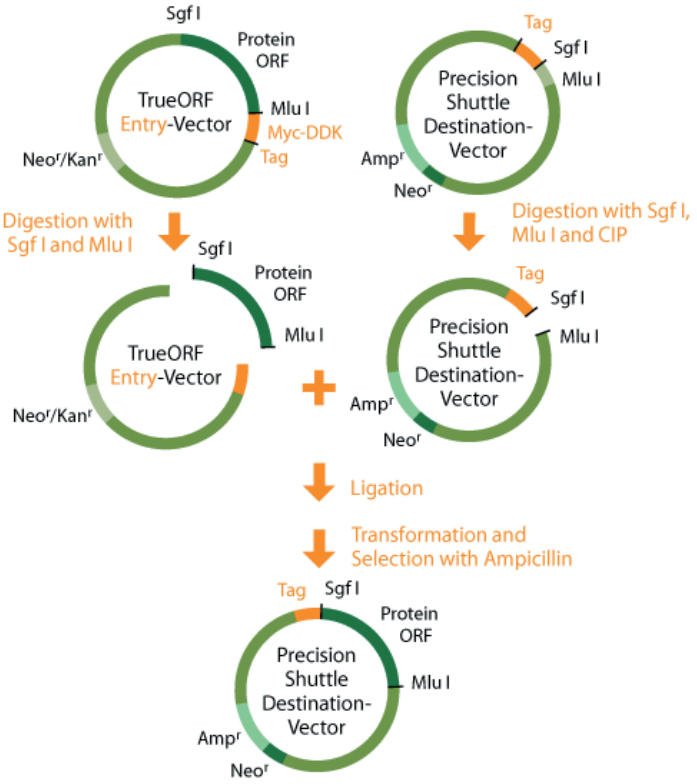


Figure 3. Schematic of the PrecisionShuttle subcloning procedure. The Entry and destination vectors are digested with Sgf I and Mlu I or other specified enzymes. After a ligation reaction, the resulting clones are grown on ampicillin-containing plates to select for successful subcloning of the ORF into the destination vector.

Two rare-cutting restriction enzymes are utilized in transferring an ORF between vectors. Most subcloning from the Entry to a destination vector involves *Sgf* I/Asis I (present in 0.37% of human ORF) and *Mlu* I (4%). In the very unusual case when *Sgf* I and *Mlu* I sites are inside the ORFs, the TrueORF vector MCS provides other rare restriction sites, such as Asc I, Rsr II, and Not I so that any ORF can be shuttled from the Entry vector to a destination vector by using some combination of these five rare restriction enzymes. Unlike site-specific recombination vector systems, the TrueORF Clone System does not append multiple amino acids to the amino or carboxy terminus of the protein of interest. The subcloning strategy maintains insert orientation and reading frame, eliminating the need to resequence the insert after each transfer. Because the Entry and destination vectors have different antibiotic resistance genes, selection after subcloning is a very simple process. Therefore, the shuttling process can be readily adapted to a 384-well format. With the availability of over 32,000 unique full-length human cDNA clones and mouse clones, OriGene is in an enviable position to develop and support such high throughput applications. The MCS of the PrecisionShuttle vectors was engineered to be compatible with most other commercially available vector systems including Gateway vectors (Invitrogen), PET vectors (Novagen) and Flexi vectors (Promega). In this sense, the TrueORF vector system is truly universal.

While the PrecisionShuttle vector system can be used for any cDNA, we have developed this system to take advantage of the largest collection of full-length cDNA clones available at OriGene. Every cDNA clone is offered in the Entry vector as a TrueORF clone, and the customer can easily transfer this ORF into any destination vector.

The PrecisionShuttle Entry and destination vectors contain the neomycin phosphotransferase gene under the SV40 promoter. Expression of the neomycin phosphotransferase gene in mammalian cells allows stable cell selection with a neomycin analog such as G418. Destination vectors with alternative selection markers (e.g. puromycin, blasticidin, hygromycin, etc., are also available. A complete and up to date listing of these vectors can be found on our website at http://www.origene.com/cdna/trueorf/destinationvector.aspx .

The development of the PrecisionShuttle vector system has gone through a rigorous quality control (QC) process. Both the Entry vector and the destination vectors have been validated for transient and stable mammalian cell transfections using a T-GFP marker (data not shown). The expression of N-terminal and C-terminal fusion tags has been validated by Western blot analysis (shown in Figure 4).

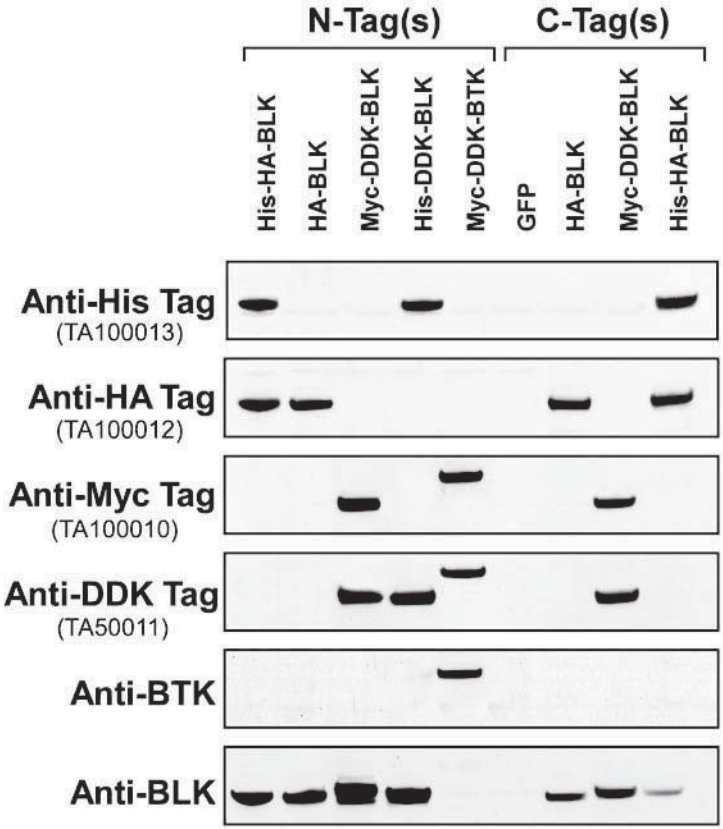


Figure 4. Western blot analysis of proteins expressed from N-terminally and C-terminally tagged PrecisionShuttle vectors. Each lane of the blot contains the whole cell lysate from an overexpression experiment using a PrecisionShuttle vector. BLK represents human B lymphoid tyrosine kinase (NM_001715); BTK represents human Bruton agammaglobulinemia tyrosine kinase (NM_000061). These two cDNAs were cloned into the destination vectors identified at the top of the blot. GFP represents one empty destination vector, pCMV6-AC-GFP, used for cloning C-terminal GFP fusion proteins. Specific antibodies against BLK and BTK detected the same size proteins as antibodies against the N-terminal and C-terminal tags.

Experimental protocols

Detect protein over-expression using anti-DDK antibody

The protein expression level can be detected using anti-DDK antibody (OriGene product number TA50011, 4C5-AntiDDK monoclonal antibody) by Western blotting. This antibody is shown to be of higher sensitivity than Sigma's M2 anti-FLAG antibody. Therefore, it is important to use TA50011 mAb when assessing protein over-expression using OriGene's TrueORF clones. When OriGene's 4C5-Anti-DDK monoclonal antibody is used, the suggested starting dilutions are 1:2000 for Western blot, 1:200 for immunoprecipitation and immunostaining, and 1:1000 for immunofluorescence.

Primer Design and PCR Amplification of ORF

The open reading frame (ORF) of the clone must be PCR amplified in order to append cloning sites to the 5' and 3' ends of the sequence. Add the target sequences of the selected restriction enzymes to the forward and reverse PCR primers; examples are shown below.

Forward primer with Sgf I
5' GAG**GCGATCGC**CNNNNNNNNNNNNNNNNNNNNNN 3'
Ns represent the sequence of the ORF beginning with the start codon, ATG. It is important to add the additional "C" base after the Sgf I site to maintain appropriate reading frames with N-terminal tags in some destination vectors.

Reverse primer with Mlu I
5' GCG**ACGCGT**NNNNNNNNNNNNNNNNNNNNNN 3'
Ns represent the reverse complement of the ORF sequence starting **with the stop codon** for N-terminally tagged or untagged destination vectors. This ensures that the expressed fusion protein will end at the native C-terminal end of the ORF. For C-terminally tagged vectors, the reverse complement of the ORF sequence should start **with the second-to-last codon**, as the stop codon must be removed to generate a fusion protein.

If the recognition sites for Sgf I or Mlu I are present internally in the ORF, another rare cutter such as Asc I, Rsr II or Not I can be used in the cloning strategy. In these cases, the sequences of these alternate restriction sites should be used in place of Sgf I and/or Mlu I (examples below).

Forward primer with Asc I:
5' GCC**GGCGCGCC**ANNNNNNNNNNNNNNNNNNNNN 3'
An extra nucleotide after Asc I is important to maintain reading frames with N-terminal tags in some destination vectors.

Reverse primer with Rsr II:
5' GCGT**CGGTCCG**CTNNNNNNNNNNNNNNNNNNNNNN 3'
Extra nucleotides after Rsr II are important to maintain appropriate reading frames with C-terminal tags in some destination vectors.

Reverse primer with Not I:
5' GCGAC**GCGGCCGC**TCACGCGTNNNNNNNNNNNNNNNNNNNNNN 3'
Mlu I is also added for downstream subcloning.

We recommend using a full-length cDNA clone as the template for ORF cloning. The success rate is low when a cDNA pool is used as the template for a PCR cloning reaction. When the GC content of an ORF (or a region of the ORF longer than 100 bp) is above 75%, a special PCR buffer with DMSO or other additive should be used to increase the success rate. The recommended PCR polymerase and buffer are available from New England Biolabs (Phusion™ High-Fidelity PCR Kit, F-553S).

PCR reaction setup:

Component	Volume
5X PCR buffer	4 μl
dNTPs (2.5 mM each)	1.6 μl
Phusion polymerase (2U/μl)	0.2 μl
Nuclease free water	11 μl
Forward primer (10 μM)	0.6 μl
Reverse primer (10 μM)	0.6 μl
cDNA template	2 μl (50-100ng plasmid)
Total volume	20 μl

All of the components should be kept on ice. When setting up multiple reactions, a master mix can be prepared without cDNA template or primers. After aliquotting the master mix, the cDNA template and primers can be added individually to each tube.

PCR cycling conditions:

The optimum Tm for annealing should be 55-60°C. The extension time depends upon the length of the ORF. The following program is generally used for ORFs from 500 bp-4000 bp.

1 cycle of	95°C	1 min
2 cycles of	95°C	10 sec
	62°C	20 sec
	72°C	4 min
2 cycles of	95°C	10 sec
	60°C	20 sec
	72°C	4 min
2 cycles of	95°C	10 sec
	58°C	20 sec

	72°C	4 min
15 cycles of	95°C	10 sec
	56°C	20 sec
	72°C	4 min
	72°C	10 min
	4°C	hold

Cloning of ORF into the Entry Vector

1. Confirm that the size of the amplification product is correct by agarose gel electrophoresis, and purify the remainder of the reaction using a purification column or similar method. Elute the DNA from the purification column in 26 µl of 10 mM Tris buffer. Set up a digestion reaction as described below, substituting other restriction enzymes as appropriate.

Component	Volume
10X restriction buffer	3 µl
Sgf I (10U/µl)	0.6 µl
Mlu I (10U/µl)	0.6 µl
Purified PCR product	26 µl
Total volume	~30 µl

Mix well, and incubate at 37°C for 3 hrs.

2. Purify the digestion reaction using a purification column and elute in 18 µl of 10 mM Tris buffer. Quantitate the DNA by UV at A260, or by OriGene's QuantiLadder (Cat# QLD200).

3. Digest pCMV6-Entry with the restriction enzymes corresponding to the sequences added to the ORF. pCMV6-Entry is available from OriGene as 10 µg lyophilized DNA (Cat# PS100001). Resuspend the lyophilized DNA in 100 µl dH_2O, and incubate for at least 30 min before use. Set up a digestion reaction as described below, substituting other restriction enzymes as appropriate.

Component	Volume
10X restriction buffer	3 µl
Sgf I (10U/µl)	0.8 µl
Mlu I (10U/µl)	0.8 µl
Nuclease free water	15.4 µl
Vector DNA	10 µl
Total volume	30 µl

Incubate at 37°C for 3 hrs, then add 1 µl Antarctic phosphatase (units used according to the manufacturer's protocol), and continue the incubation at 37ºC for another 30 min. Dephosphorylation of the digested vector is essential to eliminate self-ligation.

4. Purify the desired vector fragment by running the digestion reaction on an agarose gel, and isolating the appropriate band using a gel purification column. Elute the digested plasmid vector in 40 µl of 10 mM Tris buffer.

5. Set up a ligation reaction with the purified vector and insert fragments:

Component	Volume
10X ligase buffer	1 μl
nuclease free water	3.5 μl
T4 DNA ligase	0.5 μl
Vector fragment	2 μl (approx 10 ng)*
PCR product	3 μl (approx 30 ng)*
Total volume	10 μl

Incubate the ligation reaction at room temperature for 30-60 minutes. * Alternate ratios may need to be tested to obtain optimal ligation efficiency.

6. Transform 1 μl of the ligation mixture using 20 μl high efficiency competent *E. coli* cells (ideally $1x10^8$ CFU/ug). Following transformation, resuspend cells in 200 uL LB.

7. Plate the entire transformation reaction on standard LB-agar plates containing 25 μg/ml kanamycin. Incubate at 37°C overnight.

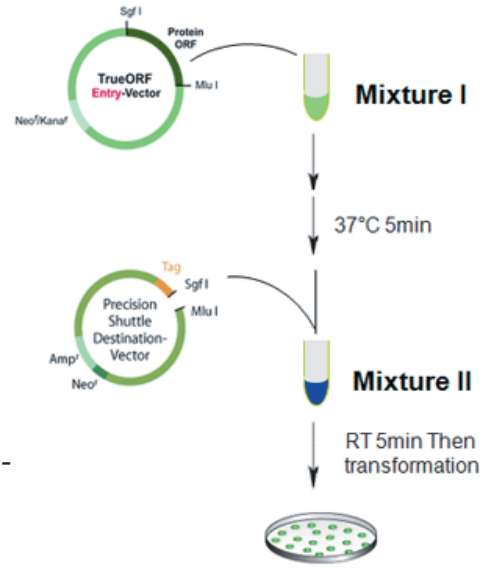
8. Pick at least 4-8 independent colonies to do a miniprep from each ligation. Confirm the insert by restriction digestion and/or vector primer sequencing (primers included in the kit).

Transfer of ORF from TrueORF Entry Vector to destination vectors

Subcloning with OriGene's RapidShuttling Kit:
OriGene has created the RapidShuttling kit to make subcloning to any of our other vectors a simple and efficient process. The RapidShuttling kit provides all required reagents and protocols allowing any TrueORF insert to be transferred to any PrecisionShuttle destination vector in a mere 30 minutes.

Kit Components Include:

- Shuttle-ready destination vector (pre-digested and dephosphorylated)
- Sgf I (Asis I) and Mlu I (20 uL) or
- Sgf I (Asis I) and Rsr II (20 uL)
- 10X Rapid digestion buffer



- T4 DNA ligase (for 10 reactions)
- 5X Rapid ligation buffer
- Water, nuclease free

Subcloning without OriGene's RapidShuttling Kit:
To transfer the protein-coding region from the TrueORF Entry Vector (donor) to a PrecisionShuttle destination vector (recipient), choose the appropriate destination vector with the desired tag options (http://www.origene.com/cdna/trueorf/destinationvector.mspx). There are three main types of PrecisionShuttle destination vectors, each of which is designed to express 1) a native (untagged) protein, 2) an N-terminally tagged protein, or 3) a C-terminally tagged protein. The translation of an N-terminally tagged protein initiates from the ATG of the tag and continues through the ORF of the gene of interest, whereas translation of an untagged or a C-terminally tagged protein initiates from the ATG of the protein of interest. The ORF of a C-terminally tagged protein is followed by either a single tag, or a double tag including a short spacer (5-6 amino acid residues). The transfer protocol between TrueORF vectors is shown schematically in Figure 3, and is detailed below.*

1. Digest the TrueORF Entry clone:

Component	Volume
10X restriction buffer**	2 µl
Sgf I (10 U/µl)	0.6 µl
Mlu I (10 U/µl)	0.6 µl
nuclease-free water	13.8 µl
TrueORF Entry vector (200 ng)	3 µl
Total volume	20 µl

Incubate at 37^{o}C for 3 hrs.

2. Digest the TrueORF destination vector:

Component	Volume
10X restriction buffer**	2 µl
Sgf I (10 U/µl)	0.6 µl
Mlu I (10 U/µl)	0.6 µl
nuclease-free water	14.8 µl
TrueORF destination vector (200ng)	2 µl
Total volume	20 µl

* For the 4% of the clones that have internal Sgf I or Mlu I sties, please use the appropriate combination of restriction sites as recommended by OriGene
** NEB buffer 3 has been shown to work well with dual digestion of Sgf I and Mlu I.

Incubate at 37°C for 3 hrs. Add 0.5 µl antarctic phosphatase (units used according to the manufacturer's protocol) to the digestion, and continue to incubate at 37°C for an additional 30 minutes.

3. Purify the digestion using a commercial PCR purification column and elute in 20 ul 10 mM Tris.

4. Set up a ligation reaction:

Component	Volume
10 x T4 DNA ligation buffer	1 µl
T4 DNA Ligase (4U/µl)	0.75 µl
nuclease-free water	3.25 µl
digested DNA from Step 1 (ORF clone)	2 µl
digested DNA from Step 2 (destination vector)	3 µl
Total volume	10 µl

Incubate the ligation reaction at room temperature for 1 hour.

5. Transform the ligation reaction into high-efficiency, competent *E. coli* cells (≥ 1×10^8 CFU/µg DNA) following the appropriate transformation protocol. Plate the transformants on LB-agar plates supplemented with 100 µg/ml ampicillin.
6. Pick at least four colonies for subsequent DNA purification and screening. Amplify and purify the selected clone(s) by growing overnight in liquid LB-amp media, then isolating the DNA using standard plasmid purification procedures.
7. Confirm the insert by restriction digestion and/or vector primer sequencing using the provided V1.5 for 5' end sequencing and XL39 for 3' end sequencing. A different set of sequencing primers are used for TrueORFs cloned in the pTUNE vector; pTUNE-F as the forward sequencing primer, pTUNE-R as the reverse sequencing primer (see FAQ).

Protocol for Transient Transfection

A sample protocol is listed here for experiments performed in 24-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate. See Table 2 for more details. OriGene recommends using TurboFectin 8.0 (TF81001) for all transfections with the broadest efficiency across most cell types. It consistently produces high transfection efficiency.

1. Preparation of cells
    a. Plate ~$5x10^4$ adherent cells or ~$5x10^5$ suspension cells per well 24 hrs prior to transfection.
2. Preparation of the Turbofectin 8.0/DNA Complexes:
    a. (Prepare immediately prior to transfection)
    b. Dilute 1 µg of DNA in 100 uL of Opti-MEM I (Gibco 51985). Vortex gently.
    c. Add 3 uL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and vortex the solution immediately for 10 seconds.

d. Incubate for 10 minutes at room temperature.

Note: We recommend starting with the ratios of Turbofectin 8.0 and DNA listed in Table 2; however, subsequent optimization may further increase the transfection efficiency.

3. Transfection
    a. Gently add the Turbofectin 8.0 / DNA mixture from step 2 to each well (already containing about 900 uL culture medium). Generally, the volume of the Turbofectin 8.0 / DNA mixture represents 1/10 of the total volume of the culture medium. Gently rock the plate to achieve even distribution of the complexes. Incubate at 37°C for 24-48 hrs.

Note: The above incubation is designed for transfection without a media change. If a media change is preferred, incubate for 30 minutes (if centrifugation is possible) or for 3-4 hrs (if centrifugation is not possible). Replace the media with the fresh complete growth media. Incubate for 24-48 hrs. Expression of the transgene can often be detected in as little as 24-48 hrs post-transfection.

Table 1. Recommended starting transfection conditions for Turbofectin 8

Tissue Culture Vessel	Growth area, cm^2/well	μg of DNA	Ratio of Turbofectin:DNA
96-well plate	0.3	0.05-0.25	3:1
24-well plate	2	0.25-1.25	3:1
12-well plate	4	0.5-2.5	3:1
6-well plate	9.5	1-5	3:1
35 mm plate	8	1-5	3:1
60 mm plate	20	2-10	3:1
100 mm plate	60	5-25	3:1

Protocol for Stable Transfection

Perform a transfection as described above (protocol for transient transfection). Twenty-four hrs post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing selective agent. A mock transfection should be performed in parallel as a control. Grow and passage the cells as necessary, maintaining selection pressure by keeping the selective agent in the growth medium. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells. Monitor the mock control to ensure the cells are dying.

Troubleshooting

For questions not addressed here, please contact OriGene's Technical Support professionals. You may dial 888-267-4436 from any US location, or 301-340-3188 outside the US. E-mail inquiries to techsupport@origene.com are also invited.

No colonies or low number of colonies from transformation

Cause	Remedy
The competent cells used in the transformation were not as efficient as necessary.	Obtain a fresh batch of competent cells and ensure that the efficiency is ≥ 1×10^8 CFU/μg DNA by performing a separate transformation reaction with a transformation-qualified control (usually a fixed amount of supercoiled plasmid such as pUC19). In some extreme cases, especially for larger inserts (>5 kb), higher efficiency cells or electroporation may be required. Should a gene be toxic to the cells, growing bacteria at a lower temperature, such as 30^0C or transforming into strains that reduce the copy number can increase the odds of obtaining colonies (i.e. ABLE-C or ABLE-K strains from Stratagene; CopyCutter from Epicentre).
Too little DNA was used in the transformation reaction.	Add more DNA (but not more than 10% of the volume of competent cells used).
The ligation of the ORF donor DNA into the recipient plasmid was not successful.	1) The ligase enzyme may not work properly. Repeat the reaction with fresh ligase and ligation buffer (which contains the temperature-sensitive component, ATP) or perform troubleshooting as recommended by the manufacturer of the ligase. 2) Change amounts and ratios of DNA (ORF insert vs vector) in the reaction.
The wrong antibiotic selection plate was used.	Make sure to use an LB-agar plate containing the correct antibotics (e.g. 25 μg/ml kanamycin for Entry vector and 100 μg/ml ampicillin for other destination vectors).

Too high self-ligation background (no insert) from destination vector

Cause	Remedy
The destination plasmid was not completely digested.	Allow the digestion reaction to continue

	for 3 hrs or overnight at 37°C.
The dephosphorylation of the destination plasmid was not complete, and the destination vector religated with its own fragment.	Increase the concentration of antartic phosphatase and/or the length of the dephosphorylation incubation as recommended by the ligase manufacturer.

Frequently Asked Questions

What is the PrecisionShuttle System?
Answer: The PrecisionShuttle System provides a restriction-enzyme-based approach to append different tags to one's open reading frame (ORF) of interest.

What is the difference between OriGene's Entry vector and the destination vectors?
Answer: The major differences are the antibiotic selection marker and the epitope tags or markers. The Entry vector carries kanamycin resistance (25 μg/ml), while all destination vectors contain the ampicillin resistance gene (100 μg/ml). This allows simple screening for successful subcloning products. All of the vectors have a unique combination of N- and C-terminal epitope tags or a fluorescent marker, as described in Table I.

How do you transfer the ORF insert I purchased into another tagging vector?
Answer: Over 60 destination vectors are designed with compatible MCS for easy shuttling of TrueORF inserts. This can be performed easily using a specific pair of restriction enzymes to cut-and-ligate subclone into the desired destination vector. OriGene simplifies this process by offering the RapidShuttling Kit (see http://www.origene.com/rapid-shuttling-kit). OriGene also provides a custom cloning service available through our website.

What are the functional aspects of the pCMV6-AC-GFP vector?
Answer: Like all OriGene vectors, the CMV promoter drives the heterologous expression of the specified open reading frame (ORF) which is in-frame with Turbo Green Fluorescent Protein (tGFP) on the C-terminus. tGFP expression permits the positive identification of mammalian cells transfected with plasmid. The neomycin resistance gene is also expressed downstream of the SV40 promoter within the same vector and permits positive selection of transfected cells as well as stable cell line production. For bacterial amplification, the ampicillin resistance gene is engineered on the opposite strand.

OriGene's GFP is listed as TurboGFP. How is this different from other available GFPs?
Answer: TurboGFP is a fully licensed, 26kDA protein product from Evrogen JSC that works well in standardized GFP assays. Excitation max is 482nm and emission max is 502nm. It yields 112% of the brightness compared to eGFP and has no known cellular toxicity. It is an isoform of the naturally occurring protein from *Pontellina plumata* that has been optimized for rapid labeling of

cells/organelles and tracking of promoter activity. It is a perfect choice for monitoring transient protein expression.

Has OriGene fully sequenced all TrueORF Gold clones?
Answer: Absolutely. The coding region and cloning site regions are fully covered by high quality sequence. Chromatograms are provided on the USB shipped with every TrueORF Gold clone. The stated consensus is fully guaranteed.

Do TrueORF clones exactly match the reference gene sequence?
Answer: All TrueORF clones are guaranteed to match the corresponding ORF sequence posted on our website. However, some clones may contain nucleotide changes compared to the published reference sequences. This is due to SNPs (single nucleotide polymorphisms) reflecting the unique differences from genes expressed in different tissues and different individuals. Published references may represent a different SNP than the OriGene transcript. Should a specific SNP be required, this can be obtained via our ExactORF cloning service (http://www.origene.com/cdna/exactorf.mspx).

Sequences of the sequencing primers, VP1.5, XL39, pTUNE-F Forward and pTUNE-R Reverse
Answer: VP1.5 (forward seq primer)
5' GGACTTTCCAAAATGTCG 3' Tm=51C

XL39 (reverse seq primer)
5' ATTAGGACAAGGCTGGTGGG 3' Tm=60C

pTUNE-F Forward
5' TAGAGTCGACCTGCAGCCGG 3' Tm=58C

pTUNE-R Reverse
5' TCGCTGATTTGTGTAGGGGA 3' Tm=52C

Can I transfer large ORFs using this system?
Answer: It has been reported that ORFs larger than 4 Kb are unstable in recombination-based systems; conversely, our restriction digest-based vector system has no real size limitation. An ORF up to 18 Kb can be readily transferred from one vector to another.

What restriction enzymes should I use if Sgf I or Mlu I sites are present in my ORF?
Answer: While 96% of all human and mouse ORFs can use the Sgf I - Mlu I combination, some ORFs do contain internal Mlu I site(s). Most of those ORFs with an internal Mlu I site can be transferred using another rare cutter (Rsr II), whose restriction site is upstream of Mlu I, or Not I, whose site is immediately downstream of Mlu I. Using one of the four different subcloning combinations, any ORF can be transferred from one vector to another. The recommended subcloning combination for every TrueORF cDNA is listed in the product information on our website.

Why does my Certificate of Analysis (COA) indicate cloning sites other than Sgf I and Mlu I?
Whenever one or both of these sites is present within the ORF of the transcript, the PrecisionShuttle vectors share other sites engineered to accommodate this, e.g. Rsr II or Asc I.

What sites should I use to transfer a TrueORF clone into the Gateway system?
Answer: There are multiple sites in pCMV6-Entry than can be used to move the insert of a TrueORF clone into any of Gateway's Entry vectors (pENTR-1A, -2B, -3C, -4, and -11). These sites are EcoR I, Sal I, BamH I and Kpn I at the 5' end, and Not I at the 3' end.

What restriction sites are available for subcloning into other vectors?
Answer: The vector maps and nucleotide sequences can be found at http://www.origene.com/cdna/trueorf/destinationvector.mspx

How many amino acids are present in the linker between my protein and tGFP?
Answer: To accommodate the Mlu I cloning site, which maintains the proper reading frame, this vector appends a threonine and arginine. This is far fewer than with other recombination-based shuttling systems.

Which vector serves the negative control for the GFP fusion clone?
Answer: We recommend pCMV6-AN-GFP (Cat# PS100019).

I cannot detect any protein expression from the TrueORF clone in a pCMV6-Entry vector. What are my options?
Answer: 1) Check your transfection efficiency. We recommend using a plasmid that expresses a fluorescent marker (pCMV6-AN-GFP PS100019). 2) AntiFLAG antibodies from other vendors are not as sensitive as OriGene's optimized 4C5-AntiDDK antibody (TA50011) when directed at the same epitope.

I cannot see any green fluorescence with the TrueORF clone in a pCMV6-AC-GFP vector. What are my options?
Answer: Your protein of interest might quench the fluorescence of tGFP. To confirm, we suggest you first run a Western Blot with a protein specific antibody or anti-tGFP. The molecular weight of the tGFP fusion protein is approximately 26 kDa larger than the endogenous protein. The tGFP antibody is available from Evrogen (AB513). The antibodies against other GFPs will not recognize tGFP.

What does your disclaimer mean?
Answer: OriGene's disclaimer for the TrueORF clones reads as follows: "Our molecular clone sequence data has been matched to the accession number below as a point of reference. Note that the complete sequence of our molecular clones may differ from the sequence published for this corresponding accession number, e.g., by representing an alternative RNA splicing form or single nucleotide polymorphism (SNP)."

The NCBI RefSeq mRNA sequences are continuously being revised, as some may have been derived from aberrantly spliced transcripts or generated by incorrect prediction of intron-exon junctions in silico. These sequences are therefore used only as a “reference” and not as a “standard”. OriGene’s clones are isolated from full-length cDNA libraries and may differ from the reference sequence for this reason.

What is the TrueORF Guarantee?
Answer: OriGene warrants that the product will meet specifications listed. At OriGene’s discretion, free replacement of any non-conforming product will be made if OriGene is notified within 30 days of product receipt. If you experience any difficulty with any OriGene product, please contact our Technical Support Staff at 888-267-4436, or 301-340-3188 outside the US.