RapidShuttling Kit

APPLICATION GUIDE

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Package Contents and Storage Conditions

Product Description

- The RapidShuttling Kit enables fast ORF insert transfer between two PrecisionShuttle vectors. It takes only 30 min from DNA digestion to transformation.
- OriGene shuttle-ready (double-digested and dephosphorylated) vectors offer optimal efficiency of ligation.

Note: The kit is designed to transfer from the Entry vectors to the destination vectors, but the component can also be used to back-shuttle into the entry vectors provided the final transformants are selected on kanamycin instead of ampicillin.

Components in kit:

- Sgf I (Asis I) and Mlu I Restriction Endonucleases (20ul at 1FDU/ul each) (90001-21 and 90001-22) for Kit Sgf I -Mlu I
- Or Sgf I (Asis I) and Rsr II Restriction Endonucleases (20ul at 1FDU/ul each) (90001-21 and 90001-23) for Kit Sgf I - Rsr II
- 10X Rapid Digestion Buffer (90001-24)
- T4 DNA Ligase (for 10 reactions) (90001-25)
- 5X Rapid Ligation Buffer (90001-26)
- Water, nuclease-free (90001-27)
- Customer-Selected, Shuttle-ready Precision-Shuttle Vector (Double-digested and 5'-dephosphorylated; 250ng)

Components needed but not included:

- Subcloning competent cells with transformation efficiency ≥ 1×10^8 CFU/μg (DH5α or similar)
- LB-AMP plates (100μg/ml)
- DNA Purification Kit (OriGene/Marligen PowerPrep HP)
- LB-Kan plates for back-shuttling into Entry vector only.

Available Destination Vectors

Table 1. Available OriGene Pre-prepared destination vectors

SKU	TrueORF Vector	Drug Selection	Transcriptional Promoter for Expression			Peptide Fusion Tag		Stable Cell line Selection
			E. Coli	Mamma-lian cells	In vitro Transcription/ translation	N-terminus	C-Terminus	
PS200001/PS300001	pCMV6-ENTRY	Kanamycin	T7	CMV	T7	-	Myc-DDK	Neomycin
PS200002/PS300002	pCMV6-AC-His	Ampicilin	T7	CMV	T7	-	His	Neomycin
PS200004/PS300004	pCMV6-AC-HA	Ampicillin	T7	CMV	T7	-	HA	Neomycin
PS200005/PS300005	pCMV6-AC-DDK	Ampicillin	T7	CMV	T7	-	DDK	Neomycin
PS200006/PS300006	pCMV6-AC-Myc-His	Ampicillin	T7	CMV	T7	-	Myc-His	Neomycin
PS200008/PS300008	pCMV6-AC-HA-His	Ampicillin	T7	CMV	T7	-	HA-His	Neomycin
PS200009/PS300009	pCMV6-AC-DDK-His	Ampicillin	T7	CMV	T7	-	DDK-His	Neomycin
PS200011/PS300011	pCMV6-AN-His	Ampicillin	T7	CMV	T7	His	-	Neomycin
PS200012/PS300012	pCMV6-AN-Myc	Ampicillin	T7	CMV	T7	Myc	-	Neomycin
PS200013/PS300013	pCMV6-AN-HA	Ampicillin	T7	CMV	T7	HA	-	Neomycin
PS200014/PS300014	pCMV6-AN-DDK	Ampicillin	T7	CMV	T7	DDK	-	Neomycin
PS200015/PS300015	pCMV6-AN-His-Myc	Ampicillin	T7	CMV	T7	His-Myc	-	Neomycin
PS200018/PS300018	pCMV6-AN-His-DDK	Ampicillin	T7	CMV	T7	His-Flag	-	Neomycin
PS200019/PS300019	pCMV6-AN-GFP	Ampicillin	T7	CMV	T7	GFP	-	Neomycin
PS200010/PS300010	pCMV6-AC-GFP	Ampicillin	T7	CMV	T7	-	GFP	Neomycin
PS200020/PS300020	pCMV6-AC-stop	Ampicillin	T7	CMV	T7	-	-	Neomycin
PS200027/PS300027	pCMV6-AC-IRES-GFP	Ampicillin	T7	CMV	T7	-	-	Neomycin
PS200034/PS300034	pCMV6-AC-RFP	Ampicillin	T7	CMV	T7	-	RFP	Neomycin
PS200031/PS300031	pEX-C-His	Ampicillin	T7	CMV	T7	-	His	
PS200033/PS300033	pCMV6-AN-RFP	Ampicillin	T7	CMV	T7	RFP	-	Neomycin

OriGene Pre-prepared Vectors:

- Digested and 5' dephosphorylated
- 250ng lyophilized. Resuspend in 25ul dH_2O to 10ng/ul, enough for 10 reactions
- Low self-ligation background (<5% compared to ligation control with actin insert)
- High efficiency (>90% by internal QC with actin insert)
- Results guaranteed (PhD scientists available at techsupport@origene.com)

Other Related Products

TrueORFs (http://www.origene.com/orf)
Destination Vectors (http://www.origene.com/cdna/trueorf/destinationvector.aspx)
PowerPrep Mini-Prep Kit (http://www.marligen.com/inserts/powerprep/11449-014.pdf)
HuSH™ shRNA Plasmids (http://www.origene.com/shRNA)

Anti-tag antibodies (http://www.origene.com/antibody/antitagantibody.mspx)
Transfection Reagents (http://www.origene.com/cdna/turbofectin.mspx)

Experimental Protocol

Description

To transfer your protein-coding region (a.k.a. ORF or CDS) from TrueORF Entry Vector to a destination vector, choose the appropriate destination vector with the desired tag option (Table 1). There are three categories of TrueORF Vectors, all containing Sgf I and Mlu I sites, expressing either a native (untagged) protein or an N-terminal-tagged or a C-terminal-tagged protein. The translation of an N-terminal-tagged protein initiates from the ATG of the tag followed by the in-frame ORF of interest, whereas the translation of a native or a C-terminal-tagged protein initiates from its own ATG of the protein of interest, followed by the tag(s), having a short spacer element of a few amino acid residues. The transfer protocol of protein-coding regions between TrueORF vectors is schematically shown in Figure 1(Sgf I-Mlu I as an example).

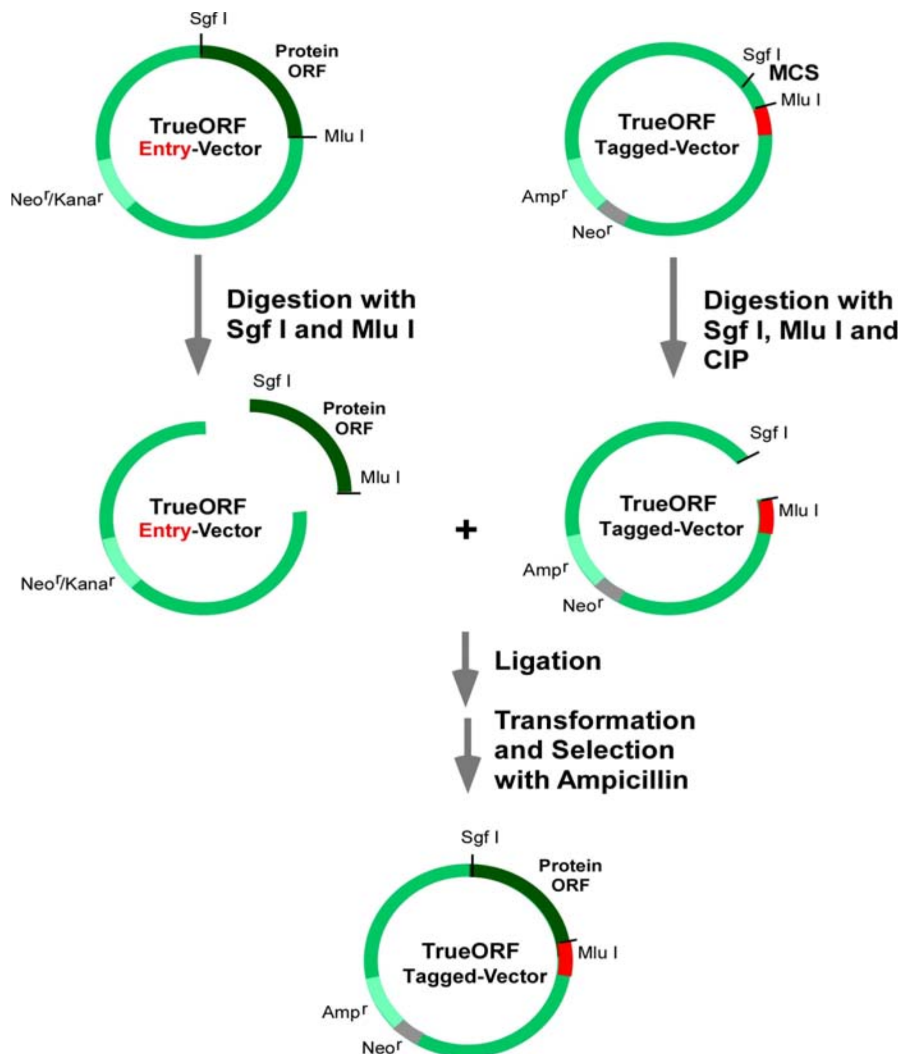

Figure 1. Diagram of transfer protocol.

Transfer Protocol of RapidShuttling Kit

1. Assemble the following reaction components to cut the TrueORF entry Vector:

Component	**Volume**
Restriction Digest Buffer	3µl
TrueORF Entry Vector (100ng/ul)	5µl
Sgf I	0.6µl
Mlu I (or Rsr II)	0.6µl
Add Nuclease-Free Water to a final volume of	30µl

2. Incubate at 37°C for 5 minutes, but be sure to quick-spin contents prior to incubation and that tube is closed tightly to avoid evaporation.)

3. Incubate at 80°C for 15 minutes to inactivate enzymes. Again, quick spin contents prior to incubation if there is any visible condensate.

4. Assemble the following ligation reaction components:

Component	**Volume**
5 x Ligation Buffer	2µl
Digested DNA from Step 3	2µl (33ng)
Pre-prepared OriGene Vector	2µl (20ng)
T4 DNA Ligase (2units/µl)	0.5µl
Nuclease-Free Water to a final volume of	10µl

6. Incubate at room temperature for 5 minutes.

7. Transform the ligation reaction into high-efficiency; E. coli competent cells (≥ 1×10^8cfu/µg DNA) following the manufacturer's transformation protocol. Selection for transformants should be on LB plates supplemented with 100µg/ml ampicillin.

8. Screen at least 4 colonies for the presence of each protein-coding region. Be sure to pick isolated colonies and grow overnight in a growth media like LB-borth containing 100ug/ml ampicillin. Purify the plasmid DNA (OriGene sells these kits separately). Digest the plasmid to ensure that Sgf I and Mlu I can cleave the appropriate protein-coding region by running the digestion reaction(s) on an ethidium bromide-stained agarose gel. DNA sequencing of the final product is not necessary since all ORF DNAs in the TrueORF Entry Vectors have been fully sequenced by OriGene Technology Inc., but sequence verification of the ligation sites is advisable. Special note: only the destination plasmids should grow in the presence of ampicillin because the entry donor constructs are Kanamycin resistant.

9. Amply and purify the selected clone to obtain the quantity and concentration of plasmid appropriate for your experiments.

Troubleshooting

For questions not addressed here, please contact OriGene's Technical Support PhD scientists. 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	1. Obtain a fresh batch of competent cells and ensure that the efficiency is $\geq 1\times10^8$ CFU/µg 2. In some extreme cases, especially for larger inserts (>5 kb), higher efficiency cells or electroporation may be required. 3. 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).
The wrong antibiotic selection plate was used.	Make sure to use an LB-agar plate containing the correct antibiotics (e.g. 25 µg/ml kanamycin for Entry vector and 100 µg/ml ampicillin for other destination vectors).
No inserts were found in any of the colonies OR all screened colonies were in the source entry vector.	Wrong antibiotic may have been used to plate out the transformation. "Snap-back" of the entry vector is quite efficient. In rare cases where the source clone's vector and the destination vector are both amp resistant, the insert must be purified prior to ligation.
My TrueORF was not cloned between the Sgf-MluI and the Sgf I-Rsr II sites.	This is a very rare occurrence within the TrueORF portfolio of clones. Please contact techsupport@origene.com for assistance.

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.

Could I use OriGene RapidShuttling Kit for subcloning with vectors other than OriGene PrecisionShuttle System?
Answer: No. OriGene RapidShuttling Kit is specially optimized for the PrecisionShuttle System. However, PCR-amplified fragments with appropriate adaptors can be cloned into any of our PrecisionShuttle vectors using the RapidShuttling kit.

Do I have to use OriGene digested/dephosphorylated vectors?
Answer: No, you don't have to. You can prepare your own PrecisionShuttle vector, but OriGene Pre-prepared vectors are pre-tested to ensure low background, high efficiency, and least amount of work of your subcloning.

What is the difference between OriGene's Entry vector and the destination vectors?
Answer: The major differences are the antibiotic selection markers and the epitope tags or markers. The Entry vector confers kanamycin resistance (25 µg/ml), while all destination vectors confers ampicillin resistance gene (100 µg/ml). This permits a 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.

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 appreciable 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; Alternative OriGene kit). OriGene also offers the Sgf I- Rsr II Pre-prepared vectors. Please be sure to choose the matching vector in combination with the enzymes contained within the kit.

The destination vector that I need to shuttle the ORF into (or the restriction enzymes for subcloning my ORF) is not included in your RapidShuttling kit offering. What are my options?

We offer RapidShuttling kits for the most popular destination vectors and the most common cloning sites. If your desired kit is not available, you have two options:

1. Purchase the desired destination vector separately (Uncut plasmid, 10ug) and perform a simple digestion-and-ligation using standard subcloning procedures.

2. Contract OriGene to provide the shuttling service. You can go the product page of the appropriate TrueORF clone and click on "Clone modification" button. Fill out your request and OriGene will send a quote within 24 hrs. The average service fee is approximately $400 in additional to the TrueORF price.

Can I use this kit to shuttle from one destination vector to another?
Answer: Yes, but there is one caveat. In this case, the insert donor vector is also ampicillin resistant. Therefore, the colonies will contain a mixture of a) empty donor vector (snap-back), b) donor construct (either undigested or re-ligated) and c) the desired construct. Either screen many more colonies (50-75), or take the time to purify the insert away from the donor vector after the digestion step 3 above.