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Subcloning Protocol

Follow this simple and efficient cut-and-ligate process to transfer a TrueORF insert into any of our PrecisionShuttle destination vectors.

Required Reagents:

- TrueORF cDNA clone
- Destination vector
- Sgf I (Asis I) and Mlu I, or Sgf I (Asis I) and Rsr II
- T4 DNA ligase
- Water, nuclease free

1) Digest the TrueORF entry clone:

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

Incubate at 37°C for 3 hrs.

2) Digest the TrueORF destination vector:

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

Note: For the 4% of the clones that have internal Sgf I or Mlu I sites, please use the appropriate combination of restriction sites as recommended by OriGene.

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 µl 10 mM Tris.

4) Set up 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 for non-Lenti vectors or 34ug/ml chloramphenicol for Lenti vectors.

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 containing the corresponding antibiotics (ampicillin for non-lenti vectors, chloramphenicol for lenti vectors), then isolating the DNA using standard plasmid purification procedures.

7) Confirm the insert by restriction digestion and/or vector primer sequencing using VP1.5 for 5' end sequencing and XL39 for 3' end sequencing (non-lenti vectors). A different set of sequencing primers are used for TrueORFs cloned in Lenti vectors; V2.0 as the forward sequencing, LR50 as the reverse sequencing primer.