Simple Cut & Paste

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1. Preparation of vector and insert

NEB Buffer	1 μl
10x BSA (if need be)	1 μl
Plasmid*	
Restriction Enzyme#	1 μl
Fill to 10 μl with H_2O	

Incubate at 37 ˚C for 1 hr (2 hr for double digestion).

* For vector, use 800 ng; for insert, use 1.5 μg.
# For double digestion, use 0.5 μl each.

2. Gel isolation

Use a gel extraction kit from Qiagen.

Elute with 30 μl of EB buffer.

3. Dephosphorylation of vector&

Add the following to the eluant in step 2.

Shrimp alkaline phosphatase (SAP) buffer	3 μl
SAP (Roche)	0.5 μl

Incubate @ 37 ˚C for 10 min.

Heat-inactivate @ 70 ˚C for 30 min.

& If the both ends of vector could not self-ligate (i.e., EcoRI and EcoRV), omit this step.

4. Ligation

Use a Rapid Ligation kit from Roche with a molar ratio of vector:insert to be 1:3.

Vector	
Insert	
Fill to 5 μl with H_2O	
Ligation Buffer (2x)	5 μl
T4 ligase	0.5 μl

Incubate at RT for 5 min.

5. Transformation

Add 4 μl of ligation reaction to 50 μl of DH5α competent cells.

Store the rest of the ligation reaction @ 16 ˚C O/N in case 5-min RT ligation did not work.

Incubate on ice for 15 min.

Heat-shock @ 42 ˚C for 90 sec.

Let stand @ RT for 2 min.

Add 150 μl of LB to the competent cells.

Incubate @ 37 ˚C for 30 min.

Spread all the cells and incubate @ 37 ˚C O/N.

6. Verification

Inoculate 4 ml of LB with a colony, grow O/N, carry out mini-prep using a Qiagen's Mini Kit.

Digest 1 μl of mini-prepped plasmids in 10 μl of reaction.

Load 4 μl of the reaction onto agarose gel.

Verify insert size under UV transilluminator.