Ligation

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:
Primary Experimenter (contact):
Other Experimenters:

Source: NEB, Dujduan Waraho

Assembled: 6/27/2012

Reagent	Details	Quantity
ddH2O (nuclease-free)		Bring total vol. to 20 µL
10X T4 DNA Ligase Buffer*		2 µL
Vector DNA		**Vector : Insert = 1 : 3
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T4 Polymerase		1 µL

*Thawed and resuspended at room temperature
** 1:3 molar ratio of vector : insert (but it can vary from 1:2 to 1:6);

Procedure:

Critical Steps:

- To be made critical

☐ **Label microcentrifuge tubes, put on ice**

☐ **Add components in listed order to microcentrifuge tubes on ice**

For numerous ligations, prepare mastermix with water and buffer

- [ ] **For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes; for blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours**
- [ ] **Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.**
    - See electroporation protocol