**Gateway LR Clonase II Reaction Protocol**

Developed by Ping in March 2011

**Materials**

 **Day 1**

* pENTR – gus
* LR Clonase Enzyme Mix
* TE pH 8.0
* SOC Media

**Day 2**

* LB+Amp plates
* proteinase k
* puc19
* NEB Competent cells
* Glass beads
* PCR tubes
* Epitubes

**Day 3**

* LB Broth

**Preliminary Steps**

 **Day 1**

* Set the Hybex to 25 degrees.

**Day 2**

* Warm LB + Amp plates up to 37 degrees.
* Put PCR tube blocks in both Hybex incubators.
* Set the other Hybex to 42 degrees.
* Thaw a tube of competent cells by resting it on top of ice. (from the box labeled NEB 12/21/10 in the -80 freezer). One tube has enough cells for 4 reactions.

**Create a 10 ul Master Mix:**

* 2 ul of LR Clonase II Enzyme Mix
* 100 ng FlexiTagII destination vector
* Enough TE at pH 8.0 reach 7 ul total volume per reaction.

**Steps**

 **Day 1**

1. Aliquot 2 ul of master mix into a PCR tube containing 50 ng source vector or pENTR – Gus to create a 1:2 ratio with the destination vector already added to the master mix.
2. Create a negative control with 2 ul of master mix and 0 ul of entry vector
3. Incubate the tubes overnight at 25 degrees in the Hybex.

**Day 2**

1. Add .5 ul of proteinase k to each sample and incubate at 37 degrees for 10 minutes.
2. Add 50 ul of competent cells into each PCR tube containing the reaction mix.
3. Add 50 ul of competent cells to two additional PCR tubes labeled positive control and negative control.
4. Add 1 ul of puc19 DNA to the positive control.
5. Tap the tubes to mix their contents.
6. **Ice for 30 minutes.**
7. **Heat shock at 42 degrees for 30 seconds, then ice for 2 minutes.**
8. **Add cells to sterile Epitubes containing 200 ul of SOC media and incubate at 37 degrees for 1 hour in the shaker.**
9. Add about ten glass beads to each LB + Amp plate.
10. Add all 250 ul of cells in SOC media to each LB + Amp plate and shake the glass beads for 20 seconds.
11. Return the glass beads to the jar of dirty beads.
12. Incubate the plates overnight at 37 degrees.

**Day 3**

1. Pick sample colonies and incubate in LB Broth overnight in preparation for Miniprep.

**Day 4**

1. Miniprep transformed colonies