Ligation into P-GEM T Vector------- AFTER INITIAL PRIMER PCR

Reaction Setup

In a clearly labeled PCR tube, make a cocktail (for n+1 reactions) of::

* 2.5 ul 2x Ligase Buffer (allow to thaw at room temperature and vortex well)
* 0.5 ul ligase
* 0.5 ul vector (200ng/ul)

Pipet up and down 5-10 times to mix (do not vortex) and aliquot 3.5 ul per tube. Add 1.5 ul finish PCR product (after verification of successful PCR by gel electrophoresis).
Total reaction volume should be 5ul.

Note: this is a very small reaction volume. As such, use of PCR tubes gives you more control when manipulating the reagents in the tube. If you are only setting up one ligation, you must set up a 10 ul ligation. At no time should you be trying to pipet 0.5 ul as our pipets are highly inaccurate below 1 ul.

Reaction Conditions

Incubate at 4^{o}C overnight.

Bacterial Transformation into JM109- AFTER LIGATION OF PCR PRODUCTS

Preparation

* **This is the easiest step to "mess up" in the entire workflow. Cells must be kept cold at all times! Chill labeled tubes, ligation mixes, etc., on ice prior to mixing of cells and DNA (ligation mix). Other hints are listed below.**

* Never thaw frozen cells in your hand; always plunge frozen cells in ice and allow to thaw there. As soon as they thaw, they start dying. The more time passes (up to the point where SOC is added) the more they die and the less transformants you'll get. Also, never heat the cells prematurely; make sure your timing for the heat shock step is precise.

* Check to make sure the water bath is holding at 42°C. If it is not, but is just slightly under (still above 40°C), that's fine. If it is too hot add a small amount of ice to bring it down; notify TAs so temperature can be adjusted. Do not adjust the temperature yourself.

* Get one LB/Carb/IPTG/Xgal plate per transformation from 4°C, label, and put on bench top.

* Obtain SOC and make sure it's sterile (it should be totally transparent). This should be prepared for you by the TAs in advance, but if you are curious about what it contains:

Reagent	Stock concentration	Final concentration	Vol/1ml
$MgSO_4$	1M	10mM	10ul
$MgCl_2$	1M	10mM	10ul
Dextrose	20%	0.36%	18ul

LB	1x	-	962ul

Reaction Conditions

* Transfer 5 ul of ligation mix into 1.7 ml eppendorf tubes. Put these ligation reactions onto ice, then ask an instructor/TA for competent cells. Tubes must be at 0 ^{o}C before adding bacteria. The instructor/TA will thaw the bacteria for you and aliquot 25 ul of JM109 competent cells per tube.

* Incubate on ice 30 minutes

* Heat shock at 42^{o}C for 45 seconds. **Timing is critical here-30 seconds or 1 minute is not OK!**

* Immediately (ie, bring your ice bucket to the water bath) place heat shocked cells back on ice for 2 minutes

* Add 400 ul SOC, pipet up and down with P200 set at 200) and incubate at 37^{o}C for 30 minutes

* Plate cells/SOC onto LB/Carb/IPTG/Xgal plates

* Dump out glass beads into trash

* Incubate plates overnight at 37^{o}C (Plates should be incubated no more than 18 hours. If you are not ready to move to the next step, you may hold them at 4^{o}C wrapped in parafilm. Whenever you are incubating or storing plates, they should be stored upside down to prevent condensation from dripping onto your plates.)