LR Reaction

Introduction

An LR reaction inserts one or more parts in pENTR vectors into a pDEST vector. Used to assemble transcriptional units from promoters and genes.

Materials

- Promoter pENTR plasmid: L4-Promoter-R1
  - Working concentration: 5 fmol/ul
- Gene pENTR plasmid: L1-Gene-L2
  - Working concentration: 5 fmol/ul
- Destination plasmid: pDEST
  - Working concentration: 10 fmol/ul
- Nuclease-free TE
- 200 μl PCR strip tubes, 1 tube per rxn
- 5x LR Clonase II
  - Stored in ~5 μl aliquots in the -80 in room 235. **Don't remove an aliquot until you're ready to use it.**
- Proteinase K
  - Stored in ~5 μl aliquots in the -80 in room 235. **Don't remove an aliquot until you're ready to use it.**

Procedure

LR Reaction Setup

1. For each LR you are doing, fill out a column in the following table:

Table1

	A	B	C	D	E	F
1	**Tube Label**					
2	**Promoter pENTR**					
3	**Gene pENTR**					
4	**pDEST**					
5						
6						

2. For each LR, label a 200 µl strip tube with your initials and tube number.

3. Into each tube, pipette:

   -- 1 µl of the promoter pENTR
   -- 1 µl of the gene pENTR
   -- 1 µl of the pDEST

4. Add 1 µl of TE to each tube

5. Retrieve an aliquot of LR Clonase from the -80.

   Bring an razor blade with you, you'll need to cut a tube from the strip tubes.

6. Pulse the LR clonase tube in the microfuge to collect the clonase at the bottom.

7. Add 1 µl of the LR clonase to each LR reaction.

   Be careful pipetting; LR clonase is viscous.

8. Cap the tubes.

9. Flick them several times to mix.

10. Pulse-spin the tubes in the microfuge to collect the liquid at the bottom.

11. Incubate at room temperature for at least 12 hours and not more than 24 hours.

    A popular strategy is to tape the tubes to the shelves over the bench, with your initials and the date.

16-24 hours later: Proteinase K kill

12. Retrieve a 5 µl aliquot of proteinase K from the -80 freezer.m n

13. Thaw in your fingers, then pulse in the microfuge to collect at the bottom of the tube.

14. Pipette 1 ul into each of the LR reactions.

15. Flick several times to mix.

16. Pulse-spin the tubes in the microcentrifuge.

17. Incubate at 37° for 15 minutes, or room-temperature for an hour.

    PAUSE POINT: You can store the reactions in the -20 indefinitely until the transformation.

00:15:00

18. Proceed to transformation. Transform 2 µl.

19. Afterwards, cap the tubes. Write the date on the caps and store in the -20 (in case your transformation failed.)