Gateway LR recombination Entered by Jun Lin
Janes Lab Protocols 4/25/11

1. In a microcentrifuge tube, add 150 ng entry clone + 150 ng destination vector in 8 μl TE buffer.
2. Remove the LR Clonase™ II Enzyme Mix (Invitrogen #11791-020) from –20° C and thaw on ice (~2 minutes).
3. Vortex the LR Clonase™ II Enzyme Mix briefly twice (2 seconds each time).
4. Add 2 μl of LR Clonase™ II Enzyme Mix to each sample. Mix well by vortexing briefly twice (2 seconds each time).
   - *Return LR Clonase™ II Enzyme Mix to –20°C immediately after use*
5. Incubate the reactions at room temperature. Take 5 μl of the sample after 1 hr and proceed to Step 6. Leave the remaining 5 μl of each sample at room temperature for up to 18 hr.
6. Add 0.5 μl of the Proteinase K solution to the 5 μl reaction and incubate for 10 minutes at 37°C.
   - *Proteinase K cleaves and inactivates the LR Clonase™ II Enzyme Mix*
7. Ethanol precipitate the reaction and resuspend in 5 μl water.
8. Transform 1 μl of the resuspended reaction into electrocompetent bacteria (see Janes_PCRcloning.pdf).
9. If the transformation is unsuccessful, repeat Steps 6–8 with the 18-hr recombination fraction.