Gateway LR Reaction

- **This protocol is for a half reaction, which is sufficient for standard cloning**

- Add reagents in the following order:
  
  \[
  \begin{align*}
  x \text{ uL} & \text{ pENTR plasmid mini-prep}^1 \\
  1 \text{ uL} & \text{ pDEST}^2 \\
  y \text{ uL} & \text{ TE (enough to bring reaction volume to 8 uL)} \\
  2 \text{ uL} & \text{ LR Reaction Buffer (5X)} \\
  2 \text{ uL} & \text{ LR Clonase Mix}^3
  \end{align*}
  \]

  - Gently tap tube several times to mix
  - Pulse spin reaction to bottom of tube
  - Incubate the reaction at 25°C for at least 4 hours, can be incubated overnight (use fly incubator)

**NOTES:**

1= Use 50 fmol of pENTR plasmid mini-prep. The equation for converting fmol to mass is as follows:

\[
\text{ng of DNA} = \left(\frac{(\text{Size of DNA in base pairs})(\text{fmol DNA})(660)}{10^6}\right)
\]

2= The concentrations of the different Destination Vectors will vary based on their size. All standard aliquots are 50 fmol of the plasmid

3= Thaw LR Clonase Mix quickly in your hand, then place on ice. Keep LR Clonase Mix on ice at all times! Do not vortex!