Gateway BP Reaction (Standard Reaction)

- This protocol is for a half reaction, which is sufficient for standard cloning.
- Add reagents in the following order:
  
  \( x \) uL TE (enough to bring reaction volume to 8 uL)
  2 uL BP Reaction Buffer (5X)
  \( y \) uL purified PCR product \(^1\)
  1 uL pDONR 201 (150 ng/uL) \(^2\)
  2 uL BP Clonase Mix \(^3\)

- Gently tap the reaction tube several times to mix
- Pulse spin the reaction to the bottom of the 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 purified PCR product. The equation for converting fmol to mass is as follows:

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

2= 150 ng of pDONR 201 is 50 fmol of the plasmid

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