In-Fusion Cloning

RECOMMENDED IN-FUSION REACTIONS FOR PURIFIED FRAGMENTS			
Reaction Component	**Cloning Reaction**	**Negative Control Reaction**	**Positive Control Reaction**
Purified PCR Fragments	10-200 ng*	-	2 ul of 2 kb control insert
Linearized Vector	50-200 ng**	1 ul	1 ul of pUC19 control vector
5x In-Fusion HD Enzyme Premix	2 ul	2 ul	2 ul
Deionized water	To 10 ul	To 10 ul	To 10 ul
* <0.5 kb: 10-50 ng; 0.5 to 10 kb: 50-100 ng; >10 kb: 50-200 ng ** <10 kb: 50-100 ng; >10 kb: 50-200 ng			

1. **Set up the In-Fusion cloning reaction:**

5x In-Fusion HD Enzyme Premix	2 ul
Linearized Vector	x ul*
Purified PCR Fragment	x ul*
dH_2O (as needed)	x ul
Total Volume	10 ul

** For reactions with larger volumes of vector and PCR insert (> 7ul of vector + insert), double the amount of enzyme premix, and add dH2O for a total volume of 20ul.*

2. Adjust the total reaction volume to 10 ul using deionized H_2O and mix the reaction.
3. Incubate the reaction for **15 minutes at 50˚C**, then place on ice.
4. Continue to the transformation procedure. You can store the cloning reactions at -20˚C until you are ready. You can use 2-5ul of the reaction to transform.