Ligation Reactions

1. Cut both vector and PCR ligation products with EcoRI and PstI restriction enzymes for 1 hour
2. Heat kill samples at 80°C for 20 minutes
3. Determine the concentration of insertion and vector samples
4. Determine volumes needed for both vector and insert while accounting for difference in base pair numbers and the 3:1 ratio of insert to vector.
5. Make 10µL using the above volumes (8.5µL) and then add 1µL of ligase buffer and 0.5µL of T4 DNA ligase.
6. Allow samples to incubate overnight at room temperature
7. Heat kill samples at 80°C for 20 minutes