

Ligation

Need:

Vector

Insert

Ligase (T4)

Ligase buffer 10X (T4)

- 1) Calculate the proportion of vector to insert needed from gel purification (usually will have at least 5 times greater amount of vector)
- 2) For ligation use a minimum total volume of 20 microliters, including:
 - X insert
 - Y vector
 - 1 microliter ligase
 - Z 1X ligase buffer
- 3) Negative control
 - X water
 - Y insert
 - 1 microliter ligase
 - Z 1X ligase buffer
- 4) Incubate ligation and negative control at 16 degrees for at least two hours (or overnight)