

Ligation Protocol

Formula to calculate the ligation ratio of vector/insert:

$$\frac{bp\ insert \times vector\ (ng)}{bp\ vector \times insert\ (ng)} = ratio$$

The ratio should be between 1:3 and 1:5 (molar) vector-to-insert.

The total amount of DNA, both insert and vector, should be 20-100ng.

Buffer 10x	2 μ L
Ligase	1 μ L
Insert	As calculated
Vector	As calculated
DDW	Complete to total volume of 20 μ L
Total Volume	20 μ L

Mix solution for 20min-1hr at 22°C, then H.I. for 5min 70°C .