Cloning: Gibson Assembly

Preparation of Gibson Assembly Master Mix

1. Set up the Gibson Assembly Master Mix as follows

Table 1: Representation of the composition of the Gibson Assembly Master Mix

Component	Concentration	Amount
5x ISO buffer	5x	100 µL
Taq DNA Ligase	40 U/µL	50 µL
T5 Exonuclease	1 U/µL	2 µL
Q5 Hi-Fi DNA Polymerase	2 U/µL	6.25 µL
Nuclease-free water		216.75 µL
Total volume		375 µL

2. Prepare 15 µL aliquots in PCR tubes. Store at -20 °C.

Gibson Assembly

1. Generate dsDNA with 20 to 40 bp overlapping ends by PCR
2. Digest the vector PCR product with DpnI for 1 hour at 37 °C to digest unamplified vector
3. Gel extraction of amplified vector with overlapping ends using the Zymoclean™ Gel DNA Recovery Kit
4. Set up the following reaction on ice

Table 2: Representation of the composition of a Gibson Assembly reaction

Component	20 µL reaction
DNA	50-100 ng for vector with 2-3-fold excess insert
Gibson Assembly Master Mix (1,3x)	15 µL
Nuclease-free water	To 20 µL

5. Incubate samples at 50°C for 60 minutes.
6. Transform 1-5 µL of the reaction into 50 µL competent cells.