Restriction protocol:

General steps:
Extaction from distribution kit → Miniprep→ Digestion → cleaning from gel → ligation → Chemical transformation.

Goal- Digest with restriction enzymes both vectors prior to ligation step.

Materials:

- **Restriction Enzymes:_____**
- **NEB buffer:_____**
- **Molecular biology water**

Procedure:
Set up the following reaction a described below:

Component	Volume
Buffer (X10)	4µl
DNA up to 4µg	Xµl
enzyme1 -HF	1µl
enzyme 2 -HF	1µl
MB water	34-X µl
Total volume	40µl

Ligation:

Goal- ligate vector and insert for creation of a circular cloned plasmid.

Materials:

T4 DNA ligase

10X T4 DNA ligase buffer

Purified, linearized vector

Purified, linearized insert

Molecular Biology Water

Procedure

1. set up following reaction in microcentrifuge tubes.
2.Your negative control is the vector with no insert. Prepare a mix with all the ingredients

below but the insert. Divide mix, and add insert to your reaction tube.

Component	Volume
T4 DNA ligase	1
10X T4 DNA ligase buffer	2
Purified, linearized vector (25-50 ng)	
Purified, linearized insert (1:3 ration*)	
Molecular Biology Water	up to 17µl
Total	20

3.Gently mix the reaction by pipetting up and down and spin down briefly.
4.Incubate for 1 hr. At 30°C in a dry bath or 16 °C overnight.
5.stor samples on ice for subsequent transformation or store at –20 °C

***How to calculate a molar ration of 1:3**

(insert amount [ng])X(plasmid length)=(plasmid amount [ng])X (insert length)

(insert amount [ng])={(plasmid amount [ng])X(insert length)X3}/(plasmid length)