Restriction Digest and Ligation

The restriction digest and ligation protocol is used to transfer DNA fragments from one plasmid to another, as long as the DNA pieces have matching restriction sites. The restriction enzymes digest the DNA at the corresponding restriction sites, which results in complementary ends of the target plasmid and the insert. The ligase finally adds together target plasmid and insert.

For this protocol the materials of NEB were used.

Restriction Digest

Materials

- Restriction Endonucleases (NEB)
- Restriction Digest Buffer (e.g. CutSmart™ Buffer)
- Plasmid DNA

Procedure

- Prepare a 25 ul reaction:

Component	**Amount**
Restriction Enonuclease	1 ul for each used enzyme
CutSmart™ Buffer	3 ul
Nuclease-free water	Up to 25 ul

- Incubation for 1h at 37°C (or at different temperature, dependent on enzyme)
- Heat inactivate restriction enzymes at 65°C to 80°C (dependent on restriction enzyme) for 20 min

Ligation

Materials

- T4 DNA Ligase Buffer (10x)
- T4 DNA Ligase
- Vector DNA
- Insert DNA
- Nuclease-free water

Procedure

For the protocol it is indispensable to know the sizes of the target vector and the insert. Normally a molar ratio of vector to insert of 1:3 is used for cohesive end ligations. A higher molar ratio of 1:4 or 1:5 can be used for ligations of DNA fragements with blunt ends

- Prepare a 20 ul reaction (T4 DNA Ligase should be added last)

Component	**Amount**
T4 DNA Ligase Buffer (10x)	2 ul
Vector DNA	Dependent on vector size, e.g. 50 ng
Insert DNA	Dependent on insert size
Nuclease free water	To 20 ul
T4 DNA Ligase	1 ul

- Gently mix by pipetting up and down
- Incubate at 16 °C overnight or for 1h at room temperature (for blunt ends: overnight at 16 °C or 2h at room temperature)
- Heat inactivate Ligase at 65°C for 20 min
- Chill on ice and possibly transform 1ul of the reaction to 50ul competent cells

References

NEB Protocol Restriction Digest:
https://international.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions

NEB Protocol Ligation with T4 DNA Ligase (M0202):
https://international.neb.com/Protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202