Technic : Double Digestion from 0,2 µg to 1,5 µg of DNA

Goals: Obtain a ready-to-ligate vector or insert, recover a gene of interest

Procedure :

1. Prepare reaction on ice for one sample (20 µL total)
2. Add 2 µL 10X restriction buffer (as appropriate for the enzymes)
3. Add 0.5 µL BSA (if required)
4. Add DNA from 0,2 to 1,5µg
5. Add 0.5 µL of each enzyme
6. Add nuclease-free water to complete the 20µL total
7. Gently mix by pipetting and keep on ice.
8. Incubate at 37°C for 1 hour.
9. Immediately inactivate enzymes by heating to 80°C for 20 min
10. Place the digestion at 4°C or on ice.
11. If needed, purify digested DNA (PCR cleanup or gel-extraction) before downstream use. If running on gel, prepare loading mixes as below.

Reagents and Solutions :

- Nuclease-free water
- Appropriate 10X restriction buffer (enzyme supplier)
- BSA (if required by enzyme)
- Restriction enzymes
- DNA template (plasmid or PCR product)

Equipment :

- Ice and ice bucket
- Heat block / dry bath or thermocycler
- Microcentrifuge tubes, pipettes/tips

Estimated time :
~2 hours

Trouble shooting :

- Be careful with the capacity of the enzymes you use, as they can only handle a certain amount of DNA: if you overload them, the digestion will not be efficient. You can find information on the maximum DNA quantity on the supplier kits.