DIGESTION

Aim: To linearize the different plasmids or create compatible ends on inserts, with appropriate enzymes.

We perform restriction enzyme digestion in order to recover linear backbones of the plasmids, and corresponding compatible ends. We choose appropriate restriction sites on the inserts based on the host plasmids. However, initial steps for creating the sites in the DNA are also required. Most of our DNA comes either from PCR production, minipreps or midipreps, or following gel extractions.

Materials:

- Molecular biology equipment: 37 °C and 65 °C water baths, pipette set, ice bucket, electrophoresis tank and power supply, imaging system
- DNA
- 10X New England Biolabs (NEB) buffer
- Nuclease-free water
- Appropriate restriction enzyme at -20 °C

Protocol:

1. Design primers with appropriate restriction sites to clone unidirectionally into a vector
2. Addition of 6 bases upstream of the restriction site is sufficient for digestion with most enzymes
3. If fidelity is a concern, choose a proofreading polymerase such as Q5 High-Fidelity DNA Polymerase (NEB #M0491) or Phusion (NEB#M0530)
4. Visit www.NEBPCRPolymerases.com for additional guidelines for PCR optimization
5. Purify PCR product by running the DNA on an agarose gel and excising the band, then followed by using a Gel extraction kit
6. Digest with the appropriate restriction enzyme

Standard Restriction Enzyme Protocol

- Restriction Enzyme 10 units is sufficient, generally 1 µl is used for 1 µg DNA.
- 10X NEBuffer 5 µl
- Nuclease-free Water to 50 µl
- Incubation time1 hour*

Incubation Temperature Enzyme dependent

* Can be decreased by using a Time-Saver Qualified enzyme.