Restriction Digests

Use 50µl reactions for plasmid digestion. Use the smaller reaction for diagnostic purposes.

- 5µl buffer #X (check with NEB)
- 0.5µl BSA (if you need it)
- 35-44µl PCR product
- water up to 50µl
- 1µl enzyme
= 50µl

- 2.5µl buffer #X (check with NEB)
- 0.25µl BSA (if you need it)
- 17-22µl PCR product
- water up to 25µl
- 0.5µl enzyme
= 25µl

1. Check for specific temperatures and times for optimal digestion of your enzyme (neb.com).
2. Vortex, spin down
3. 37 °C incubator, 30 to 60 min.
4. Heat inactivate.

TIPS:

- Always keep enzyme in -20 °C bucket.
- When doing multiple RDs: make a Master Mix (everything except DNA), vortex, divide into your reactions, and finally add DNA.