Restriction digestion

Aim

Digestion of a DNA fragment of interest for further experiments.

Procedure

Initial notes: All reaction components should be assembled on ice.
DNA should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.
Use buffer at a 1X concentration.

Thaw 10X CutSmart Buffer, and DNA sample on ice. Keep enzyme in freezer and add it as last component.

Set up the reaction as follows:

COMPONENT	50 µl REACTION
10X CutSmart Buffer	5 µl (1X)
DNA	1 µg
Restriction enzyme 1	1.0 µl (or 10 units)
Restriction enzyme 2	1.0 µl (or 10 units)
RNase-free water	To 50 µl

Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

Incubate at 37°C for 1 hour.

Heat-inactivate if enzymes allow for it. *

*For enzyme-specific incubation and heat-deactivation temperatures, please use the **NEB Cloner** feature provided by NEB®.

If heat-deactivation is not possible, the enzyme can be removed by spin column purification or phenol/chloroform extraction.

Sources

This protocol is a modified version of the original Double Digest Protocol with Standard Restriction Enzymes provided by NEB®.