DNA digestion

Aim

To digest DNA using restriction enzymes.

Equipment

- Restriction enzymes stored at –20°C (NEB : New England Biolabs)
- Plasmid to digest stored at –20°C
- 10X NEB buffer CutSmart stored at –20°C
- De-ionized water
- Water-bath at 37°C
- Heater block for deactivation at 65°C
- Timer
- Pipette p10, p20, p200 & associated cones
- Gel loading dye 6X

Name of DNA to be digested:

-
-

Restriction enzymes:

-
-

DNA digestion

.....

Protocol

DNA plasmid to digest concentration (Cross multiplication helper):

……ng	1µl
1000 ng	……µl
500 ng	……µl
50 ng	……µl

For …...ng of DNA sequence to digest: volume = ……µl

Volume of restriction enzyme needed:

Quantity of DNA to digest	Volume of restriction enzyme
1 µg	1 µl
…… µg	…… µl

Mix for a total volume of 50 µl :

DNA:……., …….. ng	……µl
Restriction enzyme:……	……µl
Restriction enzyme:……	……µl
10X NEB buffer	……µl
Sterile water	…… µl
Total reaction volume	50 µl

Mix for a total volume of 50 µl of negative control :

DNA:……., 50 ng	…… µl
10X NEB buffer	…… µl
Sterile water	…… µl
Total reaction volume	50 µl

DNA digestion

1. Mix gently by pipetting up and down 4-6 times.
2. Microcentrifuge briefly 3 s.
3. Incubate at 37°C for 15 min.
4. Stop reaction by heat inactivation: incubate at 65°C for 20 min. This step is only for specific restriction enzymes (XbaI).
5. Stop reaction by adding 10 µl of 6X gel loading dye to the 50 µl reaction.
6. Load digested DNA on an agarose gel. (see Gel Electrophoresis Protocol)