SOP: SP017

Restriction Endonuclease Digestion of DNA

Materials and Reagents:

1. DNA to be digested
2. 10X restriction endonuclease buffer (note 3)
3. Sterile MilliQ water
4. Restriction endonuclease
5. 100X BSA, if required
6. Microcentrifuge
7. Ice, ice bucket
8. Sterile 0.65 ml microcentrifuge vials
9. Pipetman (or equivalent) pipettors, 10 −200 µl
10. Pipet tips, 10 −200 µl, sterile
11. 37°C incubator, thermal cycler, or water bath (note 3)
12. 65°C heat block, thermal cycler, or water bath (note 3)

Protocol:

1. _____ Add approximately 1 µg of DNA to a sterile 0.65 ml eppendorf tube. (note 1)

2. _____ Dilute DNA with sterile MilliQ water to a volume of 44 µl, or 43.5 µl if the restriction endonuclease requires BSA. (note 2)

3. _____ Thaw restriction endonuclease buffer (see note 3)

4. _____ Add 5 µl of the 10X restriction endonuclease buffer to the DNA solution.

5. _____ If required, add 0.5 µl of 100X BSA (see note 4)

6. _____ Add 1 µl restriction endonuclease (10-20 units/µl stock) to the reaction mixture (notes 5 − 7), and mix by tapping the tube.

7. _____ Briefly spin reaction mixture in a microcentrifuge to ensure all contents are at the bottom of the tube.

8. _____ Incubate at 37°C for 1-16 hr. (note 8 and 9).

9. _____ Terminate the enzyme reaction by heating at 65°C for 10 min if enzyme is heat sensitive.

10. _____ DNA is ready for downstream application, such as visualization on agarose gel (SOP SPO018).

QC:

1. Run a subset (eg 5 µl) of the digested material on an agarose gel (SOP SP018 agarose gel electrophoresis) along with an equal aliquot of undigested DNA.
2. Run no more than 0.1-0.2 µg per well for single cut digests. If digestion liberates a drop-fragment, run 0.3-0.4 µg.

Notes:

1. This protocol details digestion of 1 to 3 µg of DNA. Smaller reaction volumes can be used by adjusting the volumes of reagents accordingly. Larger quantities of DNA can be digested by increasing the volumes of reagents. In general, DNA will digest well at 0.02-0.08 µg/µl (1 µg in 12-50 µl).
2. Before setting up the reaction, measure DNA concentration and calculate the volume of DNA and water to be added to the 50 µl digest.

3. Each restriction endonuclease requires specific conditions, (digestion temperature, buffer, cofactors such as BSA, deactivation at high temperature). Consult the enzyme product sheet. Note that suppliers provide tables of buffers and conditions to be used with specific enzymes in catalogs and on web sites.
4. BSA will not adversely affect a reaction that does not require it.
5. Good lab practice: Wear latex or nitrile gloves when handling enzymes to prevent contamination by handler. Restriction endonucleases are extremely heat labile and should be stored at –20°C and kept on ice outside the freezer.
6. Restriction endonucleases are suspended in a solution containing 50% glycerol. Since high concentrations of glycerol will interfere with restriction endonuclease activity and specificity, it is important that enzyme volume not exceed 10% of the total reaction volume. If two enzymes are added, their combined volume should not exceed 10% total volume.
7. More than one restriction endonuclease can be added simultaneously in a buffer compatible with both enzymes. Supplier catalogs and web sites list compatible enzymes and buffers. For many enzyme pairs, complete digestion can be best done by addition of one enzyme for several hours, followed by addition of the second enzyme.
8. Although 1 hr/1 ug DNA is typically listed for complete digestion in product sheets, many users find 1 hr to be inadequate. Success varies for different DNA preparations and enzymes. Digestion for 4 hr to overnight typically works well.
9. Reactions that incubate > 2-3 hr should be performed in an incubator or completely submerged in a water bath to ensure that condensate does not form on the cap of the reaction tube. Condensate indicates that the DNA is incubating in increased salt and enzyme concentrations. Submersible holders are available.

Reference:

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual (2nd Edition).