

Establishment of conditions for restriction endonuclease partial digestion of genomic DNA

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Equipment and reagents

- ◆ Restriction endonuclease (enzyme) plus appropriate buffer
- ◆ 200 mM EDTA, pH 8.0
- ◆ Water-bath or heating-block
- ◆ Gel electrophoresis apparatus

Method

- 1 Prepare a 100 μ l reaction mixture (see [Digestion of DNA by restriction endonuclease treatment](#)) optimal for the specific restriction enzyme and containing 10 μ g of genomic DNA.
- 2 Dispense 20 μ l of the mixture into microfuge tube 1 and 10 μ l into the remaining tubes 2–9. Place all tubes on ice.
- 3 Add 1 μ l (equating to a known amount of enzyme units, e.g. 1-10) of restriction endonuclease to tube 1, mix gently and place on ice.
- 4 With a fresh tip, pipette 10 μ l from tube 1 into tube 2, mix gently and replace on ice. Perform similar serial dilutions throughout the assay tubes, thereby diluting the enzyme by 50% between each tube, keeping the enzyme-DNA mixture on ice.
- 5 Incubate at 37 °C for 1 h before placing the tubes on ice.
- 6 Add EDTA, pH 8.0, to a final concentration of 20 mM to inactivate the enzyme.
- 7 Analyse each sample by gel electrophoresis alongside appropriate DNA length markers. By comparison with the DNA length markers, analyse the DNA digestion pattern in each sample to determine which quantity of enzyme produced the optimum amount of DNA fragments of the desired length.

Ethidium bromide is commonly used to stain agarose gels in order to visualise nucleic acids. It is also a carcinogen. Handle all ethidium bromide containing solutions and gels with care always using laboratory gloves. Specific waste procedures may be required for disposal of ethidium bromide containing waste.

Notes

The addition of spermidine can enhance restriction endonuclease digestion of impure DNA. Add 0.1 M spermidine to a final concentration of 4 mM and incubate the DNA mixture at 37 °C for a few minutes (spermidine may precipitate long DNA). Subsequently, add the restriction enzyme after ensuring that no DNA precipitation has occurred.