Preparation and purification of λgtWES vector for construction of genomic DNA libraries

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Equipment and reagents

- λgtWES DNA. This type of λ vector requires selective removal of the central "stuffer" DNA fragment before use in ligations to target DNA in order to construct genomic DNA libraries.
- *Eco*RI restriction endonuclease (enzyme) plus appropriate buffer
- 10–40 (w/v) sucrose gradient prepared in a suitable ultracentrifuge tube (see Large-scale preparation and purification of restriction endonuclease partial digested genomic DNA)
- Ultracentrifuge
- Fraction collector (optional)

Method

1. Carry out a complete *Eco*RI digestion of 50–100 μg λgtWES DNA (see Digestion of DNA by restriction endonuclease treatment) resulting in the appearance of a λ stuffer DNA fragment and two λ arm DNA fragments.
2. Phenol extract and ethanol precipitate the DNA (see Purification of DNA by phenol extraction and ethanol precipitation).
3. Add $MgCl_2$ to a final concentration of 10 mM to the resuspended DNA and incubate at 42 °C for 1 h. This promotes annealing of the complementary λ *cos* sites of the two λ arms generated by the restriction endonuclease digestion, and will aid the separation of the λ stuffer DNA fragment from the now enlarged λ arms DNA fragment.
4. Gently add the digested DNA onto a 10–40% (w/v) sucrose gradient.
5. Centrifuge at 120 000 rpm for 24 h at 15 °C using a swing-out ultracentrifuge rotor.
6. Collect 0.5–1.0 ml fractions carefully and analyse a sample (e.g. 20 μl) from each fraction by gel electrophoresis to determine the appropriate fractions which contain purified λ arms with no contaminating λ stuffer fragments.

7 Dialyse the appropriate fractions against sterile water overnight to remove the sucrose.

8 Concentrate the λ arms DNA by ethanol precipitation (see Purification of DNA by phenol extraction and ethanol precipitation).

9 An alternative method is to substitute NaCl gradients for the sucrose gradient:

(a) Prepare a 20% (w/v) NaCl solution in 50 mM Tris-HCl, pH 8.0, 1 mM EDTA.

(b) Freeze and thaw once at –70 °C (–20 °C is insufficient to form a correct NaCl gradient).

(c) Apply the sample and centrifuge at 270 000 rpm for 3 h at 15 °C using an ultracentrifuge.

(d) Purify the selected fractions from the gradient by dialysis against sterile water overnight at 4 °C.

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.

Phenol is a hazardous organic solvent. Always use suitable laboratory gloves when handling phenol containing solutions. Specific waste procedures may be required for the disposal of phenol containing solutions.