Preparation and purification of λEMBL3 vector for construction of genomic DNA libraries

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Equipment and reagents

- λEMBL3 DNA. This type of vector does not require selective removal of the central "stuffer" DNA fragment before the cloning ligations. Instead, by using two restriction endonucleases, it ensures that the stuffer DNA fragment cannot become involved in the cloning ligation as it will have incompatible termini when compared to the λ arm DNA fragments.
- *Bam*HI and *Eco*RI restriction endonucleases (enzymes) plus appropriate buffers
- 3 M sodium acetate, pH 5.5 (see Purification of DNA by phenol extraction and ethanol precipitation)
- TE, pH 7.4 (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, pH 8.0)
- Water-bath or heating-block
- Gel electrophoresis apparatus

Method

1. Carry out a complete *Bam*HI digestion of 50–100 μg of λEMBL3 DNA (see Digestion of DNA by restriction endonuclease treatment).
2. Phenol extract and ethanol precipitate the digested λ DNA (see Purification of DNA by phenol extraction and ethanol precipitation).
3. Resuspend the *Bam*HI-digested DNA and prepare an *Eco*RI reaction solution (see Digestion of DNA by restriction endonuclease treatment).
4. Phenol extract the *Bam*HI-*Eco*RI digested DNA (see Purification of DNA by phenol extraction and ethanol precipitation).
5. To selectively remove the short *Bam*HI-*Eco*RI polylinker that once joined the λ stuffer and arms DNA fragments, precipitate the double-digested DNA with a 0.1 volume of 3 M sodium acetate pH 5.5 and 1 volume of isopropanol. The precipitation of short DNA fragments such as the polylinker is relatively inefficient when compared to the longer λ arm DNA fragments.

6 Incubate the precipitation reaction for 15 min at –20 °C and centrifuge at 8 000 rpm in the microfuge for 10 min to collect the precipitated DNA.

7 Resuspend the λ DNA at a concentration of 0.2–0.5 μg/μl in sterile TE pH 7.4 or water. This DNA will be composed of λ arm DNA fragments (each with a *Bam*HI and *cos* terminus) and λ stuffer DNA fragments (each with *Eco*RI termini) which are incompatible with each other in terms of ligation.

8 Store at 4 °C or at –20 °C for the long term.

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.