

# Isolation of linearized BAC DNA for microinjection to produce transgenic animals

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## Equipment and reagents

- ◆ Plasmid purification kit appropriate for the isolation of large DNA constructs (e.g. KB-100 Magnum from Genome Systems Inc.).
- ◆ Sepharose matrix CL4b (Pharmacia)
- ◆ 0.25% Bromophenol Blue
- ◆ 5 ml disposable plastic pipette with cotton-wool plug
- ◆ Injection buffer: 10 mM Tris-HCl pH 7.4, 0.1 mM EDTA, 100 mM NaCl

## Method

- 1 To obtain a large quantity of BAC DNA the construct is amplified in *E.coli* similar to normal plasmid DNA and prepared using a plasmid purification kit suitable for the isolation of large DNA constructs (e.g. KB-100 Magnum, Genome Systems Inc.). BAC DNA is circular and should be linearised before injecting.
- 2 Digest about 50 µg of high-quality BAC (bacterial artificial chromosome) DNA prepared by standard methods with the restriction enzyme *NotI*, or another suitable enzyme.
- 3 To prepare a Sepharose column take a 5 ml plastic pipette and block it by blowing the cotton-wool plug into the tip of the pipette. Clamp it onto a stand and fill it gradually with Sepharose CL4b (Pharmacia) up to 1 cm below the top of the pipette.
- 4 Equilibrate the column with 30 ml of injection buffer. You can make a reservoir for the buffer by attaching a 10 ml syringe to the top of the pipette.

**Note:** Never let the column run dry!

- 5 Add 5 µl of 0.25% Bromophenol Blue to the digested BAC DNA and load the sample onto the column.

- 6 Wait until the DNA has just entered the column, then add 0.5 ml of injection buffer.
- 7 Reattach the syringe and load with 10 ml of injection buffer.
- 8 Collect 0.5 ml aliquots until the blue dye has reached the bottom of the pipette.
- 9 There will always be a slight contamination with vector DNA. To identify the fraction containing the highest concentration of high-quality insert DNA with the lowest amount of vector, load 50  $\mu$ l of each sample onto an agarose gel and run overnight under optimized conditions.
- 10 Check the DNA concentration ([Testing the exact concentration of DNA constructs used for microinjection to produce transgenic animals](#)) and, if necessary, dilute to a final concentration of 2 ng/ $\mu$ l with injection buffer.