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Microinjection DNA Purification

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We use this protocol and it's working

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Abstract

We have found the NucleoSpin Gel and PCR Clean-up Kit (available from Takara.com, catalog no. 740609.50) is a simple and fast way to obtain microinjection quality DNA. This is not to exclude other methods of DNA purification but to say that we find this is a convenient method that is less onerous than CsCl gradients.

- 1 Perform restriction digest to liberate transgene from plasmid vector sequences. Final yield should be 10-20 micrograms of transgene insert.
- 2 Separate restriction digest products with an agarose gel using either TBE or TAE. Use either low- or standard-melting temperature agarose.
- 3 Place gel on transilluminator. Cut out band(s) of interest. Use a clean razor blade or scalpel. Remove as much excess agarose as possible. Minimize DNA exposure to UV light to prevent photochemical damage (less than 1 minute).
- 4 Transfer agarose slice(s) to a preweighed, sterile, 15 ml tube. Reweigh tube to determine weight of agarose in tube.
- 5 For each 1 g of gel, add 3 ml buffer NT1. If agarose concentration is greater than 1%, add proportionately more buffer. For example, if a 2% agarose gel is used, add 6 ml buffer NT1 for each 1 g of gel.
- 6 Dissolve at 50 degrees Centigrade for 10 minutes, vigorously vortexing every 2 to 3 minutes. Check pH of dissolved agarose. If the pH is above 7.5 the DNA will not bind to the cartridge. If necessary, add 10 µl of 3M NaAcetate, pH 5.2 for every ml of solution to lower pH.
- 7 Place one or two NucleoSpin cartridges in a 2 ml micro tube and load 750 microliters dissolved gel slice onto the cartridge. Spin at maximum speed for 60 seconds in a microcentrifuge. Discard the flowthrough. The cartridge has a capacity of 2 micrograms DNA, so you can run several 750 microliters loads of dissolved gel slice through a single cartridge.
- 8 Add 750 microliters of buffer NT3 to the cartridge and spin at maximum speed for 60 seconds in a microcentrifuge. Discard the flow-through.
- 9 Replace tube with a fresh micro-tube. Spin the empty cartridge at maximum speed for 60 seconds in a microcentrifuge to completely remove buffer NT3.
- 10 Elute the DNA from the cartridge: Replace tube with a fresh micro-tube. Add 50 microliters of preheated (60 degrees Centigrade) elution buffer to cartridge. Spin at maximum speed for 60 seconds in a microcentrifuge.
- 11 If desired, repeat step 10 to increase yield. We obtain 90% of the DNA in the first elution.
- 12 Quantitate DNA solution.



- 13 Verify size and intact condition of DNA on minigel.

- 14 Store eluted DNA at -20 degrees C.

- 15 Notes. The presence of prokaryotic plasmid vector sequences will interfere with transgene expression (Chada et al. 1986, Hammer et al. 1987, Townes et al. 1985).

- 15.1 Chada K, Magram J, Raphael K, Radice G, Lacy E, Costantini F. 1985. Specific expression of a foreign beta-globin gene in erythroid cells of transgenic mice. *Nature*. 314:377-80.

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