

DNA Preparation for Microinjection

Qiagen QIAquick Gel Extraction Kit
#28704

The following protocol includes modifications made to the kit supplied protocol.

1. Digest 5 ug of plasmid DNA with appropriate enzyme to excise desired DNA fragment from vector.
2. Gel extract the desired band from an agarose gel, using a scapel.
 - Do not expose DNA to UV light or ethidium bromide
 - Minimize the size of the gel slice by removing extra agarose
3. Weigh the gel slice and add 3 volumes of Buffer QC to 1 volume of gel (100mg = 100ul)
4. Incubate at 50° C for 10 min. Mix by vortexing the tube every 2 minutes during incubation.
5. Add 1 gel volume of isopropanol to the sample and mix.
6. Place a QIAquick spin column in a provided 2ml collection tube.
7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.
 - Maximum volume of the column is 700 ul. For samples over 700 ul, load and spin again
8. Discard flow-through and place QIAquick column back in the same collection tube.
9. Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.
10. To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min. Repeat this step, for 2x total.
11. Discard the flow-through and centrifuge the column for an additional 1 min.
12. Place column in a clean 1.5 ml microcentrifuge tube.
13. Elute DNA with TE (10 mM TrisCl, 0.1 mM EDTA, pH 8.0). Add 30 ul TE to center of the column and let sit for 1 min. Centrifuge for 1 min.

14. Remove column from tube and spin down collected DNA for 10 min and remove upper ~25 ul. **KEEP THIS 25ul.** This contains your purified DNA for microinjection. The ~5 ul left in the bottom of the tube contains any debris that might clog the injection needle.

15. Load an aliquot of DNA onto an agarose gel and run against known standards to determine its concentration and quality.

16. Adjust the final volume of DNA to 6-10 ng/ul.