

Preparation of DNA for microinjection

Below you will find 4 different protocols for preparing linear dsDNA for microinjection.

Protocol #1 (Jiandong Chen 4-18-91)

If DNA is to be used for microinjection, the pellet obtained from the LMP (Low melting point agarose) protocol was resuspended in 50 μ l TE and passed through a Sephadex G-50 spun column to remove residual agarose.

The flowthrough was precipitated by adding NaCl to 0.1M and 2.5 vol ethanol.

The pellet was washed, dried and resuspended in 100 μ l of injection buffer(5mM Tris-HCl pH7.4, 5mM NaCl, 0.1mM EDTA).

Dilute 5 μ l to 50 μ l and measure OD260 using micro cuvette. Usually OD260/OD280 did not exceed 1.5. Usually ~3-6 μ g of fragment were recovered starting from 40 μ g plasmid with the insert about the same size of the vector.

Prepare 200 μ l of DNA for microinjection by diluting the concentrated solution to 4ng/ μ l using injection buffer. Keep the concentrated solution(~40ng/ μ l) for making probe for screening later.

Dialyse the diluted DNA against injection buffer in a petri dish using Millipore 0.05 μ m filter(VMWP 013 00). Float filter bright side up on the injection buffer, carefully add 100-150 μ l solution at the center of the filter, cover dish and dialyse for 1hr at RT.

Remove solution on the filter and spin at top speed in microfuge for 2min, carefully recover most of the aqueous phase, leaving behind insoluble particles. Repeat spinning twice to complete remove small particles that may clog injection pipette.

Check concentration of DNA by running 1-2 μ l on agarose gel against standard concentration controls. Store DNA in 50 μ l aliquots at -200C for microinjection.

Protocol #2 Preparation of fragments for microinjection (long)

Digest 40-50 μ g plasmid with restriction enzymes in 300 μ l volume with 2-3U enzyme/ μ g DNA for 2-3hr. Check for complete digestion. Run digested DNA on 0.8-1% low melting point(LMT) agarose

gel with TA or TBE buffer. LMP agarose has lower resolution and 40µg DNA will overload a ~15cm wide gel and result in poor separation of fragments of similar molecular weight, therefore, the unwanted fragments of similar size were usually cut to smaller pieces using restriction enzymes to improve separation. Gel is run without EtBr when overloaded and stained after electrophoresis. Desired band was cut out under UV light and excess agarose were removed as much as possible.

Estimate the volume of the excised band by its weight. Add 1/100 vol 1M Tris-HCl pH 7.5, 1/50 vol 0.5M EDTA and 1/10 vol 5M NaCl (for 1ml add 10µl Tris, 20µl EDTA, 100µl NaCl). Incubate 10min at 680C, vortex and incubate 5min at 370C. Extract twice with 2 vol phenol prewarmed to 370C. Spin at top speed at microfuge for 2min to obtain tight interphase. Extract once with ether saturated with H₂O. Heat at 680C for 5min, estimate volume, run in speed vac for 2min, remove tube and warm to 680C before returning to speed vac. Repeat several times. Add H₂O to original volume.

Hydrate NAC column with 3ml 2M NaCl, 10mM Tris pH7.0, 1mM EDTA (elution buffer). Equilibrate with 3ml of 0.5M NaCl, 10mM Tris pH7.0, 1mM EDTA (loading buffer). Pass DNA solution through column slowly. Wash with 3ml of loading buffer, elute bound DNA with 0.6ml elution buffer. The equilibration and washing steps can be done quickly using additional pressure. DNA binding and elution should be done slowly, no faster than one drop every 10 seconds. Precipitate DNA by adding 2.5 vol ethanol to eluate and store for 30min at -700C. Spin ethanol precipitate at 10K, 40C for 10min, wash pellet with 70% ethanol, dry under vacuum and resuspend in appropriate volume of TE. Sometimes, a large white pellet was recovered containing residual agarose. The agarose can be removed by passing through Sephadex G-50 filtration column.

Protocol #3 Preparation of fragments for Microinjection (short)

1. Digest 40 - 50µg of plasmid with restriction enzymes in 300µl of volume with 2 - 3 U enzyme/µg of DNA for 2 - 3 hr. Check for complete digestion.
2. Run the digested DNA on 0.8% - 1% low melting point (LMP) agarose gel with TA or TBE buffer. NOTE: LMP agarose has lower resolution and 40µg DNA will overload a ~15cm wide gel and result in separation of fragments of similar molecular weight, therefore the unwanted fragments of similar size were usually cut to smaller pieces.
3. Run gel without ethidium bromide and stain after electrophoresis.
4. Cut out desired band under long - wave UV light and remove as much excess agarose as possible.
5. Estimate the volume of the excised band by its weight, and add 1/100 volume of 1M Tris-HCl pH 7.5, 1/50 volume of 0.5M EDTA, and 1/10 volume of 5M NaCl (for 1ml add 10µl Tris, 20µl EDTA, 100µl NaCl).

6. Incubate gel slice at 68°C for 10 minutes, vortex, and incubate for 5 minute at 37°C.
 7. Extract twice with 2 volumes of phenol prewarmed to 37°C, then spin at top speed in jthe microfuge for 2 minutes to obtian a tight interphase.
 8. Extract once with H2O saturated ether. Heat to 68°C fir 5 minutes, estimate the volume in the tubeß
-

Protocol #4. Len's fragment Isolation Protocol for microinjection

1. Digest 40-50ug of plasmid with restriction enzymes until completely digested. Concentration of plasmid should be 0.2 to 0.3 ug/ul.
2. Run the digested DNA on 1% agarose gel until desired separation of fragment of interest from vector is achieved. Do Not run the gel with ethidium bromide in the gel!
3. Stain gel briefly in ethidium bromide. Do not overstain.
4. Excise desired band under long-wave UV light.
5. Transfer excised agarose slice with band into pre-poured 0.8% low melting point (LMP) agarose gel. Run gel until band entirely is within the LMP agarose. Keep gel in the dark to avoid DNA mutations while ethidium is present.
6. Excise band from LMP agarose gel under long-wave length UV light. You should not have to stain the gel again to visualize the band. Remove as much agarose as possible.
7. Weigh the gel slice in a tared tube.
8. Add 1ul 50x GELase Buffer per 50mg of gel.
9. Melt the gel slice completely at 65 degrees C (allow at least 20 min. In plastic tubes and 10 min. in glass tubes).
10. Equilibrate molten agarose to 45 degrees C (allow at least 10 min. In plastic tubes and 5 min. in glass tubes).
11. Add one unit of GELase per 300mg of gel (Note: 1U=1ul). This must be in TAE or MOPS buffer. See GELase protocol book for other buffers.
12. Incubate for at least 1 hour at 45 degrees C.

13. Add 2x volume of Elutip Low Salt Buffer to tube.
14. Purify fragment via S&S Elutip-d minicolumn protocol.
Briefly:
 1. Remove Elutip-d tip protector and cut off tip. Remove cap.
 2. Load syringe with column attached with 5mls Low Salt Buffer.
 3. Equilibrate via positive pressure--0.5 to 1.0 ml/min.
 4. Disconnect column from syringe. Reload with fragment sample.
 5. Pass sample through column slowly--1 to 2 ml/min or slower.
 6. Disconnect column from syringe. Reload with 2mls Low Salt Buffer.
 7. Wash column via positive pressure slowly.
 8. Elute DNA with 0.4mls High Salt Buffer into 1.75 ml tube.
 9. Add 2x volume of ice cold 100% ethanol. Put at -20 degrees C for >30min.
 10. Spin in microfuge 12,000x g for 25min.
15. Wash pellet with 70% ethanol. Dry.
16. Resuspend in 100ul of injection buffer.
17. Prepare 200ul of DNA for microinjection by diluting the concentrated solution to 4ng/ul.
18. Dialyze the diluted DNA against injection buffer in a petri dish using Millipore 0.05um filter (VMWP 01300). Float filter bright side up on the injection buffer, carefully add 100-150ul of solution to the center of the filter and dialyze for 1hr.
19. Remove solution from the filter and spin at top speed in a microfuge for 2min, carefully recover solution, leaving behind insoluble particles.
20. Repeat spinning twice to completely remove small particles.
21. Check concentration of DNA by running 1-2ul on agarose gel against standard concentration controls. Store DNA in 50ul aliquots at -20 degrees C for microinjection.