

Preparation of DNA construct for microinjection

There is nothing special that I use to isolate DNA construct for microinjection. However, I am always very careful when handling my DNA. I do this by working on a clean bench top and avoid vigorous pipetting. I also use filtered pipette tips and autoclaved microcentrifuge tubes.

1. Maxi prep your plasmid

I use **QIAGEN Maxi kit** and elute the plasmid with EB buffer supplied with the kit then measure DNA concentration.

2. Construct isolation

2.1. Restriction enzyme digestion

I usually linearize my plasmid and eliminate the vector backbone before injection. I usually digest about 10-15 µg of the Maxi prep plasmid in 150-µl reaction volume and digest for at least 16 hours. Remember to heat inactivate the enzymes if needed.

2.2. Gel-purify the construct

I use 0.8% agarose gel with large wells (I use scotch tape to connect several comb teeth together to make a big well that would accommodate 180 µl digestion reaction (150 µl RE digest reaction + 30 µl of 6x glycerol or ficoll-based loading buffer). Load 170 µl into the gel. I keep 10 µl for final reference gel.

Run the gel slowly to obtain nice separation between the construct and the vector backbone. I usually do not run DNA marker in the adjacent well. I skip 1 lane to prevent contamination.

Take a picture. Try to minimize the UV exposure time by taking the picture very quickly and keep the gel on its tray while taking the picture to attenuate the UV ray.

Excise the construct band with a clean blade using long wavelength UV light. Again, proceed fast and efficient to minimize UV exposure time. I usually keep the gel on its tray for additional UV protection while cutting the gel. Transfer the cut gel into a clean autoclaved tube.

Gel-purify the construct using **QIAGEN Gel Extraction kit**. Elute the DNA construct with **microinjection buffer** instead of the EB buffer that is supplied with the kit.

Microinjection buffer solution

10 mM Tris-HCl, pH8.0

0.25 mM EDTA, pH 8.0

Stock Solutions:

1M Tris-HCl, pH 8.0 (SIGMA Cat # T-2694)

0.5M EDTA disodium salt, pH 8.0 (SIGMA Cat# E-7889)

Autoclaved Milli-Q water

Note: I dedicate these stock solutions for making microinjection buffer only to prevent contamination.

I use filtered pipette tips to prepare the buffer. This buffer must be extremely clean, sterile and DNase-free. I make 10 ml of the buffer and aliquote it into autoclaved microcentrifuge tubes of 1 ml each.

After the elution step, I use filtered pipette tips and exert extra carefulness with the construct. Everything should be clean. I notice that many people are not very clean while they are handling the DNA. I usually change to a new pair of gloves (should be powder-free) at this step. Remember that this solution will be injected into fertilized eggs, so it should be extremely clean and DNase-free.

I usually elute the DNA construct using 20-30 μ l of the microinjection buffer into a clean autoclaved microcentrifuge tube.

3. Measure DNA concentration and run a reference gel.

I dilute the DNA construct to different dilutions (e.g. 1:2, 1:4, 1:8 dilutions) with the microinjection buffer and run 0.8% agarose gel.

I use **1 kb ladder** (New England Biolab Cat # N3232) for size and **High Mass Ladder** (Invitrogen Cat# 10496-016) for mass as markers. Load uncut plasmid (~500 ng/lane), cut plasmid showing the construct and backbone (10 μ l of RE digestion reaction left from step 2.2), undiluted purified construct then series of diluted purified construct.

Take a picture and label all lanes. Your construct should be of the right size, clean and show no smear or vector backbone contamination. This picture will help Lina determine your construct integrity and how much she should dilute the DNA for the injection.

4. Submit the construct for microinjection

I usually submit 30 μ l of 12-15 ng/ μ l of the purified construct for injection with the gel picture as a reference for Lina. She will further dilute the construct for the injection.

Statistics

So far, I have submitted 16 constructs for both transient and permanent transgenic analyses. The percentage of transgenics obtained per construct submission ranges from 10% - 45%. I generally get high transgenic number. On average, about 30% of the harvested embryos are transgenics.