

DNA preparation and purification for pronuclear microinjection

1. Prepare DNA using Qiagen Maxi Filter kit or Qiagen Maxi Endofree kit.
2. Digest $>10 \mu\text{g}$ of DNA with appropriate restriction enzyme to release the vector backbone.
3. Heat inactivate the enzyme.
4. Load DNA into wide gel pockets of a preparative 0.8-1.2 % TAE agarose gel. DO NOT USE ETHIDIUMBROMIDE ! Run gel (overnight) to obtain good separation of DNA bands. Use good quality agarose !
5. Proceed to gel extraction using Qiaquick gel extraction kit. All solutions from the kit MUST be filtered through a $0.02 \mu\text{m}$ syringe filter (e.g. Whatman Anotop syringe filter catalog #6809-1102). Discard the first drops before collection filtered solutions.
6. Isolate and purify the DNA from the gel as shown below; use clean scalpels and 2 ml reaction tubes:

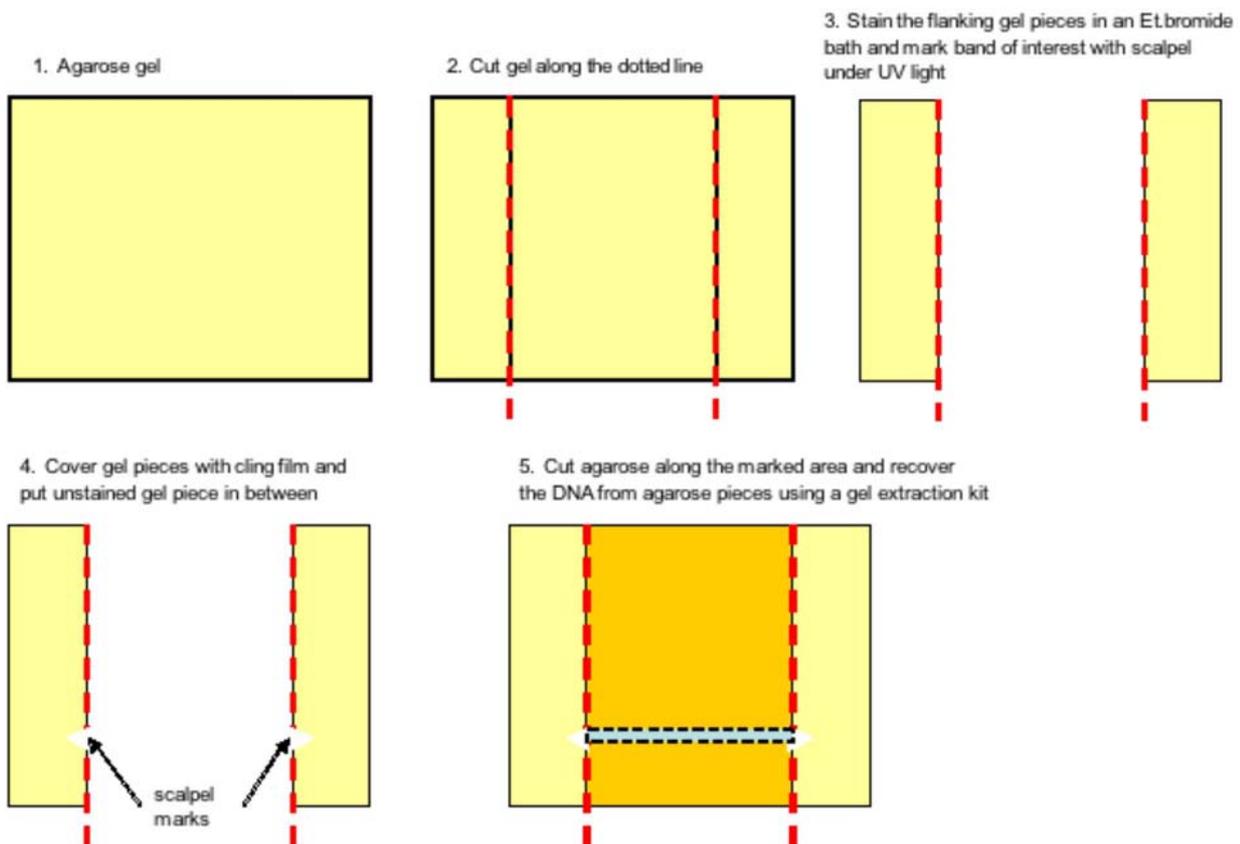


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7. For gel extraction, apply following modifications to the Qiagen protocol:
 - a) Apply the optional wash with buffer QG
 - b) Two washes with buffer PE
 - c) Elute the DNA from the column with $50 \mu\text{l}$ filtered microinjection buffer.
8. Estimate the DNA concentration of at least three different volumes on an agarose gel and measure the OD.
9. Dilute the DNA solution to a final concentration of $2 \mu\text{g}/\mu\text{l}$ with microinjection buffer and store aliquots at $-20 \text{ }^\circ\text{C}$.

Materials:

1. Qiagen Maxi Filter kit (#12262) or Qiagen Maxi Endofree kit (#12362).
2. TAE buffer
3. Seakem GTG agarose; Cambrex #50071
4. Qiaquick gel extraction kit (#28704)
5. 0.02 μ m syringe filter (Whatman Anotop10, #6809-1102; Fisher Scientific # FDP-470-080J)
6. Ethidiumbromide bath; cling film; clean scalpels; 2 ml tubes
7. Stock solutions for microinjection buffer: a) E-TOXATE® water, 30 ml (Sigma 2107-30ml) b) Tris-HCl, 1M solution pH 7.4, 1000 ml (Sigma T2663) c) EDTA, 0.5 M disodium salt solution, 100 ml (Sigma E7889)

8. Microinjection buffer (5 mM Tris-HCl; 0.1 mM EDTA; pH 7.4) for 10 ml: 9948 μ l E-TOXATE® water

50 μ l 1 M Tris-HCl pH 7.4 2 μ l 0.5 M EDTA adjust pH to pH 7.4 and filter through a 0.02 μ m syringe filter !

9. DNA marker with bands of known DNA concentration (e.g. 1 kb SmartLadder, Eurogentec # MW-170010)