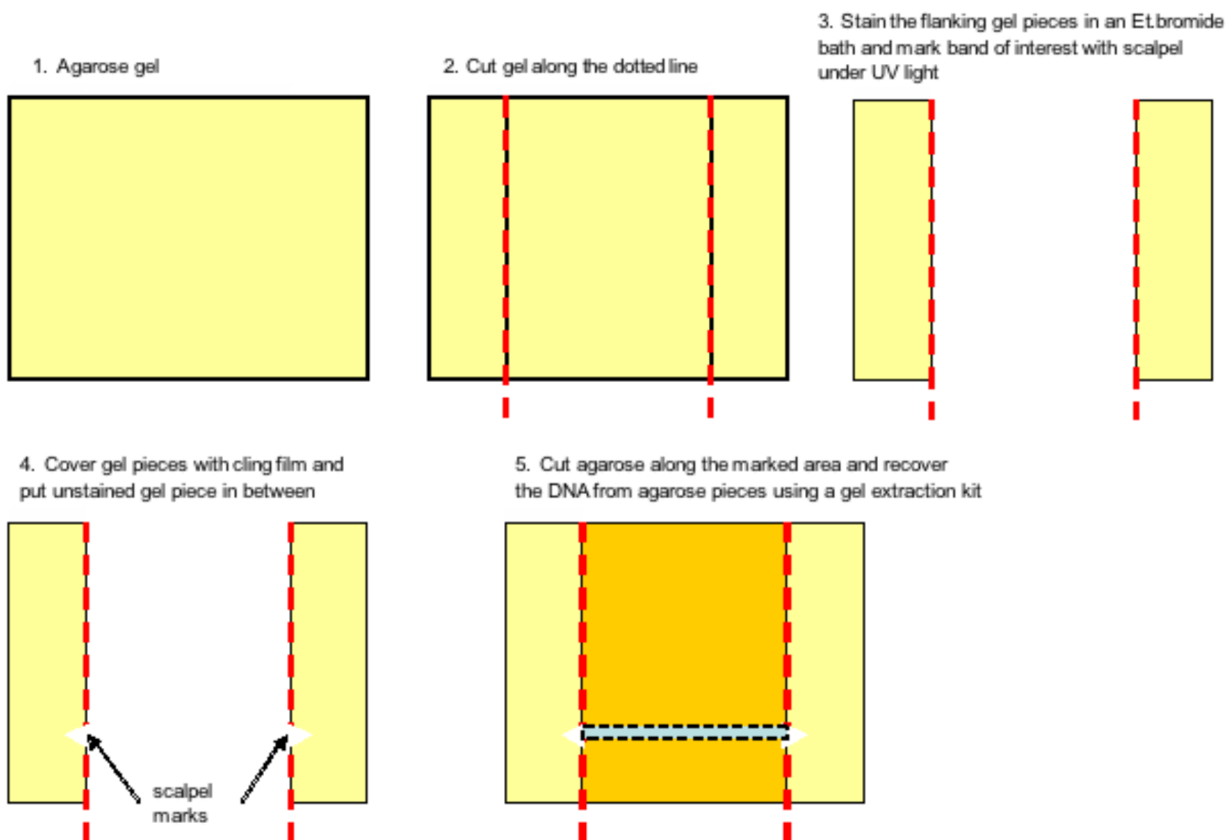


## DNA preparation and purification for pronuclear microinjection

1. Prepare DNA using Qiagen Maxi Filter kit or Qiagen Maxi Endofree kit.
2. Digest >10 µg 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 µm 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:



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 µl 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 µg/µl with microinjection buffer and store aliquots at -20 °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  $\mu\text{m}$  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  $\mu\text{l}$  E-TOXATE® water
  - 50  $\mu\text{l}$  1 M Tris-HCl pH 7.4
  - 2  $\mu\text{l}$  0.5 M EDTAadjust pH to pH 7.4 and filter through a 0.02  $\mu\text{m}$  syringe filter !
9. DNA marker with bands of known DNA concentration (e.g. 1 kb SmartLadder, Eurogentec # MW-1700-10)