

Separation of DNA from Vector Sequences by QiaexII or Qiaquick Agarose Gel Extraction Protocol

One of the most important parameters determining the success of transgenic production is the quality of the injection DNA. The DNA needs to be of the highest quality and completely free from vector sequences.

The following is provided as a guideline to assist you in the successful purification of your DNA construct for injection.

Note: *Every precaution should be taken that the DNA does not become contaminated. This is one of the major causes for lack of transgenic animals. Do not use powdered gloves. Sterility of reagents and supplies is very important. Filter sterilize all reagents using a 0.2 μ m filter.*

1. Perform a preparative restriction enzyme digest with 10 μ g of the cesium chloride gradient purified or Qiagen-purified vector containing the DNA of interest. The total volume of the digest should be 100 μ l. The choice of restriction enzymes is specific to the individual vector and insert.
2. At approximately two hours, check the digest for completeness by running 1-2 μ l of the reaction on an agarose gel. The gel should be slightly overloaded to effectively observe any low level of incomplete digestion. Photograph the gel for review. If necessary, spike the digestion mixture with additional restriction enzyme. Do not proceed with purification until the digest is complete.
3. Once the digest is determined to be complete, inactivate the restriction enzymes for 15 min at 65-70 $^{\circ}$ C.
4. Carefully load the 100 μ l of the inactivated preparative digest on a low melting temperature agarose gel (percentage of the gel depends on the size of the vector and construct). Electrophoresis should proceed until maximum resolution is obtained between the vector and the construct.
5. Follow the protocol, which comes with the Qiagen kit, to the detail. Qiaquick will extract DNA up to 10 kb and QiaexII will extract DNA fragments between 40 bp and 50 kb. Elute the DNA in 10 mM Tris pH 7.4, 0.1 mM EDTA buffer which has been prepared sterily and filtered.
6. Run a sample on a gel with appropriate molecular weight markers. It is best to overload the gel at this point to assure there are no remaining vector DNA bands present. Estimate the concentration. **A minimum of 1 microgram of DNA at a concentration of greater than 30 ng/ μ l is required.**
7. Store the DNA at 4 $^{\circ}$ C and transport immediately to the Lab in 716 CSB. We will run a gel containing quantitative molecular weight markers to determine concentration and purity. If there is sufficient pure DNA, we will do a trial Quality Control injection on 20-30 eggs to determine the action of the DNA in the injection needle, the lysis rate and the ability of the surviving injected eggs to divide to the four cell stage.