



## **DNA purification for microinjection:**

### **1. Linearize the plasmid.**

It is actually preferable to remove as much of the vector sequence as possible as the presence of bacterial sequences has been shown to decrease the expression level of the transgenic protein. However, do not cut at the immediate start of the transgenic sequence as some end degradation may occur upon injection.

### **2. Clean the fragment**

Run the fragment on an agarose gel and cut the fragment from the gel. Limit the exposure to the UV light to minimize any photochemical damage.

Clean the fragment using the Qiagen Gel Extraction Kit (or another similar method, ie ClonTech).

**Do not reprecipitate the DNA using Ethanol. Do not use phenol chloroform to clean the fragment. Trace amounts of these chemicals are toxic to the embryo.**

Redissolve the DNA in TLE (10mM Tris pH 7.4, 0.1mM EDTA). This solution should be filter sterilized using a 0.2 $\mu$  filter before adding it to the column to elute the DNA.

### **3. Quantitate the DNA**

The final amount should be between 10 – 20  $\mu$ g of transgene. Please aim for a concentration of 100 ng/ $\mu$ L or greater. Provide a gel photo and an estimate of the amount with the final prep.