

## **DNA Purification for Transgenic Injection**

### **Maine Medical Center Research Institute Core Facility**

1. Start with at least 50 $\mu$ g of Qiagen maxi prep DNA. Cut with the appropriate restriction enzyme to linearize DNA.
2. Run the digested DNA on agarose gel in 1xTAE buffer at low voltage (no higher than 50V) and excise the correct band under a long wave UV light.
3. Use Qiagen gel purification (Quickspin) kit.
  - a. Add 3 volumes of QC buffer to gel slice and incubate at 50°C until the gel slice is completely melted.
  - b. Add 1 volume of isopropyl alcohol and mix thoroughly.
  - c. Run DNA solution through the quick spin column by a 2 minute centrifugation in an eppendorf microfuge tube. I routinely use 4 quick spin columns for 50 $\mu$ g DNA.
  - d. Wash the column with 500 $\mu$ l of QC buffer once.
  - e. Wash the column with 750 $\mu$ l of PE buffer **three times**. This is a critical step to obtain very clean DNA.
  - f. After discarding the final wash, spin the column for another 3 minutes to get rid of any residual PE buffer. Also aspirate the residual PE buffer trapped in the bottom edge of the column.
  - g. Elute DNA with 25 $\mu$ l EB buffer per column (total of 100 $\mu$ l) and incubate for 10 minutes for efficient DNA recovery.
  - h. Measure O.D. at 260nm and examine DNA integrity by running it on an agarose gel. You should expect DNA with at least 80-100ng/ $\mu$ l concentration. If the concentration is very low, start from the beginning and prepare a new DNA sample. To validate the concentration of your DNA prep, run it out on a gel with using a quantification standard. Please submit this labeled gel with quantitation with your order form.
  - i. You can ship the undiluted, labeled DNA stock on dry ice. Please include the concentration on the tube.

Notes: If DNA quality is not good, based on toxicity to embryos or plugging of injection pipets, we will request that you prepare a new preparation for another injection day.