PREPARATION OF SSSSS DNA

Adapted from BV's Cookbook, TCH 1/27/02

1) Combine 3-3.3 g Salmon Sperm DNA (Sigma # S3126), 640 ml ddH20, and 40 ml 4M NaOH in a 1 liter screw cap glass flask.

2) Boil 20 minutes, shaking the flask occasionally. The DNA won't go into solution until it is boiled.

3) Put on ice until RT or colder.

4) Pour into a 2 liter flask. Add 75 ml 1M Tris pH 7.5 and 300 ul 0.5% Phenol Red.

5) pH to about 7.0 by adding about 14 ml concentrated HCl. Use phenol red as an indicator, since it turns orange/yellow at ~ pH 7. Also, check with pH paper.

6)Add 800 ml EtOH. Store overnight at 4°.

7) Pour into four 450 ml centrifuge tubes and spin 1 hour at 6K in GS3 rotor.

8) Pour off sup, then wash with 50 ml 70% EtOH per tube.

9) Dry inverted for about 30 minutes RT. Combine the pellets into one tube, then dry in a vacuum for about 1.5 hours.

10) Dissolve the pellet in 400 ml TE 10/2 pH 7.5 (20 ml 1 M Tris pH 7.5, 20 ml 0.2M EDTA, qs to 2.0 liters). Then transfer to a 2 liter flask and add 400 ml more TE.

11) Boil 20 minutes, then cool on ice.

12) Measure concentration by OD 260. First, dilute 1:100. The, take OD 260 and OD 280. An OD 260 of 1.0 is about 43 ug/ml. OD 260/OD280 should be about 1.9.

13) Add TE (usually about 500 ml) to give a final concentration of 2 mg/ml.