

# ISOLATION OF AMPLIFIED DNA

## OBJECTIVE:

To purify the amplified DNA away from the PCR reaction components. For many purposes this step is not absolutely necessary, but for further amplifications, DNA sequencing, cloning, etc., it is needed.

## INTRODUCTION:

After PCR amplification, and confirmation of amplification product, the fragment will often be used with other enzymes. Because of this, purification of the fragment away from the polymerase, buffer, primers, DNA sample and mineral oil must be performed. This can be accomplished in several ways. One is to run the whole sample on a gel and then elute the fragment from the gel (a procedure we will perform later in this class). Another way is to first extract with chloroform/isoamyl alcohol, then precipitate the DNA using one of several methods. One method of precipitation is to simply add ammonium (or sodium) acetate and then ethanol (as you have done with the CTAB method for total DNA). This may be inefficient, so another way is to go through the entire CTAB method (described in an earlier exercise). Another method is to precipitate using spermidine. The method we will be using in this exercise is precipitation using ethanol in conjunction with tRNA and glycogen (in this case purified from oysters). Using this method, nearly 100% of the amplified DNA can be recovered.

## STEPS IN THE PROCEDURE:

1. Remove the mineral oil from the top of the PCR amplified solution.
2. Add 25  $\mu$ l 0.1X TE to the PCR amplified solution.
3. Add an equal volume of chloroform/isoamyl alcohol and mix to form an emulsion. Transfer the upper phase to a new tube.
4. Add 1  $\mu$ l of the glycogen solution and 5  $\mu$ l of the ammonium acetate solution to the tube. Mix completely.
5. Add 150  $\mu$ l 95% ethanol. Mix gently.
6. Allow to precipitate at -20°C for several hours (or overnight).
7. Spin in microfuge for 15 minutes (in cold).
8. Pour off ethanol.

9. Wash pellet with 80% ethanol.
10. Spin in microfuge for a minute or two.
11. Pour off ethanol.
12. Dry pellet in desiccater or speed-vac.
13. Rehydrate pellet in 30  $\mu$ l of 0.1X TE.

### **NOTES ON STEPS IN THE PROCEDURE:**

1. Often it is easiest to remove as much of the mineral oil from the top, then remove the lower (aqueous phase) with the pipette and transfer it to a new microfuge tube.
2. Dilution for ease of handling.
3. Removes proteins (mainly *Taq* polymerase).
4. The ammonium ions form a salt with DNA similar to sodium ions described in the DNA extraction exercise.
5. Precipitation.
6. One to two hours is generally sufficient, or 5-10 min @ -80°C.
- 7-13. These steps are similar to steps in the DNA extraction exercise.

### **SOLUTIONS:**

#### **Glycogen (Oyster)**

20 mg/ml Glycogen in sterile water

#### **3 M ammonium acetate**

#### **0.1X TE**

#### **95% ethanol**

#### **80% ethanol**

#### **Chloroform/isoamyl alcohol (24:1)**