DNA Digestion Laboratory

GENERAL COMMENTS

1. It is the accepted convention that 1 unit of restriction endonuclease corresponds to the amount of enzyme required to completely digest 1 ug of lambda DNA in 1 hr under optimal assay conditions.

2. Activity of restriction endonuclease can be affected by:
   a. The pH of the reaction solution,
   b. The presence of divalent cations in the reaction solution,
   c. The presence of organic solvents in the reaction solution,
   d. The concentration of glycerol in the reaction solution (it should be no greater than 5% in the final mixture),
   e. The ionic strength of the reaction solution,
   f. The mean base composition of the nucleotides adjacent to the recognition site.

3. Repeated freezing and thawing of the DNA sample will damage the material.

4. VERY IMPORTANT!! Restriction endonucleases must be stored at -20°C. They should not be left at room temperature or on ice for long periods of time.

5. Use presterilized plastic eppendorf tubes and plastic pipette tips to provide sterile conditions.

6. Mixing several restriction endonucleases in a single reaction solution may lead to the inhibition of the reaction.

PROCEDURE:

1. Place a sterile eppendorf tube on ice. In order, add the following:
   a. Sterile water
   b. 10x high salt buffer
   c. 10x BSA
   d. DNA sample
   e. Restriction endonuclease (10 u/ul)

2. The amount of each component to be added to the reaction solution is based on the amount of DNA to be digested. The concentration of the DNA in the sample should be based upon fluorometric or spectrophotometric analysis. For the plant DNA digestions, 20 ug of DNA must be digested per reaction. For plasmid DNA, 200 ng of DNA is needed for each digestion.
As an example, if your DNA sample concentration was 1 ug/ul and you want to digest 4 ug of DNA, and your total reaction mixture volume is to be 20 ul**, then add sequentially:

a. 11 ul of sterile water  
b. 2 ul of high salt buffer  
c. 2 ul of 10x BSA  
d. 4 ul of DNA  
e. 1 ul of restriction endonuclease

**Note: Try to keep the digestion reaction to a maximum of 25 ul.  
(A larger volume will be needed for a 20 ug digest.)

3. Centrifuge the eppendorf tubes in the microfuge with a 1-2 second burst to collect the reaction mixture in the bottom of the tubes.

4. Place the eppendorf tubes in a 37ºC water bath or incubator for 2 hr or overnight. The use of Styrofoam boats or another floating apparatus keeps the tubes upright in the water.

5. After the digestion is completed, add the endonuclease reaction stop mix to the reaction solution or place at -20ºC. The stock solution is considered a 10x concentration, therefore add if you have a 20 ul volume reaction then add 2 ul of the stop mix.

QUESTIONS TO THINK ABOUT:

1. What is the purpose of each component of the reaction solution?

2. What is the purpose of the incubation period?

3. What is the function of the Endonuclease Reaction stop mix?