Molecular Biology
Lab 3
DNA analysis 101

Analysis of pGLO plasmid after purification from transformed *E.coli*

Our last lab exercise involved purifying the pGLO plasmid DNA from the *E.coli* cells following transformation with this plasmid. In this exercise we "saw" how the *E.coli* replicated the circular plasmid DNA for us and how we could separate a small, supercoiled, free-floating, circular plasmid DNA molecule, from the much larger, relaxed, membrane-tethered chromosome (also a circular DNA molecule). Although we were fairly sure that we succeeded in purifying plasmid DNA (if we followed the procedure correctly), we can now proceed to analyze this plasmid DNA in a number of ways.

A.
After a DNA purification procedure we often want to determine the quantity (concentration) of the DNA solution and to evaluate the purity.
Both quantity and quality can be determined using **spectrophotometry.** DNA absorbs light in the UV range (260 nm). Therefore when light of this wavelength shines through a sample of DNA, the amount of light absorbed is proportional amount of DNA is the solution. The absorbance of light is called **Optical Density (O.D.)** The concentration of DNA can be calculated using the following formula.

1 $O.D._{260}$ unit = concentration of 50 μg/ml double stranded DNA.

Purity can also be determined using spectrophotometry. We are usually most interested in the amount of protein that might have been inadvertently purified with the DNA. Proteins absorb light at a different wavelength (280 nm). A convenient measure of DNA purity is determined by measuring the $O.D._{260}$ / $O.D._{280}$ ratio. Pure DNA is considered to have an $O.D._{260}$ / $O.D._{280}$ = 2, although the ratio is usually slightly less in practice.

Today you will determine the concentration and purity of the DNA you purified in the last lab using spectrophotometry.

1. Obtain your DNA sample and your lab supplies
2. Dilute the DNA 1:10 in water to make a total of 20 μl in one of your microcentrifuge tubes.
3. Make a 20 μl EB solution standard or <u>blank</u> (one blank can be used for everyone).
4. Dr. Super will introduce you to the spectrophotometer and the features you will use in DNA analysis. It is called a Nanodrop ® spectrophotometer as it can analyze very small volumes of sample.
5. Start the Nanodrop software and proceed with Dr. Super's assistance.
6. Record your own OD_{260} and OD_{280}
6. Calculate the concentration and purity of your DNA.

Concetration = (OD_{260} X50 μg/ml X dilution factor)
Purity = OD_{260} / OD_{280}

B.

Once quantity and quality is determined we often want to create a "molecular roadmap" of our DNA by noting the position of some **restriction enzyme recognition sites**. (see attachment) This allows us to give the molecule some convenient markers which molecular biologists use in a variety of ways. The most basic restriction enzyme analysis shows us that DNA molecules with different sequence will have different restriction patterns. In other cases one may want to isolate a small restriction fragment of the DNA for future use.

Today you will begin to create a restriction map of the pGLO plasmid ,using 2 restriction enzymes , *Eco RV* and *Hind III* to digest your plasmid DNA.

Next week you will use DNA gel electrophoresis to separate the *restriction fragments* made by cutting the plasmid (including uncut sample and DNA molecular weight samples). Finally you will stain your DNA in your gel with ethidium bromide and visualize with U.V. light. You will make a photograph to use for analysis. The photograph should allow you to:

1. Determine the size of the DNA fragments made by the restriction enzymes.

2. Create a map of the circular pGLO plasmid molecule, showing the positions of the restriction recognition sites relative to each other.

Restriction Enzyme Digestion

Procedure:

1) Set up 4 DNA digestion tubes. Label appropriately for:
    a. pGLO DNA digested with *Eco RV*
    b. pGLO DNA digested with *Hind III*
    c. pGLO DNA digested with *Eco RV* and *Hind III*
    d. pGLO DNA undigested (-restriction enzymes)

Each tube will contain 25 μl* total volume, but the exact volume of DNA (and water) will vary depending on the concentration of DNA. Calculate the volume needed for **1μg** if possible. A 10X concentrated buffer compatible with the enzymes you will use is supplied. It should be diluted to a 1X working concentration. Distilled water is commonly used to bring the sample to the desired volume.

FYI---You could set up a DNA digestion in any volume you desired, but typically 20-30 μl is optimal for good DNA digestion and to allow you to conveniently apply your sample to an electrophoresis gel.

X μl DNA (This will vary depending on the concentration determined above)
2.5 μl 10X restriction buffer
1.0 μl enzyme
X μl H_2O (This will vary depending on the concentration determined above)
25 ul total volume

2) Place the tubes at 37 degrees C (water bath for 2+ hours).

Dr. Super will remove the tubes for you and place the tubes at 4 degrees C.
This stops the digestion and protects DNA from digestion with general DNases.
The DNA will be analyzed next week.

Materials

Micofuge tubes
Distilled water
pGLO DNA samples
Spectrophotometer

Restriction enzymes (EcoRV, Hin dIII)
10 X restriction enzyme buffers

TABLE 4.1
Some Restriction Enzymes, Their Sources and Their Recognition Sites

ENZYME	MICROBIAL SOURCE	SEQUENCE*
AluI	*Arthrobacter luteus*	5′—A—G↓C—T—3′ 3′—T—C—G↑A—5′
BamHI	*Bacillus amyloliquefaciens* H	5′—G↓G—A—T—C—C—3′ 3′—C—C—T—A—G↑G—5′
EcoRI	*Escherichia coli*	5′—G↓A—A—T—T—C—3′ 3′—C—T—T—A—A↑G—5′
EcoRII	*Escherichia coli*	5′↓—C—C—T—G—G—3′ 3′—G—G—A—C—C↑—5′
HaeIII	*Haemophilus aegyptius*	5′—G—G↓C—C—3′ 3′—C—C↑G—G—5′
HindIII	*Haemophilus influenzae* b	5′—A↓A—G—C—T—T—3′ 3′—T—T—C—G↑A—A—5′
PstI	*Providencia stuartii*	5′—C—T—G—C—A↕G—3′ 3′—G↑A—C—G—T↑—C—5′
SalI	*Streptomyces albus*	5′—G↓T—C—G—A—C—3′ 3′—C—A—G—C—T↑—G—5′

*Note: The arrows indicate the sites of cleavage on each strand.