

Laboratory 1

Your Name _____
Class period _____

An Introduction to Microvolumetrics and Pipetting

PRE-LABORATORY NOTES

I. Metric Conversions

Since the DNA experiments most commonly use microliters (μL) and nanograms (ng), it is important to become familiar with some of these small units of metric measurements.

Volumetric Conversions:

1 *milliliter* (mL) = 0.001 liter (L)

1,000 mL = 1 liter

1 *microliter* (μL) = 0.000001 liter

1,000,000 μL = 1 liter

Weight Conversions

1 *milligram* = 0.001 gram (g)

1,000 mg = 1 gram

1 *microgram* = 0.000001 gram

1,000,000 μg = 1 gram

1 *nanogram* = 0.000000001 gram

1,000,000,000 ng = 1 gram

1 *picogram* = 0.000000000001 gram

1,000,000, 000,000 pg = 1 gram

1a. Approximately 28 drops of liquid, from a medicine dropper or disposable pipette, equals 1 mL. Each drop represents what part of one mL? (Show your work.)

1b. Approximately how many μL are there in one drop of liquid?

2a. Four hairs, plucked from your head, will yield about 1 μg of DNA. How many μg of DNA can be extracted from a *single* hair root? (Show your work.)

2b. How many nanograms does this represent? (Show your work.)

Laboratory 2

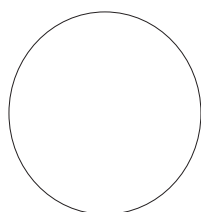
Your Name _____

Class period _____

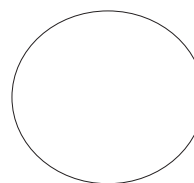
Restriction Analysis of pARA and pKAN-R

PRE-LABORATORY NOTES

1. Draw two circles, one representing pARA and the other pKAN-R. Indicate the positions of *Bam*H I and *Hind* III restriction sites and other genes. Briefly describe the function of each of these genes. *amp^r*, *kan^r*, *araC* and *rfp*.



pARA



pKAN-R

2. Which antibiotic resistant gene, is found in pARA? In pKAN-R?
3. One of these plasmids has been engineered to express a protein if the gene for that protein is inserted into a specific location. Which of the plasmids has been engineered for protein expression?
4. From what organism did the gene for mutant Fluorescent Protein come?
5. Briefly outline, in your own words, the steps required to set-up the plasmid restriction digest.
6. Why do you suppose you are asked to set up two tubes without *Bam*H I and *Hind* III?

Laboratory 2a

Your Name _____

Class period _____

**pARA-R Restriction Digest: An Introduction
to Plasmids and Restriction Enzymes**

PRE-LABORATORY NOTES

1. Restriction enzymes are purified from what type of organism?

2. A “palindrome” is a word that reads the same when read forward or backward. For example, “wow” or “ono” (a fish found in Hawaii) are palindromes. Restriction sequences are also considered to be palindromes. Explain this similarity as it relates to nucleotide sequences recognized by restriction enzymes.

3. What is a “plasmid” and what advantage might a bacterium gain by acquiring one?

4. The plasmid pARA-R has been genetically modified for what purpose?

5. What is meant by the term “restriction digest?”

Laboratory 3

Your Name _____
Class period _____

Ligation of pARA/pKAN-R Restriction Fragments **Producing a Recombinant Plasmid, pARA-R** *PRE-LABORATORY NOTES*

1. Briefly define the term “recombinant DNA.”
2. What does the term *ligation* indicate?
3. In order for two sticky ends to join together, what relationship needs to exist between them?
4. What properties of the restriction fragments produced in Lab 2 are needed to permit ligation of these fragments?
5. What is the importance **step 1** in this protocol?
6. Draw *three* possible recombinant plasmids resulting from pARA and pKAN-R fragments. Include any genes these fragments carry.

Laboratory 4

Your Name _____
 Class period _____

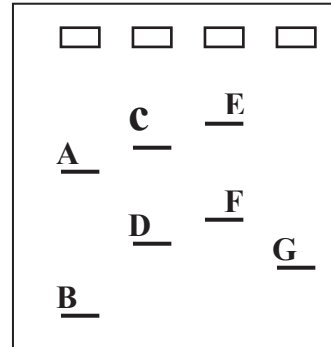
Confirmation of Restriction and Ligation

Using Agarose-Gel Electrophoresis

PRE-LABORATORY NOTES

1. Using the location of each DNA “band” in the gel, the relative size of each DNA fragment can be determined. For example, examine the figure below and arrange the letters, identifying each band, from smallest to largest.

In the space below, use the letters to list the fragments from the largest to the smallest.

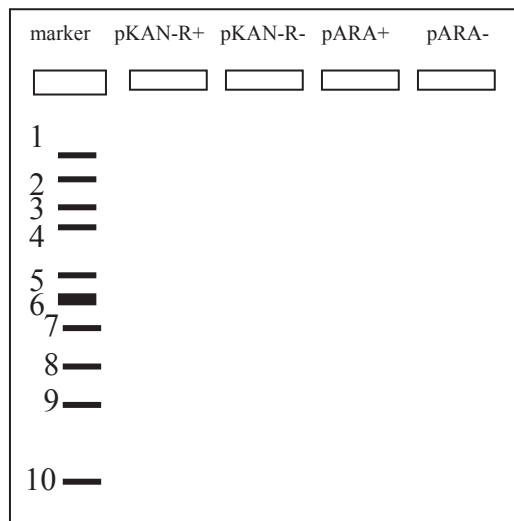


2. Besides using electrophoresis to separate DNA fragments according to their sizes, it can be used to *estimate* the actual size, in base pairs, of each fragment. For example, we might be looking for a gene and we suspect it is of a certain size; electrophoresis can be used to locate fragments in that size range. In order to do this, we would need to run a gel with a mixture of DNA fragments of known sizes. This mixture called a “marker” or “ladder,” serves as a *control* or a standard to which we can compare the positions of other DNA bands in the same gel.

In the diagram, below, the “marker” lane contains 10 DNA bands of known sizes. These sizes are given below. Using this information and the plasmid maps of pARA and pKAN-R, *predict* the positions of DNA bands produced the pARA -, pARA +, pKAN-R-, pKAN-R + samples. *Hint: first determine how many fragments should appear in each sample, and then determine the size(s) of each fragment. We will omit the “LIG” sample.*

Marker fragments:

1. 10.0 kilobase pair
2. 8.0
3. 6.0
4. 5.0
5. 4.0
6. **3.0** (thick band)
7. 2.0
8. 1.5
9. 1.0
10. 0.5



Laboratory 4a

Your Name _____

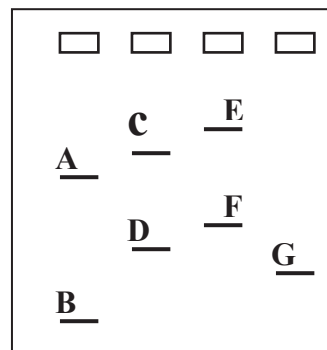
Class period _____

Confirmation of pARA-R Restriction Digest

PRE-LABORATORY NOTES

1. Using the location of each DNA “band” in the gel, the relative size of each DNA fragment can be determined. For example, examine the figure below and arrange the letters, identifying each band, from smallest to largest.

In the space below, use the letters to list the fragments from the largest to the smallest.



2. What experimental procedure will be used to confirm plasmid digestion?

3a. What is the electrical charge of a DNA molecule?

3b. To which electrode will the DNA molecules migrate?

4. Although the supercoiled and nicked-circle plasmids have the same molecular weight or number of base pairs, they will migrate at different rates. Explain the reason for this difference in migration through the agarose gel.

Laboratory 5

Your Name _____
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Transforming *Escherichia coli* with a Recombinant Plasmid *PRE-LABORATORY NOTES*

1. Beginning with the restriction digest of pARA and pKAN-R, *briefly* describe the steps required for constructing your recombinant plasmid from pARA and pKAN-R restriction fragments. Explain your answer including the manipulations of these two plasmids and their restriction fragments.

2. What is “transformation?”

3. What does it mean if cells are “competent?”

4a. How would you know if a bacterium gets transformed with a plasmid containing the amp^r gene?

4b. What would be the size of the fragment carrying this gene?

4c. From what plasmid did this fragment originate?

5. How is the P + culture treated differently from the P – culture?

What is the purpose of the P– culture?

Laboratory 5a

Your Name _____
Class period _____

Transforming *Escherichia coli*, LMG194, with pARA-R
PRE-LABORATORY NOTES

1. What is *transformation* as it relates to this laboratory?
2. What specific form of DNA is used in this lab to effect transformation?
3. During the lab, you will prepare two microfuge tubes containing non-transformed cells. One set of cells you will label *P*⁺ and the other cells *P*⁻. In what way will these cells be treated differently?
4. How will you know if the bacterial cells got transformed during the lab?

Laboratory 6

Your Name _____
Class period _____

Preparing an Overnight Culture of *E. coli*
PRE-LABORATORY NOTES

1. How does the AraC protein prevent a cell from producing mutant Fluorescent Protein when no arabinose is present?

2. How does arabinose interact with the AraC protein help to turn-on the *rfp* gene?

3. Define or identify the following terms. Use your textbook to help with these definitions or descriptions.

Transcription

Messenger RNA (mRNA)

RNA polymerase

Translation

Laboratory 7

Your Name _____
Class period _____

Purification of RFP from an Overnight Culture

PRE-LABORATORY NOTES

1. Briefly summarize how the *product* prepared in day-one activities of Lab 7 is *used* in the day-two activities.

2. Define the following terms:

Hydrophobic:

Hydrophilic:

3. Although most proteins contain both hydrophobic and hydrophilic regions, mutant Fluorescent Protein is considered to be what type of molecule?

4. How can the salt concentration, in the solution surrounding mFP, influence protein shape?

5. How can solutions of different salt concentrations be used to help purify mFP using column chromatography?

Laboratory 8

Your Name _____
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Genomic DNA Extraction from Buccal Epithelial Cells
PRE-LABORATORY NOTES

This laboratory is subdivided into two parts: the first involves a DNA extraction while the second part involves the amplification of a portion of your DNA sample using the polymerase chain reaction (or PCR). Although you will be very much involved with gathering and preparing your DNA sample, your teacher will be doing most of the second part for you.

1. Why is it important that the cells you collect from your mouth are mixed with Chelex?

2. What is the purpose of boiling the cell sample?

3. What does PCR accomplish?

4. The “primers” that are used in this PCR are unique to what locus (*location* or DNA segment)?

- 5a. What is an “*Alu* element?”

- 5b. How many base pairs is the *Alu* element?

6. Does everyone carry an *Alu* element in the region that we are amplifying by PCR? Explain.