# Bi 1x
# Lab Basics and Restriction Digests

## 1 Overview

In this lab, you will familiarize yourself with lab techniques for handling solutions of biomolecules, making spectroscopic measurements, and working with bacteria. These techniques will be used over and over again in this class and certainly beyond if you choose to embark on further studies in the biological sciences. Though we are learning basic technical procedures, these exercises will still illustrate biological principles.

In this introduction to lab practices, you will perform four exercises.

1. Practice pipetting.
2. Perform a restriction digest and use gel electrophoresis to analyze the results.
3. Practice working with *E. coli*.
4. Use spectrophotometry to measure cell density.

**A note about safety.** In this lab, we go over some of the basic techniques, but the most important thing to have in mind while in the lab is safety. Your personal safety and those of your classmates are top priority. Your instructors will go over lab safety with you on the first day. Listen carefully and follow their instructions. If at any time during this course you are unsure about a safety-related issue, please ask any of the course instructors.

## 2 Background

### 2.1 Pipetting

One of the most important instruments in a biology lab is the **pipette**, a device that allows highly accurate handling of small quantities of liquid. There are several brands of pipettes in the lab, but they all operate in the same manner, relying on air displacement to move liquid in and out of disposable plastic tips.

Pipettes are manufactured to handle different volume ranges, and it is important to choose the appropriate pipette for the volume of liquid you wish to deliver to ensure accurate results. You will be provided with a set of pipettes consisting of a combination of the following list of volume ranges:

Table 1: Varieties of pipettes

| Pipette model | Rec. volume range | Tip | note |
|---|---|---|---|
| P-2 | 0.2–2.0 µL | clear | 1 |
| P-10 | 0.5–10 µL | clear | |
| P-20 | 2–20 µL | yellow | 1 |
| P-50 | 5–50 µL | yellow | |
| P-100 | 10–100 µL | yellow | |
| P-200 | 20–200 µL | yellow | 1 |
| P-1000 | 100–1000 µL | blue | 1 |

[1)] These pipettes are the most common, and most of the pipettes we have in lab are of these varieties.

**Protecting pipettes.** Pipettes are expensive precision instruments and must be treated gently. (For example, it costs about $2000 just to calibrate the Bi 1x pipettes.) There are therefore some important rules to follow to protect them.

- Never, ever dial to a volume outside of the limits of the pipette (either too high or too low). This may result in damage to the internal mechanism.
- Do not allow liquid to enter into the pipette shaft. You can avoid this circumstance by remembering to:
  - Always keep the pipette vertical at all times that liquid is in the tip.
  - Never let the plunger snap back up quickly when you are withdrawing liquid.
  - Always use the pipette with a disposable tip of the correct size. Be sure to apply the tip with enough force to ensure an airtight seal, but not enough force that you strain the pipette shaft.

**Pipetting technique.** To get maximal benefit out of the precision of pipettes you should adhere to the following guidelines.

- Always use a fresh pipette tip for every pipetting action to minimize sample contamination (tips are cheap, contamination is not).
- It is better to pipette smaller volumes into larger volumes.
- When adding a small volume to a larger one, be sure the end of the tip is submerged. Push the plunger to the first stop position. You can then gently move the plunger up and down to mix the solution. Only when you are finished mixing, extend the plunger all the way down, hold it there, and extract the pipette. This often results in an air bubble in the solution, which can be eliminated by spinning the sample down on a desktop centrifuge.

- If you need to hold the tip steady, for example while loading a gel, rest the pipette against the index finger of your non-dominant hand just above the top of the pipette tip. This provides extra stability.

**Filter tips.** At some points during the course, you will work with purified DNA. To minimize sample contamination, you will use **filter tips** that can trap aerosols and airborne contaminants. Samples that come in contact with the filter can still be dispensed for further use.

**Serological pipettes.** When volumes in excess of 1 mL need to be handled, we typically use **serological pipettes**. These long, disposable, sterile, plastic pipettes are designed for large volume samples on the order of milliliters. The type of mechanism for suction used with these pipettes differs depending on application and personal preference. We use an electronic transfer pipette (e.g., brand names Pipet-Aid or PIPETBOY). You may see these out on bench tops, but they are used only by TAs in this course.

## 2.2 Restriction digest

As an exercise to practice pipetting, and equally importantly to introduce you to an important set of of enzymes in molecular biology, we will perform **restriction digests**. **Restriction endonucleases**, also called restriction enzymes, sever covalent bonds in the sugar-phosphate backbone of DNA, thereby "cutting," or "digesting," double-stranded DNA. Importantly, these enzymes only perform their cuts on specific DNA sequences, called **restriction sites**.

We will cut two different DNA sequences and analyze the results. We first give some background on the structure of DNA and how restriction enzymes work.

### 2.2.1 DNA and plasmids

You are probably familiar with the linear double helix of DNA. DNA consists of two polynucleotide strands coiled around each other and form a double helix. Each strand has a sugar-phosphate backbone decorated with **bases** of four types, adenine (A), thymine (T), guanine (G), and cytosine (C). The bases on opposing strands pair with each other, A with T and G with C. Each strand is directional, with a 5′ (five prime) and 3′ (three prime) end that refers to the position of the terminal groups on the sugar-ring molecule of the nucleotide backbone. Sequences are typically read 5′ to 3′. The strands in a double helix run antiparallel to each other. Sequences that can form a complete set of base pairs with each other are called **complementary**. For example, 5′−GACCTA−3′ and 5′−TAGGTC−3′ are complementary sequences.

You probably also know that a single cell contains a long piece (or multiple long pieces) of DNA that contains all of the information in its genome. In *Escherichia coli*, this long piece of DNA is actually a closed loop (often referred to as circular DNA). It consists of about 4.6 million base pairs (or 4600 kbp), and if you zoom in to a portion of the DNA, it looks like the familiar double helix. The DNA is packed inside the *E. coli* cell in a bacterial chromosome, or nucleoid.

*E. coli* also have short pieces of non-chromosomal DNA called **plasmids**. Plasmids are shorter than the genome and range in size from about $10^3$ to as large as $10^6$ base pairs. They are also almost always circular DNA. Plasmids are particularly useful in genetic engineering, as they are effective ways to introduce genes to be expressed in bacteria. In this lab, we will work with the plasmid pZE21-LacZ (see section 2.2.3).

Viruses also have genomes, though they are much smaller than those housed in cells. Viruses inject these genomes into cells, including bacteria. Viruses that infect bacteria are called **bacteriophages**, or just **phages** for short. After injection, the machinery of the cell replicates the viral genome and makes the proteins that the viral genome encodes. Viral genomes are similar in size to many plasmids. In this lab, we will work with the genome of **lambda phage**, whose genome is about 50 kbp long.

### 2.2.2 Restriction enzymes

Most useful restriction enzymes recognize four to eight base pair restriction sites. These sites are symmetric, inverted repeats called palindromes. Shown in Fig. 1 are the restriction sites of the three enzymes you will be using in this lab: EcoRI, HindIII, and KpnI. Notice how the 5′ to 3′ sequence is identical on the top and bottom strands.

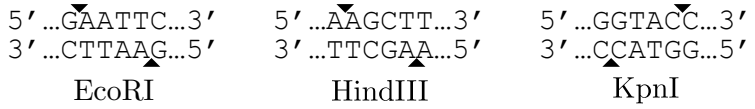


Figure 1: Restriction sites of the enzymes used in this lab. The cleavage points are indicated by triangles.

As you can see, some restriction enzymes leave single-stranded overhangs upon cleavage. Some restriction enzymes leave blunt ends, but we will not use them in this lab. It is also important to remember that restriction sequences are not necessarily unique to an enzyme—multiple enzymes often have the same recognition sequence. To look up the recognition sequences of different enzymes, you can consult the New England Biolabs (NEB) REBASE database.

Restriction enzymes are generally supplied as a given number of units. These units correspond to a metric of enzymatic activity, as specified by the manufacturer. Your digests use enzymes from NEB, which uses the following definition for a unit:

"One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl."

Lastly, restriction enzymes, like all enzymes, have certain optimal conditions that must be met for full activity. Different restriction enzymes have different requirements; salt, metal ions, detergent, and additive concentrations can all have drastic effects on activity (though many enzymes are perfectly happy in generic buffers). One common additive is acetylated bovine serum albumin (BSA). BSA levels and other reaction conditions are usually optimized by the manufacturers, who supply specific buffers with each enzyme. The enzymes you will use in this experiment are from NEB's High-Fidelity (HF) line, which means that in addition to having improved activity and specificity toward their native recognition sequence, these types of enzymes are all optimized to work in the same buffer (CutSmart), which already has BSA added. As you might imagine, this greatly simplifies planning and setting up restriction digests, especially when multiple enzymes are necessary.

### 2.2.3 Use of restriction enzymes in molecular biology

Molecular biology is modular: the machinery that translates DNA into proteins is amazingly similar across organisms. Therefore, if we would like *E. coli* to make protein encoded by a given gene (to **express** the gene), we can simply insert a plasmid with our gene of interest into *E. coli*. (Of course, this does not *always* work, but the efficacy of this strategy is remarkable.) Restriction enzymes provide a very convenient way to do this. This is best shown through an example.

We will consider the plasmid pZE21-LacZ, which we will use in our experiment. A map of its genes is shown in Fig. 2. We see that there are two restriction sites, one for HindIII and one for KpnI. Both of these restriction enzymes leave overhangs after cleavage. We could then mix the cut plasmid with a gene of interest with added overhangs that are complementary to the overhangs left by the restriction enzymes. These "sticky ends" can then pair with each other. We can then perform a **ligation** reaction which forms covalent bonds at strand breaks to give us a new plasmid. It is not surprising that restriction enzymes are ubiquitous and crucial tools in molecular biology.

### 2.2.4 Gel electrophoresis

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, among many, many other applications. An agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting, as shown in Fig. 3.

Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode. This is called **electrophoretic motion**. The DNA

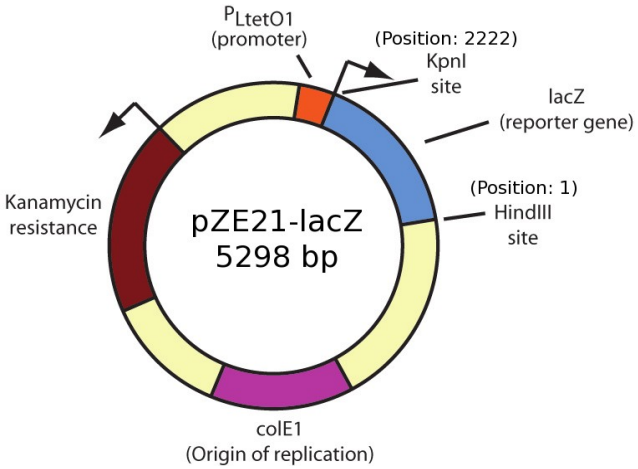


Figure 2: A gene map of the plasmid pZE21-LacZ. The **promoter** is an RNA polymerase binding site, a transcriptional regulator. The **origin of replication** is the site where plasmid replication begins prior to cell division. This regulates the **copy number** of plasmids in the bacteria. The Kanamycin gene encodes for antibiotic resistance. Therefore, bacteria with this plasmid survive in Kanamycin media, while all others die off, so we know all surviving bacteria have the plasmid.

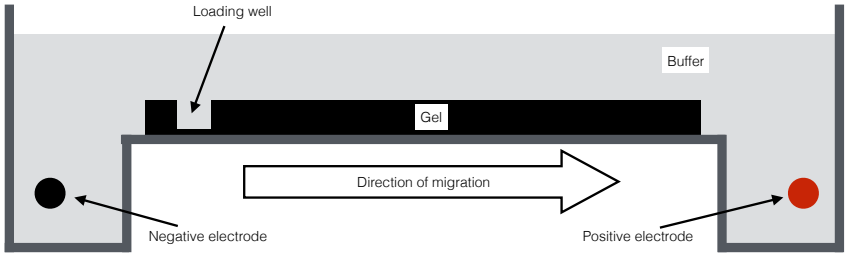


Figure 3: A schematic of gel electrophoresis. This is the side view of a gel box.

molecules are separated in the gel according to their size and shape. Linear DNA molecules are separated almost exclusively according to their size. The smaller the linear fragment, the faster it migrates due to less entanglement with the gel mesh. If the sizes of two fragments are similar or identical, they will migrate together in the gel.

Circular DNAs such as plasmids are supercoiled. Supercoiled DNA has a more compact and entangled shape (like a twisted rubber band) than its corresponding non-supercoiled forms (linear, nicked, and relaxed circles). When supercoiled DNA is cleaved by a restriction enzyme just once it unravels to its linear form. If supercoiled DNA is nicked (a phosphate bond is broken anywhere in the molecule, in either strand) it completely unravels to form a circle. Under the electrophoresis conditions being used in this experiment, supercoiled DNA migrates faster than its linear form and linear DNA migrates faster than its nicked circular form.

The samples are loaded into the wells of the gel with a **loading dye**. The loading dye contains glycerol, which helps the solution sink into the wells of the gel, and dyes that provide visualization.

After the DNA has migrated and the power is shut off, the final positions of the DNA is found by taking a picture using a **gel scanner**. The DNA will be separated by size, with each size appearing as a line (referred to as a "band") on the gel. The approximate lengths of the DNA fragments in the cell are determined by comparing the positions of the bands to those of a reference set of DNA strands called a **ladder**. Images from a gel scanner of the two ladders we use in this lab are shown in Fig. 4.

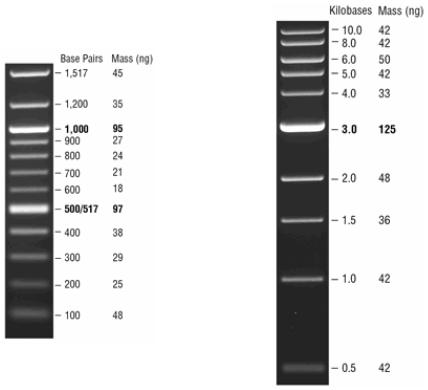


Figure 4: Left, a 100 bp ladder. Right, a 1000 bp ladder. The first set of ticks indicates the number of base pairs in the DNA in the respective bands. The mass is the number of nanograms of DNA of a given length in 500 ng of ladder solution. These images came from New England Biolabs, the supplier of our DNA ladders. The images for the 100 bp and 1000 bp ladders may respectively be accessed here and here.

## 2.3 Working with bacteria and avoiding contamination

As we will discuss in class, *E. coli* is one of the most important and widely-studied **model organisms** in all of the biological sciences. We will use *E. coli* extensively throughout the term.

When working with bacteria, it is very important to minimize contamination. Bacteria are found everywhere—on your skin, on your lab bench, and even in the air. Fortunately, by practicing sterile technique, you can easily minimize the opportunity for contamination.

In this lab, you will become acquainted with bacteria and sterile technique by performing several exercises. When labeling plates, write on the bottom plate (where the agar is resting) and not the lid. This way, if lids become misplaced, the label can still be found.

The plates we use are made of agar infused with LB (Luria-Bertani) broth, which

is a common media for growing *E. coli*. We will also use plates with supplemented with Kanamycin, an antibiotic that kills non-resistant bacteria by interfering with ribosomes and their production of proteins.

### 2.4 Spectrophotometry

We investigate spectrophotometry in more depth later in the course when we study *E. coli* growth curves. Briefly, in spectrophotometric experiments, light is shined through a solution. The intensity of the light emerging from the solution is measured, and the fraction of light that does not emerge is the **absorbance**.

It is often useful to determine how many cells one has in a culture—spectrophotometry is one technique than can be used to do this. As visible light passes through a liquid sample of cells, it will be scattered, and therefore not detected at the other side of the sample. The amount of scattering (reported as absorbance) is related to the density of cells in the suspension. At 600 nm, an optical density (OD) reading of 1 means there are approximately $10^9$ *E. coli* cells per milliliter of culture.

## 3 Protocols

### 3.1 Practicing techniques with *E. coli*

#### 3.1.1 Bacteria in air

1. Label one of your LB agar plates, take the top off, and set it agar side up on your bench. Leave this plate open for the rest of the lab period. This plate will test for microorganisms that are present on dust particles floating in the air.
2. As a negative control, label another plate and place it next to the first, except with the lid on this time. This plate will ensure that any results you see on the plate from (1) are from external particles, and not some sort of contaminated media.

#### 3.1.2 Antibiotics

1. Draw a line on the back of one plain LB plate and one LB-antibiotic plate to divide them each into two halves. Denote one half per plate as "before cleansing" and one half per plate as "after cleansing." Moisten your fingers with some tap water and place them on the "before" half of the LB plate and on the "before" half of the LB-antibiotic plate.
2. Wash your hands with soap and water (taking care not to touch the faucet knobs with your fingers) and repeat step 1 using the "after cleansing" sides of your plates.
3. Allow your plates to stand right side up, with their tops on, for the remainder of the lab period.

### 3.1.3 Streaking plates

Streaking an agar plate allows you to obtain well-isolated, discrete bacterial colonies by sequential dilution of a starting sample.

1. Examine the plate of colonies you have been given, and choose two you wish to streak out. Label the recipient plate.
2. Using a fresh disposable inoculating loop, pick up a small amount of the colony you wish to streak.
3. Immediately streak this material onto the side of the plate (streak 1 in Fig. 5), then discard the loop in the biowaste container.
4. Using a fresh loop, streak from the first area into a new area of the plate by making a series of straight lines away from (but overlapping) the edge of the previously inoculated area (streak 2 in Fig. 5).
5. Streak from the second area into a third, new area using a new loop.
6. Streak from the third area into a fourth, new area—this area should cover most of the remaining space of the plate—using a new loop.
7. Try streaking a second LB agar plate.

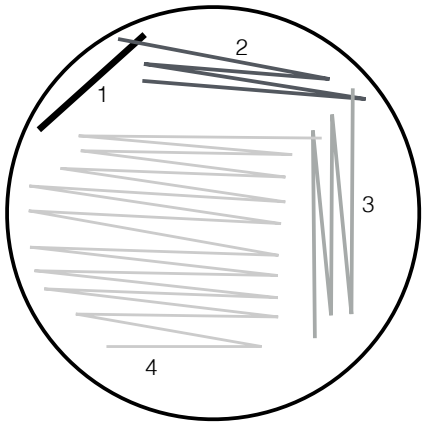


Figure 5: Sequential streaking to dilute the bacteria on a plate. Notice that for each successive streak, you only drag the inoculating loop through the previous streak once.

## 3.2 Practice with pipettes

You have been supplied with a concentrated starch-iodine complex in solution you can use to practice pipetting. Starch is a polysaccharide made up of long chains of glucose molecules. It naturally occurs in two forms: amylose, a linear chain, and amylopectin, a branching chain. Amylose reacts with iodine, soluble as part of the

compound potassium iodide (KI), to create a rich blue color, which your TAs will have prepared for you to dilute.

Your TAs will put 1.1 mL of starch-iodine solution on your bench in a 1.5 mL Eppendorf tube. You will create four 10× serial dilutions. You will also use these serial dilutions to get a handle on using a spectrophotometer, which measures absorbance of light along the solutions' path.

1. Set the volume of your P-1000 to 900 µL by rotating the plunger button until this desired volume appears in the display window. Remember that regardless of the volume, pipettes have three digit displays—the digits represent different volume increments.
2. Pipette 900 µL with provided filtered water using a P-1000 into four empty 1.5 mL eppendorf tubes.
3. Next, using a P-100 or P-200, take 100 µL of the concentrated starch-iodine solution and insert it into one of the 1.5 mL eppendorfs with filtered water.
4. Immerse the tip 2 to 4 mm into the liquid. Then, keeping your pipette vertical, mix the solution by pressing the plunger down **to the first stop** and releasing several times.
5. To fully dispense the liquid, depress to the second stop. This will "blow out" any remaining liquid in the tip.
6. Withdraw the tip from the sample, and eject it into the appropriate waste container by pushing the eject button.
7. With a new pipette tip, take 100 µL of the first dilution (not the concentrated stock) and repeat the process in the second Eppendorf tube, pipetting up and down to mix.
8. Repeat for all four solutions.

You should have a beautiful blue gradient of 1 mL dilutions!

## 3.3 Spectrophotometry

### 3.3.1 Spectrophotometry of starch-iodine serial dilutions

1. Pipette 1 mL of the concentrated starch-iodine solution and the four 1 mL 10x serial dilutions into 5 provided cuvettes.
2. Pipette 1 mL of filtered water into a 6th cuvette.
3. Set your spectrophotometer to measure optical density at 600 nm and blank it using the filtered water cuvette.

4. One by one, insert the 5 colored starch-iodine cuvettes into the spectrophotometer and record the results.

Amylose in starch is broken down by the enzyme amylase, which is found in our saliva! Talk to your TA about pipetting a small amount of your saliva into the starch serial dilutions and watching the blue color fade. This will also give you practice pipetting small amounts of viscous solution, which can be tricky.

## 3.4 Preparation of restriction digest reactions

We will prepare five mixtures for analysis of restriction digests. Three mixtures will use the pZE21-LacZ plasmid, and two will use λ DNA. The λ DNA has already been cleaved by HindIII.

| | |
|---|---|
| **$\lambda$ control**: | Predigested HindIII λ DNA |
| **$\lambda$ double digest**: | EcoRI + predigested HindIII λ DNA |
| **plasmid control**: | pZE21-LacZ with no restriction enzymes |
| **plasmid single digest**: | pZE21-LacZ + HindIII |
| **plasmid double digest**: | pZE21-LacZ + HindIII + KpnI |

Label five Eppendorf tubes for these reaction mixtures. You will add reagents to the tubes according to Tables 2 through 5. You will need to calculate the volume of each reagent to add. Note that supplied buffer is 10× concentrated, so you need to compute the volume that will give you the desired dilution.

For each reaction mixture, add the reagents in the order given in the table. Add all reagents except the enzymes. The restriction enzymes are supplied in a viscous, glycerol containing solution. Your TAs will pass around the enzymes on ice after you have added all of the other reagents. Avoid touching the bottom of the enzyme tube with your hand, as the heat from your hand can denature the enzymes. And, of course, pipette with care!

After you have assembled your reaction mixtures, you should mix them by *gently* vortexing, spin them down briefly, and place them in an incubator at 37°C for the remainder of the lab session (≈2 hours). Your TAs will freeze them afterwards for later use in gel electrophoresis.

Table 2: Recipe for $\lambda$ **control**

| Reagent | Desired amount | Added volume (µL) |
|---|---|---|
| Sterile water | fill to total | |
| Predigested λ DNA (0.5 µg/µL) | 1.5 µg | |
| CutSmart Buffer (10×) | 1× | |
| Total | 15 µL | 15 µL |

Table 3: Recipe for $\lambda$ **double digest**

| Reagent | Desired amount | Added volume (µL) |
|---|---|---|
| Sterile water | fill to total | |
| Predigested λ DNA (0.5 µg/µL) | 1.5 µg | |
| CutSmart Buffer (10×) | 1× | |
| EcoRI-HF (10 units/µL) | 1 µL | 1 µL |
| Total | 15 µL | 15 µL |

Table 4: Recipe for **plasmid control**

| Reagent | Desired amount | Added volume (µL) |
|---|---|---|
| Sterile water | fill to total | |
| pZE21-LacZ DNA (100 ng/µL) | 300 ng | |
| CutSmart Buffer (10×) | 1× | |
| Total | 15 µL | 15 µL |

Table 5: Recipe for **plasmid single digest**

| Reagent | Desired amount | Added volume (µL) |
|---|---|---|
| Sterile water | fill to total | |
| pZE21-LacZ DNA (100 ng/µL) | 300 ng | |
| CutSmart Buffer (10×) | 1× | |
| HindIII-HF (10 units/µL) | 1 µL | 1 µL |
| Total | 15 µL | 15 µL |

Table 6: Recipe for **plasmid double digest**

| Reagent | Desired amount | Added volume (µL) |
|---|---|---|
| Sterile water | fill to total | |
| pZE21-LacZ DNA (100 ng/µL) | 300 ng | |
| CutSmart Buffer (10×) | 1× | |
| KpnI-HF (10 units/µL) | 1 µL | 1 µL |
| HindIII-HF (10 units/µL) | 1 µL | 1 µL |
| Total | 15 µL | 15 µL |

## 3.5 Gel electrophoresis of restriction digests

The DNA ladders are pre-prepared by your TAs. You will prepare a mixture of your restriction digest solutions with loading dye and then load, run, and image the gel.

1. For each of your digestion reactions, add the following *in order* into a labeled Eppendorf tube.

| Reagent | Volume |
|---|---|
| Restriction digest solution | 10 µL |
| 6× DNA loading dye | 2 µL |

2. Gently vortex to mix your samples.
3. Spin down your samples briefly to get rid of any bubbles.
4. Load your samples onto the agarose gel prepared by your TAs. The gel has 15 lanes. You will share your gel with another group. One group (say group A) will use lanes 1 through 6, and the other (group B) will use 9 through 14. See the table below for lane assignments. **Be sure to write these lane assignments in your lab notebook as well.**

   For ladders, load **all** 5 µL. For restriction digest mixtures, **only** load 10 µL.

   To load the gel, place your pipette beneath the buffer surface layer, partway into your target well. **Very slowly** depress the pipette plunger to load your sample. As you depress the plunger, you will notice the sample fall into the well. Do not rush! This may cause the sample to flow out of the well. Loading a gel can be a tricky business. Make sure you see a TA do it first before attempting it yourself.

| Lane | Sample |
|---|---|
| 1 | |
| 2 | 100 bp DNA ladder |
| 3 | Group 1: λ control |
| 4 | Group 1: λ double digest |
| 5 | Group 1: Plasmid single digest |
| 6 | Group 1: Plasmid double digest |
| 7 | Group 1: Plasmid control |
| 8 | |
| 9 | Group 2: λ control |
| 10 | Group 2: λ double digest |
| 11 | Group 2: Plasmid single digest |
| 12 | Group 2: Plasmid double digest |
| 13 | Group 2: Plasmid control |
| 14 | 1 kbp DNA ladder |
| 15 | |

5. After your gel is loaded, ask your TA to help set up the power supply. Run the gel at 110V for 25 minutes.

# 4 Assignment

**Problem 0** (Summary)**.**
Write a summary of the restriction digest experiment, its objectives, and conclusions between one paragraph and one page in length.

**Problem 1** (Set-up of restriction digest reactions (**in lab**))**.**
Fill out tables 2 through 5 to determine what volumes you need to add to your Eppendorf tubes for the restriction digest reactions.

**Problem 2** (Sticky ends in restriction digests)**.**
Which of the three restriction enzymes we used in lab result in 3′ overhangs? Which result in 5′ overhangs?

**Problem 3** (Expected fragment lengths)**.**
New England Biolabs (NEB) has a guide with the cut sites of λ DNA for various restriction enzymes. You can get it from the Bi 1x course website. For the enzymes we used to cut λ DNA, use the data in that document to compute the fragment sizes for

a) Single digestion with HindIII

b) Single digestion with EcoRI

c) Double digestion with HindIII and EcoRI.

**Problem 4** (Your fragment lengths)**.**
Use your gel images to estimate the lengths of the DNA fragments you got from your restriction digests. How do these compare to the expected lengths?

**Problem 5** (Absorbance measurements)**.**
Make a plot of OD starch/iodine concentration from your serial dilution-absorbance measurements. Your concentration should be in units of the most concentrated solution. For example, a $100\times$ dilution has a concentration of 0.01. Does the plot make sense?

**Problem 6** (Safety always on the mind)**.**
List three hazards in the Bi 1x lab and how you will protect yourself from them.

**Problem 7** (Data)**.**
Attach all data and sample images not specifically asked for in the other problems. Present them neatly and clearly.