AMGEN® Biotech Experience

Scientific Discovery for the Classroom

MiniOne® Short Student Guide

30 YEARS

TSN

TEACHER SCIENTIST NETWORK

About the Amgen Biotech Experience

Genetic engineering is a branch of biotechnology that uses special procedures and techniques to change an organism's *DNA*. This ability has had a huge impact on the field of medicine, as genetically modified bacteria can make human *insulin* (the hormone responsible for regulating glucose levels in the blood) and other life-saving products. It's rare for secondary school or college students to have the chance to learn about and actually practice the procedures and techniques that are the foundation of the biotechnology industry—but in this program, you will have just that opportunity. As you work in the laboratory and carry out the very experiments that led to breakthroughs in biotechnology, you will gain hands-on experience of producing genetically modified bacteria.

The procedures in this program were developed through a series of discoveries that led to important breakthroughs in biotechnology. Some of the pioneering scientists who made these discoveries received Nobel Prizes, the highest distinction awarded to scientists in these fields from around the world. Werner Arbor, Daniel Nathans and Hamilton Smith received the Nobel Prize in Physiology or Medicine in 1978 for their work with restriction enzymes. Stanley Cohen, Paul Berg and Herb Boyer received the Nobel Prize for Chemistry in 1980 for making the first recombinant DNA molecule. Kary Mullis received the Nobel Prize for Chemistry in 1993 for his discovery of the Polymerase Chain Reaction. The work that you are about to do is based on this Nobel Prize-winning science—science that is significant and will continue to play an important role in the development of biotechnology and medicine. You will follow in the footsteps of the many scientists who have pushed and continue to push the boundaries of biotechnology. There are many advances still to be made—and students who decide to continue studying this field may contribute to those advances.

The Amgen Biotech Experience (formerly Amgen-Bruce Wallace Biotechnology Lab Program) had humble beginnings almost 25 years ago with visionary scientists and teachers who shared passion and energy for sharing their knowledge with students. Bruce Wallace, one of Amgen's first staff members, wanted all students to experience the joy of discovery and the excitement of having science at their fingertips. A desire for more robust science education at schools near Amgen's global headquarters led to involving local teachers and, later, a college professor, in developing curriculum and training educators in biotechnology. The program grew through word of mouth and teacher interest and expanded over time to other states and countries.

Cover image courtesy of embitec.com

Your Amgen Biotech Experience challenge

Your challenge in the Amgen Biotech Experience is to successfully carry out the steps of the genetic engineering process that are used to make insulin and other genetically engineered products. You will learn and practice the techniques and procedures that are part of this process. If you carry out all the steps in the program, you can create your own genetically modified bacteria.

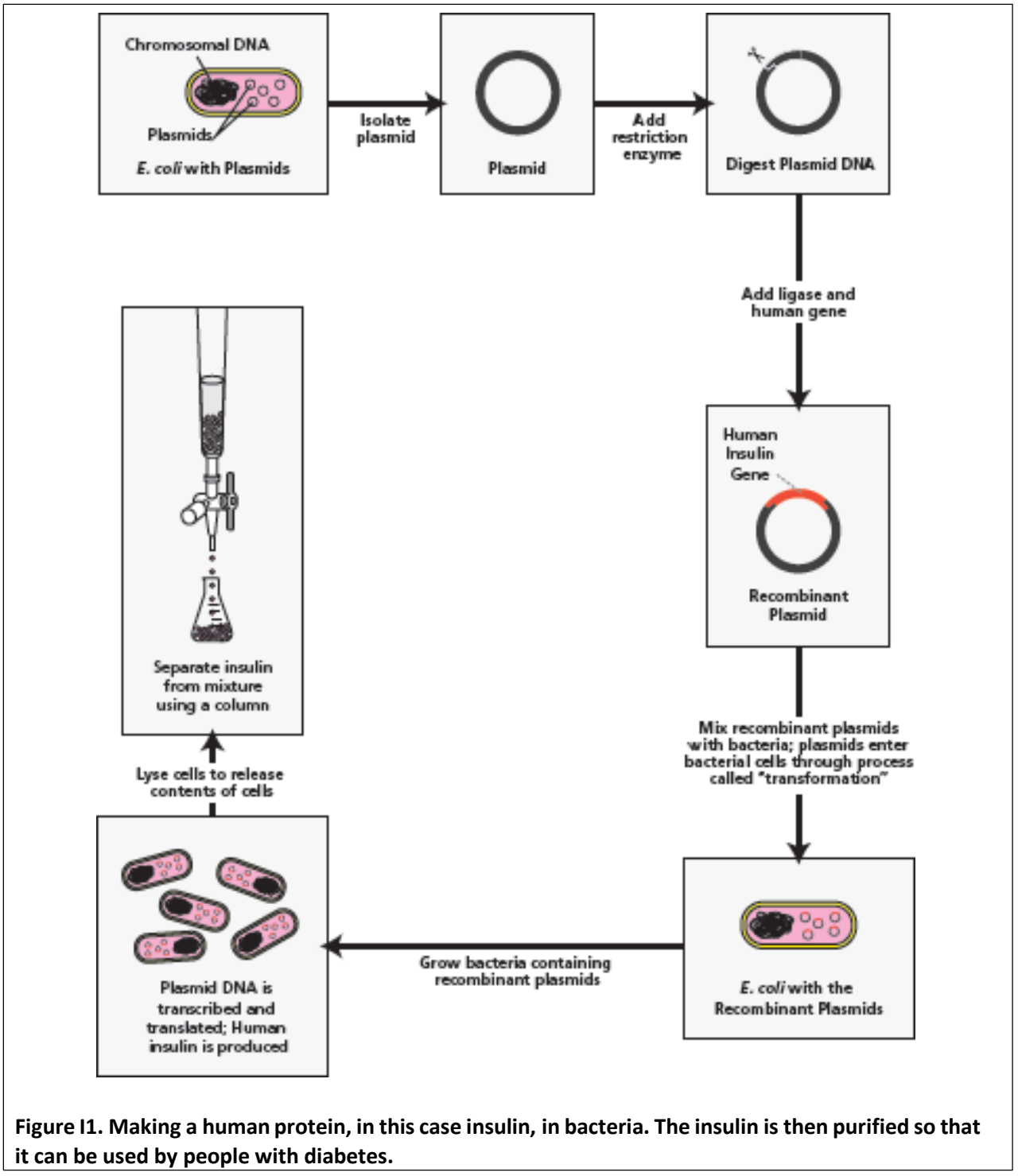


Figure I1. Making a human protein, in this case insulin, in bacteria. The insulin is then purified so that it can be used by people with diabetes.

Instead of cloning insulin or another human gene, you will work with a gene from a *sea anemone*, a soft-bodied animal related to coral and jellyfish. The gene is called *rfp* and the protein made by this gene is called red fluorescent protein. It has been altered by directed evolution to fluoresce even more brightly than the original wild-type would have done. If you are successful the bacteria you create will have a new and highly visible trait: they will produce red fluorescent protein and glow red when viewed on agar plates!

Figure I2. A fluorescing sea anemone.

Contents

Chapter 1: Some tools of the trade

Introduction

The year 1978 marked a major breakthrough in medicine. For the first time ever, scientists were able to induce bacterial cells to make human insulin by inserting human DNA into the cells. This new technology, termed genetic engineering, can be used to make products that treat the symptoms of certain genetic diseases.

To carry out genetic engineering, you need good laboratory skills. In this chapter, you'll focus on gaining practice in the use of micropipettes (instruments used to accurately transfer small volumes of liquid) and gel electrophoresis (a technique for separating and identifying biomolecules) - two critical skills for biotechnology. You can complete two practice exercises, using instruments and supplies that are identical to the ones used in biotechnology research laboratories. These practical activities are the first step in building the skills you'll need to carry out the subsequent labs and complete your challenge to create genetically modified bacteria.

The micropipette is an important tool used in genetic engineering. It is used to transfer very small and exact volumes of liquids in either millilitres (mL, 1/1,000 or one thousandth of a litre) or microlitres (µL, 1/1,000,000 or one millionth of a litre), which are the measurements of volume most often used in genetic engineering. Molecular biologists use such small volumes because of the cost of biomolecules and the difficulty purifying them from the thousands of other substances in a cell. This laboratory will give you the chance to learn how to use the micropipette to transfer very small, exact volumes of reagents and to see the relative amounts of solution measured by this very precise tool.

Gel electrophoresis is a technique used to separate a mixture of biomolecules, including DNA, by their size. The biomolecules to be separated have (or are prepared with) a negative charge, which means that they will move through a gel towards a positive electrical charge. Speed of movement through the gel varies primarily according to their weight, although molecular shape and degree of charge also influence their movement. In the genetic engineering process, gel electrophoresis is used to separate and characterise plasmids and short linear pieces of DNA.

Learning objectives

By the end of this laboratory you will be able to:

- Use a micropipette correctly
- Explain the importance of a micropipette
- Use gel electrophoresis
- Explain the purpose of gel electrophoresis

Laboratory 1.1: Using a micropipette

Aims

To use a micropipette and examine the volumes it can dispense.

Materials

EQUIPMENT & SUPPLIES

P-20 micropipette (2-20 µL)
Disposable pipette tips
Laminated practice sheet
Waste container

REAGENTS

Red dye solution (RD)

Health and safety

As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Methods

1. Check that you have all the materials
2. Review the parts of a micropipette in Figure 1.1 opposite.
3. Find the display window on the micropipette
4. Turn the plunger button on the top of the micropipette clockwise (right) to decrease the volume, or anti-clockwise (left) to increase the volume.

⚠ Never set the P-20 micropipette lower than 2.0 µL or higher than 20.0 µL or you could damage the equipment.

5. Figure 1.2 shows five micropipette volumes. Practice setting the micropipette to these volumes.

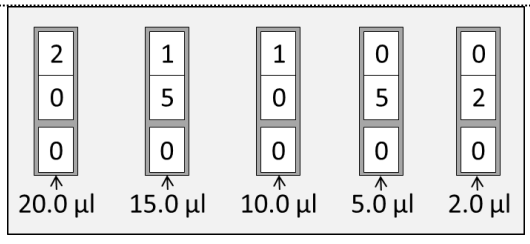


Figure 1.2 The display window for five micropipette volumes.

The display window of a micropipette shows how much fluid it will aspirate (suck up) and dispense. Five examples of displays and the corresponding amounts are shown.

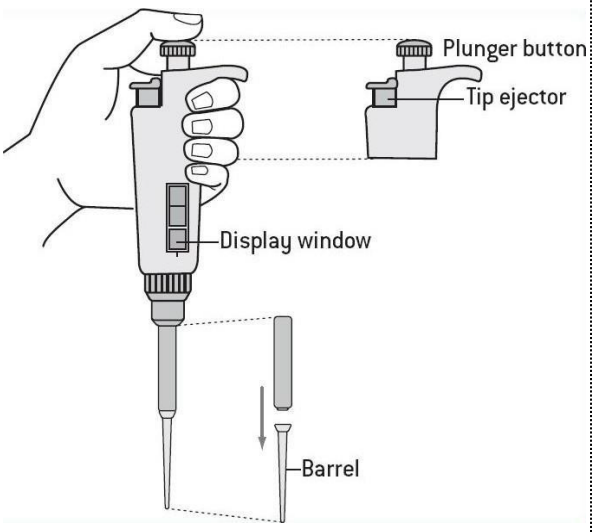


Figure 1.1 A P-20 micropipette

6. Look at the laminated micropipette practice sheet. Each group member will pipette five drops of different volumes onto the sheet. Pipetting consists of two parts: loading the liquid into the micropipette, and dispensing the liquid from the micropipette.
7. Load the micropipette with 20.0 µL of red dye solution (RD) as follows:
   a. Set the P-20 micropipette to 20.0 µL in the display window.
   b. Open the tip box. Lower the micropipette onto a tip and press down firmly (do not touch the tip with your fingers). Close the box when done.
   c. Bring the micropipette and the RD tube to eye level.
   d. Use your thumb to press the plunger to the first stop position (figure 1.3a), which is your first point of resistance.
   e. Put your pipette tip into the RD and slowly release the plunger to draw up the solution.

⚠ When loading the micropipette, only press the plunger to the first stop or you will draw too much solution into the pipette tip.

⚠ Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward because fluid could leak back into the pipette.

8. Dispense RD onto the laminated sheet by doing the following:
   a. Place the pipette tip over the 20.0 µL circle.
   b. Use your thumb to press the plunger to the first stop position and then press down to the second stop (figure 1.3b).

⚠ When dispensing liquid from the micropipette, press the plunger to the second stop in order to dispense all of the liquid.

   c. With the plunger still depressed, pull the pipette out of the liquid – this prevents you from accidentally pulling the liquid back into the tip.
9. Without setting down the micropipette, twist the plunger button to set it to 15.0 µL and repeat steps 7c–9, placing the pipette tip over the 15.0 µL circle when dispensing the liquid.
10. Without setting down the micropipette, twist the plunger button to set it to 10.0 µL and repeat steps 7c–9, placing the pipette tip over the 10.0 µL circle when dispensing the liquid.
11. Without setting down the micropipette, twist the plunger button to set it to 5.0 µL and repeat steps 7c–9, placing the pipette tip over the 5.0 µL circle when dispensing the liquid.
12. Without setting down the micropipette, twist the plunger button to set it to 2.0 µL and repeat steps 7c–9, placing the pipette tip over the 2.0 µL circle when dispensing the liquid.
13. Use the tip ejector to place your pipette tip into the waste container.

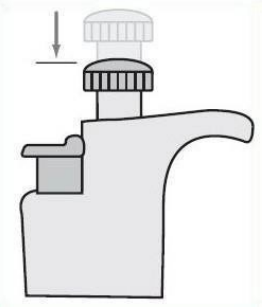


Figure 1.3a The first stop

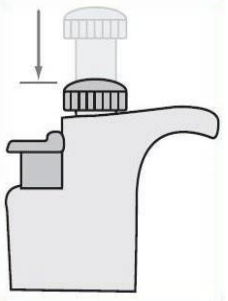


Figure 1.3b The second stop

14. Using the micropipette practice sheet, make sure that each person in your group pipettes five drops of different volumes, with each person using a new pipette tip.
15. When everyone in your group has had a chance to dispense RD onto the micropipette practice sheet, draw the approximate sizes of each drop in your notebook (or take a photograph and tape it into your notebook) and label them with the amounts.

Questions

1. When loading or dispensing a solution, why is it important to actually see the solution enter or leave the pipette tip?
2. You were instructed to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important?
3. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology?

Laboratory 1.2: Gel electrophoresis

Aims

To use gel electrophoresis and understand its purpose during the genetic engineering process.

Introduction

Gel electrophoresis involves placing an agarose gel into an electrophoresis tank of buffer, which has two electrodes either end that create an electric field across the gel when attached to a powerpack. The agarose for the gel is a type of polysaccharide (complex sugar) found in seaweed. An agarose gel looks like a lump of jelly, but it is a porous matrix (like a sponge) with lots of microscopic holes through which the solution and biomolecules flow.

DNA is negatively charged. When loaded into holes in the gel, known as wells, next to (black) negative electrodes, it will migrate towards the positive electrode (red) when an electric current is applied. As it has to move through the porous matrix of the agarose gel, smaller DNA molecules move faster and larger DNA molecules move more slowly. Figure 1.4 illustrates the separation of biomolecules by gel electrophoresis.

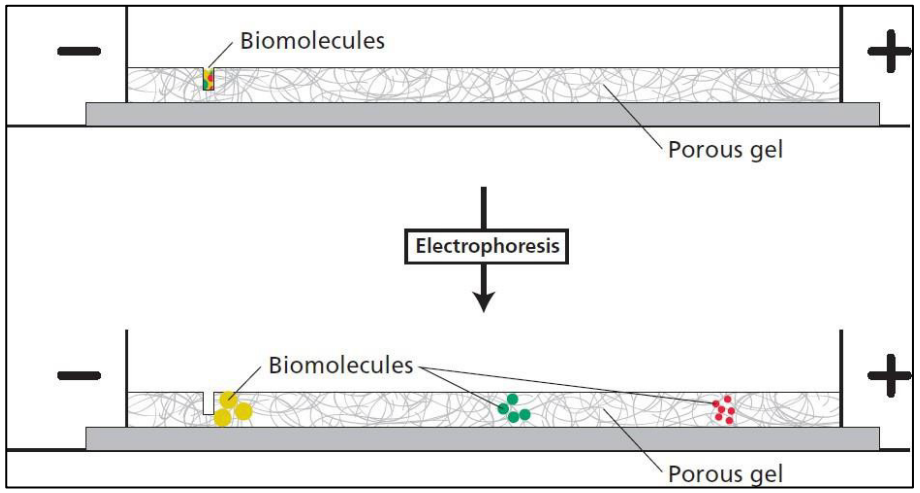


Figure 1.4 Biomolecules, including DNA, moving through the agarose gel matrix in gel electrophoresis.

Materials

EQUIPMENT & SUPPLIES

P-20 micropipette (2-20 µL)
Disposable pipette tips
Microcentrifuge
MiniOne electrophoresis tank
Waste container
Agarose plates (Lab 1.2) – prepared in advance (see p. 5.4 for detailed instructions)

REAGENTS

Microfuge tubes containing:

- Red dye solution (RD)
- Dye solution 1 (S1)
- Dye solution 2 (S2)
- Dye solution 3 (S3)

1 x TAE buffer

Health and safety

The dyes in solutions 1 to 3 do not require any special handling instructions, however they may stain the skin, so try to avoid contact with them as good practice would dictate. You should wear safety glasses and chemical-resistant gloves when using the electrophoresis buffer, to avoid contact with chemicals which may irritate your eyes or skin.

Finally, the electrophoresis chamber contains 1 X TAE buffer at approx. 40 volts. To prevent contact with the liquid during electrophoresis the tanks are designed so that the electric supply is cut off whenever the orange photo-lid is removed. The buffer sits in the gel tank, separate from the carriage (which has the electrical connections).

Methods

PART A: PIPETTING INTO WELLS

You may have a chance to practice pipetting red dye (RD) into the wells of an agarose plate.

1. Cover the agarose plate with water, so that the 'dimples' over the wells disappear.
2. Set the P-20 micropipette to 10.0 µL and put on a pipette tip.
3. Load the pipette with 10.0 µL of RD.
4. Dispense RD into a well in one of the practice plates by doing the following:
   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
   b. Lower the pipette tip until it is under the buffer but just above the well.

⚠ Be careful not to place your pipette tip into the well or you might puncture the gel and lose your sample out the hole.

   c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.
5. Repeat steps 3 and 4 until all the practice plate wells have been filled. Everyone in your group should get an opportunity to practice pipetting into the wells.
6. Eject the pipette tip into the waste container.

PART B: SEPARATING DYES WITH GEL ELECTROPHORESIS

You will use gel electrophoresis to separate different dyes. First you will add dyes into wells in the agarose gel in an electrophoresis tank. Then you will turn the electrical current on to separate the negatively charged dye molecules in the gel. (You will share the electrophoresis boxes with one other group: your teacher will tell you which wells your group should use.)

1. Check your rack to make sure that you have the three dye solutions (S1, S2, and S3).
2. Check to make sure that the wells in your gel are located near the negative electrode (figure 1.5) and that the grey gel tray platform is on the base of the gel tank.

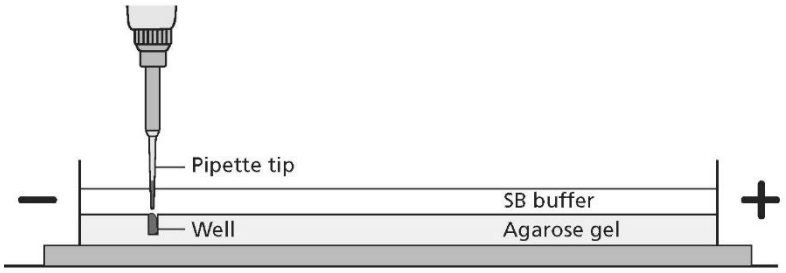


Figure 1.5 The gel electrophoresis unit

3. Fill the tank with 1x TAE buffer to the marked on the tank it should just cover the entire surface of the gel (approx. 140 ml.)
4. Use the microcentrifuge to pool the dye in the bottom of the S1, S2, and S3 tubes.

   ⚠ *Distribute the tubes evenly in the microcentrifuge so that their weight is balanced*.
5. Make a drawing in your notebook that shows which solution you have placed in each well.
6. Switch on the low-intensity blue light, this will make it easier to see the wells (the lower button in the top right corner)
7. Load 10.0 µL of S1 into the pipette.
8. Dispense the S1 into the well you've designated for that solution by doing the following:
9. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
10. Lower the pipette tip until it is under the buffer but just above the well.

    ⚠ *Be careful not to place your pipette tip into the well or you might puncture the gel and lose your sample out the hole*.
11. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

    ⚠ *Use a fresh pipette tip for each sample*.
12. Repeat steps 7 and 8 for S2 and S3, using a new pipette tip to load each solution into a new well of the agarose gel.
13. When all the samples have been loaded, place the orange photo hood on to the electrophoresis box.
14. To turn on, press the power button (bottom right-hand corner); a solid green light indicates it is working. If the light flashes please inform your teacher.
15. After two or three minutes, check to see if the dyes are moving toward the positive electrode. You should see the purple dye (bromophenol blue) beginning to separate from the blue dye (xylene cyanole).
16. In approximately 10 minutes, or when you can distinguish all three dyes, turn off the power switch.
17. Carefully remove the photo hood from the gel box and observe the dyes in the gel.
18. In your book, draw the relative location of the bands and their colours in each of the lanes containing your samples.
19. Leave the gels in the gel box.

Questions

1. What electrical charge do the dyes have? Explain how you know.
2. Study your gel electrophoresis results. Which solution sample contained a single dye: S1, S2, or S3? How do you know?
3. From your gel electrophoresis results, put the dyes into size order from largest to smallest. Explain your reasoning.
4. The molecular weights (sizes) for the dyes are 452.38 atomic units (au) for orange G, 669.98 au for bromophenol blue, and 538.62 au for xylene cyanole. How do these weights compare with your original conclusions about the sizes of the dye molecules?
5. Can you suggest any other factors that may have played a role in the separation of these dyes?
6. When might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA?

Chapter 2: Beginning to clone a gene

Introduction

Plasmids and restriction enzymes are biomolecules found in many bacteria. Their discovery was crucial to genetic engineering as they are used in the process that scientists have developed to modify the genetic material in microorganisms so that they produce useful biomolecules. The biomolecules that microorganisms produce can be medicines that save lives, such as insulin. This chapter is about plasmids, restriction enzymes and their role in cloning a gene (that is copying a gene from one location to another).

Many bacteria carry two forms of DNA: (1) a single chromosome made up of a large DNA molecule that contains all the information needed by the organism to survive and reproduce, and (2) plasmids, which are small circular DNA molecules, present in multiple copies, separate from the chromosomal DNA. This is shown in figure 2.1.

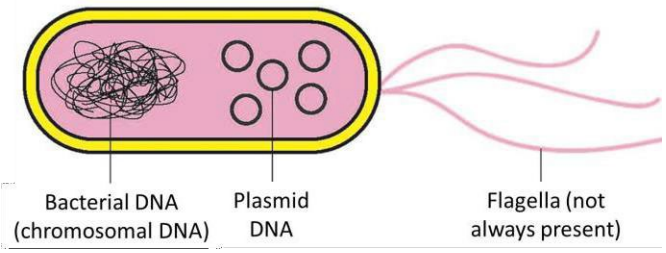


Figure 2.1. DNA in bacterial cells

Plasmids are vectors: that is vehicles for carrying DNA sequences from one organism to another. They are replicated within cells. Cells containing a plasmid can be selected by their resistance to antibiotics and if a gene of interest is inserted by the promoter sequence, the gene of interest will be expressed. So, in simple terms, if the plasmid DNA can be cut up and stuck back together to include the gene of interest, then bacteria containing the plasmid will make the gene product of interest.

The discovery of restriction enzymes in the early 1950s provided the tool for 'cutting up' the DNA sequence. Restriction enzymes are proteins that bacteria produce to recognise and destroy bacteriophage (viruses that infect bacteria) DNA without damaging the host (bacterial) DNA. Restriction enzymes from different strains of bacteria cut at specific DNA sequences, called recognition sites.

During this laboratory you will use restriction enzymes to produce the DNA fragments that will be joined to make a recombinant plasmid that can make a red fluorescent protein in bacteria. You will be supplied with 2 plasmids. Both plasmids contain the site for initiating DNA replication (*ori*). In addition, the first plasmid, pKAN-R, carries the gene that makes bacteria resistant to the antibiotic kanamycin (*kanR*), the red fluorescent protein gene (*rfp*) and a promoter sequence (*pBAD*). The second plasmid, pARA, contains the gene that makes bacteria resistant to the antibiotic ampicillin (*ampR*) and an activator (*araC*) (figure 2.2).

The kanamycin resistance gene *(kanR)* encodes a phosphotransferase enzyme that transfers a phosphate group to the kanamycin molecule destroying its antibiotic effects. The ampicillin resistance gene (*ampR*) encodes the protein beta lactamase, an enzyme that destroys the antibiotic ampicillin enabling bacteria to reproduce in the presence of ampicillin. The arabinose activator (*araC*) is a specific DNA sequence that activates a promoter when the bacteria are grown in the presence of arabinose, a five-carbon sugar that naturally occurs in various plant and bacterial carbohydrates. If arabinose is present in the bacteria, the activator causes the promoter to bind RNA polymerase, and transcription will occur. If arabinose is not present, the activator prevents the promoter from binding RNA polymerase, and transcription will not occur.

The location of these DNA sequences, restriction enzyme sites and size of the plasmids (measured in base pairs) are shown in figure 2.2. A 'base pair' is either adenine: thymine or guanine: cytosine and is the common method used to give the size of DNA molecules.

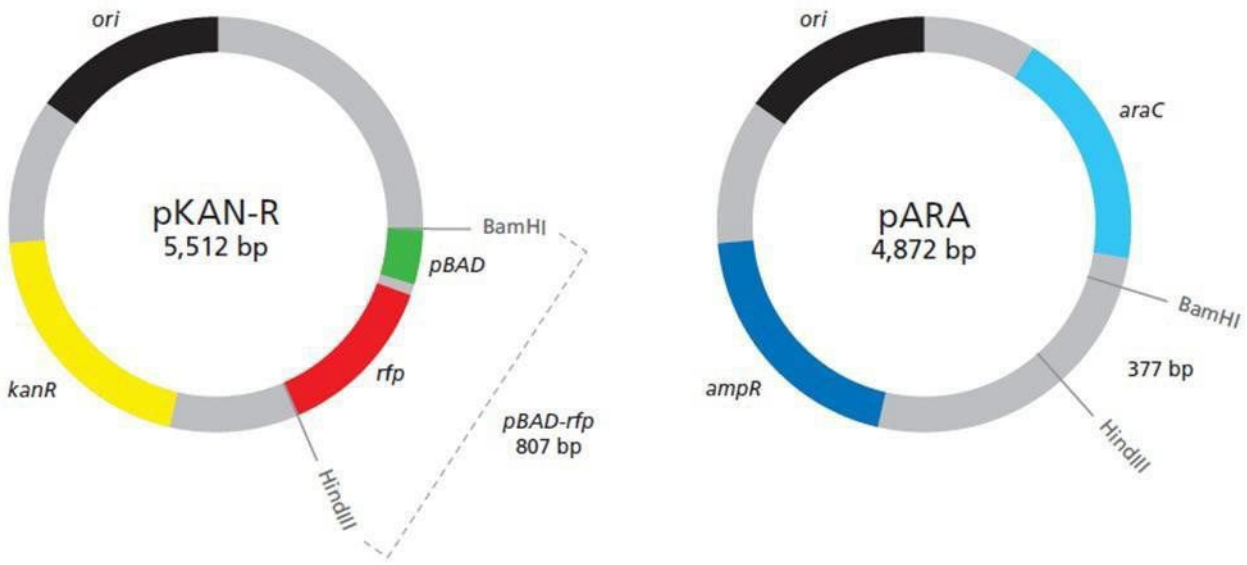


Figure 2.2 Plasmid maps showing the location of the *rfp* gene, the promoter (*pBAD*), the kanamycin resistance gene (*kanR*), the ampicillin resistance gene (*ampR*), the arabinose activator (*araC*), and the origin of replication sites (*ori*).

To clone the *rfp* gene from the plasmid pKAN-R into the expression plasmid pARA, you will use 2 restriction enzymes: *Bam*HI and *Hin*dIII. These will excise the *rfp* gene and promoter from the pKAN-R plasmid and a short 377 bp fragment from the expression plasmid pARA. Using two different restriction enzymes allows the inserted gene to be oriented in the correct position for transcribing the "sense" strand of DNA (the strand that codes for the protein), and it prevents the plasmid from reforming a circle without the inserted gene.

Learning objectives

By the end of this laboratory you will be able to:

- Describe the characteristics of plasmids
- Explain how plasmids are used in cloning a gene
- Describe the function of restriction enzymes
- Explain how to use restriction enzymes to create a recombinant plasmid

Laboratory 2: Restriction digestion of plasmids

Aims

To use restriction digestion with two enzymes (*Bam*HI and *Hin*dIII) to create linear DNA fragments containing (i) DNA sequence for gene expression (from the plasmid pARA), and (ii) the red fluorescent protein gene (from the plasmid pKAN-R).

Materials

EQUIPMENT & SUPPLIES

P-20 micropipette (2-20 µL)
Disposable pipette tips
4, 1.5 mL microfuge tubes
Permanent marker
Floating tube rack
Waste container
Microcentrifuge (shared)
37°C water bath (shared)

REAGENTS

Microfuge tubes containing:

- 2.5 X restriction buffer (RB)
- pKAN-R plasmid (K)
- pARA plasmid (A)
- restriction enzymes *Bam*HI and *Hin*dIII (RE)
- Distilled water (dH_2O)

Health and safety

In this laboratory you will be using biological materials, albeit in tiny amounts, and they should be used in a controlled way, using techniques that mirror the aseptic techniques used in microbiology. All of the materials that come into contact with the biological materials (pipette tips and microfuge tubes) will be autoclaved to denature the DNA and enzymes before disposal. As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Methods

1. Check your rack to make sure that you have all the reagents listed.
2. Use a marker to label four clean microfuge tubes as follows: K+, K–, A+, and A–. (Also include a personal identifier on each tube.)
3. Review table 2.1, which summarises the reagents that you will add in step 4.

Step	Reagent	K+ tube	K- tube	A+ tube	A- tube
4a	Restriction buffer (RB)	5.0 µL	5.0 µL	5.0 µL	5.0 µL
4b	pKAN-R plasmid (K)	5.0 µL	5.0 µL		
4c	pARA plasmid (A)			5.0 µL	5.0 µL
4d	*Bam*HI and *Hin*dIII (RE)	2.0 µL		2.0 µL	
4e	Distilled water (dH_2O)		2.0 µL		2.0 µL

Table 2.1 Reagents to add to the K+, K-, A+ and A- tubes

⚠ In step 4, be sure to use a new micropipette tip for each reagent in each tube to avoid contamination.

4. Add the following:
   a. 5.0 µL of restriction buffer (RB) to the K+, K–, A+, and A– tubes.
   b. 5.0 µL of plasmid pKAN-R (K) to the K+ and K– tubes.
   c. 5.0 µL of plasmid pARA (A) to the A+ and A– tubes.
   d. 2.0 µL of *Bam*HI and *Hin*dIII restriction enzymes (RE) to the K+ and A+ tubes. Add the enzymes directly into the solution at the bottom of the microfuge tube. Gently pump the solution in and out of the pipette 3 times, by repeatedly moving the plunger to the first stop and back up again, to mix the reagents. Cap the tubes when done.
   e. 2.0 µL of dH_2O to the K– and A– tubes. Gently pump the solution in and out with the pipette to mix the reagents. Cap the tubes when done.
5. Spin the four microfuge tubes (K+, A+, K–, and A–) in the microcentrifuge for four seconds to pool the reagents at the bottom of each tube.

⚠ Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.

6. Place all four tubes into the 37°C water bath and incubate for one hour.
7. After the incubation is complete either continue to Laboratory 3, or place all four tubes in the freezer at –20°C until you are ready to do so.

Questions

1. Using Figure 2.2, work out how many linear fragments will be produced from the plasmids pKAN-R and pARA, when they are digested with *Bam*HI and *Hin*dIII. What size will these linear fragments be?
2. Draw the linear fragments, marking on the genes and important sequences. Using table 2.2 below, include the nucleotide sequence of the sticky ends.

Source	Restriction enzyme	Recognition site
Bacillus amyloliquefaciens	*Bam*HI	5′ G↓GATCC 3′ 3′ CCTAG↑G 5′
Haemophilus influenzae	*Hin*dIII	5′ A↓AGCTT 3′ 3′ TTCGA↑A 5′

Table 2.2 The restriction enzymes used in this laboratory.

Information on the bacterial strains they were isolated from and the DNA sequences that they cut. In the examples shown, the enzymes cut asymmetrically on the strands of DNA, leaving single-stranded overhanging sequences at the site of the cut. Cuts are shown by
arrows. For example, a cut (or digestion) with *Bam*HI will leave an GATC overhang (or “sticky end”) on one strand and a CTAG sticky end on the other strand.

3. In order to create a plasmid that can produce the red fluorescent protein in bacteria, what components are needed in the plasmid?
4. Bacteria can be killed by an antibiotic unless they carry a plasmid that has the gene for resistance to that antibiotic. Biotechnologists call these genes *selectable markers* because only bacteria that carry the gene will survive an antibiotic. If the uptake of DNA by bacteria is inefficient, why is a selectable marker critical in cloning a gene in bacteria?
5. What role do restriction enzymes have in nature?
6. Why might the enzymes work best at 37°C? Why should the unused enzymes then be placed in the freezer?
7. In step 4, you are asked to set up two tubes without the restriction enzymes, *Bam*HI and *Hin*dIII. What is the purpose of this step, and why is it important?
8. Using your understanding of evolution, why would bacteria retain a gene that gives them resistance to antibiotics? How is the existence of bacteria with antibiotic resistance affecting medicine today?
9. Bacteria, sea anemones, and humans seem, on the surface, to be very different organisms. Explain how a gene from humans or a sea anemone can be expressed in bacteria to make a product never before made in bacteria.
10. Due to a mishap in the lab, bacteria carrying a plasmid with a kanamycin-resistant gene and bacteria carrying a plasmid with an ampicillin-resistant gene were accidentally mixed together. Design an experiment that will allow you to sort out the two kinds of bacteria. (Hint: Make sure that you do not kill off one of the kinds of bacteria you are trying to sort out!)

Chapter 3: Building a recombinant plasmid

Introduction

Armed with ways to transfer genes between cells using plasmids as vectors and to generate fragments of DNA using restriction enzymes, the final essential tool required to make recombinant plasmids was a way to 'glue' the DNA fragments together. In the early 1960s the discovery of DNA ligase, an enzyme that catalyses the joining of DNA fragments, provided that tool for utilising in genetic engineering.

DNA ligase is one of several enzymes involved in the replication of DNA in all cells. During replication the two parent DNA strands separate at origin of replication, or *ori* sites. An enzyme called DNA polymerase adds new nucleotides complementary to the single-stranded parent DNA to form base pairs held together by hydrogen bonds. On one parent DNA strand the new, complementary daughter strand forms continuously. On the other parent strand of DNA the new, complementary nucleotides are added discontinuously. It is DNA ligase that then catalyses the formation of covalent bonds between these discontinuous DNA fragments to make a complete second daughter strand of DNA. Replication produces two new DNA molecules, each consisting of one parent strand and one daughter strand that are exact replicas of the original DNA molecule.

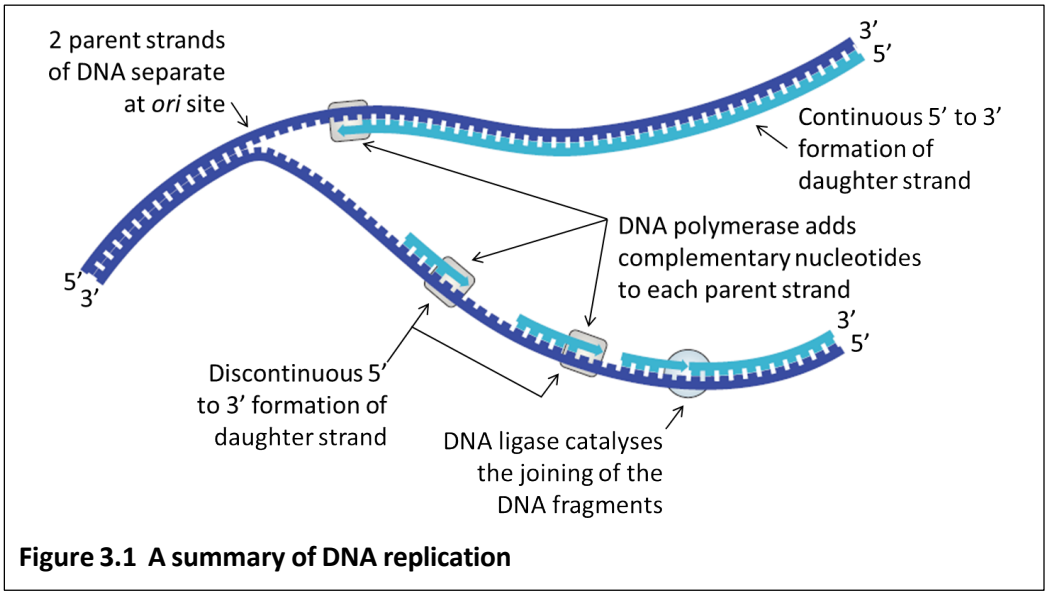


Figure 3.1 A summary of DNA replication

During the procedures used to make recombinant plasmids, DNA ligase is used to bond linear DNA fragments from restriction digestion together. Any two fragments that have ends cut with the same restriction enzyme can be ligated together. First the unpaired bases at the sticky ends form hydrogen bonds to each other, and then the ligase catalyses the formation of covalent bonds between the sugar and phosphate groups of adjacent nucleotides. If you have multiple fragments, the ligation procedure can lead to a number of possible products.

In this laboratory you will use DNA ligase to ligate the DNA fragments you produced during Laboratory 2 together, making new recombinant plasmids. These recombinant plasmids will contain the four restriction fragments from Laboratory 2 recombined in different ways to produce new sets of DNA. The ligation process will result in several different plasmids, but the plasmid that you are interested in will contain the *ori* sequence for the initiation of DNA replication, the gene for ampicillin resistance (*ampR*), the arabinose activator sequence (*araC*), a promoter sequence for initiating transcription (*pBAD*) and the red fluorescent protein (*rfp*) gene. This desired recombinant DNA plasmid is called the pARA-R plasmid and is shown in Figure 3.2.

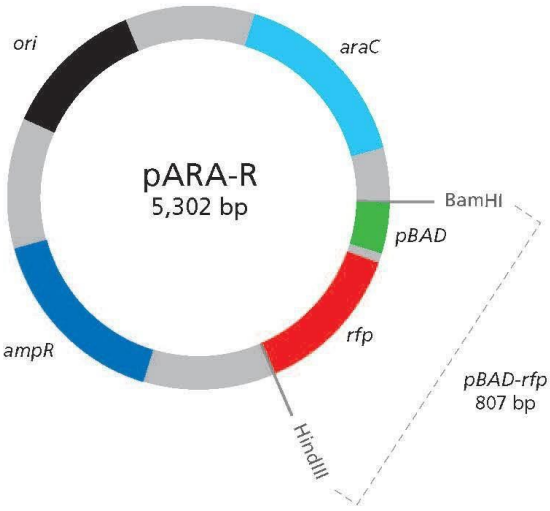


Figure 3.2 The pARA-R plasmid

Learning objectives

By the end of this laboratory you will be able to:

- Describe the role of a DNA ligase in replication
- Explain how DNA ligase is used to create a recombinant plasmid
- Describe possible recombinant plasmids that form when ligating your restriction digests

Laboratory 3: Ligating to build the pARA-R plasmid

Aims

To use the enzyme DNA ligase to catalyse covalent bond formation between the linear fragments with sticky ends formed by restriction digestion in laboratory 2, making new recombinant plasmids.

Materials

EQUIPMENT & SUPPLIES

P-20 micropipette (2-20 µL)
Disposable pipette tips
Permanent marker
Floating tube rack
Waste container
Microcentrifuge (shared)
70°C water bath (shared)

REAGENTS

Microfuge tubes containing:

- Digested pKAN-R plasmid from lab 2 (K+)
- Digested pARA plasmid from lab 2 (A+)
- Ligation buffer (LB)
- DNA ligase (LIG)
- Distilled water (dH_2O)

Health and safety

In this laboratory you will be using biological materials, albeit in tiny amounts, and they should be used in a controlled way, using techniques that mirror the aseptic techniques used in microbiology. All of the materials that come into contact with the biological materials (pipette tips and microfuge tubes) will be autoclaved to denature the DNA and enzymes before disposal. As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Methods

1. Check your rack to make sure that you have all the reagents listed.
2. Place K+ and A+ tubes from Laboratory 2 in a floating microfuge tube rack in the 70°C water bath for 30 minutes. This heat exposure will denature the restriction enzymes.
3. Label the LIG tube with your personal identifier.
4. After 30 minutes, remove the K+ and A+ tubes from the water bath and place them in your rack.
5. Add the following solutions directly into the 2.5 µL of DNA ligase solution at the bottom of the LIG tube:
   a. 6.0 µL of A+
   b. 6.0 µL of K+
   c. 4.0 µL of LB
   d. 2.0 µL of dH_2O

⚠ In steps 5a–d, be sure to use a new micropipette tip for each reagent to avoid contamination.

6. After adding the dH_2O, gently pump the solution in and out with the pipette to mix the reagents. Cap the tube when done.
7. Spin the LIG tube in the microcentrifuge for four seconds to pool the reagents at the bottom of the tube.

⚠ Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.

8. Place your LIG, A+, and K+ tubes in the microfuge racks designated by your teacher. (Your LIG tube will incubate at room temperature overnight then be stored in the fridge (4 °C) until the next class. Your A+ and K+ tubes will be returned to the –20°C freezer for Laboratory 5.)

Questions

1. What is the function of enzymes in reactions?
2. Based on your understanding of DNA replication, explain the role of DNA ligase in replication.
3. Describe what happens when two DNA fragments with complementary sticky ends join, and speculate how the activity of DNA ligase ensures that the join is permanent.
4. What are the roles of hydrogen bonds and covalent bonds in the structure of DNA?
5. Using your description of the fragments that formed from the digestion of pKAN-R and pARA with *Bam*HI and *Hin*dIII draw three possible recombinant plasmids resulting from the joining of two pARA and pKAN-R fragments. For each plasmid, identify the genes, other important sequences, and the number of base pairs.
6. Why is it important to inactivate the *Bam*HI and HindIII restriction enzymes before ligating the fragments? What might happen if you did not perform this step?

Chapter 5: Checking you've created a recombinant plasmid

Introduction

As part of the gene cloning process, molecular biologists have to check that they have completed each stage successfully and created the recombinant plasmid they need: one with the gene of interest as well as all the necessary components for the protein of interest to be made. The multiple combinations of recombinant plasmids possible, as well as the sources of potential error in any procedure, make it important that the DNA products from each stage of the gene cloning process are carefully checked. Gel electrophoresis is the procedure used to check the size of DNA molecules.

The technique of gel electrophoresis involves placing DNA samples, combined with a loading dye, into the wells of an agarose gel. This gel is then submerged in electrophoresis buffer within a gel electrophoresis tank, and an electric field applied across the gel to separate the DNA. Phosphate groups in the backbone of DNA create a negative charge, so it is loaded near the negative electrode and will migrate through the porous matrix of the agarose gel towards the positive electrode. The DNA molecules are separated primarily according to their molecular size, although molecular shape and degree of charge also influence their movement. Small DNA molecules move through the agarose matrix more easily, so they migrate faster than larger DNA molecules (figure 5.1).

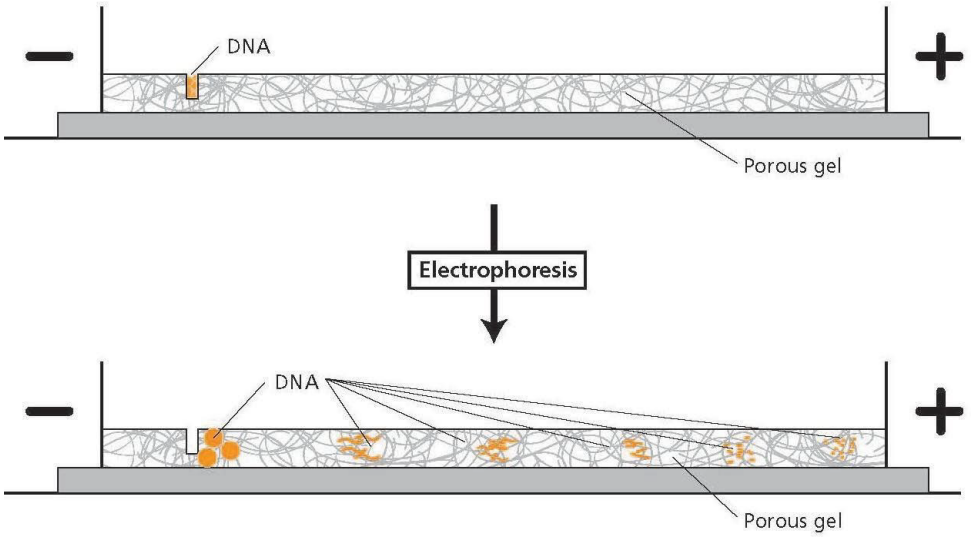


Figure 5.1 Separation of DNA by size using agarose gel electrophoresis.

Although linear pieces of DNA will separate by size as expected, the different configurations (shapes) of plasmids will alter their migration through the gel. There are three different plasmid configurations, which are shown in figure 5.2. The most common plasmid configuration is supercoiled. Supercoiling of DNA is catalysed by an enzyme in bacteria. It is the natural configuration in bacteria and is only seen in plasmids that have been replicated in bacteria. Supercoiling results in a very compact molecule, one that will move through the gel very quickly for its size. The second plasmid configuration is nicked circle. A break in one of the covalent bonds located in the sugar-phosphate backbone of one of the DNA strands along one of the two nucleotide strands means that this circular plasmid configuration will not move through the agarose gel as easily as the supercoiled configuration. The third plasmid configuration is a multimer. This configuration of two or more plasmids that are connected is only seen in plasmids that have been replicated in bacteria, because multimers form when plasmids are replicated so fast that they end up linked together. Unsurprisingly, given their size and shape multimers migrate very slowly through the gel.

Using gel electrophoresis you will examine the size of the pKAN-R and pARA plasmids and the restriction digest fragments (laboratory 2), the products from the ligation (laboratory 3) and the products from the PCRs (laboratory 4). The sizes of the DNA molecules will be determined by comparing them to a DNA ladder: a mixture of DNA fragments of known sizes. (When the DNA ladder is run on gel electrophoresis and stained, the bands that show the fragments look like the rungs of a ladder.) You will use the size of the DNA fragments to judge whether your procedures have been successful in creating the pARA-R recombinant plasmid that contains the *rfp* gene.

In order to visualise the DNA we will use a special stain that interacts with the DNA called GelGreen. Then a photographic record of your gel will be made to document this vital step.

Supercoiled Nicked circle Multimer

Figure 5.2 The three different plasmid configurations

Learning objectives

By the end of this laboratory you will be able to:

- Describe why it is important to check the products at each stage of the genetic engineering process
- Predict the relative speed of DNA restriction fragments and plasmids through a gel during gel electrophoresis
- Separate and identify DNA molecules using gel electrophoresis

Laboratory 5: Visualisation of products from restriction digestion and ligation

Aims

To check whether each of the experimental stages so far have been successful, using agarose gel electrophoresis to determine the size of the DNA products and compare them to the expected sizes.

Materials

EQUIPMENT & SUPPLIES

P-20 micropipette (2-20 µL)
Disposable pipette tips
5, 1.5 mL microfuge tubes
Permanent marker
Waste container
Microcentrifuge (shared)
MiniOne electrophoresis unit with 0.8% agarose gel (shared)

REAGENTS

Microfuge tubes containing:

- Undigested pKAN-R from lab 2 (K-)
- Digested pKAN-R from lab 2 (K+)
- Undigested pARA from lab 2 (A-)
- Digested pARA from lab 2 (A+)
- the ligation reaction from lab 3 (LIG)
- loading dye containing GelGreen (LD*GG)
- distilled water (dH_2O)
- DNA ladder (1 kb)

1 X Tris-acetate EDTA buffer (1 x TAE)

Health and safety

In this laboratory you will be using biological materials, albeit in tiny amounts, and they should be used in a controlled way, using techniques that mirror the aseptic techniques used in microbiology. All of the materials that come into contact with the biological materials (pipette tips, microfuge tubes and agarose gels) will be autoclaved to denature the DNA and enzymes before disposal.

The loading dye is low hazard, but can stain skin, so try to avoid contact with it as good practice would dictate. You should wear safety glasses and chemical-resistant gloves when using the electrophoresis buffer, because the buffer contains substances that can irritate your eyes and skin.

The electrophoresis chamber contains 1 X TAE buffer with an electric current passing though (at a low voltage). To prevent contact with the liquid during electrophoresis the tanks are designed so that the electric supply is cut off whenever the photo hood is removed.

GelGreen is used to stain the DNA. Whilst **not** mutagenic like some stains, this is a substance that can bind to DNA (of which you have lots), therefore you will need to wear chemical resistant gloves, a lab coat and eye protection when using the loading dye containing GelGreen.

As good laboratory practice you should clear up any spills immediately and wash your hands well with soap after completing the practical work.

Using MiniOne Electrophoresis system

How to Cast a Gel

- The number of samples to be electrophoresed will determine whether you use a 6 or 9 tooth comb
- Place the MiniOne® Gel casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.
- Use approximately 11 mL of your molten agarose per gel tray but prepare more than this (eg. 50 ml).

- Preparation of the agarose solution
  Wearing eye protection and chemical-resistant gloves, make up 200 ml of 1x TAE buffer in a measuring cylinder, labelled "TAE", by mixing 4.0 ml of 50x TAE buffer with 196.0 ml of distilled or deionized water (dH2O)
- For each 0.8% agarose gel to be poured, measure 0.088 g agarose and add 11 ml 1x TAE buffer into a glass flask with 3 x the capacity of the volume used (as it will bubble when heated). We do not recommend making single gel quantities, scale this up according to the number of gels you may need eg. 0.4g agarose + 50ml 1 x TAE (0.8%), should allow you to pour 4 gels (with a bit left over) – pour by-eye and do not use a measuring cylinder.
- Do not swirl as the agarose will be deposited on the walls and not dissolve in the liquid.
- Cover the flask opening with pierced plastic wrap to prevent excessive loss of water vapour.
- Heat the flask in a microwave on high for 15 seconds. Using a heatproof glove, remove the flask and gently swirl.
- Continue microwaving for 5 to 15 second periods until all of the agarose has dissolved. When held to the light and swirled there should be no agarose crystals suspended in the liquid.
- Don't forget to cool the agarose after it has melted. If the agarose is poured when it is too hot then it will warp the gel tray. If the solution begins to re-solidify, reheat it in the microwave. Leave to solidify for about 10 minutes.
- When the agarose solution has cooled to the point where you can safely touch the flask containing it, pour about 11 ml of the solution into each gel tray, with the comb in (6 or 9 wells)
- Carefully remove comb when gel has set. Remove gel tray with solidified gel from casting stand and wipe off any excess agarose from the bottom of the tray before placing in the Gel Tank.

- Gels can be stored for several days if wrapped and stored in the fridge. If combs are removed, ensure a small amount of buffer runs into the wells so that they do not dry-up

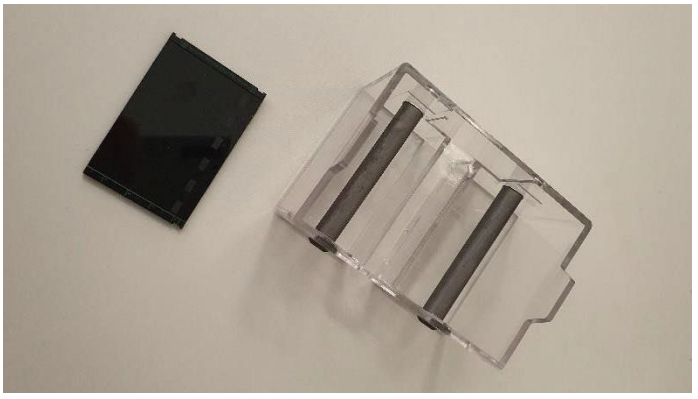
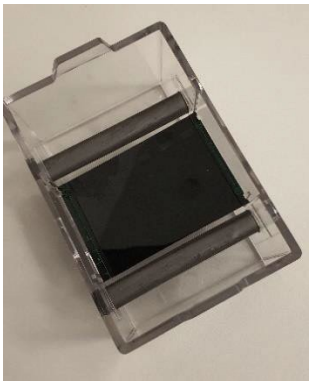
.

Using MiniOne Electrophoresis system

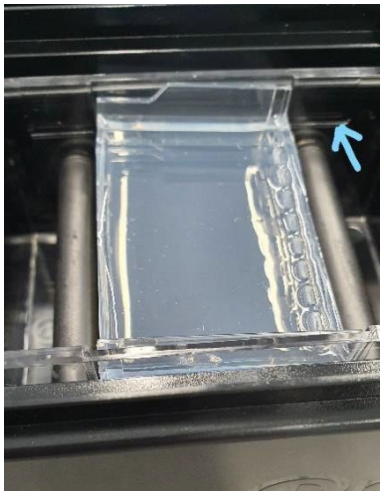
Setting up the MiniOne gel tank

- ⚠ **Wear eye protection (safety glasses are sufficient) and chemical-resistant gloves when handling the gel or TAE buffer**

1. Ensure the black viewing platform is in the tank if it is not already installed.

a.  

2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage (arrowed in Fig 5.4).
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.
5. Pour the x1 TAE buffer into one side of the tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the other side of the gel tank until the fill line. Do not overfill (max. volume = 140 ml)



The green power LED will not turn on or will flash if:
- The tank is not properly placed inside the carriage
- There is no buffer in the tank
- The buffer is too concentrated or too diluted
- The photo hood is not on the carriage
- There is too much or too little running buffer
- The power supply is not plugged in. Check by turning on the blue LED

6. Place photo hood on the carriage.
7. Press the power button which should now be a solid green light. If green light is solid, turn off the unit remove the photo hood and proceed to preparing and loading samples.

Preparing samples

1. Check your rack to make sure that you have all the reagents listed.

   Distilled water, loading dye containing GelGreen , K-, K+, A-, A+, LIG. and 1 kb

   1. Use a marker to label five clean microfuge tubes for the gel electrophoresis samples as follows: gelK-, gelK+, gelA-, gelA+ and gelLIG. (Also include a personal identifier on each tube.)
   2. Review table 5.1, which summaries the reagents that you will add in steps 4-7 to make up the samples for gel electrophoresis.

Step	Reagent	gelK- tube	gelK+ tube	gelA- tube	gelA+ tube	gelLIG tube
3	Distilled water (dH2O)	3.0 µL	3.0 µL	3.0 µL	3.0 µL	3.0 µL
4	Loading dye containing GelGreen (LD*GG)	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL
5a	Undigested pKAN-R (K-)	5.0 µL				
5b	Digested pKAN-R (K+)		5.0 µL			
5c	Undigested pARA (A-)			5.0 µL		
5d	Digested pARA (A+)				5.0 µL	
6	The ligation reaction (LIG)					5.0 µL

Table 5.1 Reagents to add to the tubes for gel electrophoresis samples In steps 3-7, be sure to use a new

⚠ micropipette tip for each reagent in each tube to avoid contamination.

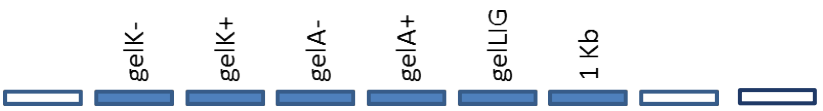
⚠ Wear eye protection (safety glasses are sufficient) and chemical-resistant gloves when handling the loading dye (it contains gelGreen)

3. Add the following:
   a. 3.0 µL of distilled water (dH_2O) to the gelK-, gelK+, gelA- and gelA+ tubes.
   b. 3.0 µL of distilled water (dH_2O) to the gelLIG tube.
4. Add 2.0 µL of loading dye (LD*GG) to each of the tubes.
5. Add the following:
   a. 5.0 µL of undigested pKAN-R (K-) to gelK– tube.
   b. 5.0 µL of digested pKAN-R (K+) to gelK+ tube.
   c. 5.0 µL of undigested pARA (A-) to gelA- tube.
   d. 5.0 µL of digested pARA (A+) to gelA+ tube.
6. Add 5.0 µL of the ligation reaction (LIG) to the gelLIG tube.
7. Add 2.0 µL of the loading dye containing GelGreen to the 1 kb Tube (if it does not already include it)
8. Spin the six microfuge tubes (gelK-, gelK+, gelA-, gelA+ gelLIG and 1 kb) in the microcentrifuge for four seconds to pool the reagents at the bottom of each tube.

⚠ Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.

Loading samples

1. Check your rack to make sure that you have all the reagents listed. gelK-, gelK+, gelA-, gelA+, gelLIG. and 1 kb



The sample loading order into the wells of the agarose gel.

2. Turn the low intensity blue light on by pressing the button 2nd from top on the carriage to help visualize the wells when loading
3. Using a fresh pipette tip for each sample, dispense 10.0 µL of the DNA ladder (1 kb), gelK-, gelK+, gelA-, gelA+ and gelLIG into their designated wells. For each sample, do the following:
    a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
    b. Lower the pipette tip until it is under the buffer but just above the well (see Figure 1.5, page 1.7).

⚠ Be careful not to place your pipette tip into the well or you might puncture the gel and lose your sample out the hole.

⚠ Only press the micropipette plunger to the first stop when dispensing samples to avoid getting air bubbles in the buffer. The sample will then sink into the well.

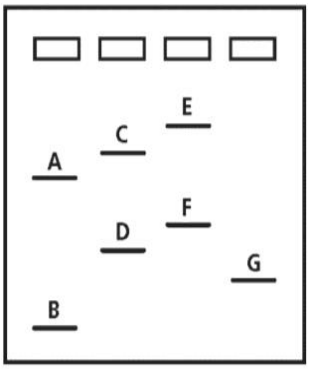
Running the gel

1. Once the gel is loaded, do not move it. Place the photo hood on the carriage. Turn on the unit by pressing the power button (bottom right). The green LED next to the button will turn on and should not flash – constant green light. (see p5.5 Troubleshooting)
2. Check the migration of the bands (~every five minutes) by briefly switching on the low intensity blue light.
3. Allow the gel to run 20 minutes or until the bands have separated sufficiently. Keep in mind small molecules run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the power button. Never leave the blue light on while the gel is running.

Observing and recording your results

1. Turn on the high intensity blue light.
2. Place your cell phone or camera directly on the photo hood to take a picture of the DNA.
3. DO NOT zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)

Questions



1. Why do DNA restriction fragments and plasmids separate when analyzed by gel electrophoresis?
2. Why is it important to check each stage in the preparation of a recombinant plasmid?
3. When DNA fragments are joined with a DNA ligase an array of products are created. How does this happen?
4. After different DNA fragments and plasmids have been separated by gel electrophoresis, the bands of DNA in the gel indicate the location of each kind of fragment and plasmid. The drawing of a stained gel opposite shows a series of bands that have been labelled with letters. The locations of the wells are also shown. What is the order of the fragments, from smallest to largest?
5. The loading dye contains three dyes that you may have separated in Laboratory 1.2. Why is it useful to use the loading dye in this lab?
6. If you used gel electrophoresis to separate the same plasmid that has all three configurations, the supercoiled plasmid would move the fastest, while the multimer would move the slowest. Why do the different plasmid configurations move the way they do through the gel?
7. The pKAN-R and the pARA plasmids you digested in Laboratory 2 were replicated in a bacterial cell. What configurations— supercoiled, nicked circle, and multimer—might these two plasmids have before digestion?
8. The ligation you carried out in laboratory 3 can result in a number of plasmids, but none of these have been replicated in a bacterial cell. What configurations—supercoiled, nicked circle, and multimer—might the ligated plasmids have?
9. What products might you expect to see from the gelK-, gelK+, gelA-, gelA+ and gelLIG tubes? Create a table that shows all the possible fragments and plasmids by tube. Include the length (bp size) of each fragment or plasmid, and arrange the products found in each microfuge tube by size, from smallest to largest. Include any possible plasmid configurations and arrange them first by size and next by speed through the gel, from fastest to slowest.
10. The DNA ladder serves as a standard because it contains a mixture of DNA molecules of known sizes. By running your samples and the DNA ladder side-by-side in your gel, you can estimate the actual size in base pairs of unknown DNA molecules. The DNA Ladder Diagram below shows 10 DNA bands of known sizes. Their sizes are given in kilobase (kb) pairs. 1 kb is 1,000 base pairs (bp). On the diagram predict the positions of DNA bands produced by the possible products found in the gelK-, gelK+, gelA-, gelA+ and gelLIG tubes by indicating their position on the DNA Ladder Diagram (figure 5.5).
11. When you have had a chance to examine your gel photograph, analyse how your actual gel results compare to your gel predictions.
12. Do you see any bands that are not expected? What could explain the origin of these unexpected bands?
13. Does the gel show that your restriction digest and ligation procedures were successful? Describe the evidence you used to make this assessment.

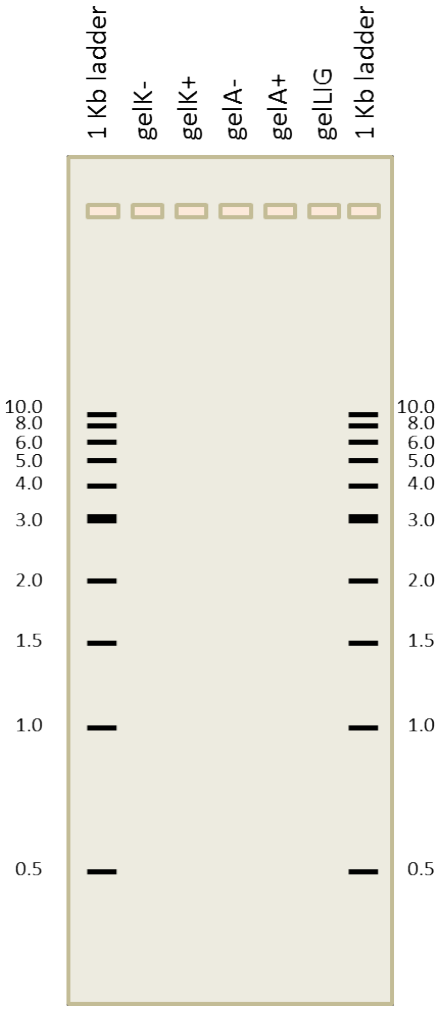


Figure 5.5 The DNA ladder diagram.

Glossary

Activator A protein that increases gene(s) transcription. Often a DNA-binding protein affecting activity at a promoter.

Adenine (A) A nitrogen-containing base, one member of the base pair AT (adenine-thymine). Adenine is found in both DNA and RNA.

Agar A polysaccharide extracted from the cell walls of some seaweed or red algae species. Frequently used as a culture medium in microbiology**.**

Agarose A highly purified polysaccharide (complex carbohydrate) extracted from seaweed. Commonly used to create a gel matrix for electrophoresis.

Aliquot A sample of some part of a whole (eg., a sample of plasmid or a sample of cells).

Allele An alternative form of a genetic locus; a single allele for each locus is inherited from each parent.

***Alu* element** A 300 base pair piece of DNA that has been randomly inserted throughout the genome. It no longer appears to have any function in the human genome. This specific DNA fragment is called the '*Alu*' element because it carries a recognition sequence for the *Alu* restriction enzyme.

Amino acid Any of a class of 20+ molecules that are combined to form proteins. The sequence of amino acids in a protein and hence protein function are determined by the genetic code.

Ampicillin A commonly used antibiotic of the penicillin family. Ampicillin prevents new cell wall material from linking properly in bacteria. This weakened cell wall will prevent the growth of new bacteria.

Amplification An increase in the number of copies of a specific DNA fragment.

ampR Symbol used to designate the gene for ampicillin resistance. This gene is located in the pARA plasmid.

Annealing Hydrogen bonding between complementary base pairs of two single stranded DNA molecules (usually of a primer and template during PCR).

Antibiotic A substance that kills or prevents the growth of cells.

Arabinose A five carbon sugar that occurs naturally in some plants and bacteria.

AraC protein A protein that is required for expression of mutant fluorescent protein in cells transformed with pARA-R.

Aspirate To draw in or suck in.

Autosome A chromosome that occurs in homologous pairs in both males and females and that does not bear the genes determining sex.

ß-lactamase An enzyme encoded by the *ampR* gene that destroys ampicillin.

Base One of the nitrogen-containing molecules that distinguish one nucleotide from another. In DNA, the bases are adenine, guanine, cytosine and thymine.

Base pair Two nitrogenous bases (adenine and thymine or guanine and cytosine) held together by hydrogen bonds. Two strands of DNA are held together in the shape of a double helix by the bonds between base pairs. The sum of the base pairs in a DNA molecule is frequently used to express the size of the molecule.

Base sequence The order of nucleotide bases in a DNA molecule; determines structure of proteins encoded by that DNA.

Biotechnology A set of biological techniques developed through basic research and now applied to research and product development. In particular, biotechnology refers to the use by industry of recombinant DNA, cell fusion and new bioprocessing techniques.

Buffer A solution used to maintain an optimal physical chemical environment for a chemical reaction. Buffers are used in restriction digests, ligations and for PCR.

Cell The basic unit of any living organism that carries on the biochemical processes of life.

Cell membrane A selectively permeable membrane that regulates movement of substances in and out of a cell.

Cell wall A structure that provides cells with physical support; surrounds some bacterial cells.

Chromosome In eukaryotes, a linear strand composed of DNA and protein, located in the nucleus of a cell, that contains the genes; in prokaryotes, a circular strand composed solely of DNA. In humans, there are 23 pairs of chromosomes in body cells.

Cloning Using specialized DNA technology to produce multiple, exact copies of a single gene or other segment of DNA. A second type of cloning exploits the natural process of cell division to make many copies of an entire cell. The genetic make-up of these cloned cells, called a *cell line*, is identical to the original cell. A third type of cloning produces complete, genetically identical animals or plants.

Cloning vector DNA molecule originating from a virus, a plasmid or the cell of a higher organism into which another DNA fragment of appropriate size can be integrated without loss of the vector's capacity for self-replication; vectors are engineered to introduce foreign DNA into host cells, where the DNA can be reproduced in large quantities. In the Amgen Labs, pKAN is used to clone many copies of the *rfp* gene.

Co-factor A non-protein compound that is needed for a protein's biological function. Often the co-factor binds to an enzyme assisting in its biochemical activity.

Codon Group of three mRNA bases that encodes a single amino acid.

Competent Cells capable of taking up plasmid DNA.

Complementary base pair Nitrogen-containing bases that are found opposite each other in a double-stranded DNA molecule. Complementarity is the result of size (a large base must be opposite a small base) and number of hydrogen bonds between the adjacent bases in the pair (A and T form two hydrogen bonds, G and C form three). Adenine is complementary to thymine and guanine is complementary to cytosine.

Covalent chemical bond One of the forces that holds atoms together in a molecule. It is considered to be a strong bond and forms from the sharing of electrons between two atoms.

Cytosine (C) One of the nitrogen-containing bases found in DNA and RNA. It is complementary to guanine.

Degrade To lower in quality; to convert into a more simple compound; to decompose.

Denaturation The melting of DNA at high temperatures into single nucleotide strands; changing the three-dimensional shape of a protein molecule.

Deoxyribose A type of five-carbon sugar found in DNA.

Dimorphic Having two forms.

Diploid A double set of chromosomes present in the somatic cells of plants and animals. Humans have a diploid number of 46.

Directed evolution An experimental procedure to evolve a desired trait. It involves using PCR to produce a library of variants, screening the library for organisms expressing the desired trait, then replicating lots of the desired variant.

DNA (deoxyribonucleic acid) The molecule that encodes genetic information. DNA is a double stranded molecule held together by hydrogen bonds between base pairs of nucleotides. The material of heredity.

DNA ligase An enzyme that joins DNA strands by forming covalent chemical bonds in the sugar-phosphate backbone.

DNA polymerase An enzyme used to replicate DNA molecules. PCR uses a DNA polymerase from the bacterium *Thermus aquaticus* and is called *Taq* polymerase.

DNA replication The use of existing DNA as a template for the synthesis of new DNA strands.

Double helix Structure in which two strands of DNA are twisted spirally around each other.

Electrophoresis Movement of charged molecules toward an electrode of the opposite charge; used to separate nucleic acids and proteins. When used to separate DNA fragments, agarose gel electrophoresis will separate the fragments by size, with smaller fragments moving faster than smaller fragments.

Enzyme A protein that acts to speed-up chemical reactions.

Escherichia coli Common bacterium used in numerous molecular biology protocols. The strain of *E. coli* used in the Amgen protocols is relatively harmless and does not grow well outside the laboratory environment.

Eukaryote An organism that shelters its genes inside a nucleus and has several linear chromosomes.

Exon Segment of a gene that encodes regions of a protein.

Express To make a protein.

Expression vector A plasmid genetically engineered specifically to express genes (e.g., pARA).

Extension The third stage in the PCR thermal cycling during which DNA polymerase catalyse the addition of nucleotides to the primer.

Fluorescence The production of light by a molecule (e.g., red fluorescent protein will release red light when exposed to ultraviolet light). The protein used in the Amgen Labs can been seen with ambient light.

Forensics Specializing in the application of scientific knowledge to legal matters. DNA is frequently used as evidence in legal matters.

Gamete Mature male or female reproductive cell.

Gene The fundamental physical and functional unit of heredity; an ordered sequence of nucleotides located in a locus that encodes a specific functional product.

Gene expression The process by which a gene's coded information is converted into the structures present and operating in the cell. Expressed genes include those that are transcribed into mRNA and then translated into protein.

Genetic code The sequence of nucleotides, coded in triplets (codons) along the mRNA, that determines the sequence of amino acids in protein synthesis.

Genetic engineering Altering the genetic material of cells or organisms to enable them to make new substances or perform new functions.

Genetic polymorphism Difference in DNA sequence among individuals, groups or populations (e.g., alleles for the *tPA* locus amplified by PCR, or height in pea plants-tall and short).

Genetics The study of inheritance.

Genome All the genetic material in the chromosomes of a particular organism; its size is generally expressed as its total number of base pairs.

Genotype The combination of alleles carried by an individual for a specific trait.

Green fluorescent protein A protein produced by the marine jellyfish, *Aequoria victoria*; protein encoded by the *gfp* gene.

Guanine (G) One of the nitrogen-containing bases found in DNA and RNA. It is complementary to cytosine.

Haploid A single set of chromosomes present in the gametes of plants and animals. Humans have a haploid number of 23.

Heterozygous The two different copies, or alleles, of the same gene.

Homologous chromosomes A chromosome containing the same linear gene sequence as another, each derived from one parent. While the gene sequence is (generally) the same, the precise alleles may differ between chromosomes.

Homozygous Having two identical copies, or alleles, of the same gene.

Hydrogen bond A weak force resulting from the attraction of a positive hydrogen atom to negatively charged regions of other atoms. This is the force that holds the two strands of nucleotides together in the DNA molecule. The GC base pairs form three H-bonds while AT pairs for two.

Hydrophilic Water loving; dissolves in water; polar. Some examples are sugar and salt.

Hydrophobic Water fearing; does not dissolve in water; non-polar. Some examples are oil, wax and mutant fluorescent protein.

In situ Whilst staying in the same place.

In vitro 'In glass'; experimental work performed in equipment, not living organisms.

In vivo 'In life'; experimental work performed in living organisms, not equipment.

Intron Segment of a gene that does not code for a protein. Introns are transcribed into mRNA but are removed before being translated into a protein.

Kanamycin An antibiotic that kills non-resistant cells by inhibiting proteins synthesis.

kanR Symbol for the kanamycin resistant gene found in the plasmid pKAN; encodes an enzyme called phosphotransferase that inactivates kanamycin.

Kilobase (kb) Unit of length for DNA fragments equal to 1,000 nucleotide pairs.

Kilobase ladder A set of standard DNA fragments with lengths differing by one kilobase; used as a size standard in electrophoresis.

Ligase The enzyme required to covalently join two fragments of DNA.

Ligation The reaction that chemically joins two fragments of DNA resulting in a recombinant DNA molecule.

Locus A place or location on a chromosome, it may be a gene or just any site with variations which can be measured. (e.g., Alu+, Alu- in the *tPA* intron).

Lyse To break open.

Marker See: *kilobase ladder.*

Messenger RNA (mRNA) The nucleic acid molecule that carries genetic information from the genes to the rest of the cell for protein synthesis.

Nitrogen-containing base A molecular component of DNA and RNA nucleotides. In DNA there are four nitrogen-containing bases: A (adenine), G (guanine), T (thymine), C (cytosine).

Nuclease A family of enzymes that will degrade nucleic acids.

Nucleic acid A large molecule composed of nucleotide subunits; a polymer of nucleotides.

Nucleotide A subunit or monomer, of DNA and RNA. Each nucleotide consists of a nitrogen containing base, a five-carbon sugar and a phosphate group.

Operon A cluster of genes transcribed together to give a single molecule of mRNA. The arabinose operon is used in the pARA expression vector to transcribe the *rfp* gene.

Phage A virus for which the natural host is a bacterial cell.

Phenotype Characteristic due to the expression of our genes; usually refers to visible properties but may refer to characteristics revealed by laboratory tests.

Plasma membrane Another name for the cell membrane.

Plasmid Circular molecule of DNA which replicates independently of the host's genomic DNA (e.g., pKAN, pARA).

Polymer A large molecule made of similar or identical subunits linked together (e.g., DNA, RNA, proteins).

Polymerase Chain Reaction (PCR) A chemical procedure used to amplify a DNA sequence by repeated cycles of replication and denaturation.

Polymorphism Difference in DNA sequence at a particular genetic locus. The differences may or may not result in a different phenotype.

See also: *locus, phenotype.*

Primer Short, pre-existing polynucleotide chain to which new nucleotides can be added by DNA polymerase. Two different primers complementary to the DNA sequence bracketing the region to be amplified are used in PCR.

Prokaryote A cell or organism with a single chromosome and no nuclear membrane; bacteria.

Promoter Region of DNA in front of a gene that binds RNA polymerase and so promotes gene expression; in the *ara* operon, the region of pARA that binds the AraC protein-arabinose complex and RNA polymerase prior to *rfp* expression.

Protein A large polymer of amino acids. Examples are enzymes, mutant fluorescent protein and some hormones.

Reagent A substance or compound that is added to a reaction to assist in that reaction.

Recognition sequence (recognition site) Specific nucleotide base sequence recognized by a restriction enzyme. The enzyme will cut the DNA within this nucleotide sequence.

Recombinant DNA molecule A combination of DNA molecules of different origin that are joined using recombinant DNA techniques.

Restriction enzyme An enzyme that binds and cuts DNA at a specific base sequence (e.g., *Bam*H I and *Hin*d III).

Restriction fragment A piece of DNA that results from the cutting of a DNA molecule with a restriction enzyme. Fragments are often separated on a gel using electrophoresis.

Restriction map Diagram of pieces of DNA, such as a plasmid, showing the restriction sites for restriction enzymes.

Ribose Five-carbon sugar found in RNA nucleotides.

Ribosome Site within the cell that assembles amino acids into protein molecules.

RNA polymerase An enzyme that catalyses the synthesis of RNA, using DNA as a template. Involved in transcription of genes.

SB buffer Sodium borate solution used as an electrophoresis buffer. It is a solution that conducts an electric current and maintains a relatively constant pH.

Somatic cells Cells making up the body which are not sex cells or gametes. These cells are usually diploid having two sets of chromosomes.

Sticky ends Ends of a DNA molecule cut with certain restriction enzymes. These ends have unpaired bases.

Supercoiling Higher level of twisting of DNA often found in plasmids.

Supernatant The liquid on top of a pellet deposited by centrifugation.

***Taq* polymerase** A heat stable enzyme commonly used in PCR; polymerase found in the bacterium *Thermus aquaticus.*

TAE (Tris-Acetic acid-EDTA) A solution used for gel electrophoresis and in the preparation of agarose gels. The solution helps conduct an electric current while maintaining a constant pH. An alternative to SB buffer.

Thermophilic 'Heat-loving'.

Thymine (T) One of the bases found in DNA; the base that is complimentary to adenine.

Transcription A chemical process that converts a DNA nucleotide sequence into an mRNA nucleotide sequence; process that uses RNA polymerase to convert a DNA template into a mRNA strand.

Transformation A process that places foreign DNA, like a plasmid, into a cell.

Translation A chemical process that converts an mRNA nucleotide sequence into an amino acid sequence; site of this reaction is the ribosome.

Vector A DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and expressed.

Wild type The original or naturally occurring version of a gene or protein.