October 2023

ABE for Lower Secondary School:
Engaging Younger Students in the Amgen Biotech Experience

By Susan Senior, ABE Australia

ABE Master Teacher Fellowship Program

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

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The projects designed by the 2022–23 ABE Master Teacher Fellows are a compilation of curricula and materials that are aligned with Amgen Biotech Experience (ABE) and prepare students further in their biotechnology education. These projects were created over the course of a 1-year Fellowship in an area of each Fellow's own interest. Each is unique and can be adapted to fit the needs of your individual classroom. Objectives and goals are provided, along with expected outcomes. Projects can be used in conjunction with your current ABE curriculum or as an extension.

As a condition of the Fellowship, these classroom resources may be downloaded and used by other teachers for free. The projects are not edited or revised by the ABE Program Office (for content, clarity, or language) except to ensure safety protocols have been clearly included where appropriate.

We are grateful to the ABE Master Teacher Fellows for sharing their work with the ABE community. If you have questions about any of the project components, please reach out to us at ABEInfo@edc.org, and we will be happy to connect you with the author and provide any assistance needed.

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

Australia

Master Teacher Fellowship
ABE for Lower Secondary School

AUTHOR: Susan Senior

PROGRAM SITE: ABE Australia

TIME FRAME: 6–8 hours for Biotechnology First-Hand Investigation, Others 1–2 hours

SUGGESTED AGE RANGE: 12–16 (Depending on the activity)

SUGGESTED COURSE OR CONTENT AREA:

CONNECTION DESCRIPTIONS:

☒ Data analysis/Data literacy

☒ Project or problem-based learning

☒ Professional skills in STEM/Profiles in STEM

Learning Activity Detailed Guide (To be included for each individual lesson)

1. **Overview**
   Introduce students to the idea of small volumes and their application and have them use a device that accurately measures small volumes.

2. **Learning Goals**
   - Students can accurately convert between units of volume.
   - Students can accurately set and use a micropipette.

3. **Assessed Outcome**
   Teacher will mark worksheet and observe pipetting techniques during class.

4. **Key Vocabulary**
   Micropipette; microliters; volume

5. **Materials and LabXchange Pathway(s)**
   - https://www.labxchange.org/library/items/lb:LabXchange:1e7712e1:lx_assignment:1
   - https://www.labxchange.org/library/items/lb:LabXchange:1ceef27f:video:1
   - Measuring Small Volumes Worksheet (attached)

6. **Teacher Preparation**
   Lab 1.1 From ABE Teacher Guide

7. **Lab Safety Considerations**
   As per Lab 1.1 From ABE Teacher Guide

8. Sequence of Activities

Activity Description	*Time*	*Materials*
1. Discuss small units of volume and their application	5 min	Nil – Class Discussion
2. Students complete Measuring Small Volumes online activity from LabXchange	5 min	LabXchange access on a device
3. Watch Video on using a micropipette on Lab	8 min	LabXchange access on a device
4. Students Complete Lab 1.1 from ABE & Worksheet	40 min	• Red Dye – 1.3 mL per pair • Laminated pipetting practice sheet P-20 & P-200 • P-20 & P-200 pipettes • Box of Yellow pipettes tips • Microfuge tube rack • Waste container • Gloves • Worksheet - attached

Learning Activity Detailed Guide (To be included for each individual lesson)

1. **Overview**
   Students will complete the abridged ABE program (Labs 1.1, 1,2, 2A & 4A) to gain an understanding of the basics of the biotechnology and to collect first hand data to produce a scientific report using appropriate scientific language.

2. **Learning Goals**
   - Conduct a controlled experiment to collect first-hand data.
   - Communicate science ideas for specific purposes and construct evidence-based arguments using appropriate scientific language, conventions, and representations.

3. **Assessed Outcome**
   Students will be marked on the accurate completion of the sections indicated in blue, including photographs of them completing the lab components in the attached booklet (to be completed electronically).

4. **Key Vocabulary** none

5. **Materials and LabXchange Pathway(s)**
   ABE Lab Program (Labs 1.1, 1.2, 2A, 4A)

6. **Teacher Preparation**
   As per ABE Lab Program (Labs 1.1, 1.2, 2A, 4A)

7. **Lab Safety Considerations**
   As per ABE Lab Program (Labs 1.1, 1.2, 2A, 4A)

8. Sequence of Activities

Activity Description	*Time*	*Materials*
1. Research – Questions on genes, DNA, proteins & biotechnology	2 hours (not class time)	Student research – using some suggested websites: • Genes, proteins, and cells | Genes, cells, and organisms | High school biology | Khan Academy • DNA cloning and recombinant DNA | Biomolecules | MCAT | Khan Academy • Overview: DNA cloning (article) | Khan Academy • Gel Electrophoresis
2. Lab 1.1	1 hour	As per ABE teacher guide
3. Lab 1.2	1 hour	As per ABE teacher guide
4. Lab 2A	1 hour	As per ABE teacher guide
5. Lab 4A	1.5 hours	As per ABE teacher guide
6. Lab 5A	2 hours	As per ABE teacher guide
7. Writing Up of Report	2-4 hours	Student to complete independently

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

Australia

Biotechnology First-Hand Investigation

This laboratory notebook must be completed electronically.

Introduction

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 of the field of medicine, as genetically modified bacteria can make human insulin that treats diabetes and other lifesaving products.

Over the coming weeks, it will be your task to investigate whether organisms can be engineered to make different protein products and have new traits.

You will be working in the laboratory to carry out experiments that led to breakthroughs in biotechnology and gain hands-on experience with producing genetically modified bacteria.

Laboratory Notebook

In science, the ability to keep track of what you are doing and communicate your work is extremely important. To demonstrate that you performed an experiment, either so it can be duplicated and verified by others or if you want to apply for a patent, you need to have a very accurate record of what you have done. As you complete your experiments, carefully record your observations with photographs and complete the activities as indicated in blue text.

Research

Use the following information and any other relevant resources to complete the answers to the following questions - ***use a different coloured font to enter your answers using in-text referencing in Harvard formatting. Remember to include a reference list at the end of your answers.***

1. How does the expression of a gene result in the traits of an organism?
2. What is the relationship between DNA, genes, proteins, and traits?
3. How is information encoded in DNA?
4. How is this information decoded?
5. What is the product of the decoded message?
6. How does this product result in traits?
7. What are the consequences to an organism if the product is altered or not made?
8. What is genetic engineering?
9. How does the process of gel electrophoresis work and what does it allow scientists to do?
10. Why can DNA fragments be separated by gel electrophoresis?
11. What are plasmids and how are they used in cloning a gene?
12. What are restriction enzymes and how are they used to create a recombinant plasmid?
13. Why is it important to verify products created in the genetic engineering process?
14. What is the purpose of using a DNA ladder when verifying the products created?
15. Describe the role of transformation using bacteria in the gene cloning process.

Starting Resources:

- Genes, proteins, and cells | Genes, cells, and organisms | High school biology | Khan Academy
- DNA cloning and recombinant DNA | Biomolecules | MCAT | Khan Academy
- Overview: DNA cloning (article) | Khan Academy
- Gel electrophoresis

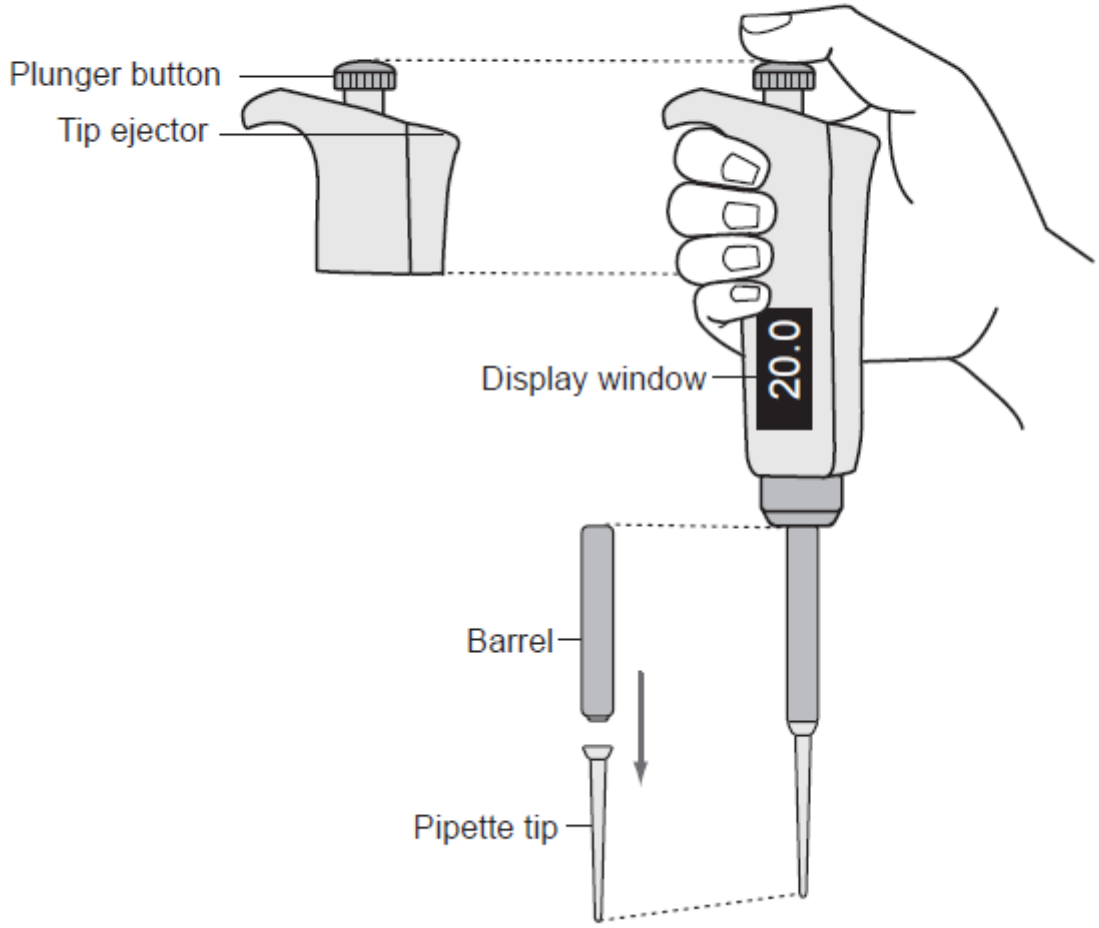
Lab 1.1 - Micropipetting

Introduction:

To carry out genetic engineering, you need good laboratory skills. In this section, you will focus on gaining practice in the use of micropipettes (instruments used to transfer small volumes of liquid), a critical skill for biotechnology. This is the first step in building the skills you'll need to carry out the subsequent labs and complete your challenge in this program.

The purpose of this laboratory is to introduce you to an important tool used in genetic engineering: the micropipette, shown in Figure 1.1. A micropipette is used to transfer very small and exact volumes of liquids in either millilitres (mL, thousandths of a litre) or microlitres (µL, millionths of a litre), which are the measurements of volume most often used in genetic engineering. This laboratory will give you the chance to learn how to use the micropipette and to see the relative size of different amounts of solution measured by this very precise tool.

Figure 1.1: A P20 micropipette



Materials:

- A plastic microfuge tube rack with a microfuge tube of red dye solution (RD)
- P20 micropipette (measures 2.0–20.0 µL)
- P200 micropipette (measures 20–200 µL)
- Yellow box of yellow disposable pipette tips
- Two laminated micropipette practice sheets
- Waste container for used tips and microfuge tubes (will be shared among groups)

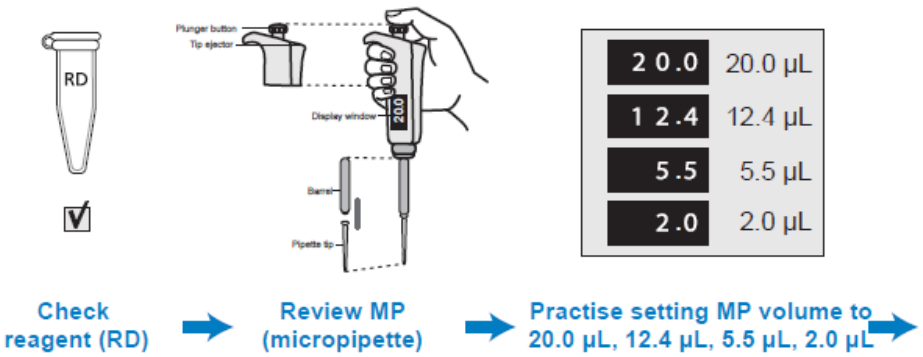
Safety:

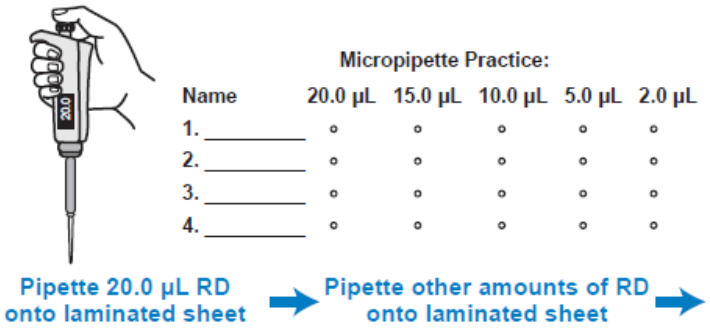
- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles, lab coat & gloves.
- Wash your hands well with soap after completing the lab.

Method:

1. Check your bench to make sure that you have the required equipment.
2. Review the parts of the micropipette shown in Figure 1.1.
3. Watch the video on micropipetting How to Use a Micropipette - LabXchange or Micropipetting
4. Follow the instructions in the flowchart:

Laboratory 1.1 Flowchart

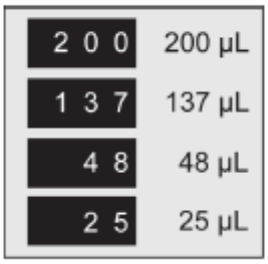




5. Swap to the P200 micropipette.

6. Figure 1.3 shows four micropipette volumes. Practice setting the P200 micropipette to these volumes.

Figure 1.3: Four micropipette volumes



7. Dispense RD onto the laminated P200 micropipette practice sheet as directed.

Results: Photographs - insert photographs of your laminated sheets

P20 Micropipette:

P200 Micropipette

Questions: *Answer the following questions using a different coloured font*

1. When loading or dispensing a solution, why is it important to actually see the solution enter or leave the pipette tip?

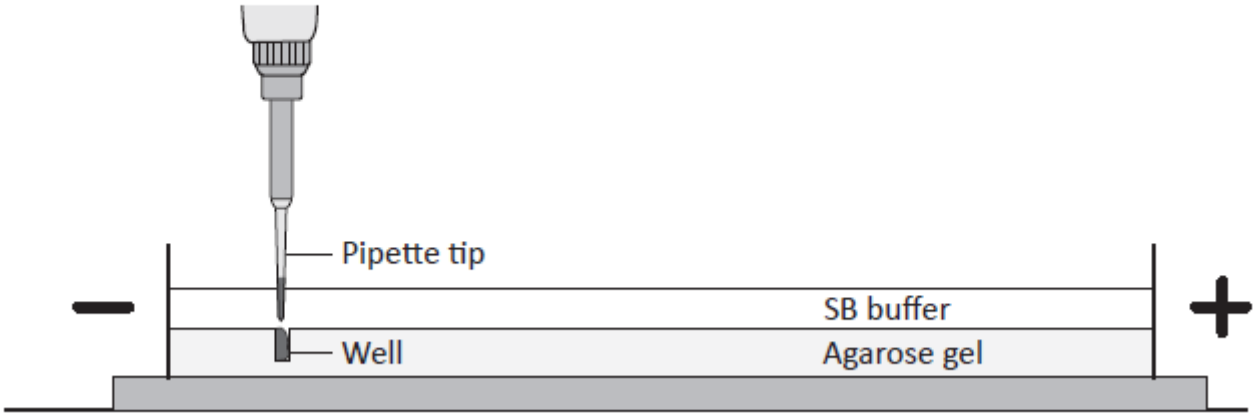
2. You were instructed to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important?

Lab 1.2 - Gel Electrophoresis

Introduction:
The purpose of this laboratory is to give you experience with gel electrophoresis, which is used to separate and identify a mixture of biomolecules including DNA; the components of each mixture can then be identified by their location in the gel.

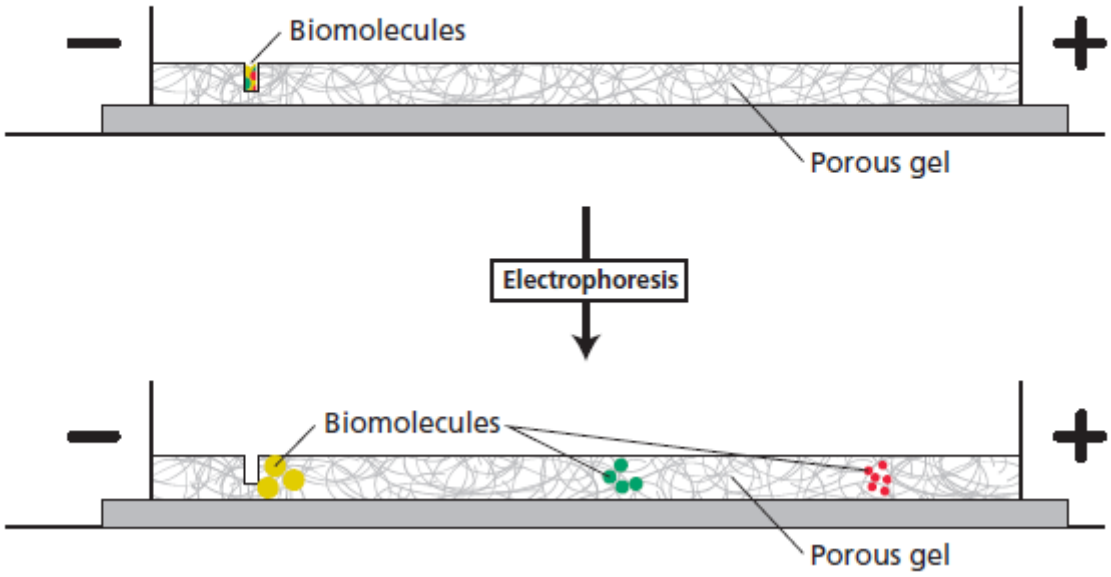
The electrophoresis setup consists of a box containing an agarose gel and two electrodes that create an electric field across the gel when the box is attached to a power supply. The negative electrode is black, and the positive electrode is red. Samples of biomolecules are pipetted into wells near the negative (black) electrode. The samples move through the gel toward the positive (red) electrode, as shown in Figure 1.5.

Figure 1.5: The gel electrophoresis unit



The gel that the biomolecules move through is composed of agarose, a polysaccharide (complex sugar) found in seaweed. Its structure is a porous matrix (like a sponge) with lots of holes through which the solution and biomolecules flow. See Figure 1.6.

Figure 1.6: How biomolecules, including DNA, move through the agarose gel matrix in gel electrophoresis



Materials

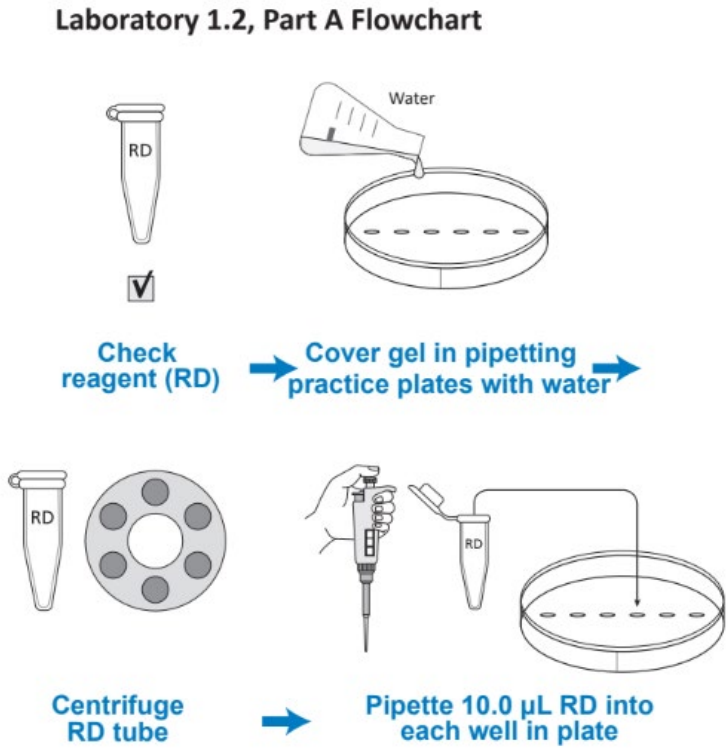
- A plastic microfuge tube rack with the following:
  - Microfuge tube of red dye solution (RD)
  - Microfuge tube of dye solution 1 (S1)
  - Microfuge tube of dye solution 2 (S2)
  - Microfuge tube of dye solution 3 (S3)
- Flask containing 3000 mL of 1x sodium borate buffer (1x SB) (shared with another group)
- Flask containing water
- P20 micropipette (measures 2.0–20.0 µL)
- Yellow box of yellow disposable pipette tips
- 1 pipetting practice plate loaded with 0.8% agarose gel (shared with another group)
- Electrophoresis box loaded with 0.8% agarose gel (will be shared among groups)
- Microcentrifuge (will be shared among all groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

Risk Assessment: *Complete a risk assessment for Parts A & B either using Student Risk Assess (and insert the file here) or construct a suitable table*

Method:

Part A: Pipetting Into Wells

1. Ensure all the required materials are on the lab bench
2. Watch the video Loading an Agarose Gel - LabXchange
3. Follow the Flowchart 1.2 Part A



4. Use the following steps to pipette:

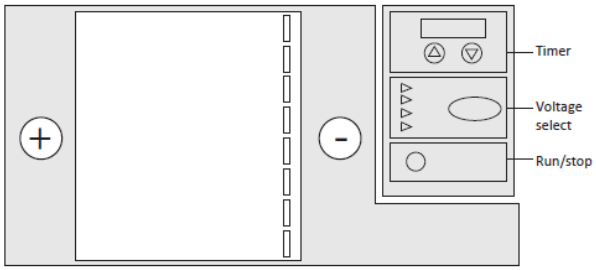
    1. Place one elbow on the table to steady the pipette hand and if needed use the other hand to support the pipetting hand.
    2. Lower the pipette tip until it is under the buffer but just above the well.
    3. Gently press the plunger to the first stop to slowly dispense the sample
    4. While the plunger is still depressed, pull the tip out of the buffer

5. Repeat steps 4 and 5 until all the practice plate wells have been filled.
6. Eject the pipette tip into the waste bin

Part B: Separating Dyes With Gel Electrophoresis

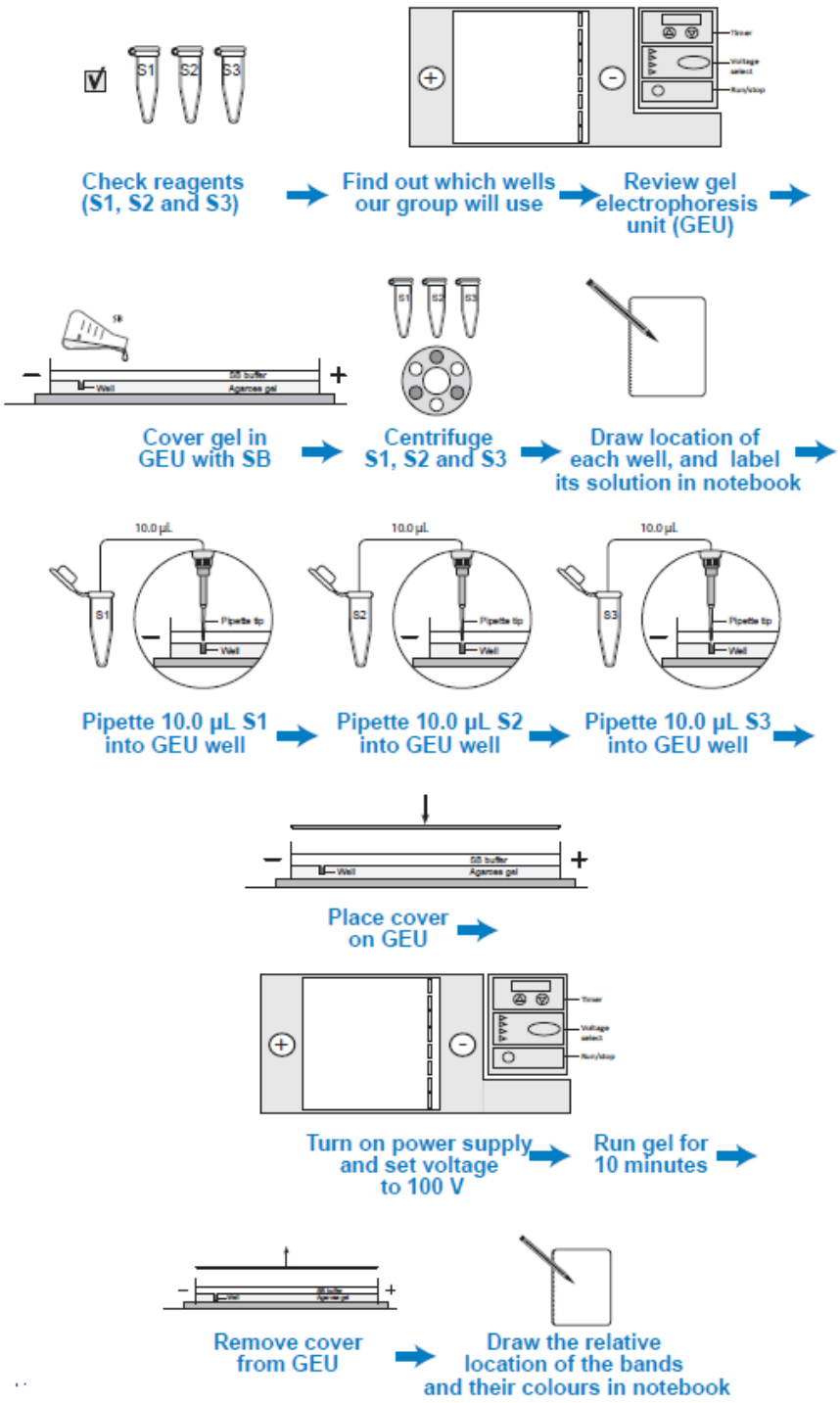
1. Review Figure 1.7 and check to make sure that the wells in the gel are located near the negative electrode.

Figure 1.7: Electrophoresis



2. Follow the Flowchart 1.2 Part B

Laboratory 1.2, Part B Flowchart



Results:

Insert a photo labelling it with the location of each solution in the wells in the electrophoresis box before running the gel

Insert a photo off the relative location of the bands and their colours in each of the lanes containing the samples.

Questions: *Answer the following questions using a different coloured font*

1. Study the gel electrophoresis results. Which solution sample contained a single dye: S1, S2, or S3? How do you know?

2. What electrical charge do the dyes have? Explain your reasoning.

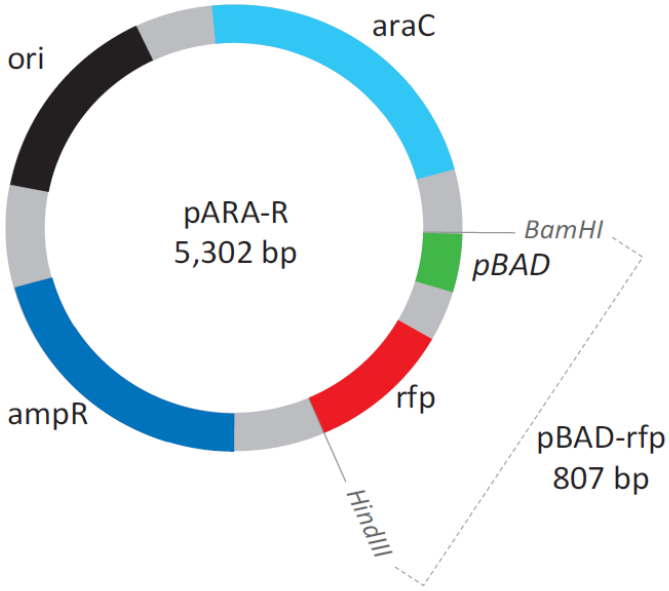
3. The dyes that you are separating are orange G (yellow), bromophenol blue (purple), and xylene cyanole (blue). If the molecular shape and electric charge of all three dyes are similar, what is the order of the dyes from heaviest to lightest molecules, based on your initial results? Why do you think this is the correct order?

Lab 2A: Restriction Digest - Digesting the Plasmid

The purpose of this lab is to ensure that the recombinant plasmid, pARA-R, used is the correct one for making the red fluorescent protein in bacteria. To do this you will use restriction enzymes to cut the plasmid (see Figure 2A.3), which will generate DNA fragments of lengths characteristic of the pARA-R plasmid. This procedure is called a restriction digest and the lengths of the fragments can be determined by gel electrophoresis (which you will do next).

The recombinant DNA plasmid pARA-R contains the gene for ampicillin resistance, the red fluorescent protein (rfp) gene, a promoter sequence for initiating transcription, and the ori site for the initiation of DNA replication. The pARA-R plasmid also contains a DNA sequence that activates the promoter when the bacteria are grown in the presence of arabinose, a five-carbon sugar that naturally occurs in various plant and bacterial carbohydrates. This sequence is called the arabinose activator (araC). The activator controls the promoter. If arabinose is present in the bacteria, the RNA polymerase will bind the promoter, and transcription will occur. If arabinose is not present, polymerase will not bind the promoter, and transcription will not occur.

Figure 2A.3: The pARA-R plasmid



The relevant components on the plasmid are the red fluorescent protein (rfp) gene, the promoter (pBAD), the ampicillin resistance gene (ampR), and the arabinose activator (araC). In addition to showing the relevant components, Figure 2A.3 also shows the size of the plasmid (the number in the centre, which indicates the number of base pairs [bp]) and the sequences where it can be cut by the restriction enzymes that will be used in the lab. The sites labelled “BamHI” and “HindIII” represent recognition sites for these two restriction enzymes.

Activity:

1. On the Plasmid Diagram (Worksheet 2A.1):
    - Use scissors to cut out the plasmid sequence and tape the ends together to make a paper model of the plasmid.
    - Locate the positions of the *ori* site, the promoter site and the genes for antibiotic resistance.
    - Locate the positions of each restriction enzyme recognition site.
2. Choose the restriction enzyme that should be used to cut the plasmid. Verify that the restriction enzyme meets all the following criteria:
    - The ori site on the plasmid is intact.
    - The promoter site is intact.
    - At least one of the antibiotic resistance genes is intact.
    - The enzyme cuts the plasmid only once
    - The cut is close to the promoter sequence.
3. Review Table 2A.1 and use scissors to cut the plasmid at the recognition site exactly as the restriction enzyme would cut it. Write the sequences of the nucleotides that are left on each end of the plasmid.

Table 2A.1: Restriction enzymes used in this laboratory

Source	Restriction enzyme	Recognition site
Escherichia coli	EcoRI	5′ G↓AATTC 3′ 3′ CTTAA↑G 5′
Bacillus amyloliquefaciens	BamHI	5′ G↓GATCC 3′ 3′ CCTAG↑G 5′
Haemophilus influenzae	HindIII	5′ A↓AGCTT 3′ 3′ TTCGA↑A 5′

The symbols ↑ and ↓ indicate where the DNA is cut.

4. On the Human DNA Sequence (Worksheet 2A.2), scan the human DNA sequence and determine where the three restriction enzymes, BamHI, EcoRI and Hindi II, would cut the DNA.
5. Determine whether the restriction enzyme you chose in step 2 is a good choice for cutting out the insulin gene from the human DNA by verifying that it meets all the following criteria:
    - It does not cut within the insulin gene.
    - It cuts very close to the beginning and end of the gene.
    - It will allow the insulin gene to be inserted into the cut plasmid.
6. Review Table 2A.1 and use scissors to cut the human DNA at the recognition site exactly as the restriction enzyme would cut it. Write the sequences of the nucleotides that are left on each end of the insulin gene after it is cut from the human DNA.
7. Use tape to insert the insulin gene into the cut plasmid. Verify that the sticky ends will connect in the correct orientation (in the lab, a third biological tool, DNA ligase, is used to permanently connect the sticky ends together).

Results:

Insert a photo of your completed plasmid and left over pieces below.

Questions: *Answer the following questions using a different coloured font*

1. Which restriction enzyme did you choose? Why did you choose that one?
2. Where would you insert the insulin gene and why?
3. Which antibiotic would you use to determine if the recombinant DNA was taken in?

CLONE THAT GENE: PLASMID DIAGRAM

Restriction Enzyme Recognition Sites

BamHI	GGATCC CCTAGG
EcoRI	GAATTC CTTAAG
HindIII	AAGCTT TTCGAA

WORKSHEET 2A.2

CLONE THAT GENE: HUMAN DNA SEQUENCE

Restriction Enzyme Recognition Sites

BamHI	GGATCC CCTAGG
EcoRI	GAATTC CTTAAG
HindIII	AAGCTT TTCGAA

Materials

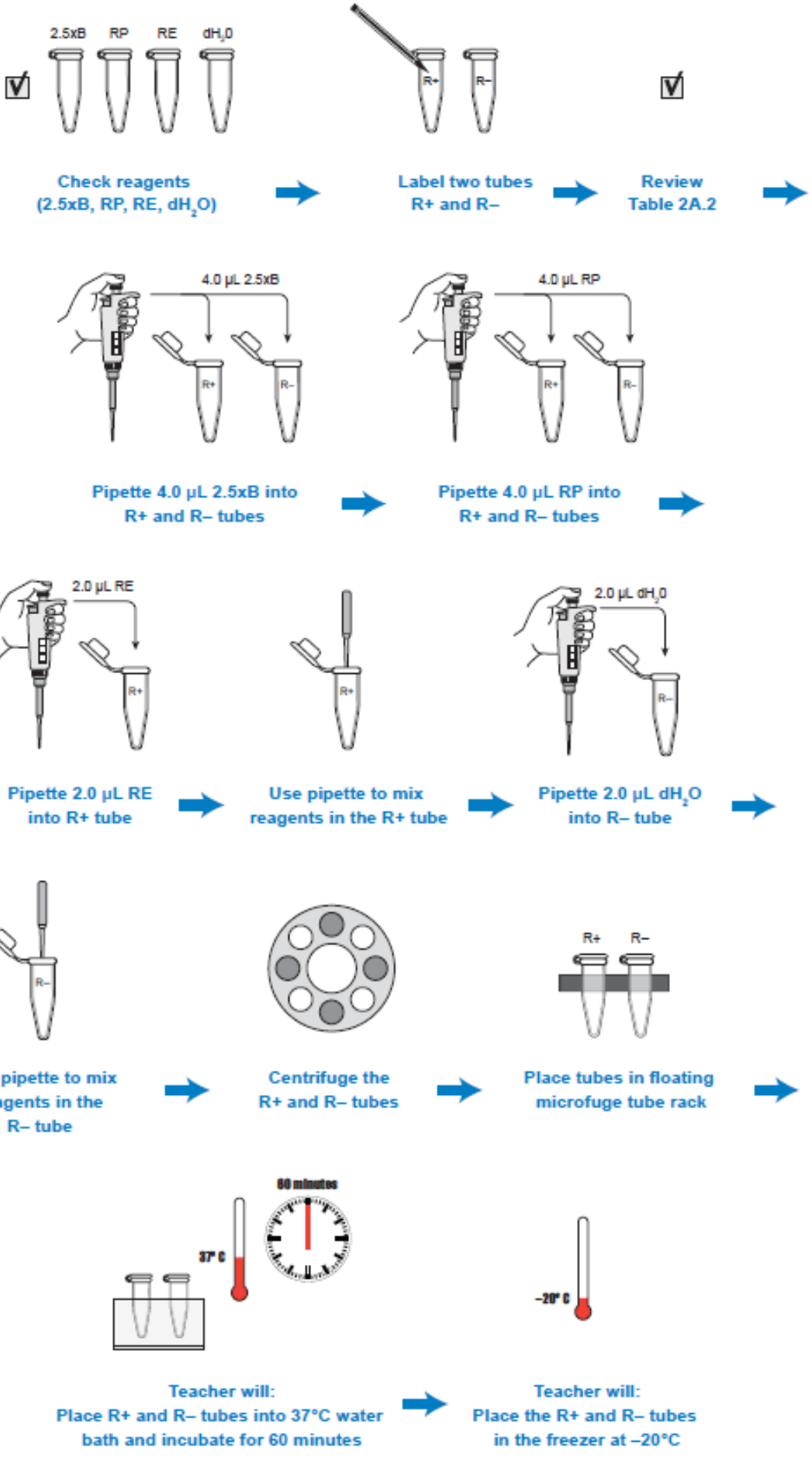
- A rack with the following:
    - Microfuge tube of 2.5x restriction buffer (2.5xB)
    - Microfuge tube of pARA-R (RP)
    - Microfuge tube of restriction enzymes BamHI and HindIII (RE)
    - Microfuge tube of distilled water (dH_2O)
- P20 micropipette
- Box of disposable pipette tips
- 2 x 1.5 mL microfuge tubes
- Permanent marker
- Microcentrifuge (will be shared among all groups)
- 37°C water bath with floating microfuge tube rack (will be shared among all groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

Safety:

- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.
- Wash your hands well with soap after completing the lab.

Method: ***Write a method using the flowchart below as a guide.***

Laboratory 2A Flowchart



Lab 4A Verification of Recombinant Plasmid Using Gel Electrophoresis

Introduction

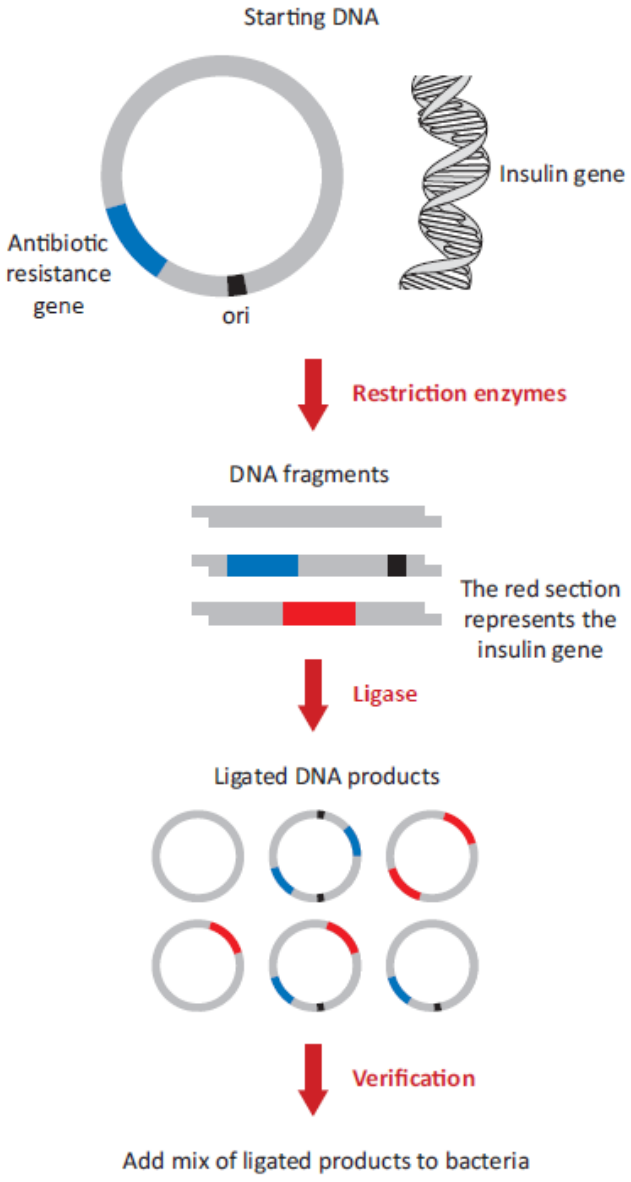
It is important to verify work in the lab—there are many sources of potential error in any procedure, including the procedures used in cloning a gene. In gene cloning, there is also the problem that some procedures are not selective. For example, when a DNA ligase is used to ligate—bind together—DNA fragments, many different combinations result from the ligation process. Unless you verify your work, you do not know if you have made the recombinant plasmid that is needed.

How To Verify The Recombinant Plasmid

Figure 4A.1 shows how to verify your results when making a recombinant plasmid. You verify that the restriction digest and ligation procedures worked by comparing the products of both procedures with each other and with what you started with.

Figure 4A.1: Verification method when making a recombinant plasmid

Verify that the restriction digest and ligation procedure was successful by comparing the products of the restriction digest, the products of the ligation procedure, and the starting materials



Activity

Read the information at Plasmids in Gel Electrophoresis - LabXchange and construct a labelled diagram to show the 3 plasmid configurations - insert a photo of your drawing here.

Questions: *Answer the following questions using a different coloured font*

1. The pARA-R plasmid you digesting in Lab 2A was replicated ina bacterial cell. What configurations (supercoiled, nicked circle and/or multimer) might the plasmid have before digestion?
2. Complete the table below to show all the possible fragments and plasmids by tube:

Tube	*Fragment name/s*	*Fragment Length/s (bp)*	*Configuration of Plasmid if Applicable*
R-			
R+			

Materials

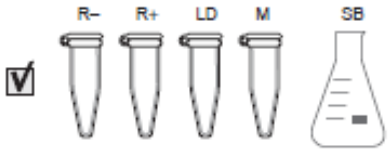
- A rack with the following:
  - Microfuge tube of non-digested pARA-R from Laboratory 2A (R–)
  - Microfuge tube of digested pARA-R from Laboratory 2A (R+)
  - Microfuge tube of loading dye (LD)
  - Microfuge tube of DNA ladder (M)
  - 500 mL flask containing 300mL of 1x SB (shared with another group)
- P20 micropipette
- Green box of white disposable pipette tips
- Microcentrifuge (will be shared among all groups)
- Electrophoresis box loaded with 0.8% agarose gel (will be shared among two groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

Safety:

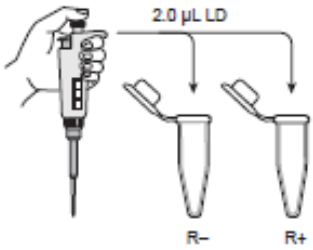
- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.
- Wash your hands well with soap after completing the lab.

Method: ***Write a method using the flowchart below as a guide***

Laboratory 4A Flowchart



Check reagents
(R–, R+, LD, M in rack, 1x SB flask)



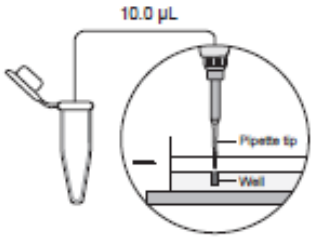
Pipette 2.0 µL LD into
the R– and R+ tubes

Centrifuge
the two tubes

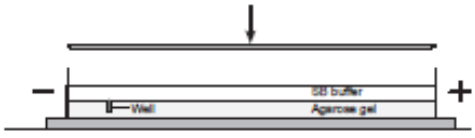
Review gel
electrophoresis
unit (GEU)

Cover gel in
GEU with 1x SB

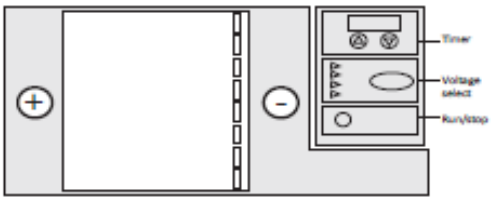
Draw location of
each well, and label
which solution is loaded
in each in notebook



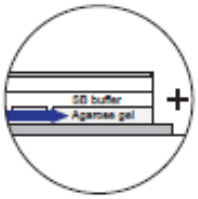
Pipette 10.0 µL each of
DNA ladder (M), R–, and R+
into designated wells in GEU



Place cover
on GEU



Turn on GEU and set voltage
to 100 V



Check to see if the
loading dye is moving toward
the positive (red) electrode

Teacher will explain
final steps

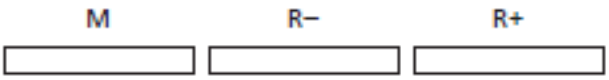
Questions:

1. Carefully remove the cover from the gel box and transfer the gel in the transparent gel tray into the PrepOne™ Sapphire gel illuminator. Turn on the gel illuminator to view your DNA bands. Use the photo hood and capture an image of your gel. - Insert your photo here
2. The DNA Ladder Diagram (Worksheet 4A) shows 10 DNA bands of known sizes. Using this information, predict the positions of DNA bands produced by the possible products found in the R– and R+ tubes (see table you completed above) by drawing their position on the DNA Ladder Diagram - insert a photo of your completed sheet here.
3. Measure the distance that each piece of DNA in the gel has travelled from the back of the well to the front edge of the band using a ruler and record this information in the table below (add additional rows for more fragments.

DNA Ladder Fragment	Size (bp) (predicted for R-/R+)	Distance Travelled (mm)
1	10,000	
2	8,000	
3	6,000	
4	5,000	
5	4,000	
6	3,000	
7	2,000	
8	1,500	
9	1,000	
10	500	
R- Band #1		
R+ Band # 1		

4. Make a copy of the template provided for the standard curve (bp v Distance travelled in mm) for the DNA ladder fragments and enter the distance travelled for your fragments from R- and R+ (Base Pairs v Distance Travelled in Gel) Insert a copy of your data and the graph generated here
5. How close were the calculated values compared to the expected values in your table in the previous activity? Discuss any sources of error in your calculations/measurements.
6. In the R- lane do you see evidence of multiple configurations of plasmids? Explain your answer
7. In the R+ lane do you see evidence of complete digestion? Explain your answer
8. Compare the lanes that have linear fragments with the lanes that have plasmids. Is there a difference in the shape of the bands between these two DNA forms?

LABORATORY 4A: DNA LADDER DIAGRAM



1	10,000 bp
2	8,000 bp
3	6,000 bp
4	5,000 bp
5	4,000 bp
6	3,000 bp
7	2,000 bp
8	1,500 bp
9	1,000 bp
10	500 bp

Lab 5A TRANSFORMING BACTERIA WITH THE pARA-R PLASMID

Introduction

So far, the presence of the pARA-R plasmid containing the rfp gene that can make the red fluorescent protein has been verified. Once a recombinant plasmid is made that contains a gene of interest (in this cases rfp), the plasmid can enter bacterial cells by a process called transformation. In this laboratory another step of the gene cloning process will be completed, which is to transform *E. coli* bacteria with this plasmid.

The *E. coli* bacteria used have been pretreated with calcium chloride and will be heat shocked in this lab to increase the usual uptake of plasmids by the bacteria. The bacteria will be divided into two groups: a control group to which no plasmid is added, and a treatment group to which the pARA-R plasmid will be added. After heat-shocking both groups of cells, they will then be grown under several different conditions:

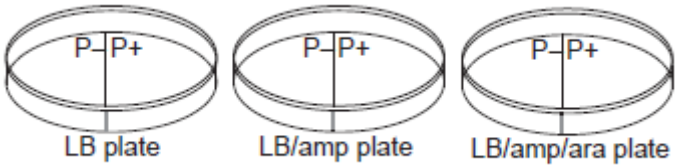
- A control group (P-) grown in the presence of Luria Broth (a medium that supports bacterial growth).
- A control group (P-) grown in the presence of Luria Broth and the antibiotic ampicillin.
- A control group (P-) grown in the presence of Luria Broth, ampicillin, and the sugar arabinose
- An experimental group (P+) grown in the presence of Luria Broth
- An experimental group (P+) grown in the presence of Luria Broth and the antibiotic ampicillin
- An experimental group (P+) grown in the presence of Luria Broth, ampicillin, and the sugar arabinose

By examining the growth of bacteria under these conditions, the success of the procedure can be assessed and the bacteria transformed with the pARA-R plasmid can be identified as these bacteria will have a new and highly visible trait - they will produce red fluorescent protein, which makes the cells red or bright pink.

The relevant components of this plasmid are the rfp gene, the promoter (pBAD), the ampicillin resistance gene (ampR), and the arabinose activator protein gene (araC). The ampR gene confers resistance to the antibiotic ampicillin (biotechnologists call these genes selectable markers because only bacteria having the gene will survive in the presence of an antibiotic).

The araC gene controls the promoter. If arabinose, a simple sugar, is present in the bacteria, the activator protein made by the araC gene turns on the promoter, which then binds RNA polymerase, and transcription of the rfp gene occurs. Activator proteins are used in some recombinant plasmids to control production of the protein of interest.

In the lab, samples of the control group P– and the experimental group P+ will be added to plates that contain various combinations of Luria Broth (LB), ampicillin, and the sugar arabinose. The plates will be arranged as follows:



Activity:

Write an aim and hypotheses for this section, identifying the dependent and independent variables and including your predictions for the growth you would expect for each combination in your hypotheses.

Aim:
Hypotheses:
Independent Variables (there are more than one):
Dependent Variables (there are more than one):

Safety:

- All appropriate safety precautions and attire required for a science laboratory should be used. Please refer to your teacher's instructions.
- Use caution when handling *E. coli* bacteria and use aseptic technique. Aseptic technique is a set of procedures that ensure protection of the lab worker and protection of a bacterial sample, which is necessary for the experiment to be successful. Specifically:
    - Do not touch anything that has been or will be in contact with *E. coli* bacteria. Students handling equipment that comes into contact with bacteria should wear gloves.
    - Try to avoid spills or contamination of surfaces with anything that has been in contact with *E. coli* bacteria. Immediately inform your teacher if a spill or contamination occurs.
    - When you have finished using microfuge tubes, pipette tips, and cell spreaders, place them immediately into the biohazard bag or waste container, as directed by your teacher.
    - When directed to do so, place your Petri plates in the biohazard bag.
    - Wash your hands well with soap after completing the lab

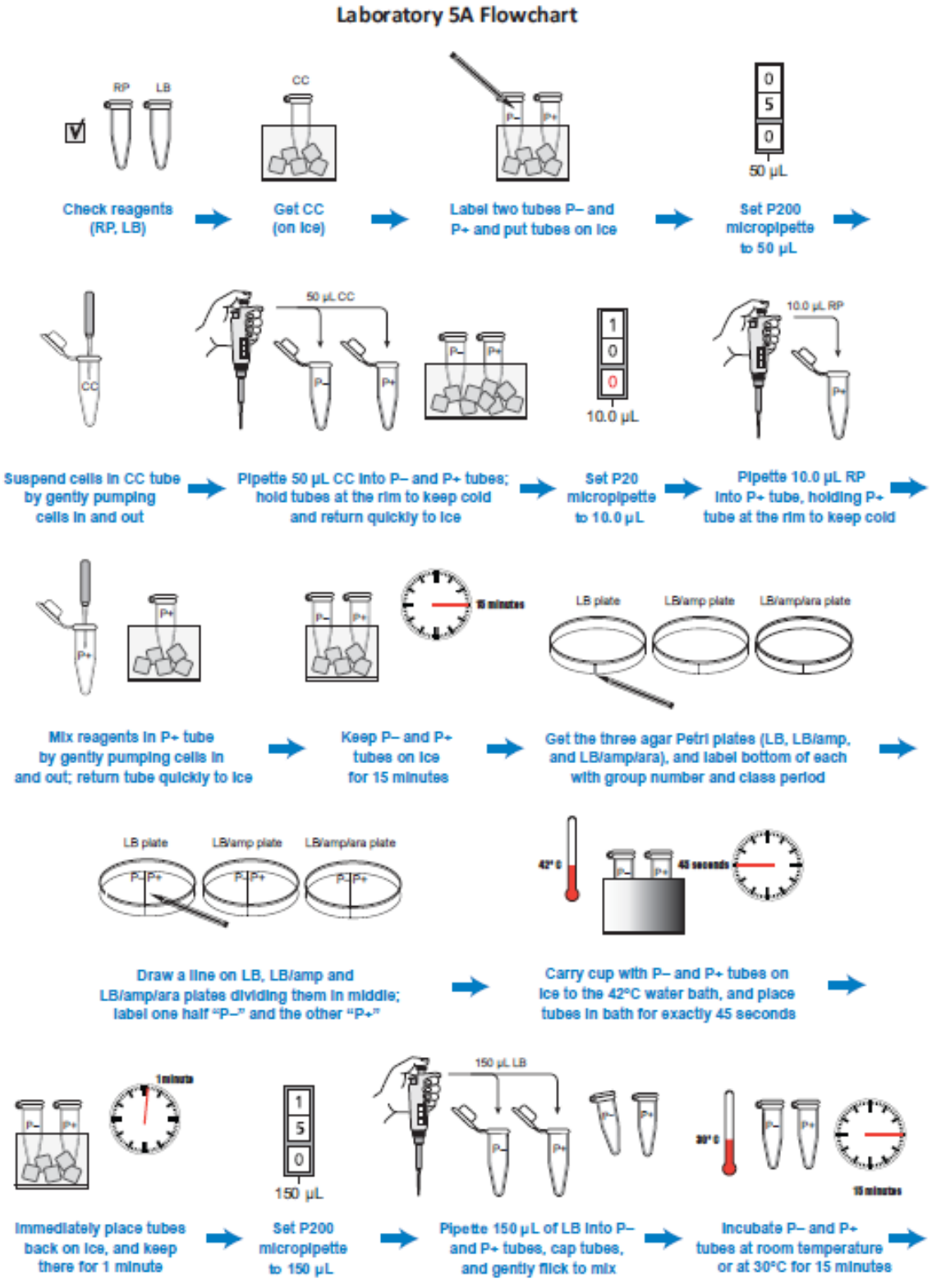
MATERIALS

Reagents

- A rack with the following:
    - Microfuge tube of pARA-R plasmid (RP)
    - Microfuge tube of Luria Broth (LB)
    - Microfuge tube of 100 µL of chilled competent *E. coli* cells (CC) Note: The CC tube must be kept on ice at all times.
- 3 Petri plates with agar:
    - 1 of LB
    - 1 of LB/amp
    - 1 of LB/amp/ara
- Container of crushed ice Note: Fill a cup with some of the crushed ice from the container holding the CC tubes before taking a CC tube. You'll need to keep the CC tube on ice at all times.
- 2 1.5-mL microfuge tubes
- Permanent marker
- Disposable gloves
- P20 micropipette
- P200 micropipette
- Green box of white disposable pipette tips
- Yellow box of yellow disposable pipette tips
- Pack of cell spreaders (will be shared among groups)
- 42°C water bath (will be shared among all groups)
- Timer or clock (will be shared among groups)
- Coloured tape (will be shared among groups)
- 30°C incubator (will be shared among all groups)

- Biohazard bag for materials that come into contact with *E. coli* cells (will be shared among groups)
- Waste container (will be shared among groups)

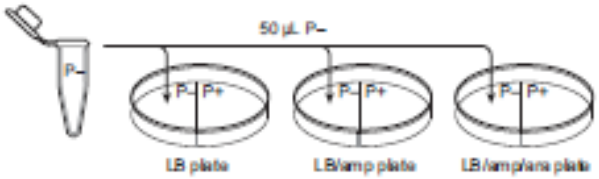
Method: *Follow the method in the student guide, using the flowchart to help you*



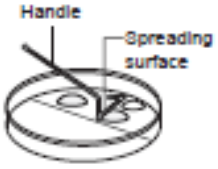
Laboratory 5A Flowchart (Continued)

Set P200 micropipette to 50 µL

Gently pump pipette a couple of times in P– tube

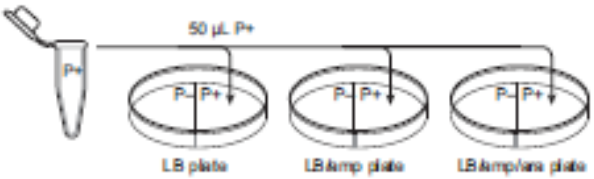


Pipette 50 µL of P– onto P– halves of LB, LB/amp and LB/amp/ara plates and close lids immediately after dispensing P–

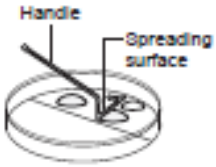


Use one cell spreader to spread P– cells on P– halves of plates in this order: LB (1st), LB/amp (2nd), LB/amp/ara (3rd), and close lids

With new pipette tip, gently pump pipette a couple of times in P+ tube



Pipette 50 µL of P+ onto P+ halves of LB, LB/amp and LB/amp/ara plates, and close lid after dispensing P+



Use one cell spreader to spread P+ cells on P+ halves of plates in this order: LB (1st), LB/amp (2nd) and LB/amp/ara (3rd), and close lids

Let plates sit right side up for 5 minutes

Tape all three plates together, and label tape with group number and class period

Place plates in the 30°C incubator upside down

Incubate plates for 24–36 hours at 30°C

Examine plates and record the amount of growth on each

Results & Conclusions:

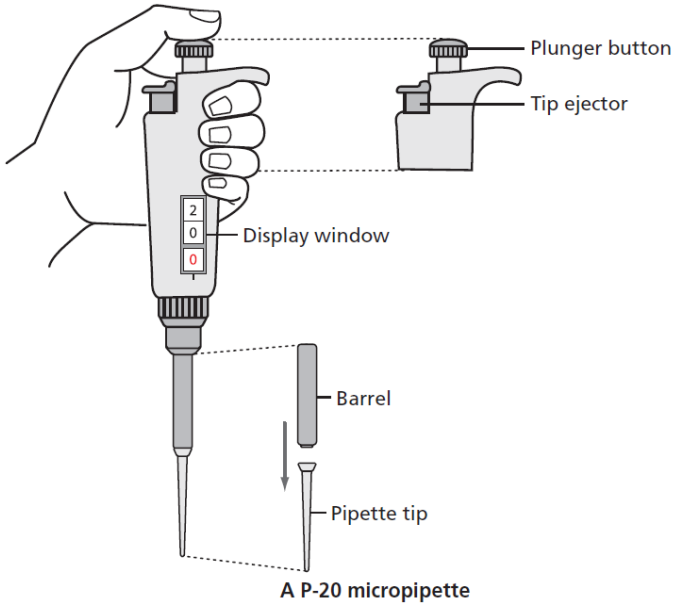
1. Take photographs of your plates and insert them here.
2. Complete the tables below summarising your results.

Plate	Amount of Growth	Colour of Colonies
P- Luria Broth (LB)		
P- Luria Broth ampicillin (LB/amp)		
P- Luria Broth ampicillin arabinose(LB/amp/ara)		
P+ Luria Broth (LB)		
P+ Luria Broth ampicillin (LB/amp)		
P+ Luria Broth ampicillin arabinose(LB/amp/ara)		

3. Compare these results to the predictions made in your hypotheses and write a suitable conclusion accepting or rejecting your hypotheses.
4. Compare the results obtained with those expected and/or of other students and discuss possible reasons for these results and improvements if appropriate.

Measuring Small Volumes

The purpose of this laboratory is to introduce you to an important tool used in genetic engineering: the micropipette, shown in the figure below. A micropipette is used to transfer very small and exact volumes of liquids in either millilitres (mL, thousandths of a litre) or microliters (µL, millionths of a litre), which are the measurements of volume most often used in genetic engineering. This laboratory will give you the chance to learn how to use the micropipette and to see the relative size of different amounts of solution measured by this very precise tool and how precise the amounts that you can measure with it are.



A P-20 micropipette

Image Taken from Amgen *Foundations of Biotech* Student Guide, 2019, p. 19

Activity 1: Measuring Small Volumes

Complete the activity Measuring Small Volumes and enter your score _____ /6

Activity 2: How to Pipette

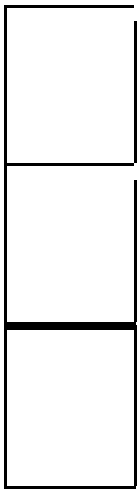
Watch the video Introduction to the Micropipette - LabXchange then complete the table below to put the steps into the correct order.

Steps	Correct order (write the numbers)
1. Push the plunger down to the first stop	
2. Push the plunger all the way to the bottom	
3. Set the volume you need to deliver	
4. Put the pipette (with tip on) into the solution you want to deliver	
5. Put the correct tip on	
6. Release the plunger and allow the solution to suck up into the tip	
7. Touch the side of the tube/surface you want to deliver the solution to	

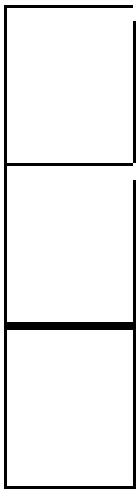
Activity 3: Using the P20 & P200 Micropipettes

Write the settings on the pipette for the following volumes:

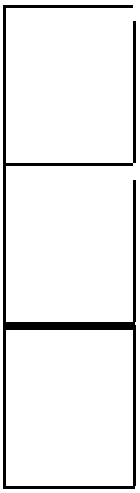
20 µL



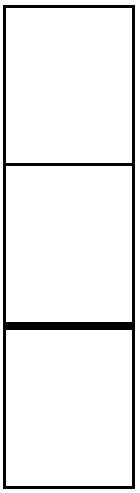
12.4 µL



5.5 µL



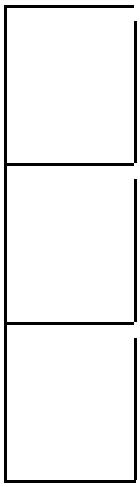
2.0 µL



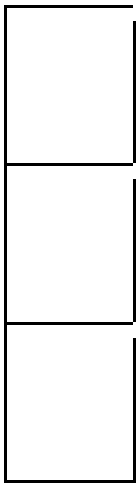
Sketch a diagram in the space below showing the relative size of the samples placed onto the laminated **P20** practice sheet:

Write the settings on the micropipette for:

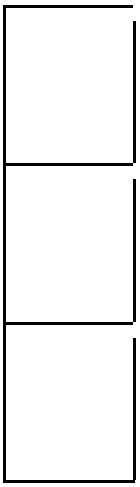
200 µL



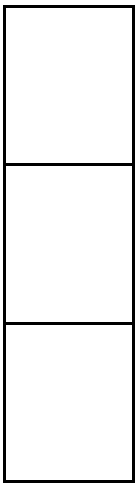
137 µL



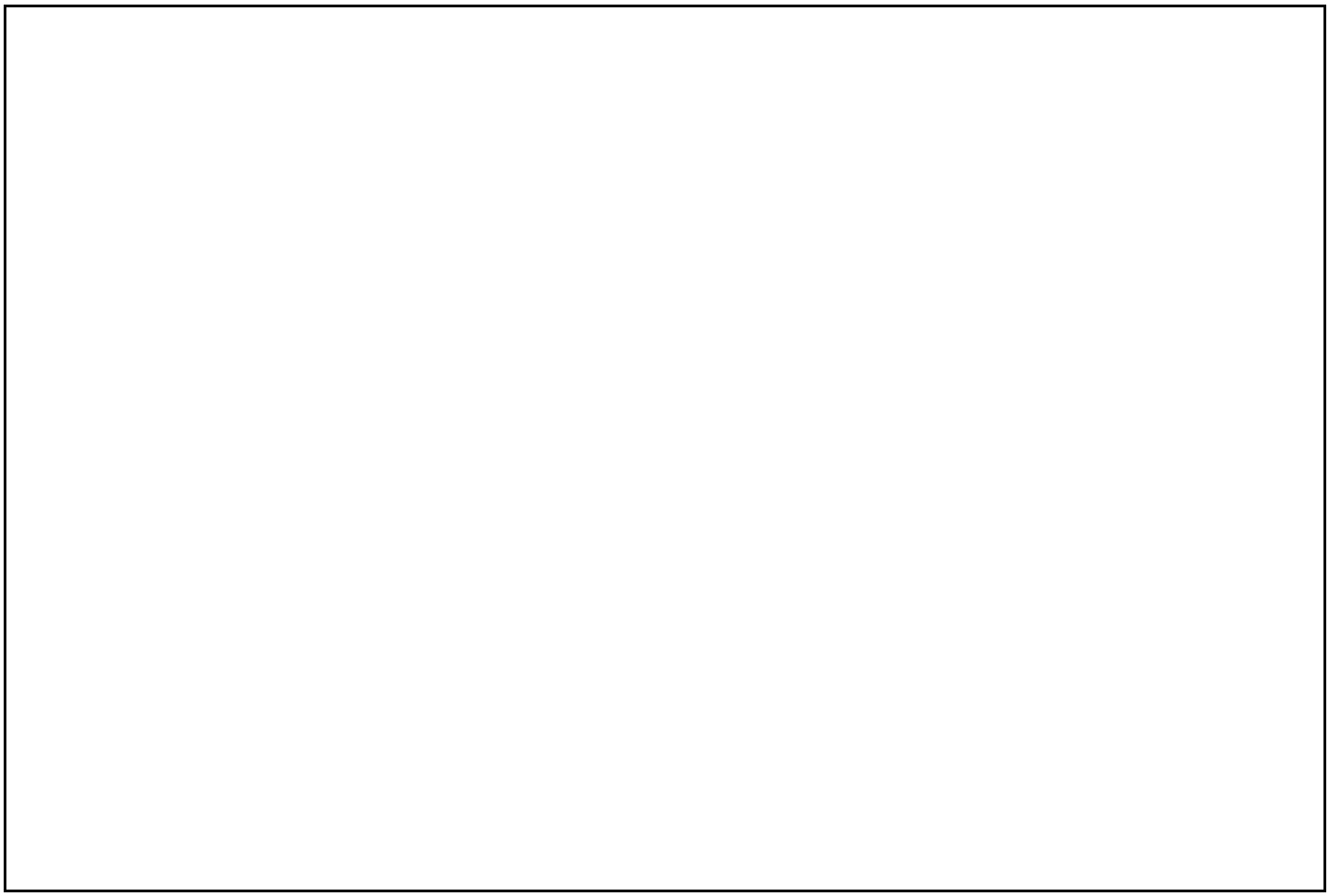
48 µL



25 µL



Sketch a diagram in the space below showing the relative size of the samples aliquoted onto the laminated **P200** practice sheet:



Activity 4: Post Lab Knowledge Check

1. What volume would the following micropipette settings deliver?

P20	P200	P20	P200	P20	P200
0	0	1	1	2	0
7	7	0	0	0	2
5	7	0	0	0	0
_____ µL	**_____ µL**	**_____ µL**	**_____ µL**	**_____ µL**	**_____ µL**

2. What setting(s) would you use to deliver 25 µL using a P200 and a P20? *Hint you might have to pipette more than once to deliver the volume with one of the pipettes.*

   a. **P200:** _____

   b. **P20:** _____

3. Fill in the table below with the ranges and settings for each of the pipettes you have used

Type of Micropipette	**Volume range**		**Tip Colour**	**Settings used**
	(mL)	(µL)		
P20				

P200				

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Marking Criteria for First-Hand Investigation

Outcome	Criteria	Marks
SC5-4WS Hypothesis	formulated a testable hypothesis based on prior research/previous observations	3
	proposed a hypothesis based on prior research or previous observations	2
	proposed a hypothesis either loosely or not related to the background information collected	1
	not attempted	0
TOTAL		/3

Outcome	Criteria	Marks
SC5-5WS Planning	completed a valid and well-planned scientific investigation over a period of time	4
	completed a well-planned scientific investigation over a period of time	3
	completed a scientific investigation with moderate planning	2
	submitted a project with limited planning	1
	not attempted	0
SC5-5WS Aim	has well-defined aims and clearly expressed the subject of the investigation	3
	had some tentative aims and adequately described the subject of the investigation	2
	had no clear aim and vaguely described the subject of the investigation	1
	not attempted	0
SC5-5WS Risk Assessment	identified procedures and performed a risk assessment prior to experimentation	2
	considered experimental risks but did not conduct a formal risk assessment	1
	no attempted	0
SC5-5WS Variables	identified independent and dependent variables and took deliberate steps to keep controlled variables constant	3
	controlled some variables	2
	did not recognise or control variables	1
	no attempted	0
TOTAL		/12

Outcome	Criteria	Marks
SC5-6WS Repetition Replication Observation	accurately made relevant observations in replicated trials using appropriate technologies	3
	gathered experimental data over a number of trials using appropriate technologies	2
	gathered some first-hand data without replication	1
	no attempted	0
SC5-6WS Logbook	included a comprehensive logbook, detailing the investigative process, from brainstorming, through data collection, to the final conclusion	3
	included a logbook detailing the different stages of the investigative process	2
	provided limited or disorganised documentation in the accompanying logbook	1
	not attempted	0
TOTAL		/6

Outcome	Criteria	Marks
SC5-7WS Background Research	included a concise and comprehensive summary of relevant prior research in the field and assessed its reliability	3
	included a summary of current relevant background information and checked its reliability	2
	included some relevant background research	1
	not attempted	0
SC5-7WS Data Recording	recorded data in an organised, sequential, and logical manner using correct units	4
	recorded data in a systematic manner using correct units	3
	recorded data using incorrect or no units	2
	did not present the data clearly	1
	not attempted	0
SC5-7WS Data Analysis	analysed and evaluated trends, patterns and relationships in the data collected	4
	analysed and explained trends, patterns and relationships in the data collected	3
	identified occasional trends, patterns and relationships in the data	2
	loosely identified trends, patterns and relationships in the data	1
	not attempted	0
TOTAL		/11

Outcome	Criteria	Marks
SC5-8WS Communication	exhibited deep understanding of related science concepts	4
	demonstrated good understanding of the science concepts used in the investigation	3
	demonstrated minimal understanding of the science concepts used in the investigation	2
	demonstrated inadequate understanding of the science concepts used in the investigation	1
	not attempted	0
SC5-8WS Discussion	suggested worthwhile directions for future research clearly	3
	suggested modifications to procedures and ideas for future research	2
	put forward some ideas for future improvements	1
	not attempted	0
TOTAL		/7

Outcome	Criteria	Marks
SC5-9WS Creativity	was innovative and creative in approach, content, methodology or communication to audience	2
	had some innovative or creative ideas but did not develop them	1
	not attempted	0
SC5-9WS Conclusion	used critical thinking to synthesise information and argue the merits of conclusions	4
	used critical thinking to derive conclusions	3
	formulated conclusions that were not fully supported by experimental data	2
	manufactured conclusions lacking supporting information and scientific accuracy	1
	not attempted	0
SC5-9WS Communication	used clear, concise, consistent, and meaningful language, visuals, and sequencing to effectively communicate to the intended audience; bibliography using Harvard referencing style	4
	communicated the report with effective use of language, visuals and sequencing; bibliography using Harvard referencing style	3
	communicated the report with adequate use of language, visuals and sequencing, appropriate to the intended audience; bibliography	2
	communicated the report with poor expression and inadequate use of visuals; bibliography	1
	not attempted	0
SC5-9WS Acknowledgment	acknowledged the source and type of any assistance given	3
	acknowledged some assistance but did not provide details of the type of assistance given	2
	did not acknowledge assistance given	1
	not attempted	0
TOTAL		/13