IGEMer Day
CUHK-Hong Kong-SBS ATREUS

Introduction

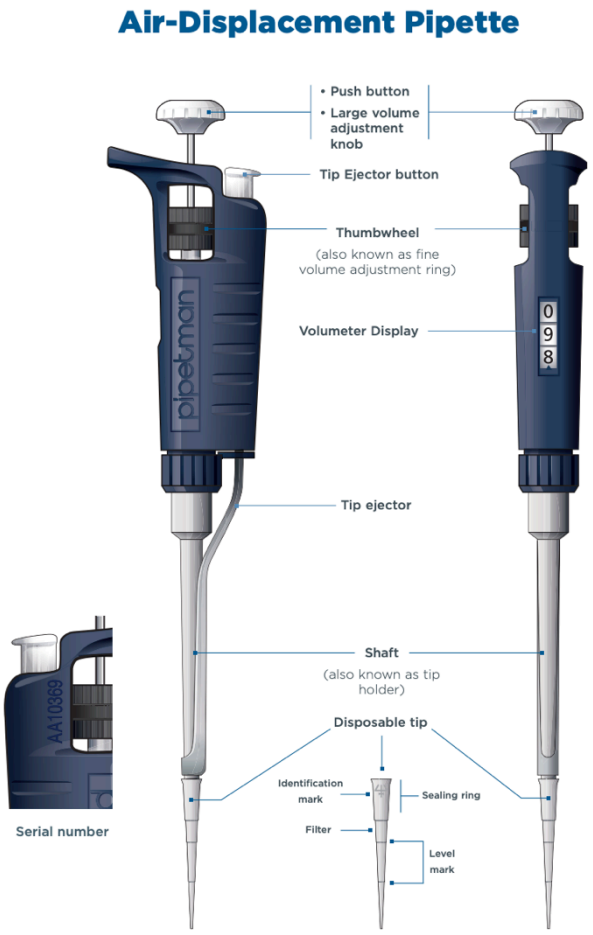
Synthetic biology is a field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities. It can be widely used to solve problems in medicine, manufacturing and agriculture. Today we will teach you some essential techniques that are often used in the field of synthetic biology.

There are two parts in this workshop: 1) pipetting and 2) gel electrophoresis. They are very common and useful techniques used in experiments.

Part 1: Pipetting

Micropipette is an equipment that can transfer, measure and inject very small and precise amount of liquid. This is an extremely important technique as we always have to deal with tiny amount of samples.

1.1 Structure of a micropipette



1.2 Types of micropipettes

There are different types of micropipettes, such as P20, P200 and P1000. The number after ‘P’ represents the maximum amount of liquids that can be transferred by this micropipette. Different types of micropipettes handle different ranges of volumes and they all fit into specific model of pipette tips. Before pipetting, make sure to choose the right pipette for the right volume.

Micropipette	Range of volume to be pipetted
P20	2-20µL
P200	20-200µL
P1000	100-1000µL

*1000µL = 1mL

1.3 Procedures

Overview

Step 1 Select an appropriate pipette – Choose the right pipette for the right volume

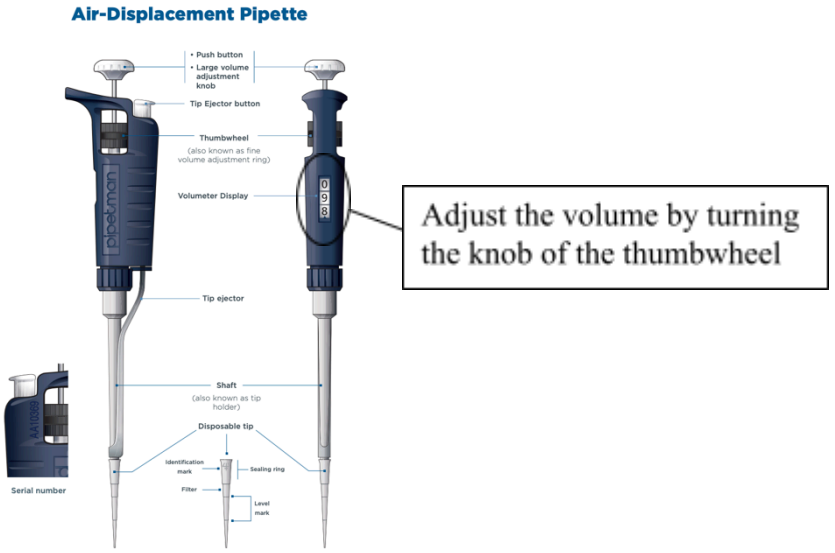
Step 2 Set the volume – Turn the dial

Step 3 Pipetting – Fit into the right tip; aspiration and dispensation

I. Select an appropriate pipette

Volume	15	*35	175	*300	800
P20					
P200					
P1000					

II. Set the volume



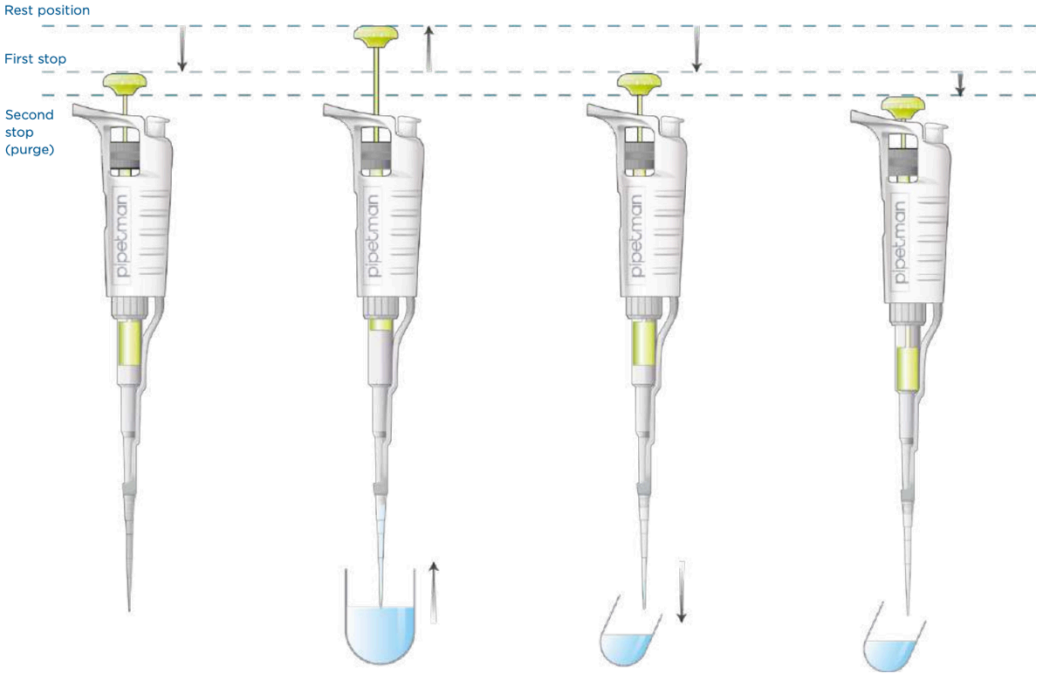
III. Pipetting

Step 1 Fit the pipette into appropriate model of pipette tip

Step 2 Push the button to the **first stop** and aspirate solution

Step 3 To dispense the solution, push the button all the way to the **second stop**
**DO NOT release the button until the tip has left the solution

Step 4 Eject the tip into the waste bottles using the ejector
**To avoid contamination, remember to change tips when we pipette different solutions



1.4 Practical I: Pipetting accuracy and precision

Overview

Task 1 Select an appropriate pipette for pipetting 200ul of water into 1.5ml microcentrifuge tube and measure the mass of water in the tube (Repeat for 3 times)

Task 2 Repeat Task 1 for 3 times again but this time use P1000

Result

Task 1 Pipette that has been chosen: P20 / P200 / P1000 (circle the answer)			
Trials	Trial 1	Trial 2	Trial 3
Weight (g)			

Task 2			
Trials	Trial 1	Trial 2	Trial 3
Weight (g)			

According to your results, which micropipette gets a more accurate result?

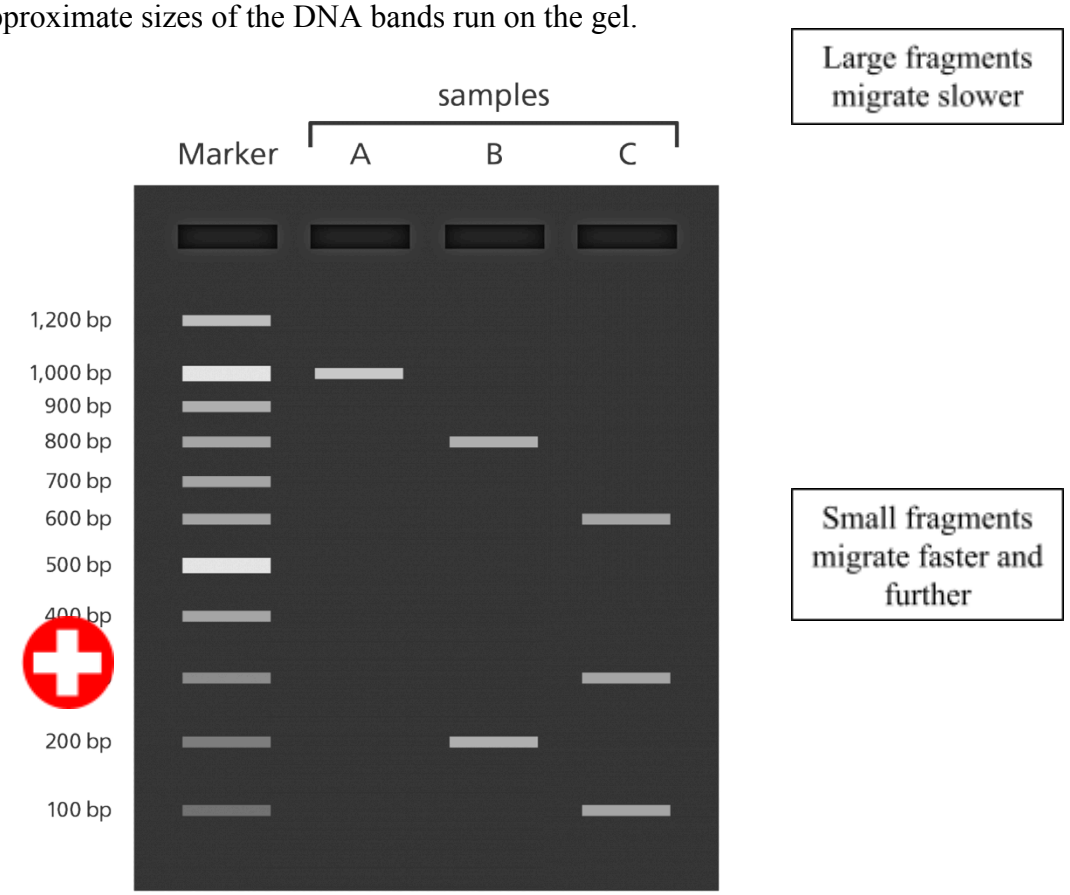
Part 2: Gel electrophoresis

Gel electrophoresis is another common lab technique that can be used for separating fragments of nucleic acids and identifying the presence of certain charged nucleic acids based of their sizes.

2.1 Working principle of gel electrophoresis

DNA which is negatively charged move through the pores in an agarose gel to the positive pole when an electric current is applied. The smaller is the molecular size of that particular molecules, the further will its band goes, and vice versa. This is because the smaller molecules can easily pass through the pores in the agarose gel to

the opposite pole. DNA ladder is used as a reference so that we can know the approximate sizes of the DNA bands run on the gel.



2.2 Procedures

Overview

Step 1 Set an agarose gel

Step 2 Prepare the samples –mix the samples with loading dye

Step 3 Load the sample onto the gel

I. Set an agarose gel

The percentage of agarose gel prepared can highly affect the resolution (what size of nucleic acids can you separate). This is because the higher the percentage of the gel, the finer is the pore in the gel. It will be harder for larger DNA fragments to pass through the pores and almost impossible for them to be separated. Therefore we first have to know the size of the nucleic acids that we want to visualise on the gel. Then determine which percentage of agarose gel we are going to prepare.

II.

Agarose (%)	Resolution (bp = base pair)
0.5	1000-30000 bp
1.0	500-10000 bp
1.5	200-3000 bp
2.0	50-2000 bp

For example, gene encoding green florescence protein (GFP) is around 720 bp. Any percentage between 1-2% is good enough to separate the gene fragments of GFP.

Protocol for casting a 1% agarose gel (for practical session)

Step 1 Weigh the 0.3 g of agarose powder

Step 2 Dissolve agarose powder using 30 ml 1x TAE buffer in a conical flask

Step 3 Microwave the mixture for 1 – 1.5 minute for better dissolution
**It will be very hot so be careful when taking it out from the microwave

Step 4 Swirl the conical flask and flush the outside of the flask with tap water to cool down the solution until you are able to touch the flask without discomfort from heat
**DO NOT cool down to very low temperature to prevent solidification of solution in this step

Step 5 Add 2 µL of GelRed into the solution for staining the gel

Step 6 Swirl the conical flask to mix everything well

Step 7 Pour all the solution into the rack provided and wait till it is solidified
**Avoid making any air bubbles as they can hinder the migration of DNA bands; if there is any air bubble, use pipette to either poke it or aspirate it

III. Prepare the samples

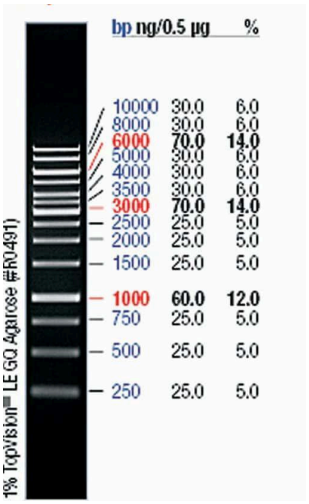
The concentration of loading dye is usually 6x which means that the concentration is 6-fold of the working solution. Therefore we have to dilute the loading dye to 1x.

	DNA sample	6x Loading dye	Total
Volume (µL)	10	2	12

IV. Loading the sample

3 µL of DNA ladder is loaded into the first well. Then pipette the samples into the subsequent wells.

DNA ladder



2.3 Practical II: Looking for Green Fluorescence Protein (GFP)

GFP is a protein extracted from jellyfish which can exhibit green fluorescence when it is exposed to light in the blue to ultraviolet (UV) range. This protein is often used as a marker protein to see if the gene of interest is inserted into cells so it is frequently used in the field of synthetic biology.

Overview

Task 1	Perform a Gel Electrophoresis by following the protocol provided above
Task 2	Identify which sample contains the gene encoding GFP

Result

Which sample contains GFP gene? (X / Y)

Thanks for joining our workshop!

Please fill out the survey and follow our Instagram for more information about synthetic biology!

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