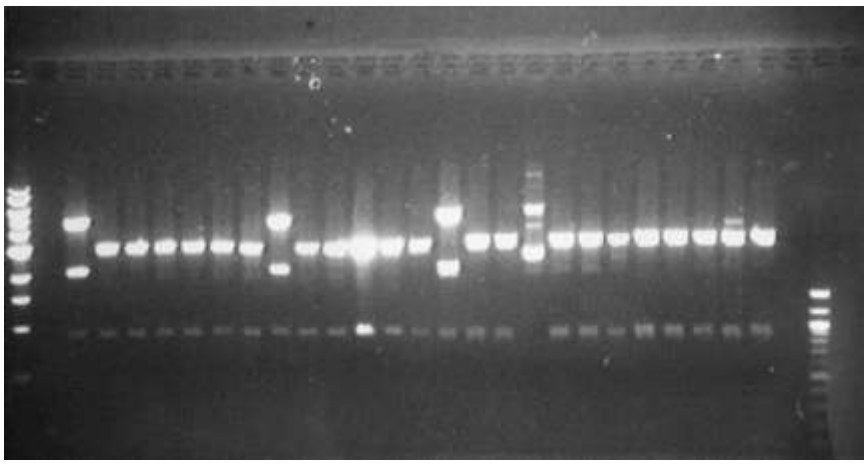
SPARQ-ed

Introduction to Molecular Biology Techniques : DNA Restriction and Gel Electrophoresis



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SPARQed is a collaboration between The University of Queensland's Diamantina Institute and The Queensland Government's Department of Education and Training. It exists due to the hard work of the SPARQ-ed Regional Reference Group (Regan Neumann, Associate Professor Nigel McMillan, Associate Professor Brian Gabrielli, Dr Peter Darben, Cheryl Capra, Peter Ellerton, Andrew Rhule, Michael Sparks, and Patrick Trussler).

The DNA Techniques project is based on the Polo-box Cloning project developed by Associate Professor Brian Gabrielli. The experimental program and all supporting materials were adapted for student use by Dr Peter Darben, under the supervision of Associate Professor Brian Gabrielli and Stephanie Le.

Risk assessments were developed with the assistance of Paul Kristensen, Maria Somodevilla-Torres and Jane Easson.

Many thanks to all in the "Gab Lab" whose patience and support made this happen.

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Introduction

Deoxyribonucleic acid (DNA) is the molecule which carries the genetic instructions for almost every living thing. Its unique chemistry not only allows this information to be copied and passed on to an organism's descendents, it also allows scientists opportunities to investigate and manipulate an organism at a molecular level. As a result, molecular biology techniques are at the forefront of most cutting edge scientific research. In this project you will investigate a number of commonly used molecular biology techniques involving DNA.

What is Molecular Biology ?

Molecular biology is the study of living things at the level of the molecules which control them and make them up. While traditional biology concentrated on studying whole living organisms and how they interact within populations (a "top down" approach), molecular biology strives to understand living things by examining the components that make them up (a "bottom up" approach). Both approaches to biology are equally valid, although improvements to technology have permitted scientists to concentrate more on the molecules of life in recent years.

Molecular biology is a specialised branch of biochemistry, the study of the chemistry of molecules which are specifically connected to living processes. Of particular importance to molecular biology are the nucleic acids (DNA and RNA) and the proteins which are constructed using the genetic instructions encoded in those molecules. Other biomolecules, such as carbohydrates and lipids may also be studied for the interactions they have with nucleic acids and proteins. Molecular biology is often separated from the field of cell biology, which concentrates on cellular structures (organelles and the like), molecular pathways within cells and cell life cycles.

The molecules which form the basis of life provide scientists with a more predictable and mechanistic tool for scientists to study. Working with whole organisms (or even just whole cells) can be unpredictable, with the outcome of experiments relying on the interaction of thousands of molecular pathways and external factors. Molecular biology provides scientists with a toolkit with which they may "tinker" with the way life works. They may use them to determine the function of single genes or proteins, and find out what would happen if that gene or protein was absent or faulty. Molecular biology is used to examine when and why certain genes are switched "on" or "off". An understanding of each of the factors has granted scientists a deeper understanding of how living things work, and used this knowledge to develop treatments for when living things don't work so well.

Common Molecular Biology Techniques

The following list covers some of the more commonly used molecular biology techniques – it is by no means exhaustive.

Electrophoresis – a process which separates molecules such as DNA or proteins out according to their size, electrophoresis is a mainstay of molecular biology laboratories. While knowing the size of a molecule might not seem like all that much information, it can be used to identify molecules or fragments of molecules and as a check to make sure that we have the correct molecule present.

Polymerase Chain Reaction (PCR) – a process used to amplify very small amounts of DNA to amounts which can be used in further experiments. It is used as a basic tool in molecular biology to ensure that we have sufficient DNA to carry out further techniques such as genetic modification, however it has wider practical uses such as in forensics (identification using DNA profiling) and disease diagnosis. PCR can also be used to introduce small point mutations into a gene in a process called site-directed mutagenesis.

Restriction Digest – the process of cutting DNA up into smaller fragments using enzymes which only act at a particular genetic sequence.

Ligation – the process of joining two pieces of DNA together. Ligation is useful when introducing a new piece of DNA into another genome.

Blotting – a technique used to specifically identify biomolecules following electrophoresis. The molecule of interest is indicated using either a labeled probe (a complementary strand of nucleic acid) or a labeled antibody raised against a specific protein.

Cloning – the technique of introducing a new gene into a cell or organism. This can be used to see what effect the expression of that gene has on the organism, to turn the organism into a factory which will produce large quantities of the gene or the protein it codes for, or (within the inclusion of a label) to indicate where the products of that gene are expressed in the organism. Insertion of genetic material into a bacterium is called transformation, while insertion into a eukaryotic cell is called transfection. If a virus is used to introduce this material, the process is called transduction.

Each of these techniques is used in conjunction with other techniques to help scientists solve a particular research question. For example, following using ***PCR*** to create large quantities of a particular gene a scientist may ***ligate*** a gene for a particular protein into a plasmid vector (a short circular strand of DNA which acts as a carrier), perform a quick ***restriction digest*** and ***electrophoresis*** to ensure that the gene has been inserted properly, and then use that plasmid to ***transform*** a bacterial cell which is used to produce large quantities of the vector. After purification of the vector from the bacteria, it is then used to ***transfect*** a mammalian cell in culture. The scientist then uses protein ***electrophoresis*** and ***western blotting*** to demonstrate the expression of the gene product.

The Project

Many molecular biology techniques take a significant amount of time to complete. Many of the enzyme-based reactions which underpin these techniques require incubation periods of an hour or more, while cloning and transformation often requires overnight incubation to allow the transformed cells time to recover and multiply. In the limited time we have available, we cannot hope to cover all molecular biology techniques, however you will undertake a mini-project which will expose you to some of the more important DNA-based techniques.

In this project you will be provided with a sample containing a plasmid vector. This vector has had a gene for a short region of the protein PLK1 inserted into it through ligation. Due to the nature of DNA, that gene may have been inserted correctly or back-to-front. Using a small sample of the plasmid, you will perform a restriction digest to "drop out" the insert and use electrophoresis to check its orientation.

Getting Started

Before you begin, make sure that you are familiar with the relevant theory behind the techniques we will be performing. This manual contains several appendices which will provide you with this information. Make sure you read this information before proceeding.

Appendix A : DNA

Appendix B : Using a Micropipette

Appendix C : Glossary of Terms

Other molecular biology techniques are provided at the SPARQ-ed website at :

http://www.di.uq.edu.au/sparqed-services#background

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Theoretical Basis of the Project

Using Plasmids for Cloning

One of the more common techniques available to scientists working in molecular biology is cloning. In this technique, sequences of DNA containing genes of interest are inserted into vectors which are then used to introduce these genes into cells or organisms to study the effects of the expression of the genes.

Vectors are based on bacterial plasmids – short circular pieces of DNA separate to the main bacterial chromosome which may be transferred between bacteria. Scientists source plasmid vectors from biological supply companies, which create them by ligating together pre-existing genes and sequences of DNA built from scratch using sequencing technology. An example of a commercial vector is the pGEM-T Easy system.

Prior to today's project, a gene for a fragment of a protein called pololike kinase 1 (PLK1) has been inserted into a pGEM-T Easy vector by another group of students. The Diamantina Institute's Cell Cycle research group hopes to use these vectors to study where PLK1 localises in the cell during the cell cycle in order to find out how changes to the cell cycle might lead to cancer.

DNA is a symmetrical molecule, meaning that during the process of transcription, either strand could act as a template for the creation of the mRNA molecule. As each strand runs in an opposite direction to the other, a gene could in theory be transcribed forwards or backwards. In the cell nucleus, genes are only transcribed in one direction due to the placement of promoter sequences upstream of the gene. In our vector, however, the promoters are found on the vector itself rather than the insert. Therefore there may be some of the samples where the vector has been inserted the right way around, and others where it has been inserted backwards. We only want the samples where the vector has been inserted the right way around. Luckily, we can use a restriction digest to find out this information.

Restriction Digests to Check Orientation

Restriction enzymes cut the DNA strand at very specific locations, normally given by sequences of half a dozen or so base pairs. The pGEM-T Easy vector has been created with a number of restriction sites on either side of the insertion point. Some of these restriction sites are only found on one side of the insertion point, and so can be used to linearise the vector to make it ready for electrophoresis. Others are found on both sides, and so may be used to "drop out" the insert to check its size.

Electrophoresis separates fragments of DNA into its component sizes. Fragments of the same size travel through the gel in one region, resulting in a "band" of DNA. When we compare the band to bands of known size, we can estimate the size of the fragments in our band. Consider the following example :

> The enzyme EcoRI has restriction sites on both sides of the insertion point. This means that if we expose our sample to EcoRI, we should end up with two sorts of fragment – one representing the vector (around 3000 bases long, or 3kb) and one representing our insert, which in this case is 700 bases long (0.7kb). This will appear on the gel as indicated in Figure 1 over :

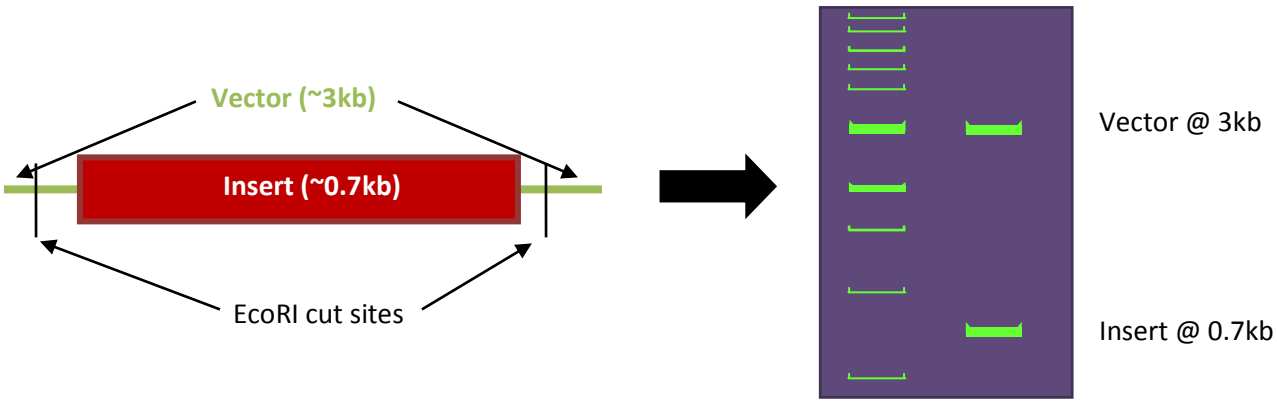

Figure 1 – EcoRI Digestion to check Insertion

This technique allows us to check whether the insert has gone into the vector (otherwise you would just see a band at 3kb) for the vector. However, it does not tell us which way around the insert is oriented. To do this we need to use a different restriction enzyme.

SalI has a single cutting point just downstream of the insertion point in the vector. Normally, a digest using this enzyme would result in a linearised plasmid and a single band at 3.7kb (3kb vector + 0.7kb insert). As luck would have it, however, the inserted gene also contains a SalI cutting point close to the 5' end. This means that there are two possibilities, depending on how the insert is oriented (see Figure 2 below).

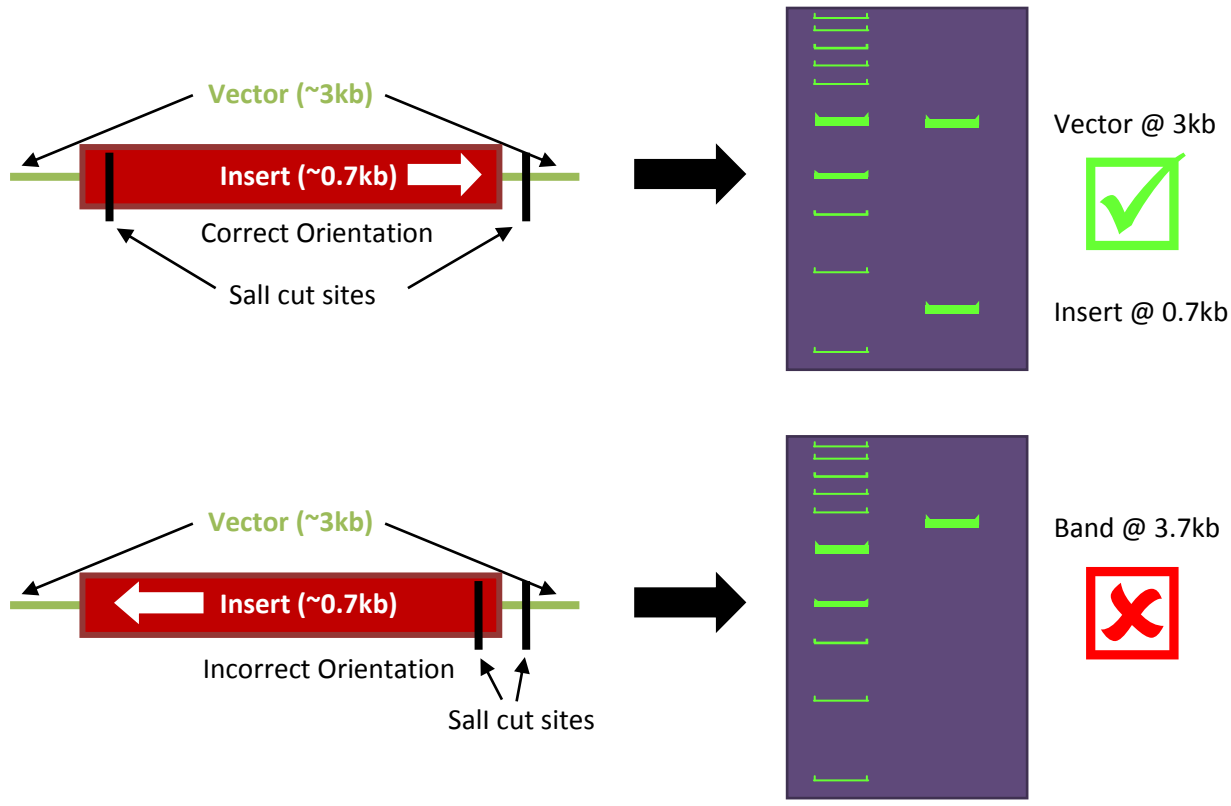

Figure 2 – Using SalI digestion to check the orientation of inserts

In the incorrect orientation example above, the SalI sites are so close together that the fragment dropped out will be too small to resolve using this gel.

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Experimental Protocol

How to Use this Manual

Throughout this section you will see a series of icons which represent what you should do at each point. These icons are:


Write down a result or perform a calculation.


Prepare a reaction tube.


Incubate your samples.

When you are asked to deliver a set volume, the text will be given a colour representing the colour of the micropipette used:

e.g.	750µL	Use the blue P1000 micropipette (200-1000µL)
	100µL	Use the strong yellow P200 micropipette (20-200µL)
	15µL	Use the pale yellow P20 micropipette (2-20µL)
	2µL	Use the orange P2 micropipette (0.1-2µL)

Restriction Digest

Our first procedure will be to prepare restriction digests of our DNA samples. Each person will receive a sample of the plasmid vector containing our gene insert. Make sure that you write down the identity of the sample.

Sample tested : _____

Restriction digests require very small amounts of reagents to be added. Since this exercise is a check of our samples and we do not want to use all of them up, we will work with the barest minimum volumes.

The reagents required for our digest are :

DNA Sample – generally added in the same proportion as the restriction enzyme

Restriction Enzyme – generally added at 10% of the final volume

10X Buffer – a solution of salts which maintain the correct pH for the enzyme to function. "10X" refers to the fact that it is ten times more concentrated than it needs to be and so must be diluted by the addition of the other reagents. The volume of buffer used must be 10% of the final volume. There may be different buffers required for each restriction enzyme

BSA – "Bovine Serum Albumin" a solution of protein derived from the blood of cows. This reagent stabilizes the enzyme reaction mixture, although it is not needed for all restriction enzymes

Water – used to make up the reaction mixture to the final volume

We will be performing a SalI restriction digest on our sample to check the orientation of our inserts.

Use the following table to calculate the volumes needed for our restriction digest :

Tube	Sample DNA (10% of Total)	SalI Enzyme (10% of Total)	10X Buffer (NEB3) (10% of Total)	10X BSA (10% of Total)	Water (Remaining Volume)	Total
SalI Digest						10µL

Once you have calculated the volumes needed and checked these with your tutor, prepare a tube containing the reagents.

Incubate the tubes at 37°C for 1-2 hours

Electrophoresis

In order to check whether the orientation of our insert is correct, we need to examine the size of the DNA fragments which result from our restriction digest. We do this using agarose gel electrophoresis.

Preparation of TAE Buffer

Electrophoresis uses an electric field to "push" DNA fragments through the gel. To ensure that this occurs efficiently, all of the DNA must have a negative charge (to ensure that it is pushed away from the negative terminal). This is done using a buffer which keeps the experiment at a pH where all of the DNA is negatively charged.

The buffer most commonly used in DNA analysis is TAE, which stands for Tris – Acetate – EDTA (where EDTA stands for Ethylenediaminetetracetic acid). The buffer is usually made up at 50X concentration and then must be diluted when needed (this allows us to make and store large amounts of the buffer without having to remake it as often).

For the whole group, we will only need around 500mL of TAE buffer. You will need to prepare 500mL of 1X TAE buffer from the 50X stock solution provided. Perform the following calculations :

Total volume = 500mL

1/50 of 500mL = 500 ÷ 50 = ____ mL

∴ volume of 50X stock needed is ____ mL

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Volume dH_2O needed = Total volume - Volume stock needed

= 500mL – ____ mL

= ____ ml

Dilute ____ mL stock in ____ mL of dH_2O

Use the calculations above to prepare 500mL of 1X TAE buffer

Preparation of Gel

The gel used to studying DNA is made from agarose, a jelly-like substance derived from seaweed. This material is supplied in powder form, and must be dissolved in the TAE buffer. For our experiment, we require a gel containing 0.8% agarose, ie. 0.8g of agarose powder dissolved in 100mL of buffer.

To make sufficient agarose gel for the entire class, we need 4 x 40mL, or 160mL. Since 100mL requires 0.8g of agarose, 160mL requires 160/100 x 0.8 = 1.28g

Weigh out 1.28g of agarose powder and suspend in 160mL of TAE buffer in a conical flask.

- Microwave the solution on HIGH for 2 minutes (for a small gel)for 30 second bursts). Make sure that the agarose is completely dissolved by swirling the heated mixture roughly every 30 seconds. Allow it to cool for 3 minutes.

TAKE CARE: Do not put a lid on the flask while microwaving, otherwise the flask may explode.

TAKE CARE: The agarose solution is quite hot. Use gloves and be careful not to spill any of the solution.

- Wipe a plastic gel tray and comb with 70% ethanol and place in the electrophoresis tank so that the rubber tubing forms a seal with the sides of the tank.

- Add 16µL of SYBR-Safe into the melted agarose and swirl to mix. This substance is a dye which binds to the DNA and glows green under ultraviolet light – it allows us to see where the DNA has migrated in the gel.

- Pour the melted agarose into the gel trays. Place the comb into the right position and allow it to set for approximately one hour (this can be done faster by placing the gel tray in the refrigerator.

- Carefully remove the comb from the gel. Rotate the gel tray so that the wells are toward the negative (black) terminals (the top of the tank, assuming that the electrodes are on the right hand side). Cover the gel with 1X TAE running buffer.

Loading the Gel

The samples must now be loaded into the wells in the gel left by the comb. To make this process easier, we mix the samples with a blue dye and glycerol. The dye migrates before all of the DNA and we can use this to tell when to stop running the gel. The glycerol increases the density of the sample so that it sinks to the bottom of the well on loading. The dye is provided at 6X the required concentration. This means that we have to add it to the sample in a proportion which dilutes it 1 in 6 (ie. five times as much sample as dye). Use the following calculation to find out how much dye is needed to add to a given volume of sample :

- We are going to use all 10µL of our digest product
- if the volume of dye added is "x" :

  x + Volume of DNA = 6x

Volume of dye needed to add to 10µL of digest product = _____ µL

Prepare loading solutions for each of your samples and DNA ladder.

Load all of the loading solutions into separate wells in the gel (loading the DNA ladder last into a separate well on the left or right hand side of your gel). Use the table below to keep track of where you have loaded each sample:

Loading End - Negative (Black) Electrode							
Sample ID #1	Sample ID #2	Sample ID #3	Sample ID #4	Sample ID #5	Sample ID #6	Sample ID #7	Sample ID #8

Running the Gel

- Run the gel at 80V. There must be small bubbles rising from both ends of the electrophoresis chamber. Check after 5 minutes to make sure the gel is running (i.e. the dye front has moved, is relatively straight and has run the correct direction). Then allow the gel to run for the necessary amount of time (about 1 hour however, check that the dye front has almost run through the gel).

> TAKE CARE: While the electrophoresis tanks are well insulated, they still feature high voltages and conductive solutions. Ensure that the power pack is switched off and the leads unplugged before opening the tank.

- Switch off the power pack and take the gel to the transilluminator.

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- Pour away the buffer from the electrophoresis tank and rinse well with water. Rinse the gel tray and comb as well.

Interpreting Your Gel

Whenever we run a gel, we should always include a DNA "Ladder" which features fragments of DNA of known size. This ladder serves as a reference point to indicate the size of the DNA fragments in our sample. A map of the ladder we are using in this exercise is provided in Figure 4.

In order to visualize the DNA bands, we need to place the gel on a UV Transilluminator. The SYBR-Safe dye we added to our gel binds to the DNA and fluoresces green under ultraviolet light.

- Place your gel on the transilluminator, close the plastic shield and switch it on
- Observe the location of DNA bands in the lane that you loaded.
- You should either see a single band at 3.7kb (incorrect orientation) or bands at 3kb and 0.7kb (correct orientation). Was the insert in your sample oriented correctly or incorrectly ?

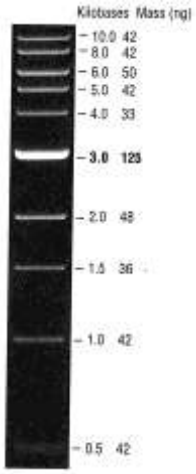

Figure 3: Map of 1kb DNA Ladder

Your tutors will take your gel to a transilluminator with a camera attached to photograph it. The image will be forwarded to your teacher for a permanent record of the experiment (Note that the photograph will be in grayscale, as colour cameras are not sensitive enough to detect the signal from the SYBR-Safe).

Appendix A : DNA

- Deoxyribonucleic acid (DNA) is a large molecule which stores the genetic information in organisms. It is composed of two strands, arranged in a double helix form. Each strand is composed of a chain of molecules called ***nucleotides***, composed of a phosphate group, a five carbon sugar (pentose) called deoxyribose and one of four different nitrogen containing bases.

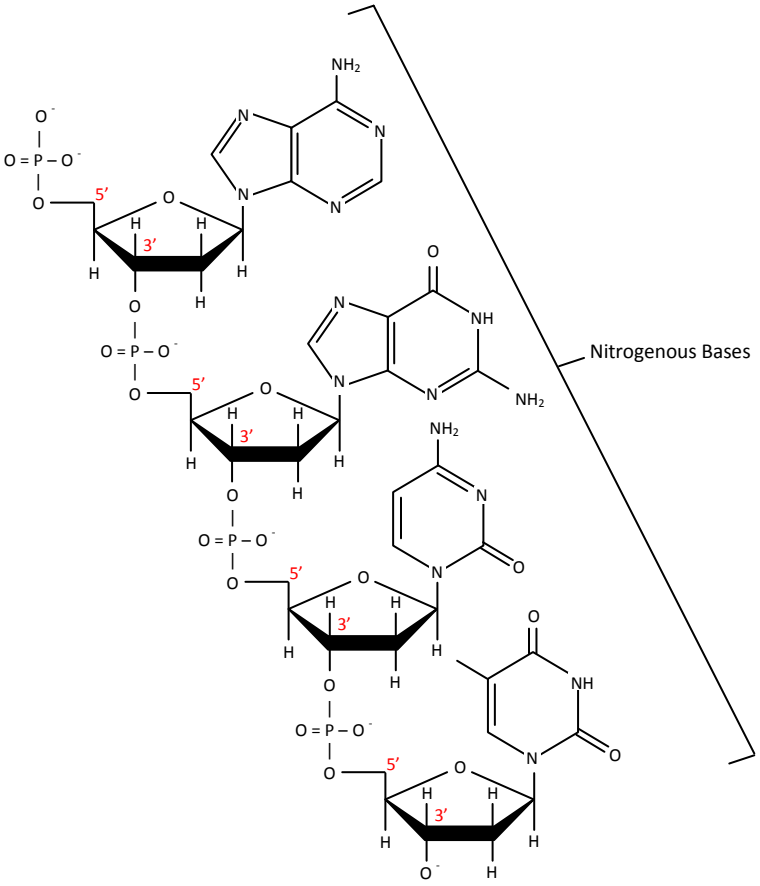

Figure A1 – The Structure of a Single Strand of DNA

- Each nucleotide is connected to the next by way of covalent bonding between the phosphate group of one nucleotide and the third carbon in the deoxyribose ring. This gives the DNA strand a "direction" – from the 5' ("five prime") end to the 3' ("three prime") end. By convention, a DNA sequence is always read from 5' $\rightarrow$ 3' ends.

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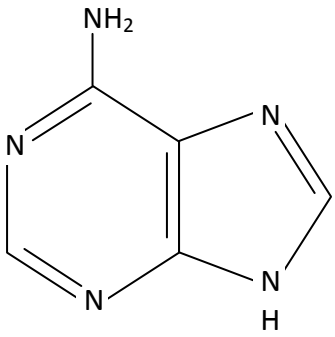
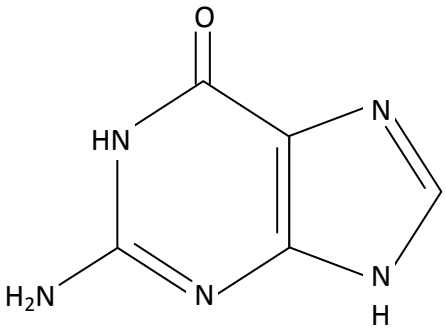
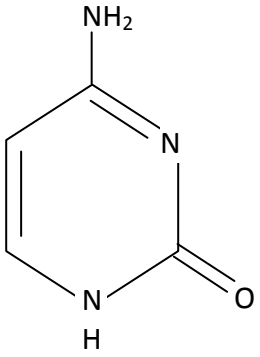
- DNA nucleotides contain one of four different nitrogenous bases:



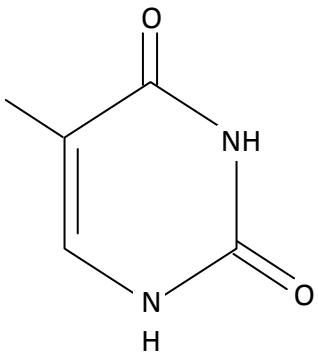
Adenine



Guanine



Cytosine



Thymine

Each of these bases jut off the sugar-phosphate "backbone". If the double helix of the DNA molecule can be thought of as a "twisted ladder", the sugar-phosphate backbones form the "rails", while the nitrogenous bases form the "rungs".

- The two strands of DNA are bound together by hydrogen bonding between the nucleotides. Adenine always binds to thymine and guanine always binds to cytosine. This means that the two strands of DNA are ***complementary***. The complementary nature of DNA is allows it to be copied and for genetic information to be passed on - each strand can act as a template for the construction of its complementary strand.

- The order of bases along a DNA strand is called the ***DNA sequence***. It is the DNA sequence which contains the information needed to create proteins through the processes of transcription and translation.

- Each strand of DNA is ***anti-parallel***. This means that each strand runs in a different direction to the other – as one travels down the DNA duplex, one strand runs from 5' $\rightarrow$ 3', while the other runs 3' $\rightarrow$ 5'.

- An animation of the structure of DNA can be found at:
  http://www.johnkyrk.com/DNAanatomy.html

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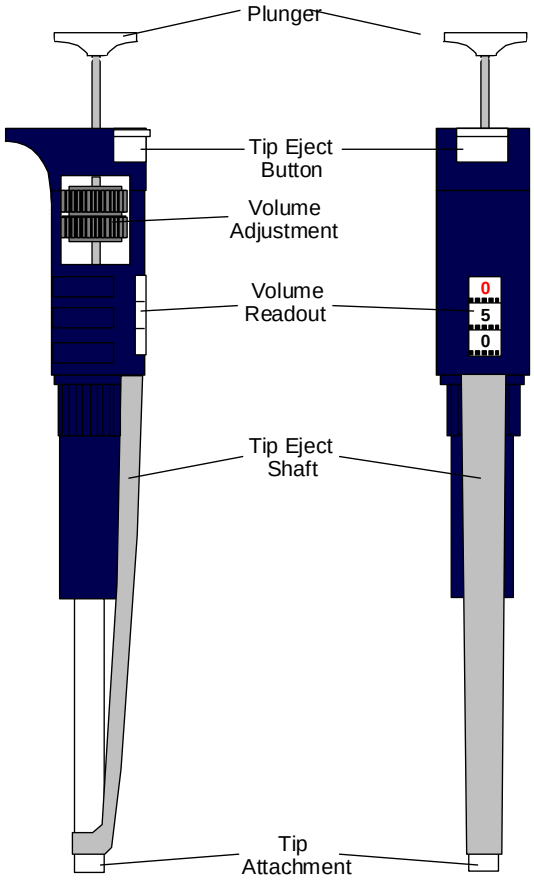
Appendix B : Using a Micropipette

When scientists need to accurately and precisely deliver smaller volumes of a liquid, they use a *pipette* – a calibrated glass tube into which the liquid is drawn and then released. Glass and plastic pipettes have been mainstays of chemistry and biology laboratories for decades, and they can be relied upon to dispense volumes down to 0.1mL.

Molecular biologists frequently use much smaller volumes of liquids in their work, even getting down to 0.1µL (that's one ten thousandth of a millilitre, or one ten millionth of a litre!). For such small volumes, they need to use a *micropipette*.



Micropipettes are called a lot of different names, most of which are based on the companies which manufacture. For example, you might hear them called "Gilsons", as a large number of these devices used in laboratories are made by this company. Regardless of the manufacturer, micropipettes operate on the same principle: a plunger is depressed by the thumb and as it is released, liquid is drawn into a disposable plastic tip. When the plunger is pressed again, the liquid is dispensed.

The tips are an important part of the micropipette and allow the same device to be used for different samples (so long as you change your tip between samples) without washing. They come in a number of different sizes and colours, depending on the micropipette they are used with, and the volume to be dispensed.

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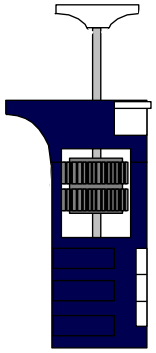
The most commonly used tips are:

- Large Blue – 200-1000µL
- Small Yellow – 2-200µL
- Small White - <2µL

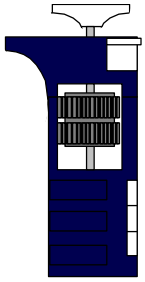
They are loaded into tip boxes which are often sterilised to prevent contamination. For this reason tip boxes should be kept closed if they are not in use. Tips are loaded onto the end of the micropipette by pushing the end of the device into the tip and giving two sharp taps. Once used, tips are ejected into a sharps disposal bin using the tip eject button. Never touch the tip with your fingers, as this poses a contamination risk.

The plunger can rest in any one of three positions:

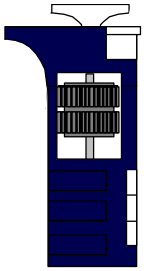
Position 1 is where the pipette is at rest



Position 2 is reached by pushing down on the plunger until resistance is met



Position 3 is reached by pushing down from Position 2



Each of these positions plays an important part in the proper use of the micropipette.

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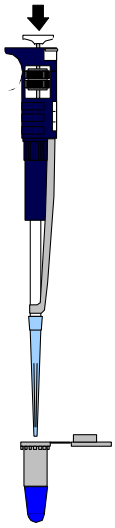
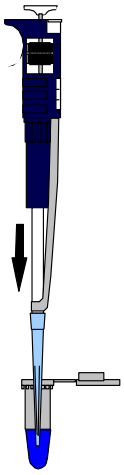
To Draw Up Liquid:

- Hold the micropipette with the thumb resting on the plunger and the fingers curled around the upper body.

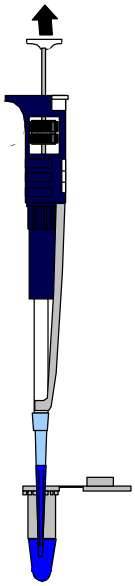
Push down with the thumb until Position 2 is reached.



Keeping the plunger at the second position, place the tip attached to the end of the micropipette beneath the surface of the liquid to be drawn up. Try not to push right to the bottom (especially if you are removing supernatant from a centrifuged pellet), but ensure that the tip is far enough below the surface of the liquid that no air is drawn up.



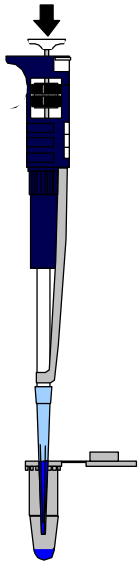
Steadily release pressure on the plunger and allow it to return to Position 1. Do this carefully, particularly with large volumes, as the liquid may shoot up into the tip and the body of the micropipette. If bubbles appear in the tip, return the liquid to the container by pushing down to Position 3 and start again (you may need to change to a dry tip).



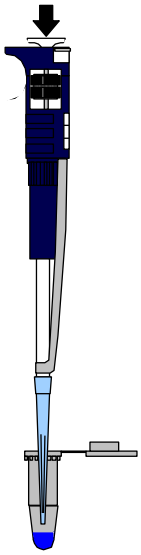
To Dispense Liquid:

- Hold the micropipette so that the end of the tip containing tip is inside the vessel you want to deliver it to. When delivering smaller volumes into another liquid, you may need to put the end of the tip beneath the surface of the liquid (remember to change the tip afterwards if you do this to save contaminating stock). For smaller volumes you may also need to hold the tip against the side of the container.

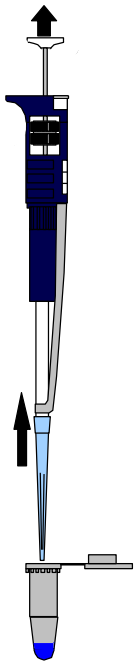
Push the plunger down to Position 2. If you wish to mix two liquids together or resuspend a centrifuged pellet, release to Position 1 and push to Position 2 a few times to draw up and expel the mixed liquids



To remove the last drop of liquid from the tip, push down to Position 3. If delivering into a liquid, remove the tip from the liquid before releasing the plunger



Release the plunger and allow it to return to Position 1



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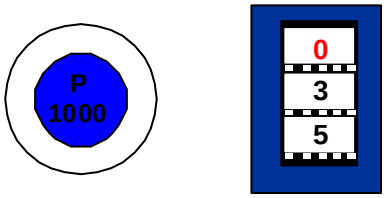
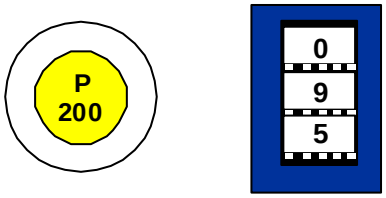
Changing the Volume:

Some micropipettes deliver fixed volumes, however the majority are adjustable. Each brand uses a slightly different method to do this – Gilsons have an adjustable wheel, others have a locking mechanism and turning the plunger adjusts the volume. All have a readout which tells you how much is being delivered and a range of volumes which can be dispensed. Trying to dipense less than the lower value of the range will result in inaccurate measurements. Trying to dispense over the upper range will completely fill the tip and allow liquid to enter the body of the pipette. Do not overwind the volume adjustment, as this affects the calibration of the micropipette.

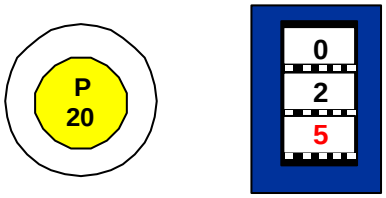
The way to interpret the readout depends on the micropipette used:



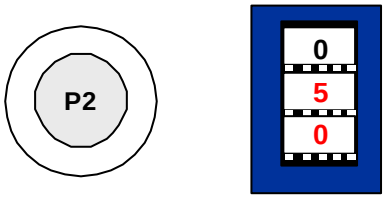
In a 200-1000µL micropipette (e.g. a Gilson P1000) the first red digit is thousands of µL (it should never go past 1), the middle digit is hundreds, while the third is tens. Therefore 1000µL would read as 100, while 350µL would read as 035.



In a 20-200µL micropipette (e.g. a Gilson P200) the first digit is hundreds of µL (it should never go past 2), the second is tens and the third is units. Therefore, 200µL would read as 200, while 95µL would read as 095.



In a 2-20µL micropipette (e.g. a Gilson P20) the first digit is tens of µL (it should never go past 2), the second is units and the third red digit is tenths. Therefore 20µL would read as 200, while 2.5µL would read a 025.



In a 0.2-2µL micropipette (e.g. a Gilson P2) the first digit is units of µL (it should never go past 2), the second red digit is tenths and the third red digit is hundredths. Therefore, 2µL would read as 200, while 0.5µL would read as 050.

Appendix C : Glossary of Terms

Agarose – a substance derived from seaweed which forms a gel when dissolved in water. Agarose gels are used in DNA electrophoresis.

Antibiotic – a chemical agent which kills or inhibits the growth of microorganisms. Antibiotics are sometimes used as selective agents in bacterial culture.

Bacterium – a microorganism with a cell wall but which lacks membrane-bound organelles.

Band – a region of a gel containing DNA or protein fragments of a particular size.

Bases – the four organic molecules which are found in nucleotides. The bases found in DNA are adenine, thymine, guanine and cytosine. In RNA, thymine is replaced by uracil.

Biochemistry – the study of the chemistry of living things.

Biomolecule – a complex organic compound which is made as the result of a biological process. Also called ***macromolecules***, because most are quite large.

BSA – bovine serum albumin – a protein solution derived from the blood of cows which is used to stabilize restriction digests.

Buffer – a compound which helps to keep the pH of a solution stable and constant.

Cancer – a condition characterized by abnormal cell growth and multiplication, as well as migration of affected cells throughout the body.

Cell – the basic unit of all living things. Cells are metabolically active membrane bound bodies capable of reproduction.

Cell Biology – the study of processes which cells use to survive.

Cell Cycle – the progression of stages which a cell passes through in its growth and development. It consists of G1 (Gap 1) phase, where organelles are produced and the cell starts to increase in size, S (Synthesis) phase, where DNA is replicated so that each daughter cell has a complete copy of the genome, G2 (Gap 2) phase, where the cell checks that all is in order for division, and M (Mitosis) phase, where the chromosomes are separated (mitosis) and the cell divides into two daughter cells (cytokinesis). Following M phase, cells return to G1 phase should they need to divide again. Most cells go from G1 phase into G0 phase, where they carry out their normal cellular functions, as most cells do not need to constantly divide. Changes to the cell cycle can lead to a situation where the cells are constantly dividing, a state which may progress to cancer. An understanding of the processes which control the cell cycle can lead to ways to treat cancer, either by stopping the cell cycles of cancerous cells, or preventing cells from turning cancerous in the first place.

Chromosome – A length of DNA. Human cells have 46 linear chromosomes, while bacteria have a single circular chromosome.

Cloning – the process of introducing a new gene into a test cell for the purposes of producing multiple copies of that gene.

Comb – a device used to create the wells in a gel into which the samples are loaded.

Culture – the practice of growing cells by providing them with the right temperature and nutrient requirements.

Dilution – reducing the concentration of a solution by adding more solvent.

DNA – deoxyribonucleic acid – the biomolecule which stores the genetic information in most living things. DNA consists of two strands of deoxynucleotides linked by phosphodiester bonds. The bases in the two nucleotide strands bind in complementary pairs (adenine to thymine, cytosine to guanine) through hydrogen bonds. This gives the molecule the appearance of a twisted ladder, with the sugar-phosphate chains forming the runners and the base pairs forming the rungs. The sugar in the nucleotides which make up DNA is deoxyribose.

Downstream – towards the 3' end of a strand of nucleic acid.

Electrophoresis – a technique which uses an electric field to separate DNA fragments or proteins by size through a gel.

Enzyme – a protein which acts as a biological catalyst – it speeds along reactions which would normally be too slow to be useful.

Fragment – a piece of DNA.

Gel – a semi-solid material used to separate DNA fragments or proteins by size during the process of electrophoresis.

Gene – a small section of DNA which contains the information used to produce a protein, or which controls and regulates the expression of other genes.

Incubation – a waiting period, to allow a reaction time to take place, or organisms time to grow and multiply.

Insert – a small sequence of DNA (eg. a single gene) placed inside another piece of DNA (eg. a plasmid).

Kilobase – a unit representing 1000 bases along a strand of DNA or RNA.

Ladder – a collection of bands in a gel produced by including a standard sample of DNA of known sizes. Used to estimate the size of DNA in test samples.

Medium – a combination of salts and nutrients dissolved in a liquid (broth) or semi-solid material (plate) in which cells are grown.

Micropipette – a device used to accurately and precisely deliver small quantities (<1mL) of liquid.

Molecular Biology – the study of how chemical processes contribute to living systems. Molecular biology concentrates largely on the nature of DNA and proteins.

Nucleic Acid – a biomolecule consisting of a chain of nucleotides connected by phosphodiester bonds. DNA and RNA are nucleic acids.

Nucleoside – a combination of one of the nitrogenous bases (adenine, guanine, thymine, cytosine or uracil) and a five carbon (pentose) sugar – deoxyribose in DNA or ribose in RNA.

Nucleotide – a nucleoside joined to a phosphate (PO_4) group. Nucleotides make up nucleic acids.

pH – the degree of acidity (low pH) or alkalinity (high pH) of a solution.

Plasmid – a small, circular "satellite" chromosome found in bacteria and capable of genetic exchange between bacteria.

PLKI – pololike kinase I – an enzyme which plays an important regulatory role in the cell cycle.

Polobox Domain – a region within PLKI which allows it to attach to other proteins and sub-cellular components.

Protein – a biomolecule consisting of polypeptide chains folded up into three dimensional forms. Proteins play many roles in organisms, including being the building blocks of cellular structures, control and regulation of chemical reactions (enzymes), recognition and communication between cells (receptors and hormones) and defense (antibodies).

Promoter – a region in the DNA upstream of a gene which encourages the transcription and expression of that gene.

Restriction Digest – an enzyme-mediated reaction which cuts DNA at specific base pair sequences.

Stock Solution – a concentrated solution used to store reagents. Stock solutions are usually made to be a certain number of times more concentrated that the working solutions and so must be diluted by the factor to create the working solution. eg. 50X stock must be diluted 1 in 50 before it can be used.

TAE – tris-acetate-EDTA – a buffer used to run DNA gels because it keeps the solution at a pH where all of the DNA is negatively charged.

Transformation – the process of introducing genes into prokaryotic cells.

Upstream – towards the 5' end of a strand of nucleic acid.

Vector – something which is used to introduce a new gene into a cell. Plasmids are commonly used to introduce genes into bacteria.

Well – a "hole" cast in a gel using a comb into which the sample is loaded for electrophoresis.

Working Solution – the solution which is used in a chemical solution. Working solutions may be made up fresh or diluted from stock solutions. They are normally given the name "1X" to differentiate them from their stock solutions.

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