11/1/2006 La Trobe University, Melbourne
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Title: A week's work experience at La Trobe.

Right are pictures of La Trobe Melbourne

Aim:

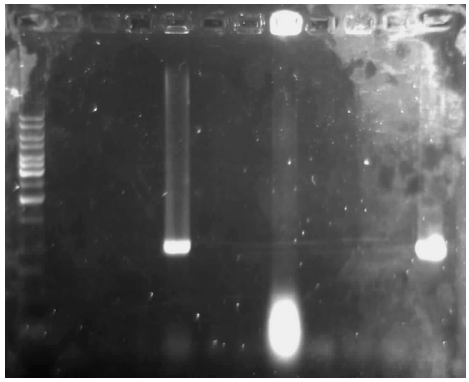
- To get an understanding of how a laboratory operates.
- To be able to safely conduct accurate experiments.
- To understand how malaria parasites look and behave.
- To experience what it is like in a working environment.

Results:

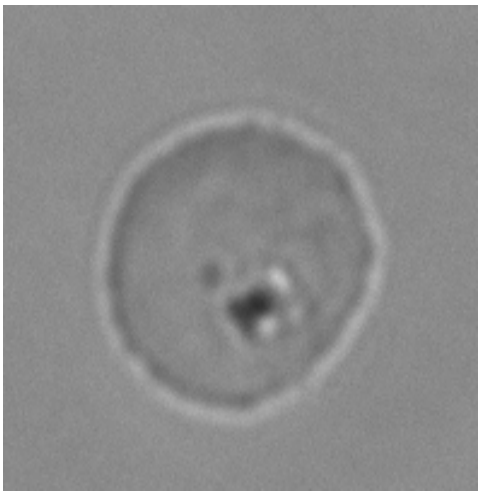
Monday: Monday was a day of listening and learning. I was given a book on Malaria to read, which proved to be useful in further tasks, I then read through the first chapters and took some notes. Carmela showed me some safety procedures and precautions around the laboratory. She showed me the fume cabinets, the MSDS's (Material Safety Data) and the culture room. We went to the cool room and we looked at how to tell if oxygen levels are down and what to do if they were, then we were off to the freezer room where I was shown some freezers that are kept at -80°C. Carmela took me through the first aid kit and explained when to use it and then the wash area in which you can rinse your eyes out or any part of your body exposed to a corrosive or toxic chemical. After lunch Akin told me to make a phosphate-buffered saline solution (PBS). This is done by mixing sodium chloride (NaCl) with di-sodium hydrorthophosphate (Na_2HPO_4 (anhydrous)) and mono-sodium phosphate (NaH_2PO_4 (anhydrous)) PBS is made to be a substitute to a bodily fluid such as blood or tears. It is made to contain a similar pH and salt content of the particular fluid. After making the PBS, Akin brought out some slides of *Plasmodium falciparum* parasite for me to look at. It was interesting to see what these parasites actually looked at having read about them that morning. The day was ended with the relaxing and certainly less mentally taxing job of filling pipette tip boxes.

Tuesday: Tuesday started with Akin, who showed me how to determine protein concentration using reaction with a copper based substance to give a coloured product. Light is passed into the threw the sample and the amount of colour is proportional to the amount of protein By the lightness or darkness of the colour you can determine the amount of protein in it. This although had to grasp originally, was very interesting. After the experiment I started a PCR (Polymerase Chain Reaction) with Kath Jackson. The first part of the experiment was done to change the genetic makeup of a bacterial cell (E coli). We did this by taking a section out of the DNA plasmid (section A) then replacing it was a different DNA fragment insert. We took 2µl of cloning reaction into a vial, followed by incubating it on ice for thirty minutes, then heat shocked the cells for thirty seconds at 42°C (to put holes in the E coli so that the new plasmids can enter into the cells).We then put the tubes back on ice for a further two minutes (to reseal the E coli to increase the chances that the E coli couldn't reject the new plasmid) I then placed my transformed bacteria onto selective plates, then incubated the samples overnight at a temperature of 37°C, so that the bacteria could grow. The method for this experiment is written at the bottom of this report. After the experiment I was taught how to use a computer program called 'Endnote' which is program designed to help store references for documents, papers etc. and was given a small job to do on that.

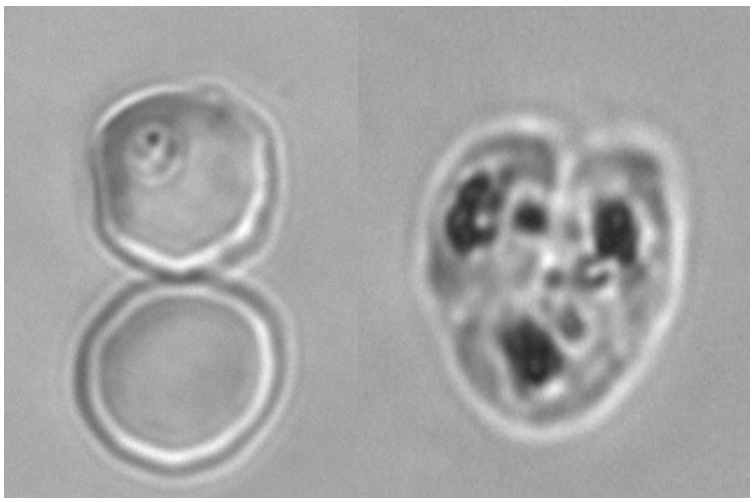
Wednesday: Wednesday started with finishing the next step of creating a PCR with Kath. We took the selective plates out of the incubator and observing the bacterial colonies. We then made up a 'PCR cocktail' from a PCR buffer, dNTP (the different bases present in DNA), distilled water (H_20), forward and reverse primers (complementary to insert A) and TAQ (an enzyme for amplifying DNA.)
We then placed 20µl of the PCR combination into ten separate tubes, then took samples of bacteria from ten different bacterial colonies into the tubes. We then put the samples of DNA into the GeneAmp PCR system and put them through three stages; denaturation, a cycle run at 94°C, followed by annealing which ran at 54°C, then extension a cycle set at 72°C. The three stages of a PCR make copies of a particular DNA segment, so that the particular section is amplified. Whilst I was waiting for the PCR process to take place, Catherine Li took me up to an epifluorescence microscope. Here I was shown ultra violet imagery of malaria parasites and was able to get an accurate insight of what a Malaria parasite looks like. After lunch we continued with the PCR. First off was to make an agarose gel (1%) by placing 1 gram of DNA grade agarose powder into 100ml of TAE buffer, and then add ethidum bromide, a toxic substance. We then placed the agarose mixture into an electrophoresis container and allowed for it to cool over ten minutes. Once the gel has set we took 3 to 4 µl of PCR product and added 1µl of SLB (sample loading buffer, mainly containing glycerol and blue dye) to weigh the PCR product down and to show where the PCR product has gone. We then place the combined solution into the agarose gel and then placed the container into the electrophoresis machine. After forty minutes we took the samples to the gel documentation system were it took images of the PCR product to create an image document data sheet. It showed that only one of the ten samples had insert A. I then finished the day with some more reading.



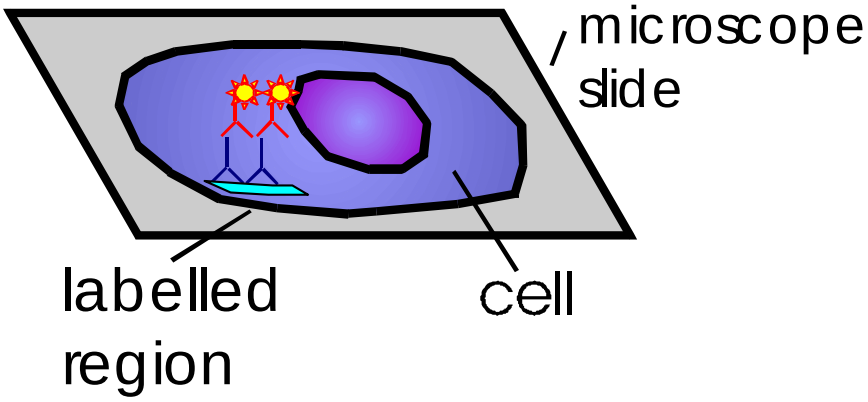
Above PCR results.



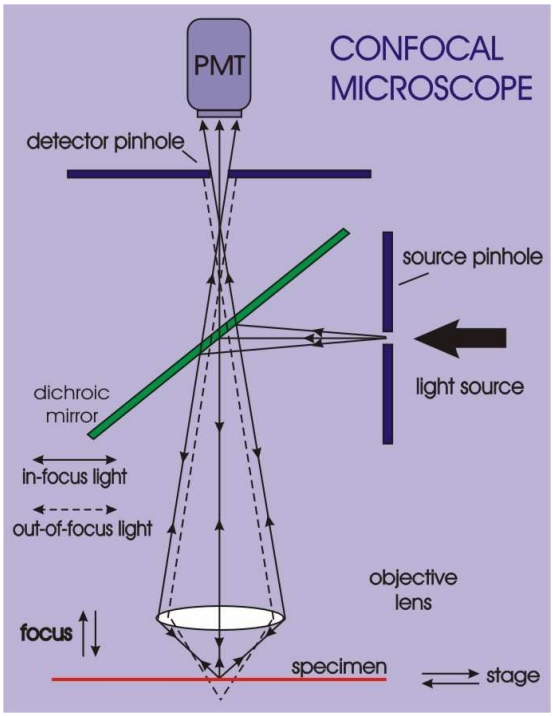
RBC infected by malarial parasites and various stages of development. Far right shows three parasites in one cell, right shows the top cell with an immature parasite, left immature parasite.



Thursday: Today Akin and I did an Immuno Florescence Assay, which highlights different parts of a parasite cell. It is done be smearing parasite infected blood onto a cell, which Akin had done yesterday, then soaking the slides in acetone and methanol to allow minimal movement of the cells. We then made a blotto, a mixture of skim milk and saline, which blocks non-specific bonding of the antibodies. After the acetone and methanol had ten minutes to take effect they are taken out, and dried. Once they were dry, the primary antibodies were added (to attach themselves to particular parts of the cell. In the case of these antibodies (anti-Rex) they attach to Maurer's clefts). These antibodies were left in a humid box for around one hour, then taken out and washed. The secondary antibodies (which contain a florescent probe and attach themselves to the primary antibodies attached to the cells) are then added and left for forty minutes before being washed off. After that an anti-fade was added, (to minimise the bleaching from the intense lights cause by the confocal scanning light microscope) then a cover slip, which had nail polish painted on around the outside to keep the slip in place and to make sure that no moisture was lost.

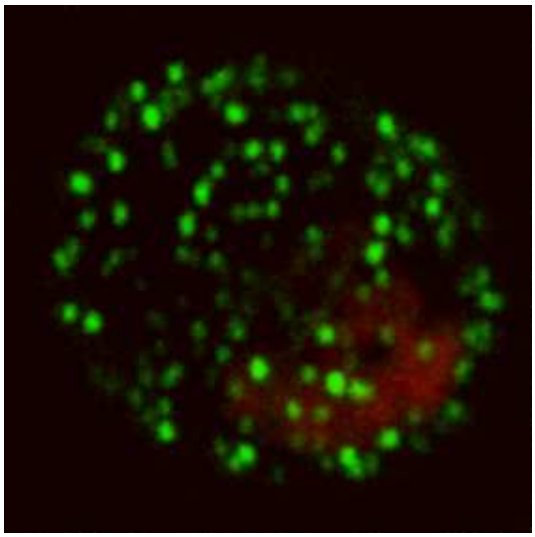


After we had done all this I was taken up to the confocal scanning light microscope to look at the particular parts of the cell. This was an experience as you could see distinct parts of the parasite in the cell. Today was finished off with some report writing and endnote work using a drawing package called Corel draw.



Friday: Today I did a flow cytometry assay with Sarah. A flow cytometry assay is done to observe the levels of cells containing PfEMP1 (*Plasmodium falciparum* erythrocyte membrane protein 1) and the amount of mature parasites that occupy the RBC (Red Blood Cells). This is done by recording the amounts of ethidium bromide recorded (because the ethidium bromide attaches to the nucleus) and RBCs have no nucleus.

Flow cytometry assays start with placing ten different lots of infected blood cells in tubes then placing the samples into a centrifuge (this is to pellet the RBC.) The excess fluid is then disposed and the RBC are washed with PBS with 1% BSA. It is put through the centrifuge, then the antibody agent PFEmP1is added and the solution is stored at 37°C in an incubator for an hour. Once the samples are ready they are taken to a flow cytometer which then determines the PfEMP1 fluorescence (green) and the ethidium bromide levels fluorescence (red). To the right is a picture of a RBC cell with the surrounding dots created by the illumination from the layered effect from the PFEmP1 antibody.



Conclusion: The week of work was very interesting and has furthered my enthusiasm in medical science. I found the experiments more appealing than the work that I have done in science at school over the last few years. It has given me a far better insight on how cells behave, look and react to different enzymes and processes. I have learnt importance of accuracy and safety in a laboratory and how to keep thing sterile and safe. I would like to thank the Center of Excellence for Coherent X-Ray Science for the summer scholarship and also Leann and the lab for allowing me to have this enlightening opportunity. I would strongly recommend for someone else looking for work experience or even a day in the lab to take the opportunity as it is very worthwhile.

Title: Transformation of bacteria (E coli)

Aim: To determine if insert A was contained in the plasmid

Safety: In this experiment various bacterial and chemical substances are used. So to ensure safety, gloves and a lab coat should be worn at all times when handling these substances. Ethidum bromide is toxic chemical and if handled, gloves, a lab coat or overalls and eye protection (such as lab glasses) should be worn. Vapors from ethidum bromide are also harmful and should be avoided.

Note: Sterol equipment should be used (tips etc.) to ensure no other bacteria mixes with the experimental bacteria to allow for a valid test.

Method:

- Add 2µl of cloning reaction into a vial;
- Incubate on ice for thirty minutes;
- Heat shock the cells for thirty seconds at 42°C (to put holes in the e coli) ;
- Put the tubes back on ice for a further two minutes(to reseal the e coli);
- Place transformed bacteria onto selective plates (agar gel);
- Incubate overnight at a temperature of 37°C;

Overnight some bacterial colonies should appear.

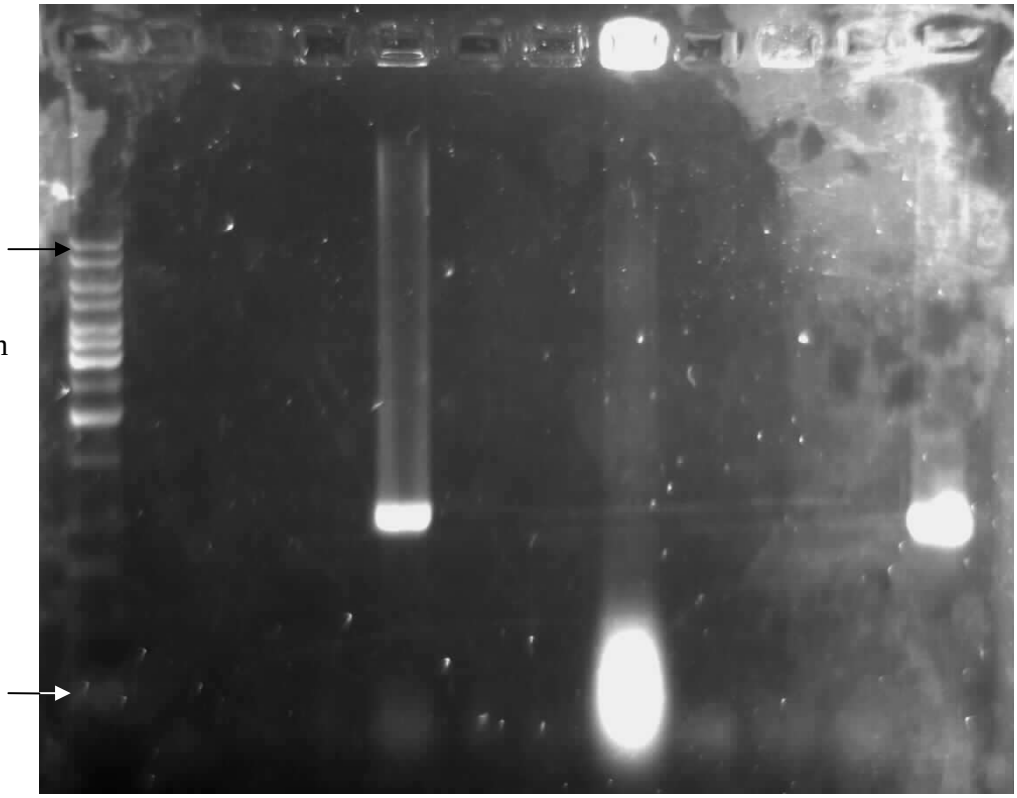
- Combine a PCR (Polymerase Chain Reaction) buffer, dNTD (the different bases are present in DNA - adenine (A), thymine (T), cytosine (C), and guanine (G).), H_2O, forward and reverse primers (complimentry to insert A)and TAQ (enzyme for amplifying DNA);
- Place 20µl of the PCR combination into ten separate tubes, then take samples of bacteria from ten different bacterial colonies into the tubes;
- Amplify the insert DNA using a PCR GeneAmp system (cylcles of heating/denaturation and cooling/annealing);

This process will take a few hours.

- Make an agarose gel (1%) by placing 1 gram of DNA grade agarose powder into 100ml of TAE buffer, stir and then add ethidum bromide. Take the mixture and place it into an electrophoresis container and allow to cool over ten minutes;
- Once the gel has set take 3 to 4 µl of PCR product and add 1µl of SLB (sample loading buffer, mainly containing glycerol and blue dye) to weigh the PCR product down and to show where the PRC product has gone. Place the combined solution into the agarose gel then place container into the electrophoresis machine.

Ladder c1 c2 c3 c4 c5 c6 c7 c8 c9 neg. pos.

Results: As seen on the right, there was only 1 positive bacterial colony (colony 4) along with the positive control. It was marked between 900 and 1000 base pairs as shown by the ladder on the left. (Each mark indicates the amount of base pairs from 10,000 top to 250 as pointed out below



Conclusion: It was shown that there was only one positive colony which means that only one of the ten samples contained insert A.