Gene Regulation and Chromatin Remodelling
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For my placement, I went to United Arab Emirates University, Al Ain, where I observed multiple laboratory experiments being conducted. A series of experiments were done to answer the hypothesis of the research. The researches conducted in the lab were directed towards DNA formation, repair and damage. One of the main key points were DNA double strand breaks. A DNA double strand break was conducted in the DNA of yeast cells. DNA damage was done at different locations to see what kind of effect it would have on the yeast genome and what type of enzymes were generated to repair that specific damage.

This placement has had a huge impact on since I got an amazing insight on how research laboratories work. I was able to understand the purpose of many different equipment and instruments used. Since this was my first time working in a laboratory it also gave me a better perspective on what research based work is like and how this industry keeps evolving. It also helped me acknowledge the amount of patience required to do research based work since there is always a probability that the experiment didn't give accurate results. A lot of experiments took hours and days and in the end they weren't successful so the methodology had to be done again. Overall, this was an eye opening experience and it will help me appreciate the amount of effort, time and resources it takes into research.

Although not related to the research of DNA damage, I learned a lot of other things such as →

- Primers are to amplify the region of DNA and they are used during PCR
- Nano-drop is used to check the concentration of plasmids after plasmid isolation has occurred
- Salmon sperm DNA is as a carrier for DNA during isolation
- BSA → Bovine serum albumin is a protein where you know the concentration and it is used to create a standard curve. After plotting the standard curve it can be used to find the concentration of any unknown sample
- Brad ford is used for protein estimation. It is a reagent (dye) that binds to the amino acids of the protein.

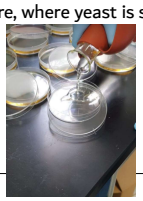
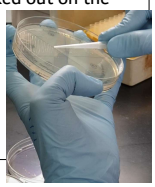
The other protocol I followed was the total DNA isolation from yeast cells →

To isolate genomic DNA from yeast, we need to first make YPD media. After making the media it gets autoclaved to ensure the YPD media is not contaminated with other microorganisms. Then prepare 50mM of EDTA (pH 8.0) and take lyticase along with Nuclease-free water (It a type of water that has been removed of RNase which is very stable and can hinder with the procedure of DNA isolation) and resuspend till a final concentration of 75 units/µL. Now that all the solutions are ready, we can carry on with the protocol.

Add the 1ml of a culture grown for 24 hours in the YPD media, then take the solution and dispense it into a 1.5ml microcentrifuge tube. Take the tube and centrifuge it for 2 minutes at the speed of 13,000 - 16,000 × *g*. This causes the pellet to collect at the bottom of the tube; after the centrifuge remove the supernatant. Resuspend the pellet with 289 µl of 50Mm EDTA buffer. Then add 7.5 µl of 75 units/ µl lyticase and mix it. Afterwards incubate the sample for 30 to 60 minutes at 37 °C. This will allow the digestion of the cell wall. Again centrifuge the sample at the speed of 13,000 - 16,000 × *g* for 2 minutes and remove the supernatant. Then add 300 µl of Nuclei Lysis solution to the pellet collected and gently mix it using a pipet. Then add 100 µl of protein precipitation solution and vortex the solution for 20 seconds. Then again centrifuge it at the same speed as before for 3 minutes. Transfer the supernatant containing the DNA to a clean 1.5 ml microcentrifuge tube containing 300 µl of isopropanol. Gently invert the mixture until the thread-like strands of DNA form a visible mass. Again centrifuge it at the same speed for 2 minutes. Drain the supernatant and add 300 µl of room temperature 70% ethanol. Again centrifuge the mixture at the speed of 13,000 - 16,000 × *g* for 2 minutes. Drain the tube and let the pellet collected air-dry for 10-15 mins. Add 1.5 µl of RNase solution to the purified DNA sample. Followed by vortexing the sample for 1 second. Centrifuge the sample briefly for 5 seconds to collect the liquid and incubate at 37 °C for 15 minutes.

One the very first steps in the process of making a DNA double strand break was making a special selective media for the yeast to grow in. The media used for the culture of yeast was YEP – Lactate → Yeast Extract Peptone – Lactate. This media was used for ChIP (chromatin immunoprecipitation). ChIP is an antibody – based technology where specific DNA-binding proteins are enriched with their DNA targets. It is used to investigate the interaction of particular protein-DNA interactions.

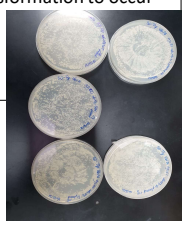
During the process of making YED, space is left for sugars (glucose, lactose etc) to ensure the sugar doesn't get caramelized with the media during autoclaving. After autoclaving the media is spread on the plates left to become semi-solids. After 3-4 hours the media would be ready for the culture, where yeast is streaked out on the media and left overnight for colonies to grow.

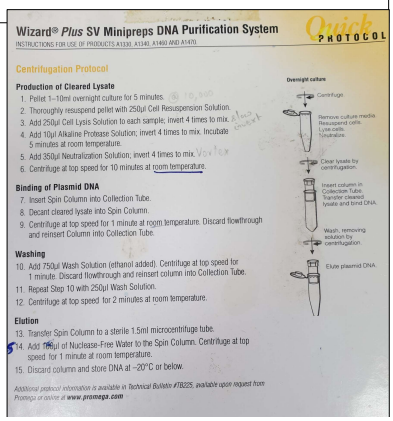
After the yeast has been cultured overnight, the following procedures are done for transformation →

1. Going through the process of Total DNA Isolation from yeast
2. Plasmid transformation into E. Coli
3. Isolation of plasmid from bacteria
4. Quantified using nano-drop
5. Transformation of plasmid to yeast
6. Streaking out colonies on selection

Transformation → is the process of putting pure DNA into a cell. The plasmid DNA is removed (the DNA is already modified to have a cut at a particular point) and then put into another cell that will allow you to study the phenotype of that double strand break (mutation). During the process of transformation DMSO (dimethyl sulfoxide) is used to make the cell more permeable which allows for the transformation to occur more successfully.



The main procedure I observed during the transformation process was the DNA purification system. The protocol can be seen in the image below→


Wizard® *Plus* SV Minipreps DNA Purification System

Quick PROTOCOL

Centrifugation Protocol

Production of Cleared Lysate

1. Pellet 1–10ml overnight culture for 5 minutes.
2. Thoroughly resuspend pellet with 250µl Cell Resuspension Solution.
3. Add 250µl Cell Lysis Solution to each sample; invert 4 times to mix.
4. Add 10µl Alkaline Protease Solution; invert 4 times to mix. Incubate 5 minutes at room temperature.
5. Add 350µl Neutralization Solution; invert 4 times to mix.
6. Centrifuge at top speed for 10 minutes at room temperature.

Binding of Plasmid DNA

7. Insert Spin Column into Collection Tube.
8. Decant cleared lysate into Spin Column.
9. Centrifuge at top speed for 1 minute at room temperature. Discard flowthrough and reinsert Column into Collection Tube.

Washing

10. Add 750µl Wash Solution (ethanol added). Centrifuge at top speed for 1 minute. Discard flowthrough and reinsert column into Collection Tube.
11. Repeat Step 10 with 250µl Wash Solution.
12. Centrifuge at top speed for 2 minutes at room temperature.

Elution

13. Transfer Spin Column to a sterile 1.5ml microcentrifuge tube.
14. Add 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at top speed for 1 minute at room temperature.
15. Discard column and store DNA at –20°C or below.

Overnight culture
Centrifuge
Remove culture media. Resuspend cells. Lyse cells. Neutralize.
Clear lysate by centrifugation.
Insert column in Collection Tube. Transfer cleared lysate and bind DNA.
Wash, removing solution by centrifugation.
Elute plasmid DNA.

Additional protocol information is available in Technical Bulletin #TB325, available upon request from Promega or online at www.promega.com