From Genes to Proteins to Behaviors

PREPARATION OF GENOMIC DNA FROM A MUTANT RED YEAST STRAIN

In the **first week** of lab you subjected a white yeast strain to ultraviolet light. After the UV treatment some of you observed a red colony amidst the thousands of white colonies.

In the **second week** of lab you learned that representative red and white yeast strains have additional differences beyond just color. The two types of strains also exhibit a difference in their nutritional requirements for growth. The red strain is auxotrophic and unable to grow in minimal media unless adenine is added, while the white strain is able to grow in minimal media in the absence of adenine or other growth factors (i.e. prototrophic).

UV treatment is known to alter DNA so the difference in colony color and nutritional requirements could be due to an alteration in the DNA. *A particular DNA change could convert a wild-type, white, adenine-synthesizing strain into a mutant, red, adenine-requiring strain.* ***This is our working hypothesis.*** If our hypothesis is correct then we should be able to identify a difference (i.e. mutation) in the DNA when we compare the genomic DNA of the red and white strains. To study the DNA we must first separate it from all of the other cellular constituents (e.g. proteins, carbohydrates). This week you will prepare a purified sample of genomic DNA from cells that formed red colonies after being subjected to UV light. DNA from cells that form white colonies has already been analyzed and made available following an international multi-million dollar collaboration called the yeast genome project, similar to the much-touted human genome project. We can therefore compare the DNA sequence from cells that formed red colonies after UV exposure to the DNA sequence from the cells that form white colonies, further testing our hypothesis.

METHODS:

A. Yeast genomic DNA isolation

The following protocol has many steps and is like a complex recipe. ***Read through the entire*** **step before attempting it** to make sure you understand the proper technique. Also make sure you **perform each step completely** before moving onto the next step.

1) Obtain a microfuge tube containing liquid culture of a representative red yeast strain (HB2), which was obtained by inoculating a red colony into liquid culture just like you practiced last week. Harvest the red yeast cells by centrifuging for 5 minutes at 2000 RPM (revolutions per minute) in a microcentrifuge. Please coordinate and share the microcentrifuges with other groups, making sure to evenly balance your samples in the rotorprior to spinning.

2) Gently pour the liquid out of the tube into the sink. Use a P1000 pipet to add 600 uL of "Sorbitol buffer" to the pellet, gently pipetting the mixture up and down to resuspend the cells. Use a P20 to add 20 uL of a solution called "lyticase". Invert the tube several times to mix the cells with the lyticase and incubate the tube in the 30°C waterbath for 30 minutes.

Yeast cells have a very sturdy cell wall that will prevent the release of the DNA. In order to disrupt the cell wall you are adding an enzyme, lyticase, which will catalyze the destruction of the cell wall.

3) Pellet the cells by centrifuging for 3 minutes at 2000 RPM in a microcentrifuge. Use a P1000 to pull off the bulk of the supernatant (liquid layer) above the pellet. Then use a P200 to remove the small amount of remaining liquid above the pellet.

After the initial centrifugation the pellet may have a "tail" up the side of the tube. If the tail flops over into the liquid while you are trying to remove the liquid, re- spin the tube in the microcentrifuge for 15 seconds. Remove the remaining liquid with a P200.

4) Once you have removed all of the liquid, resuspend the pellet in 180 uL of the buffer labeled "ATL." With a P200 set at 120 uL, gently pipet the mixture up and down to homogenize the solution.

5) Add 20 uL of the enzyme Proteinase K, mix by vortexing for approximately 5 seconds, and incubate in the 55°C waterbath for 30 minutes.

ATL buffer provides the optimum conditions for the enzyme Proteinase K to work. The buffer also contains detergent to permeabilize the cell membrane. Proteinase K is an enzyme that catalyzes the breaking of peptide bonds that hold together proteins. Some proteins help hold the cell together and others are wrapped closely around DNA, and Proteinase K can remove these proteins.

6) *After the 30 minute incubation in the previous step, vortex the sample for 15 seconds. Then add 200 uL of solution "AL" and vortex briefly but thoroughly (15 seconds) to mix the sample. Incubate the sample in the 70°C waterbath for 10 minutes.*

A white precipitate may form upon addition of "AL", but this is reversed after the 70°C incubation.

7) After the 10 minute incubation, add 200 uL of ethanol. Vortex to mix the solution.

At the front of the lab, you'll find two small tubes (one tube nested inside the other) provided by the Qiagen DNA extraction kit. The tube on top is not just a tube (in fact it's called a column). The column has a white membrane that will absorb DNA under some conditions but release it under others. You will apply the solution of DNA and other macromolecules onto this column under a condition that will trap the DNA but other molecules will wash through. Then you will wash the column under a variety of conditions to ensure that all macromolecules other than DNA have been washed through the membrane. Finally, you will wash the membrane under a condition that will cause the DNA to be released.

8) Label the cap of the column <u>and</u> the side of the collection tube with your group initials. Pipet the DNA-containing solution onto the white membrane, making sure not to stab the membrane with the pipet tip.

9) Wash the contents through the column by applying centrifugal force---that is, place the column nested in its collection tube in a microcentrifuge and centrifuge at maximum speed for one minute. Dump the fluid in the collection tube into the sink.

At this point the DNA is stuck on the white membrane.

10) Place the column back into the emptied collection tube and add 500 uL of a buffer labeled "AW1" and, as in the step before, centrifuge. Again, dump the fluid in the collection tube into the sink.

11) Place the column back into the emptied collection tube and add 500 uL of a buffer labeled "AW2" and, as in the step before, centrifuge. However, this time centrifuge the sample for 3 minutes at full speed. This will eliminate almost all liquid from the column in preparation for eluting the DNA. Dump the fluid in the collection tube into the sink. SAVE THE COLUMN!

12) Place the column back into the emptied collection tube and use the microcentrifuge to spin the column again, this time for 1 minute.

This second centrifugation will ensure that all liquid has been removed from the surface of the membrane. Although dry, the membrane has DNA stuck to its surface.

13) Get a **fresh** plastic collection tube for this step. Label the collection tube with your lab group's initials. Place the column inside the collection tube and then add 200 uL of "AE" directly onto the membrane of the column without stabbing the membrane. Let the "AE" incubate on the membrane for 1 minute at room temperature.

14) Place the column and collection tube into the microcentrifuge. Centrifuge the sample for one minute at full speed. Save the liquid sample; this has your purified DNA! Transfer the liquid sample to a small 1.5 ml microfuge tube with a lid for safekeeping. Save the column and the collection tube for the next step.

15) In order to maximize the amount of DNA recovered, put the column back in the now-empty collection tube. Add another 200 uL of "AE" to the column, incubate for one minute, then centrifuge as in the step above. Add the solution in the collection tube to the contents of the microfuge tube from the step above, giving you ~400 uL of total volume. Label the microfuge tube containing the genomic DNA with your lab group's intitials and "red yeast DNA." Place the tube into a microfuge rack at the front of the room to be stored in the freezer.

Voilà! This microfuge tube has the DNA from the cells that you harvested ~2 hours ago. The DNA is now ready for analysis, which will take place **next lab period**.

B. Analysis of nutritional requirements of red yeast

Last week you tested the **nutritional requirements** of the white and red yeast strains by streaking them onto YPD plates, minimal plates, and minimal + adenine plates. Observe the growth phenotypes on the plates and construct a data table listing "growth" (+) or "no growth (-) of the white (HAO) and red strains (HB2) under the various conditions tested (i.e. YPD, minimal, minimal + adenine). **You will need to include a similar data table in your lab report**. Are these results consistent with your hypothesis for the difference between white and red strains as they relate to nutritional requirements?

C. Assessment of inoculation experiment

Look at the tubes of liquid media you prepared last week. Did you succeed in growing a yeast culture similar to the one you used today? Is the uninoculated "control" tube free of contamination by bacteria or fungi? This is a check on how well your sterile technique is working. Consult with your instructor if you have any problems.