THE YEAST TWO-HYBRID ASSAY

***John Mordacq*[1] *and Roberta Ellington*[2]**
Program in Biological Sciences
Northwestern University
Hogan Hall 2-100
2205 Tech Dr.
Evanston, IL 60208
[1]j-mordacq@northwestern.edu
[2]r-ellington@northwestern.edu

Abstract: This laboratory exercise investigates the yeast two-hybrid assay as it is used to identify protein-protein interactions. The two proteins being tested are called the bait and the prey. The cDNA that codes for the bait protein has been subcloned into a plasmid vector containing the coding sequences for the DNA-binding domain for the yeast GAL4 transcription factor. The cDNA that codes for the prey protein has been subcloned into a separate vector containing the coding sequences for the activation domain of GAL4. If these chimeric proteins interact, they result in the reconstitution of the GAL4 transcription factor as the DNA-binding domain and activation domain are held together by the protein-protein interaction between the bait and the prey proteins. Interactions are observed in a strain of yeast that is auxotrophic for histidine and adenine biosynthesis. This yeast strain contains histidine and adenine reporter genes found downstream from the yeast UAS sequence (UAS is the binding site for GAL4). This selection allows for the screening of protein interactions on media lacking histidine and/or adenine.

Introduction

This laboratory exercise is used at Northwestern for the introductory biology sequence during the quarter that covers the topics of Biochemistry and Molecular Biology. This is the second quarter of the introductory biology sequence. The majority of the students take this course during their sophomore year and most are either biology majors or students taking the pre-medicine curriculum. The students are taught in groups of twenty-four and work in pairs. This laboratory is relatively inexpensive to run and spans three laboratory sessions. It concentrates on some important microbial techniques including sterile technique. It also emphasizes important molecular topics such as transcription, translation, and protein interactions. The bait and prey plasmid DNAs can be obtained by contacting John Mordacq.

Student Outline

Introduction

The proteome is defined as all of the proteins produced by a cell. It is estimated that there are approximately 25,000-30,000 genes in the human genome. It also has been estimated that approximately sixty percent of these genes encode alternatively spliced mRNAs which can lead to the translation of multiple proteins from a single gene. So, one can argue that the proteome is more complex than the genome. In addition, many of these proteins interact with other proteins or molecules leading to a wide array of interactions. It is these interactions that have a range of effects which can control the developmental fate of a cell or the signal transduction events that tell a cell what to do or how to respond. In this exercise, we will look at an experimental approach that identifies such protein-protein interactions.

Transcription Factors

The mechanism by which RNA polymerase transcribes a particular gene into RNA is complicated and not completely understood. If this was an unregulated process, all genes would be transcribed, and subsequently, all mRNA would be translated into protein. This does not happen due to the presence of a region of DNA that controls transcription. The nucleotide sequences that control the rate of transcription are collectively called the promoter and are found in the region upstream (within approximately 200 base pairs) from the start site for RNA synthesis. The promoter contains various conserved nucleotide sequences that affect the rate of transcription. These sequences are the recognition sites for regulatory proteins called transcription factors. The transcription factors bind to their recognition sequences and act together with the TATA binding factor and RNA polymerase II to activate transcription. Transcription factors have two functional domains: one for binding to DNA and one for activation of RNA polymerase. Most promoters activate transcription by having multiple recognition sequences for one or more transcription factors, which work cooperatively to activate transcription. Many transcription factors contain protein domains for interactions with other transcription factors that are required for the activation of a given promoter. One such example is the leucine zipper that allows for the dimerization of a transcription factor that is required for DNA binding. Another example is the homeodomain sequence that consists of three alpha helices, the first two being required for protein-protein interactions with other transcription factors and the third for interaction with DNA.

When researchers are interested in studying a particular protein, they will at some point want to know something about the location and timing of its expression within a certain cell type. The best way to answer these questions is to study the transcriptional regulation of the gene. The experimental approach for this analysis is to isolate and characterize the promoter for that gene. This form of study usually includes the use of a reporter gene, which encodes a protein that is not normally found in the cell type being tested. The reporter gene is subcloned into a plasmid using restriction enzymes. Then, the promoter is inserted in front of the reporter gene and the entire plasmid is inserted into cells. The cells are then assayed for the presence of reporter protein. The reporter protein verifies that the promoter is working to activate transcription of the reporter gene, which in

turn gets translated in reporter protein. The genes used as reporters usually code for a very stable protein and therefore the lack of reporter protein infers lack of transcription.

Two-Hybrid Assay

The two-hybrid assay was developed in the 1980s as a means of identifying protein-protein interactions. Traditionally, this assay is done in *Saccharomyces cerevisiae* (yeast) although there are now versions available for bacteria and mammalian cells. This assay relies on the ability of two proteins to activate the transcription of a reporter gene. Initially the system was developed using the lacZ gene from the bacterial lac operon. Recombinant DNA technology was used to fuse the lacZ reporter gene to the upstream activating sequences (UAS) commonly found in yeast gene promoters. A transcription factor called GAL4 binds to these DNA sequences and activates expression of the lacZ gene (Figure 1).

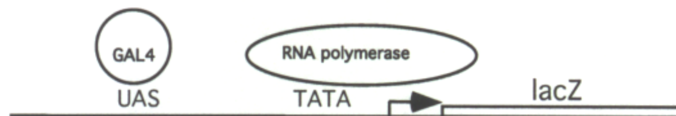


Figure 1. Upstream activating sequences.

When the UASs are bound by GAL4, RNA polymerase transcribes the lacZ gene which codes for the b-galactosidase protein. This protein can be easily detected by growing the yeast in the presence of the substrate X-a-Gal. b-galactosidase is an enzyme that converts the colorless X-a-Gal substrate to a blue product. Thus, blue yeast colonies are an indication of reporter gene activity.

A chimera is defined as a hybrid of two distinct individuals. The goal of the two-hybrid assay is to generate a chimeric GAL4 transcription factor and test for the ability of this transcription factor to activate transcription. As discussed above, every transcription factor has two domains: one for DNA binding and one for activation. We will be employing two different plasmid vectors in this experiment. The first vector called the bait contains the gene encoding the GAL4 DNA-binding domain (DBD) linked to one of our test genes (X) (Figure 2). This chimeric gene is found downstream from a strong promoter (ADH1). Thus, when this plasmid is transformed into yeast, the GAL4-DBD/protein X chimeric gene is transcribed and translated generating abundant amount of this protein. A second plasmid, the prey, contains the GAL4 activation domain fused to a second test gene (Y) (Figure 2). The GAL4 transcription factor has essentially been cut in half and neither the DNA-binding domain nor the activation domain is able to activate transcription without the other. The only way to "reconstitute" GAL4 is if proteins X and Y interact, thus bringing the two portions of GAL4 into close proximity (Figure 3). When the bait and prey proteins interact, the two portions of GAL4 are brought into proximity and thus activate transcription of the reporter gene. You will notice from the plasmid maps that the bait vector encodes the resistance gene for

kanamycin and the prey vector encodes the resistance gene for ampicillin. These two genes are the selectable markers used to amplify these DNA vectors in bacteria.

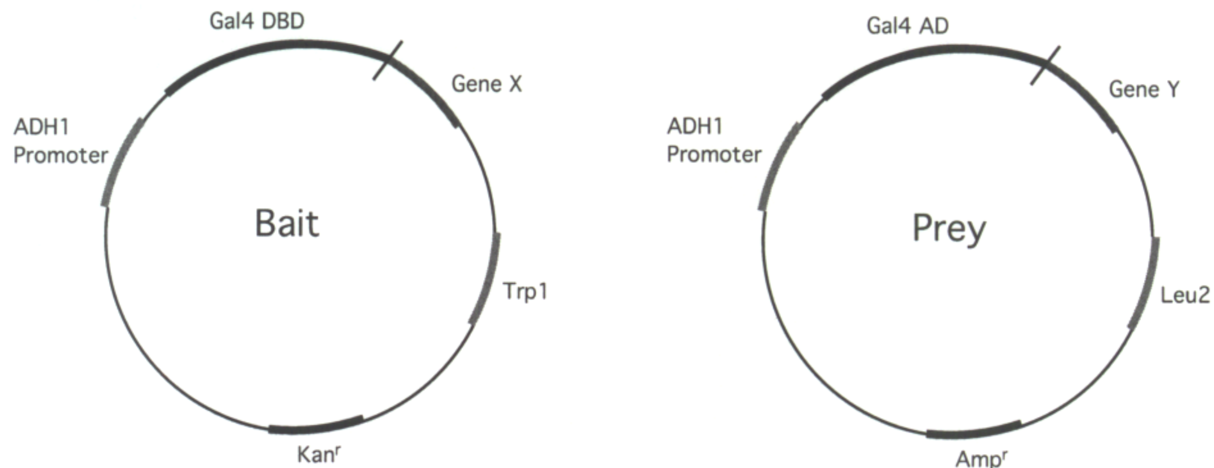


Figure 2. Plasmid Vectors

Screening Interactions

The relative strength of interaction between two proteins can be evaluated using the two-hybrid assay. Originally, when the lacZ gene was used as a reporter, this would result in the presence of various amounts of blue color from the X-a-Gal staining. Researchers found this method of detection to be very unreliable and switched to a genetic screening procedure. In our experiment, we will be using the yeast strain AH109, which is auxotrophic for tryptophan, leucine, histidine, and adenine. A wild type yeast strain is prototrophic, meaning that it can synthesize these four compounds.

As you can see from Figure 2, the bait vector contains the TRP1 gene. Therefore, any yeast that contains this plasmid will be able to synthesize tryptophan. Likewise, the prey vector contains the LEU2 gene which codes for the synthesis of leucine. These two genes are used as selectable markers; if you plate the yeast on growth medium that does not contain (minus) tryptophan and leucine, the AH109 yeast will not grow while AH109 yeast cells that have both plasmids will grow. This allows you to identify which yeast cells have been transformed with both plasmids (Figure 4).

The AH109 strain contains several reporter genes. The two that we will be using are the GAL UAS linked to the HIS3 and ADE2 genes (Figure 5). The HIS3 and ADE2 genes are part of the histidine and adenine biosynthetic pathway respectively. Since the yeast contains these two reporters, they have the ability to grow in the absence of histidine and adenine if the GAL promoter has been activated. And, we know that this activation can only occur if the bait and prey proteins come together reconstituting the DNA-binding and activation domains of the GAL4 transcription

factor. An important aspect of the selection is that the ADE2 reporter provides a stronger nutritional selection compared to the HIS3 promoter.

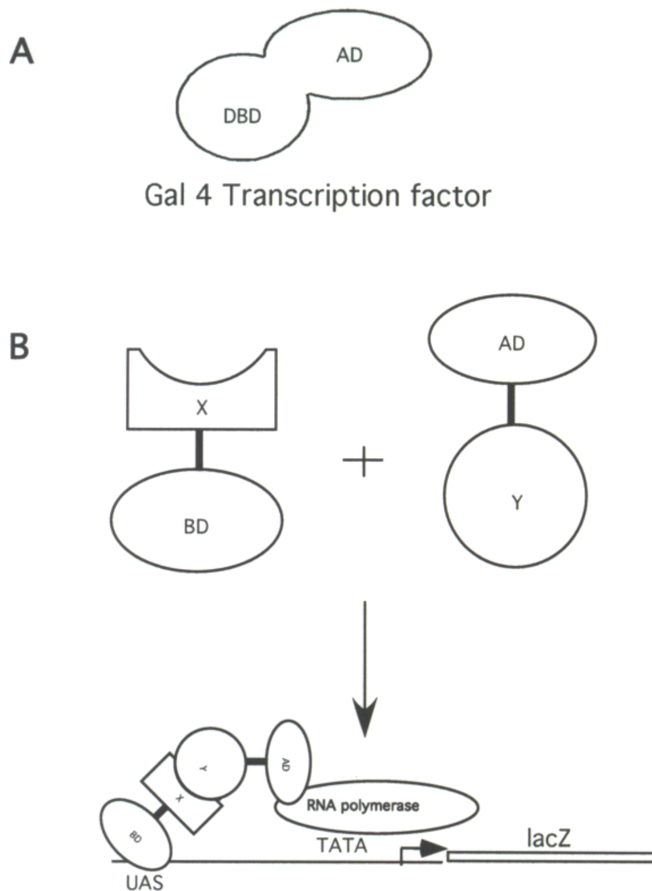


Figure 3. A) Wild type Gal4.
B) Detection of protein-protein interactions.

In this experiment, we will be using three different nutritional stringencies for the growth of the yeast. Low stringency, tryptophan minus/leucine minus, will detect which yeast cells have taken up both the bait and the prey plasmid vectors. Medium stringency, tryptophan minus/leucine minus/histidine minus, will be used to test for weak interactions between the bait and the prey proteins. And, high stringency, tryptophan minus/leucine minus/histidine minus/ adenine minus will be used to test for strong interactions between the bait and prey proteins. This experiment will take place over several weeks. During the first week you will transform AH109 yeast with the prey and bait plasmids and select for growth on tryptophan minus/leucine minus plates. During week two, you will streak any transformed colonies on tryptophan minus/leucine minus/histidine minus plates and tryptophan minus/leucine minus/histidine minus/ adenine minus plates. During the third week

you will check for growth and determine whether the bait and prey proteins can interact. You and your partner will be given one of six different combinations of bait and prey proteins to work with. Some of these will be positive controls (two proteins that are known to interact), negative controls (proteins that do not interact), as well as several test proteins. The positive control proteins are p53 and SV40 T antigen. The negative control proteins are Lamin C and SV40 T-antigen. The test proteins are Pitx2c, Smad2, and Smad3. Coincidently, all of these proteins are transcription factors. Be careful not to be confused by this, while these proteins are transcription factors, they are not activating transcription from the UAS in the promoter. It is only their interaction that reconstitutes the GAL4 transcription factor that will activate transcription.

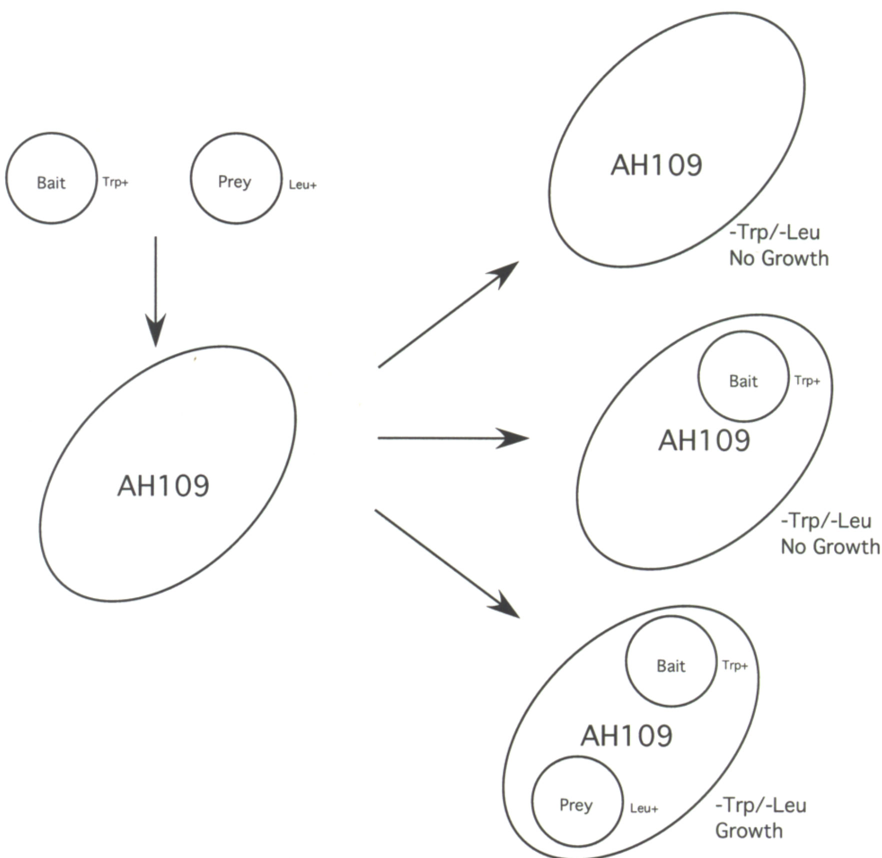


Figure 4. Yeast Transformation

Activin Signaling

Activin is a member of the TGFβ superfamily of ligands and acts through a dimeric receptor complex to stimulate target genes. Most ligands of this family are characterized by dimerization of two subunits through a covalent disulfide bond to form a biologically active protein. In its physiologically active state, activin is a dimer of two b-subunits, while inhibin is a dimer of a b-subunit with a a-subunit. The dimers of interest are activin (βA/βA), inhibin A (βA/α), and inhibin

B (βB/α). In the female reproductive axis, activin is produced by pituitary gonadotroph cells, and acts in an autocrine or paracrine fashion to stimulate the secretion of follicle stimulating hormone (FSH). FSH travels to the ovary and binds to receptors on granulosa cells. It promotes follicle development, and concurrently induces production of the inhibin a-subunit, leading to formation of mature inhibin dimers. Inhibin then travels back to the pituitary, where it binds to activin receptors on gonadotrophs, and antagonizes activin stimulation of FSH in a classic endocrine negative feedback manner.

When activin binds to its receptor, the receptor serine/threonine kinase phosphorylates itself. The phosphorylated receptor then recruits and activates the Smad regulatory proteins. The Smad proteins are a family of proteins that when activated bind other Smad proteins and get translocated to the nucleus where they interact with other transcription factors to regulate genes. Some of the Smad proteins are positive regulators while others are negative regulators. In the case of FSH regulation, the Smads interact with the Pitx2c transcription factor to activate transcription. Pitx2c is a bicoid-related homeodomain protein involved in embryonic development of certain tissues and most significantly the pituitary gland.

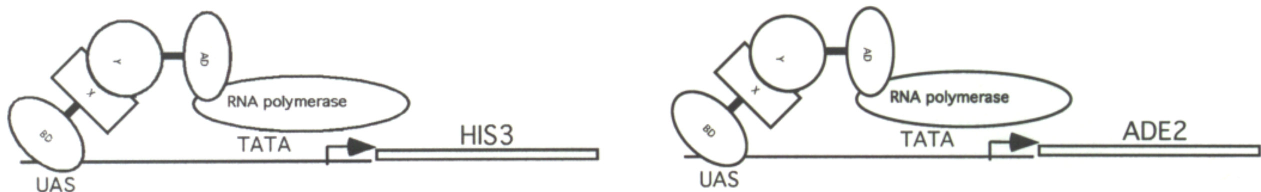


Figure 5. Medium and High Stringency Selection.

Laboratory Procedures

Part I: Yeast Transformation

1. Your bench will be marked with the bait and prey plasmids that you will be testing (see list below). Prior to lab a log phase culture of yeast was grown up and made competent for transformation using lithium acetate and polyethylene glycol. Proceed to the TA bench and get a 10 µl aliquot of your two plasmids, 10 µl of carrier DNA, and 100 µl of yeast competent cells. All reactions will be done in microcentrifuge tubes. Make sure that the tube containing the yeast is clearly labeled with your initials.

   p53 (bait) with TAg (prey)
   Lamin C (bait) with TAg (prey)
   Pitx2c (bait) with Smad3 (prey)

Pitx2c (bait) with Smad2 (prey)
Pitx2c homeodomain deletion of helices 1, 2, and 3 (bait) with Smad3 (prey)
Pitx2c homeodomain deletion of helix 2 (bait) with Smad3 (prey)

2. Add 10 μl of bait and 10 μl of prey plasmid to the yeast cells.

3. Add 10 μl of carrier DNA and mix by vortexing for 15 seconds.

4. Add 600 μl of polyethylene glycol/lithium acetate solution (side bench) to the yeast/DNA mixture and vortex at high speed for 30 seconds.

5. Incubate the tube at 30°C for 30 minutes with shaking.

6. Put on a pair of Latex gloves and add 70 μl of Dimethylsulfoxide (DMSO) to the reaction. Mix well by gently inverting the tube (DO NOT VORTEX).

7. Heat shock for 15 minutes in a 42°C water bath.

8. Chill on ice for 2 minutes.

9. Centrifuge the transformed cells for 5 seconds at maximum speed (hold down the short spin button on the front of the centrifuge). Make sure that you use a balance tube when using the centrifuge.

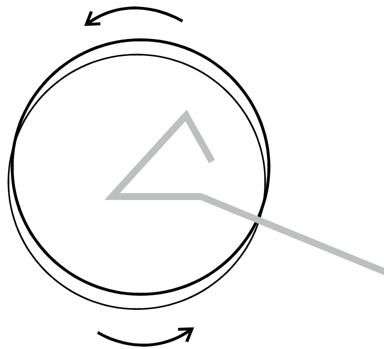
10. Use a glass pipet to remove the supernatant without disturbing the pellet. The supernatant will be thick so allow residual supernatant from the tube walls to fall to the bottom of the tube and remove it as well. Make sure that you do not remove any yeast, as the pellet will be very loose. Place the tube back in the centrifuge and spin an additional 5 seconds. Use a P200 pipetman with a yellow tip to remove any residual supernatant. Any residual transformation solution will inhibit cell growth.

11. Resuspend the pellet in 500 μl of Tris/EDTA (TE) solution. Pipet up and down several times to make sure that the pellet is completely resuspended.

12. Label two Trp minus/Leu minus plates with your name and partner's name, your lab section number, and date of inoculation. Plates should be labeled near the edge to make it easier to see colonies once they have grown. Plates are always labeled on the bottom and never on the cover. This prevents the label from becoming separated from the preparation.

13. Transfer 200 μl of the yeast to the center of the plate. Lift the lid of the petri dish over the bottom and only high enough to add the yeast to the media. Plates should be kept covered at all times except when transferring inoculum. At these times, the cover should be held over the bottom to prevent air-born bacteria and fungal spores from settling down onto the surface of the agar.

14. You will be using a bent metal or glass rod to spread the yeast over the surface of the media. First, dip the rod into a beaker containing ethanol. Hold the rod at a slight downward angle so that the ethanol does not drip onto your hand. Next, place the rod briefly in the flame of your Bunsen burner until the ethanol ignites. Cool the rod in the air for several seconds. Lift the lid of the petri dish and touch the glass rod to the agar surface (away from the yeast) to cool the rod. Using the rod, spread the yeast from one side of the plate to the other. Without setting down the rod, replace the lid and rotate the plate approximately 90 degrees. Use the rod to again distribute the yeast across the surface of the plate.



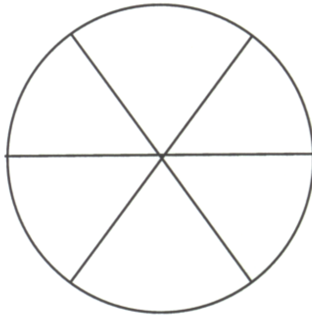
15. When the yeast has dried onto the surface of the media, invert the plate and place it on a tray at the side of the room. If the lid is placed on the top side and condensation forms, it could drip on to the surface of the media and wash some cells from one colony into another colony. The staff will place the yeast in a 30°C incubator for 5 days to allow colonies to form.

16. Before leaving lab make sure that you know what other bait and prey combinations were done by your group of six.

Part II: Growth on Medium and High Stringency Media

1. Retrieve your plates from the tray at the side of the room.

2. Each person will be completing Part II. At your bench you will find a medium (-Trp, -Leu, -His) stringency plate and high (-Trp, -Leu, -His, -Ade) stringency plate. Take a marker and draw lines on the bottom of the plate dividing it into 6 identical regions (see below). You will be streaking one of the six different conditions from your group in each of the regions. Label the bottom of the plate with your initials. In small print near the edge of the bottom, label what condition you will be streaking in each section. Also, fill out the data sheet with this information.



3. Pass the end of a platinum loop through the flame of the Bunsen burner until it glows red. Allow it to cool for approximately 15 seconds. Touch the loop to the agar surface on the medium stringency plate to dissipate any residual heat. Touch one of the colonies from the week one plate with the tip of the sterile loop. Do not scoop up the entire colony; a little yeast will go along way. Slide the loop across the appropriately labeled region on the medium stringency plate using the following pattern. Flame the loop again, cool it and pick a new colony. Streak the same region on the high stringency plate.

4. Repeat this streaking procedure for each of the remaining five conditions remembering to heat and cool the loop before each transfer.

5. Label the bottom of the plates with your initials and section number and tape them together. Put your plates on a tray at the side of the room. The staff will place the yeast in a 30°C incubator for 5 days to allow colonies to form.

Part III: Data Analysis

1. Retrieve your plates from the tray at the side of the room.

2. Analyze the growth on your plates and make a record of it on the Data Page.

Report

The twelve laboratory partners that form a group will share the data collected for this experiment. While some data are shared, the data analysis is expected to exhibit a degree of uniqueness that represents the individual thoughts, views, and conclusions of each student. While it is expected that laboratory partners will discuss the implications of the experiment in detail, it is expected that the narrative parts of the analysis will be unique. Any questions involving students who have not followed these procedures and who are suspected of academic misconduct will be referred to the WCAS Office of Studies.

The report must be typed.

Use several pages to describe the results of the experiment. It may be useful to use your textbook as well as other references.

The following questions should be discussed.

Did the controls work properly? What did they tell you?

Is there an interaction between Pitx2c and Smad3 and/or Smad2? Is one of these "partners" preferred over the other?

Did the various deletions have any effect? If so, why? What does this tell you about the interaction of these proteins and their ability to activate transcription?

Diagram the signaling pathway (based on your data) for the activation of FSH by activin, beginning with activin binding to the receptor and ending with the transcription factors binding the FSH promoter.

Data Sheet

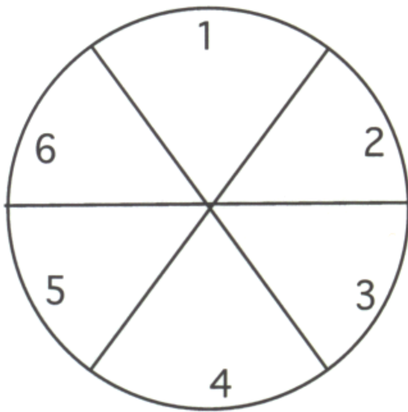
Name ___________________________

Section _____

Part I

Number of colonies on plate ______________

Part II



Yeast streaked on plate	Growth-Medium Stringency (Yes or no – some or abundant)	Growth–High Stringency
Region 1 ____________	Growth _______	Growth _______
Region 2 ____________	Growth _______	Growth _______
Region 3 ____________	Growth _______	Growth _______
Region 4 ____________	Growth _______	Growth _______
Region 5 ____________	Growth _______	Growth _______
Region 6 ____________	Growth _______	Growth _______

Acknowledgements

This laboratory exercise is a result of work carried out in Professor Theresa Woodruff's Laboratory at Northwestern University.
Relevant Journal Articles:

Suszko et al. 2003 Regulation of Rat Follicle-Stimulating Hormone b-Subunit Promoter by Activin. Mol Endocrinol 17(3):318-332

Suszko et al. 2005 Smad3 Mediates Activin-Induced Transcription of Follicle-Stimulating Hormone b-Subunit Gene. Mol Endocrinol 19(7):1849-1858

The protocols for the yeast transformation can be found in the Clonetech Yeast Protocols Handbook.

About the author(s)

John received his B.S. in 1984 from the University of Illinois and Ph.D. in 1991 from Northwestern University. He is a College Lecturer and Director of Undergraduate Laboratories at Northwestern in the Program in Biological Sciences.

Roberta received her B.A. in Biology from Barat College, Lake Forest, Illinois, and her M.S. from Loyola University, Chicago. Since 1986, she has been a Lecturer/Preparator at Northwestern in the Program in Biological Sciences

©2007 *John Mordacq and Roberta Ellington*

APPENDIX A: RESULTS AND REPORT ANSWERS

Two-Hybrid Report

Data

Part I – subtract points for no colonies

Part II – subtract points for no growth on + control or growth on – control or growth where there shouldn't be any.

	B (medium)	C (High)
P53/T-Ag	+++	+++
Lamin C/T-Ag	-	-
Pitx2C/Smad3	++	++
Pitx2C/Smad2	++	+
Pitx2C DHD1,2,3/Smad3	-	-
Pitx2C DHD/2Smad3	++	++

Discussion of questions

First, they should say something about the controls. P53 and T-antigen are transcription factors known to interact strongly to activate transcription from the SV40 virus. Lamin C is part of the nuclear lamin (intermediate filament) and as such should not interact with a transcription factor (p53).

Smad 3 mediates the activin dependant stimulation of the FSH gene. Co-transfections of Smad 3 and Pitx2c leds to the stimulation of the FSH promoter. The Smads are members of a family of proteins that mediate signal transduction and transcriptional activation/repression. Pitx2 is a transcription factor that is required for pituitary development. Binding sites for both Smad3 and Pitx2 are found in the FSH promoter and are required for transcriptional activation. Because this activation is cooperative, Pitx or Smad alone have much less intense effects. Both Smad 2 and Smad3 interact with Pitx2c although the Smad 3 interaction appears stronger.

Homeodomains are a structural motif found in a family of transcription factors (Essential Cell Biology textbook page 272). It contains 3 helices. Transcription factors that contain this sequence are usually found to be critical for the control of proper development and differentiation. As it turns out, this DNA-binding motif is also important for protein/protein interactions. We see that deletion of the entire homeodomain disrupts the interaction between Pitx2c and Smad3. So, we have mapped the site of interaction between the two proteins. If they write that we do not see any growth because we have deleted the DNA-binding domain then it is wrong – Gal4 is what binds to the promoter. The second deletion of helix 2 shows wild type activity so the site of interaction must be helix 1 or helix 3.

Signaling Pathway (Essential Cell Biology textbook page 565). Activin – heteromeric receptor – receptor phosphorylates Smads – translocation to nucleus - bind promoter near Pitx2c on the FSH promoter – activate transcription (presumably through protein-protein interactions).

APPENDIX B: PREPARATORY GUIDE

Before Lab:

The week before lab, grow up yeast (AH 109) from freezer on YEPD agar plates.

Part 1—Transformation of Yeast.

Start the day before lab. This protocol is per section (24 students). Do not increase volume. Do it separately for each section.

1. Inoculate 1 ml of YPDA (this is YEPD + Adenine) with several (2-3 mm in diameter) AH 109 colonies. Use a microcentrifuge tube.
2. Vortex vigorously to disperse cell clumps.
3. Transfer cells to a 250 ml flask containing 50 ml YPDA.
4. Incubate at 30° C for 16-18 hours with shaking (250 RPM) to stationary phase, i.e., ON ($OD_{600} > 1.5$).
5. Transfer the overnight culture (this should produce an $OD_{600} = 0.2$-0.3) to a final volume of 300 ml. Have 275 ml YPDA in a 1 liter flask and add 25 ml of yeast ON culture.
6. Incubate cells at 30° C for 3 hours with shaking (250 RPM) to $OD_{600} = 0.4$-0.6. Note that for early morning lab sections the cultures can be partially grown in advance. Grow the ON culture the previous Thursday night. On Friday, grow for 2 hours. Monday morning at 7:30 am grow for 1 hour and go to step 7.)
7. Transfer cells to 2 sterile centrifuge bottles (150 ml/bottle). Centrifuge at Room Temp at 1000 X G (RPMs = 2600) for 5 minutes.
   Note that step 7 can be started up to 2 hours prior to lab.
8. Discard supernatant. Resuspend cell pellets in 50 ml 1 X TE buffer. Use only 50 ml for both fractions. Now there is one pooled fraction.
9. Centrifuge the pooled cell pellets at Room Temp at 1000 X G (RPMs = 2600) for 5 minutes.
10. Decant supernatant.
11. Resuspend the cell pellet in 1.5 ml freshly prepared I X TE/LiAC (These are the competent cells. These cells can be at Room Temp for a couple of hours prior to transformation.)
12. Place the 1.5 ml competent yeast cells in microcentrifuge tube rack on TA desk at Room Temp. Each student will use 100μl of cells.

ON LAB BENCH PER PAIR OF STUDENTS:

Yellow tips
Blue tips
P-200 pipetman
P-1000 pipetman
Microcentrifuge tube rack
Container of microcentrifuge tubes
Alcohol jar
Spreader

Small plastic waste beaker for liquid waste
Test tube rack with 2 tubes of sterile Pasteur pipets
1 low stringency plate (the "a" plates) per student, i.e., 2 plates per pair
Trash bag
Kimwipes
Ice bucket with one set of bait and prey plasmids.
DNA concentration = 250 ng/10 µl
Vortexer
Plastic waste beaker labeled "Sharp Waste"

ON SIDE BENCH:

Water bath set to 42° C with floats for microcentrifuge tubes
Lined tray with DMSO in orange capped jar
Yellow tips
P-100 set to 70 µl
Trash bag for DMSO tips
Labeled tray for student's plates
2 microcentrifuges set to 14,000 RPM
Tray with PEG/LiAC solution in orange capped jar
Blue Tips
P-1000 set to 600 µl
Small trash bag
Tray with 1 X TE Buffer
Blue Tips
P-1000 set to 500 µl
Small trash bag

ON TA DESK:

Have ice bucket with:
Bait
Prey
Carrier DNA – Herring Testes DNA (10 mg/ml), Clontech #630440
The carrier DNA must be boiled for 10 minutes and then placed on ice.
Boil each time before use
2 p20s
Yellow tips
Small trash bag
The Competent Yeast--1.5 ml—at room temp. TA will aliquot 100 ul per pair
P-100
Yellow tips
Small trash bag

2-500 ml beakers for the 30 minute shaking—6 tubes in one and 6 tubes in the other.

IN EQUIPMENT ROOM

Shaker set to 30° C with 3 microcentrifuge racks taped down.

Incubators set to 30° C

Have centrifuge set at Room temp and 5 minutes and 1000 x g.

RECIPES

1. YEPD (Yeast Extract, Peptone, Dextrose) Broth

For 1 liter: 10 g Yeast Extract
20 g Peptone
900 ml deionized water

a. Pour 900 ml deionized water into a flask and add the Yeast Extract and Peptone.
b. Autoclave
c. Add 100 ml of sterile 20 % Glucose solution when cool (This equals 20 g Glucose per liter of broth).

2. YEPD AGAR PLATES

a. For 1 liter, follow the above step a as for YEPD broth.
b. Add 20 g Difco Bacto agar.
c. Autoclave.
d. Add 100 ml of sterile 20 % Glucose solution.
e. Pour plates.

3. YEPD + ADENINE BROTH

For 1 liter:

20 g Peptone
10 g Yeast Extract
885 ml Deionized water
Autoclave

ADD:

0.2% Adenine Hemisulfate—15 mls
20 % Glucose—100 ml

4. TE Buffer 10 X

Tris-HCl 0.1 M + 10 mM EDTA; pH 7.5
For 300 mls:

a. 0.1 M Tris-HCl FW 121.1
Use 30 ml of 1 M Tris-HCl pH 7.5
b. 10 mM EDTA pH 7.5
Use 15 ml of 0.2 M EDTA pH 7.5

c. Add 255 ml Dei water.
d. Check pH to 7.5.
e. Autoclave

Make 1 liter (at a time) of 1X dilution: 100 mls of 10X and 900 mls deionized water.

5. 1 X TE/LiAC

Make 10 mls at a time.

1 ml 10X TE
1 ml 10X LiAC
8 mls sterile deionized water

6. Lithium Acetate (Sigma L-6883)

Make 1M. This is 10X
FW = 102 Use 10.2 g/100 mls deionized water. Adjust to pH 7.5 and autoclave.

7. PEG + LiAC Solution

Make stocks of each ahead of time. Mix at the last minute.

Chemical	Final Conc	For 10 mls
PEG	40%	8 ml of 50% PEG
TE buffer	1 X	1 ml of 10 X TE
LiAC	1 X	1 ml of 10 X LiAc

8. PEG 50% (Polyethylene Glycol 3350) (Sigma- P-3640)

50 g of PEG into sterile deionized water for a total volume of 100 ml.
or
500 g PEG into sterile deionized water for a total volume of 500 ml.
Heat to dissolve.

9. DMSO (Sigma D-8779)

10. SD MEDIUM

For each drop out solution (a, b, and c), make 1 liter/section of 24 students. That will be 1 plate per student. **These plates need about 40 ml each instead of the usual 25 ml.**

Use yeast nitrogen base (YNB) without amino acids. Do not use YNB without amino acids and without ammonium sulfate.

Note that there is an easier but more expensive way to do this. Clontech sells dropout media: -leu, -trp # 630417, -leu, -trp, -his # 630419, -leu, -his, -trp, -ade #630428. These dropout mixes must be added to YNB without amino acids (Sigma Y0626)

Nutrient	1 liter
YNB without amino acids	6.7 g
Bacto agar	20 g
Deionized water	800 ml
Autoclave.	

ADD:

20% Glucose	100 ml
Drop Out Solution	100 ml
Tyrosine (2 mg/ml in 0.2 M NaOH)	15 ml
Threonine (40 mg/ml stock)	5 ml
3-AT	2.5 ml

11. 3-AT 1 M (3 amino-1,2,4-triazole) Sigma A-8056)

FW = 84. Make 100 mls = 8.4 g of 3-AT in 100 mls deionized water.

Filter sterilize. Add to cooled medium. It is heat-labile. Store at 4°C.
Use 2.5 ml of 1 M stock to make 2.5 mM in 1 liter SD medium.

12. 10 X DROP OUT (DO) SOLUTIONS

a. –leu; -trp
b. –leu; -trp; -his
c. –leu; -trp; -his; -ade

Make threonine and tyrosine separately

Nutrient	10X 500 ml	Sigma #
L-Adenine hemisulfate salt	100mg	A-9126
L-Arginine HCl	100 mg	A-5131
L-Histidine HCl monohydrate	100 mg	H-9511
L-Isoleucine	150 mg	I-7383
L-Lysine HCl	150 mg	L-1262
L-Methionine	100 mg	M-9625
L-Phenylalanine	250 mg	P-5030
L-Threonine *	1000 mg	T-8625
L-Tyrosine **	150 mg	T-0254
L-Uracil	100 mg	U-0750
L-Valine	750 mg	V-0500

13. * Threonine—Make a 40 mg/ml stock in deionized water. Filter sterilize. Add 5 ml per liter to the SD media.

14. ** Tyrosine—Make a 2 mg/ml stock in 0.2 M NaOH. Filter sterilize. Wrap in tin foil and keep in the dark at room temp. Add 15 ml per liter to the SD media.

Part 2:

Students will streak Medium and High Stringency (the “b” and “c”) plates.

ON LAB BENCH:

Bunsen burner
2 Loops
Lab Marker
1 Medium and 1 High Stringency plate (b and c) per student.

ON SIDE BENCH:

Tray with student’s Part 1 plates.
Empty tray for new plates

Part 3:

Students will read plates.

Student’s Part 2 plates

Large Biohazard Bag