BICD111 - Cell Biology Laboratory
Fall 2009

Tuesdays and Thursdays 11:00am – 5:30pm

Instructor: Colin Jamora (cjamora@biomail.ucsd.edu)

TAs: Pedro Lee (pelee@ucsd.edu)
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Office hours with the TA or instructor can be scheduled by appointment as needed

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Evaluation/Grading:

Quizzes (15%)
Quizzes will be given **every Tuesday** and consist of short technical questions. The quizzes are designed to test your understanding of the protocols carried out during the prior week – they will focus on the underlying principles of why you carried out various steps in the experiment. They will commence *promptly* at 11 am and you will be given 10 min to complete it without the use of notes so please arrive to class on time.

Problem Sets (15%)
Questions about the previous week's experimental data will be handed out at the end of class on Tuesday and will be **due at the beginning of class on Thursday**. These questions will be designed to guide your analysis of the results you obtain from the protocols and will prepare you for the types of questions on the midterm.

Experimental Techniques (10%)
In order to assess your mastery of the techniques employed in the class you will be graded on the successful outcome of your experiments and/or the correct interpretation of the data derived from them.

Midterm (25%)
The midterm will be open notes, consisting of conceptual questions and requiring short essay answers and experimental design. They will also contain questions similar in scope to the problem sets as well as questions dealing with making solutions. Note that the questions will not be testing your ability to memorize the

information presented during the course but application of techniques and concepts to new situations.

Final (35%)
The final will be open notes, comprehensive, and similar in format to the midterm.

The grading system will be based on the absolute score you receive (i.e. there is no curve):

Grade	**Percentage**
A+	95-100
A	90-94
A-	85-89
B+	80-84
B	75-79
B-	70-74
C+	65-69
C	60-64
C-	55-59
D	50-54
F	below 50

Please note:

Many experiments will have some preparatory work so please **read all protocols in advance** and write any notes that may help you work more efficiently. Moreover, you will be required to make many of the solutions that you will use in your experiments. If these are not made correctly, they can adversely affect the success of your experiment and the data on which you base your conclusions. If you are unsure about how to make a solution (or any other aspect of an experiment) **feel free to ask your TA or your professor for help.**

Due to the limited amount of functional microscopes, they will be available for use on a first-come, first-serve basis. However, there will be a twenty minute time limit for the use of the microscope per group. If you would like to view your samples for more than 20 minutes, you will have to wait until all of the other groups have had an opportunity to use the microscope. You will be required to sign in with your group number and time prior to entering the microscope suite.

09/24/09 - Thursday

General Introduction

1. Overview of class, grading and expectations
2. Safety information video and safe lab practices
3. Pipetting techniques
4. Preparation of 10mLs each of solutions P1, P2, and N3 for day 2 of Protocol #1.

P1: 50mM Tris-Cl pH8, 10mM EDTA, 100ug/ml RNase
P2: 200mM NaOH, 1%SDS
N3: 3 M Potasium Acetate pH 5.5, 2.875% glacial acetic acid

09/29/09 - Tuesday

Protocol #1 – Day 1 of 4

Transformation of Mannosidase II-GFP into DH5α bacterial cells

Work in groups of two:

You will be given one aliquot of a 200µl suspension of competent DH5α bacterial cells per two groups (four people). **Please keep on ice to thaw. Do not thaw at room temp. or in your hands**

1. Make sure bacteria are resuspended before starting. Add 40µl of bacteria to the tube containing 1µl pEGFP-Mann II. To a second tube, (no DNA), add 40µl bacteria only. Tap tube lightly to mix, and incubate on ice for 30min.
2. Heat shock bacteria – *Ask the TAs for the time and temperature of the heat shock*
3. Immediately put back on ice for 2 min.
4. Add 300µl LB, mix by tapping tubes.
5. Incubate at 37°C for 30 min
6. Plate 200µl of each sample on separate LB-Kan plates, let sit upright for 5 min to dry
7. Label plates with group number and sample and put in the 37 °C incubator room upside down, overnight.

You will be graded on whether you have colonies growing on the plate which is a sign of successfully transforming your DNA into the bacteria.

10/01/09 - Thursday

Protocol #1 – Day 2 of 4

Purification of pEGFP-Mann II from bacterial cells
To save time, we have started the liquid cultures for you.

1. Pour 1.5ml of the culture into 2 microfuge tubes.
2. Spin tubes at 14,000rpm for 1 min, in tabletop centrifuge, to pellet bacteria.
3. Carefully aspirate supernatant without sucking up your pellet.
4. Resuspend the pellet in 100µl buffer 1 (**P1**) (50mM Tris-Cl pH 8, 10mM EDTA, 100µg/ml RNase). Resuspend the bacteria completely by vortexing and/or pipetting.
5. Add 200µl solution 2 (**P2**) (200mM NaOH, 1% SDS). Mix by inverting 4-5 times; DO NOT VORTEX. Put on ice for 2 min (no longer).
6. Neutralize the reaction with 200µl solution 3 (**N3**) (3 M Potasium Acetate pH 5.5, 2.875% glacial acetic acid). Mix by inverting 4-5 times; DO NOT VORTEX. Let sit on ice for 5 min. At this point you should see a white precipitate form.
7. Spin at 14,000rpm for 10min
8. Transfer supernatant (which contains your plasmid) to a new tube.
9. Add an equal volume of Phenol:Chloroform, vortex to mix.
   Phenol is very toxic and can cause painful burns: wear gloves and goggles.
   Chloroform is toxic and volatile: work under the hood.
10. Spin at 14,000rpm for 4min to separate aqueous from organic phase. You should see the aqueous phase (containing the DNA) on the top and the organic phase (Phenol:Chlorophorm, containing the proteins) on the bottom.
11. Remove the aqueous phase (which contains the DNA) and put in new tube.
12. Add 2.5X vol of 100% EtOH, and 1/10 vol of Sodium Acetate (pH 5.7, 3M stock).
13. Keep on ice for 10 minutes
14. Spin DNA pellet at 14,000rpm for 10 min
15. Wash with ice-cold 70% EtOH, and re-spin for 5 min.
16. Aspirate off EtOH, be careful not to suck up DNA pellet.
17. Let eppendorf lay flat on benchtop to dry, then add 30µl TE-RNase (10mM Tris-Cl pH 8, 1mM EDTA, 100µg/ml RNase)

DNA should dissolve over time. Mix by taping tube.

Visualize DNA on agarose gel:

1. In a 100 mL flask make 50 mL of a 1% agarose gel using TAE as the solvent.
2. Heat until agarose is melted, about 1 min in microwave.
3. Add 5 ul of Sybr Safe dye.
4. Pour into gel mold, insert comb and let cool to solidify.
5. During this time, prepare your samples for electrophoresis. In a clean tube, mix 2µl of DNA sample, 3µl water and 1µl 6X DNA loading buffer.
6. When gel is solidified, cover with TAE.
7. Load 5µl 1kb DNA ladder in one lane, and each of your samples into separate lanes.
8. Run electrophoresis at 100V watching for dye front to migrate half-3/4 the way down gel.
9. Visualize purified DNA on UV box (**wear gloves when handling gel; wear goggles, UV light is harmful).** *Show your gel to the TA as you will be graded based on the successful extraction of the plasmid DNA*
10. choose the 2 best minipreps for purification on the QiaSpinPrep columns.
11. add 150 µl of buffer PB, mix and load onto labeled spin column
12. spin in microfuge for 1 min at 9000 rpm, discard flowthrough.
13. wash column with 750 µl buffer PE,
14. spin for 1 min as before, discard flowthrough
15. spin again to remove any remaining PE
16. place column into clean 1.5 ml microfuge tube
17. elute by adding 20 µl EB buffer (10 mM Tris-Cl pH 8.5)

To check DNA concentration:

Dilute 3µl of your purified miniprep DNA into 600µl H_2O). Measure optical density (OD) at 260nm in a 1ml quartz cuvette (Why do we use quartz?)

Hint: 1µg/µl DNA diluted to 1:200 gives an OD_{260} of 0.1

Record concentration of your DNA for the next experiment.

10/06/09 - Tuesday

Protocol # 1 – Day 3 of 4

Transfection of pEGFP-Mann II into NRK cells.

Today we will be using the DNA:lipid complex method of transfection. All work should be done under a sterile environment and using sterile techniques.

1 set of transfections per group.

NRK cells grown on coverslips will be provided to you. Take three coverslips and place each into a separate well of a 24 well plate. Each well should have 500 µl of DMEM.
Perform the following transfection reactions in the tissue culture hood.

1st well

1. Add 1.2µl TransIT-LT1 to 24µl of OPTI-MEM, incubate for 5 min at room temp
2. Add 0.4µg pEGFP-Mann II (positive control) that will be provided to you to the TransIT-LT1/OPTI-MEM solution. Mix gently by tapping the tubes.
3. Incubate for 15 min at room temp.
4. Add the DNA-TransIT-LT1 solution drop-wise to the cells. Each well should have 500µl DMEM.
5. Incubate at 37 °C for 24-48hrs.

2nd well

1. Add 1.2µl TransIT-LT1 to 24µl of OPTI-MEM, incubate for 5 min at room temp
2. Add 0.4µg of one of your favorite prep from Thursday to the TransIT-LT1 /OPTI-MEM solution. Mix gently by tapping the tubes.
3. Incubate for 15 min at room temp.
4. Add the solution dropwise to the cells. The well should already have 500µl DMEM in it.
5. Incubate at 37 °C for 24-48hrs.

3rd well

1. Add 1.2µl TransIT-LT1 to 24µl of OPTI-MEM. Incubate for 20 min at room temp
2. Add the solution dropwise to the cells. The well should already have 500µl DMEM in it.
3. Incubate at 37 °C for 24-48hrs.

General protocol for immunofluorescence

1. Fix coverslips for 10 min with 4 % Formaldehyde in PBS (at RT[Room Temperature]) or for 10 min with ice-cold MeOH at –20° C, depending on your primary antibody.

2. Block coverslips for 15 min with blocking buffer (2.5 % FCS, 0.1 % Triton X-100, 0.02 % NaN3 in PBS)

3. At no time should you allow the coverslips to dry out.

4. Primary antibody – Make a 1mL stock of the appropriate dilution. This will be the only stock that you will get for the class. Incubation:

   add 20 ml/well (diluted appropriately in blocking buffer)
   (save primary antibody dilutions, they can be reused)

5. wash 2x with PBS

6. secondary antibody incubation:

   add 20 ml/well (1:500 of secondary antibody and 1:5000 of Hoechst in blocking buffer)

7. wash 2 x with PBS

8. Mount coverslips

General Rules:

- Always keep the diluted primary antibody, it can be reused.
- Wash with PBS between every step
- Make sure coverslips don't float
- Perform secondary antibody staining (and following washes) always in the dark
- When staining one coverslip with 2 primary antibodies, incubate primary antibody separately, then stain with a mixture of the corresponding secondary antibodies.
- For staining actin: incubating cells with phalloidin-Rhodamine (1:100) should be performed during the incubation with the secondary antibody.

10/06/09 – Thursday

Protocol #2 – Day 4 of 4

Visualization of organelles in NRK cells – Golgi, endoplasmic reticulum, and mitochondria.

Visualize cells at the light microscopy level. You should always make sure you have cells before starting.

Transfer 3 coverslips/group from the master tissue culture plate to your 24-well dish (will be shown to you by the TAs).

Wash the cells with PBS (add 500µl of PBS/well, aspirate and repeat). **Never expose cells to air for too long and avoid letting the cells get completely dry**

Fix coverslips #1 and 2 with formaldehyde at room temperature (RT) by adding 500 µl of 4% formaldehyde in PBS/well. Wait for 10 min at room temperature, then remove fixative by aspiration. Wash cells 2 times with PBS (3 minutes each time).

Incubate cells with blocking buffer (2.5% FCS, 0.1% Triton X-100, 0.02% NaN_3 in PBS) for 10 min at room temperature.

In a separate 24 well dish, **fix coverslip #3** with ice-cold methanol. Put at -20°C for 10 min (no longer). Wash cells 2 times with PBS.

Incubate cells with blocking buffer (2.5% FCS, 0.1% Triton X-100, 0.02% NaN_3 in PBS) for 10 min at room temperature as before.

Remove the blocking buffer and discard.

Staining with primary antibody: Recycle and save all primary antibodies, store at 4°C

Coverslip#1: Add 20µl of anti-giantin antibody (rabbit) (diluted 1:500 in blocking buffer) and leave for 30 min at room temperature.
Coverslip#2: Add 20µl of anti-Calreticulin antibody (rabbit) (diluted 1:500 blocking buffer) and leave for 30 min at RT.
Coverslip#3: Add 20µl of anti-Hsp60 antibody (mouse) (diluted 1:100 in blocking buffer) and leave for 30 min at RT.

Wash all coverslips 2 times with PBS as before.

Staining with secondary antibody and Hoechst:

Coverslip#1: Add 20µl of goat anti-rabbit-Rhodamine (1:500 in blocking buffer), Hoechst (1:5000 in blocking buffer) mixture and incubate in the **dark** for 20 minutes at room temperature.

Coverslip#2: Add 20µl of goat anti-rabbit-Rhodamine (1:500 in blocking buffer), Hoechst (1:5000 in blocking buffer) mixture and incubate in the **dark** for 20 minutes at room temperature.

Coverslip#3: Add 20µl of goat anti-mouse-AlexaFluor448 (1:500 in blocking buffer), Hoechst (1:5000 in blocking buffer) mixture and incubate in the **dark** for 20 minutes at room temperature.

Remove the antibody and discard.

Wash cells 2 times with PBS.

Mount the cover slips on glass slides (will be shown to you by the TAs).

Visualize by fluorescence microscope (will be shown to you by the TAs).

Store slides in the dark when not visualizing.
You will be graded on whether your staining works.

Record what you see:

How do the structures differ from each other?

Where are they localized in the cell?

How do these structure compare to what you've been taught in other biology classes?

Similarities? Differences?

It is recommended to draw what you see.

10/08/09

Protocol #2 – Day 4 of 4

Visualization of transfected Mannosidase II-GFP by Immunofluorescence

If you are unsure of the protocol, verify it with your TA or instructor

3 coverslips/group (from your transfection experiment)

Perform immunofluorescence looking at the endogenous ER protein (in well 1), endogenous Golgi protein (for well 2 and 3) and compare its localization to that of Mannosidase II-GFP (exogenous).

You will be graded on the following:

What percentage of the cells are transfected?
What are the expression levels like of GFP-Mann II?
Is Mann II-GFP localized in the correct place?
Is MannII-GFP expressed in the ER or in a compartment other than the Golgi?

10/13/09 – Tuesday

Protocol #3

<u>The impact of microtubules and actin on cell shape and organelle organization.</u>

6 coverslips/group

Drug treatments:

Cytochalasin B: (cyto B) — stock: 2 mg/ml, treat cells with a final concentration of 2 ug/ml Cyto B in complete medium supplemented with 25 mM Hepes pH 7.4 for 60 min at 37 °C.

Nocodazole: (Noc) — stock: 10 mg/ml, treat cell with a final concentration of 25 ug/ml Noc in complete medium supplemented with 25 mM Hepes pH 7.4 for 60 min at 37 °C.

No drug: — complete medium supplemented with 25 mM Hepes pH 7.4 for 60 min at 37 °C.

MTs = microtubules

Coverslip 1	Coverslip 2	Coverslip 3	Coverslip 4	Coverslip 5	Coverslip 6
No drug	No drug	Cyto B	NOC	CytoB	NOC
Stain Golgi	Stain Golgi	Stain Golgi	Stain Golgi	Stain Golgi	Stain Golgi
Stain Actin	Stain MTs	Stain actin	Stain MTs	Stain MTs	Stain actin

Fixation: 4% formaldehyde for coverslips 1,3, and 6
100% cold methanol for coverslips 2,4, and 5

Note: Refer to the "General Rules" section of this syllabus for information on staining for actin.

Your grade will be determined on your record of any effect the drugs have on:
1) Golgi morphology and localization
2) cytoskeletal elements
3) cell morphology

10/15/09 - Thursday

Protocol #4

<u>The effect of Brefeldin A on the organization of the Golgi apparatus.</u>

4 coverslips/group.

Drug treatment:

Brefeldin A: stock: 10 mg/ml, treat cells at a final concentration of 2 mg/ml for 60 min in complete medium supplemented with 25 mM Hepes pH 7.4 at 37 °C.

No drug: complete medium supplemented with 25 mM Hepes pH 7.4 for 60 min at 37 °C.

<table>
<tr><td>Coverslip 1</td><td>Coverslip 2</td><td>Coverslip 3</td><td>Coverslip 4</td></tr>
<tr><td>No drug</td><td>BFA</td><td>BFA</td><td>BFA</td></tr>
<tr><td colspan="2">Fix after drug/control incubation</td><td colspan="2">Washout BFA</td></tr>
<tr><td colspan="2">Block</td><td>Recover for 60 min
at 15 °C</td><td>Recover for 60 min
at 37 °C</td></tr>
<tr><td colspan="4">Visualize Golgi, ER and DNA</td></tr>
</table>

FYI: You could block TGN to endosome trafficking by incubating the cells at 20^{o}C.

Your grade will be based on:

Visualize the morphology of Golgi membranes ER and of DNA and describe the effects BFA has on the organization of the Golgi apparatus.

What effect does temperature have on the recovery of Golgi membranes after BFA treatment?

10/20/09 – Tuesday

Protocol #5

Test the role of Microtubules in the Reassembly of Golgi from BFA

Design an experiment to test whether microtubules are required for the reassembly of the Golgi during the recovery from BFA treatment. Note that microtubules are NOT required for BFA to cause the cis-trans cisternae to fuse with the ER nor is it required for the BFA stimulated fusion of the TGN to the endosome.

You will be given 6 coverslips/group (though you will not need them all).

Midterm Review

10/22/09

Midterm

10/22/09 - Thursday

Protocol #6 – Day 1 of 3

Transfection of NRK cells with pcDNA3-FLAG-MEK1.

3 wells of cells per group.

NRK cells grown in a 6 well plate will be provided for you.
Perform the following transfection reactions in the tissue culture hood. Maintain sterile conditions!!!

Well #1: No Transfection

Well #2: Control Transfection (pcDNA3 vector control)
1. Add 6ul of TRANSIT-LT1 to 75ul of OPTI-MEM in a tube. Incubate for 5 min.
2. Add 2ug of pcDNA3 to the TRANSIT-LT1/OPTI-MEM solution. Mix by tapping.
3. Incubate for 20' at room temperature.
4. Add the solution dropwise to cells. Each well should have 5mL DMEM.

Well#3: pcDNA3-FLAG-MEK1 Transfection
1. Add 6ul of TRANSIT-LT1 to 75ul of OPTI-MEM in a tube. Incubate for 5 min.
2. Add 2ug of pcDNA3-FLAG-MEK1 to the TRANSIT-LT1/OPTI-MEM solution. Mix by tapping.
3. Incubate for 20' at room temperature.
4. Add the solution dropwise to cells. Each well should have 5ml DMEM.

Incubate cells at 37^0C for 48 hours.

10/27/09 – Tuesday

Protocol #6 Day 2 of 3

Electrophoresis of total cell lysate from transfected and mock-transfected NRK cells.

Preparation of a polyacrylamide gel: (assemble gel apparatus as shown to you by your TA)

Running gel:

2.4 ml H2O
1.5 ml 1.5 M Tris pH 8.8
2 ml Acrylamide solution
60 ul 10 % SDS
60 ul 10 % APS

mix well
get the gel set up ready!

→ add 15 ul of TEMED,
pour gel (as shown to you by the TA)
overlay with 200 ul isopropanol
wait until polymerized

CAUTION:
Acrylamide is carcinogenic!!!
Always wear gloves!!!

Remove isopropanol and wash 2X with deionized water

Stacking gel:

6 ml H2O
2.5 ml 0.5M Tris pH 6.8
1.3 ml Acrylamide solution
100 ul 10 % SDS
100 ul 10 % APS

mix well
get comb ready!

→ add 15 ul of TEMED
pour stacking gel and place comb in between glass plates, **wait until polymerized**

Sample preparation:

Add 100 ul 2x sample buffer to each well and use a cell scraper to detach cells from the well.
(NOTE: Each group will receive one cell scraper. Thoroughly rinse scraper and dry it with a kimwipe before using it in different wells to avoid cross-contamination.)

Transfer cell solution into an eppendorf tube.
Boil for 4 min on 95ºC heat block. Vortex.
Centrifuge for 2 min at full speed in table top centrifuge.
The samples are now ready to be loaded onto the gel.

Loading, running and transfer of an SDS-polyacrylamide gel:

Carefully remove comb
place gel into setup
add running buffer:

1x running buffer (from 4x stock)
0.1 % SDS
H_2O to volume

wash the well with running buffer

load samples in the following order:

- 5 ul rainbow marker
- 20 ul lysate from mock-transfected cells
- 20 ul lysate from FLAG-transfected cells
- 20 ul lysate from non-transfected control cells
- 20 ul lysate from control sample (given to you)
- 5 ul rainbow marker
- 20 ul lysate from mock-transfected cells
- 20 ul lysate from FLAG-transfected cells
- 20 ul lysate from non-transfected control cells
- 20 ul lysate from control sample (given to you)

run gel at 35 mAmps until dye front runs off.

While gel is running, prepare nitrocellulose, Whatman paper and transfer buffer:

1x running buffer
20 % methanol

Transfer to nitrocellulose: assemble 'sandwich' as shown to you by the TAs

Transfer for 45 min at 200 mAmps (put ice block and stir bar)

Diassemble transfer setup and place nitrocellulose in small plastic box

Stain with red Ponceau for 1 min, remove (but don't discard)

Rinse with H_2O

Dry nitrocellulose between 2 pieces of Whatman paper

Mark the lanes with a pen. Wrap nitrocellulose in plastic wrap and store at 4^oC.

10/29/09 - Thursday

Protocol #6 Day 3 of 3

Western Blot analysis

Cut nitrocellulose into 2 pieces (in the middle of the 2nd rainbow marker) and analyze lanes 1-5 for the presence of the MEK1 protein and lanes 6-10 for the presence of the FLAG epitope

Block nitrocellulose for 10 min with 3 % milk, 0.01 % Tween in PBS

Primary antibody: 1 ml of anti-MEK1 (rabbit) antibody in blocking buffer (1:500) for lanes 1-5)
1 ml of anti-FLAG (mouse) in blocking buffer to a final concentration of 10ug/mL (lanes 6-10) 40 min at RT on shaker

wash blot 3x 5min with PBS

secondary antibody: 10 ml of goat-anti-rabbit HRP in blocking buffer
10 ml of goat anti-mouse HRP in blocking buffer

15 min at RT on shaker

wash blot 3x 5 min with PBS

TMB Stabilized Substrate for Horseradish Peroxidase

Add enough TMB (3,3',5,5'-tetramethylbenzidene) to cover membrane.

Incubate at room temperature until desired amount of color development is obtained.
(Can be up to 15min)

Stop color development with 3x 5 min washes with distilled water.

Your grade will be determined on your ability to:
1. Identify each protein band that appears.
2. How do the proteins bands compare from lane to lane, differ?
3. What information can you obtain about your protein using SDS-PAGE/Western Blotting and how does that differ from the information obtained by immunofluorescence?

11/03/09 - Tuesday

Protocol #7

Prepare an acrylamide gel for purification experiment (see previous experiment for directions)

Affinity Purification of pQE30-His-GFP from bacteria with Ni-Agarose Beads

5ml of bacteria expressing His-GFP protein were pelleted
Resuspend pellet in 200ul of CellLytic (lysis) buffer
Resuspend the pellet completely by vortexing (1-2 min).
Put 10ul into new tube. Add 10ul 2X Sample Buffer. Boil 5 min. (Label this tube total lysate)
Spin 2min 14,000 rpm
Take out 10ul of the supernatant and add 10ul of sample buffer. Boil for 5 min (Label tube supernatant)
Put 50ul of Ni-Agarose bead slurry into new tube. Wash 2x with 1ml PBS (spin 30sec 2000rpm)
Add remaining supernatant from lysate and put into new tube. Mix by inverting.
Rotate samples 45min. While this is going, make 100ul each of 10mM. 30mM, 100mM, 200mM imidazole
Spin 1000rpm 30sec.
Take 10ul of supernatant and place into a new tube. Add 10ul 2X Sample Buffer and boil for 5 minutes. (This is your unbound sample).
Remove the rest of the supernatant from the Ni-agarose beads and discard. **Be careful not to suck up beads.**
Wash beads with 1ml PBS. Pellet beads as before. Take 10ul of supernatant and add 10ul 2X Sample Buffer. Boil and label tube "PBS wash"
Remove the remaining PBS from the Ni-agarose beads and discard. **Be careful not to suck up beads.**
Wash with 50ul 10mM Imidazole. (mix by tapping tube). Pellet beads as before. Take 10ul of supernatant and add 10ul 2X Sample Buffer. Boil and label tube "10mM Imid wash"
Wash with 50ul 30mM Imidazole. Pellet beads as before. Take 10ul of supernatant and add 10ul 2X Sample Buffer. Boil and label tube "30mM Imid wash"
Wash with 50ul 100mM Imidazole. Pellet beads as before. Take 10ul of supernatant and add 10ul 2X Sample Buffer. Boil and label tube "100mM Imid wash"
Wash with 50ul 200mM Imidazole. Pellet beads as before. Take 10ul of supernatant and add 10ul 2X Sample Buffer. Boil and label tube "200mM Imid wash"
Remove as much liquid from the Ni-agarose without sucking up the beads. Add 20ul of 2X sample buffer and boil. Label tube "Beads"

Load 5 ul rainbow marker and 15ul of each sample onto 12% Acrylamide gel in the following order

Rainbow marker
Total Lysate
Supernatant
Unbound
PBS wash

10mM Imidazole wash
30mM Imidazole wash
100mM Imidazole wash
200mM Imidazole wash
Beads (10ul)

Add Coomassie Blue to gel and wait for bands to appear. Destain gel to remove background

Your grade will be based on the quality of the purification.

11/05/09 - Thursday

Protocol #8 Day 1 of 2

Adherens Junction Dynamics

MDCK cells will be treated accordingly:

Coverslip	1	2	3	4	5	6
2mM EGTA	none	25 min	25 min	25 min	25 min	25 min
Recovery (hr)	none	0	2	2	4	4
During recovery cytochalasin	none	none	none	2ug/ml	none	2ug/ml

2mM EGTA treatment is done in DMEM (WITHOUT SERUM) at 37^{o}C

Recovery is carried out in complete DMEM (WITH SERUM) at 37^{o}C

Fix cells with formaldehyde and leave in blocking solution at 4^{o}C until Thursday.

11/12/09 – Thursday

Protocol #8 Day 2 of 2

Visualization of Adherens Junctions

Stain cells for actin, E-cadherin (dilute antibody 1:100), and DNA

Your grade will be based on your staining of intermediary structures of AJ formation and the role actin plays in this process.

11/17/09 – 11/24/09

Protocol #9

Design your own experiment

You will be given two tubes of media, one of which contains an antibody.
First you will identify which tube has the antibody in it.
Second, you will definitively demonstrate what antigen it recognizes:
Hint – here are the molecular weights of some of the proteins you worked with
Actin = 42kD
Tubulin = 55kD
E-cadherin = 120kD
GM130 = 130kD
Calreticulin = 48kD
Giantin = >300kD
GFP = 27kD

11/26/09 – Thursday

The effect of tryptophan on cellular metabolism

Go home and eat turkey

12/01/09 – Tuesday

Review for Final Exam/Potluck

12/03/08 - Thursday

Final Exam