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)\*!,3'!>/)R#/J!D,/&\$,&/'A!!L3'!/)UODOR'D!/'2&(#/(J!F0\$<!0\*!,3'! I#\*FI DJ\*,3'D)-'!,3'!>O(J>'>,)F'!\$3#)\*!,3/0&23!,3'!R'RU/#\*'!OH!,3'!XKA!!L3)D!#((OID!HO/!HO(F)\*2!#\*F!ROF)H)\$#,)O\*!OH! ,3'!>0(J>'>,)F'!,0!0\$\$&/A!!Y\*\$'!,3'!>/0,')\*!)D!>/0>'/(J!H0(F'F!#\*F!\$0R>(','(J!DJ\*,3'D)-'F!),!)D!RON'F!H/OR!,3'! A!!GH!, 3' !>/O, ')\*!)D!)\*, '\*F' F!HO/! XK!,0!#\*0,3' /!D&U\$' ((&(#/!\$0R>#/,R' \*,!0/!0&,!0H!,3' !\$' ((!N)#! 'Z>0/,!#/0R!,3'!\$'((5!),!,J>)\$#((J!R0N'D!#/0R!,3'!XK!,0!,3'! !! 3' /' !!\&/,3' /!ROF)\!)\$#,)0\*D!,#<' ! >(#\$' A!!L3' !>/O>' /!,/#HH)\$<)\*2!OH!>/O,')\*D!)D!N),#(!HO/!H&\*\$,)O\*)\*2A!!4' N' /#(!F)D' #D' D!)\*\$(&F)\*25!QC45!/',)\*),)D! >)2R' \*,OD#!#\*F!V)(DO\*!F)D' #D' !3#N' !U' ' \*!#DDO\$)#,' F!I ),3!>/0,')\*!R)DS(O\$#()-#,)O\*!I ),3)\*!,3' !\$' ((A?!L3' !R)DS

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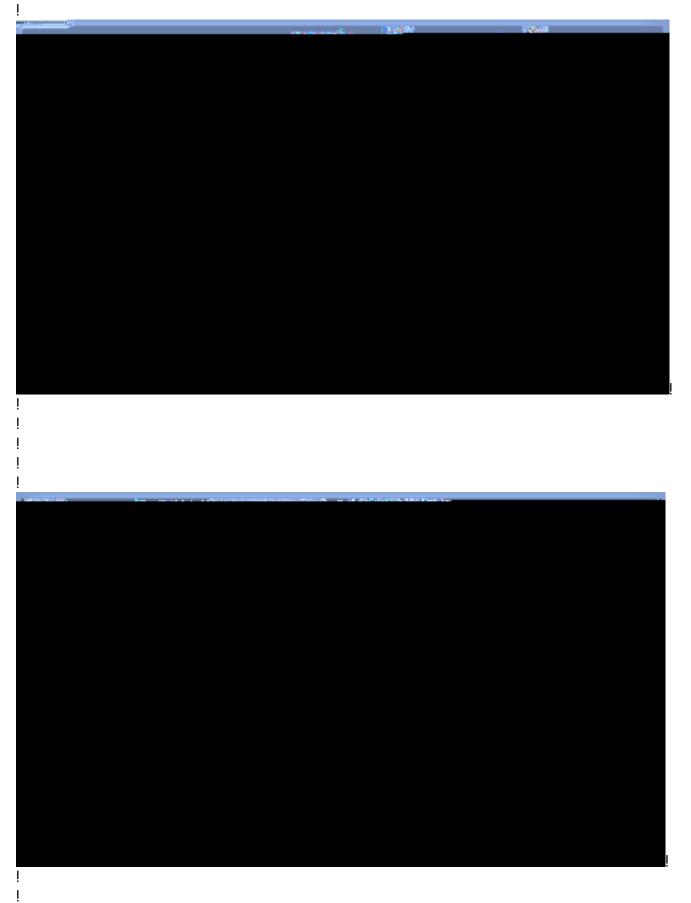
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- \$' ((&(#/!\$0R>#/, R' \*, D!#\*F!, 3' !' HH' \$,)N' \*' DD!OH!, 3' !2/0&>TD!>/' D' \*, #,)O\*!OH!, 3' !ROF' (A!!! @A !4,&F'\*,D!D30&(F!F'R0\*D,/#,'!,3')/!&\*F'/D,#\*F)\*2!OH!I'D,'/\*!U(0,,)\*2!#\*F!\$'((!H/#\$,)0\*#,)0\*!>/)0/!,0!
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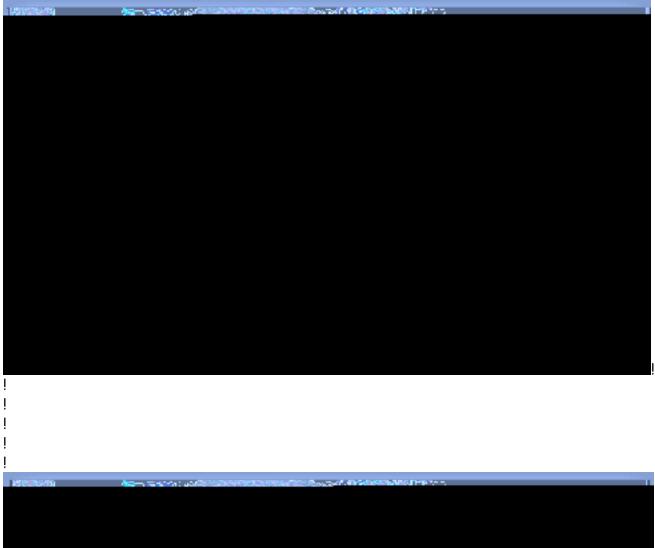
S de Seci Whe e did all eig An Overview of Protein Trafficking

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The second step of cell fractionation typically includes equilibrium density-gradient centrifugation.

#### What physical property do you think this method is based on?\_

In order to carry out this process, the impure organelle fraction from differential centrifugation is placed on top of a gradient of sucrose or glycerol. The tube is then spun at high speeds for several hours until each organelle migrates to equilibrium. Equilibrium is reached when the density of the organelle matches the density of the sucrose gradient, as seen in the image to the right.

The endoplasmic reticulum has a density of 1.20 g/cm<sup>3</sup> and the plasma membrane has a density of 1.12 g/cm<sup>3</sup>. Include arrows to show where these organelles would appear after centrifugation.

#### E e i e all De e i e he I acell la Pah a ake b MHC Cla I Cla II

Major Histocompatibility Complex (MHC) proteins are human cell surface proteins vital for the presentation of antigen (protein on the surface of an invader) and initiation of a specific immune response. MHC proteins are divided into two classes based on their structure, MHC Class I and MHC Class II. The two different protein classes are recognized by different immune cells which allows for a slightly different immune response. This is beneficial because certain immune cells are better for fighting bacteria, while others are better for fighting viruses.

It was experimentally determined that MHC Class I and Class II move through cell differently. In this lab activity you will carry out a western blot of cell fractionation extracts to determine the likely pathway followed by each of these proteins.

In order to accurately analyze the gel, we run, you will need to use marker proteins to verify that each

## Part B: Blotting onto a Nitrocellulose Membrane

While the gel is running:

- 12. Soak membrane (one per gel) in methanol for 2-3 minutes
- 13. Soak mesh pads and 6 pieces of filter paper in cold SDS transfer buffer (stored at 4°C)
- 14. Set up antibody dilutions in blocking solution: (each group will be responsible for one dilution)
  - a. Anti-Calreticulin= 1:1000
  - b. Anti-GM-130 = 1:400
  - c. Anti-MHC I = 1:5,000
  - d. Anti-MHC II = 1:5
- 15. Fill the gel cartridge
  - a. Lay down the black side of the cassette
  - b. Place in one mesh pad
  - c. Lay down 3 pieces of filter paper
  - d. Lay down nitrocellulose membrane (make sure there are no bubbles)
  - e. Remove gel from running apparatus and lay on top of the membrane
  - f. Lay down 3 pieces of filter paper
  - g. Place in one mesh pad
  - h. Close gel cartridge with sliding clamp
  - i. Repeat this process for second gel
- 16. Slide gel cartridge into transfer apparatus with black side towards red electrode
- 17. Place ice pack in apparatus
- 18. Fill apparatus with cold transfer buffer
- 19. Run at 100V for 35 minutes

## **Part C: Detect proteins**

- 20. Remove membrane from transfer apparatus and place in blocking solution on rocker for 30 minutes
- 21. Cut the membrane into 5 sections, each containing a MW marker and the four extracts.
- 22. Incubate each section of membrane with a different antibody overnight at 4°C
- 23. Pour off antibody and wash each membrane 3x with PBS Tween
- 24. While washing, dilute the anti-rabbit HRP secondary to 1:2000 in blocking solution
- 25. Incubate each membrane at room temperature for 1 hour in secondary antibody
- 26. Pour off antibody
- 27. Wash three times with PBS Tween

# Part D: Develop Image

- 28. Dilute the Amplified Opti-4CN Substrate according to directions in kit
- 29. Develop membranes in Amplified Opti-4CN Substrate for 30 minutes on rocker
- 30. Reassemble membranes and take picture

Analysis (Please type following)

Lab Re R b ic

Category	<b>Total Points</b>	Points Earned & Comments
Introduction		
<ul> <li>Start with the purpose of the lab</li> <li>Provides background needed to understand results (see above)</li> <li>Clear and concise; one topic flows into the next</li> </ul>	15	
Procedure		
<ul> <li>Written in paragraph form (NO STEPS)</li> <li>Describes all procedures performed</li> <li>Bolded materials</li> <li>Contains sufficient detail to allow a reader who works in the field to understand what you did to collect data</li> </ul>	10	
Results		
<ul> <li>Written description of results</li> <li>Properly labeled membrane image</li> </ul>	15	
Discussion		
<ul> <li>Refers to specific data points/figures (incorporates the images that outline MHC movement)</li> <li>Includes interpretation of results (see above)</li> <li>Includes explanations of unexpected results (if applicable)</li> <li>Includes potential next steps</li> <li>Summary statement</li> <li>One topic flows to the next</li> </ul>	20	
Works Cited		
<ul> <li>Includes all sources consulted</li> <li>Aligned with in text citations</li> </ul>	5	
<ul> <li>Formatting &amp; Conventions</li> <li>Each section is labeled</li> <li>Written in past tense</li> <li>Few grammar and spelling mistakes</li> <li>Document is properly titled when uploaded to Google Drive</li> </ul>	5	Total Pointer /70

Total Points: \_\_\_\_/70

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