

Welcome to Protocol Walkthrough 1!

- **Today's Playlist**

- ghost – MisterWives
- Tape Machine – STRFKR
- All The Stars – Kendrick Lamar, SZA

- **Notes for Today's Format**

- Please let me know if you have any constructive criticism!

- **Upcoming BMES Events**

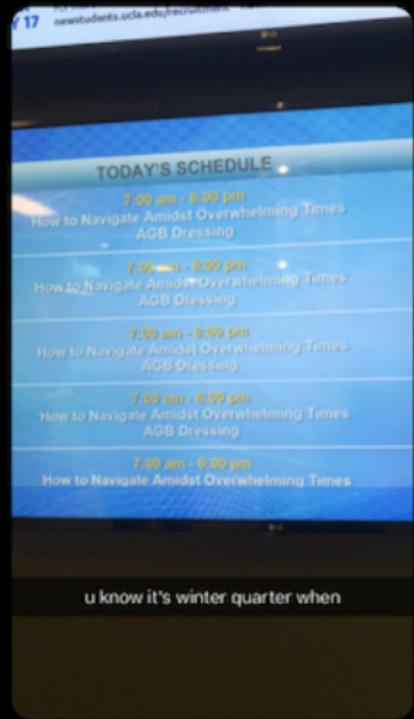
- Eagle Workshop – Sunday 1/17 3:00 – 4:00 PM
- Technical Presentation Workshop – Monday 1/25 6:30 – 8:30 PM



A note on winter quarter:

- Reach out if you need help!
- Form study groups and
(virtually) study together!

Los Angeles, CA
January 14, 2019, 6:19 PM



Protocol Walkthrough 1: Western Blotting

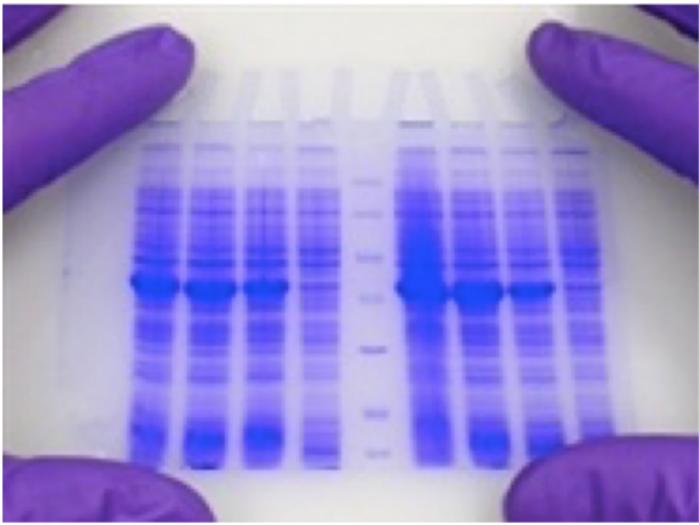
BMES Cell Team

Winter 2021



Outline

- Protocol Background
- Step-by-Step Walkthrough
 - BCA Assay
 - Western Blot
- Codenames Social!



Protocol Background

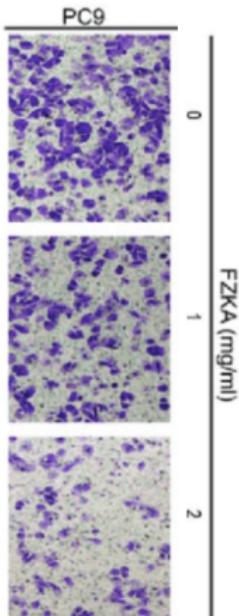
- Originally, this protocol was part of a larger experiment on AXL inhibition research in Meyer Lab
 - Adapted for Cell Team by Alison Tran
- Three day protocol
 - Day 1: BCA Assay, SDS Gel Electrophoresis, Protein Transfer, and Primary Antibody Incubation
 - Day 2: Secondary Antibody Incubation
 - Day 3: Imaging of Western Blot



Dr. Aaron Meyer

Protocol Background

- **Definition:** PC9 is a cancer cell line that expresses the AXL receptor.
- Human lung adenocarcinoma
- Isolated in 1989
- Utility: testing of cytotoxic drugs
 - Ex: FZKA
- Utility: study of tumorigenicity
 - Ex: Association between cigarette smoke and lung cancer development



Protocol Background

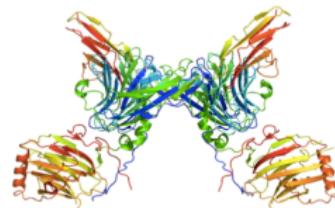
- **Definition:** The *AXL* gene is associated with metastatic progression.

- AXL receptor tyrosine kinase

- Cell surface receptor (ECM signals → cytoplasm)
 - Found on numerous types of aggressive cancer

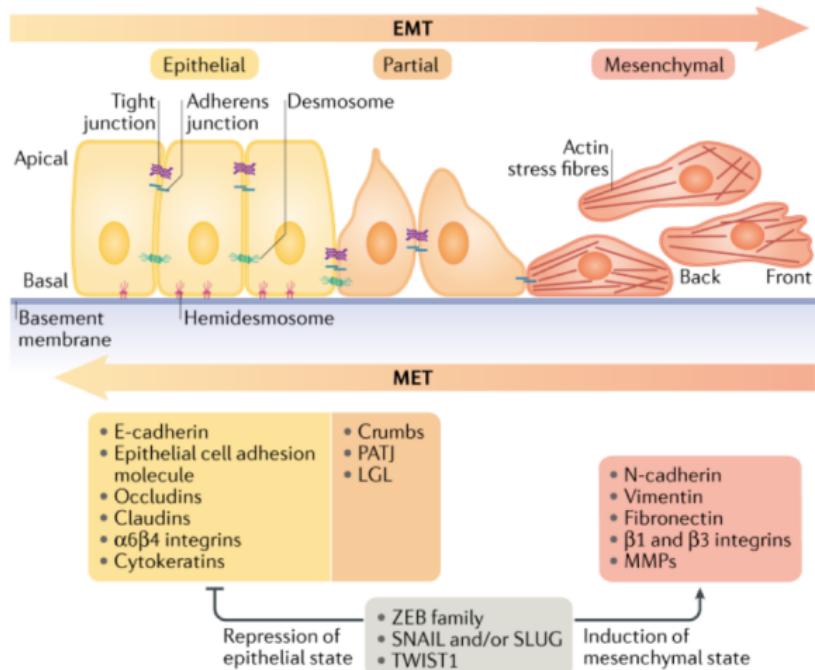
- *AXL* gene

- Isolated in 1988
 - Classified as an oncogene after detection in leukemia



Why do we care about AXL?

Expression of the AXL gene has been associated with **epithelial-mesenchymal transition**, which leads to metastasis.



Leveraging AXL In Cancer Therapy

The spread of aggressive cancers can be limited by inhibiting the behavior of the AXL receptor tyrosine kinase

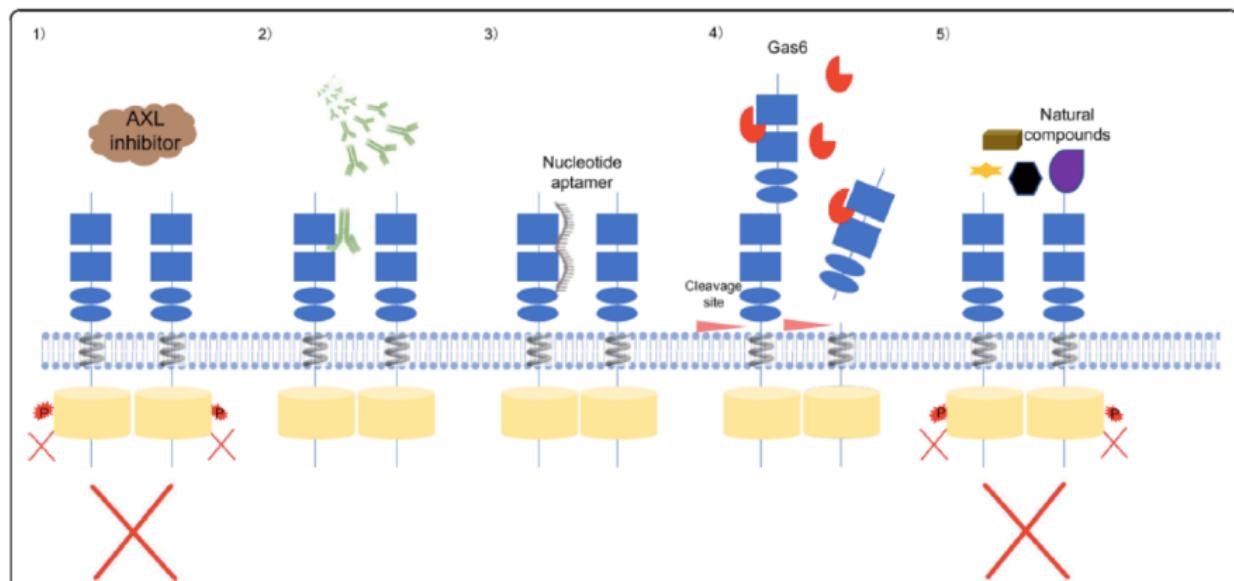


Fig. 2 Different therapeutic agents targeting AXL have been developed, including 1) small molecule inhibitors that block AXL auto-phosphorylation and kinase activities; 2) anti-AXL monoclonal antibodies (mAbs); 3) nucleotide aptamers; 4) soluble receptors; and 5) several natural compounds

The Meyer Lab Experiment

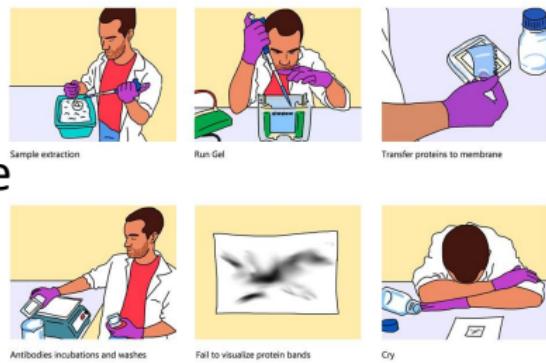


- **Engineering AXL Inhibition**
 - Use CRISPR to remove the AXL gene in PC9 cells
 - Compare behavior of unaltered PC9 cells and PC9 AXL KO
- Our Protocol Walkthrough
 - BCA Assay: confirm presence of proteins in PC9 and PC9 AXL KO samples and determine their concentration
 - Western Blot: confirm that the PC9 cells have the AXL gene and the PC9 AXL KO cells do not have the AXL gene

Review: Basic Protocol for a Western Blot

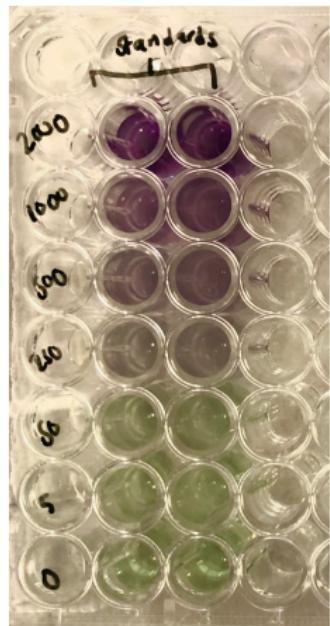
1. Lyse cells and collect proteins
2. BCA Assay
 - Calculate sample protein concentration
3. SDS Gel Electrophoresis
 - Separate proteins by size
4. Transfer proteins to membrane
5. Immunoblot target proteins
 - Antibody Binding
6. Image membrane

Western Blot Protocol



BCA Assay: Layout for 96 Well Plate

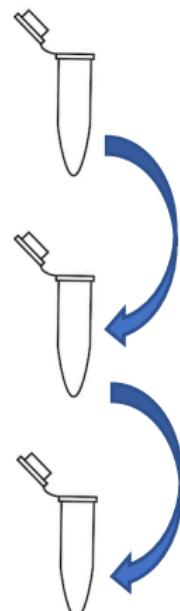
Setup triplicates of standards and duplicates of samples



	Standards			samples								
	1	2	3	4	5	6	7	8	9	10	11	12
A	2000 µg/mL	2000 µg/mL	2000 µg/mL									
B	1000 µg/mL	1000 µg/mL	1000 µg/mL			PC9	PC9					
C	500 µg/mL	500 µg/mL	500 µg/mL									
D	250 µg/mL	250 µg/mL	250 µg/mL			PC9	PC9	AXLKO	AXLKO			
E	50 µg/mL	50 µg/mL	50 µg/mL									
F	10 µg/mL	10 µg/mL	10 µg/mL									
G	0 µg/mL	0 µg/mL	0 µg/mL									
H												

BCA Assay: Serial Dilution to Make the Standards

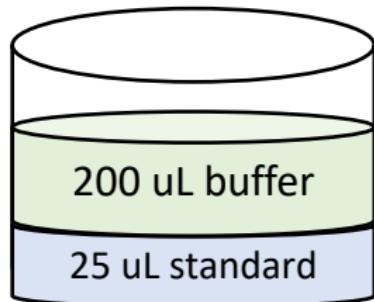
Use $M_1V_1 = M_2 V_2$ to generate the standards



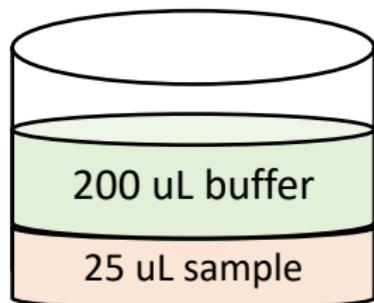
BSA Concentration	BSA Solution	Water (uL)	Total Volume after well (uL)	Total Volume at end (uL)
2000 ug/mL	160 uL of 2000 ug/mL	0	160	80
1000 ug/mL	80 uL of 2000 ug/mL	80	160	80
500 ug/mL	80 uL of 1000 ug/mL	80	160	80
250 ug/mL	80 uL of 500 ug/mL	80	160	128
50 ug/mL	32 uL of 250 ug/mL	128	160	144
5 ug/mL	16 uL of 50 ug/mL	144	160	160
0 ug/mL	0 uL	80	80	80

BCA Assay: Make reaction buffer & Load well plate

Vortex assay reagents



Standard well content



Sample well content

BCA Assay: Measure Absorbance with Plate Reader

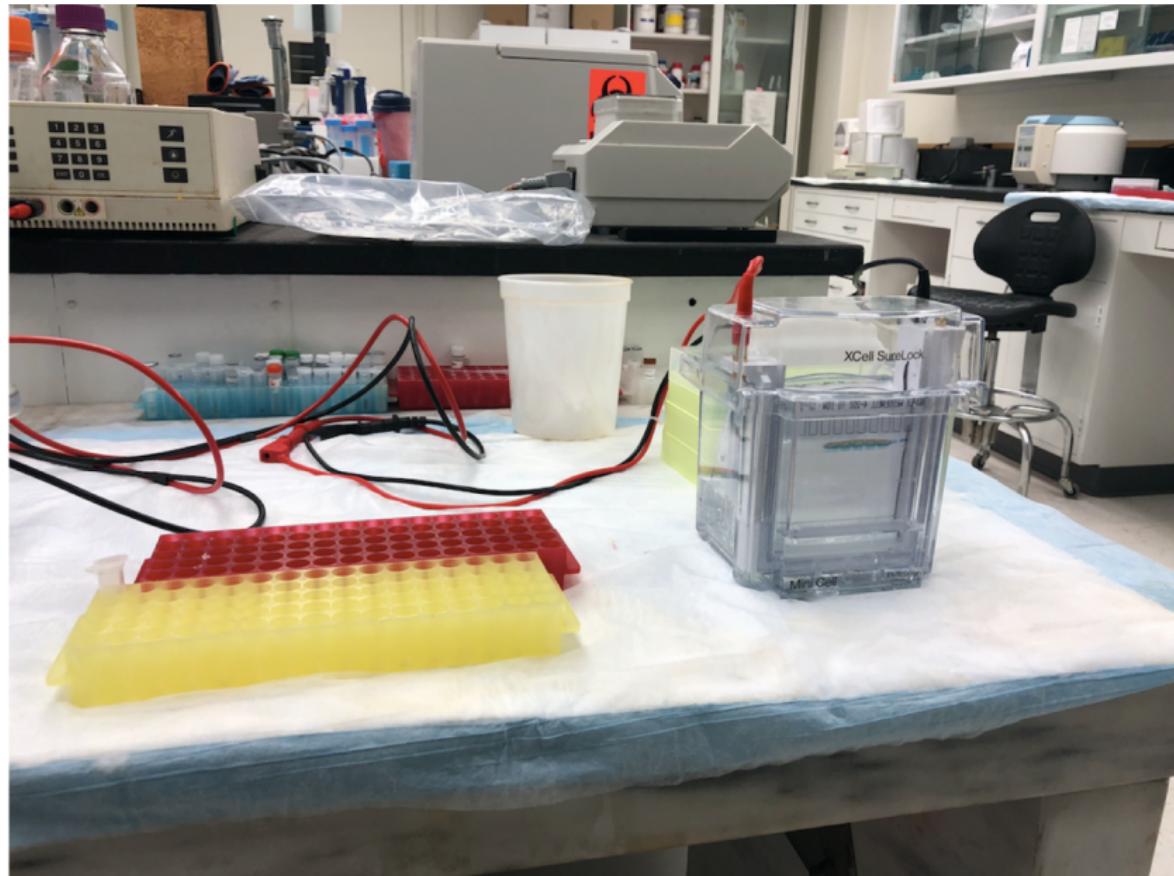
Load 96 well plate into
plate reader

		1	2	3	4	5	6	7	8	9	10	11	12
		A	15	10	9	10	7	8	6	5	7	7	7
		B	5	4	4	4	4	3	4	3	4	4	5
		C	3	5	4	3	3	3	4	2	3	4	4
		D	3	3	2	3	3	4	3	2	6	4	6
		E	3	2	3	4	3	5	5	3	3	4	3
		F	2	2	5	5	4	4	3	1	3	3	4
		G	2	3	2	2	2	2	3	2	5	2	5
		H	4	3	3	2	3	3	4	3	4	4	4

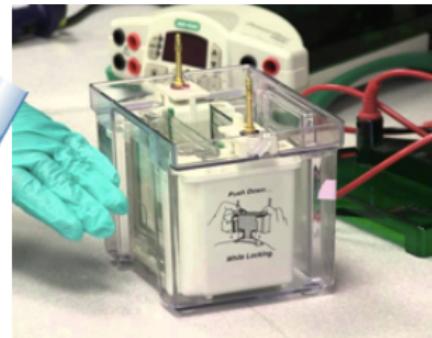
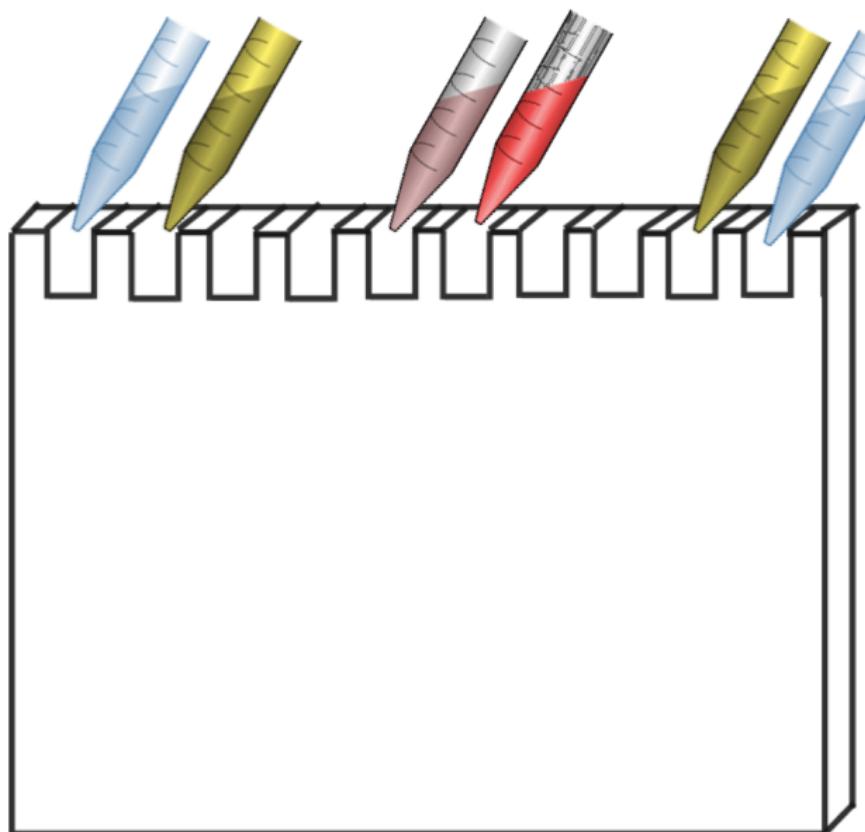


We will do the absorbance
analysis **next Tuesday**

Western Blot: Gel Electrophoresis Setup

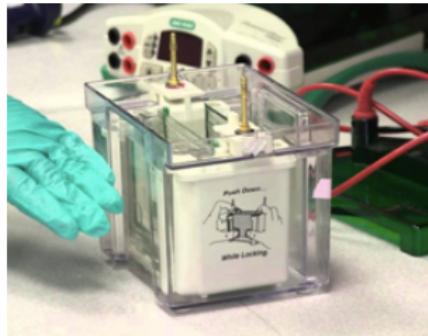
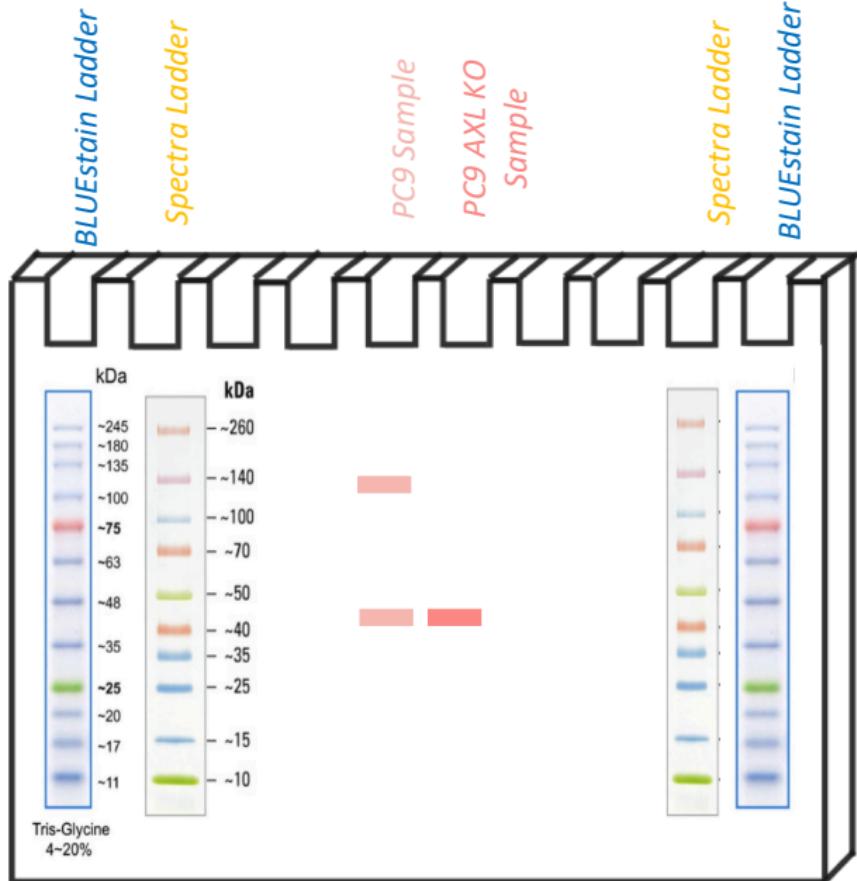


Western Blot: SDS PAGE



- BLUEstain Protein Ladder*
- Spectra Multicolor Ladder*
- PC9 Sample*
- PC9 AXL KO Sample*

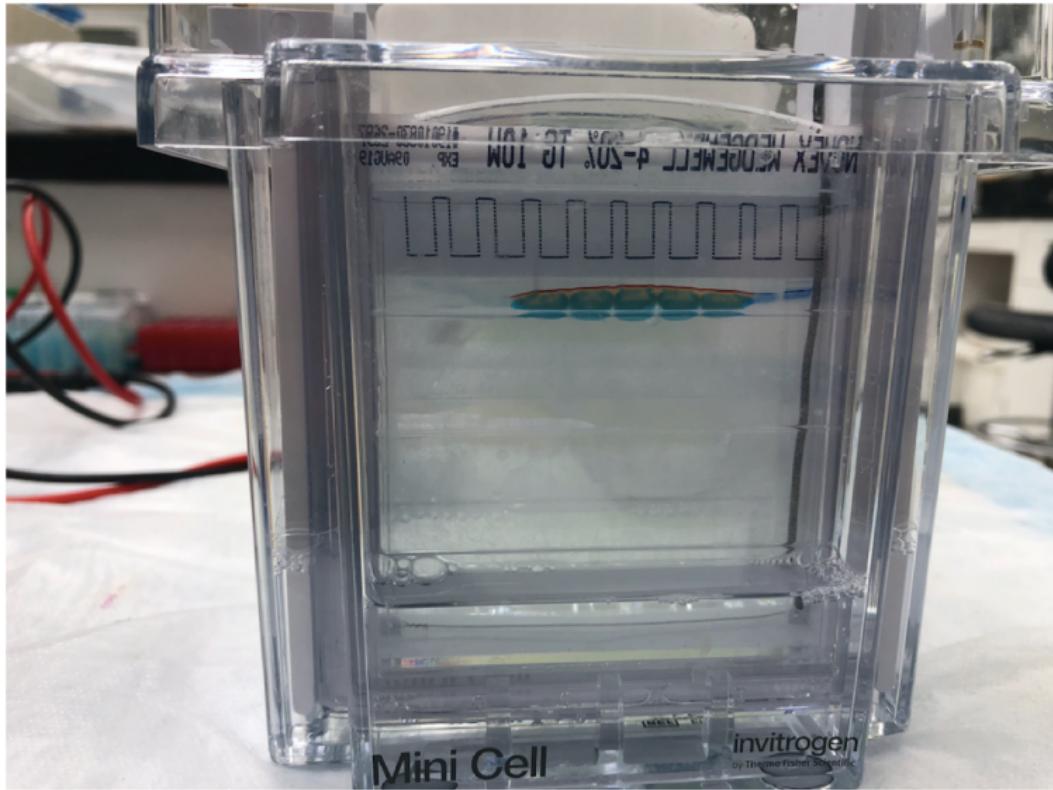
Western Blot: Run the Gel



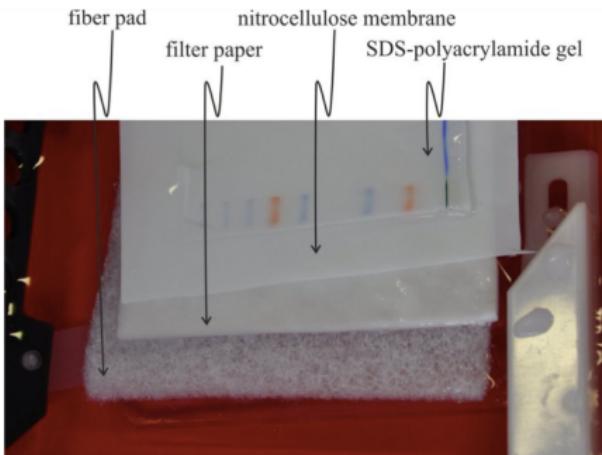
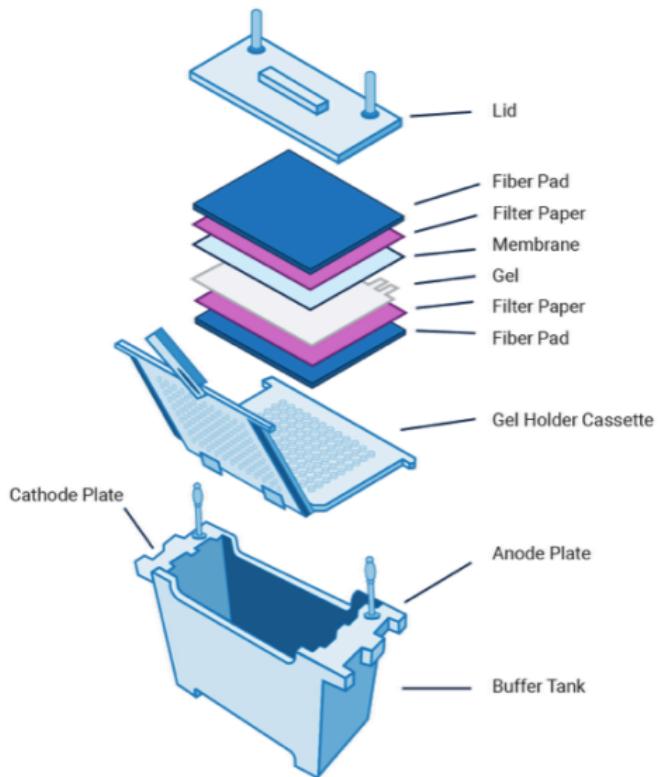
Actin bands:
42 kDa

AXL band:
135 kDa

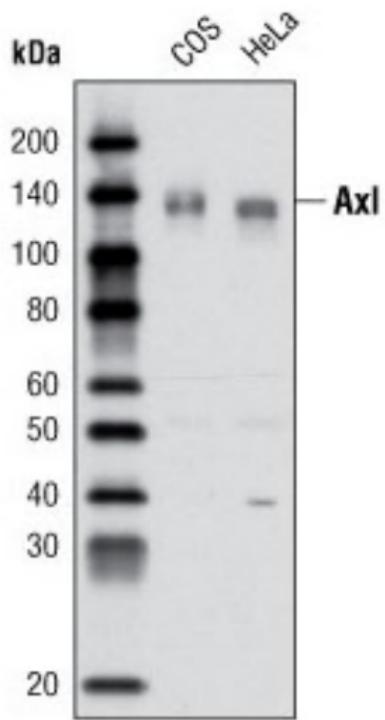
Western Blot: Close Up on the Gel



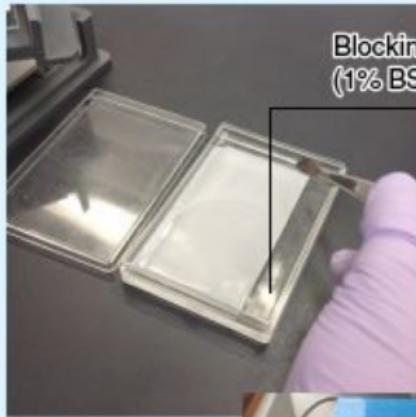
Western Blot: Protein Transfer



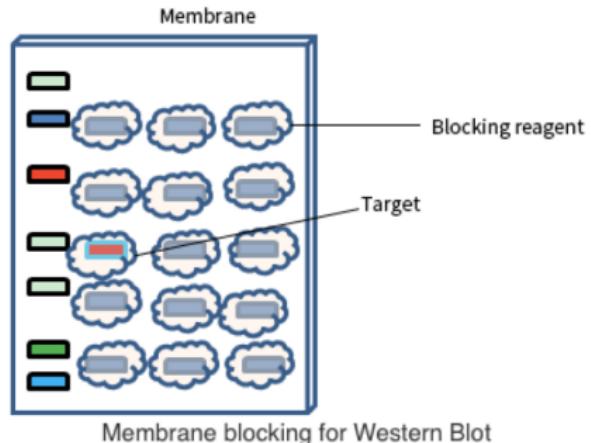
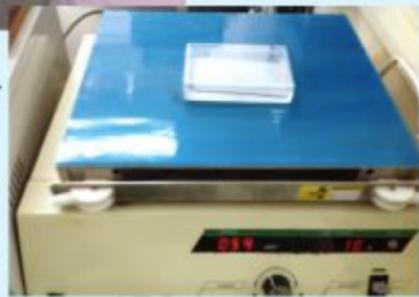
Western Blot: Protein Transfer



Western Blot: Blocking Buffer Incubation

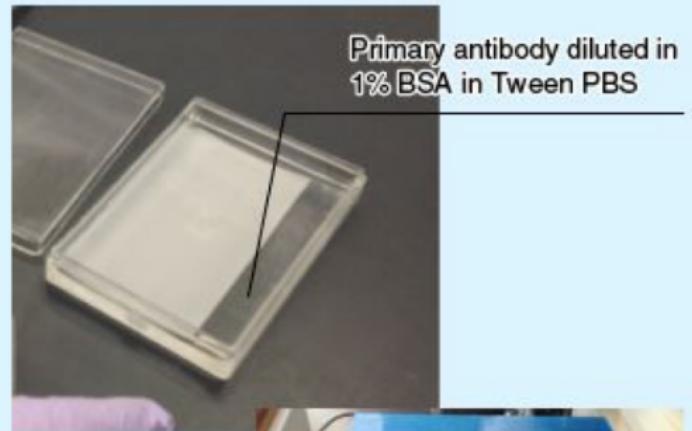


With shaking ➤



Membrane blocking for Western Blot

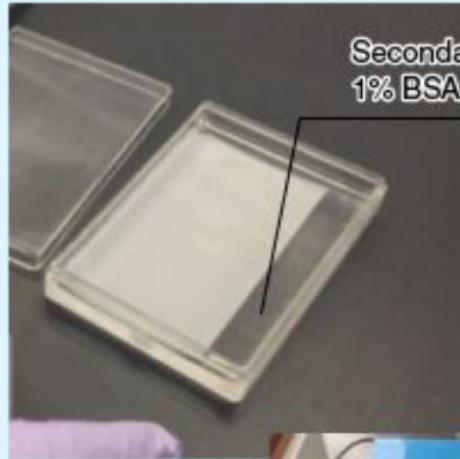
Western Blot: Primary Antibody Incubation



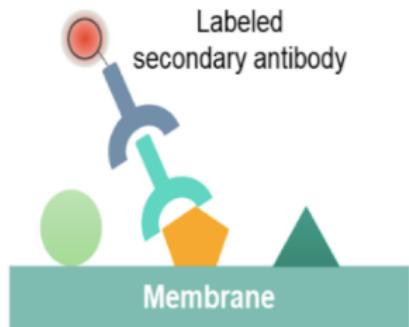
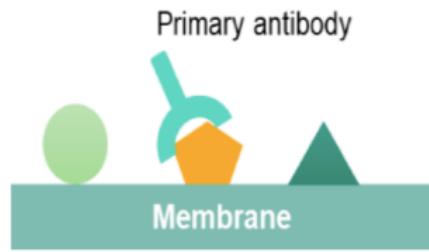
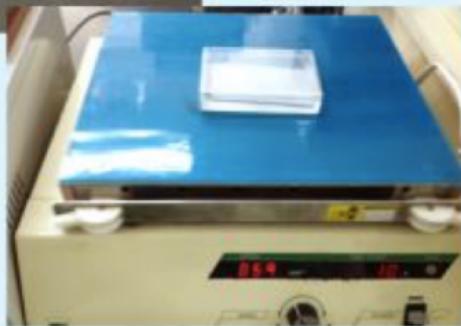
With shaking ➤



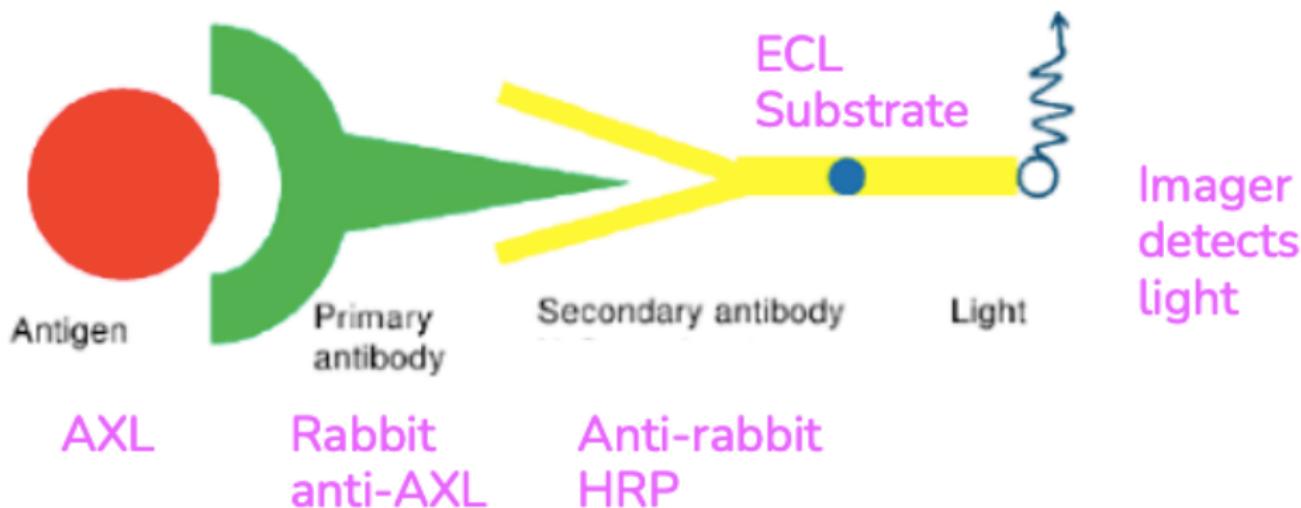
Western Blot: Secondary Antibody Incubation



With shaking ➤



Western Blot: Imaging with Chemiluminescence



Codenames Social!

- **While I set the game up, form project groups!**
 - Reference the [Group Projects slides](#) on the Cell Team site
 - **If you already have a group,** hop in a breakout room and discuss which topic you want to do / how you want to divide up the work.
 - Go to the breakout room that corresponds to your group number on the Sign up Sheet
 - **If you don't have a group yet,** stay in the main session and talk about which topics you're interested in and see if you have a shared topic of interest with anyone else.
 - Join a breakout room once you have a group.
 - **Type your topic and breakout room number in the Zoom chat**

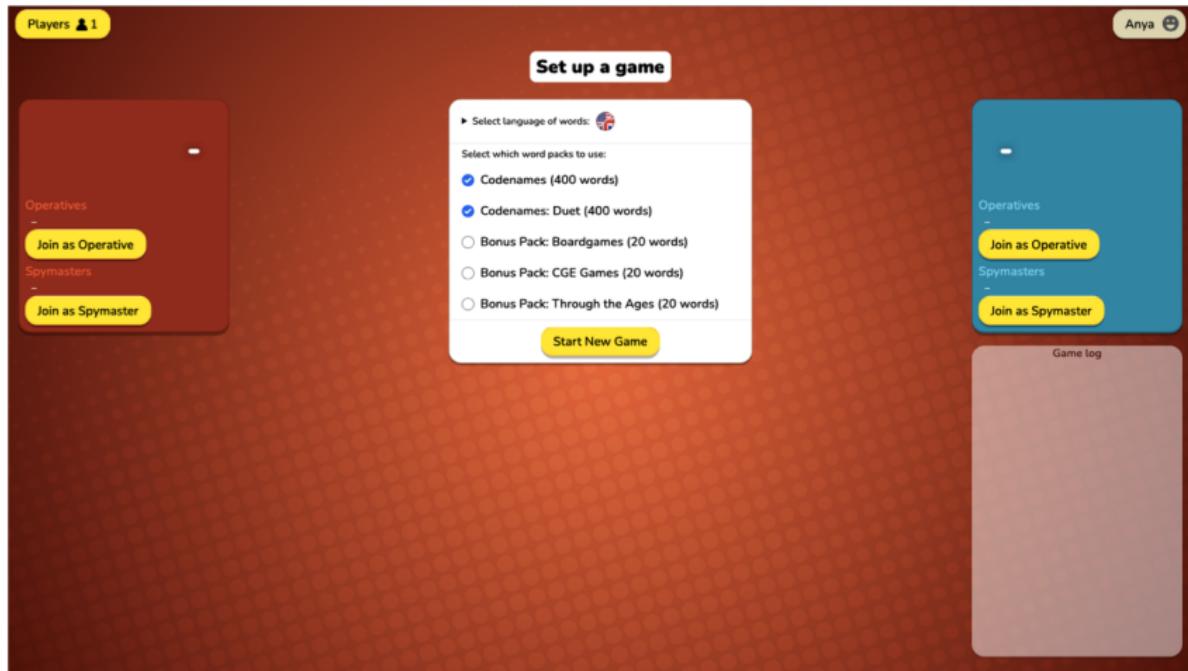
Codenames Social!

- **How this will work:**

- <https://codenames.game/room/dress-port-deck>
- Breakout rooms 2-3 will be on Anya's Team (Blue Team)
 - Anya will join as a Spymaster for the first game
- Breakout rooms 5-6 will be on Justin's Team (Red Team)
 - Justin will join as an Operative for the first game
 - Justin will also screen share
- If you want to be one of the people to type in clues / give the clues, click the link and join your team!

Codenames Social!

- <https://codenames.game/room/dress-port-deck>



Codenames Social!

- <https://codenames.game/room/dress-port-deck>

Players 1

Red spymaster is playing. (To play, you need to join a team.)

9

Operatives

Join as Operative

Spymasters

Join as Spymaster

8

Operatives

Join as Operative

Spymasters

Join as Spymaster

Game log

MOON	AXE	PRINCESS	BALLOON	BULB
SPOON	BIKINI	GERMANY	WHIP	PIPE
KID	TEACHER	SPINE	SHOP	BRUSH
BRICK	TRACK	FLOOR	MAGICIAN	CRUSADER
SIGN	PANTS	MOHAWK	CHEESE	SQUARE

Codenames Social!

- <https://codenames.game/room/dress-port-deck>

Players 1

The opponent spymaster is playing, wait for your turn...

MOON	AXE	PRINCESS	BALLOON	BULB
SPOON	BIKINI	GERMANY	WHIP	PIPE
KID	TEACHER	SPINE	SHOP	BRUSH
BRICK	TRACK	FLOOR	MAGICIAN	CRUSADER
SIGN	PANTS	MOHAWK	CHEESE	SQUARE

9

8

Operatives

Spymasters

Anyा

Reset Game

Game log

Codenames Social!

- <https://codenames.game/room/dress-port-deck>

Players 1

Give your operatives a clue.

?

THUMB	MUD	LEPRECHAUN	CHECK	CARROT
BEACH	SALOON	SWAMP	FUEL	ICE CREAM
TURKEY	HAIR	BAR	PAD	KNOT
LUCK	ALIEN	CHOCOLATE	RECORD	tie
PIT	MAPLE	LOCK	CARD	STAFF

Type your clue here - Give Clue

Reset Game Anya

Operatives - Spymasters

Operatives - Spymasters Anya

Game log

8

9

The image shows a screenshot of the Codenames Social game interface. At the top, it says "Give your operatives a clue." Below the grid, there's a text input field "Type your clue here" and a yellow "Give Clue" button. The game board is a 5x5 grid of words. The word "LEPRECHAUN" is highlighted in orange. Other words include THUMB, MUD, CHECK, CARROT, BEACH, SALOON, SWAMP, FUEL, ICE CREAM, TURKEY, HAIR, BAR, PAD, KNOT, LUCK, ALIEN, CHOCOLATE, RECORD, tie, PIT, MAPLE, LOCK, CARD, and STAFF. On the left, player 8 (Operative with sunglasses) has selected BEACH, TURKEY, LUCK, and PIT. On the right, player 9 (Operative with blonde hair) has selected SALOON, HAIR, ALIEN, MAPLE, CHOCOLATE, LOCK, CARD, and STAFF. A large blue button labeled "Game log" is visible on the right side of the screen.

Codenames Social!

- <https://codenames.game/room/dress-port-deck>

Players 1

Reset Game Anya

Give your operatives a clue.

?

Operatives

-

Spymasters

-

9

Operatives

-

Spymasters

Anya

Game log

8

THUMB

MUD

LEPRECHAUN

CHECK

CARROT

BEACH

SALOON

SWAMP

FUEL

ICE CREAM

TURKEY

HAIR

BAR

PAD

KNOT

LUCK

ALIEN

CHOCOLATE

RECORD

TIE

PIT

MAPLE

LOCK

CARD

STAFF

TREE

2

Give Clue