

# 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

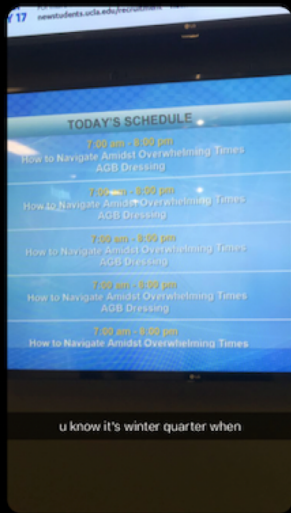


## A note on winter quarter:

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

Los Angeles, CA

January 14, 2019, 6:19 PM



u know it's winter quarter when

# Protocol Walkthrough 1: Western Blotting

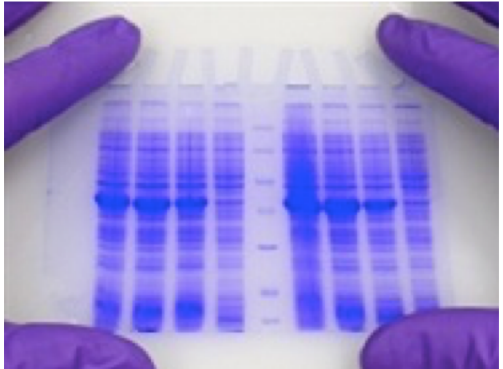
BMES Cell Team

Winter 2021



# Outline

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





# Protocol Background

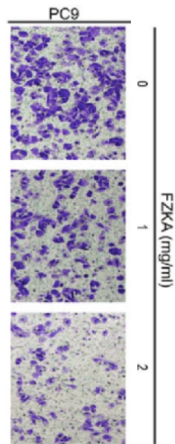
- Originally, this protocol was part of a larger experiment on AXL inhibition research in Meyer Lab
  - Adapted for Cell Team by Alison Tran
- Three day protocol
  - Day 1: BCA Assay, SDS Gel Electrophoresis, Protein Transfer, and Primary Antibody Incubation
  - Day 2: Secondary Antibody Incubation
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



**Dr. Aaron Meyer**

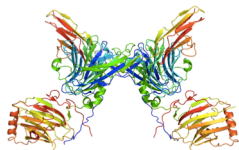
# Protocol Background

- **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

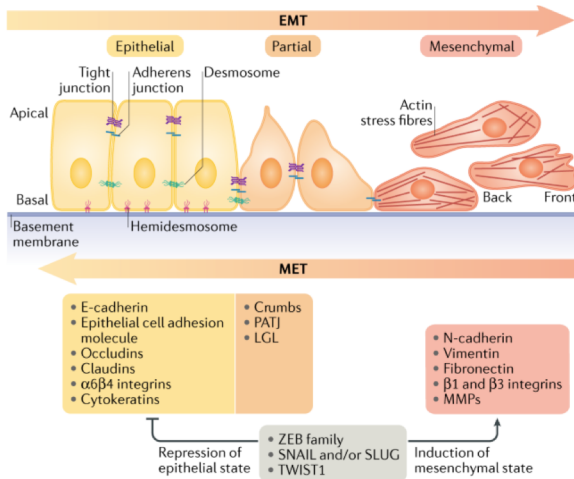
- **Definition:** The *AXL* gene is associated with metastatic progression.



- 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

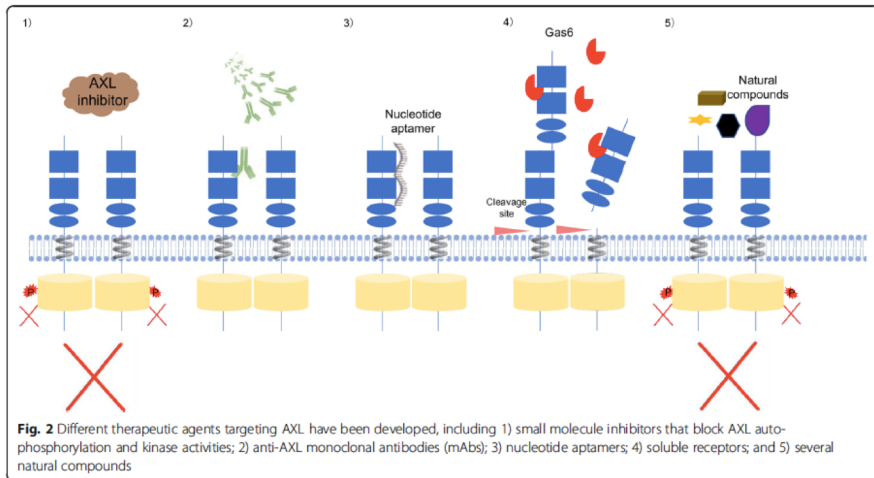
# Why do we care about AXL?

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

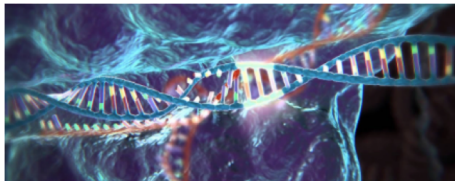


# Leveraging AXL In Cancer Therapy

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



# 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



Sample extraction



Run Gel



Transfer proteins to membrane



Antibodies incubations and washes



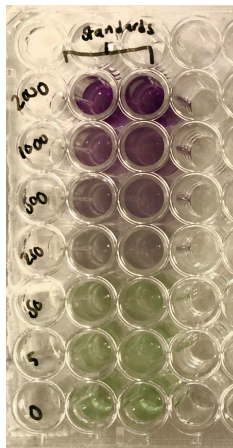
Fail to visualize protein bands



Cry

# 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 AXL KO	PC9 AXL KO					
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												

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# BCA Assay: Serial Dilution to Make the Standards

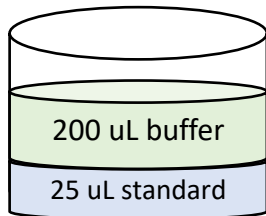
Use  $M_1 V_1 = M_2 V_2$  to generate the standards



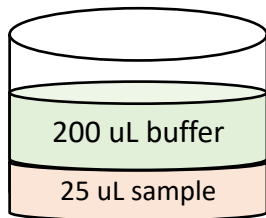
BSA Concentration	BSA Solution	Water (uL)	Total Volume after well (uL)	Total Volume at end (uL)
2000 ug/mL	160 uL of 2000 ug/mL	0	160	80
1000 ug/mL	80 uL of 2000 ug/mL	80	160	80
500 ug/mL	80 uL of 1000 ug/mL	80	160	80
250 ug/mL	80 uL of 500 ug/mL	80	160	128
50 ug/mL	32 uL of 250 ug/mL	128	160	144
5 ug/mL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



Plate1

Matrix Statistics

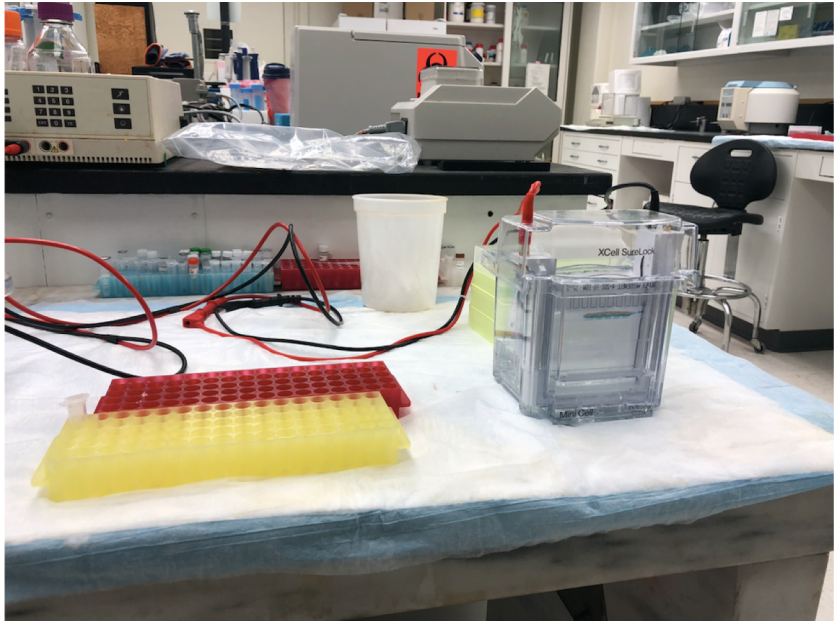
Data: Read 3 Lum Edit Matrix Read #: Show

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

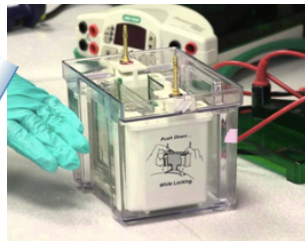
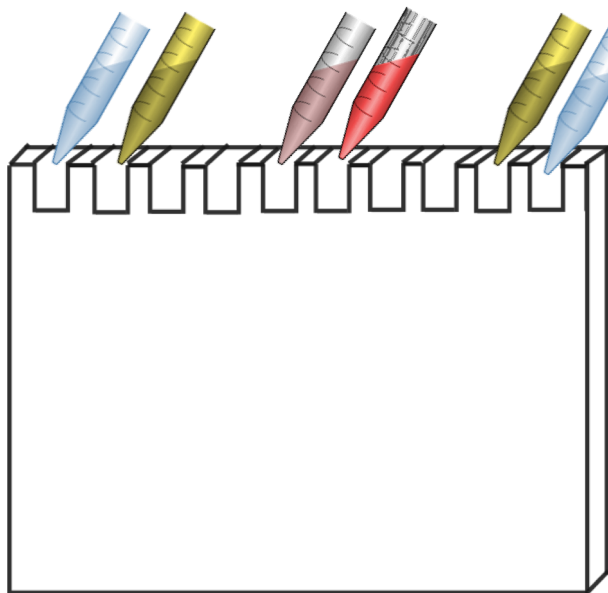
Edit Mask Help

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

# Western Blot: Gel Electrophoresis Setup



# Western Blot: SDS PAGE



*BLUEstain Protein Ladder*



*Spectra Multicolor Ladder*

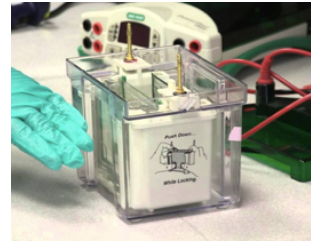
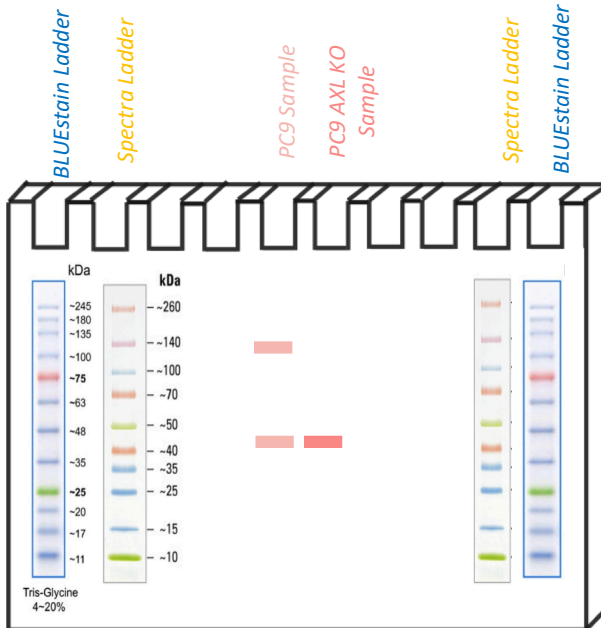


*PC9 Sample*



*PC9 AXL KO Sample*

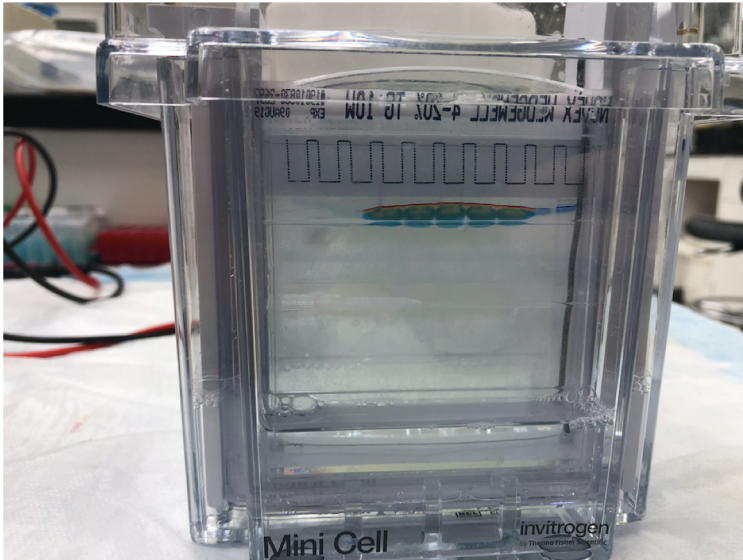
# Western Blot: Run the Gel



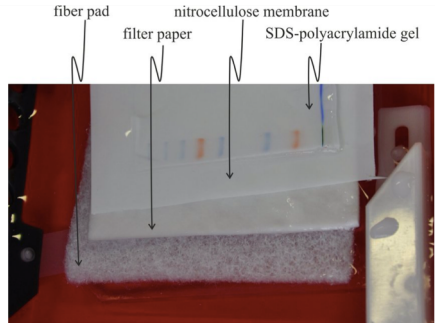
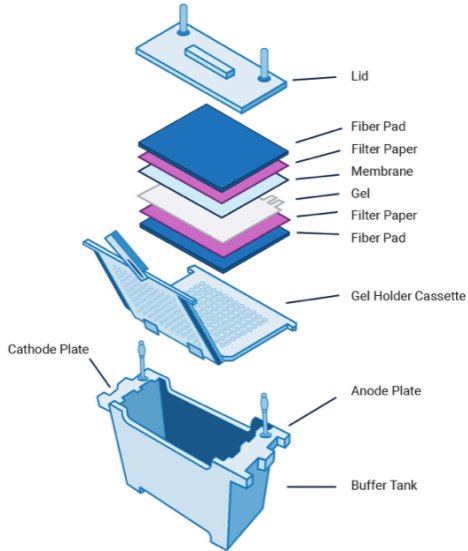
Actin bands:  
42 kDa

AXL band:  
135 kDa

# Western Blot: Close Up on the Gel

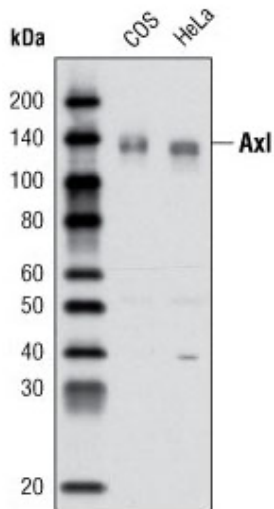
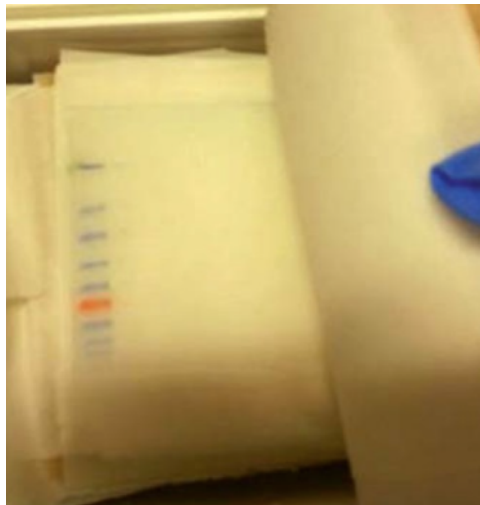


# Western Blot: Protein Transfer

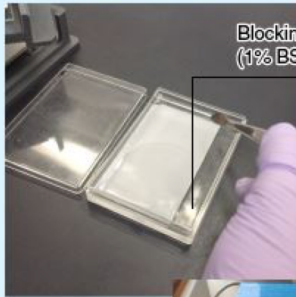




# Western Blot: Protein Transfer

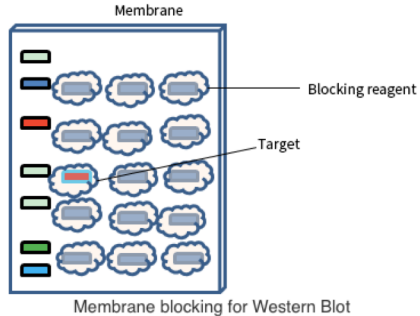
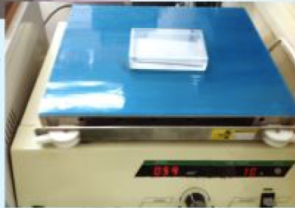


# Western Blot: Blocking Buffer Incubation

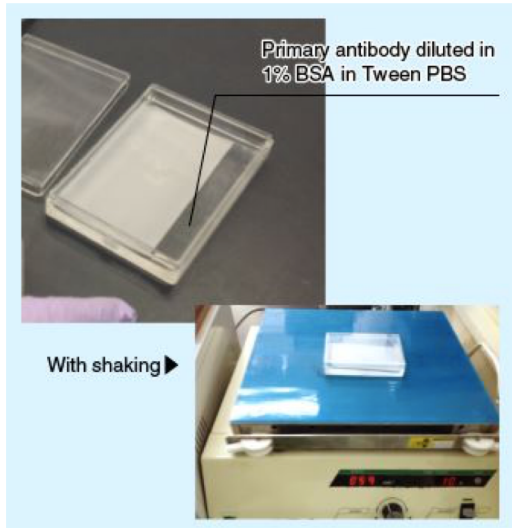


Blocking buffer  
(1% BSA in Tween PBS)

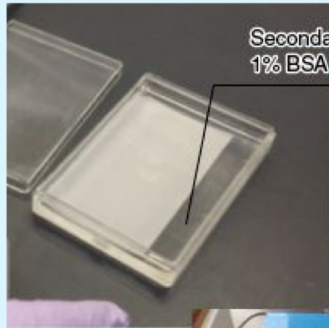
With shaking ►



# Western Blot: Primary Antibody Incubation

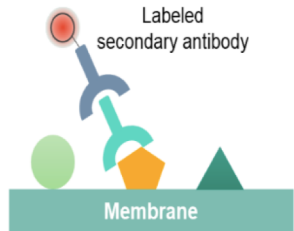
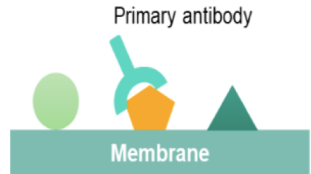
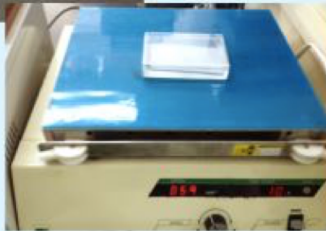


# Western Blot: Secondary Antibody Incubation

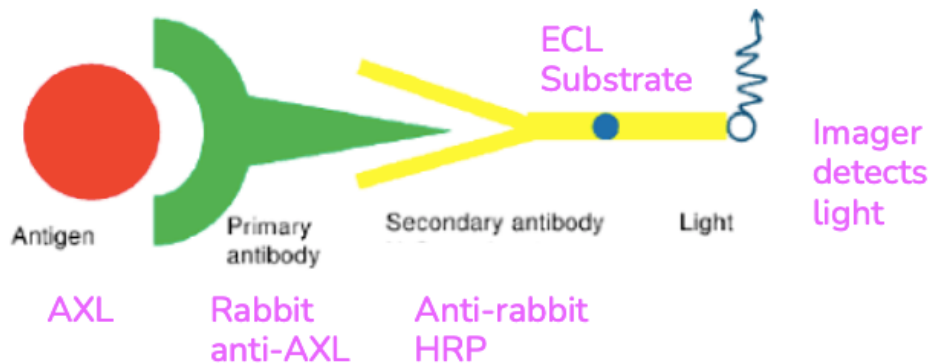


Secondary antibody diluted in  
1% BSA in Tween PBS

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**

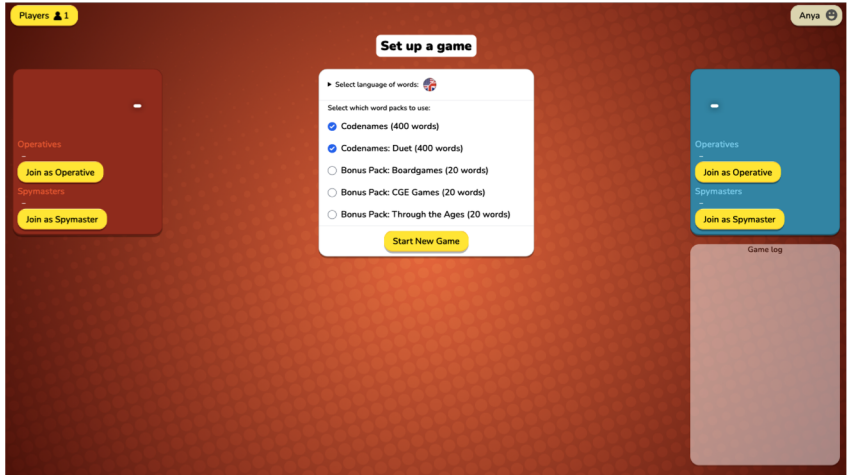
# Codenames Social!

- **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

Reset Game Anya

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

**9**

Operatives  
Join as Operative

Spymasters  
Join as Spymaster

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

**8**

Operatives  
Join as Operative

Spymasters  
Join as Spymaster

Game log

# Codenames Social!

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

Players 1

Reset Game Anya

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

9

Operatives  
-  
Spymasters  
-

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

8

Operatives  
-  
Spymasters  
Anya

Game log

# Codenames Social!

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

The screenshot displays the Codenames Social! game interface. At the top, a yellow button indicates 'Players 1'. A central instruction box says 'Give your operatives a clue.' with a question mark icon. The main area features a 5x5 grid of word cards. Each card has a colored background (blue, orange, or tan) and a small icon in the top right corner. The words on the cards are: 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, and STAFF. On the left, a player's profile is shown with a red background, a character image, the number 8, and the roles 'Operatives' and 'Spymasters'. On the right, another player's profile is shown with a blue background, a character image, the number 9, and the roles 'Operatives', 'Spymasters', and 'Anya'. A 'Reset Game' button and a 'Give Clue' button are also visible.

Players 1

Reset Game

Anya

Give your operatives a clue.

8

Operatives  
-  
Spymasters

9

Operatives  
-  
Spymasters  
Anya

Game log

Type your clue here - Give 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

# Codenames Social!

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

Players 1

Reset Game Any

Give your operatives a clue. ?

8

Operatives  
-  
Spymasters

9

Operatives  
-  
Spymasters  
Any

Game log

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