HOST-PATHOGEN INTERACTIONS LAB
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TITLE: WESTERN BLOT ANALYSIS:

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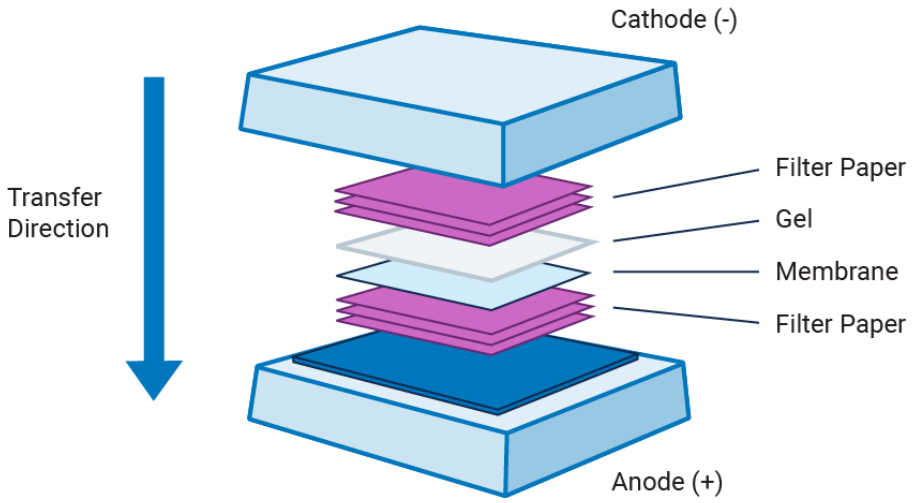
Name: Ipsita Nandi

1. Prepare the 500 ml 1X transfer buffer (300 ml DDW, 100 ml 10X transfer buffer stock solution, 100 ml methanol)
2. Soak a sandwich of blotting paper and nitrocellulose membrane (3 blotting paper on top of its nitrocellulose membrane and on top of it three blotting paper) for 10 mins.
3. After the gel running stack the gel and the membrane on the transfer cassette as:



4. The gel is transferred to nitrocellulose membrane either by Biorad system (2.5A, 25V, 10min)/ Hoefer system (15V, 45min)
5. The membrane is then washed with TBST for 5 mins in shaking for one time.
6. The membrane is blocked using TBST+ 1% skimmed+ 2.5% BSA for 1 hour at 22 ºC (For probing phosphorylated proteins the blocking is to be done using TBST+ 5% BSA) with shaking.
7. Then the membrane is washed with TBST- 3 X 5 mins each with shaking

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8. Then the membrane is probed with primary antibody with specific dilution (dilution is made in TBST+ 5% BSA) @ 22 ºC for 1.5 hr/ 4 ^{0}C for O/N depending upon the primary antibody
9. The membrane is washed with TBST- 3 X 5 mins each with shaking.
10. The membrane is incubated with secondary antibody (anti mouse or anti rabbit) with dilution 1:10000 (1 ul in 10 ml) for 1 hr at 22 ºC
11. The membrane is washed with TBST- 3 X 5 mins each with shaking.
12. For developing the blot ECL substrate mixture is made using sol. A and B (1:1 V/V, kept at 4 ^{0}C) and vortexed the mixture, the blot was incubated for 2 mins with the mixture before developing in Gel doc (chemiluminescence) and save the pictures in folder (Documents- Aroeti-Your name)

10X transfer buffer: 500 ml	
Tris base	15 gr
Glycine	72 gr
Adjust pH-8.8 with HCl	

1X transfer buffer: 500 ml	
DDW	350 ml
10X transfer buffer	50 ml
Methanol	100 ml

TBST: 1000 ml

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DDW	960 ml
1M Tris-HCl (pH-8.0)	10 ml
5M NaCl	30 ml
20 % Tween-20	0.5 ml

Blocking Buffer: TBST + 1% skimmed milk + 2.5% BSA: 500 ml	
TBST	500 ml
Skimmed milk	5 gr
BSA	12.5 gr
Aliquot 10 ml in each 15 ml polypropylene tubes and store at -20 ^{o}C	

Antibody dilution Buffer: TBST + 5% BSA: 500 ml	
TBST	500 ml
BSA	25 gr
Aliquot 10 ml in each 15 ml polypropylene tubes and store at -20 ^{o}C	