E-Blotter operation

MATERIAL

- BSA (1 mg/ml; 0.05 g BSA (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.) dissolve in 50 ml ddH_2O, aliquot to 1 ml in eppendorf tubes and freeze in -20°C)
- NK92 cell lysate (Emo Biomedicine Corp.)
- Pre-stained marker (Bio-Rad, Hercules, CA, U.S.A.)
- ProMarker (Wealtec, Taipei, Taiwan)
- 12% SDS-PAGE resolving gel (0.75 mm); 2.31 ml ddH_2O, 2.8 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 1.75 ml 1.5 M Tris-Cl, pH 8.8 (Sigma), 70 µl 10 % SDS (Bio-Rad), 70 µl 10 % APS (Bio-Rad), 2.8 µl TEMED (Bio-Rad)
- 5 % SDS-PAGE stacking gel (0.75 mm); 1.7 ml ddH_2O, 0.415 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 0.315 ml 1 M Tris-Cl, pH 6.8 (Sigma), 25 µl 10 % SDS (Bio-Rad), 25 µl 10 % APS (Bio-Rad), 2.5 µl TEMED (Bio-Rad)
- 10X Tris-Glycine buffer (Sigma)
- V-GES Casting module (Wealtec)
- Alignment card (Wealtec)
- V-GES Electrode module and electrophoresis tank (Wealtec)
- Block Cooler (Wealtec)
- Block Heater (Wealtec)
- Glass plate separator (Wealtec)
- E-Blotter
- Coomassie Blue Staining Buffer (0.1 % Coomassie Blue R-250 (Bio-Rad) in Water/Methanol/Acetic acid (45:45:10))
- Destaining Buffer (Water/Methanol/Acetic acid (45:45:10))

PROCEDURE

- A 12% SDS-PAGE gel was prepared. 5 ml of the resolving gel solution and thereafter 1 ml EtOH was pipetted into the assembled casting module and the gel was polymerised for 60 minutes. By placing the pipette in the middle of the

glass plates between both wing releasers, and slowly emptying the contents of the tip, the build up of bubbles can be minimised. After polymerisation, the EtOH was removed and the stacking gel solution was prepared and poured on top of the polymerised resolving gel. A 0.75 mm 10 well comb was gently inserted into the space between the plates, ensuring no air bubbles were trapped. The stacking gel was left for polymerisation for 60 minutes.

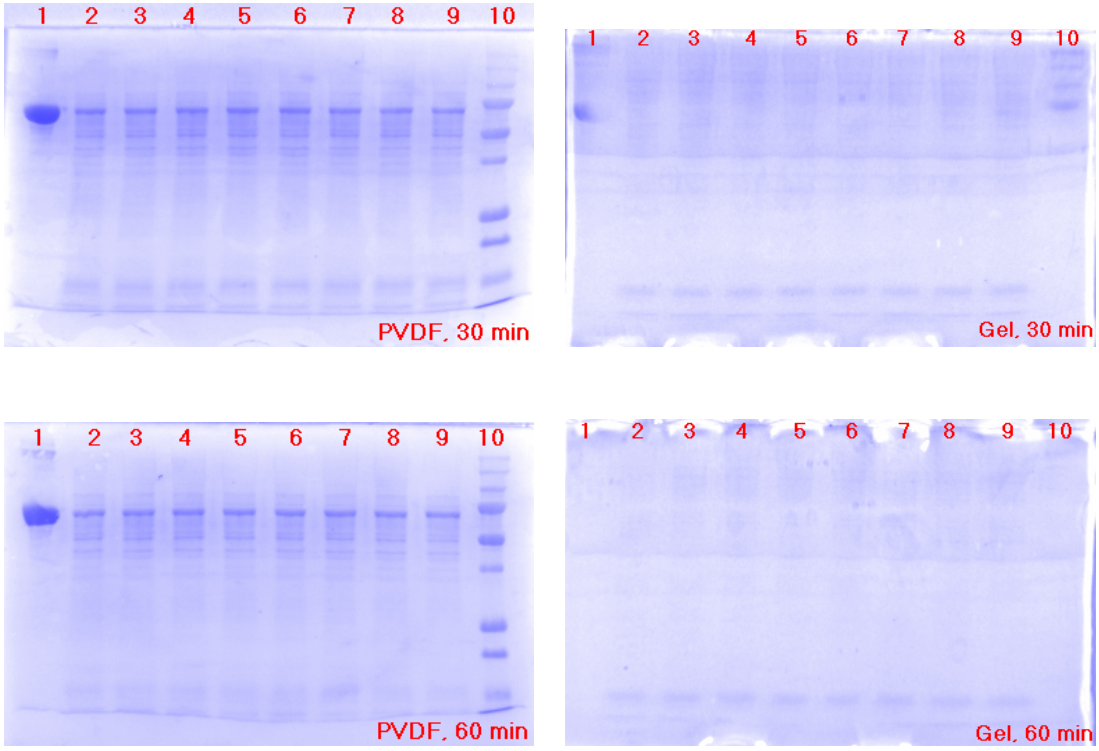
- The SDS-PAGE running buffer was prepared by diluting 100 ml 10 X buffer stock in 900 ml ddH_20. To the SDS-running buffer, SDS was added to a final concentration of 3.5 mM.
- The gel sandwich was released from the casting module by applying equal force to both wing-releasers and gently pulling upwards, and then pressing the back of the gel sandwich.
- Frozen BSA (1 mg/ml) and NK92 cell lysate were thawed by inserting a tube into the Block Cooler (4°C). Then 25 µl 4X protein loading dye was added to 75 µl thawed sample solution.
- The mixed BSA sample, the NK92 cell lysate, a Pre-stained marker, and ProMarker were all boiled for 5 minutes in 95°C on a block heater.
- Load 10 µl BSA sample, Pre-stained marker, ProMarker and 30 µl NK92 cell lysate sample per well.
- The lid was placed on top of the tank and the electrode wires were connected to the power supply.
- The electrophoresis was run as follows; 60 minutes at 90 V, and then 60 minutes at 130 V.
- After electrophoresis, the gel was removed from the glass plates by gently inserting a glass plate separator between the glass plates and gently wriggling it until the plates separate. The upper stacking gel was removed and the resulting resolving gel was put into the transfer buffer. The transfer buffer was prepared by diluting 100 ml 10 X buffer in 200 ml methanol and 700 ml ddH_20.
- Put the gels into the transfer buffer gently, Pre-wet the PVDF membrane by methanol and equilibrium by ddH_20. Rinse two filter paper and sponges for each gel by transfer buffer.
- Open the E-Blotter cassette, put one wet sponge on the black cassette and then one wet filter paper on the sponge, thereafter put the gel on the filter paper.
- Put the PVDF membrane onto the gel and remove the air bubbles gently. Cover

the filter paper and then the sponge. Put the red cassette and close the transfer sandwich.

- The electrophoresis was run as follows; 30 minutes at 100 V, and 60 minutes at 100 V.
- After electrophoresis, the gel after transfer and the PVDF membrane were stained by Commassie Blue for 15 min and then distained over night.
- Use 90% methanol and 10% acetic acid to wash the PVDF membrane to remove the background.
- Capture the gel and the PVDF membrane images by Dolphin-Doc+.

RESULTS

- Although the high molecular weight bands of the marker shows less transfer efficiency of 60 minutes transfer, the major bands are much clearer than the 30 minutes transfer.
- Because the volume for BSA, NK92 cell lysate and the prestained marker are not all the same, the width of different sample lane is not equivalent.



Lane 1, BSA; lane 2 to 9, NK92 cell lysate; lane 10, pre-stained marker (Bio-Rad)

REMARKS

- The height of the tank might need to increase since the buffer surface would be very closer to the edge of the tank while pouring the buffer to cover the E-Blotter module.
- The E-Blotter module needs to be soaked by transfer buffer in the tank to make a good cooling system.
- The E-Blotter has good transfer efficiency and less time consuming.

APPENDIX

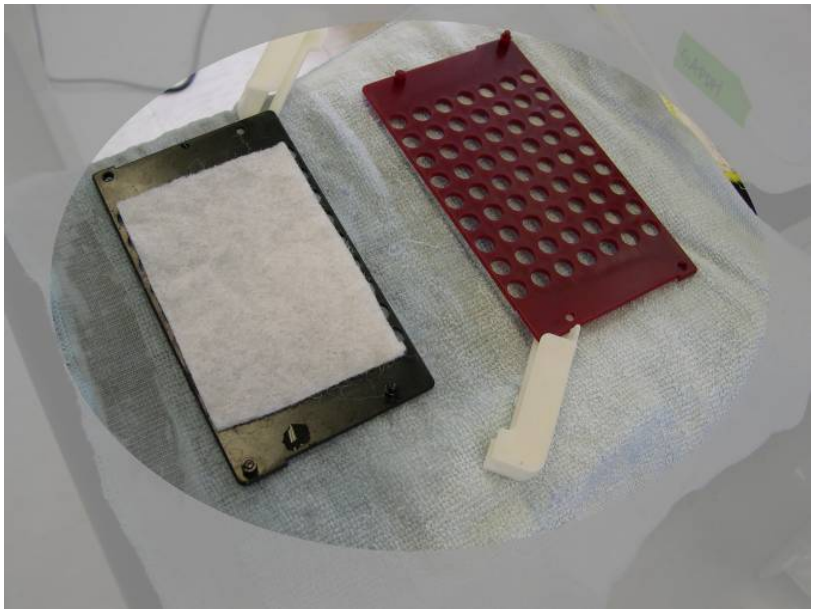


Figure 1: *Rinse two sponges by transfer buffer. Place one sponge on the black blotting cassette. The clamp of the cassette should be pulled and released out on both sides.*

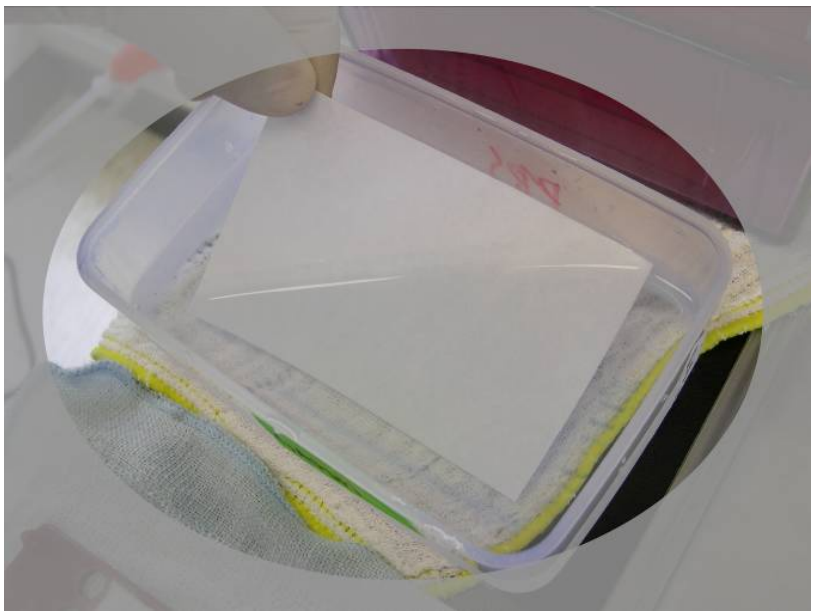


Figure 2: *Pre-wet the PVDF membrane by methanol and equilibrium by ddH_2O. Rinse two filter papers, the PVDF membrane and the gel for transfer by transfer buffer.*

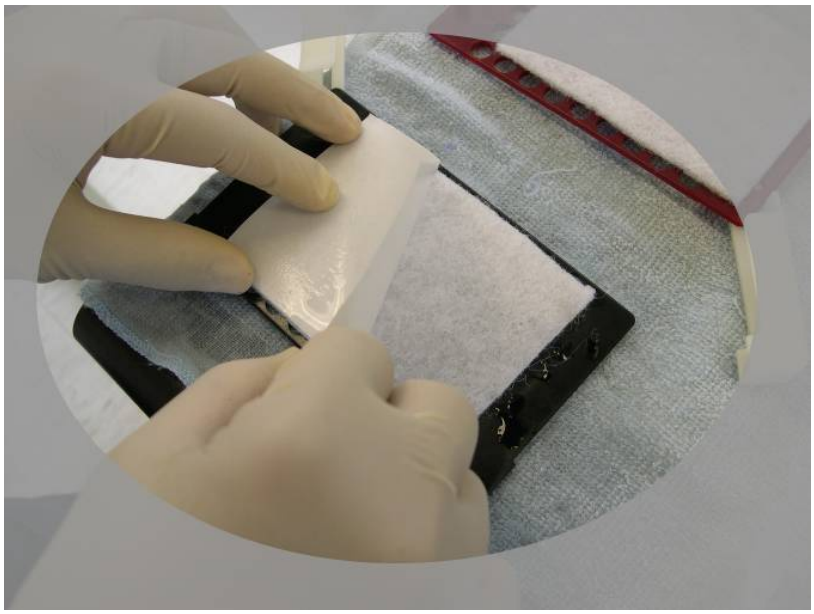


Figure 3: *Put one wet filter paper on the sponge.*

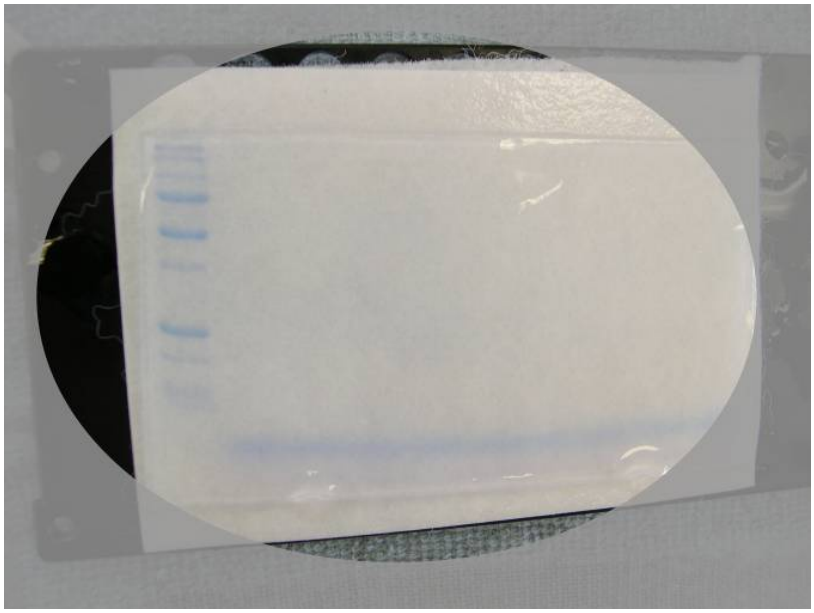


Figure 4: *Spread some transfer buffer on the filter paper. Gently put the gel on top of the filter paper. Do not tear the gel.*

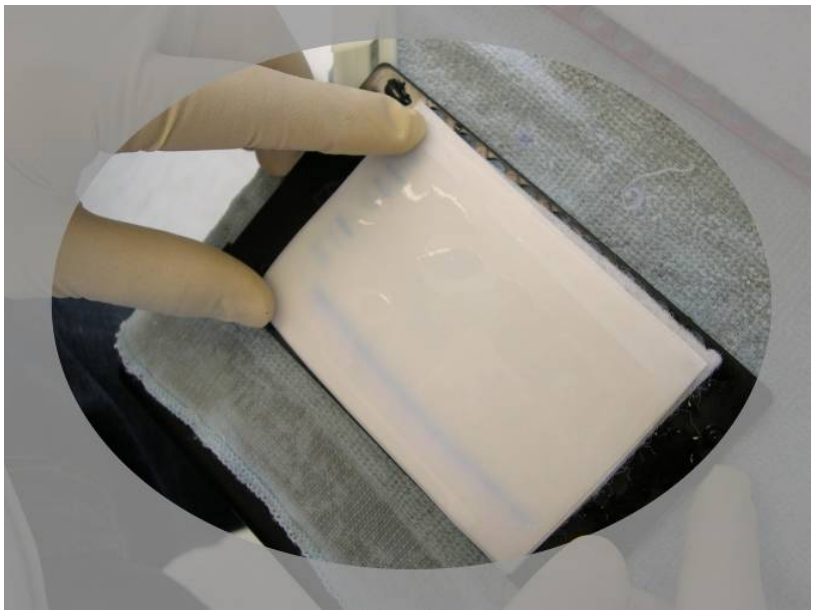


Figure 5: *Carefully put the PVDF membrane on the gel. Do not generate too many air bubbles.*

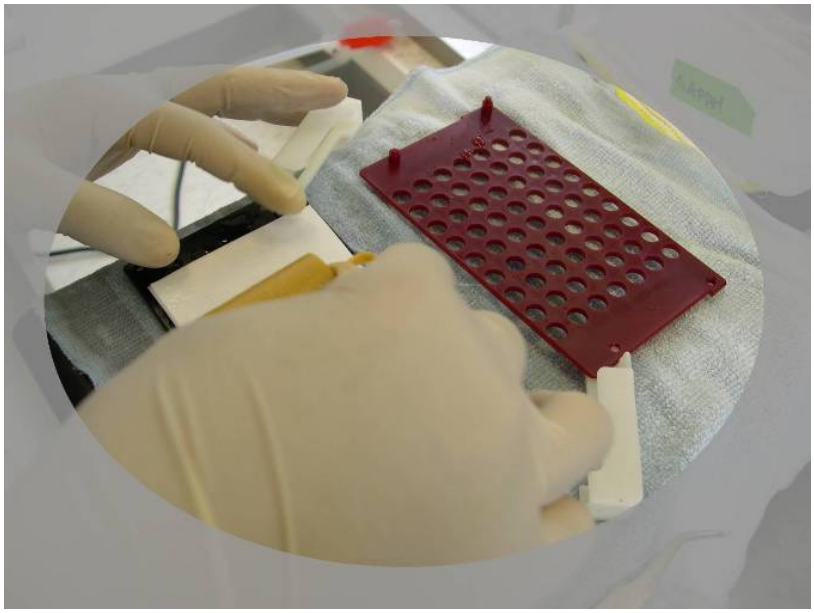


Figure 6: *Slightly remove the air bubbles by the roller.*

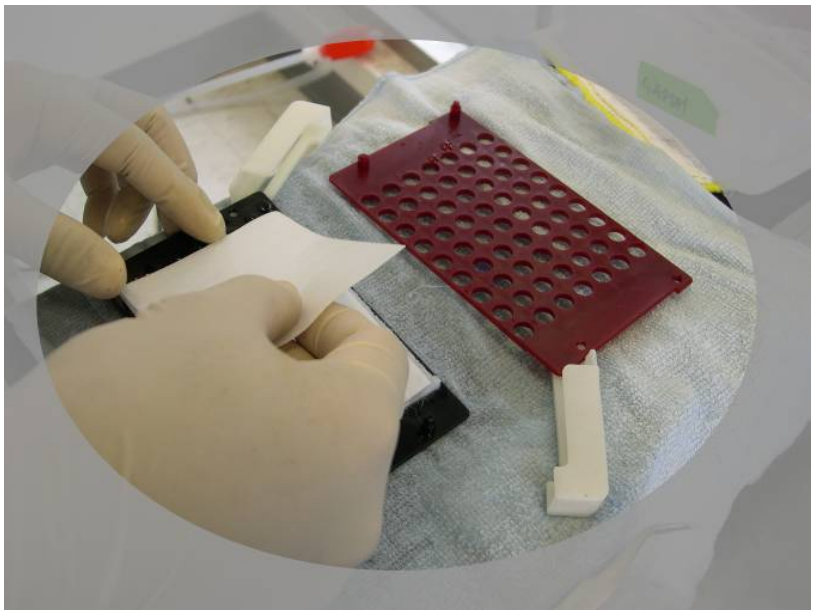


Figure 7: *Put the other wet filter paper on top on the PVDF membrane. Use the roller if there is any air bubble.*

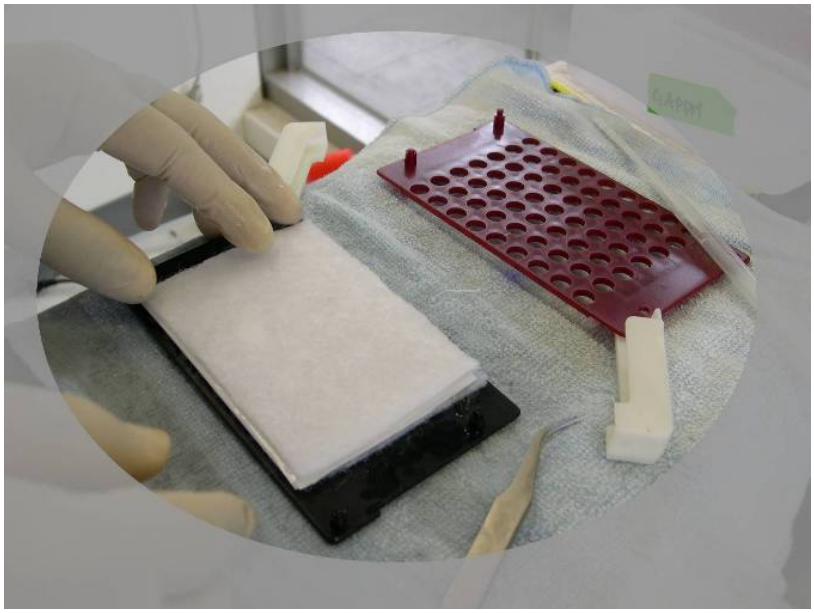


Figure 8: *Put the other wet sponge. All the components have to be placed centre within the two sponges. This assembly is also called "sandwich".*

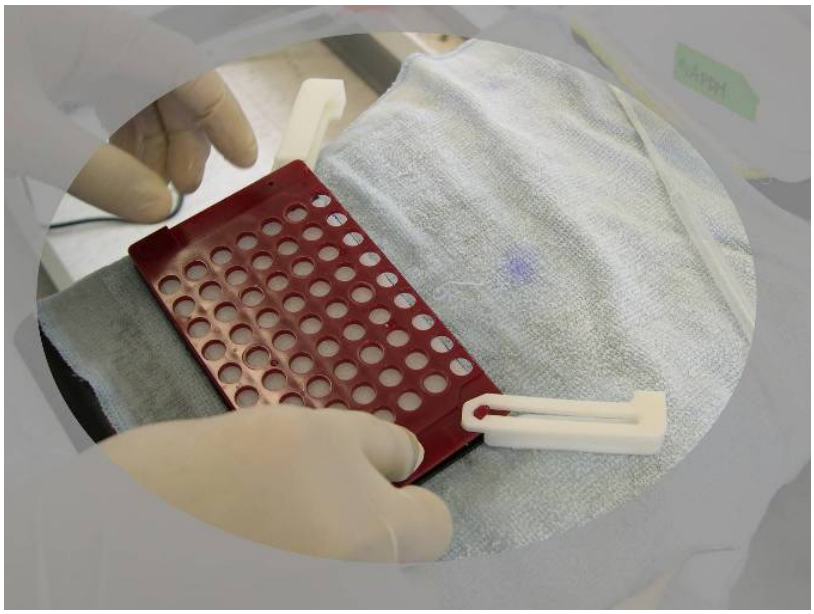


Figure 9: *Place the red blotting cassette on the sponge. Make sure the tenon of the red blotting cassette correctly placed in the corresponding hole of the black blotting cassette.*

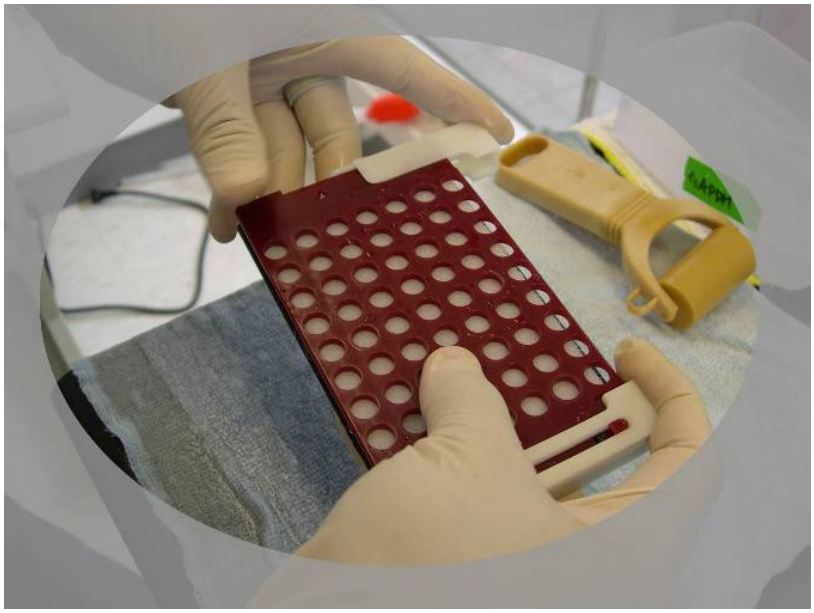


Figure 10: *Push and lock the clamp on both sides.*

Figure 11: *Gently place the blotter cassette assembly in the E-Blotter cassette holder. The red blotting cassette should face to the red electrode plate of the holder.*

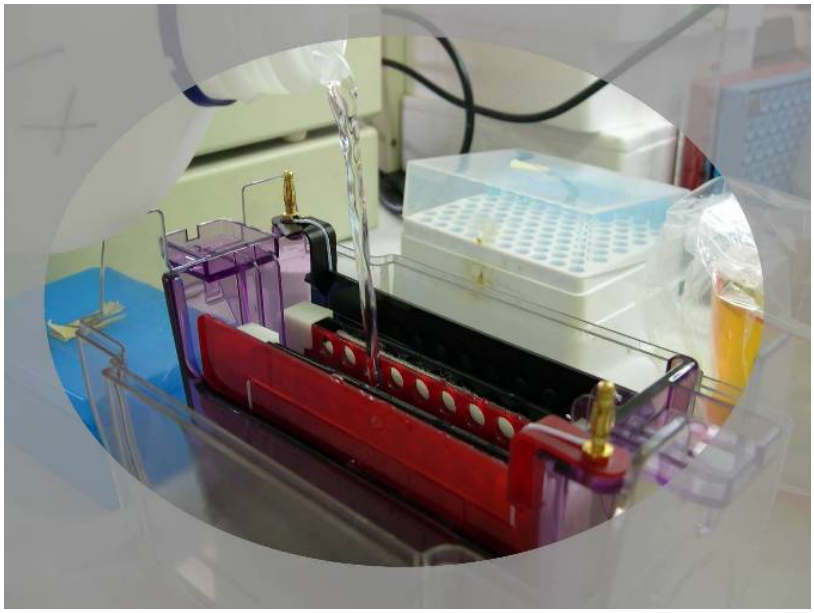


Figure 12: *Pour the transfer buffer and fill the tank until the buffer covers the entire blotter cassette assembly.*

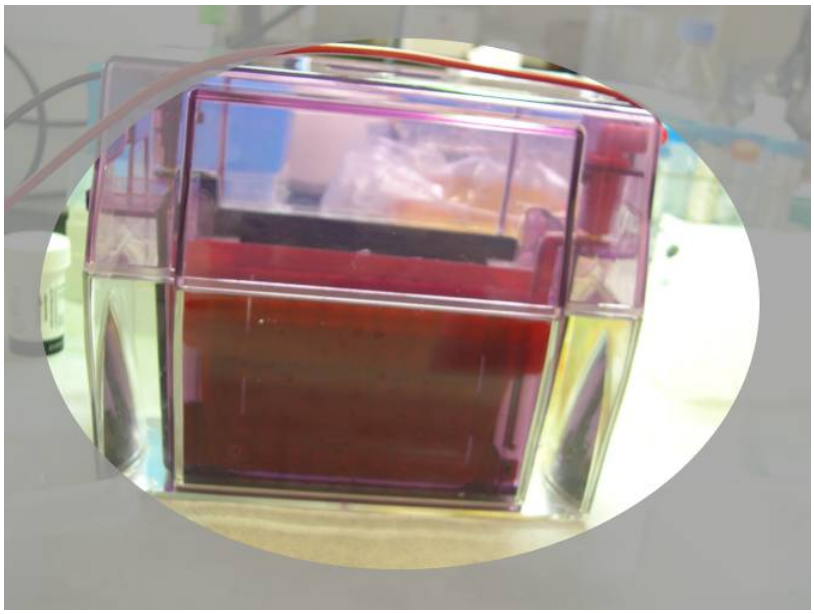


Figure 13: *Close the upper lid and connect the E-Blotter system to the power supply. Switch on the power supply and start to run.*

Note: *All the information can be found in E-Blotter manual.*

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