The optimal condition of Yrdimes semi-dry blotter

MATERIAL

- 10X Tris-Glycine buffer (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.)
- SDS (Bio-Rad, Hercules, CA, U.S.A.)
- PVDF membrane (Millipore, Billerica, MA, U.S.A)
- Filter paper (Amersham Biosciences Corp, Piscataway, NJ; U.S.A.)
- BSA (Sigma-Aldrich, 1 mg/ml diluted in PBS).
- 4X sample loading dye (;0.25M Tris-HCl, pH6.8, 8% SDS, 10% 2-ME, 0.3M DTT, 30% Glycerol, 0.02% Bromphenol blue (Bio-Rad))
- Prestained Marker (Bio-Rad)
- Promarker (Wealtec, Taipei, Taiwan)
- 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))
- 12% resolving SDS-PAGE (0.75mm); 2.31ml ddH_2O, 2.8ml 30% Acrylamide/Bis (29:1) (Bio-Rad), 1.75ml 1.5M Tris-Cl, pH8.8 (Sigma), 70ul 10% SDS(Bio-Rad), 70ul 10% APS (Bio-Rad), 2.8ul TEMED (Bio-Rad)
- 5% stacking gel (0.75mm); 1.7ml ddH_2O, 0.415ml 30% Acrylamide/Bis (29:1) (Bio-Rad), 0.315ml 1M Tris-Cl, pH6.8 (Sigma), 25ul 10% SDS (Bio-Rad), 25ul 10% APS (Bio-Rad), 2.5ul TEMED (Bio-Rad)
- V-GES casting module (Wealtec)
- V-GES electrode module and electrophoresis tank (Wealtec)
- Thick glass plate, U-shaped glass plate, 0.75 mm spacers, 0.75 mm 10 teeth comb (Wealtec)
- Block heater (Wealtec)
- Yrdimes Semi-dry blotter (Wealtec)
- Dolphin-Doc Plus Image system (Wealtec)
- UV/White light converter plate (Wealtec)

- ELITE 300 plus power supply (Wealtec)

PROCEDURE

- SDS-Running buffer was prepared by diluting 100 ml 10 x buffer stock in 900 ml ddH_20. The transfer buffer was prepared by diluting 100 ml stock in 700 ml ddH_20 and 200 ml methanol. To the SDS-Running buffer, SDS was added to a final concentration of 3.5 mM.
- 6 pieces of filter paper and PVDF membranes were cut to 6 cm x 10 cm.
- Sample preparation; 25 µl 4x loading dye was added to 75 µl 1mg/ml BSA in PBS.
- The electrophoresis glass plate sandwich was assembled in the V-GES casting module and the gel solution 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. Thereafter 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 gel sandwich was inserted into the electrode module mounted inside the vertical electrophoresis tank. A one gel gate was inserted into the empty side of the module. SDS-PAGE running buffer was poured into the space between the gel sandwich and the one-gel gate up until approximately 1 cm from the upper edge of the glass plates. The assembly was checked for leakage, and when no leakage could be detected, 1 l SDS-PAGE running buffer was poured into the tank surrounding the gel assembly.
- Protein samples to be loaded onto the gel were initially boiled at 95°C for 5 minutes using a block heater. Thereafter the comb was removed and a pre-stained protein marker and an unstained protein marker were loaded at both outer wells of the gel. BSA samples stained with loading dye were loaded onto the 8 inner wells. The lid was placed on top of the tank and the electrode was connected to the power supply.
- The electrophoresis was run under conditions as follows; 60 minutes at 90 V, and then 70 minutes at 130 V.

- Gel transfer: (*for figures, see appendix*)
  After electrophoresis, the gel was removed from the glass plate sandwich and left to equilibrate in transfer buffer while the semidry blotter was assembled. Pre-cut PVDF membranes were wet in methanol for 15 seconds and then transferred to dd$H_2$0. Thereafter they were left to equilibrate in transfer buffer. 6 pieces if filter paper were soaked in transfer buffer. The transfer sandwich was assembled as follows; 3 pieces of filter papers were put on the bottom cathode plate (*fig 2*). Thereafter the PVDF membrane (*fig 3*) and the gel (*fig 4*). 3 pieces of filter papers (*fig 5*) were put on top of the gel. Extra transfer buffer was poured on top of the sandwich and then a spacer was swiftly drawn across the upper filter paper in order to get rid of any trapped air bubbles (*fig 5*). Thereafter the upper anode plate was gently placed on top of the transfer sandwich (*fig 6*) and the assembly was securely locked by applying equal force onto both sides of the lid of the semidry blotter and pressing together (*fig 7*); making sure the locking mechanism was joined correctly (*fig 8*). The electrode wires were connected to the power supply (*fig 9*) and constant current was chosen. The current was set to 150mA (2.5mA/cm^2) and the time was set to 60 minutes.
- After transfer, the transfer sandwich was disassembled and both gel and membrane were soaked in coomassie blue staining buffer for at least 10 minutes. Thereafter, the gel and the membrane were destained in destaining buffer with agitation over night.
- After destaining, the gel and the membrane were photographed in Dophin-Doc plus image system using a UV/white light converter plate and epi-illumination.
- After transfer, the transfer sandwich was disassembled and both gel and membrane were soaked in coomasie blue staining buffer for at least 10 minutes. Thereafter, the gel and the membrane were destained in destaining buffer with agitation over night.
- After destaining, the gel and the membrane were photographed in Dophin-Doc plus image system using a white light converter plate and epi-illumination.

RESULTS

- When comparing the gel and PVDF membrane (*fig 1 B and A)*); 2.5mA/cm^2 for 60 minutes results in a transfer efficiency of 90% to 95%. Almost 100% of the prestained marker is transferred onto the membrane. Some BSA and unstained marker remain in the gel.
- The transfer of small molecular weight proteins is more efficient than large molecular weight proteins.
- Even though an apparent large amount of BSA is transferred on to the membrane, some BSA appears to be left in the gel after transfer. This is likely due to saturation of the membrane since a very high concentration of BSA is used.
- The "sandwich" complex and the electrode plates are a slightly hot after transfer
- For results, see appendix

REMARKS

- 2mA/cm^2 results in a good transfer efficiency of proteins. Increasing the current further up to 2.5mA/cm^2 increases the transfer efficiency slightly, however the maximum voltage allowed by the Yrdimes semidry module is 25V, and a current setting of 2.5mA results in voltages reaching values very close to 25V.
- Based on previous blotting experiments, the current vs voltage changes recorded during semidry blotting transfer using Yrdimes are; 90V: 33mA down to 19mA, 130V: 29mA down to 21mA, 150mA: 12V up to 24V.
- According to estimations based on previous data, the transfer efficiency reaches 85% of 2mA/cm^2 for 1hr and up to 90% when using 2mA/cm^2 for 1.5hr
- Extended time or current would both increase the transfer efficiency; however, the voltage should not get higher than 25V. Care must be taken when setting time and current, not to exceed the maximum allowed settings of the module.
- Nitrocellulose (NC) membranes can also be successfully used with Yrdimes as a transfer membrane with transfer efficiencies similar to PVDF, however since the CBB staining result of NC is too poor to compare with the gel, those results are not included here

- The signal on coomassie blue stained PVDF membranes seems to decrease with time, it might be a good idea to document results prior to drying the gel and the PVDF membrane
- This protocol is valid for creating a transfer efficiency of around 90-95 % based on the proteins and marker used here. In order to optimise transfer of specific proteins, the protocol might need modifications. In general small proteins require less transfer time and current than large proteins. Proteins of extreme pH values or charges might need longer or shorter transfer times and buffer-modifications in terms of pH and SDS-contents.
- The signal on coomassie blue stained PVDF membranes seems to decrease with time, it might be a good idea to document results prior to drying the gel and the PVDF membrane.

APPENDIX

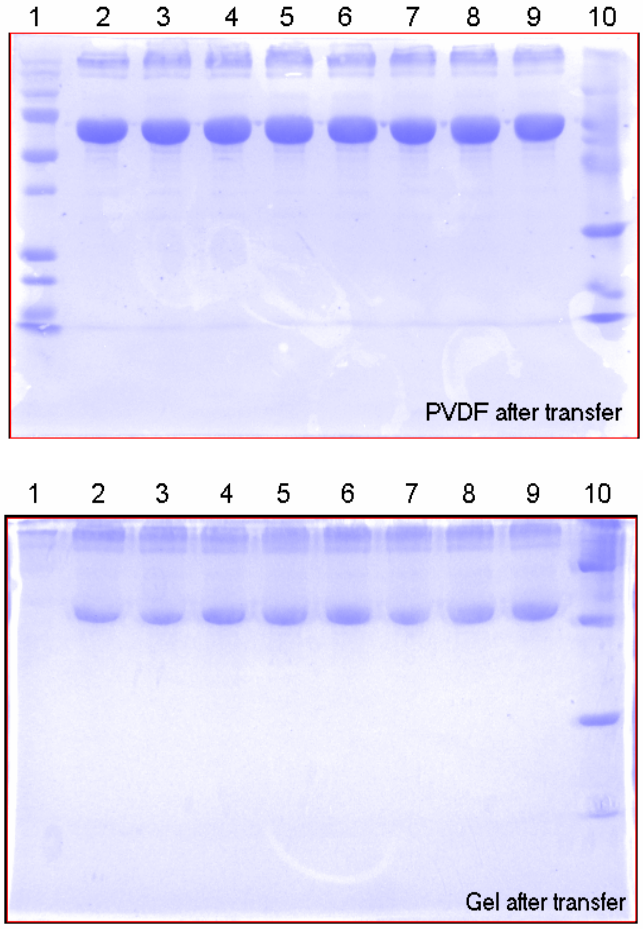

Figure 1(A and B). *Blotting membrane (A) and gel (B) after semidry protein transfer. Lane1: Prestained Marker (Bio-Rad), Lane2-9: BSA (1mg/ml), Lane10: Promarker (Wealtec)*

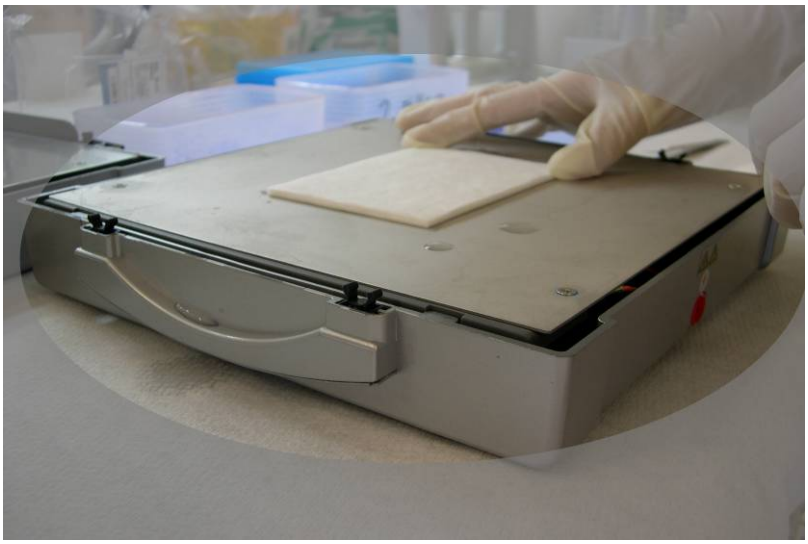

Figure 2: *Place 3 filter papers soaked in buffer on the bottom plate (cathode).*

Figure 3: *Position the wet membrane on top of the filters.*

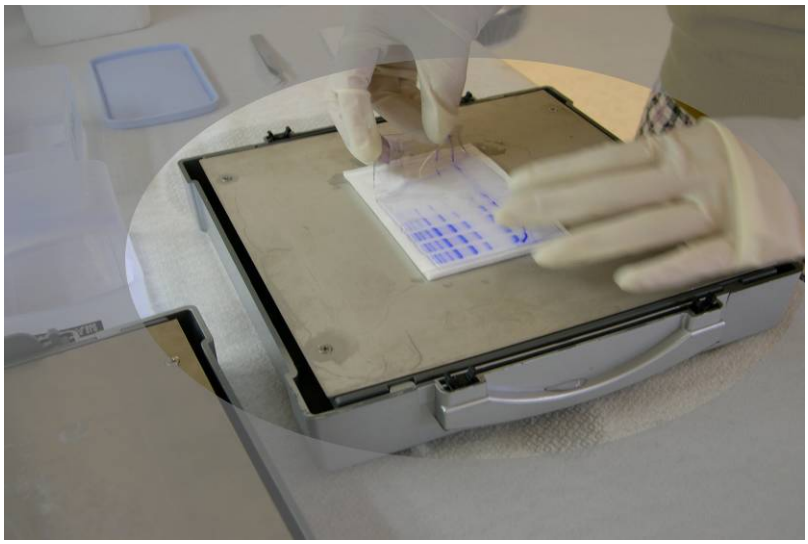

Figure 4: *Place the gel on top of the membrane, make sure no air bubbles are trapped and that the gel is flat against the membrane.*

Figure 5: *Place an additional 3 soaked filter papers on top of the gel and swiftly drag a spacer across in order to get rid of trapped air.*

Figure 6: *Place the top anode plate on top of the gel sandwich, making sure the locking mechanism is correctly positioned.*

Figure 7: *With both hands, apply equal force to both sides of the blotter and press together (Same procedure as for unlocking after transfer).*

Figure 8: *When the plates are correctly adjoined, the gap between the plates is equal all around the blotter. The adjoining points are not visible.*

Figure 9: *Connect the electrode wires to the power supply and start transfer.*

Joy Lee
Product Specialist
Email: info@wealtec.com

Wealtec BioScience Co. Ltd.
27Fl. No. 29-1 Sec.2, Jungjeng E. Rd., Danhuei Jen, Taipei, Taiwan 25170
TEL: +886-2-8809-8587 FAX: +886-2-8809-8589; http://www.wealtec.com