Lazzara Lab
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Trans-Blot Turbo Transfer System Protocol

1. Prepare 1L of 1X transfer buffer by mixing 200 mL of 5X BioRad TransBlot Turbo transfer buffer with 600 mL of MilliQ water and 200 mL of ethanol (reagent or molecular biology grade; what we use for preparing 70% EtOH for cell culture is fine).

2. Put 30 mL 1X transfer buffer in a bin large enough to accommodate the stacks and gels. Equilibrate two transfer stacks (separated by blue sheets) and one nitrocellulose membrane by submerging completely in the transfer buffer for 2-3 min. Handle the membrane carefully and at the edges as much as possible to avoid marring the surface to which proteins will be transferred.

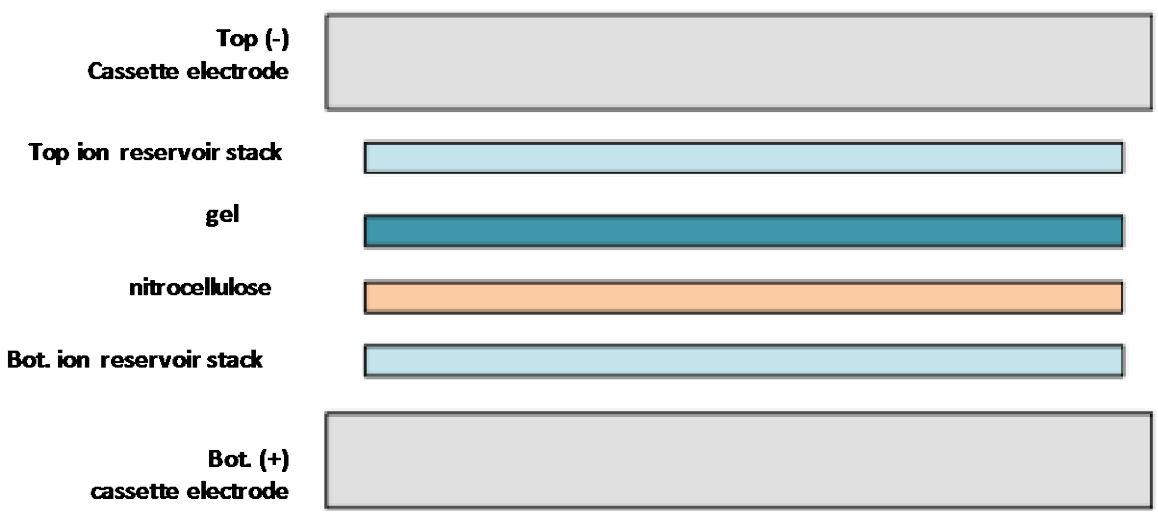
3. Place one wetted stack on bottom of cassette this will serve as bottom of ion reservoir stack.

4. Place wetted membrane on top of wetted stack in the cassette.

5. Place gel on membrane. Do not equilibrate the gel before transfer. If needed, remove any air bubbles with blot roller.

6. Place second wetted transfer stack on top of gel. This will serve as the top ion reservoir stack.

7. Roll the assembled sandwich with the blot roller to expel trapped air bubbles. Please refrain from adding any extra transfer buffer to the cassette. Saturated transfer stacks provide ample transfer buffer.



8. Close and lock cassette lid by turning the green dial to the locked position. Insert the cassette in the instrument.

9. Set transfer conditions -- LIST>USER DEFINED>EDIT:

    a. 1 x 1.5mm gel = 1.3A, 25V, 15min
    b. 2 x 1.5 mm gels = 2.5A, 25V, 15 min

10. Run protocol – RUN.

11. After run is complete and stacks are removed, rinse trays thoroughly with DI water and leave to dry on bench.