Western blotting.

1. Cut a piece of PVDF membrane approximate to the size of the gel.

2. Soak them about 10 min in methanol at room temperature.

3. Soak the sponges and filter paper in the transfer buffer in a separate tray.

4. Once the gel run is over take the gel from PAGE set up carefully.

5. Assemble the blot in the following order Sponge- filter paper- gel- membrane- filter paper- sponge.

6. While assembling the membrane should be placed in transparent side and gel in black side

7. Transfer for 1 hr at 80 Volts in a tank. Place the cold pack inside the buffer tank. By using magnetic stirrer the buffer should be mixed. Change the ice pack after half an hour.

7. After one hour take out the set up and take the membrane.

8. Incubate the membrane in blocking buffer (5% not fat dry powdered milk in TBS) for one hour at room temperature or $4^{o}C$ at overnight.

9. Wash with 3 times 10 min with TBST (0.05% in TBS).

10. Incubate with primary antibody diluted in PBS for one hour at room temperature or $4^{o}C$ overnight.

11. Wash with 3 times 10 min with TBST

12. Incubate with secondary antibody diluted in PBS for one hour at room temp.

13. Wash with 3 times 10 min with TBST
14. Develop the blot using chemiluminescence (ECL) substrates.