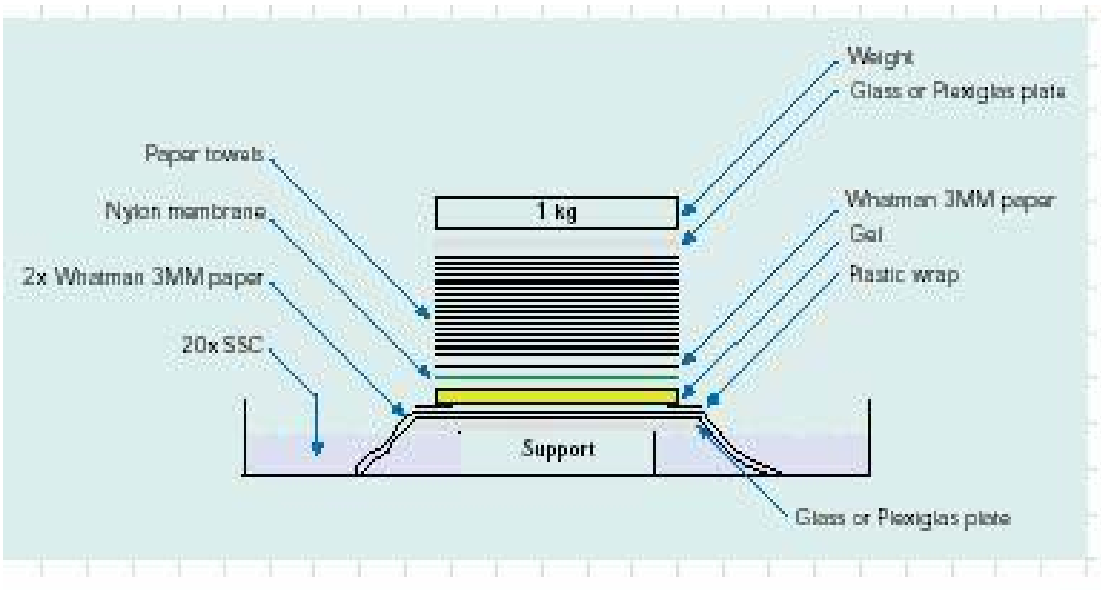
Blotting Protocol

1] Soak gel in 0.5M NaOH for 30min on rocker
2] Soak gel in 10X SSC for 15min on rocker
3] Cut one sheet of nylon membrane and two sheets of Whatmann 3MM paper to the size of the gel
4] Cut two lengths of whatmann paper wider than the gel and long enough to fit from end to end in transfer buffer tray
5] Wet the nylon membrane in water
6] Soak the nylon membrane and whatmann paper in 20X SSC for 1-2min
7] Set up the capillary transfer

-fill large pyrex dish (cleaned with RNAseZap) with 20X SSC
-place a support in the middle
-place the two long whatmann wicks over the support and remove air bubbles
-place gel upside down on whatmann wick
-place plastic wrap around the edges of gel to prevent short circuits
-place presoaked nylon membrane over gel and remove air bubbles
-place the two presoaked sheets of whatmann over nylon membrane and remove air bubbles
-place a stack of 15-20cm of dry/cut to gel size paper towels
-place a piece of glass on top of paper towels
-place 1kg weight on top of glass
-let proceed for 12-18hr, replacing the wet paper towels at least once

8] Disassemble the capillary transfer set up
9] Carefully peel away the gel from the membrane
10] Mark the lanes with a blunt ended pencil
11] Check transfer on uv box and mark in pencil the ladder positions
12] Soak membrane in 3X SSC for 10min
13] Autocrosslink with stratalinker in Proctor Lab
14] Place in seal a meal bag if not using right away and store at 4C


Weight
Glass or Plexiglas plate
Paper towels
Nylon membrane
2x Whatman 3MM paper
20x SSC
1 kg
Whatman 3MM paper
Gel
Plastic wrap
Support
Glass or Plexiglas plate