Procedure for Electroblotting onto ProBlott Membranes for Sequencing

**Problott Membrane:** Applied Biosystems:
- 20 cm x 20 cm 20 / pack  P/N 400994
- 10 cm x 10 cm 10 / pack  P/N 401194

**Electroblotting**

- **Stock Buffer:** CAPS 3-[cyclohexylamino]-1-propanesulfonic acid buffer
  - 10X CAPS (100mM, pH 11): Dissolve 22.13 g of CAPS in 900 mL of D.I. water. Titrate with 2N NaOH (approximately 20 mL) to pH 11, and add D.I. water to a final volume of 1 liter. Store at 4 degrees C.

- **Electroblotting buffer (1X stock buffer in 10% MeOH):**
  - Prepare 2 liters of buffer by mixing 200 mL of Stock 10X CAPS buffer with 1.6 liter D.I. water then with 200 mL of methanol.

1. Wet ProBlott membrane with methanol for a few seconds, and place the membrane (the size of a gel) in a dish containing blotting buffer.
2. Remove the gel from the electrophoresis cell and soak it in electroblotting buffer for 5 minutes.
3. Assemble the transblotting sandwich, and electroblot at constant voltage of 50 volts (170 mA-100 mA), at room temperature for 30 minutes.
4. Remove ProBlott membrane from transblotting sandwich and rinse with D.I. water prior to staining.

**Protein Detection**

Protein samples blotted onto ProBlott membrane can be detected with conventional staining techniques using Coomassie brilliant blue or Ponceau S.

**Procedure for Coomassie Blue Staining**

1. Remove ProBlott membrane from the transblotting sandwich and rinse with D.I. water.
2. Saturate the ProBlott membrane with 100% MeOH for a few seconds.
   - Stain the ProBlott membrane with 0.1% Coomassie Blue R-250 in 40% MeOH/ 1% acetic acid (protein bands should appear within one minute.
   - Note: Staining for more than 1 minute may result in longer destaining times)
3. Remove the ProBlott membrane from staining solution and destain with 50% MeOH until the bands are visible but background staining has diminished.
4. Rinse extensively with D.I. water.
5. Excise the bands of interest.

**Procedure for Ponceau S Staining**

1. Remove ProBlott membrane from the transblotting sandwich. Rinse in DI water.
2. Stain ProBlott membrane in 0.2% Ponceau S in 1% acetic acid with gentle swirls. Protein bands should appear within one minute.
3. Destain with simple rinse of D.I. water.
4. Excise the bands of interest.