Center for Workshops in the Chemical Sciences and the RCMI Clinical Proteomics Core Facility

Fundamentals of Proteomics Workshop
University of Puerto Rico Medical Sciences Campus
June 21- 26, 2009

2D-DIGE LABORATORY EXERCISE

DAY 3

Equilibration of focused Immobiline DryStrips for ANALYTICAL GEL

1. Remove focused Immobiline DryStrips from the first dimension apparatus or if the strips have been frozen, allow them to warm to room temperature.

2. Incubate each strip in 10 mL equilibration solution containing DTT for 15 min with gentle agitation (orbital shaker).

Second dimension for ANALYTICAL GEL

1. Heat up agarose solution 90 °C in waterbath.

2. Prepare 1X SDS and 2X SDS and the Ettan Dalt Twelve for loading.

    - Position the Dalt Twelve drain valve to circulate and add ~ 7.5L of 1xSDS to lower tank.
    - Start the pump and decrease the temperature to 20°C.

3. Briefly rinse the Immobiline DryStrip by submerging in a measuring cylinder of 1x SDS electrophoresis running buffer.

4. Place the gel on the rack with the tall glass on the back and the short glass facing you.

5. With forceps, carefully place the Immobiline DryStrip between the two glass plates of the gel. Lay the back of the strip against the taller glass side and then gently push down using the thin ruler. By convention, the acidic (+) end of the Immobiline DryStrip is on the left.

6. Gently position the strip so that it rests on the surface of the polyacrylamide gel. Avoid trapping air bubbles between the strip and gel. Handle the strips carefully to avoid damage of the first and second dimension gels.

7. Slowly pipette the molten agarose sealing solution between the glass plates at the top of the second dimension gel; take care not to introduce bubbles.

8. Allow the agarose to cool and solidify.

9. Load the gel plates into the Ettan Dalt Twelve electrophoresis tank filled with 1x SDS electrophoresis running buffer in the lower tank.

10. Add 2x SDS electrophoresis buffer in the upper tank.

11. Set the circulator to 20 °C and cover the tank with the black box to prevent light exposure.

12. Run the gels at constant current of 12 mAmps/gel overnight. In the morning if the bromophenol blue has not reached the end the current can be turned up to 20-30 mAmps/gel.