Isoelectric focusing for 11cm strips (Criterion Gels)

Materials:

- 11 cm IPG strips (Amersham-Biosciences’ Immobiline DryStrip or Bio-Rad’s ReadyStrip IPG Strip) with desired pH range
- IPG buffer with matching pH range (Amersham-Biosciences)
- DeStreak Rehydration Buffer (Amersham-Biosciences)
- Paper wicks (Bio-Rad)
- Mineral oil or DryStrip Cover Fluid (Amersham-Biosciences)

Method:

1. Prepare each sample so that the final volume of sample plus rehydration buffer is 200 µl. Add 1 µl IPG buffer to give a final concentration of 0.5%. The amount of protein in the sample is generally 150-200 µg. Mix and spin in a microcentrifuge at 2000 RPM for 2 minutes (if necessary to break any bubbles)

2. Place paper wicks over each electrode of the ceramic strip holders (Amersham-Biosciences). Wet each wick with 8 µl of Milli-Q H₂O just prior to adding the sample/rehydration buffer mix. Pipet the sample/rehydration buffer mix between the electrodes of the strip holder being careful not to introduce bubbles.

3. Peel the protective plastic from the IPG strip. The positive end of the IPG strip will be placed in the pointed end of the ceramic strip holder. While holding each end of the strip with forceps and the gel surface facing down, bend the strip so the center curves down and begin placing it in the sample. As one lowers the ends of the strip, the sample generally flows across the length of the strip holder. It may be necessary to raise and lower the end of the strip to have the sample flow all the way across and to remove air bubbles. If small bubbles have been introduced, one can usually remove them by gently tapping on the strip with forceps.

4. Cover strips with mineral oil and place cover on ceramic strip holder. Place strip holders in IPGhor focusing instrument (Amersham-Biosciences) and focus as follows:
   - 50 V for 11 hrs (active rehydration)
   - 250 V gradient 1 hr
   - 500 V gradient 1 hr
   - 1,000 V gradient 1 hr
   - 8,000 V gradient 2 hr
   - 8,000 V 48,000 V/hr

5. Remove IPG strips and carefully blot strip with damp filter paper to remove oil. Freeze strips at -80° C if 2nd dimension is not immediately performed.

http://bioinfo.utmb.edu/proteomics/NHLBI/
http://www.utmb.edu/brf v1.0