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# Nevada Proteomics Center

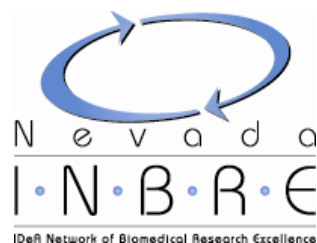
## 2-D Gel Materials and Methods

**Materials** All reagents and solutions for 2-D electrophoresis and protein assays, IPG strips, and SDS polyacrylamide gels were obtained from Bio-Rad (Hercules, CA) except as noted.

**Two-dimensional electrophoresis** 195 ul of each extract was loaded onto a 3-10L 11 cm IPG strip by overnight passive rehydration. Isoelectric focusing was carried out on a Bio-Rad Protean IEF cell using a program as follows: 250 V, linear ramp for 20 minutes; 8000 V, linear ramp for 2 hours 30 minutes; and 8000 V for a total of 20,000 Vhr (all steps with a maximum current of 50 uA per gel). Strips were stored at  $-80^{\circ}\text{C}$ , then thawed and incubated twice for 10 minutes each in 8M urea, 2% SDS, 0.05 M TrisHCl, pH 8.8, 20% glycerol. The first incubation contained 2% DTT and the second contained 2.5% iodoacetamide. The strips were then layered on 4-20% Criterion Tris HCl gradient gels and embedded in place with 0.5% agarose, along with Invitrogen BenchMark Protein Ladder molecular weight markers. Electrophoresis was performed at a constant current of 200 mA until the dye front ran off the gel. Gels stained overnight with either Bio-Safe Coomassie or Sypro Ruby stain.

**Imaging and comparison of protein spots** Stained gels were imaged on a Bio-Rad VersaDoc imager. Images of gels were compared using Bio-Rad PDQuest version 8.0.1 software and spot sets were created. The spots in these sets were excised from gels using a Bio-Rad EXQuest Spot Cutter. Gel spots were then trypsin digested on the Investigator ProPrep Digestion and Mass Spec Preparation Station (Genomics Solutions, Ann Arbor, MI) and spotted onto ABI MALDI plates in alpha-cyanohydroxycinnamic acid.

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