Directions for in-gel tryptic digestions of coomassie-stained 1D Bands and 2D Spots.

NOTE: Although nearly any SDS-PAGE system can be utilized upstream of an LC-MS analysis, the DPCF recommends Invitrogen's NuPAGE Bis-Tris mini-gel system. A good general purpose gel covering a large MW range (6-200 kDa) is the 4-12% gradient gel (Cat. No NP0321) using MES running buffer. These pre-cast gels provide excellent resolution, fantastic staining sensitivity (10 ng BSA using colloidal blue staining kit, Cat. No. LC6025), and are highly compatible with downstream LC-MS analysis. Additional product information can be found at: http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Protein-Expression-and-Analysis/Protein-Gel-Electrophoresis/1D-Electrophoresis/NuPAGE.html.

A couple of very important things to avoid keratin contamination:

1. Any gel manipulation prior to trypsin digestion should be done in a BSC or laminar flow hood.
2. Wear nitrile (not latex) gloves.
3. Wear a lab coat and make sure there is no gap between your coat sleeve and the gloves (lab tape works well).

Procedure:

1. After aspirating off liquid that sample is stored in, cut into equal size pieces using either a scalpel (cutting plug on the side of the Epi tube) or pinch it between the tongs of small tweezers to break it apart.

2. Wash gel pieces with 200-500 µL (depending on the volume of the gel pieces) of a mix of 40% acetonitrile and 60% 50 mM ammonium bicarbonate ("AmBic"), pH 8, for 15-30 minutes on rotator. Repeat with a second or third wash if necessary to remove stain.

3. Discard washes and shrink pieces with neat acetonitrile (pieces will turn white and stick together). Discard acetonitrile and dry pieces with tubes open at 50°C.

4. Swell gel pieces in 50 mM AmBic containing 10 ng/uL trypsin. Use Promega sequencing grade modified trypsin (porcine). Prepare stock from 20 µg vial at 0.1µg/µL by diluting with 50 mM AmBic. This can be stored at -20°C for 6 months. Dilute this stock 1:10 with 50 mM AmBic pH 8.0 to give 10 ng/µL. Gel pieces should be just covered, but not in a large excess of volume (for a single 2D gel spot, use 25-30 µL of 10 ng/µL trypsin).

5. Digest overnight for 16-18 hours at 37°C.

6. Following digestion, centrifuge condensate to bottom of vial. Add extraction solution of 1% formic acid, 2% acetonitrile in water. The amount needed will vary with the gel volume, but typically you can use the same amount as the volume of trypsin solution you added (25 to 30 µL for a 2D gel spot). Vortex pieces in extraction solution occasionally over 30 minutes (optional: after adding extraction solution, sonicate the gel for 5 minutes. Be careful of contamination).

7. Remove supernatant containing peptides and pipette into LC vial. Depending on amount of protein expected, more peptides can sometimes be recovered by shrinking gel pieces in ~50 µL of neat acetonitrile, then adding this to the LC vial. Speed vac to dryness and resuspend in LC buffer (0.2% formic acid, 2% acetonitrile in water) if last acetonitrile shrinking step is used.

In-gel digest protocol is based on the procedure from M. Mann's lab: "Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry." M. Wilm, A. Shevchenko, T. Houthaeve, S. Breit, L. Schweigerer, T. Fotsis, M. Mann. Nature. 379, *1996*, 466-469.