In-gel Digestion protocol

Destaining procedure

1. Remove the stacking buffer portion from the gel
2. Stain the gel using coomassie or silver staining
3. Rinse the gel in DI water (1 minute)
4. Cut the gel bands and put them in 1.5 ml Eppendorf tubes. Add 1 ml destaining solution
5. Rinse the gel pieces three times with the destaining solution (10 minutes each run, use vortex). Coomassie stained slices may require additional rinses
6. Rinse/vortex for 10 minutes with 1 ml of 100mM ammonium carbonate
7. dehydrate the gel with acetonitrile (1 ml) for 10 minutes (vortex)
8. Re-hydrate gels with 1 ml 100 mM ammonium bicarbonate for 10 minutes (vortex)
    a. At this step you can the gels placed in eppendorf tubes in the freezer for the next step, protein digestion or extraction

9. To start protein digestion, dehydrate gels with 1 ml acetonitrile for 10 minutes (vortex), remove the acetonitrileand dry the gels (30 minutes using the speedvac or 3 hrs using the in-house vacuum)

Reduction and alkylation (not required for protein ID)

1. Place the dry gel pieces in 0.2 ml tubes, add 50-100 ul of 20 mM DTT in 100 mM NH_4HCO_3 and incubate them for 1 hr at 60 deg C
2. Remove the DTT solution and add 50-100 ul of 55 mM iodoacetamide (IAM) in 100 mM NH_4HCO_3 (freshly prepared)
3. Incubate at room temperature for 45 minutes in the dark
4. Remove the IAM solution and rinse/vortex the gels for 10 minutes with 100 mM NH4HCO3 and then for another 10 minutes with acetonitrile. Repeat this procedure
5. Dry the gels using the speedvac (30 minutes) or in-house vacuum (3 hours)

Protein digestion

1. Prepare a cold solution of the digestion buffer containing the enzyme: 12.5 ng/ul trypsin in 50 mM ammonium bicarbonate. Add 50 ul of this solution to each vial and incubate for 45 minutes on ice bath. The solution enzyme should penetrate inside the gel, but will not start the cleavage reaction due to the low temperature
2. Remove the digestion buffer containing the enzyme and add 25 ul of 20 mM ammonium bicarbonate (without enzyme) and digest the samples overnight at 37 deg C
3. In the morning, spin down the solutions. Sonicate the samples in liquid bath or shake in a thermomixer for 15-20 minutes, then spin down again and collect the digestion liquid
4. Incubate the gel slice 3 x 20 minutes each with 5% formic acid, 50% CH_3CN
5. Dry the collected supernatant by speedvac to about 20 ul, not completely dry