

## Preparation of Protein Samples from Gel Electrophoresis for Mass Spectrometry for Coomassie-stained gels

Excise protein spots using disposable transfer pipettes (produces ~1mm diameter plugs).

Wash gel plug with 50mM ammonium bicarbonate, centrifuge, remove solution and discard.

Wash gel plug with 50% acetonitrile/50% 50mM ammonium bicarbonate, centrifuge, remove solution and discard. Repeat as necessary to remove stain.

Completely dehydrate gel with 100% acetonitrile.

Rehydrate in 25mM ammonium bicarbonate containing 12.5ng/uL Promega trypsin at 4°C for 1 hour.

Remove excess solution and add just enough 25mM ammonium bicarbonate to cover gel plugs.

Incubate overnight at 37°C (alternatively, incubate at 50°C for 4hr).

Add 1 uL of 50% formic acid to remaining solution and analyze by MALDI-TOFMS or LC-MS/MS.