In-solution digestion using pepsin agarose beads v1.0

1. Denaturation and reduction buffer: 3 M Guanidine hydro chloride (GdnHCl) 0.25 M TCEP:

- Take an Eppendorf containing 3mmol GdnHCl powder (found in the orange box in the -80ºC) and suspend the powder in 500µL of miliQ water to make a 6M solution (vortex until the powder is fully dissolved).
- Find an Eppendorf tube containing 0.75M TCEP (found in the green box in the -80ºC) and dilute to 0.5 M (eg. take 333.3 µL of 0.75 M TECP and add miliQ water to a final volume of 500 µL)
- Mix both solutions 1:1 to get the final solution

2. Digestion of 500pmol of protein:

- Take the protein and dilute it 1:10 with the 3 M GdnHCl 0.5 TCEP and incubate for 45 minutes at 60ºC with mild agitation
- Take 50µL of pepsin beads and transfer them into a spin cup containing a 0.45µm cellulose acetate filter and centrifuge it at 400G for 1 minute
- Wash the beads by adding 80 µL of buffer A to the spincup and centrifuge at 400G for 1 minute (repeat 3 times) (do not let the beads dry for more than 2 minutes, they can be left in buffer A)
- Acidify your protein sample by adding TFA to a final concentration of 0.1%
- Add your protein sample to the clean dry beads in the spin cup and let it incubate for 10 minutes at room temperature with agitation.
- When the 10 minutes have passed centrifuge at 2000G for 1 minute.
- Add 50uL of buffer A onto the beads in the spin cup and centrifuge again at 2000G or 1 minute, to make sure all peptides go through the filter
- The peptic peptides are now in the supernatant collected at the bottom of the spin cup (they still contain 3M GdnHCl and 0.25 TCEP thus stage tipping is necessary for MALDI analysis.)

Protocol log:

Date	Version	comments	Responsible
26/4/16	1.0	New protocol	GC