## Digestion of Smt3-containing recombinant proteins with Ulp1

1. Add 0.5µL of Ulp1 protease in 50µL of the recombinant protein (concentrated in e.g. NaPi buffer, preferably no salt or low salt concentration). Optionally a final concentration of 1mM DTT may be added in the mixture.
2. Incubate at RT for at least 10 minutes. (Determine the suitable digestion time by taking samples at different time points and running them on an SDS-PAGE.)

## Preparation of an SDS-PAGE gel and electrophoresis

1. Build the equipment: place two glass plates against each other in a gel caster.
2. Prepare a mixture for the separating gel (here 15%):

| | |
|---|---|
| 4X separating buffer (1.5M Tris-HCl, 0.4% SDS, pH 8.8) | 1.25mL |
| Milli-Q $H_2O$ | 1.875mL |
| 40% Acrylamide | 1.875mL |
| 10% APS | 20µL |
| TEMED | 5µL |
| Total | 5.025mL |

**NOTE:** Add APS and TEMED at the end, then mix the solution and pipet it between the glass plates. Add isopropanol on top of the gel to remove bubbles and make the surface even.

3. Prepare a mixture for the stacking gel (here 3%):

| | |
|---|---|
| 2X stacking buffer (0.5M Tris, 0.2% SDS, pH 6.8) | 0.95mL |
| Milli-Q $H_2O$ | 0.8mL |
| 40% Acrylamide | 0.15mL |
| 10% APS | 10µL |
| TEMED | 2.5µL |
| Total | 1.9125mL |

After the separating gel has polymerized, remove isopropanol. Pipet the stacking gel mixture on top of it and place a comb between the glass plates. Let the stacking gel polymerize.

4. Place the polymerized gel(s) in a running tank and fill up the space between the gels with the 1x SDS-PAGE running buffer containing diluted from 10X SDS-PAGE stock solution of 25 mM Tris, 192 mM glycine, 0.1% SDS . Add a sufficient amount of buffer in the tank surrounding the gels.
5. Remove the comb(s) and pipet a ladder (used: ThermoFisher Scientific PageRuler Prestained Protein Ladder, 10 to 180 kDa, Catalog number: 26616) and the samples (**NOTE:** sample processing*) into the wells.
6. Run the electrophoresis at 110 V for ~1-1.5 hours until the samples have run almost to the bottom of the gel.
7. Rinse the gels with Milli-Q $H_2O$ and stain them with Coomassie blue for 30-45 minutes with shaking.
8. Keep the gels in the destaining solution (30%methanol, 10%acetic acid and water) for ~1 hour (or more) until the bands become visible and most of the blue background staining is lost. Destaining solution may be changed during this time.
9. Image the gels.

### Sample processing for SDS-PAGE:

1. After adding SDS Loading Dye into the samples, heat them at +95°C for 10 minutes.