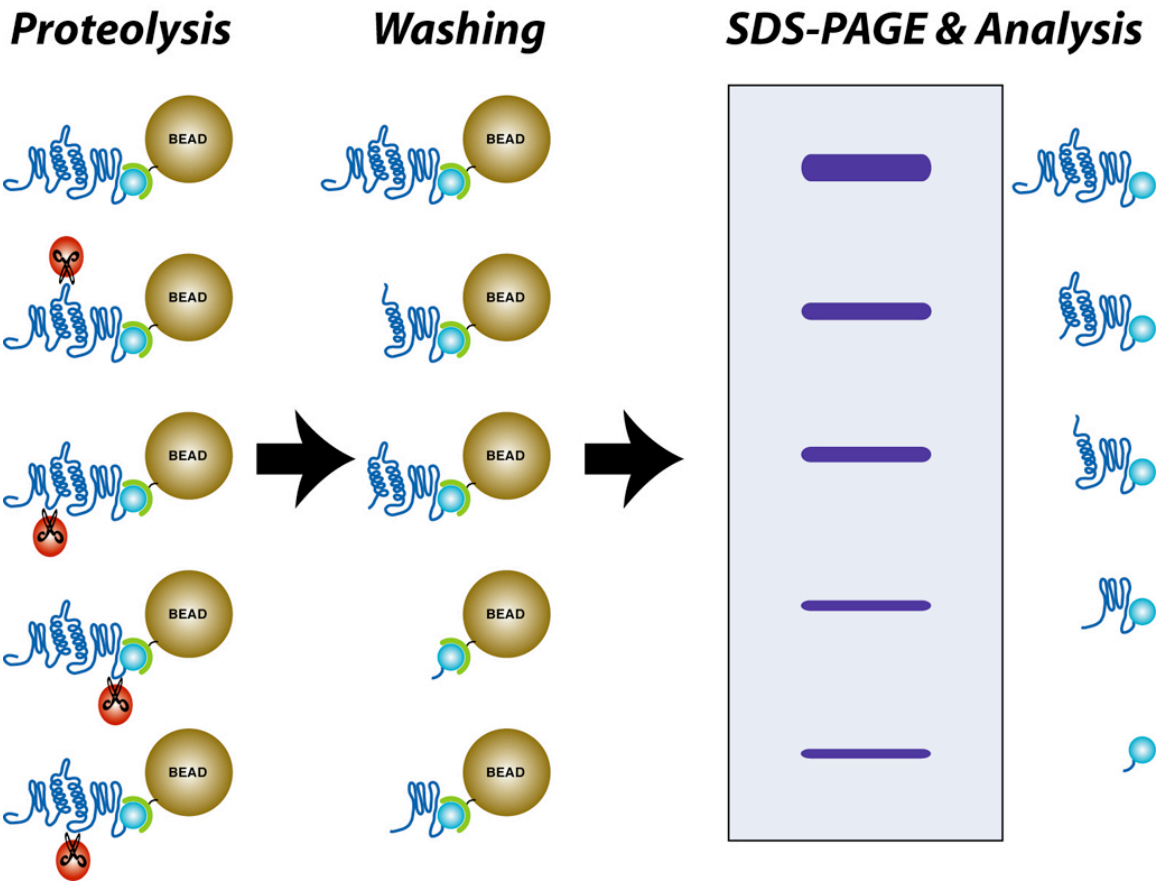
Protease Mapping

This protocol is demonstrated schematically in the figure below. Tagged proteins are attached to a resin, and a partial protease digest is done. Unbound fragments are washed away, and the bound fragments are eluted and run on a gel. This method has many applications, including domain mapping (as in Devos et al, PLoS 2004). The following protocol assumes that you're starting with already-purified protein. You can also start from yeast lysate, in which case pick up this protocol from step 7 after you've done your pullout, bound your protein to the beads and washed them.



1. Transfer 25uL (1mg) of Dynabeads to each of 5 microcentrifuge tubes. The Dynabeads are conjugated with the appropriate binding partner to the tagged protein. (We have done this successfully with PrA, GFP, and 6xhis tagged proteins).

2. Add 700uL binding/wash buffer (as appropriate to your tag), and vortex to equilibrate beads. Apply to magnet and aspirate off supernatant. Repeat wash once.

3. Resuspend beads in 100uL binding/wash buffer. Add at least 2-3ug tagged protein to the tubes, and bring volume up to 700uL with binding/wash buffer.

4. Incubate on nutator 10 minutes at room temperature to bind protein to beads.

5. Place tubes on magnet, and aspirate off supernatant.

6. Wash beads 4x 700uL binding/wash buffer. Each wash add buffer, vortex, put beads on magnet, and aspirate off supernatant.

7. Meanwhile, make 1ng/uL dilution of your protease in the appropriate buffer (see below).

8. To each tube of beads, add the appropriate amount of diluted protease (you'll have to do test runs to see how much enzyme gives a good ladder). Bring to 50uL with buffer. Add 50uL buffer alone to the control.

9. Incubate digests on the TOMY shaker in the 37 degree C incubator for the appropriate times (we've used times ranging from 1 second to 75 minutes). After that time, put the tube on the magnet, and aspirate off supernatant. Wash the beads with 700uL binding/wash buffer. Vortex, put on magnet, and aspirate off supernatant. For the 1 second time point, do this immediately after adding the protease. (5 tubes including the control gives four time points).

10. Elute the protein. This can be done in many ways. Two suggestions follow:

    a. This method was used for his-tagged proteins on Dynabeads TALON: Add 60uL 2x Morris buffer to each of the Dynabead tubes after the wash. Vortex, and heat 10 minutes at 95degC. (keep the control tube without protease at 37degC for the full 30 minutes). Store on ice until ready to run gel.

    b. This method was used for PrA and GFP tagged proteins on antibody-conjugated Dynabeads: Wash Dynabeads with 0.1M NH_40Ac, 0.1mM$MgCl_2$ 0.02% Tween 20.(Make fresh and filter). Add 1ml, incubate on nutator 5 min-Vortex-Place on magnetic bar. Take off supernatant. Elute bound protein with 1mL of 0.5M NH_4OH 0.5mMEDTA (pH ~10.9), incubate on nutator 30 minutes at room temperature. Place beads on magnetic bar, and transfer supernatant to a fresh tube. Flash freeze elution in liquid nitrogen, and allow to evaporate in Speedvac overnight. Resuspend pellet in gel sample buffer (10uL solution A, sonicate, 10uL solution B). Heat 10 minutes to 95degC. Store on ice until ready to run gel.

See next page for recipes…

Here are the proteases that we've used, and their appropriate buffers.

a) endoproteinase Asp-N (Roche, 1 420 488 or 1 054 589),
b) endoproteinase Lys-C (Roche, 1 420 429 or 1 047 825),
c) trypsin (Roche 1 521 187 or 1 418 025)

Preparation of proteinases.
a) Asp-N (1:200)
2ug in 50 ul = 40ng/ul (stock solution).
For 3 ug of protein need 15 ng of proteinase (1/200).
Dilute stock solution 2.5 times with 1x reaction buffer.
Use 1 ul of 16ng/ul solution in reaction

Asp-N reaction buffer: 50mMsodium phospate buffer, pH8, 0.01%SDS
Prepare 10 ml of 10x buffer. Filter.

b) Lys-C (1:200)
5ug in 50 ul = 100ng/ul (stock solution)
For 3 ug of protein need 15ng of proteinase (1/200).
Dilute stock solution 6.5 times with 1x reaction buffer.
Use 1 ul of 15.3ng/ul solution in reaction

Lys-C reaction buffer: 25mM Tris, pH8.5 buffer, pH8.5, 1mMEDTA, 0.01%SDS
Prepare 10 ml of 10x buffer. Filter.

c) Trypsin (1:200)
25 ug in 50ul = 500ng/ul
For 3 ug of protein need 15ng of proteinase (1/200).
Dilute stock solution 33 times with water.
Use 1 ul of 15.15ng/ul solution in reaction

Lys-C reaction buffer: 100mM Tris, pH8.5 buffer, pH8.5, 0.01%SDS
Prepare 10 ml of 10x buffer. Filter.

Gel sample buffer:
Solution A = 0.5M Tris base, 5% SDS
Solution B (50mL) = 37.5mL glycerol, 12.5mL ddH2O, 960mg DTT, 0.05% bromophenol blue