mTEV Digestion

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Materials/Reagents/Equipment	Vendor
mTEV protease	prepared in-house
dialysis buttons	Hampton Research
dialysis tubing	prepared in-house Spectrum
dialysis tubing closures	
50 ml conical tube	
buffers	prepared in-house
1 – 2 L beaker	

Procedure

I. Small Scale Trials.
Prior to cleaving a protein with mTEV, small scale trials should be conducted to avert large scale precipitation. These could involve cleavage with dialysis into various buffers representing a range of possible salt concentrations or pHs depending on the characteristics of the protein. If the protein solution is about 2 mg/ml or greater, precipitation may be seen if the dialysis solution is incompatible with the protein. If the protein solution is less than 2 mg/ml, the precipitation may not be visible. In all cases, the protein solution should be centrifuged and the concentration of the supernatant should be determined by Bradford for comparison (see next paragraph).

Hampton Research dialysis buttons are a convenient small scale method of testing cleavage/dialysis conditions. By using the 50 ul buttons, a quick 1:1000 dialysis can be obtained in 50 ml conical tubes.

Determine the amount of mTEV required to cleave the desired mass of protein. This will depend on the mTEV preparation used. Load the protein/mTEV solution into the button and seal it with dialysis tubing of the correct porosity cut into ~2 cm x 2 cm pieces and an o-ring. Put the button in to the 50 ml conical tube containing the dialysis buffer. Seal the tube and place it on a rocker for 1-2 hours at room temperature. These trials can also be performed in a cold room if protease degradation is expected. After the incubation observe the button(s) for precipitation which would indicate an undesirable condition. The protein solution can be removed from the dialysis button by piercing the membrane with a pipette tip and aspirating the sample, which can be centrifuged to pellet insoluble material. The supernatant can be analyzed by the Bradford method for protein concentration to compare with the starting solution. The buffer condition with the most soluble protein is the best condition for dialyzing during cleavage with mTEV.

The following are some suggestions for testing.
1. Reduce the protein concentration to ~ 2 mg/ml if it is too high.
2. Maintain 50 to 100mM Imidazole in the dialysis buffer if the protein is eluted at >400mM Imidazole. Those proteins that are eluted at high Imidazole concentration tend to have problems when cutting off the tags.
3. Include a condition with a higher salt concentration in the dialysis buffer, such as 0.3M or 0.5M.
4. Add 5-10% glycerol in the dialysis buffer.

II Large scale. Make sufficient buffer selected from the small scale trials to dialyze the volume that the protein will be cleaved in. A 1:20 ratio should be the minimum. It is often helpful to first dilute the protein to ~2 mg/ml prior to cleavage in order to reduce the incidence of post-cleavage precipitation. After addition of mTEV to the protein, place the solution into a dialysis bag and place the bag in the beaker containing the buffer. Mix the buffer with a stir bar during the digestion to aid the dialysis.