FcRn Purification (Jennie Johnson)

Harvesting cells:

The soluble form I have purified was grown on the Cell Pharm by David. I filter the harvests through a 0.45 µm filter, add azide to 0.05%, and add EDTA to 1 mM final concentration. Store the harvests in the cold room until they can be purified over the rat IgG column. The harvests I have purified yield 20-25 mg/L protein.

The PI-linked form is grown either in large tissue culture flasks (25 ml media/flask) or on plates (10 ml/plate). To harvest the protein with PI/PLC in flasks, remove 15 ml media, and add 10 µl of 0.1 mg/ml (dilute the PI/PLC stock [see David for its location] in the hood with autoclaved water just before it is used--**do not dilute large quantities of enzyme; it is not stable for long periods at low concentration**!). For plates, just add 10 µl of the 0.1 mg/ml enzyme. Incubate the cells for 2 hours at 37ºC, remove the media containing protein, and add the appropriate amount of fresh media (25 ml for flasks, 10 ml for plates). The cells can be harvested every other day until they get contaminated (roughly four weeks--this is highly variable depending on how careful you are in your manipulations; also, do immunoprecipitations to make sure the cells are still producing protein). Filter through a 0.45 µm filter, add azide to 0.05%, and add EDTA to 1 mM final concentration. Store the harvests in the cold room until they can be purified over the rat IgG column. I have never purified this form of the protein, so I do not know the yields.

Purification:

The rat IgG column will bind FcRn at pH 6.0 and will release it at pH 8.0. Prepare 50 mM $NaPO_4$ buffers at pH 6.0 and pH 8.0 (both with azide added to 0.05%) for washing and eluting the column. The harvests must be brought to pH 6.0 before they are run over the rat IgG column. Add monobasic $NaPO_4$ to the harvests to a final concentration of 50 mM. The harvests will turn yellow as the pH is lowered. Check the pH of the harvest; if it is not 6.0, add acid to lower the pH to 6.0. Run the harvest over the column, wash with 5-10 column volumes of pH 6.0 $NaPO_4$, and elute with pH 8.0 $NaPO_4$. Collect the eluate into 1 ml fractions. Bring the pH of the column back to 6.0 by running 5-10 column volumes of pH 6.0 $NaPO_4$ over the column. Concentrate the fractions containing protein in a vacuum dialysis apparatus with a 25 kDa MWCO bag. Dialyze the protein into 25 mM PIPES (plus azide to 0.05%) at the pH of interest. For long-term storage, the protein should be kept at pH 6.0-6.5. Vacuum the protein sample to 500 µl, remove the sample, and wash the dialysis bag with 500 µl buffer. Determine the concentration of the protein by BCA assay, and run it on a 13% SDS PAGE gel.