% Solution	0%	10%	20%	25%	30%
70% Sucrose	0 ml	6.43 ml	12.9 ml	16.1 ml	19.3 ml
500mM Tris pH 8.5	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml
1M KCl	1.125 ml	1.125 ml	1.125 ml	1.125 ml	1.125 ml
1M $MgCl_2$	0.45 ml	0.45 ml	0.45 ml	0.45 ml	0.45 ml
dH_2O	38.925 ml	32.495 ml	26.025 ml	22.825 ml	19.625 ml

% Solution	40%	45%	50%	55%	60%
70% Sucrose	25.7 ml	28.9 ml	32.1 ml	35.3 ml	38.6 ml
500mM Tris pH 8.5	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml
1M KCl	1.125 ml	1.125 ml	1.125 ml	1.125 ml	1.125 ml
1M $MgCl_2$	0.45 ml	0.45 ml	0.45 ml	0.45 ml	0.45 ml
dH_2O	13.225 ml	10.025 ml	6.825 ml	3.625 ml	0.325 ml

Buffered conditions are 200mM Tris pH 8.5, 50mM KCl and 25mM MgCl2 and appropriate concentration of sucrose as shown above in table. Notice that I make up 45 ml of each concentration.

MAKING GRADIENTS

Make gradients the day before. For a total volume of 11.5 ml gradients in polyallomar 12 ml, make 5 gradients of 2.3 ml each. To do this, slowly load 2.3 ml of each gradient into the tube at a tilt starting with 60% sucrose and freeze at –70°C for one hour. Repeat for the remaining 4 gradients making sure you leave 0.5 ml remaining for worm extract. I typically do two different concentration gradients simultaneously: 60%, 55%, 50%, 45%, and 25% for one gradient and 60%, 50%, 40%, 30%, and 20% for the other gradient. Frozen gradients should be left at out overnight to thaw and diffuse. The next day cool gradients at 4°C for one hour before adding worm extract.

WORM EXTRACT

Lyse your worms via sonication in the 0% sucrose buffer above adding protease inhibitors if required. You can either lyse two separate preps or lyse one prep and aliquot half of the lysis into another tube so that you will have two separate insoluble pellets. After lysis, spin at 4K, 4°C for 10 minutes to remove debris and then spin at 14K, 4°C for 10 minutes to separate soluble and insoluble pellet. You may want to spin again at 14K or higher (ultracentrifugation) to remove as much soluble protein contamination as possible. I

typically save the soluble portion and do a protein assay to guestimate how much protein is in the insoluble pellet. If you assume that 1/3 of all proteins are insoluble, then you can divide your soluble protein concentration by three to get a rough estimate. At this point, I resuspend the insoluble pellets in 450 µl lysis buffer (or 0% sucrose) and sonicate the pellets until I get most of the pellet resuspended. I then add the worm extract to the gradients and measure both gradients on the balance to make sure the weight is the same since the SS-41 rotor is sensitive. Add drops of lysis buffer until both gradients have the same weight.

ULTRACENTRIFUGATION

Spin gradients in an ultracentrifuge at 36K for 18 hours at 4°C using the SS-41 rotor. The rotor and the ultracentrifuge are owned by the Pallanck lab. The rotor is kept in the cold room on the 4th floor and the centrifuges are in the basement. I typically do the spin overnight so that I can collect fractions in the morning.

FRACTION COLLECTION

After the spin, carefully collect fractions of 1 ml , 200 µl at a time in a cold room. I typically have all my microcentrfuge tubes ready and labeled. This takes forever and you will need to take breaks so you don't become an icicle. Some labs use a pump that makes the gradients and collects the fractions but I have not found a lab that has this apparatus. Once you have your fractions you can either TCA precipitate the protein or do a methanol-chloroform precipitation to analyze on a gel or to digest for mass spec.