

## Preparing and Loading a Sucrose Gradient

Clark Lab

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1. Get a 16 x 102 mm polyclear centrifuge tube, insert into the metal SW28.1 tube holder and use the upper edge of the tube holder to draw a line marking the center of the tube.
2. Using a cannula-fitted syringe, add the lower percentage sucrose solution to the tube so that it is ~1 mm above the centerline. Make sure to avoid forming bubbles.
3. Using a different cannula syringe, underlayer the higher percentage sucrose:
  - a. Wipe cannula and insert it down the side to the bottom of the tube with no drips.
  - b. Slowly but steadily force out the sucrose solution, keeping the tip of the cannula near the phase boundary. Stop when this boundary is at the centerline.
4. Take a rubber cap with a short stem and place it on the tube. Make sure the side of the cap with the air hole is the last to go in, insuring no bubbles.
5. Turn on gradient Master and place level on top of circular plate. Use the control pad dial to adjust the front/back balance, and the hand screw on the right side to adjust left/right balance. Press DONE when surface is level.
6. Place the matching tube holder on the surface and put the prepared tube in the tube holder.
7. On the Gradient Master control panel, choose GRAD, then RCNT. Next choose the appropriate rotor, SW 28.1 in this case.
8. What follows is a list of gradients. Scroll through using the control dial and select based on the low and high sucrose gradient concentrations chosen, for example 10% and 30% or 15% and 45%. Once the appropriate gradient has been located, press USE and the Gradient Master will go to work.
9. Remove gradient, put tube holder away and turn off Gradient Master.
10. Remove lid from tube and pipette sample to be separated on to inside edge of tube just above the surface of the sucrose gradient. Usually 300-400  $\mu\text{L}$  will fit.
11. Balance tubes and spin appropriately.