

## Making Linear Sucrose Gradients with the Bio-Comp Gradient Master

Patricia L. Clark

last updated: April 4, 2002

### Preliminary considerations:

\* The Gradient Master is pre-programmed for making sucrose gradients in several different ranges (10-30%, 15-45%, etc). It is easier to use one of the pre-existing programs than to optimize a novel program; however, if a novel program is required, the Gradient Master manual has guidelines for program development.

\* Gradients should be made as close as possible to the time of use (centrifugation).

### Sucrose solutions:

\* Make sure you understand the difference between (w/v) and (w/w)!! Most of our gradients are made with (w/w) sucrose solutions. 100 ml of a 15% (w/w) sucrose solution will therefore have 15 g of sucrose mixed with 85 g of buffer (not water! ...use whatever buffer your sample is in).

\* For high-percentage sucrose solutions, speedy solubilization can be achieved by gently warming the stirring solution. The old steel stir plates are ideal for this procedure.

\* Sucrose solutions <60% will support bacterial growth over time. To combat this, filter sterilize your solutions, and keep them in the cold room. Do not autoclave.

### Loading tubes with sucrose solutions:

1. Decide whether to use the SW28 (shorter, fatter) or SW28.1 (longer, thinner) rotor and tubes. Make an even number of gradients, so the rotor will be balanced (or make 3).
2. Mark tubes using the aluminum gig provided for each tube size: stick tube in gig, make ultra-fine tip sharpie mark around tube using either upper or lower edge of gig. Upper edge is for smaller caps/smaller sample volume; lower is for large caps/samples.
3. Pipet lower percentage sucrose solution into tubes, ~1 mm *above* mark (does not need to be exact, or equivalent to other tubes).
4. Use syringe with cannula to layer higher percentage sucrose solution under lower percentage: fill syringe with solution, slide cannula down inside edge of tube until hit bottom of tube, *gently* displace lower percentage solution with higher. Keep adding sucrose until boundary is *exactly at* gig mark. Remove cannula.
5. Put caps (small or large) on tubes: find overflow valve on cap. Push cap onto tube, with the valve side slightly higher, so the air and excess sucrose escapes through the valve. There should be no air between the cap and the solution. Blot excess solution in cap center with kimwipe.

### Gradient Master procedure:

1. Turn on Master. Check if platform is level (use bubble level). If not, use dial on control panel and thumbscrew next to control panel to adjust. When level, press 'DONE'.
2. Press 'GRAD' to enter the gradient menu.

3. 'LAST' will display the last gradient used. 'RCNT' will display recently used gradients, and 'LIST' will display the full list of gradients. If using 'RCNT' or 'LIST', select the rotor you will be using (SW 28 or 28.1).
4. Turning the control knob will scroll through the available gradient programs. Turning the knob more will increase the scrolling speed.
5. Gradients are listed by:
  - Short/Long: this refers to the caps on the gradients
  - Sucr: sucrose
  - % range: many ranges are pre-programmed
  - 1/2St: refers to one- or two-step gradient forming program
6. For example, if you want to separate a 200 ul sample on a 15-45% sucrose gradient using the SW28.1 rotor, you would select "Short Sucr 15-45% 1St" . When you find the correct program, press 'USE'.
7. The screen will show a summary of the run program, including the name of the gradient, the first and total number of steps, and the time and angle used for the first step.
8. Place appropriate tube holder (magnetic base) on platform; put tubes *with caps* in holder.
9. Press 'RUN'.