

Fractionating Gradients Using the Bio-Comp Fractionator

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Preliminary Considerations:

* The Fractionator manual is quite clear and complete; please refer to it for answers to detailed and advanced questions. Section 7, "Instrument Operation" and "Practical Manual" are particularly useful.

* The Fractionator and the Gilson fraction collector live in the cold room. If you want to remove it from the cold room, consult Patricia Clark.

* The highest resolution (least smearing) between fractions is achieved by washing between fractions. The easiest method is to add these washes to the preceding fraction, although this will dilute the fraction some. Fractionation without washes is possible, but resolution will suffer due to laminar capillary flow (i.e., solution in center of tubing will move faster than solution at edge of tubing). The longer the tubing, the larger the problem.

Getting Started:

1. In addition to your gradient to be fractionated, you will need:
 - buffer (whatever you used for your gradient)
 - two small beakers, for Fractionator and fraction collector waste ports
 - spare centrifuge tube
 - sterile good water
 - 70% ethanol
2. Turn on the Fractionator and fraction collector (switches in back, by power cords).
3. Retract the piston completely: 'STUP', then use 'MOTR' to move piston.
4. Remove the tube holder by turning it CCW (window towards buffer reservoir) and sliding it off its base. Fill the tube holder with water up to the mark.
5. Fill buffer reservoir with buffer, and prime the lines: flip toggle to down, and wait until buffer runs out into waste (you can use the RINSE key to encourage it, but gravity alone is sufficient). Flip the toggle up, and push RINSE until buffer runs out of the fraction collector port.

Selecting a Rinse Program:

1. Press 'RINS' on the main menu
2. The Fractionator might beep if the control knob is not in the appropriate starting position (center) for the rinse menu. Turn the knob so that " ^ ^ " shows on the display before proceeding.
3. The rinse program shown will be the last one used. There are 10 rinse programs available, named 0-9 (only 0-7 are currently being used). 0-5 are pre-programmed by Bio-Comp, and are described in detail in the manual (page 19-21). If you wish to modify an existing rinse protocol, please use program 8 or 9 rather than write over an existing

program. *Program 6* has been optimized for our setup; this protocol provides sufficient rinsing of the line to the fraction collector, and contains an automatic trigger to advance the fraction collector after each fraction.

4. Press 'EXIT' to return to the main menu. The rinse program displayed when you press 'EXIT' will be the one used for the fractionation.

Preparing to Fractionate:

1. Remove the cap (black ring) from the top of the tube holder (twist and lift).
2. Insert the tube into the cap. Insure that the cap is on correctly; the tubes have a tendency to catch on the notch in the front. You can tell when this happens because the cap will not be level. Correct seating of the cap can be achieved by pressing gently on the tube with your thumb, just below the notch, and encouraging the cap to seat.
3. If the cap is on correctly, it should hold the tube, and you can set the tube in the holder and lock it in place by twisting the cap.
4. Reposition the tube holder on the Fractionator (slide on base and twist CW).
5. Load the fraction collector with tubes (at least 35 if collecting 30 fractions). Put some tubes on the far right side of the tube rack, and position the retaining bar.
6. Press 'START' on the fraction collector to position the tubing over the first tube. You may have to manually adjust the position of the tubing tip.

Fractionation:

1. Press 'FRAC' on the main menu. You will be prompted to turn the knob to full CCW.
2. Turn on the light under the tube holder. This will make it easier to watch the piston tip.
3. Lower the piston into the gradient until the first drop of the gradient appears at the tip of the fraction collector tubing: turn the knob CW (turn more to move faster). Try to go pretty fast until the piston tip enters the gradient, then slow down a lot (turn CCW) and cover the STOP button with your finger. Press STOP when the first drop appears.
4. Turn off the light under the tube holder, to avoid excessive warming of the gradient.
5. Press 'RSET' to set this position as the top of the gradient.
6. Press 'FRAC', then 'SNGL' to automatically collect fractions of a defined size.
7. Press 'SPED' to select the piston speed (I use 1 mm/sec); 'DIST' to set the piston distance for each fraction (I use 3 mm) and press 'NUMB' to set the number of fractions (I use 30). The total distance to be covered will be displayed to the right (should be 90 mm for SW28.1 tubes).
8. Press 'START' from this window to start the fractionation. Insure that the fractions drip into the tubes, and that the collector is advancing properly. There may be a little liquid lost between fractions as the collector advances; do not worry about this, as it contains only the last bit of rinse buffer.
9. When the piston reaches the bottom of the tube, it will retract automatically. This may be at fraction 29 instead of 30, and indicates that the piston tip was further down in the gradient at the start of the fractionation.
10. Press 'ADV' on the fraction collector to advance to the next tube, then 'RINSE' on the control panel for a few seconds, to flush the lines with buffer (this will be collected in the next tube).

11. Remove the tube holder, and cap. There will still be some sucrose in the tube. This corresponds to the first few drops (which often seep past the trumpet tip) and the last few drops in the curved bottom of the tube.
12. Load another gradient tube, fresh fraction collector tubes, and repeat,
13. OR: clean up by dumping the water in the tube holder, washing the holder cap, and loading the spare tube, filled with sterile good water. Dump the remaining buffer in the reservoir, and fill with water, priming as before. Fractionate the water just like your gradients (if you press 'END', then turn off the fraction collector before you begin, all of the liquid will go to waste).
14. Dump the water out of the reservoir, and blow a little air through the lines using 'RINSE' (toggle down, then up) and then 'AIR'.
15. Replace the tube holder and cap, and lower the piston completely. Turn off the Fractionator.

Complete and thorough cleaning is required after each use!!