* **Sucrose Gradient**
  + Prepare ~7 ml of both 45% and 15% sucrose solution per sample
    - By diluting premade 60% sucrose

|  |  |  |  |
| --- | --- | --- | --- |
| Final Con. | Total Volume | Volume of 60% sucrose | Volume of polysome buffer |
| 15% | 15 mL | 3.75 mL | 11.25 mL |
| 45% | 15 mL | 11.25 mL | 3.75 mL |
| 15% | 25 mL | 6.25 mL | 18.75 mL |
| 45% | 25 mL | 18.75 mL | 6.25 mL |

* + - Add freshly prepared CHX (1/1000), SuperseIn (1/1000) and DTT (2mM)
  + Mark the centrifugation tubes with the mark block
    - Tube, Thinwall, Polypropylene, 13.2 mL, 14 x 89 mm (Beckman)
  + Fill the tubes with 15% sucrose solution first with needle and syringe-fill to just slightly above the marked line
  + Add 45% sucrose solution starting at the bottom of the tube slowly to the marked line
    - lift up slowly as you add to displace weight
    - Should get a “bulb” at the top of the tube with the 15% bubbling almost over.
  + Close of tube with black rubber stopper, keeping the air hole at the top during closing
  + Turn on the gradient master
    - make sure the gradient master is level
  + Put tubes into the magnetic holder and place it on the gradient master
  + Program
    - go to gradient->recent (double check that this is what you want)->sw41->use->run.
      * If a different gradient is required, adjust accordingly
  + While gradient master is running, clean the needle with methanol and DEPC
  + When gradient is done, remove stoppers
  + Remove 600 µl off top of tubes with tips
  + Gently load 550 µl of sample to top
  + Weigh the tubes so that they are balanced (within 0.05g) for centrifugation
  + Slowly add add samples into the holders of the SW41 centrifuge rotor
  + centrifuge at 38000 rpm for 2 hours and 38 min at 4 °C
* **Fractionation**
  + During centrifugation wash and set up fractionator
    - Wash fractionator
      * Turn on the components and allow the lamp to warm-up for at least 15 min.
      * Turn on the pump to manual.
      * Place the small tubing into 50 ml methanol, and reverse rapid into the syringe of the pump.
      * Remove all air bubbles from syringe and tubing by tilting the machine carefully vertically and forward pump out bubbles
      * connect tubing to the needle.
      * Slowly pump till methanol comes out of the needle and all bubbles are gone.
      * Turn bottom of apparatus to puncture the bottom of the empty sample tube.
        + Try to get the needle in the middle of the tube.
        + Continue Puncturing tube until both black lines on needle are visible
      * pump the methanol into the tube until methanol reaches the wast
      * Remove methanol from tubing by reverse pumping.
      * Than transfer methanol back to 50ml tube storage tube
      * Repeat this twice with DEPC treated water
    - Set-up fractionator
      * Select the following settings on the chart recorder: Set Sensitivity dial to 'SET LAMP AND OPTICS'; set Noise filter switch to '1.5'; set Peak separator dial to 'OFF'; set Chart speed dial to '30 cm/hr' (5 cm/10 min); set Baseline Adjust dial (on top of the spectrophotometer unit) to 'MAX OPEN'.
      * Place the syringe and barrel in the pump and tighten into place (use provided screws and Allen key).
      * Install an ultracentrifuge tube.
      * Fill the syringe with DEPC-treated water by using the ‘‘REV’’ command at RAPID speed. Place the pump in upright position and chase air through the tubing using the ‘‘FORWARD’’ command. Only use RAPID for this function and washes.
      * start syringe pump on ‘‘Manual’’ at 6.0 ml/min
      * As the water passes through the flow cell (when chase solution fills 1/3 of the tube), switch speed to variable and set to 1.0 ml/min.
      * At a flow rate of 1.0 ml/min, adjust the Baseline Adjust dial on the spectrophotometer until the voltage on the chart is at zero (+/- 0.5 units).
      * Set the desired Sensitivity to '0.5'
      * Push the Auto Baseline button and adjust the baseline setting to the 10% mark on the chart recorder by turning the Recorder offset dial
      * Once a stable baseline is achieved, turn the pump switch to 'OFF' and the Chart Speed dial off
      * Recover DEPC-treated water by switching pump to 'REV' position at RAPID
  + Fractionating sucrose gradients
    - Prepare ice bucket with premade holes
    - After centrifugation, remove tubes from holders with a forceps and place them on ice
    - Fill syringe with ±25 ml of 60% sucrose solution
    - Attach seal apparatus on sample tube.
    - Pump forward on normal speed to remove all bubbles from needle
    - place the needle in line with the sample tube until the apparatus is sealed tight
    - slowly wind the needle into sample until both black lines on needle are visible
    - Remove cap of red chart marker
    - Set needle marker at about 30 on graph
    - Place Eppendorf tube in collection platform
    - Turn Brandel pump to “remote”
    - Settings on “forward”, “normal”, and “1.5”
    - Press run
    - Mark the graph paper as the peaks come out to correspond with tubes.
    - Label tubes and place on ice
    - When profiling is done or ready to be aborted, go to manual on Brandel go and reverse sample back.
    - Forward pump into a waste tube
  + Clean fractionator system
    - Remove graph
    - Cap graph marker
    - go back and forth with methanol 3x trough tubing
    - Rinse system with DEPC-treated water
    - Shut down with last DEPC-treated water wash still in tubing

**Relevant publications**

* Faye, Mame Daro, Tyson E. Graber, and Martin Holcik. "Assessment of selective mRNA translation in mammalian cells by polysome profiling." JoVE (Journal of Visualized Experiments) 92 (2014): e52295-e52295.
* <http://www.jove.com/video/52295/assessment-selective-mrna-translation-mammalian-cells-polysome>