Centrifugal Elutriation of NB4 Cells

This protocol uses centrifugal elutriation to enrich for cells at G1, S, and G2/M cell cycle phases. This protocol is optimized for the NB4 suspension cells.

Reagents

2 x 10^8 NB4 cells (~300 mL spinner culture)

PBS + 1% FBS ("elutriation buffer", RT, 750 mL)

70% ethanol

10 ml syringe

18G needle

Procedure

Prepare the elutriator

With a high flow of elutriation buffer, i.e. Flow Setting (FS) of ~150, eject all air bubbles from tubing by squeezing and releasing the tubing at ~10 psi of backpressure. Remove bubbles from chamber by repeating the same procedure with the chamber held upright. Turn off flow. Turn the Trap Valve to Pos 1 and the Inject Valve to Pos 1. Start the centrifuge at 1000 rpm to remove bubbles trapped in the elutriation chamber. Stop centrifuge and flow rate. Check the tubing near the elutriation chamber. Ensure that the tubing is taut between the chamber and the metal bracket on the top right corner.

Elutriation

1. Pellet (1000 rpm, 4 min). Re-suspend in 5 mL elutriation buffer and transfer to 50 mL falcon tube.
2. Load cells into syringe.
3. Pass slowly through 18 gauge needle into same falcon tube to produce monodispersion.
4. Repeat step 3 three times.
5. Start centrifuge at 1800 rpm.
6. Set FS to 102.
7. If the back pressure increases above 5 psi, stop centrifuge, and reposition tubing.
8. Synchronize strobe speed to view the elutriation chamber.
9. Load cells into syringe, leaving a small aliquot in 50 mL tube for asynchronous control.
10. Remove bubble trap from flow (Trap Valve Pos 1). Insert syringe into luer lock. Secure the position of the syringe barrel with a finger. Turn the Inject Valve so that the bubble trap is isolated (Inject Valve Pos 2). Slowly inject cells into the bubble trap. Close the Inject Valve (Inject

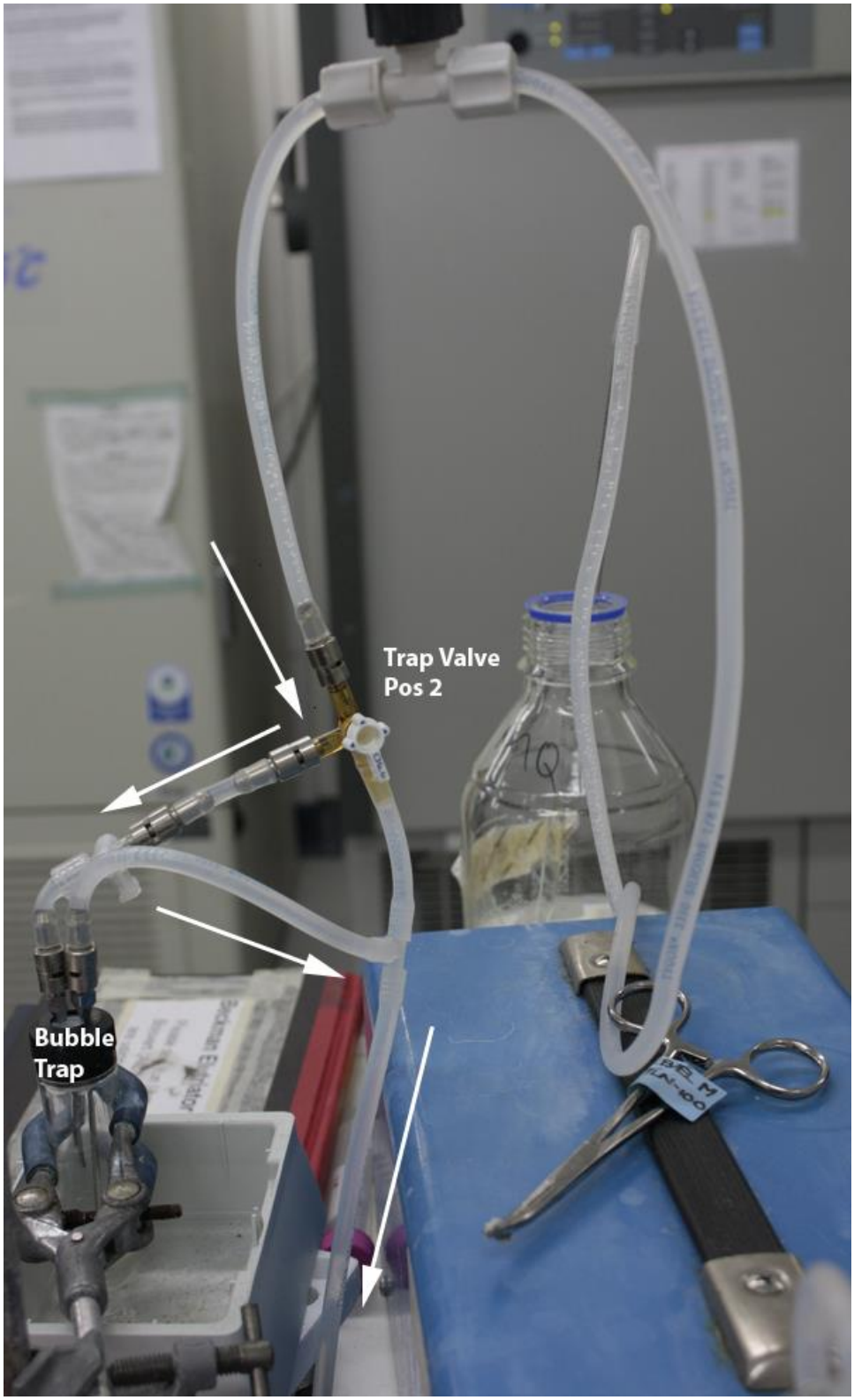
Valve Pos 1). Reconnect the bubble trap to the flow (Trap Valve Pos 2). View the chamber and ensure that cells are filling the chamber. Adjust the flow if necessary.

11. Wait until all cells are loaded into the chamber. You may have to flick the bubble trap to re-suspend cells.
12. Switch Trap Valve to Pos 1.
13. Collect remaining 50 mL fractions. For NB4 cells, collect at 112 (G1), 118, 122 (S), 126, 130, and 150 (G2/M). Stop spin, and collect last 50 mL fraction ("Post"). Replace elutriation buffer with 70% ethanol. Purge the tubing and chamber with 70% ethanol.
14. Remove glass pipet, allowing air to enter. Flow until all liquid is removed.
15. Turn off strobe light and centrifuge.

Notes

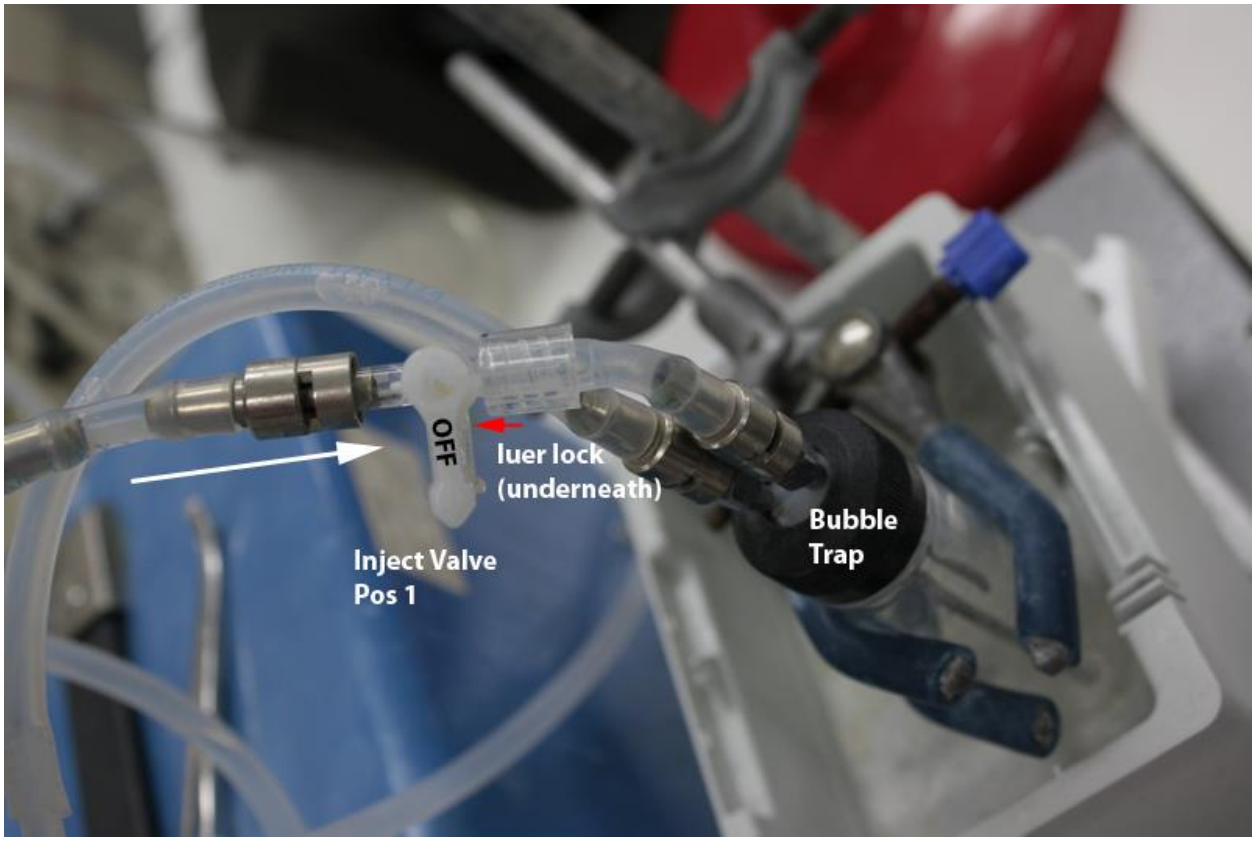
- **Trap Valve**
  - Pos 1: The T-valve is turned such that the flow goes through the bubble trap
  - Pos 2: Bypass the bubble trap
- **Inject Valve**
  - Pos 1: The "OFF" end is pointing towards the luer lock.
  - Pos 2: The "OFF" end is pointing towards the T junction with the peristaltic pump.

Trap Valve



Trap Valve Pos 1: “OFF” is pointing towards bubble trap

Inject Valve



Inject Valve Pos 2: “OFF” is pointing to left (peristaltic pump)