Protocol#5 - French Press (Thermo)

Aim

To quickly, efficiently and carefully lyse large prep of bacterial pellet. Unlike sonication, the French press is aimed at high volume (>10ml) cell extrusion due to high pressure forcing suspension flow through a thing orifice. The piston should be cooled before usage due to temperature buildup.

<u>Important note to outside users:</u> you should contact Noam Grinberg, tel# 28447, before using the French press in Raz Lab.

Materials & Equipment

- Piston
- Cylinder
- Piston "Head"
- Needle-Valve
- Flowthrough pipe + cap
- Ice
- Tripod

Experiment procedure

1. Wash parts with DW & submerge in ice for a minimum of 10' (while incubating mince cell pellet).
2. Insert piston into cylinder up to the line mark "Max fill" (use direct thrust without any "screwing" movements)
3. Turn cylinder up-side-down and place it on the tripod.
4. Screw needle-valve into piston head (not tight).
5. Screw flowthrough pipe tightly.

6. Pour well minced bacterial suspension into the cylinder leaving approximately 1cm of space.
7. Connect the empty 50ml falcon tube to the cap of the flowthrough pipe.
8. While supporting the piston handle with one hand, fit the piston head over the filled cylinder.
9. With both hand holding the cylinder, turn the complex so the piston handle points upward and place the complex at the correct position in the pressure chamber. Verify that the needle-valve is slightly pointing to the left side.
10. Secure the complex and tight the needle-valve on the piston head.
11. Turn the device on and move lever to the medium position.
12. See that the pressure is stabilizing at 1500psi; use the right side dial to adjust to the required pressure.
13. If there is no flowthrough dropping into the tube, move handle to high position.
14. Use a screwdriver's handle to tap the needle-valve to the point in which a small and drizzle can be seen in the tube.

    Note: pressure should always be maintained at 1500psi – if the pressure drops, slightly close the needle-valve by tapping.
15. At the end, when the piston is driven into the piston, lower the complex down by moving the lever to "down" position.
16. Unscrew falcon tube and place it on ice.
17. Remove the complex from the French press and pull the piston all the way to the max fill line; place it up-side-down on the tripod.
18. Repeat steps 6-17 twice.

Cleaning the device

- Clean all parts as soon as possible
- Wash parts with copious amounts of DW.
- Using DW sprinkler, thoroughly wash tubing and small parts.
- Pass a paper towel through the cylinder and make sure it is clean.

- Using a 95% EtOH sprinkler, wash all parts and place it at the chemical hood for **no more** than 2hrs or till all parts are completely dry
- Place all parts, large first-small last, in the plastic basket and return them to the cabinet