

# Bead beating

## Aims of the experiment

Bead beating is used for lyses the cells in order to obtain cell extract efficiently. It is proceed according Sun, Z. Z et al., 2013.

## Materials

- Cell pellets from the Cultivation Protocol
- 15ml Falcon tubes
- S30A buffer
- Beads
- 5ml pipet
- Bead-beating tubes
- Micro-centrifuge tubes

## Procedure

1. Weight required 50ml Falcon tubes with pellet and record mass. Calculate pellet mass, S30A buffer volume needed (pellet mass \* 0.9) (ml), and mass of beads (pellet mass \* 5.0) (g).
2. Add suitable amount of S30A buffer calculated in step 1 to each Falcon tube, vortex until homogenous, and return to ice.
3. Add beads intermittently to a single Falcon tube in three aliquots, each using 1/3 of the total beads, then vortex for 30 seconds. Avoid air bubbles and distribute beads evenly to achieve efficient extract. Alternate Falcon tube between ice and vortex step.
4. After last aliquot is added, ensure beads are uniformly distributed. A thick paste should be formed.
5. Place bead-beating tubes on ice.
6. Verify high viscosity of cell-bead solution using cut 5mL pipet with 3-4 mm opening. It should be viscous to the point of barely exiting the pipette tip during ejection. (If too viscous, re-adjust pipette tip; if not viscous enough, beads can be added in increments of (pellet mass \* 0.05), to a maximum mass of (pellet mass \* 5.1).
7. After each addition of beads, vortex for 30 seconds and return to ice.

8. Transfer bead-cell solution by modified pipet into a sterile bead-beating tube, with three-quarters full.
9. Spin extremely briefly (1s) to remove air bubbles without redistributing beads.
10. Finish adding bead-cell solution to form a concave meniscus.
11. Add a very small drop of bead-cell solution onto the inside of a bead-beating tube cap for closing the tube more sufficiently. Take sure there are no air bubbles.
12. Cap the bead-beating tube with the bead-beating cap from the previous step.
13. The cap should be tightly sealed and no air bubbles should be visible.  
(Redo if air bubbles are visible or the cap does not fully close.)
14. Vortex Falcon tube from step 3 with the remaining bead-cell solution to ensure even distribution of beads. Repeat steps 8 to 12 until Falcon tube is empty.
15. Place filled bead-beating tubes from step 12 and place on ice. Once two filled tubes have been collected and have been on ice for at least 1 minute, begin bead beating.
16. Beat one tube for 30 seconds at 46 rpm. Place upside down on ice for 30 seconds while beating the other tube.
17. Repeat previous step until it has been beat for 1 minute total.
18. Repeat steps 15 to 17 until all filled bead-beating tubes have been processed.
19. Remove cap from processed bead-beating tube and press micro-chromatography column firmly onto end of processed bead-beating tube until completely sealed. Snap off elution end of micro-chromatography column, and place micro-chromatography column, elution end down, into empty bead-beating tube.
20. Place this complex into 15ml Falcon tube with bead-beating tube cap placed up-side-down as support inside. Keep on ice when complete.
21. Centrifuge required filter apparatuses, with Falcon tube uncapped, at 6,000g for 5 minutes at 4°C.

22. Discard all turbid tubes, and transfer the pellet-free supernatant from non-turbid tubes into individual 1.75ml micro-centrifuge tubes. Keep on ice until all bead-beating tubes have been processed.
23. Centrifuge micro-centrifuge tubes at 12,000g for 10 minutes at 4°C.
24. Transfer supernatant into empty bead-beating tubes using a pipet, consolidating 500µl into a new bead-beating tube.
25. Place in a tissue culture tube and incubate with bead-beating caps removed, at 220 rpm, 37°C for 80 minutes.
26. Refer to Dialysis Protocol for completing the cell extract.