

# G1 arresting cells for block and release experiments

## Notes

- Strains must be prototrophic for this to work well. Some sick strains will not arrest effectively in G1.

- Arrested cells show mainly a 1C (G1) population but about 10-30% cells are 2C (G2). On release the G1 cells will do S phase first, but there is second burst of S phase (not usually obvious by flow cytometry) when the G2 population reaches S phase.

1. Grow strain in EMM to log phase ( $OD < 1$ )
2. Wash x2 in sterile water and resuspend in EMM-N at  $OD_{600}$  0.15-0.3. Measure OD.
3. Grow overnight (16h) at 26°C.
4. Next day measure OD - should have approximately doubled. Also check under the microscope for lack of contamination. Cells should all be v small.
5. Spin down cells and resuspend in EMM (or YES) and release at appropriate temperature. G1 arrested cells do S phase about 2.5 h after release in YES, EMM will be a bit later.