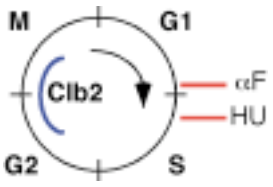
S. cerevisiae cell-cycle arrests



NOTE 1: While you can in theory use any of these arresting agents for cell cycle synchronization, in practice α-F is preferable since the cells release a lot cleaner. However all of these approaches are used in the literature (look at the cell cycle analyses on SGD; http://www.yeastgenome.org/).

α-Factor (as used in: Keogh *et al* (2006) *Genes Dev* **20:**660-5)

NOTE 2: α–Factor mating pheromone (THTLQLKPGQPMY) is available from Zymo Research (4mg: 10mM in 0.1M NaAc pH 5.2, 240µl). Store in aliquots at –20°C. For BAR strains this is used at ≈100µM; for *bar*Δ strains at ≈15nM (see MCK notebook pH13.127).

NOTE 3: To avoid blowing through the α-F, it is **STRONGLY** advised to make the MATa *ura3*⁻ strain *bar1*Δ. Prepare a qiagen prep of plasmid pΔ*bar1*::URA3 (YV143 / b2562, Amp^R). NB. The yield from this plasmid sucks, but you only need a few $URA3^+$ colonies and the HR efficiency is ≈ 100%. Cut plasmid with BamH1/BglII and high-efficiency transform appropriate yeast strain. Check URA3+ colonies by Halo assay.

Halo Assay: (see MCK notebook pH13.107) Innoculate test colony into 1ml YPD and grow 4hrs at 30°C (unless strain is *ts*) Collect by centrifugation and wash pellet with ddH_2O. Resuspend pellet (≈ 50µl packed cell volume) to 1ml. Dilute 1/100 and spot 50µl x 2 onto a YPD plate. Allow to dry and place a 7mm filter in the center of the spot. Place 10µl of 6 or 60µM α-F onto the filter, allow to dry and incubate 48hrs at 30°C. Compare the zone of clearance to a WT strain : *bar1*Δ strain should be much more sensitive to αF arrest.

Strains used for α-Factor (αF) synchronization were deleted for Bar1, the aspartyl protease used by *S.cerevisiae* to degrade the mating pheromone. αF was added (25nM final) to exponentially growing MATa *bar1*Δ cells in YPD (OD_{600} ≈ 0.3) and the culture incubated for two hours at 30°C. >95% G_1 arrest, as indicated by cells with small buds, was confirmed by microscopy (these cells are known as "schmoos"). Cells were collected by centrifugation at 4°C, washed twice with medium, and resuspended at OD_{600} ≈ 0.4 in 30°C YPD. Cultures were incubated at 30°C with 10ml samples taken for TCA extraction and western analysis every seven minutes for 126 minutes (WT cells should complete a cycle in ≈ 90 minutes under these conditions) (**NB. This is a long day**). Synchronized passage through the cell cycle was confirmed by immunostaining for the G_2/M cyclin Clb2 (kind gift from Adam Rudner, Cell Biology, HMS).

Hydroxyurea (depletes NTP levels, causing cells to arrest during DNA replication : S phase)

> **NOTE 4:** HU is also used as a DNA DSB inducing agent: with the lengthened S-phase there is a greater likelihood of a collapsed replication fork.

Make a 2M stock in ddH_2O: final is 100mM. Add to exponentially growing cells in YPD (OD_{600} ≈ 0.3) and incubate culture for two hours at 30°C. Confirm arrest by microscopy. Wash culture extensively to release.

Nocodazole (destabilizes microtubules preventing sister chromatin separation: G_2/M phase)

> **NOTE 5:** Nocodazole escape can occur (particularly with checkpoint mutants). Arresting cells for too long is eventually lethal. (Sigma M1404 >99%TLC; 10mg $54, 50mg $215). Nocodazole arrest is strain dependent (too much can induce break-through, results are also problematic at 37°C). For best results titrate your interest strain. In some cases it helps to add 50% more an hour or so into the experiment.

Make a 1.5mg/ml stock in DMSO; this is 100x. Make exponentially growing culture (OD_{600} ≈ 0.3) 1% DMSO before adding Nocodazole and incubate culture for two hours at 30°C. Confirm arrest by microscopy. Wash culture extensively to release.

Cyclin mutants (since different cyclins are required for cell cycle progression, their removal cause specific arrests).

Available:
YF159, WT (W303)
YF178, *cdc15-2* (Elion Lab; *ts*, G_2/M block, arrest 37°C 90m)
YF179, *cdc28-4* (Elion Lab; *ts*, G_1 block before START, arrest 37°C 90m)

Confirm appropriate strains and lack of revertants by spotting. Grow cultures in exponential phase at RT° (OD_{600} ≈ 0.5). Add hot media (65°C) to immediately shift temperature to 37°C and incubate culture: 37°C, two hours, 90m. Confirm arrest by microscopy. Wash culture and resuspend in RT° media to release.