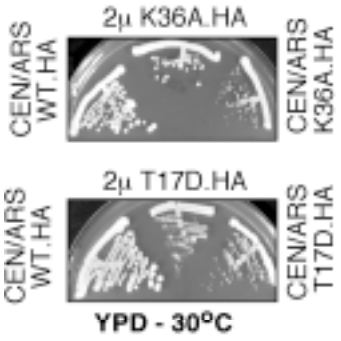
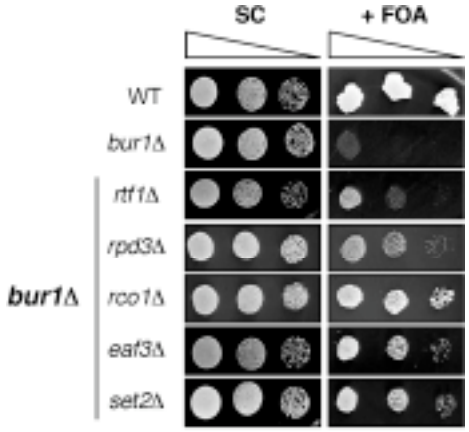
Spot testing *S.cerevisiae*

Note 1: Used to analyze growth phenotypes, spotting takes longer than streaking, but is preferable since it gives much better pictures. Compare the streaks in: Keogh *et al* (2002) *MCB* **22:**1288 with the spots in Keogh *et al* (2006) *Cell* **123:**593.





Note 2: Spotting requires relatively dry plates, especially important since multiple add-backs (see the **Media Recipes** page) are generally required. Move 10cm plates from 4°C to RT° a few days before the spotting, and do the add-backs the day before.

ADE, LYS, TRP, URA	300μl / plate
HIS	100μl / plate
LEU	500μl / plate

1. Harvest cells from **fresh plates** (large loopful; about 50μl packed cell volume (pcv)) or exponentially growing cultures. For the latter grow 5ml culture to $OD_{600} \approx 0.8$; assuming WT-like growth, 10AM: inoculate a loopful of a relatively fresh strain into YPD, should be right by 4PM. Collect by centrifugation: 5min, 2000rpm in a tabletop centrifuge (should get ≈ 100μl pcv).

2. Transfer the loopful of cells (or resuspend the pellet) into an ependorff containing 1ml ddH_2O. Flash spin to collect the cells by centrifugation (up to 10K, then stop) and resuspend pellet to 500μl.

Note 3: This is the time to balance multiple samples for cell density based on the pcv: 50μl to 500μl, 40μl to 400μl, etc

3. I generally spot four to six ten-fold dilutions (six is recommended although it's more work). Dilutions are done in a 96-well plate: aliquot 200μl ddH_2O per well, resuspend the cells from **step #2** and dilute 10 fold into the top well: 20μl cells into the 200μl ddH_2O. Ensure complete mixing and repeat the 10 fold dilution into the 2nd well immediately below. Repeat until you have six dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) for each test strain.

4. Align the first 10cm plate on the template (see over). Ensure the cells are completely resuspended in each well and spot 10μl (using a 20μl Gilson) of the highest dilution (10^{-6}) to the appropriate location on the grid: if you spot from highest to lowest dilution then you can use the same tip within each sample set. Once each plate is finished, gently replace the lid and move aside until spots have dried in.

Note 4: Spot drying shouldn't take longer than 10 minutes, although if the plates are still quite wet it can take much longer. Placing the plates lids-off in a laminar flow or beside a lit Bunsen will speed up the drying significantly.

5. Incubate plates inverted at the appropriate temperature. Examine and photograph at ≥40h.

Note 5: Multiple photographs are generally taken of each plate as appropriate
WT strain, YPD, 30°C or 37°C; optimal 40-48h (usually overgrown by 72h)
WT strain, YPD, 25°C; optimal spots 48-72h
WT strain, YPD, 12-14°C; check at 6-10 days
When testing genotoxins (check the **media** and **media additives** protocols) a good rule of thumb is to take the first photograph at 48-72h and every 24h thereafter until you have a feel for the optimal exposure time.

Note 6: see Hampsey M (1997) *Yeast* **13:**1099. This paper describes a compendium of phenotypes (and the plates to assay them) that can be easily screened to identify pleiotrophic defects associated with a mutation. In many cases a particular phenotype (or set thereof) can suggest a function for the gene under study.

Spot Templates: Copy these to size and align plates as appropriate for spotting.