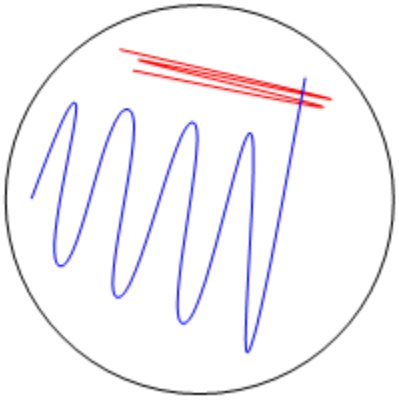
Yeast selection

AJ 21.05.19

Selection protocol: Gene knockout using Ura selection cassette

1) Plate cells on Ura-minus plates (select for presence of Ura cassette)
   -If you do not need to select for the Ura cassette, use YPD plates
   -If growing from glycerol stock, use a toothpick to streak in a C shape (do not let glycerol stock melt!)
2) Grow for 2-4 days at 30°C
3) Pick 4-8 colonies and re-streak onto fresh plates – grow for 2-3 days.
   a) Streak first in one direction
   b) Use a new tooth pick to streak in 2nd direction.



Notes:

- It is important to use a new tooth pick or otherwise growth will be too dense to obtain individual colonies.
- You often see some background growth at stage 2, in addition to individual colonies. Streaking from background growth failed to produce subsequent colonies (Book7, pg 62). This suggests that as long as the colonies were well separated all colonies from second streaking should be identical.

4) Assay for correct incorporation of construct at this stage.

Notes

- It is **important** to design the PCR test reactions in advance: assemble a sequence of the expected products and use it to work out what sized products you expect
- Include positive and negative controls that will tell you if PCR is working ok. (Same PCR from wt strain, same PCR from plasmid containing the region of interest, PCR which gives different length products depending on whether the insertion has occurred or not.
- Consider doing test assays on wt strain + one of the colonies you picked in 3 first to check if the assay is working, before trying it out on many colonies.
- At the end you have to check that the Ura has been hopped out: best way to do this is to have PCR primers that are outside the region that you used as a construct.

This should give a product of the right size. As a control you could do one of the reactions with a primer that points out from the Ura gene (A128, A129).

- Life becomes more complicated if you have many different constructs to assay. If for example I add lots of different tags to wt dynein and then want to make deletion constructs, it is better to carry out the deletion on the tagged strains rather than redoing the tags in the deletion background.

5) Pick a positive colony: grow 10ml o/n or during course of day
6) Pellet cells and plate onto 5'FOA plates (select for loss of cassette)
7) After 2 days re-plate cells on 5'FOA plates again.
8) Test a few colonies for correct gene modification:
   a) PCR direct from colonies – followed by sequencing.
   b) gDNA prep – PCR – followed by sequencing.
   c) Replica plate onto Ura^- and 5'FOA, and using frogger.

Notes:

- Keep the earlier plates, so that if there is a problem at this stage you can go back and try popping the ura gene cassette out from another construct.
- Cells kept on FOA plates become difficult to grow up after a long time. Store the cells on YPD plates.

Future : Ura replacement, by a second transformation/ FOA selection
: Kan selection