

Use of the Singer MSM system for determination of yeast generational life spans.

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Normally it is sufficient to measure the life-spans of 60-80 virgin cells in order to determine the mean and the maximal life span of any strain. At first you will find it easiest to grid these as 40 cells on each of 2 plates. As with tetrad dissection, the plates should not be too wet (i.e. dried at least 2-3 days on the bench)

Initially, thinly spread cells from a freshly-grown culture in the inoculum spread area to the left side of the plate, allowing these to grow a little. As soon as they are detachable from their mothers, 40 newly-formed buds (small virgin, first generation cells) are micromanipulated away from these mothers and gridded on the MSM matrix at positions A1-10, C1-10, E1-10 and G1-10.

These 80 cells are then observed **at regular intervals**, each bud (daughter cell) they form being manually detached, micromanipulated to another position on the matrix and recorded as a new daughter cell. It is convenient to move the daughters of the cell at A1 to B1; the daughters of the cell at A2 to B2; etc. (i.e. to use rows B, D, F and H of the matrix as depositories for daughter cells). We have found a sheet of A3-scale graph paper adjacent to the MSM to be convenient for recording the daughters as they are produced by each mother cell (the latter numbered according to its position on the matrix (A1, A2, etc.)).

Between each session on the MSM (involving viewing of mother cells, then detachment and scoring of daughters they produce) the plates are sealed with parafilm and incubated 1-2h at 30 °C. Alternatively they can be placed at 4 °C overnight (again sealed with parafilm), so as to arrest their growth until such time as you wish to continue with the life span analysis. **Do not forget to view your dividing mother cells after regular 1-2h intervals of growth**, or the cells overgrow and it is then difficult to identify the mother and count the number of daughters it has produced. Provided the mother cells have not undergone more

than 1-3 divisions there is usually no problem in identifying the mother and counting the number of daughters produced, provided it is always remembered that :

- (i) the mother is usually larger than the daughter; and
- (ii) the mother will enter the next cell cycle and therefore form a bud in advance of the last daughter that it has produced.

Eventually, after producing a variable number of daughters, each old mother cell becomes senescent – a state characterised by the cessation of budding. Sometimes it lyses. On other occasions the last bud produced is inviable, fails to enlarge and cannot be detached from the mother (only viable progeny should be counted). Thus towards the end of a life-span determination one is mainly using the MSM to scan from one senescent cell to the next, looking for the last few old mother cells that are still undergoing division.

With a little practice, it is possible to increase the number of cells whose life spans are analysed on a single plate. This can be done in various ways :

- (i) the spacing of cells analysed in rows A, C, E, G can be reduced from 6mm to 4mm; and
- (ii) at each position on the grid, the field of vision provided by the MSM is actually sufficient for more than one mother cell to be followed – placing upper and lower virgin cells in this field is a simple way of doubling the number of cells whose life spans are being analysed.

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