

# COMPETITION GROWTH ASSAY

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## DAY 1

- Set up 5ml O/N in appropriate Hv-broth (YPC or Ca, +Thy if needed)

## DAY 2

- Dilute O/N in fresh media, use 10  $\mu$ l (1/500), 5  $\mu$ l (1/1000) and 2.5  $\mu$ l (1/2000) for 5ml O/N

## DAY 3

- When OD = 0.4 ( $\sim 10^8$  cells), make serial dilutions of  $10^0$  to  $10^{-6}$  in 18% SW
  - For different OD values, see this [table](#)
- Either: Spot out 20 $\mu$ l of  $10^{-4}$  to  $10^{-6}$  of each strain on YPC (+ thy)
- Or: Plate out 100 $\mu$ l of  $10^{-5}$  ( $\sim 10^2$  cells) of each strain on YPC (+ thy)
  - Use  $10^{-5}$  for wild-type-like strains, for slower-growing strains use lower dilutions , e.g.  $10^{-4}$
- Inoculate the same 5ml Hv-broth (YPC or Ca, +Thy if needed) with:
  - 100 $\mu$ l WT strain at  $10^{-4}$  ( $\sim 10^3$  cells) and 100 $\mu$ l of slower-growing mutant at  $10^{-3}$  ( $\sim 10^4$  cells)
    - If mutant is not slow-growing, use 100 $\mu$ l at  $10^{-4}$  to inoculate culture
    - Optional: Plate out 100 $\mu$ l of combined culture on YPC (+ thy)
- Incubate at 45°C for two days

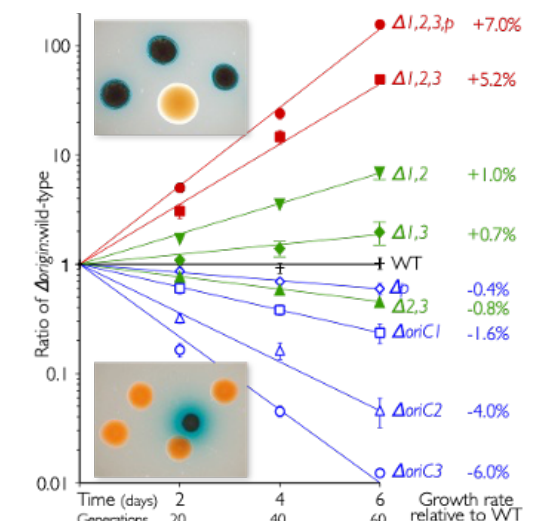
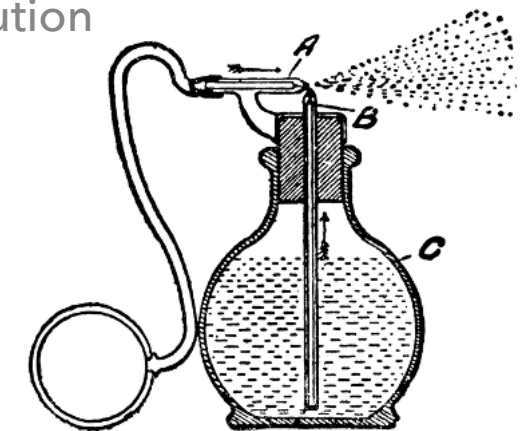
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## DAY 5

- When culture is at OD ~0.4 (~ $10^8$  cells) make serial dilutions of  $10^0$  to  $10^{-6}$
- Plate out 100 $\mu$ l of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  on YPC (+ thy), incubate at 45°C for 5 days
  - Normally, 100 $\mu$ l of  $10^{-5}$  (~ $10^2$  cells) is best, but depends on OD and growth rate of mutant
- Inoculate 10ml YPC (+thy) with 100 $\mu$ l of  $10^{-3}$  or  $10^{-4}$  dilution ( $10^4$  or  $10^3$  cells)
  - Depends on growth rates, if mutant is not slow-growing, always use 100 $\mu$ l of  $10^{-4}$  dilution

## DAY 7, 9, 11, ETC.

- Repeat dilutions and plating as on Day 5
  - At later timepoints, it will probably be necessary to use 100 $\mu$ l of  $10^{-4}$  dilution
- When plates have been incubated for 5 days at 45°C, spray with X-gal
  - Xgal is dissolved in 1-methyl-2-pyrrolidinone at 20 mg/ml
  - Spray in fume hood, solvent is harmful
- Incubate at 45°C overnight and count blue vs red colonies



CELLS PER ML @ OD650 VALUE

OD650	Cells/ml
0.022	9.00E+05
0.05	3.00E+06
0.09	1.20E+07
0.15	2.40E+07
0.25	8.00E+07
0.39	1.40E+08
0.86	3.50E+08
0.1	2.50E+07
0.2	5.00E+07
0.3	7.50E+07
0.4	1.00E+08
0.5	1.75E+08
0.6	2.50E+08
0.7	3.25E+08
0.9	4.00E+08

