GRADIENT PLATES

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DAY 1

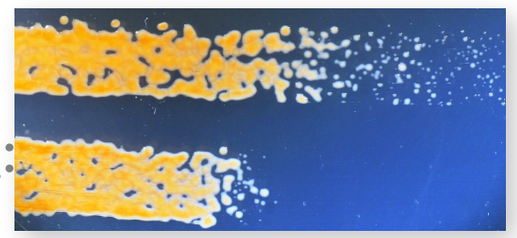
- Set up 5ml O/N in appropriate Hv-broth (YPC or Ca, +Thy if needed)

DAY 2

- Dilute O/N in fresh broth, use 10 µl (1/500), 5 µl (1/1000) and 2.5 µl (1/2000) for 5ml O/N

DAY 3

- When OD = 0.4 (~10^8 cells), make serial dilutions in 18% SW to 10^{-4} and 10^{-5}
  - For different OD values, see this table
- Use an autoclaved paintbrush to paint a line of cells on gradient plate:

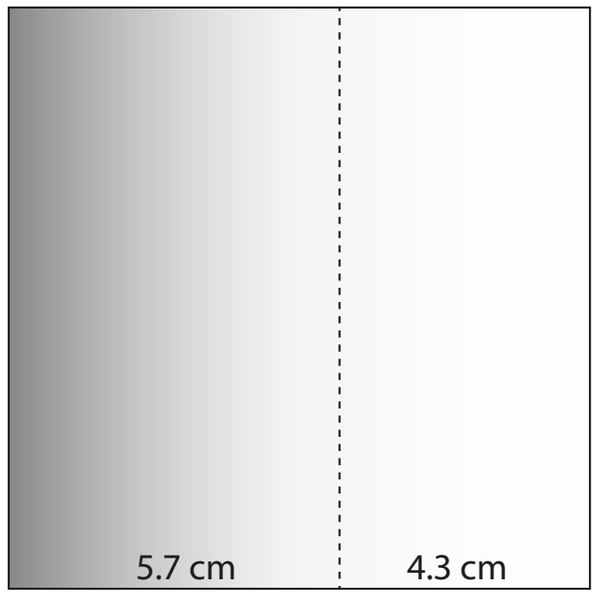


  - Autoclaved paintbrush must first be dipped into 18% SW to wet bristles
  - Paintbrush is then dipped into 10^{-5} dilution and painted in one direction across the gradient plate
  - Same paintbrush is dipped into 10^{-5} dilution again and painted over top of first streak in other direction
  - Use the same paintbrush for 10^{-4} dilution, in same manner, but on another gradient plate
- Incubate at 45°C for 4 or 5 days

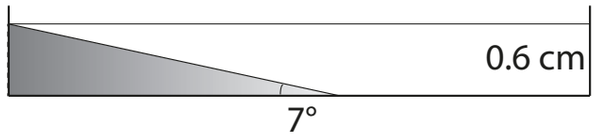
Bryson & Szybalski (1952); Hawkins et al (2013)

GRADIENT PLATES CONTINUED . . .
DAY 3

Square 10x10 cm
Total volume = 60 ml,
depth = 0.6 cm



- Prepare Hv-Ca+ura agar
  - Meanwhile warm shelf for casting square plates in plate dryer for >1 hour
- Prepare 16 square 10x10cm plates (4 plates for each Trp concentration)
  - Draw a line in the square plates to divide into 5.7 cm and 4.3 cm halves:
- Add tryptophan (10 mg/ml) to Hv-Ca+ura for 4 different concentrations:
  - 0.25 mM (1.7 ml to bottle of 333 ml)
  - 0.5 mM (3.4 ml to bottle of 333 ml)
  - 1 mM (6.8 ml to bottle of 333 ml)
  - 2 mM (13.6 ml to bottle of 333 ml)



17 ml Hv-Ca+ura+trp
43 ml Hv-Ca+ura

- Set up square plates on pre-heated shelf with 7° inclination (use upright plastic tube rack as prop), then pour a wedge of 17 ml of Hv-Ca+ura+trp on the left side (5.7 cm portion) of each plate
  - First pipette a small amount of agar along dividing line (drawn above), to wet the plate and break surface tension on the plastic, then carefully pipette remainder of 17 ml of of Hv-Ca+ura+trp on left side of plate to create wedge
- Once Hv-Ca+ura+trp agar wedge is solid, make shelf level and pipette 43 ml of Hv-Ca+ura agar on the right side of each plate, until the agar level is the same across the whole plate
  - Full gradient plates should be used ASAP; plates with just 17 ml Hv-Ca+ura+trp agar wedge may be stored at 4°C

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