DNA DAMAGE ASSAYS

MMC PLATES

- Add dH_2O to MMC vial to a final concentration of 1mg/ml, store at -20˚C
  - Inject dH_2O through rubber stopper to dissolve powder. Take care, MMC is very toxic
- Make YPC plates as usual
- After autoclaving and cooling, but before pouring, add $CaCl_2$, and MMC (1mg/ml stock solution) to following concentrations:

+ µl	Final concentration (µg/ml)
33.3	0.02
24.9	0.015
16.6	0.01
8.3	0.005
4.16	0.0025

- Pour plates
  - Note: use freshly made MMC plates within 2 days

MMC ASSAY

- Set up 5ml pre-overnights
  - Dilute overnight into fresh Hv-YPC (5ml) and grow to an A_{650} of ~0.4
- Make serial dilutions (10^{0}-10^{-7}) of cells in 18% SW
- Spot duplicate 20µl samples onto Hv-YPC with 0-0.02 µg/ml MMC
  - Air dry plates for 30 minutes
- Incubate at 45˚C for 4-7 days, counting colonies every day
- Note: use freshly made MMC plates, no more than 2 days old

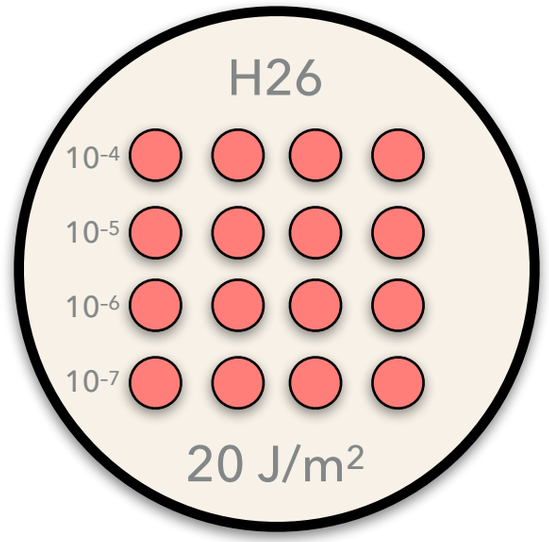
UV ASSAY

- Set up 5ml pre-overnights from plates
- Dilute overnight into fresh Hv-YPC and grow overnight to an A_{650} of ~0.4
  - Use 20µl into 5ml for strains with wild-type growth rate, 50µl into 5ml for slow-growing strains
- Make serial dilutions (10^{0}-10^{-8}) of the cells in 18% salt water
  - Dilute 100µl into 1ml of 18% SW, invert tube ~10 times to mix
- Spot 4x 20µl samples onto Hv-YPC (1 plate for each irradiation, including '0 sec' control)
  - See figure and text on next page for appropriate dilutions
- Air dry plates for 30 minutes, turn UV on to warm up
  - You can use the UV Cross-linker instead. Turn on a couple of minutes prior to use and do a dummy 150J/m^2 dosage first
- Irradiate batches of plates for 100, 80, 60, 40, 20, and 0 secs
  - If using cross-linker, select "ENERGY" and then type desired UV dose, then hit "ENTER". Finally select "START"
  - Units are calibrated as J/m^2; so typing in 100 will provide a 100 J/m^2 dosage
- Incubate in a black bag at 45˚C for 4-7 days, counting colonies every day

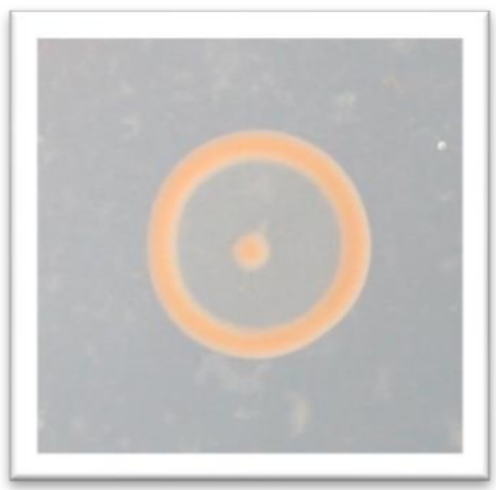
UV ASSAY: POINTS TO NOTE

- Suggested dilutions to spot at each UV dose

0	20 J/m²	40 J/m²	60 J/m²	80 J/m²	100 J/m²
10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}
10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}



- Using cross-linker is a good alternative to UV lamp
  - Irradiation is done at a high dosage for a short period of time
- However, *Haloferax* rings appear above 100J/m²
  - Makes counting of single colonies difficult (see Callum's thesis)



4NQO DILUTIONS

- Add DMSO to 4NQO vial to final concentration of 1mg/ml, store at -20˚C
- Make the 10-40 µg/ml stock solutions in DMSO
- Add 20µl of stock solutions to 1 ml culture to get the following final concentrations
- Carry out acute exposure in liquid assay

Stock solution (µg/ml)	Final concentration (µg/ml)
10	0.2
20	0.4
30	0.6
40	0.8

MMC LIQUID ASSAY DILUTIONS

- Add dH_2O to MMC vial to a final concentration of 2mg/ml store at 4˚C
- Make the 4-200 µg/ml stock solutions in dH_2O
- Add 12.5µl of stock solutions to 5 ml culture to get the following final concentrations

- Carry out acute exposure in liquid assay

Stock solution (µg/ml)	Final concentration (µg/ml)
4	0.01
10	0.025
20	0.05
40	0.1
100	0.25
120	0.3
160	0.4
200	0.5

ACUTE EXPOSURE IN LIQUID

- Grow cells to an OD of 0.4 - 0.6
- Add DNA damaging reagent (MMC or 4NQO) at a variety of concentrations, including 0 µg/ml control (DMSO or H_2O)
- Incubate for 1 or 3 hours
  - To see transcription and translation effects respectively
- Make serial dilutions (10^{-1} to $^{-8}$), spot 20 µl onto YPC plates
  - Air dry plates for 1 hour
- Incubate at 45˚C for 4-7 days, counting colonies every day

MMS DILUTIONS

MMS IS HIGHLY VOLATILE AND VERY TOXIC. USE CORRECT PPE!

- Take MMS vial (Sigma M4016, cas: 66-27-3) and prepare 5% stock in 18% SW
  - 50 µl of MMS + 950 µl of 18% SW
  - This must be carried out fresh, in fume hood!
- Then dilute MMS:

Dilution	5% MMS stock (µL)	18% SW
1:5	20	80
3:10	30	70
2:5	40	60
1:2	50	50

MMS ASSAY

MMS IS HIGHLY VOLATILE AND VERY TOXIC. USE CORRECT PPE!

- Day 1: Set up 5ml pre-overnights from plates
- Day 2: 1/10 dilution of overnight into fresh Hv-YPC (5ml) in the morning
  - In the afternoon, dilute cultures so they grow to an A_{650} of ~0.6 by Day 3
- Day 3: Aliquot overnight cultures (OD600 of ~0.6) into 4x1ml (in 4ml culture tubes)
  - Add 20µl of diluted MMS to 1mL of aliquoted culture and incubate for 1 hour
  - Spin cells at 6000 rpm at RT for 8 mins in 2mL eppendorfs
  - Resuspend cells in 1mL YPC
  - Make serial dilutions (10^{0}-10^{-7}) of the cells in 18% salt water
  - Spot duplicate 20µl of cells onto Hv-YPC
  - Air dry plates for 1 hour
  - Incubate at 45˚C for 4-7 days, counting colonies every day