Overnight culture start at **1300**. Perform serial dilution (x1, x5, x25, x125, x625, x3125).

x125 was in log phase (0.55).

Get out at **0900**.

Put in spectrophotometer around **1200**.

0.55 OD working yeast stock.

The log phase wasn’t lasting long enough when yeast stock was diluted to 0.05 (x1). To find optimal initial yeast stock density, x5, x10, x20, x50 and x100 dilutions were performed. One of these should extend the log phase sufficiently to around 2-4h.

For pipetting – Add media, then treatment, then finally yeast stock and mix. Shake (vortex) yeast stock falcon tube before each yeast stock dispensing.

Don’t cover with parafilm. (increases OD blank too but didn’t want to risk it).

Repeat this expt on 5 separate days so n=5. Take mean of 4 replicates from each day. Use replicate means for statistical analyses (SD, p value).

**For control (replicates = 4):**

**10µL yeast stock + 90 µL media**

**For DMSO 1% (Treatment 1):**

**1µL DMSO + 10µL yeast stock + 89 µL media**

**For Methanol 1% (Treatment 2):**

**1µL Methanol + 10µL yeast stock + 89 µL media**

**For DMSO 1% + Methanol 1% (Treatment 3):**

**1µL DMSO + 1µL Methanol + 10µL yeast stock + 88 µL media**

Start 1030. End 0230 (16h)

BMG PlateReader Settings:

195 cycles, cycle time 300s, shaking double orbital 200rpm time 20s before each cycle. (16h 15min (cycles) + 65min (shaking) = 17h 20min)