DATE: 6/8

Toxicity estimation of PCB 6/8

Culture we need:

200 µl /well * (3*12 + 6 = 42 wells) = 8400 µl = 8,4 ml

OD of overnight WT culture:
0,244* 10 → OD_600 = 2.44

2,44 * x = 0,1 * 20 ml => x= 0,8196 = 819,6 µl

YPD= 19,1804 ml

OD_600: 0.142 OK!

We decided to use the following dilutions of PCB.

D1:

5µl PCB (original PCB: 0,2 µg/µl)
195 µl H2O
tot: 200 µl ---> conc. 5000*10^-6 µg/µl

D2:

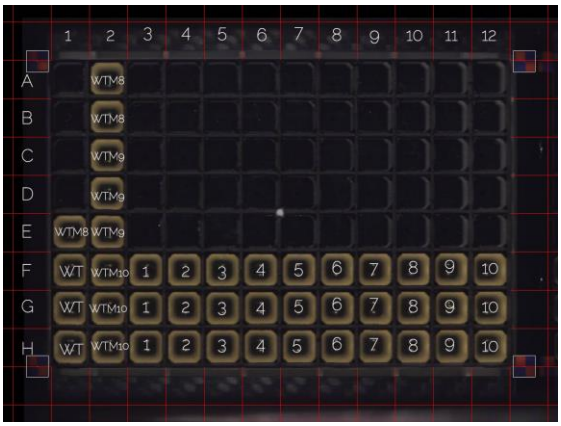
4µl from D1
196 µl H2O
tot: 200 µl ---> conc. 100*10^-6 µg/µl

D3:

5µl from D2
195 µl H2O
tot: 200 µl ---> conc. 5000*10^-6 µg/µl

Label	Concentration PCB (*10^-6 µg/µl)	Methanol (µl)	PCB (µl)	From dilution	Mq water(µl)	Cells (µl)
1	0,01	-	2,5	D3	47,6	200
2	0,1	-	25	D3	25	200
3	0,5	-	1,25	D2	48,8	200
4	1	-	2,5	D2	47,6	200
5	10	-	25	D2	25	200
6	100	-	5	D1	45	200
7	500	-	25	D1	25	200
8	1000	-	1,25	PCB bottle	48,8	200
9	5000	-	6,25	PCB bottle	43,8	200
10	10000	-	12,5	PCB bottle	37,6	200
WT	-	-	-	-	50	200
WTM8	-	1,25	-	-	48,8	200
WTM9	-	6,25	-	-	43,8	200
WTM10	-	12,5	-	-	37,6	200

Here is how we loaded the wells:



After 1,85 days the measurement was done and data were collected in a specific excel document (Drive: data). The cells had nice growth curves for all concentrations of PCB.

Some notes about PCB measurement in the future:

- Don't touch the bottom of the special rack, if this is done. Wipe the bottom softly with some tissue.
- PCB should be kept from light.
- The fumehood in balance room is not sterile. Add everything else in another fume hood and then move to the balance room when adding PCB.

DATE: 9/8

Toxicity estimation of PCB 9/8
The culture used for the experiment was the same culture as the last time. The falcon tube with culture was taken from the 4C room after being kept there for 3 days.
The OD was measured for the culture with a 5x dilution:
OD600= 0,065 which means that the culture without any dilution have OD600=0,325.

Wanted a final volume on 16 ml just to be sure, if something happens.
0,325*X=0,1*16000µl
X=4923 µl from original culture.

11077µl YPD and 4923 µl original culture made the new culture and a new OD measurement of:
OD600= 0,093 which is close to 0,1, good enough. 1 ml of new culture was used for OD which means we have 15 ml left.

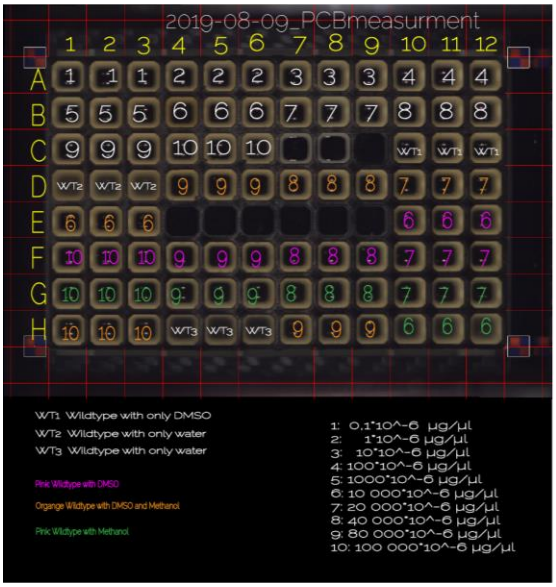
Two different dilutions of PCB mixture was made:

D1*: concentration of PCB: 1000*10^-6µg/µl
Made of 1 µl PCB and 199µl DMSO.

D2*: concentration of PCB: 10*10^-6µg/µl
Made of 2 µl from D1* and 198µl DMSO.

Cells, water, Methanol and DMSO was added to the special rack in sterile fume hood. PCB was diluted and added in fume hood balance room (note not sterile).
The fume hood in balance room is not sterile but the cells should be fine because they are in the exponential phase.

The solutions were added to the rack in according order (see below). Total volume 250µl.



Label	Concentration PCB (*10^-6 µg/µl)	PCB (µl)	From dilution	Mq water(µl)	Cells (µl)
1	0,1	2,5	D2*	147,5	100
2	1	25	D2*	125	100
3	10	2,5	D1*	147,5	100
4	100	25	D1*	125	100
5	1000	1,25	PCB bottle	148,5	100
6	10 000	12,5	PCB bottle	137,5	100
7	20 000	25	PCB bottle	125	100
8	40 000	50	PCB bottle	100	100
9	80 000	100	PCB bottle	50	100
10	100 000	125	PCB bottle	25	100

Label	DMSO	Methanol	Mq water(µl)	Cells (µl)
WT1 (DMSO)	100	-	-	100
WT2 (water)	-	-	100	100
WT3 (water)	-	-	100	100
WTM 10	-	125	25	100
WTM 9	-	100	50	100
WTM 8	-	50	100	100
WTM 7	-	25	125	100
WTM 6	-	12,5	137,5	100
WTDM 10	25	125	-	100
WTDM 9	50	100	-	100
WTDM 8	100	50	-	100
WTDM 7	125	25	-	100
WTDM 6	137,5	12,5	-	100
WTD 10	125	-	25	100
WTD 9	100	-	50	100
WTD 8	50	-	100	100
WTD 7	25	-	125	100
WTD 6	12,5	-	137,5	100

After two days (12/8) the OD measurment was done and everything (mostly) failed. Really weird growth profiles and only a few WT (without PCB succeeded). This may be an effect from that the culture was in 4C room for 3 days and the cell were dead before the experiment started. This experiment should be repeated and we should replate the WT strain so we have one plate (patch/single colony) where we can take repeated cultures from.

DATE: **26/8**

Overnight culture for PCB experiments

Wild type strains of both URA and G418 yeat wild types were inoculated for transformation next day

DATE: **27/8**

PCB growth experiment

In order to find out the cause of failure in previous growth profiler experiment, this experiment was designed. 1 ml culture was inoculated in a cuvette pre-cleaned with ethanol.

PCB concentration in methanol - 10 ng/µl

time (hr)	URA WT + 50 µl PCB + 300 µL DMSO	URA WT + 200 µl methanol + 300 µl DMSO	NAT WT + 50 µl PCB + 300 µL DMSO	NAT WT + 200 µl methanol + 300 µl DMSO
0	0.1.5	0.105	0.107	0.107
0.5	0.115	0.115	0.103	0.103
2.5	0.169	0.169	0.168	0.168
3.5	0.129	0.08	0.075	0.059
5.5	0.146	0.106	0.150	0.1

DATE: **28/8**

Toxicity estimation of PCB again (Moa, Ellen)

Used two different cultures: WT ?? and WT ??, calles WT1 and WT2, respectively. The cells' OD was measured and they were resuspended in new YPD media in the morning according to table 1:

Table1

	WT1	WT2
cell culture		

YPD was added to reach a total volume of 15 ml. After having been kept in the 30 degree room for approximately 3-4 hours the OD was measured again which resulted in:

Table 2

	WT1	WT2
OD	0.513	0.698

Two different dilutions of PCB mixture were made:

D1*: concentration of PCB: 1000*10^-6µg/µl
Made of 1 µl PCB and 199µl DMSO.

D2*: concentration of PCB: 10*10^-6µg/µl
Made of 2 µl from D1* and 198µl DMSO.

Cells, water, Methanol and DMSO were added to the growth profiler rack in sterile fume hood. PCB was added in fume hood balance room. See table 3 for volumes regarding the PCB concentrations (WT1+PCB, WT2+PCB):

Table 3

Label	Concentration PCB (*10^-6 µg/µl)	PCB (µl)	From dilution	Mq water(µl)	Cells (µl)
1	0,1	2,5	D2*	147,5	100
2	1	25	D2*	125	100
3	10	2,5	D1*	147,5	100
4	100	25	D1*	125	100
5	1000	1,25	PCB bottle	148,5	100
6	10 000	12,5	PCB bottle	137,5	100
7	20 000	25	PCB bottle	125	100

See table 4 for the cells with DMSO and methanol (WT1+DMSO+MetOH, WT2+DMSO+MetOH):

Table 4

Row	MetOH [microL]	DMSO [microL]	Cells [microL]
A	100	50	100
B	50	100	100
C	25	125	100

See table 5 for cells with MetOH and water (WT2+MetOH, WT2+MetOH):

Table 5

Row	MetOH [microL]	MQ [microL]	Cells [microL]
F	25	125	100
G	25	125	100

See table 6 for cells with DMSO and water (WT1+DMSO, WT2+DMSO):

Table 6

Row	DMSO [microL]	MQ [microL]	Cells [microL]
D	25	125	100
E	25	125	100

For the wells labeled only WT1 and WT2 (A,B,C,11,12) 100 microL of cells were added to 150 microL of MQ-water.

TABLE 7

	Type ➡	WT1+ PCB	WT1+ PCB	WT1+ PCB	WT2+ PCB	WT2+ PCB	WT2+ PCB	WT1+ DMSO +MetOH	WT1+ DMSO +MetOH	WT2+ DMSO +MetOH	WT2+ DMSO +MetOH		
[PCB]	Column ➡ / Row ⬇	1	2	3	4	5	6	7	8	9	10	11	12
0.1	A	50 mL DMSO+ 147.5 L H20										WT1	WT2
1	B											WT1	WT2
10	C											WT1	WT2
100	D											WT1 + DMSO	WT1 + DMSO
1000	E											WT2 + DMSO	WT2 + DMSO
10000	F											WT1 + MetOH	WT1 + MetOH
20000	G							0.1 WT1+ PCB				WT2 + MetOH	WT2 + MetOH

The well A1 was messed up and instead the well G7 was used for WT1-cells with 0.1*10^-6 microg/microL. There were no cells added towell A1.

See table 7 for placement in the growth profiler rack. The rack was put in the growth profiler at 4 pm on the 28th.

Overnight culture

Wild types strains both URA marker and Nourseothricin yeast were inoculated for next day