**PBR ICL Exp4: Experimental set-up**

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**Chlamydomonas preparation:**

(Start at least 10 days before the start of the PBR)

* Inoculate a scoop of Chlamy from a TAP agar plate into a flask containing 50mL of TAP media.
* Incubate 3 days under continuous shaking and constant illumination.
* Determine the cell concentration using a 40 uL aliquot in the Beckman cell counter.
* Collect the volume required to get 3250000 cells.
  + 3250000 = 65000 cells/mL \* 50 mL
* Centrifuge and wash twice using TP to remove TAP traces.
* Resuspend the Chlamy pellet into a flask containing 50 mL of TP.
* Incubate 7 days under continuous shaking and constant illumination.

**SynCom preparation:**

(Start at least 5 days before the start of the PBR)

* Scoops of bacteria were transferred to 250uL of TSB50% in a 96-well (from plates that were incubated in the cold room for couple of days)
* Incubate for 48h in shaker
  + In exp4 we incubated the plate for 72h
* All bacteria that grew over OD=0.75 were refreshed by adding 50uL of bacterial culture to 200uL of fresh media in a new 96-well plate. **Note\_1: We usually use OD=0.5 but as we incubated the plate 24h extra we will use 0.75**
  + Using multistep 200 uL of TSB 50% were added to each well that would receive the 50 uL
  + Then the 50 uL of bacterial culture were added
* All bacteria that grew below OD<0.75 (low density; see Note\_1 above) were re-transferred by adding 200uL bacterial culture to 50uL of fresh media. In this way, we hope to have all bacterial strains actively growing by inoculation day
  + 200 uL of bacterial culture were added to their corresponding empty wells
  + Afterwards 50 uL of TSB were added to each of the low density wells.
* Incubation for 24h in incubator (25°C, 180rpm)
* The list of bacteria used, plate set-up (\biodata\ dep\_psl\grp\_rgo\ICL\PBR\exp4\PBR\_ICL\_exp4-Syncom\_plate.tsv) and OD measurements (\biodata\ dep\_psl\grp\_rgo\ICL\PBR\exp4\PBR\_ICL\_exp4-Syncom\_plate-Pre\_1st\_dilution.xlsx)

**INOCULATION DAY**

**BACTERIA**

* Measure OD600nm using Tecan
* Wash cells two times by:
  + Centrifugation <3200g x 10 min
  + Resuspension in TP
* Incubate for 4-24h (25°C, 180rpm)
* Measure OD600nm using Tecan
* Harvest an aliquot corresponding to an OD 0.002 for each strain and pool in a single tube.
* Determine the cell concentration using a 40uL aliquot in the Beckman Cell counter

**CHLAMY**

* Wash two times Chlamy cells by centrifugation at 6000rpm for 5min, and resuspension in 10 mL of TP
* Measure cell counts by using a 40uL aliquot in the Beckman Cell counter
* Dilute Chlamy cells to X
* The final bact:Cham ratio to use will be (**1:3.2**)

INOCULATION

* In the mixing bottle add in the following order:
  + TP
  + Syncom
  + Chlamy
* Distribute 85 mL of the mix to each tube