P(3HB) Synthesis Labbook

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Contents

12/08/13 - Preparation of P3HB synthesis -phabc

Used inoculating loop to streak ABC transporter (BBa_K258008) on 1 AMP plate. no cells

Streak phaA (BBa_K338003) on 2 KAN plates. cell grew.

Streaked phaBC (BBa_K338004) on 2 KAN plates. no cells.

All plates are cultured at 37 degree C for O/N culture

13/08/13- Preparation of P3HB synthesis

Mistakes made, phaBC should be on AMP plate. No need to streak ABC transporter, as Margarita and Jemma already have overnight culture for this. Streak ABC recognition domain LARD1 on AMP plate instead.

Wrong:

- streaked phaBC (BBa_K338004) on 1 KAN plate, without stabbing the agar.
- streaked phaBC on 1 KAN plate, stabbed the agar.
- streaked ABC transporter (BBa_K258008) on 1 AMP plate, without stabbing the agar.
- streaked ABC transporter on 1 AMP plate, stabbed the agar.
- all plates are cultured at 37 degree C for day culture.

Correct:

- streaked phaBC (BBa_K338004) on 1 AMP plate, stabbed the agar.
- streaked LARD1 (BBa_K258001) on 1 AMP plate, stabbed the agar.
- all plates are cultured at 37 degree C for day culture.

O/N culture

- LARD1 (BBa_K258001) in AMP
- phaA (BBa_K338003) in KAN
- phaBC (BBa_K338004) in KAN/AMP/CHLORA - Throw me a bone HERE, c'mon!

14/08/13

Sample Name	Sample Type	Biobrick Number	DNA conc (ng/ul)	Person who made sample	Absorbance
LARD1	miniprep	BBa_K258001	9.4	Matthew Chin	0.57
phaA	miniprep	BBa_K338003	19.7		1.76

Transformed T10 competent cells with 3µl of part phaABC (BBa_K934001).

15/08/13

Picked 2 colonies of the transformed cells, day culture. Not enough cell grew.

Picked another 2 colonies of the transformed cells, overnight culture.

Used LARD1 and phaA glycerol stock for overnight culture, because the previous ones have grown for 20 hours.

16/08/13

Made glycerol stock for phaABC colonies 3 and 4, LARD1 and phaA, and miniprepped them.

LARD1, phaA and phaABC colony 4 have relatively good quality and will be sent for sequencing.

19/08/13 - J - Growth Media

300mL in LB

300mL in LB with waste conditioned media

prepared 6 flasks for sterile filtration of media from each of above for 100mL in each

Also: 1g SRF in 25mL water raises volume to 27mL.

O/N culture for phaABC glycerol stock

O/N culture for stress response MG1655 colonies DEF

20/08/13

Prepared waste conditioned media (WCM), protocol see wiki page.

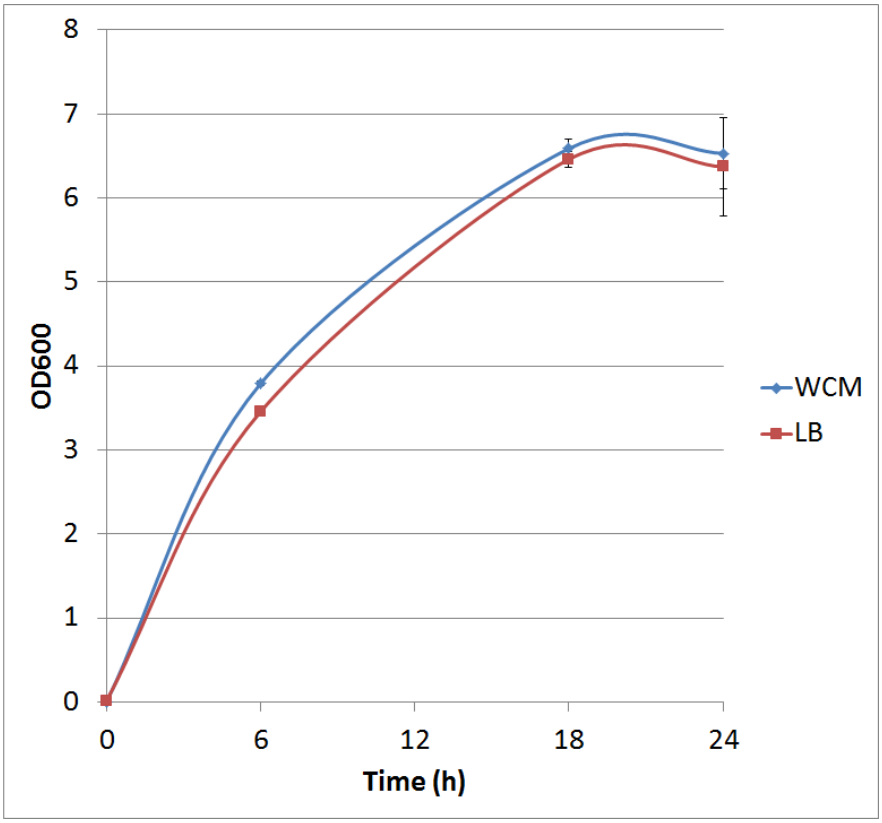
Added 1 ml stress response colony D E F to 6 flasks, 3 flasks contained 100 ml WCM, the other 3 contained 100 ml LB. Measured absorbance for time points 0 and 1.

For measuring absorbance, WCM and LB are both diluted by factor 2 at time point 1. Blanks are WCM and LB without cells. Results are shown below:

OD600	0 h	6 h	18h	24h
WCM (D)	0.009	3.902	6.515	6.428
WCM (E)	0.003	3.71	7.02	5.14
WCM (F)	0	3.766	6.23	8.028
LB (D)	0.019	3.558	6.83	8.032
LB (E)	0.017	3.452	6.225	4.096
LB (F)	0.016	3.364	6.325	6.984

The stress response cell cultures are mixed in the bottle containing SRF.

	0h	6h	18h	24h
WCM	0.004	3.792667	6.588333	6.532
LB	0.017333	3.458	6.46	6.370667



Growth curve - phaABC cell growth on glucose

Prepared 4 different concentrations of glucose in LB.

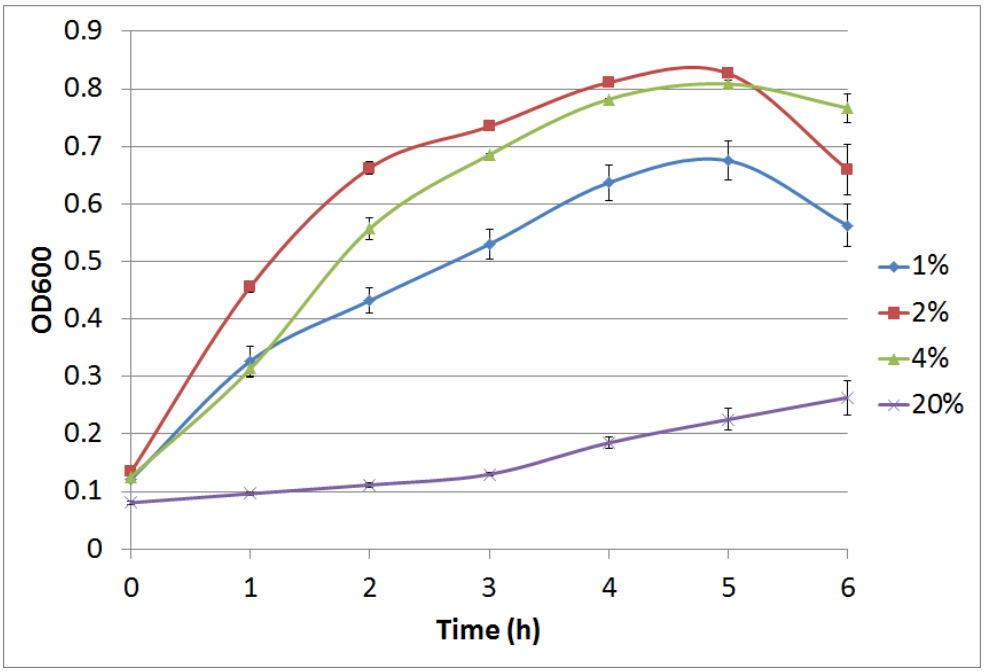
- LB(already contains 1% glucose) + 0 glucose
- LB + 0.2 g glucose
- LB + 0.4 g glucose
- LB + 2 g glucose

Added 5 microlitre of chloramphenicol in each tube.

Used LB as the blank, obtained OD600=3.26, diluted by the factor of 3.26 to obtain OD600=0.05

Therefore, within 800 microlitre to be pipetted in each well on the 96 well plate, there should be 245.4 microlitre of phaABC culture + 554.6 microlitre of LB.

Results are shown in another doc.



21/08/13

Measured OD600 of WCM containing colonies DEF and LB control.

Serial dilutions of stress response cells to work out CFU.

Dilution	LB (microlitre)	SR cells(microlitre)
10^{-2}	1980	20 from stress response cells from O/N culture
10^{-4}	1980	20 from 10^{-2}
10^{-5}	4500	500 from 10^{-4}
10^{-6}	4500	500 from 10^{-5}
10^{-7}	4500	500 from 10^{-6}

Pipetted 200 microlitre of each dilution on a C plate and cultured them at 37 degrees C overnight. result: obtained 59 colonies for dilution 3.

To convert to CFU/mL $= \frac{\text{Colonies}}{\text{volume pippeted from each dilution on plates}} \times \text{dillution} = \frac{59}{0.2} * 100,000 = 2,950,000\text{CFU/mL}$.

22/08/13 - linearise the DNA and cut phaA out from phaABC

- phaB forward primer ™= 60.9 degree C
- phaC reverse primer ™= 59.9 degree C

for Pfu, T= 59.9-5= 55 degree C

Forgot to cancel gradient, but the tubes were in the middle, so should be fine.

Target size= 4200 bp (phaABC) - 1000 (phaA) + 2000 (plasmid) = 5200 bp

Time= 15 * (5200/1000)=75 sec

made gel, stored in fridge.

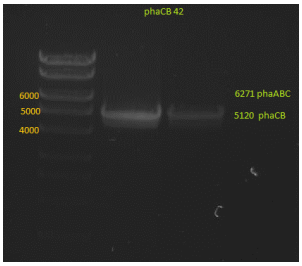
stored DNA in fridge.

23/08/13

Gel electrophoresis

gel electrophoresis of phaBC sample 1. Extracted DNA from the the gel.

sample phaBC 41: 8ng/microL, A260/A280=1.2.



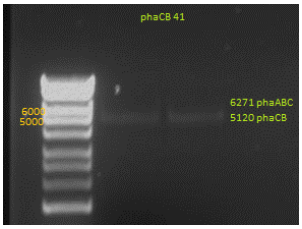
PCR and gel electrophoresis of phaBC sample 2. Extracted DNA from the gel.

Sample phaBC 42: 12.6 ng/microL, A260/A280=1.86

Ligation of phaBC to form circular DNA

plasmid 2070+ phaBC 2950= 5120

plasmid 2070+ phaABC 4201= 6271



Nile red plates observation

Looked at the 3 existing phaABC nile red plates - these have been incubated for 36 hours - no PHB so far. They are now in the fridge - will look at them again in 2-3 days

Iain streaked pha ABC at 12pm 22/08 - these will need to be out of the incubator and into the fridge 9am tomorrow

26/08/13

Transformation

Transformed circularised phaBC 41 and 42 in NEB 10 cells. Only phaBC41 plate has colonies.

27/08/13

Transformation

Transformed phaABC4 and phaBC41(ligation product) and MCherry in MG1655 cells. Colony merged on phaABC4 plate. No colony on phaBC41 plate. Many colonies on MCherry plate.

O/N culture

Prepared overnight cultures for MCherry (MG1655 using the glycerol stock), phaABC4 (T10), pha41 (NEB10), PLC 7 and 11 (NEB10). Controls are PLC 4 (NEB10) and promoter and RBS construct (T10, from glycerol stock)

28/08/13

Used 500 uL for PBS + waste.

Prepared glycerol stock for phaABC(T10), MCherry(MG1655) and phaBC41(NEB10).

Miniprepped Mcherry(MG1655) and phaBC41(NEB10).

Plated out PhaABC4(T10) and promoter and RBS construct(as control) onto nile red plate with 3% glucose(incubated at 1.30pm, take out at end of day on 29/08)

Transformed promoter+RBS, phaABC4 and phaBC41 into MG1655.

PhaABC4 colonies were viewed under the fluorescent microscope and images taken, a control was compared. The control was a colony without PhaABC4(not specified which) on a non-nile red plate which is not really comparable. Hence why we plated new PhaABC4 and the promoter/RBS on nile red plates for comparison. On the slides they were in the order control, Jemma's plate from two days before mine and then mine, this is also the order they were photographed in.

29/08/13

Spread the rest of phaABC4 and phaBC41 transformation mixture on plates.

Prepared O/N culture for phaABC4 colonies A and B, phaBC41 colonies A and B, promoter+RBS colony A, MCherry colony A.

PhaABC4 vs. Promoter/RBS on nile red plates. PhaABC4 colonies fluoresce orange whereas the promoter/RBS colonies do not. The plates were imaged on a plate reader, Richard has the of the wavelength and machine name.

Impranil, PHB, Polydega and PLA plates were made. Within each 3 sets of plates were made, some induced by arabinose, some by xylose and some uninduced. The Impranil plates were induced with less xylose than they should have been, 0.22g instead of 2.2g.

30ml Impranil emulsion and 20ml Polydega, PHB and PLA emulsions were added to 300ml LB agar. Before dividing this into the 3 sets below, 165ul chloranphenicol was added to Impranil and 160ul to the rest to give a 1 in 2000 dilution.

100mL	Arabinose(g)	xylose(g)	control
Impranil	0.23	2.3	0
Polydega	0.21	2.1	0
PHB	0.21	2.1	0
PLA	0.21	2.1	0

30/08/13

Shaker did not work. Cells in the O/N cultures might be dead.

O/N culture

Prepared O/N culture for phaABC4 colonies C and D, phaBC41 coloneis C and D, promoter and RBS construct colony B

31/08/13

Glycerol stock

Prepared glycerol stock for the O/N culture from 30-08-13.

Growth assay

Produced 6-timepoint growth assay for multiple concentrations of 3HB, P(3HB), acetoacetate, and ethylene glycol, and one concentration of glucose and PLA.

The results are shown in the graph.

3HB solutions were prepared in advance. An initial stock of 100mM 3HB was made from 0.315g 3HB dissolved in 25 mL ddH2O. This was then diluted down to 10mM, 1mM, 100µM, 1µM working solutions in LB. Furthermore, 3% glucose in LB was prepared with solutions made from 3g glucose in 100 mL LB.

O/N cultures were prepared, these were diluted to OD=0.05 by measuring $\frac{O/NOD}{0.05}$. With this complete the required dilutions of cells were made into 1.5 mL reaction tubes to give a total volume of 800 µL, this was then divided up into 4 wells for quadruplicate technical repeats. Cells used were phaABC, which is involved in P3HB synthesis from glucose, phaBC which synthesises P3HB from acetoacetate and EV empty vector which was used as a growth control.

	LB pha ABC	10mM 3HB LB pha ABC	1 mM 3HB LB pha ABC	100µM 3HB LB pha ABC	1µM 3HB LB pha ABC	LB phaBC	10mM 3HB LB phaBC	1 mM 3HB LB phaBC	100µM 3HB LB phaBC	1µM 3HB LB phaBC	LB	10mM 3HB LB
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												
	1 mM 3HB LB	100µM 3HB LB	1µM 3HB LB	3% glucose phaBC	3% glucose	3% glucose pha ABC	3% glucose EV	LB EV	10mM 3HB LB EV	1 mM 3HB LB EV	100µM 3HB LB EV	1µM 3HB LB EV

With the plate loaded, it was then placed in a shaking incubator at 700 rpm, 37°C for 30 minutes. After this, we took readings using a robotic plate reader for t=0 at OD600, then repeated this over a further 7 hours.

A second plate was prepared to measure acetoacetate as a sole carbon source. Lithium acetoacetate was prepared by placing 10 mg in 9.2 mL water to give a stock concentration of acetoacetate of 10 mM. Dilutions were made to 100 µM and 1µM. Ethylene glycol growth assays were prepared by diluting 1.11 g/L stock solution by 1000 and 500 to give final concentration of 100 mM and 200 mM respectively. Emulsions were prepared as described below. These were further diluted from stock solutions of 1.25 g/L to working solutions of 31 mg/L and 31 µg/L.

	10 mM acetoacetate + pha ABC	100 µM acetoacetate + pha ABC	1 µM acetoacetate + pha ABC	10 mM acetoacetate + phaBC	100 µM acetoacetate + phaBC	1 µM acetoacetate + phaBC	10 mM acetoacetate	100 µM acetoacetate	1 µM acetoacetate	LB	10 mM acetoacetate + EV	100 µM acetoacetate + EV
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												
	1 µM aceto-acetate + EV	31 mg/L PLA + LB + EV	31 mg/L P3HB + LB + pha ABC	31 mg/L P3HB + LB + phaBC	31 mg/L P3HB + LB + EV	31 µg P3HB + LB + pha ABC	31 µg P3HB + LB + phaBC	31 µg P3HB + LB + EV	100mM EG LB EV	200mM EG LB EV	water	

03/09/13

O/N culture

Prepared O/N cultures for phaABC (4D) and phaBC (41D), both in MG1655 from glycerol stock prepared on 31/08/13.

Waste media

Weighed 6g SRF and put into 300ml PBS and 300ml LB respectively.

03/09/13

Serial dilutions of PhaABC1655 cells to work out CFU.

Dilution	LB (microlitre)	SR cells(microlitre)
10^{-2}	1980	20 from PhaABC MG1655 cells from O/N culture
10^{-4}	1980	20 from 10^{-2}
10^{-5}	4500	500 from 10^{-4}
10^{-6}	4500	500 from 10^{-5}
10^{-7}	4500	500 from 10^{-6}

Pipetted 130 microlitre of each dilution on a C plate and cultured them at 37 degrees C overnight.

Created large O/N colony for purification of PHB next day.

Waste conditioned media

Prepared 2 bottles of waste in 300 mL water and 50 ML PBS, and 2 bottles of waste in 300 mL water and LB.

Added 2mL of phaABC into PBS and LB bottles respectively. Same for phaBC.

05/09/13

Overnight culture

Prepared O/N culture for phaABC, phaBC, promoter and RBS.

06/09/13

Growth assay

Produced 6-timepoint growth assays for multiple concentrations of 3HB and acetoacetate in two different media LB and M9.

The results are shown in the graph.

3HB solutions were prepared in advance. An initial stock of 100mM 3HB was made from 0.315g 3HB dissolved in 25 mL ddH2O. This was then diluted down to 10mM, 1mM, 100µM, 1µM working solutions in M9.

An initial stock of 5.64g of acetoacetate was dissolved in 9.2 mL ddH2O, and then diluted down to 1mM, 100 µM and 1 µM working solutions in M9 and LB.

O/N cultures were prepared, these were diluted to OD=0.05 by measuring $\frac{O/NOD}{0.05}$. With this complete the required dilutions of cells were made into 1.5 mL reaction tubes to give a total volume of 800 µL, this was then divided up into 4 wells for quadruplicate technical repeats. Cells used were phaABC, which is involved in P3HB synthesis from glucose,

phaBC which synthesises P3HB from acetoacetate and EV empty vector which was used as a growth control.

Plate 1	LB + phaABC	1mM acetoacetate in LB + phaABC	100uM acetoacetate in LB + phaABC	1uM acetoacetate in LB + phaABC	LB + phaBC	1mM acetoacetate in LB + phaBC	100uM acetoacetate in LB +phaBC	1uM acetoacetate in LB +phaBC	LB + EV	1mM acetoacetate in LB + EV	100uM acetoacetate in LB + EV	1uM acetoacetate in LB + EV
A												
B												
C												
D												
E												
F												
G												
H												
	LB	1mM acetoacetate in LB	100uM acetoacetate in LB	1uM acetoacetate in LB	M9	1mM acetoacetate in M9	100uM acetoacetate in M9	1uM acetoacetate in M9				

Plate 2	M9 + phaABC	1mM acetoacetate in M9 + phaABC	100uM acetoacetate in M9 + phaABC	1uM acetoacetate in M9 + phaABC	M9 + phaBC	1mM acetoacetate in M9 + phaBC	100uM acetoacetate in M9 + phaBC	1uM acetoacetate in M9 + phaBC	M9 + EV	1mM acetoacetate in M9 + EV	100uM acetoacetate in M9 + EV	1uM acetoacetate in M9 + EV
A												
B												
C												
D												
E												
F												
G												
H												
	M9 + phaABC	10mM 3HB in M9 + phaABC	1mM 3HB in M9 + phaABC	100uM 3HB in M9 + phaABC	1uM 3HB in M9 + phaABC	M9 + phaBC	10mM 3HB in M9 + phaBC	1mM 3HB in M9 + phaBC	100uM 3HB in M9 + phaBC	1uM 3HB in M9 + phaBC		

Plate 3	M9 + EV	10mM 3HB in M9+ EV	1mM 3HB in M9+ EV	100uM 3HB in M9+ EV	1uM 3HB in M9+ EV	10mM 3HB in M9	1mM 3HB in M9	100uM 3HB in M9	1uM 3HB in M9			
A												
B												
C												
D												
E												
F												
G												
H												
	LB	Arabinose in LB	ESTCS2 + arabinose in LB	ESTCS2 + LB	pulA + arabinose in LB	pulA + LB						

10/09/13

3% MASSIVE ASSAY

Dilutions were made:

3% glucose - 300 mg in 10 mL - 166.5 mM

3% acetoacetate - 125 mg in 4.16 mL - 278 mM - too high, increases OD600 to >1, so will re-test at lower concentration of AA.

3% 3HB - 300 mg in 10 mL - 237.9 mM

These were made into both LB and M9 media. The required amount of chloramphenicol was added to these solutions to stop growth of other bacteria.

phaCAB (41C), phaCB (4D) and EV were grown in O/N cultures. In the morning their OD was measured at OD600. The calculations were then performed in order to allow for measuring of the correct volume required for dilution to 0.05 OD.

These were then diluted into 800 µL final volumes, including the reagent being tested. From this, 200 µL was pippetted into a 96 wells plate in quadruplicate, then placed in a shaking incubator as per previous growth assays (37C, 700 rpm shaking). Every hour measurements of growth were taken to see how cells were growing.

Results, see: "10-09-13 Data"

Serial Dilution

Dilution	LB (ul)	SR cells(ul)	OD R1	Colonies OD R1
$1x10^{-2}$	1980	20 from phaCAB cells from O/N culture		
$1x10^{-4}$	1980	20 from 10^-2		
$1x10^{-5}$	4500	500 from 10^-4		
$1x10^{-6}$	4500	500 from 10^-5		115
$1x10^{-7}$	4500	500 from 10^-6		

Original OD 1.572, overall CFU/ml = 884,615,384

Dilution	LB (ul)	SR cells(ul)	OD R1	Colonies OD R1
$1x10^{-2}$	1980	20 from J23104+B0034 cells from O/N culture		
$1x10^{-4}$	1980	20 from 10^-2		
$1x10^{-5}$	4500	500 from 10^-4		

$1x10^{-6}$	4500	500 from 10^-5	173
$1x10^{-7}$	4500	500 from 10^-6	

Original OD 2.64, overall CFU/ml = 7,984,615,385

Fluorescent Microscopy

Imaged phaCAB under the microscope. Images in the experimental data file of the same date.

In summary, placed drops of Nile Red stained cells in media onto slide and viewed this, this is preferable to the agar pad technique.

Bright field images good, however no real difference between controls and phaCAB or phaCB containing cells when viewed under fluorescence for Cy3(excited with green light).

Stained 4ml of O/N culture with 3.2µl Nile Red in DMSO(25mg/ml). WIll try with a more concentrated solution as this is much lower than the concentration which was on the the plates.

Cultures for biomass vs. OD calibration

Made three 300ml cultures of phaCAB, phaCB and J23104+B0034.

11/09/13

M9 assay

Preparation of both M9M (minimal media) and M9S (supplemented media).

Made stocks of MgSO4 (246.48 g/mol), CaCl2 (147.02 g/mol), and thiamine hydrochloride (337.3 g/mol):

- MgSO4: 100 mM - 2.46g in 100 mL ddH2O
- CaCl2: 20 mM - 0.29g in 100 mL ddH2O
- Thiamine hydrochloride: 50 mM - 0.84g in 50 mL ddH2O

The bottles used were pre-autoclaved. When all media was prepared, it was sterile filtrated, as autoclaving results in the formation of magnesium precipitate.

M9M [final]	1L	500 mL	M9S [final]	1L	500 mL
1X M9 salts	200 mL 5X M9 salts	100 mL	1X M9 salts	200 mL 5X M9 salts	100 mL
2 mM MgSO4	20 mL [100 mM stock]	10 mL	2 mM MgSO4	20 mL [100 mM stock]	10 mL
0.1 mM CaCl2	10 mL [20 mM stock]	5 mL	0.1 mM CaCl2	10 mL [20 mM stock]	5 mL
0.4% C source	4g	2g	0.4% C source	4g	2g

Water	770 mL	385 mL	Water	750 mL	375 mL
			0.2% casein hydrolysate	2g	1g
			1 mM thiamine hydrochlo-ride	20 mL [50 mM stock]	10 mL

Growth assay on M9M & M9S

EV and phaCAB were grown in M9M and M9S over a 5h period with 700 rpm shaking, 37C.

Results see: "11/09/13 Data"

Conclusion: M9M grows less than M9S.

Dilution Plating

Made dilution plating for phaCAB and J23104+B0034. Only plated 10^{-5}, 10^{-6} and 10^{-7} as we didn't have enough chloranphenicol plates. 10^{-6} plates for phaCAB may be 10^{-5} insted, compare this to other plates.

Plastic Cultures

Growing two 300ml cultures of phaCAB and phaCB respectively and one 300ml of J23104+B0034 Grown in shaking incubator at 30°C and shaking at 120rpm. Started to grow at 14.00. Incubated for 72h. phaCAB and J23104+B0034 will have their ODs measured and plastic content extracted and calculated. phaCB will only have its plastic extracted.

Dry Mass Calculations

	phaCAB	J23104+B0034
container mass 1(g)	12.87	12.93
container mass 2(g)	12.90	12.88

	Dilution	OD	dry biomass in 300ml(g)	dry biomass(g/l)
phaCAB	1	2.132	0.32	1.07
	0.4	0.811		0.428
	0.25	0.533		0.266
	0.1	0.215		0.107
J23104+B0034	1	4.013	0.61	2.03

	0.15	0.602	0.3045
	0.1	0.437	0.203

O/N phaCB 41D

Made 4ml overnight cultures of colony D on plates made by Margarita today. It will be PCRd and sequenced tomorrow.

12/09/13

Colony PCR

Plate colonies (numbered)phaCB 41D (glycerol) **1**=1, **2**=2, **3**=3, **4**=4, **5**=1, **6**=2, **7**=3, **8**=4

PK **9**=1(2), **10**=2(2), **11**=3(2)

PKK **12**=1(3), **13**=2(3), **14**=3(3)

PCR - results see: "12-09-13 colonyPCR2.JPG"

Primers used:

- G1005 & phaCSeq2_FW for phaCB testing
- VF2 & VR for phaCB, PK & PKK

Serial Dilution

Results

Unfortunately too many colonies grew for phaCAB at 10^-6 and too few at 10^-7.

Dilution	LB (ul)	SR cells(ul)	OD R1	Colonies R1	Colonies R1
$1x10^{-2}$	1980	20 from J23104+B0034 cells from O/N culture			
$1x10^{-4}$	1980	20 from 10^-2			
$1x10^{-5}$	4500	500 from 10^-4			
$1x10^{-6}$	4500	500 from 10^-5			
$1x10^{-7}$	4500	500 from 10^-6		99	35

CFU/ml = (no.colonies/plated volume)*dilution factor

Original OD of 4.013. CFU/ml of 7,615,384,615 and 2,692,307,692.

18/09/13

Induction assay

Testing induction of completed constructs - in SB1C3 and MG1655.

These are:

- PueB - xylose - LB/OD
- PUR-ESTCS2 - arabinose - LB/OD/GFP
- CLE - xylose - LB/OD/GFP
- bdh2 - arabinose - OD
- PulA - arabinose - OD
- EV

25/09/13

Dilution Plating phaCAB and EV

phaCAB OD : 1.564, 30 colonies , CFU = 375,000,000

EV : 1.704, 103, CFU = 1,287,500,000

80ul added to plates of 10^-5 and 10^-6

CFU/ml = (no.colonies/plated volume)*dilution factor

27/09/13

Producing plastic with the hybrid promoter

Grown using 300ml 3% glucose LB

	natural phaCAB	Hybrid promoter phaCAB
Vol/L	1.2	1.2
OD	2.90	5.64
Dry Biomass Concentra-tion(g/l)	1.5	2.9
Dry Biomass(g)	1.8	3.48

Using the information from

Plastic from both dates was combined so that all that produced from phaCAB(biomass dry mass=1.59g) was together and all that from the hybrid promoter(biomass dry mass=2.16g) was together.

	hybrid promoter-phaCAB	native phaCAB
Dry Biomass(g)	3.48	1.8
P3HB mass(g)	2.05	0.09
P3HB mass/dry mass cells(%)	57.5	5
Tokyo Tech Highest P3HB mass/dry mass cells(%)*		9.9
PHB concentration(g/L)	1.66	0.075
Tokyo Tech Highest PHB concentration cells(g/l)*		0.204