Group 3 Notebook: September

SATURDAY, 01/09/2018

Plasmid Extraction

THURSDAY, 13/09/2018

Xylose growth experiment 1.0

1. Inoculate BL21*, BL21*-pQE80L-XylR, and BL21*-pQE80L-XylR* in 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm overnight.

FRIDAY, 14/09/2018

Xylose growth experiment 1.0

1. Transfer 1% culture to 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm for 3-4 hours or when OD reaches 0.8-1.0.
3. Add 5µL of 1 mM IPTG to all samples.
4. Incubate at 20°C, 220 rpm overnight.

SATURDAY, 15/09/2018

Xylose growth experiment 1.0

1. Measure OD and dilute to OD=0.2 using M9 medium.
2. Add and mix 800uL of M9 with 200uL of 20% glucose (0.2% glucose), or 200uL of 20% xylose (0.2% xylose), or 100 ul of 20% glucose and 100 ul of 20% xylose (0.1% glucose 0.1% xylose).
3. Transfer 150uL of each mixture into 9 separate tubes.
4. Add 150 ul of diluted cultures into each mixture and mix.
5. Transfer 100 ul of each mixture into a 3 wells of a 96-well plate, with 100 ul of M9 as blank.
6. Perform steps 3-5 with double the volumes such that 200 ul of samples can be added.
7. Measure absorbance at 600nm .
8. Incubate at 37°C, 220rpm.
9. Measure absorbance at 600nm every 1 hour.

Subsequent runs will use 100 ul of reaction mixture only, as 200 ul samples took too long to grow and so meaningful analysis cannot be conducted.

MONDAY, 17/09/2018

Xylose growth experiment 1.1

1. Inoculate BL21*, BL21*-pQE80L-XylR, and BL21*-pQE80L-XylR* in 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm overnight.

TUESDAY, 18/09/2018

Xylose growth experiment 1.1

1. Transfer 1% culture to 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm for 3-4 hours or when OD reaches 0.8-1.0.
3. Add 5µL of 1 mM IPTG to all samples.
4. Incubate at 20°C, 220 rpm overnight.

WEDNESDAY, 19/09/2018

Xylose growth experiment 1.1

1. Measure OD and dilute to OD=0.2 using M9 medium.
2. Add and mix 800uL of M9 with 200uL of 20% glucose (0.2% glucose), or 200uL of 20% xylose (0.2% xylose), or 100 ul of 20% glucose and 100 ul of 20% xylose (0.1% glucose 0.1% xylose).
3. Transfer 150uL of each mixture into 9 separate tubes.
4. Add 150 ul of diluted cultures into each mixture and mix.
5. Transfer 100 ul of each mixture into a 3 wells of a 96-well plate, with 100 ul of M9 as blank.
6. Measure absorbance at 600nm.
7. Incubate at 37°C, 220rpm.
8. Measure absorbance at 600nm every 1 hour.

Biosynthesis 2.0

1. Inoculate BL21, BL21-F3′H, BL21-FNS, BL21-FF in 10 ml of LB broth with the relevant antibiotics.
2. Incubate at 37°C, 220 rpm overnight.

THURSDAY, 20/09/2018

Biosynthesis 2.0

1. Transfer 1% culture into 10 ml TB, 10 ul trace elements, and the necessary antibiotics.
2. Incubate at 37°C, 220 rpm until OD reaches 0.6.
3. Add respective inducers: 10 µl of 200 µM ATc for constructs containing PTet, and 100 µl of 20% arabinose for constructs containing PBAD.
4. Incubate at 20°C, 220 rpm in the dark overnight.
5. Aspirate 2 ml of culture for SDS-PAGE analysis.
6. Centrifuge remaining culture at 5000 rpm for 15 min.
7. Discard supernatant and resuspend pellet in 10 ml of M9.
8. Add 100 µl of 20% glucose, 10 µl of 0.2M naringenin, and 10 µl of trace elements.
9. Transfer mixture to flask. For co-culture, mix 5 ml of each culture.
10. Incubate at 30°C, 220 rpm for 36hrs.

FRIDAY, 21/09/2018

Biosynthesis 2.0

1. Harvest and extract samples, and send samples for HPLC analysis.

SUNDAY, 23/09/2018

Xylose growth experiment 1.2

1. Inoculate BL21*, BL21*-pQE80L-XylR, and BL21*-pQE80L-XylR* in 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm overnight.

MONDAY, 24/09/2018

Xylose growth experiment 1.2

1. Transfer 1% culture to 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm for 3-4 hours or when OD reaches 0.8-1.0.
3. Add 5µL of 1 mM IPTG to all samples.
4. Incubate at 20°C, 220 rpm overnight.

Transformation in BL21*

Transformation of Brep-F3′H

Transformation of Brep-FNS

Transformation of Brep-F3′H-Brep-FNS

Co-transformation of Brep-F3′H and Brep-FNS

TUESDAY, 25/09/2018

Xylose growth experiment 1.2

1. Measure OD and dilute to OD=0.2 using M9 medium.
2. Add and mix 800uL of M9 with 200uL of 20% glucose (0.2% glucose), or 200uL of 20% xylose (0.2% xylose), or 100 ul of 20% glucose and 100 ul of 20% xylose (0.1% glucose 0.1% xylose).
3. Transfer 150uL of each mixture into 9 separate tubes.
4. Add 150 ul of diluted cultures into each mixture and mix.
5. Transfer 100 ul of each mixture into a 3 wells of a 96-well plate, with 100 ul of M9 as blank.
6. Measure absorbance at 600nm.
7. Incubate at 37°C, 220rpm.
8. Measure absorbance at 600nm every 1 hour.

For subsequent runs, OD will be measured using the microplate reader to minimize variability.

Xylose growth experiment 2.0

1. Inoculate BL21*, BL21*-pQE80L-XylR, and BL21*-pQE80L-XylR* in 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm overnight.

Biosynthesis 2.1

Transfer 1% culture into 10 ml TB, 10 ul trace elements, and the necessary antibiotics.

1. Incubate at 37°C, 220 rpm until OD reaches 0.6.
2. Incubate at 20°C, 220 rpm in the dark overnight (induction).
3. Aspirate 2 ml of culture for SDS-PAGE analysis.
4. Centrifuge remaining culture at 5000 rpm for 15 min.
5. Discard supernatant and resuspend pellet in 10 ml of M9.
6. Add 100 µl of 20% glucose, 10 µl of 0.2M naringenin, and 10 µl of trace elements.
7. Transfer mixture to flask. For co-culture, mix 5 ml of each culture.
8. Incubate at 30°C, 220 rpm for 36hrs.

WEDNESDAY, 26/09/2018

Xylose growth experiment 2.0

1. Transfer 1% culture to 10mL of LB broth with the necessary antibiotics.
2. Incubate at 37°C, 220 rpm for 3-4 hours or when OD reaches 0.8-1.0.
3. Add 5µL of 1 mM IPTG to all samples.
4. Incubate at 20°C, 220 rpm overnight.

Biosynthesis 2.1

1. Centrifuge cell cultures at 5000 rpm for 6 min.
2. Supernatants are discarded and cell pellets resuspended to OD600 of 2.0 using M9 culture medium supplemented with 6 nM Thiamine and any necessary antibiotics.
3. Addition of Substrate naringenin at final concentration 0.2 uM.
4. Continue incubation at 30°C, 300 rpm for 36 hours.
5. Centrifugation at 10,000 rpm for 3 mins to collect the supernatant.
6. Filter-sterilisation of supernatant is carried out in BSCs using a 0.22 um filter.

THURSDAY, 27/09/2018

Xylose growth experiment 2.0

1. Measure OD and dilute to OD=0.2 using M9 medium.
2. Add and mix 800uL of M9 with 200uL of 20% glucose (0.2% glucose), or 200uL of 20% xylose (0.2% xylose), or 100 ul of 20% glucose and 100 ul of 20% xylose (0.1% glucose 0.1% xylose).
3. Transfer 150uL of each mixture into 9 separate tubes.
4. Add 150 ul of diluted cultures into each mixture and mix.
5. Transfer 100 ul of each mixture into a 3 wells of a 96-well plate, with 100 ul of M9 as blank.
6. Measure absorbance at 600nm.
7. Incubate at 37°C, 220rpm.
8. Measure absorbance at 600nm every 1 hour.

Biosynthesis 2.1

1. Harvest and extract samples, and send samples for HPLC analysis.