Group 3 Notebook: October

TUESDAY, 02/10/2018

Xylose growth experiment 3.0

1. Transformation of pQE80L-XylR and pQE80L-XylR* into new batch of competent BL21*.

WEDNESDAY, 03/10/2018

Xylose growth experiment 3.0

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

THURSDAY, 04/10/2018

Xylose growth experiment 3.0

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.

FRIDAY, 05/10/2018

Xylose growth experiment 3.0

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

MONDAY, 08/10/2018

Xylose growth experiment 3.1

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

Bioreactor synthesis

1. Inoculate BL21*-Brep-F3′H and BL21*-pBAD-FNS.
2. Prepare sufficient TB and M9 medium.

CUHK collaboration experiment

1. Transform lpp-iSpi and lpp-Spi2 into BL21*.

TUESDAY, 09/10/2018

Xylose growth experiment 3.1

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.

Bioreactor synthesis

1. Transfer 1% of overnight culture into four separate 250 mL flasks each containing 50 mL TB containing trace elements.
2. Incubate at 30°C, 220 rpm.
3. Induce at OD=0.6. Keep BL21*-Brep-F3'H in the dark, and add arabinose to BL21*-pBAD-FNS to a final concentration of 0.2%.
4. Incubate at 20°C, 220 rpm overnight.

CUHK collaboration experiment

1. Patch colonies.
2. Inoculate from the same colony, in 3 tubes of 10 ml LB broth with chloramphenicol.
3. Incubate at 30°C, 37°C, and 45°C, 220 rpm overnight.

WEDNESDAY, 10/10/2018

Xylose growth experiment 3.1

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

Bioreactor synthesis

1. Centrifuge the cultures at 4000 rpm, 4°C for 20 min and remove the supernatant.
2. Resuspend cells in M9 medium, and mix both cultures to a volume of 2 L.
3. Transfer the mixture to a 5 L bioreactor.
4. Add 2 mL of 0.2 M naringenin.
5. Carry out the one-pot reaction under microaerobic condition with stirring at 220 rpm for 36 hours.

CUHK collaboration experiment

1. Dilute culture to OD=0.2 using M9 medium.
2. Add DFHBI to final concentration of 200 uM.
3. Incubate at the respective temperatures (30°C, 37°C, and 45°C) for 45 min.
4. Add 100 ul of each sample to 96 well-plate with M9+DFHBI as blank.
5. Repeat step 4 twice for triplicates.
6. Measure fluorescence at 447/501.

FRIDAY, 12/10/2018

Bioreactor synthesis (extraction)

1. Centrifuge samples at 8000 rpm for 30 min and retrieve the supernatant.
2. Add concentrated HCl to a final concentration of 0.1 N.
3. Transfer 250 mL of acidified samples to 1 L separatory funnel and treat with equal volume of ethyl acetate.
4. After observing clear separation of the solvent and aqueous layers, discard the aqueous layer.
5. Repeat steps 13 and 14 until all samples are transferred.
6. Centrifuge at 8000 rpm for 20 min to remove impurities.
7. Retrieve the solvent layer and mix with equal volume of ethyl acetate.

8. Transfer to separatory funnel, and discard aqueous layer after 10-15 min.
9. Collect the solvent layer and centrifuge at 8000 rpm for 30 min.
10. Separate the solvent layer and concentrate it using a rotary evaporator.