Part I: Plasmid Construction

Q5 polymerase was used for PCR amplification of assembly fragments, while Pfu polymerase was used to perform PCR site-directed mutagenesis. Gel extraction or PCR purification was performed on amplified products. Assembly was done via either Gibson assembly or restriction digestion followed by ligation. Heat shock was conducted to transform assembly products into chemically-competent cells. Colony PCR was performed using Taq polymerase. Successful colonies were inoculated and plasmid extraction from overnight cultures were done. The extracted plasmids were subjected to restriction digestion check if necessary, and sent for sequencing.

A. *PCR amplification* (Q5)

Reagent	*Volume* (µL)
Plasmid	1.0
Forward primer	0.5
Reverse primer	0.5
2.5 mM dNTP	1.0
5X Q5®Reaction buffer	10.0
Q5® High-Fidelity DNA Polymerase	0.5
Nuclease-free water	36.5
Total	50.0

	Temperature (°C)	*Time* (s)
Initial denaturation	95	
Denaturation*	95	
Annealing*	Varies with primers	
Elongation*	72	30 s per 1 kbp
Final elongation	72	
Hold	12	∞

*The process of denaturation, annealing and elongation was repeated for 30 cycles.

B. PCR mutagenesis

Reagent	*Volume* (µL)
Plasmid	0.5
Forward primer	1.0
Reverse primer	1.0
2.5 mM dNTP	4.0
5X TranStart Fast Pfu Buffer	10.0
TranStart Fast Pfu DNA polymerase	1.0
Nuclease-free water	32.5
Total	50.0

	Temperature (°C)	*Time* (s)
Initial denaturation	95	120
Denaturation	95	20
Annealing	Varies with primers	20
Elongation	72	30 s per 1 kbp
Final elongation	72	300
Hold	12	∞

*The process of denaturation, annealing and elongation was repeated for 30 cycles.

C. Gel extraction

Protocol provided by Qiagen for 'QIAquick Gel Extraction Kit Protocol' in the QIAprep® Miniprep Handbook was followed for all gel extractions.

1. Following gel electrophoresis, excise the DNA fragment from the agarose gel with a clean scalpel.

2. Weigh the gel slice in a 1.5 mL Eppendorf tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl).

3. Incubate at 50°C for 10 min. To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.

4. After the gel slice has dissolved completely, add 1 gel volume of isopropanol to the sample and mix.

5. Place a QIAquick spin column in a provided 2 ml collection tube.

6. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.

7. Discard flow-through and place QIAquick column back in the same collection tube.

8. To wash, add 0.75 mL of Buffer PE to QIAquick column and centrifuge for 1 min.

9. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 13,000 rpm.

10. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

11. To elute DNA, add 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H2O to the center of the QIAquick membrane and centrifuge the column for 1 min at maximum speed. Alter- natively, for increased DNA concentration, add 30 µl

elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min.

D. PCR purification

1. Add 50 µL of PCR product to 250 µL of PB buffer and mix.
2. Transfer mixture to spin column. Centrifuge for 30 s at 13000 rpm and discard flow-through.
3. Add 750 µL of PE buffer to spin column. Centrifuge for 30 s at 13000 rpm and discard flow-through.
4. Centrifuge for 2 min at 13000 rpm and discard residual wash buffer as flow-through.
5. Transfer spin column to new tubes. Add 40 µL of elution buffer or nuclease-free water and let it stand for 2min.
6. Centrifuge for 1-2 min at 13000 rpm and collect the flow-through.
7. Measure concentration of purified PCR product using NanoDrop.

E. Gibson assembly

Assembly was carried out following recommended protocol from New England Biolabs: https://www.neb.sg/protocols/2012/12/11/gibson-assembly-protocol-e5510.

	2-3 Fragment Assembly	*4-6 Fragment Assembly*
Total amount of fragments	0.02–0.5 pmols* X µL	0.2–1 pmols* X µL
Gibson Assembly Master Mix (2X)	10 µL	10 µL
Deionized H_2O	10-X µL	10-X µL
Total volume	20 µL	20 µL

1. Spin down Eppendorf tubes for 10 minutes to mix reagents thoroughly.
2. Incubate at 50°C for 1 h and hold sample at 12°C indefinitely.

F. Restriction digestion

Reagent	*Volume (µL)*
DNA (~1µg for plasmid, ~0.2µg for PCR product)	X
10X FastDigest Green Buffer	2.0
FastDigest restriction enzyme 1	1.0

FastDigest restriction enzyme 2	1.0
Nuclease-free water	16.0-X
Total	20.0

1. Add and mix the above.
2. Incubate at 37°C. Incubation time depends on the star activity of the restriction enzymes.

G. Ligation

Reagent	*Volume (µL)*
Plasmid	X
Insert	Y
T4 DNA ligase	1.0
T4 DNA ligase buffer	2.0
Nuclease-free water	17.0-X-Y
Total	20.0

1. Add and mix the above. The volume of plasmid and insert to be added is determined by NEB Ligation Calculator based on a 3:1 ratio.

2. Incubate at 25°C for 1 hour or overnight.

H. Preparation of competent cells

1. Grow an overnight culture of cells carrying plasmid of interest at 37°C, 220 rpm.

2. Inoculate 1% of the overnight culture into fresh LB and the appropriate antibiotics, if necessary. Grow to OD 0.6.

3. Once OD 0.6 is reached, spin down culture at 6, 400 rpm for 6 min. Discard the supernatant.

4. Resuspend the cell pellet in 1 mL of cold sterile water.

5. Add another 9 mL of cold sterile water into the mixture.

6. Repeat Steps 3-5.

7. Spin down culture at 6, 400 rpm for 6 min. Discard the supernatant.

8. Resuspend the cell pellet in 1 mL of cold 10% glycerol.

9. Add another 9 mL of cold 10% glycerol into the mixture.

10. Spin down culture at 6, 400 rpm for 6 min. Discard the supernatant.

11. Add 60 µL of cold 10% glycerol to the cell pellet. Resuspend the pellet.

12. Aliquot 60 µL of competent cells into 1.5 mL Eppendorf tubes each.

I. Transformation

(a) Heat-shock

Condition	*Duration*
Ice	30 mins
Heat at 42 °C	35s
Ice	2 mins

1. Subject cells to the conditions as specified in the above table.
2. After transformation, recover cells in LB at 37°C for 30 mins.

(b) Electroporation

1. Place recovery media (LB) at 37°C.
2. Place electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. Thaw electrocompetent cells on ice and mix cells by flicking gently. Transfer 50 µL of the cells to a chilled microcentrifuge tube. Add 1 µL of the DNA solution.
4. Carefully transfer the cell/DNA mix into a chilled cuvette and make sure that the cells deposit across the bottom of the cuvette.
5. Wipe off excess moisture from outside of cuvette using a Kim wipe. Electroporate. The typical time constant is ~5.0 milliseconds.
6. Remove cuvette from the chamber and immediately add pre-warmed LB.
7. Transfer LB-cell mixture to an Eppendorf tube.
8. Incubate tube in 37°C shaker for at least 45 min.
9. Place LB-agar plate(s) supplemented with appropriate antibiotic in 37 °C incubator to warm.
10. Plate transformation onto pre-warmed LB-agar plate.

J. Colony PCR

Reagent	*Volume (µL)*
Forward primer	1.0

Reverse primer	1.0
10 mM dNTP	1.0
10X Std Taq Buffer	5.0
Taq polymerase	0.5
Nuclease-free water	41.5
Total	50.0

1. Add and mix the above.

2. Dispense 10µL into separate tubes.

3. Pick a colony using a pipette tip and swirl in the mixture.

	Temperature (°C)	*Time* (s)
Initial denaturation	95	30
Denaturation	95	10
Annealing	Varies with primers	30
Elongation	72	60 / kb
Final elongation	72	300
Hold	12	∞

*The process of denaturation, annealing and elongation was repeated for 34 cycles.

K. Plasmid extraction (Miniprep)

Protocol provided by Qiagen for 'QIAprep Spin Miniprep Kit and a Microcentrifuge' in the QIAprep® Miniprep Handbook was followed for all plasmid DNA purification.

1. Spin 1-2 mL of overnight culture(s) at 5000 rpm for 5 min.

2. Resuspend pelleted bacterial cells in 250 µL Buffer P1 and transfer to micro-centrifuge tube.

3. Add 250 µL Buffer P2 and mix thoroughly by inverting the tube 4-6 times.

4. Add 350 µL Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.

5. Centrifuge for 10 min at 14,680 rpm in a table-top microcentrifuge.

6. Apply the supernatants from Step 4 to the QIAprep spin column by decanting or pipetting.

7. Centrifuge for 30 s. Discard the flow-through.

8. Wash the QIAprep spin column by adding 0.5ml Buffer PB and centrifuging for 30 s. Discard the flow-through.

9. Wash the QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30 s.

10. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.
11. Place the QIAprep column in a clean 1.5ml microcentrifuge tube. To elute DNA, add 50 µL Buffer EB (1 mM Tris-Cl, pH 8.5) or water to the centre of each QIAprep spin column, let stand for 1 min, and centrifuge for 30 s.

Part II: Naringenin Biosynthesis

There are 3 enzymes within the plasmid (to be referred to as naringenin plasmid henceforth); 2 of them (OsPKS & MCS) are under the P_{lac} promoter and 1 of them (4CL) is under a constitutive promoter. This plasmid carries the AmpR gene.

1. Grow an overnight culture of (a) BL21* and (b) BL21*- de novo plasmid at 37°C, 220 rpm.

2. Inoculate the overnight cultures (1:1000 ratio) into fresh LB (containing 100 µg/mL ampicillin if needed) and grow up to OD 0.6 at 37°C, 220 rpm.

3. Once at OD 0.6, add 0.1 mM IPTG into the culture that is to be induced and incubate overnight for protein expression at 25°C, 220 rpm.

4. After induction, spin culture at 6400 rpm, 10°C for 6 min to pellet cells.

5. Resuspend cells in M9 media + 20 mM glucose (containing 100 µg/mL ampicillin if needed). Add 0.75 mM coumaric acid and 1 mM malonic acid if necessary.

6. Add 0.1 mM IPTG to the cultures and shake for 24 hours at 25°C, 220 rpm for biosynthesis.

7. For subsequent extraction and analysis, please refer to ‘High Performance Liquid Chromatography (HPLC)’.

Part III: Luteolin Biosynthesis

1. Grow an overnight culture from the relevant stock culture by inoculating into 5 mL LB medium with any necessary antibiotics (e.g. 50 ng/ µL Kanamycin; 25ng/ µL Chloramphenicol).

2. Incubate overnight at 37°C, 220 rpm.

3. Inoculate the overnight culture into fresh LB medium with any necessary antibiotics in a 250 mL flask.

4. Incubate at 37°C, 300 rpm, and grow up to OD_{600} of 0.6.

5. Add respective inducers. Induction of constructs carrying promoter P_{Tet} with 200 nM ATC; induction of constructs carrying promoter P_{BAD} with 2% arabinose. Induce at 30°C, 300 rpm for 3 hours.

6. After induction, spin down cell cultures at 5000 rpm for 6 min to pellet cells.

7. Discard supernatant and resuspend cell pellets to OD_{600} of 2.0 using M9 culture medium supplemented with 6 nM Thiamine, and any necessary antibiotics.

8. Add naringenin to a final concentration 0.2 µM.

9. Continue incubation at 30°C, 300 rpm for 36 hours.

10. Centrifuge at 10,000 rpm for 3 mins to collect the supernatant.

11. Filter supernatant using a 0.22 µm filter. Carry this step out in a BSC.

12. For subsequent extraction and analysis, please refer to 'High Performance Liquid Chromatography (HPLC)'.

Part IV: Luteolin Bioreactor Synthesis & Extraction

1. Inoculate BL21*-Brep-F3'H and BL21*-pBAD-FNS from the stock culture in 10 mL of LB broth with any necessary antibiotics.

2. Transfer 1% of overnight culture into four separate 250 mL flasks each containing 50 mL TB containing trace elements.

3. Incubate at 30°C, 220 rpm.

4. 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%.

5. Incubate at 20°C, 220 rpm overnight.

6. Centrifuge the cultures at 4000 rpm, 4°C for 20 min and remove the supernatant.

7. Resuspend cells in M9 medium, and mix both cultures to a volume of 2 L.

8. Transfer the mixture to a 5 L bioreactor.

9. Add 2 mL of 0.2 M naringenin.

10. Stop the reaction after 36 hrs.
11. Centrifuge samples at 8000 rpm for 30 min and retrieve the supernatant.
12. Add concentrated HCl to a final concentration of 0.1 N.
13. Transfer 250 mL of acidified samples to 1 L separatory funnel and treat with equal volume of ethyl acetate.
14. After observing clear separation of the solvent and aqueous layers, discard the aqueous layer.
15. Repeat steps 13 and 14 until all samples are transferred.
16. Centrifuge at 8000 rpm for 20 min to remove impurities.
17. Retrieve the solvent layer and mix with equal volume of ethyl acetate.
18. Transfer to separatory funnel, and discard aqueous layer after 10-15 min.
19. Collect the solvent layer and centrifuge at 8000 rpm for 30 min.
20. Separate the solvent layer and concentrate it using a rotary evaporator.

Part V: High Performance Liquid Chromatrography (HPLC)

A. *Sample Preparation*

Spin down all samples at 6400 rpm, 10°C for 6 min to collect their supernatants. Filter supernatants through a 0.22 µm filter before organic extraction (see 'Organic Extraction' below).

B. *Organic Extraction*

1. Add 1 mL of 6N HCl to 10 mL of the supernatant sample in a 15 mL Falcon tube.
2. Add 2 mL of ethyl acetate to the mixture and shake thoroughly.
3. Centrifuge the mixture at 6000 rpm, 10°C for 3 min.
4. Pipette the top organic solvent layer out carefully into 2 mL Eppendorf tubes. *Be careful to not pipette the bottom layer!*
5. Evaporate the solvent in the tubes for 35 min with the caps open using a SpeedVac.
6. Add 1.5 mL of ethyl acetate to the remaining sample left in the 15 mL Falcon tube and shake thoroughly.

7. Centrifuge the mixture again for 3 min.
8. Pipette the top layer to the same 2 mL Eppendorf tube.
9. Evaporate the solvent for 40 min with the caps open.
10. Repeat steps 6-9.
11. Dissolve the organic solute obtained in 1 : 1 DMSO : Methanol and transfer them to HPLC vials.

C. *HPLC Run Specifications*

Action	*Time (min)*	*Volume (µL)*	*Remarks*
Flush	15	1	Methanol
Equilibrate	15	1	Methanol
Blank	46	10	-
Equilibrate	15	1	Methanol
Sample 1	46	10	-
Equilibrate	15	1	Methanol
Sample 2	46	10	-
Equilibrate	15	1	Methanol
...	46	10	-
Equilibrate	15	1	Methanol
Flush	15	1	Methanol

Part VI: SDS-PAGE

1. Prepare separating and stacking gels.
2. Prepare samples.
    i. Spin down cultures to pellet cells.
    ii. Resuspend cells in 300 µL of 1X Binding Buffer and transfer into an Eppendorf tube.

iii. Put Eppendorf tube into a beaker filled with ice and lyse cells via sonication. Settings are as such: amplitude: 10; process time: 1 min; pulse ON time: 5 secs; pulse OFF time: 15 secs.

3. After lysing, samples were spun down for 10 min at 4°C.

    i. After centrifugation, transfer supernatant into a separate tube and resuspend cell pellet in 300 µL 1X Binding Buffer.

4. Prepare 2 Eppendorf tubes per sample: one for the supernatant component, the other for the cell pellet component. Fill tubes with 10 µL of 2X Loading Dye.

5. Transfer 100 µL of supernatant and cell pellet that has been resuspended in binding buffer to the respective Eppendorf tubes (filled with loading dye).

6. Put all tubes on a heat block at 95°C for 5 mins. After 5 mins, spin down mixture for a few seconds to let mixture settle to the bottom of the tubes.

7. Load samples into gel. Run gel for 60 mins at 150 V.

8. After electrophoresis, carefully remove gel from the gel casting apparatus.

9. Remove the stacking gel layer and submerge the separating gel in water. Swirl in the orbital shaker at 50 rpm per min. After 5 min, change the water in which the gel is submerged.

10. Repeat *Step* 9 2 more times. Rinse away the water.

11. Pour BLS into the container until the gel is submerged. Heat in microwave for 1 min.

12. Swirl gel in the orbital shaker at 50 rpm per min for 1 hour.

13. Pour away BLS and rinse gel thoroughly with water.

14. Fill container with water until gel is fully submerged. Leave to shake in the orbital shaker at 50 rpm per min overnight.

Part VII: Blue-Light Characterization

1. Grow an overnight culture from the relevant stock culture by inoculating into 5 mL LB medium with any necessary antibiotics (e.g. 50 ng/ µL Kanamycin; 25 ng/ µL Chloramphenicol).

2. Incubate overnight at 37°C, 225 rpm (standard incubation condition, otherwise as indicated). Blue light inducible cell cultures are required to be protected from light exposure (i.e. covered with aluminum foil).

3. Inoculate an overnight culture into fresh LB medium with any necessary antibiotics and grow up to OD_{600} of 0.05.

4. Transfer 1 mL of overnight cell culture into wells in 12-well plate.

5. Take hourly OD_{600} and fluorescent readings. For measurement of red fluorescence from RFP, set excitation to 535 nm and emission of 600 nm in BioTek Synergy H1 Hybrid Multi-Mode Reader.

Part VIII: Stress Reporter Characterization

1. Inoculate BL21* PCDF-RFP, BL21* Pcon-GFP, and BL21* deNovo and BL21* P_{BLrep}-FNS and P_{BAD}-FNS in 4 mL of LB broth with the respective antibiotics.

2. Incubate at 37°C, 220 rpm overnight.

3. Transfer 1% culture to 7 mL of LB broth with the respective antibiotics in a 50 mL Falcon tube at 37°C in the shaking incubator at 220 rpm. Prepare LB, added with respective antibiotics (streptomycin and/or kanamycin and/or ampicillin), to be used as controls. Subject controls to the same conditions by shaking at 37°C, 220 rpm.

4. To induce the de novo plasmid, add of 1 mM of IPTG. To induce P_{BLrep}-FNS and P_{BAD}-FNS, keep sample in the dark and add 0.02% arabinose respectively.

5. Extract 100 µL of each sample, including controls at 0, 2, 4, 5, 7 h time points in triplicates to measure fluorescence (GFP/mRFP) and OD₆₀₀ using microplate reader (BioTek).

Part IX: Xylose Growth Experiments

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.

3. Transfer 1% culture to 10mL of LB broth with the necessary antibiotics.

4. Incubate at 37°C, 220 rpm for 3-4 hours or when OD reaches 0.8-1.0.

5. Add 5 µL of 1mM IPTG to all samples.

6. Incubate at 20°C, 220 rpm overnight.

7. Measure OD and dilute to OD 0.2 using M9 medium.

8. Add and mix 800 µL of M9 with 200 µL of 20% glucose (0.2% glucose), or 200 µL of 20% xylose (0.2% xylose), or 100 µL of 20% glucose and 100 µL of 20% xylose (0.1% glucose 0.1% xylose).

9. Transfer 150 µL of each mixture into 9 separate tubes.

10. Add 150 µL of diluted cultures into each mixture and mix.

11. Transfer 100 µL of each mixture into 3 wells of a 96-well plate, with 100µL of M9 as blank.

12. Measure absorbance at 600nm under (BioTek).

13. Incubate at 37°C, 220rpm.

14. Measure absorbance at 600nm every 1 hour.

Part X: Naringenin Growth Experiments

1. Inoculate BL21* in 5 mL of LB broth.

2. Incubate at 37°C, 225 rpm, overnight.

3. Transfer 1 µL of culture into 5 mL of LB broth.

4. Refresh culture until it reaches OD_{600} = 0.100.

5. Aliquot 1 mL of culture each into 4 Eppendorf tubes.

6. Add naringenin of varying concentration into each tube as follows:

    a. Tube 1 (Control): No naringenin
    b. Tube 2: 0.1 mM
    c. Tube 3: 0.2 mM
    d. Tube 4: 0.4 mM

7. Pipette up and down to mix well.

8. Transfer 300 µL of culture from each tube into each well of a 96 well-plate.

9. Create triplicates for each naringenin concentration.

10. Add 300 µL of LB broth into a well to act as blank.

11. Measure OD_{600} periodically over a period of 10 hours according to the following measurement protocol in microplate reader (BioTek Synergy H1):

    i. Incubate and shake the loaded 96 well-plate for 8 minutes.

ii. Measure OD_{600} every 10 minutes.