Week 39 - Overview

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Weekly summary:

During Week 39, we repeated the expression of ci2350-MBP and also expressed our new constructs: ci2350-MBP under the hypoxia promoter, and the single mutant ci2350-MBP R215A under the T7 promoter. Hypoxia induction was achieved by stopping the shaking, and sealing the flask with plastic foil and parafilm, allowing the culture to deplete the oxygen. The cells were stored as pellets in the -80ºC freezer until purification in Week 40.

Day 55: [September 23, 2026], Wednesday

Responsibles: -
Participants: Cecilia

Daily overview of experiments

Inoculation of starter couture (the day prior) and protein expression in E.coli BL21(DE3) of ci2350_MBP, ci2350_MBP + PfnrS and ci2350_MBP + R215A. Induction was done with 0.8mM IPTG for ci2350_MBP and ci2350_MBP + R215A, and induction via hypoxic conditions (stop shaking and cover with foil and parafilm) for ci2350_MBP + PfnrS.

Activity	***Protocol File***	***Comments***
Inoculation (22 September)		ci2350_MBP, ci2350_MBP + PfnrS, ci2350_MBP + R215A
Protein expression via IPTG or hypoxia induction		

Results and interpretation

Inoculation (22 September)

For the overnight starter culture, the following constructs were inoculated: ci2350_MBP (plate from July 4), ci2350_MBP + PfnrS (hypoxia promoter), ci2350_MBP + R215A (single mutant).

5 mL of LB medium was mixed with 5 µL of 100 mg/mL ampicillin stock, and one colony was inoculated. Samples were incubated overnight at 37°C, 150 rpm (~15 h).

Expression (23 September)

Starter cultures were placed on ice, and OD600 was measured (1:10 dilution in LB medium) to calculate the inoculation volume needed for the main culture, based on 0.5 mL of starter culture at OD600 = 3 per 100 mL main culture.

	OD600 (1:10)	OD600	Volume to add (mL)

ci2350_MBP + PfnrS	0.182	1.82	0.98
ci2350_MBP + R215A	0.147	1.47	1.23
ci2350_MBP	0.137	1.37	1.31

Per culture, 120 mL terrific broth (A+B) was mixed with 120 µL ampicillin (100 µg/mL) and inoculated with the appropriate volume of starter culture.

After inoculation, a first measurement (t0) was taken at OD600. Further measurements were then taken at intervals of 30 minutes to 1 hour (exact data in the Excel overview, protein expression logging sheet): Protein expression logging sheets.
If OD > 0.3 the cultures were diluted with TB media.
Note: the temperature setting was per mistake not started and therefore for the first hour there was no cell growth. (start of cultivation 11:04; turning on temperature to 37°C 12:35, which was therefore set as the new starting point)

OD600 ->	ci2350_MBP + P-fMRS	ci2350_MBP + R215A	ci2350_MBP
T0 (12:35 PM)	0.018	0.013	0.014
T1 (1:40 PM)	0.04	0.04	0.034
T2 (2:40 PM)	0.15	0.163	0.124
T3 (3.40 PM)	1.11	1.01	0.82
T4 (4.10 PM)	1.45	1.56	1.34
T5 (5.20 PM)	1.48	1.60	0.81*

*for the ci2350_MBP sample at T5 there must have been a mistake with taking the sample since OD600 drastically decreased.

Induction

Before induction, 1 mL of sample was taken for later SDS-PAGE analysis and stored at 4°C.

Induction (at T5 = 5.20 PM) was carried out when OD600 = ~1.5, using 0.8 mM (960µL) IPTG (stock = 100mM) for ci2350_MBP and ci2350_MBP + R215A, and via hypoxic conditions (shaking stopped, cultures covered with plastic foil and parafilm) for ci2350_MBP + PfnrS.

After induction, cultures were incubated overnight for production at 17°C: ci2350_MBP and ci2350_MBP + R215A at 170 rpm, and ci2350_MBP + PfnrS static (no shaking).

Day 56: [September 24, 2026], Thursday

Responsibles: -
Participants: Cecilia

Daily overview of experiments

Harvesting of overnight cultures of ci2350_MBP, ci2350_MBP + PfnrS, ci2350_MBP + R215A at 17°C.

Activity	***Protocol File***	***Comments***
Harvest cells from expression the day prior		ci2350_MBP, ci2350_MBP + PfnrS, ci2350_MBP + R215A

Results and interpretation

Harvest

After overnight cultivation at 17°C, cultivation was stopped. Before centrifugation, 1 mL was taken from each sample for SDS-PAGE analysis and stored at 4°C. Samples were transferred to 50 mL falcon tubes, centrifuged at 5000 × g for 10 minutes at 4°C, and the supernatant was discarded. Pellets were weighed and stored at -80°C.

Pellet weights:

	Pellet A (g)	Pellet B (g)
ci2350_MBP	2.21	2.08
ci2350_MBP + PfnrS (hypoxia)	0.66	0.9
ci2350_MBP + R215A (single mutant)	1.52	1.67