

System Engineering
M2D6

11.01.11

Three part lab today

Part 1: SDS-PAGE
Part 2: seq analysis of mutants
Part 3: photo?

- Measure OD600 of 1:10 of bacterial photography strain, Candidate 1, Candidate 2
- Harvest 2 OD e.g. if 0.5 OD, harvest 4 ml of 1:10 or 0.4 ml of undiluted
- Isolate protein with lysis kit (enzymatic lysis of cells, spin out debris)
- Mix supernatant with loading dye
- Boil
- Load for SDS-PAGE along with markers, + control lysate

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Lane	Sample	Volume to load
1	"Kaleidoscope" protein molecular weight standards	10 ul
2	H6-EnvZ positive control protein	40 ul
3	wild type light sensor	40 ul
4	mutant candidate 1	40 ul
5	mutant candidate 2	40 ul
6	"Kaleidoscope" protein molecular weight standards	10 ul
7	H6-EnvZ positive control protein	40 ul
8	wild type light sensor	40 ul
9	mutant candidate 1	40 ul
10	mutant candidate 2	40 ul

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Thursday's lab!

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1. Retrieve the sequences




Sequence File : **wildtype-NO296.seq**

```
>wildtype-NO296_G02.ab1
NNNNNNNNNNNNNNNNNNNNNCGGCTGGTGTAAAGCNCTGGCGGATGA
ACTTGGCGACGCCGCTGACGCGTATTCGCCTGGCGACTGAGATGATGA
AATAAAGATATCGAAGAGTGAACGCCATCATTGAGCAGTTTATCGAC
AATGGCGGATCTTAATGCACTACTCGTGAGGTGATTGCTGCCGAAAC
```

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1. Retrieve the sequences
2. Compare your sequence (= query) vs pCph8 (= NB466)



```
WT CACGCTGCTGATGGCGGGGTAAGT-CACGACTTGCACGCCGCTGA 95
MUT CACGCTGCTGATGGCGGGGTAAGTGC-CGACTTGCACGCCGCTGA 99
```

How will you interpret mismatches?

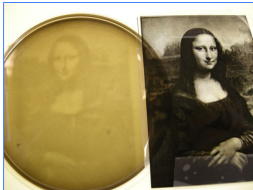
How will you understand action of amino acid changes (if you have any)?

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Refer to M2D2 protocol

Can compare to wild type (NB466) that Jingjing has grown for you



Finally: if there are other experiments you'd like to run Thursday, let us know!

This week in lab: