11/25/14

① Protein Purification:- with diff. protocol. → Buffer.

Bacteria growth:-

200 ml + Kan
+ 500 µl inoculum. ← 8:30 AM
↓
11:30
OD_{600} = .502
↓
3:10 PM

~~[illegible]~~ = ~~[illegible]~~

.047g IPTG

$\frac{m \times 1000}{W \times V}$ = $\frac{.0478 \times 1000}{238 \times 200}$ 5 = $\frac{.235 \times 10^3}{238}$ ≈ 1 mM

② Run Protein gel: 96 Samples.

Lib 2, Plate 1
only (+)ve gel 1;
only (+)ve gel 2;

Lib2, Plate 2
only (+ve)
only (+ve)

		120 Rxn
4X Buffer	5	600
10X DTT	2	240
Bug Buster	13	1560
	20	2400

↓
20 µl.

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Ceftazidime hydrate

0.1 g = 100 mg in 10 ml H_2O

$$\frac{100\ mg}{10\ ml} = 10\ mg/ml$$

$$= 10\ \mu g/\mu l$$

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grow WT cells.

200ml LB + Kan] 8:30 AM
+ 500 µl inoculum]
↓
11:30 OD_{600} = ·5
↓
add IPTG

IPTG : ~~[illegible]~~ = 1x ~~200~~ml 0.047 g in 200ml.

Harvest
~~3:16~~ → 4100

Cephalothin :- 6 Plates, Lib 2.

Plate#1	WT, A1, A3, A4, A5, A8, A10, A11
2	WT, B2, B4, B7, B9, B10, B11, B12
3	WT, C3, C4, C7, C9, C10, C11, C12
4	WT, D4, D8, D11, D12, E1, E3, E4.
5	E5, E8, E10, E11, F1, F3, F4, F6
6	G2, G3, G9, G10, G11, H2, H3, H4

① 100 ml LB + Kan
+ IPTG 1ml (of 100mM)

1mM IPTG.

100mM V = 1 x 100
V = 1ml

15 ml of #1
add ~~54~~ µl Cephalothin (of 200 µg/µl)
180 µl
180 x 200 = 36000 µg / 15 ml
= 2400 µg / ~~ml~~ ml