

### 6.7-6.13

Primer design

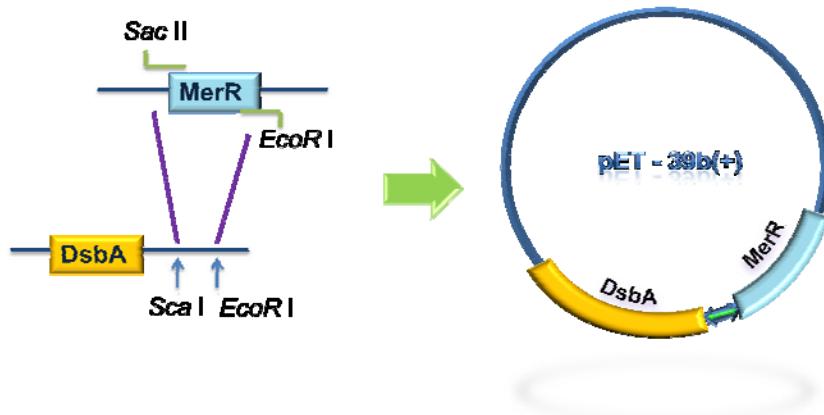
For expression test	pET21a-MBP-His	pET 39b DsbA-MBP	pMAL p4X-MBP-MBP
	pET21a-MerR-His	pET 39b DsbA-MerR	pMAL p4X-MBP-MerR
For standard part	MBP_SD	DsbA-MBP_SD	MBP-MBP_SD
	MerR_SD	DsbA-MerR_SD	MBP-MerR_SD

### 6.14-6.20

Literature Review & Experimental Design for Periplasmic MBP

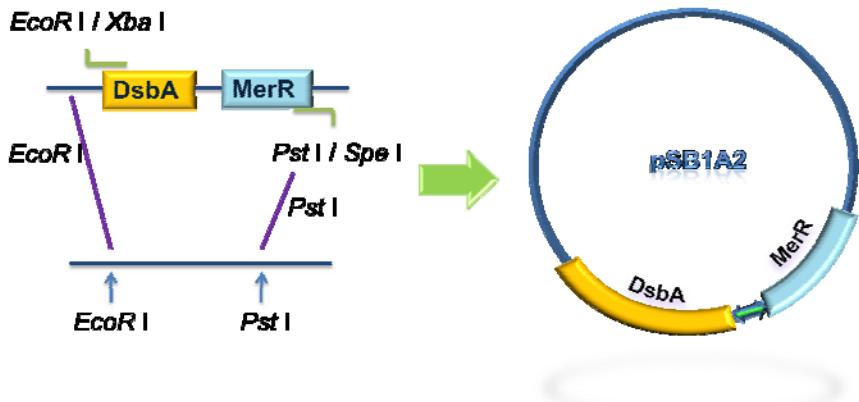
### 7.5-7.11

- DsbA-MerR (Periplasmic MerR) construction



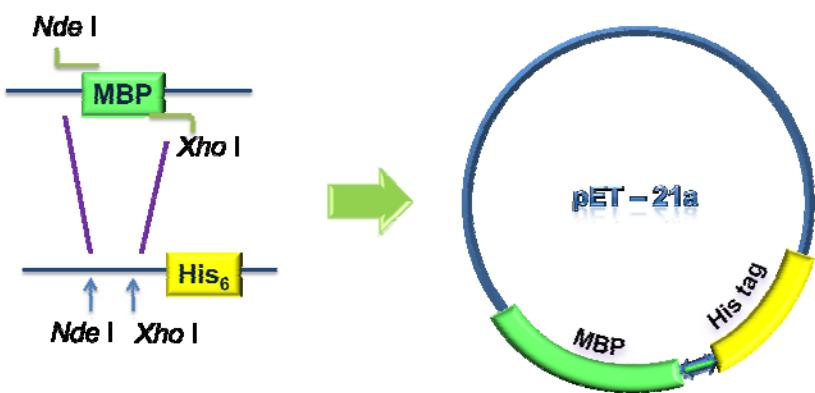
Successful clones have been verified by sequencing.

- Standardization for DsbA-MerR



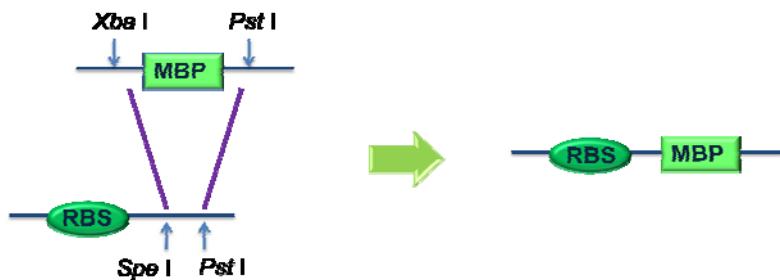
Double digest shows the right result. Need to be sequenced.

- MBP-His (cytosol MBP for expression test) construction



Double digest result shows that clone have failed. No band of right molecular weight can be detected.

- MBP (cytosol MBP for mercury binding test) construction



Clones have been constructed, but need to be verified by double digestion.

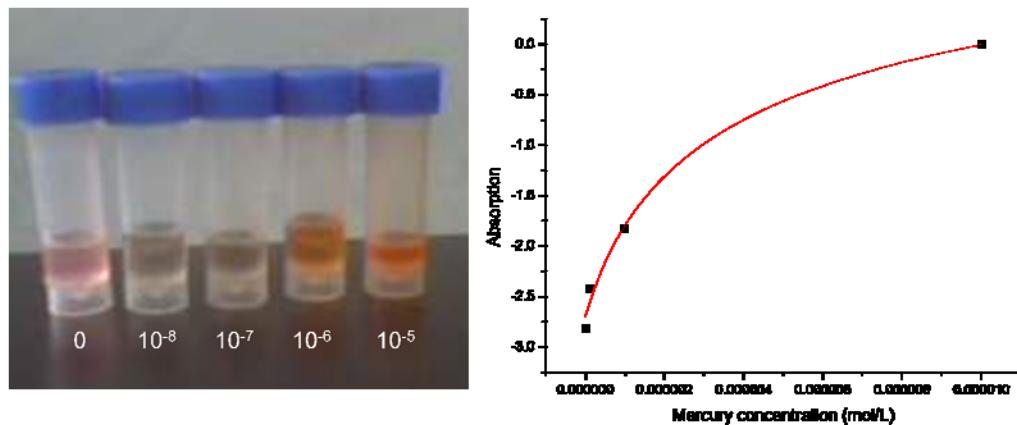
- Mercury Semiquantitative Detection

❖ Solution Preparation

- 1 g/L Dithizone Chloroform solution
- $1.038 \times 10^{-3}$  mol/L  $\text{HgCl}_2$
- Dithizone Eluant
- $\text{Na}_2\text{S}$  detoxic solution
- 1 mol/L NaOH
- Concentrated  $\text{HNO}_3$

❖ Standard Mercury Concentration Determination

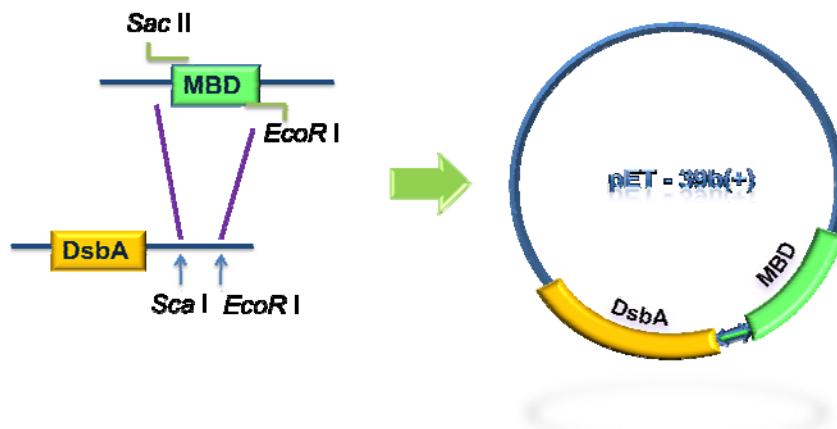
Mercury concentrated 25 times



Color change can be easily seen, but out of linear range of the standard curve.

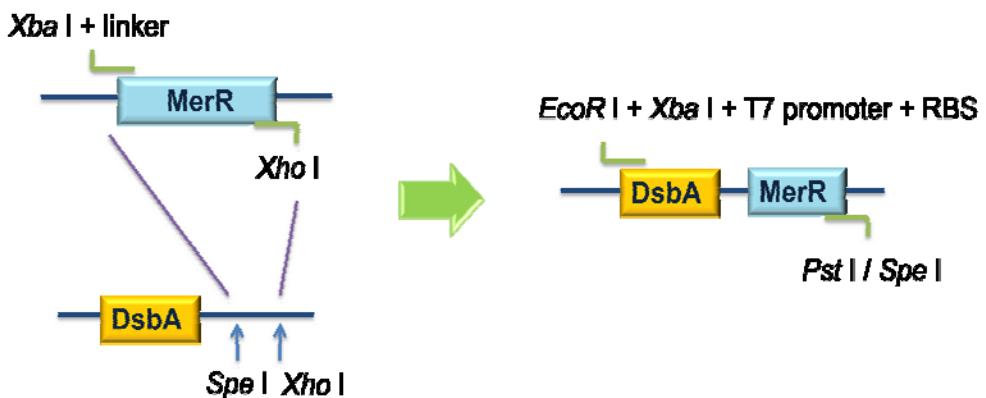
### 7.12-7.18

➤ DsbA-MBP (periplasmic MBP for expression test) construction



Band of expected molecular weight can be detected by double digest. Need to be sequenced.

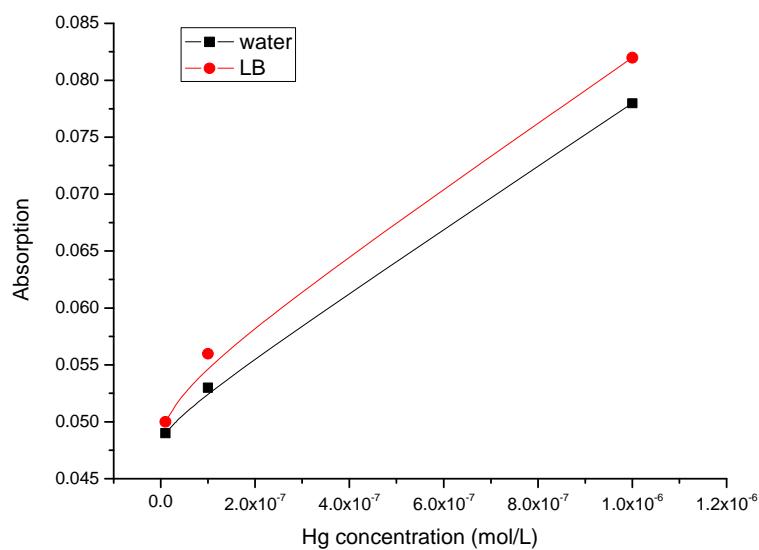
- Re-design the construction strategy for DsbA-MerR(MBP)



- MBP-His & MBP construction  
Failed.

- Mercury Semiquantitative Determination

Standard solution concentrated 10 times.



There is no significant difference between detected mercury concentration prepared in LB or water. But the absolute value is too low.

➤ DsbA-MerR construction. Need to be verified.

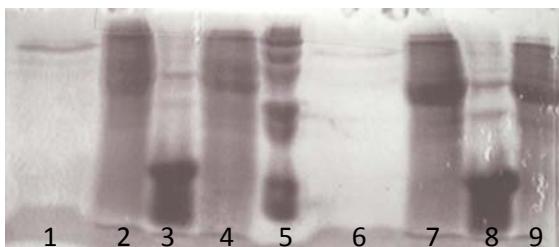
➤ DsbA-MBP construction. Need to be verified.

➤ SDS-PAGE & Western Blot

❖ Buffer preparation

- 5 × SDS-PAGE buffer
- Tris – HCl pH 6.8
- Tris – HCl pH 8.8
- 30% Acr / Bis-Acr
- 5 × TBS
- 5 × Transfer Buffer
- 1 × TTBS

❖ SDS-PAGE result of DsbA-MerR



Lane 1-4: before induction

Lane 6-8: after induction

Lane 1&6: supernatant

Lane 2&7: cytosol & pellet

Lane 3&8: periplasm

Lane 4&9: whole bacteria

Overexpression band can be detected at the expected molecular weight (40 kD).

## 7.26-7.28 & 8.3-8.8

➤ Double digest verification for DsbA-MBP, MBP-His & MBP.

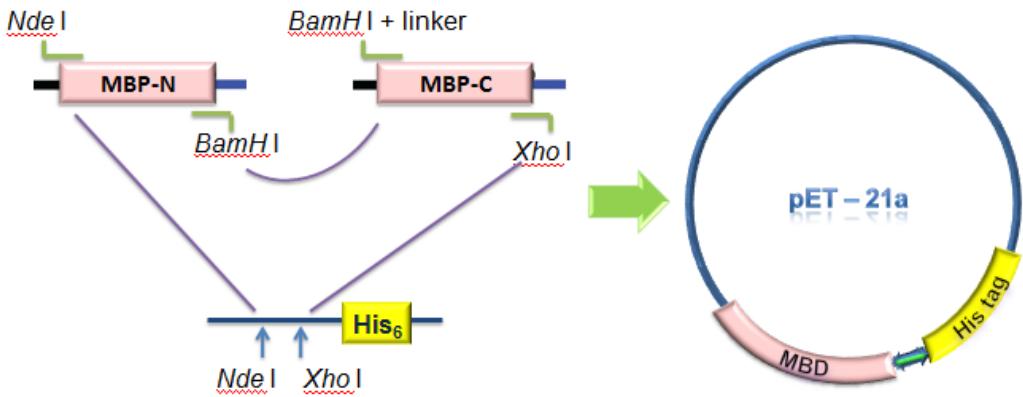
Failed.

➤ Check the template of MBP by sequencing.

➤ Re-design for MBP & DsbA-MBP construction.

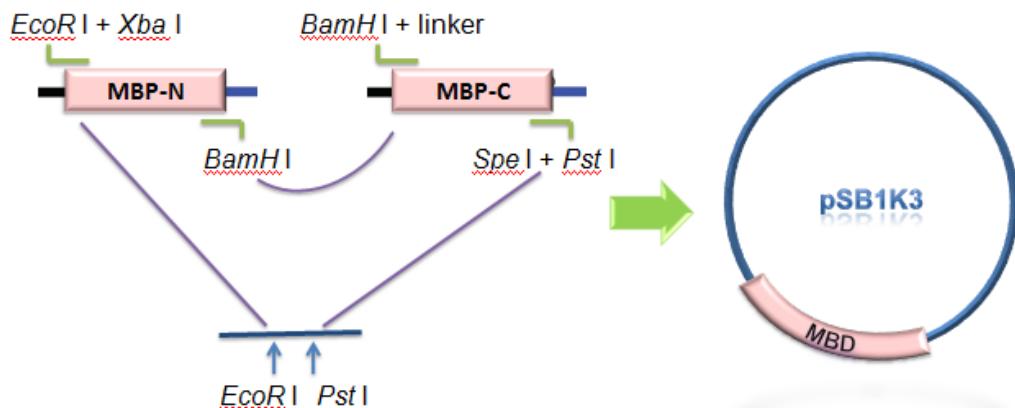
## 8.9-8.15

➤ MBP-His construction



Sequencing.

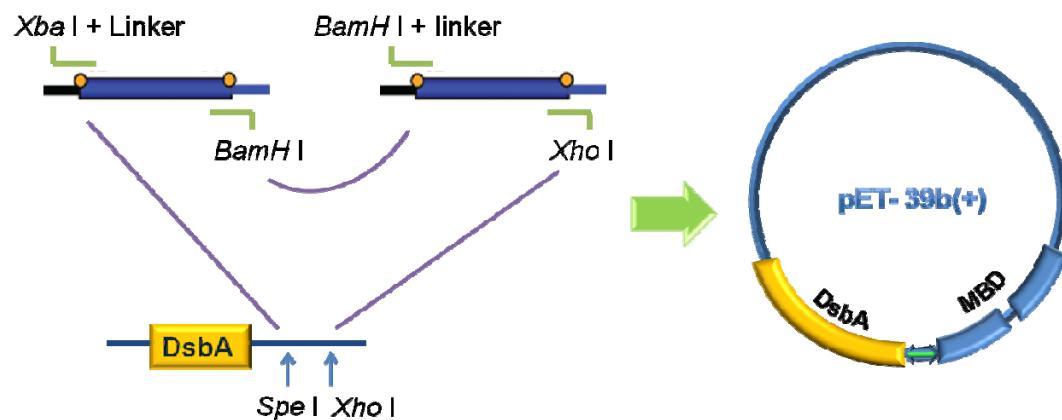
➤ MBP construction



Sequencing.

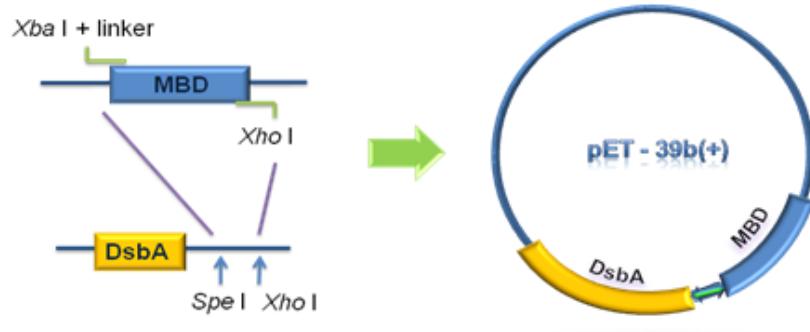
➤ DsbA-MBP construction

✧ strategy I



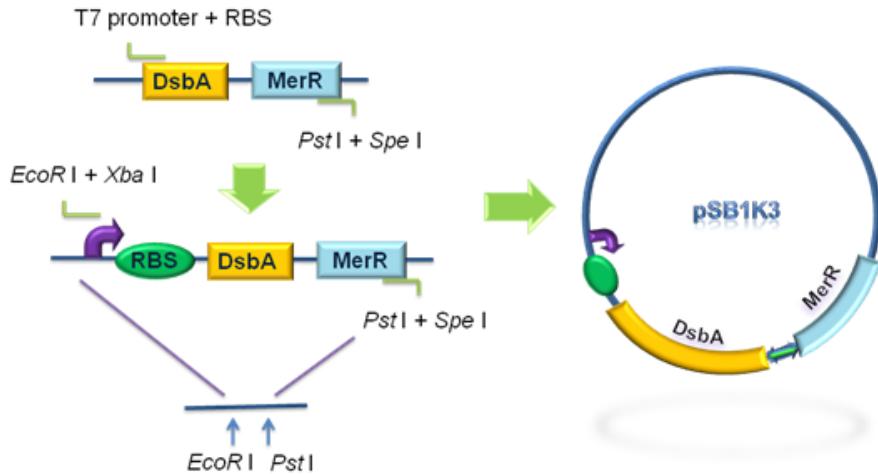
Sequencing.

❖ Strategy II



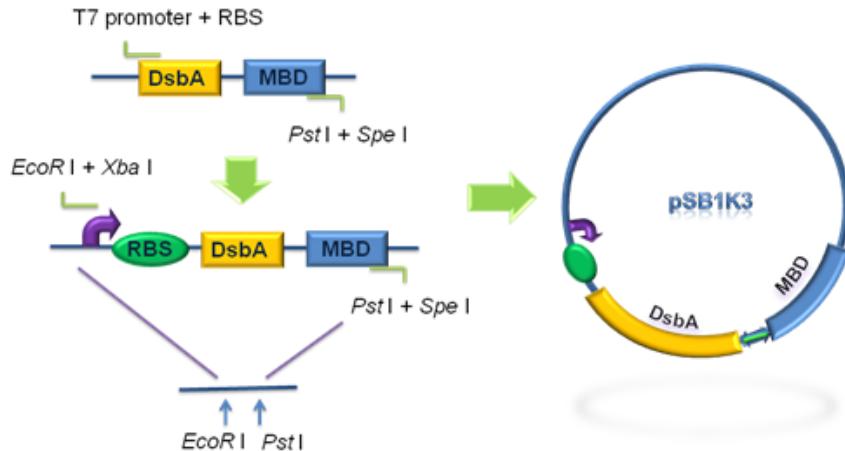
Sequencing.

➤ Standardization for DsbA-MerR



Sequencing.

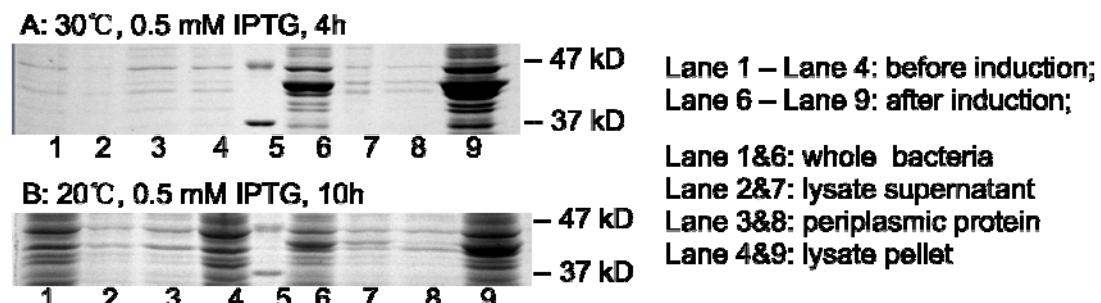
➤ Standardization for DsbA-MBP



➤ DsbA-MerR sample preparation for SDS-PAGE

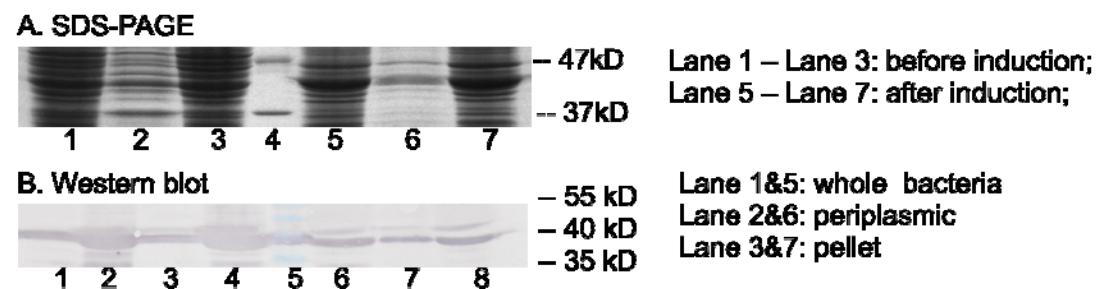
## 8.16-8.22

- DsbA-MerR standardization finished.
- Re-construct DsbA-MBP
- Expression condition optimization for DsbA-MerR

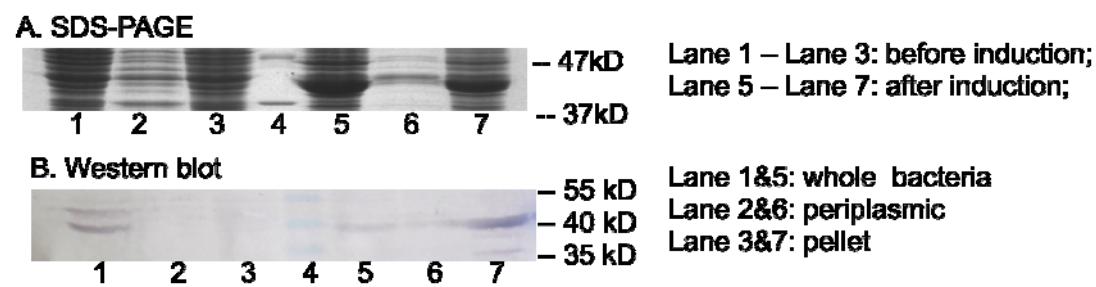


## 8.23-8.29

- SDS-PAGE & Western Blot for DsbA-MerR



- SDS-PAGE & Western Blot for DsbA-MBP

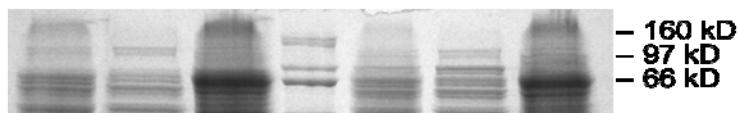


The ratio of the primary antigen to secondary antigen should be optimized to get better figures.

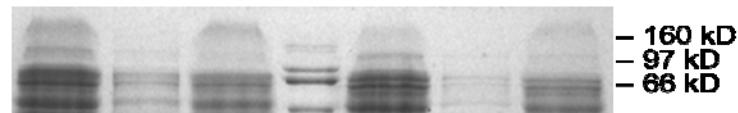
### 8.30-9.5

- SDS-PAGE for T3 polymerase

**A. WTY's T3pol**

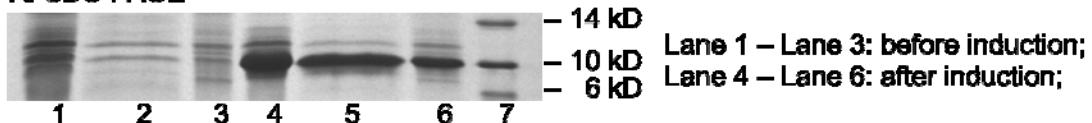


**B. DsbA-MBD**



- SDS-PAGE & Western Blot for MBP

**A. SDS-PAGE**



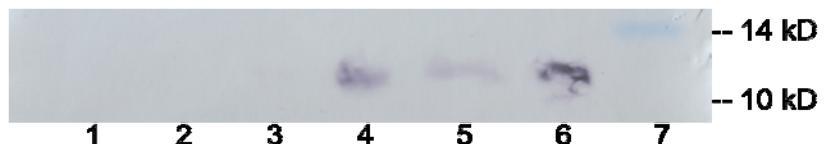
**B. Western blot**



The ratio of the primary antigen to secondary antigen should be optimized to get better figures.

### 9.6-9.12

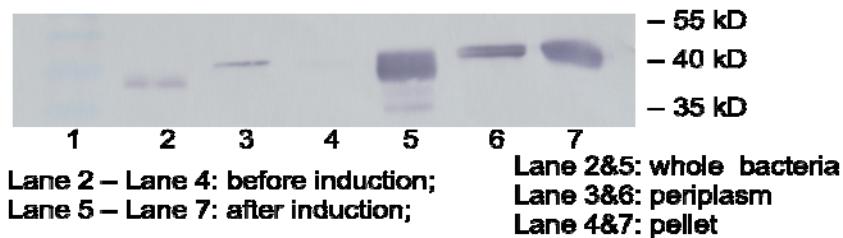
- Western Blot for MBP



Lane 1 – Lane 3: before induction;  
Lane 4 – Lane 6: after induction;

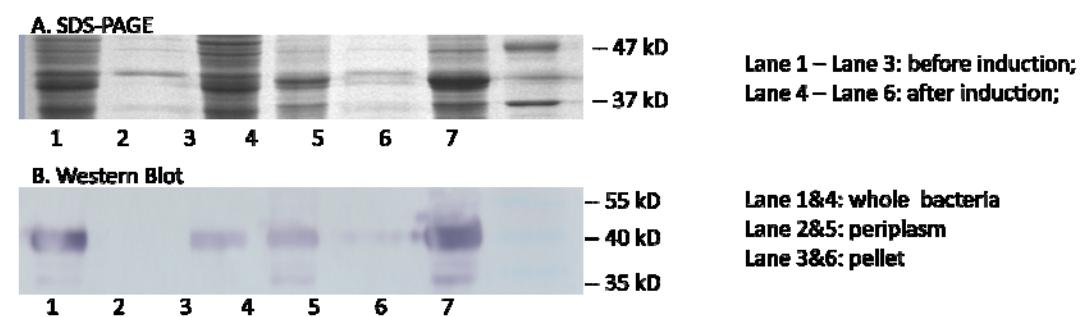
Lane 1&4: whole bacteria  
Lane 2&5: cytosol  
Lane 3&6: pellet

- Western Blot for DsbA-MBP

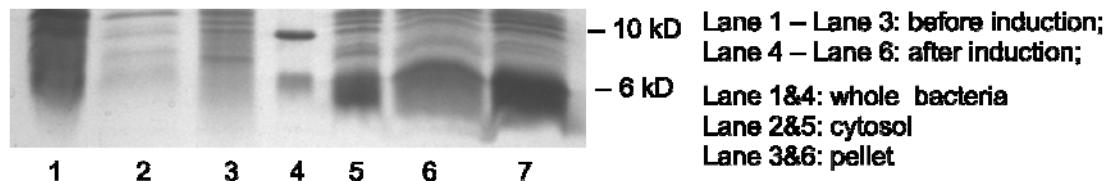


### 9.13-9.19

- SDS-PAGE & Western Blot for lead DsbA-MBP



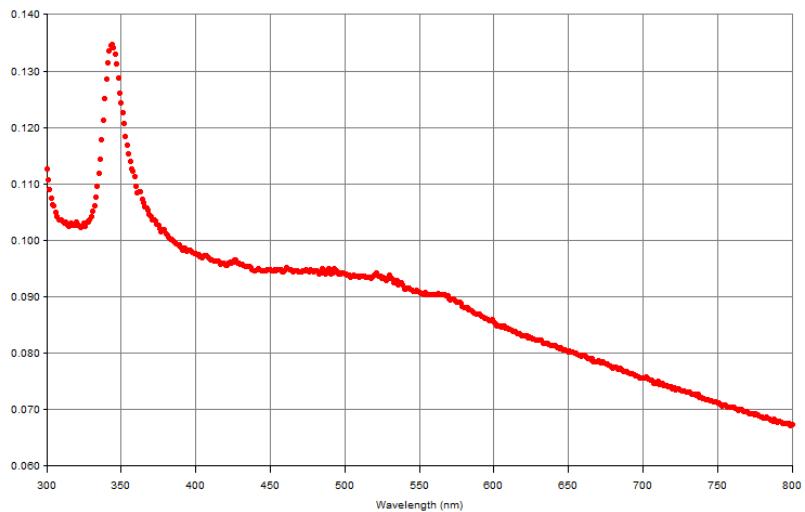
- SDS-PAGE for lead MBP



The molecular weight of the overexpression bands does not meet our expectation.

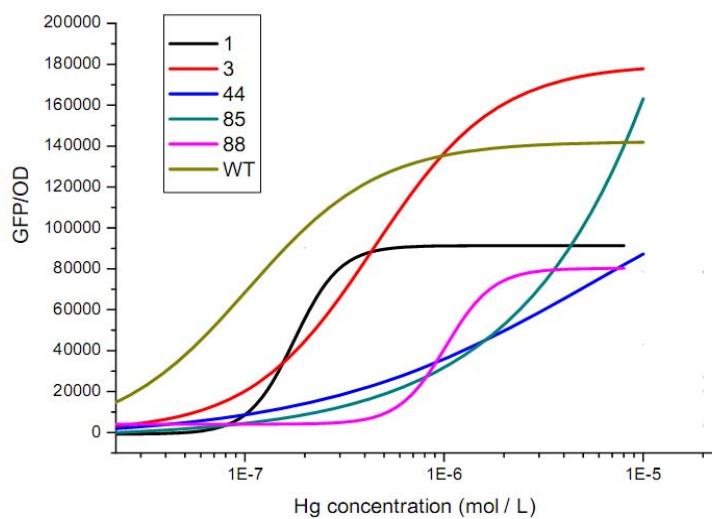
Check the plasmid.

- Scan the spectrum of Crt EBI



### 9.20-9.26

- Characterize the mutation of PmerT.



### 9.27-10.3

- 1-18I-pag, 2-2M-pag, 1-18E-pag construction

### 10.4-10.10

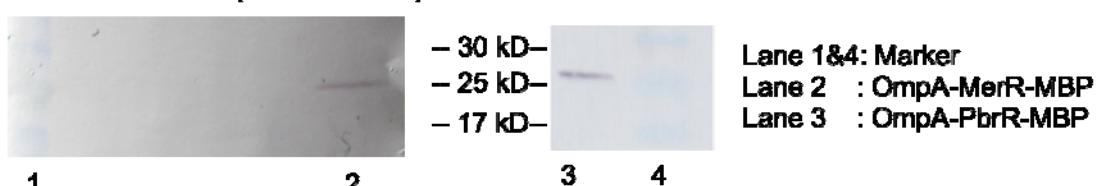
- western blot for mercury Lpp-OmpA-MBP

**A. SDS-PAGE**



– 37 kD  
Lane 1: OmpA-MerR-MBP cytosol  
Lane 2: OmpA-MerR-MBP membrane  
Lane 3: OmpA-PbrR-MBP cytosol  
Lane 4: OmpA-PbrR-MBP membrane  
Lane 5: Marker  
– 14 kD

**B. Western Blot (after induction)**



– 30 kD—  
Lane 1&4: Marker  
– 25 kD—  
Lane 2 : OmpA-MerR-MBP  
– 17 kD—  
Lane 3 : OmpA-PbrR-MBP

**10.11-10.17**

- Cigarette digestion for our biokit.  
Failed.