Notebook October

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Date 10.7

Awaken of the Glycerin bacteria BL-21 containing KmAdh on the plasmid PET22b and BL-21

Recorder: Xiaoyu Zhang

Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

Date 10.8

His-tag purification of KmADH

Recorder: Shihan Zhu

Procedure:

1. Pure out 20% ethyl inside of the tube;
2. Wash the tube with 10mL 1M imidazole 3 times;
3. Wash the tube with 10mL H2O;
4. Balance the tube with 1xPBS buffer 3 times;
5. Load my sample;
6. Inoculate protein with Ni-NTA，4°C，200rpm，1h;
7. Wash the tube with 10 mL 30mM imidazole 2 times and collect the sample;
8. Wash the tube with 10 mL 300mM imidazole and collect the sample;
9. Wash the tube with 10 mL 1M imidazole three times and collect the sample;
10. Wash the tube with 10 mL H2O;
11. Wash the tube with 20% ethanol and seal the tube.;

PCR of KmAdh

Recorder: Xiaoyu Zhang

1.Prepare 3 PCR tubes and sequentially add：

sample	**1**	**2**	**3**
Sterilized ddH2O	7 μL	7 μL	7 μL
2×Primer Star	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL
KmAdh-r(10 μM)	1 μL	1 μL	1 μL
KmAdh-f(10 μM)	1 μL	1 μL	1 μL
total	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	54 s
step 5	72	10 min
step 6	4	--

30 cycles

3.Agarose gel electrophoresis mixed with X μL 6× DNA loading buffer each X μL sample;XX V, XX min Result:

lane1-4: Marker,WT,KmAdh,Postive control

Enzyme activity test

Recorder: Xiaoyu Zhang

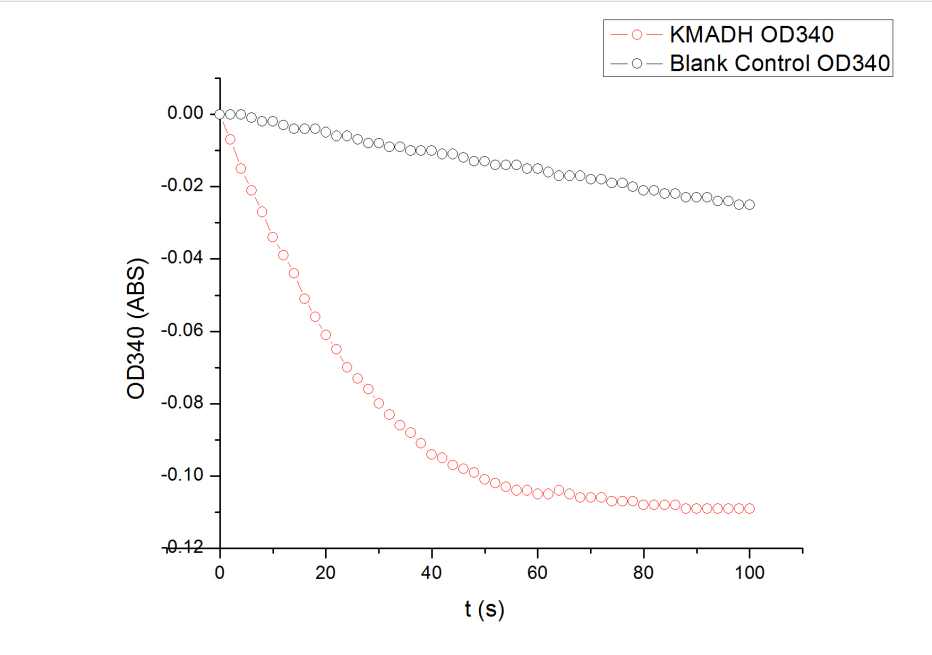
Procedure: The components of enzyme active reaction system are shown below.

System components	Volume
0.1mM Tris-HCl	100μL
3mM NADH	30μL
40% acetaldehyde	22μL
ddH2O	873μL
KmAdh	75μL

The components of blank control are shown below.

System components	Volume
0.1mM Tris-HCl	100μL
3mM NADH	30μL
40% acetaldehyde	22μL
ddH2O	873μL
PBS	75μL

Scan the 340nm UV absorption value over time. Draw the OD340-t curve. Compare the changes of OD340 over time.



We can obviously see the rapid increase in the absorption value after adding enzyme, indicating that NADH is drastically consumed. This shows the purified enzyme function is normal and the KmAdh is successfully expressed in E. coli.

> **Date 10.9**

Awaken of the Glycerin bacteria BL-21 containing CmCR on the plasmid PYYDT and BL-21

Recorder: Xiaoyu Zhang

Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

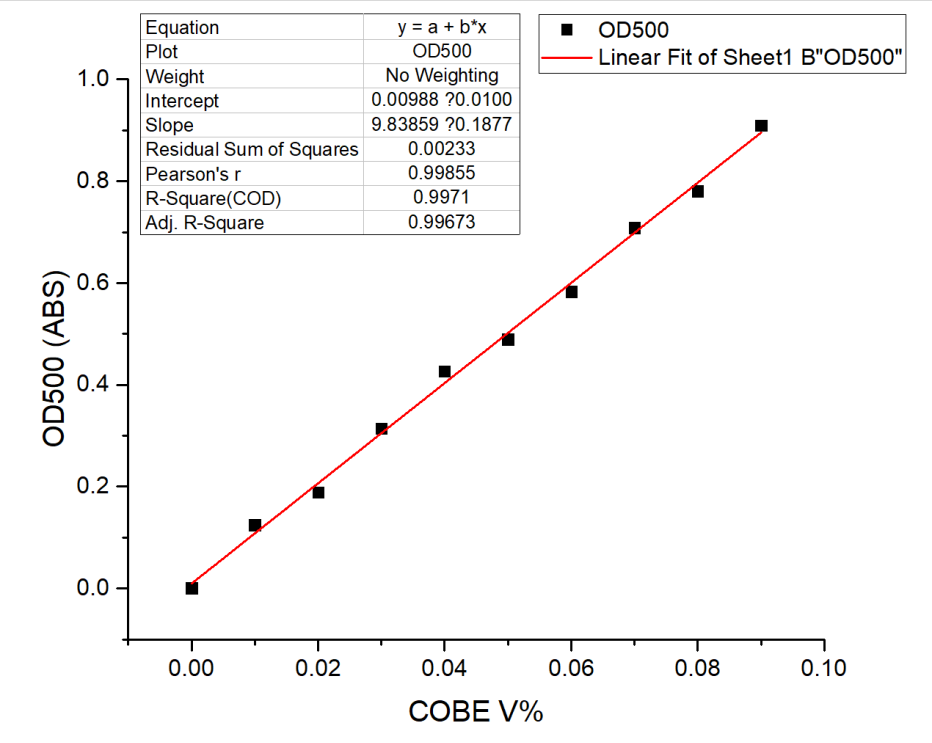
Standard curve of COBE

Recorder: Xiaoyu Zhang

The components of enzyme CmCR's active reaction system are shown below.

Sample number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
FeCl3	390μL	390μL	390μL	390μL	390μL	390μL	390μL	390μL	390μL	390μL	390μL
COBE	0μL	1μL	2μL	3μL	4μL	5μL	6μL	7μL	8μL	9μL	10μL
ddH2O	10μL	9μL	8μL	7μL	6μL	5μL	4μL	3μL	2μL	1μL	0μL
ethanol	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL	3600μL

Scan the 500nm UV absorption value .



Standard curve of COBE

Date 10.15

CysDes function analysis--acid labile sulfide analysis

Recorder: Shihan Zhu

Preparation

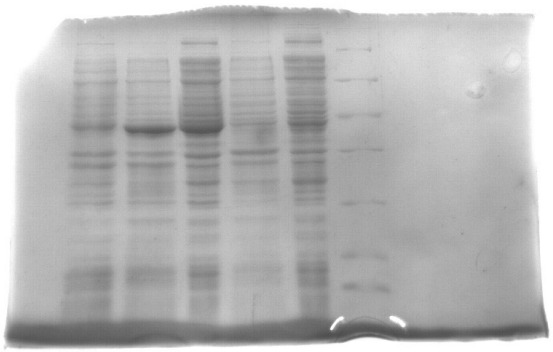
1. Inoculate 2 mL of BL21 with CysDes into 200 mL LB media and add cysteine to final concentration 1 mM and cultivate for about 3 hours, 37°C,250rpm.
2. When OD600 is about 0.4-0.6, add AHL to the media to final concentration 250nM.
3. Cultivate for 3 hours.

Procedure

1. 2 mL of culture samples are centrifuged at 17,000 g for 2 min;
2. The supernatant is removed and the cell pellet is resuspended in 1mL of 0.75 M NaOH;
3. The suspension is then transferred to a EP tube and incubated at 95 degree centigrade for 15 min;
4. Vortex the suspension vigorously and take 25 μL of the suspension to mix with 375 μL of 0.75 M NaOH and 250 μL of 2.6% zinc acetate dihydrate ; 125 μL 0.1% N,N-dimethyl-p-phenylenediamine dihydrochloride in 5 M HCl (freshly prepared) are added to and the solution is vortexes until clear;
5. 50 μL of 11.5 mM FeCl3 in 6M HCl are added and the solution is vortexes and incubated at RT for 30 min;
6. 425 μL of deionized water are added and the OD of the samples are recorded at 670 nm. (Solutions of 0-0.2 mM sodium sulfide in 0.75 M NaOH served as calibration standards.)

SDS-PAGE

帮助



(lane left to right: before induction, after induction , raw enzyme , wt, wt)

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