Lab Notebook 20170717－20170723 (1)

A. Experiment: Transformation in E.coli of the two genes MSMEG_5998、FGD

B. Schedule:

20170711—FGD(pET29a)→BL21

20170712—MSMEG_5998→DH5α

20170716—MSMEG_5998→BL21、FGD(pET29a)→DH5α

20170717—FGD (pET14b)→DH5α

20170720—FGD (pET14b)→BL21

C. Objective: to express and amplify the DNA from Dr.Taylor Matthew, Autralia.

D. Instruments and materials：

1. Plasmid：MSMEG_5998(pDESTIN)、FGD(pET29a、pET14b)
2. Competent cells：BL21-DE3、DH5α.
3. Transformation related instruments.

E. Methods:

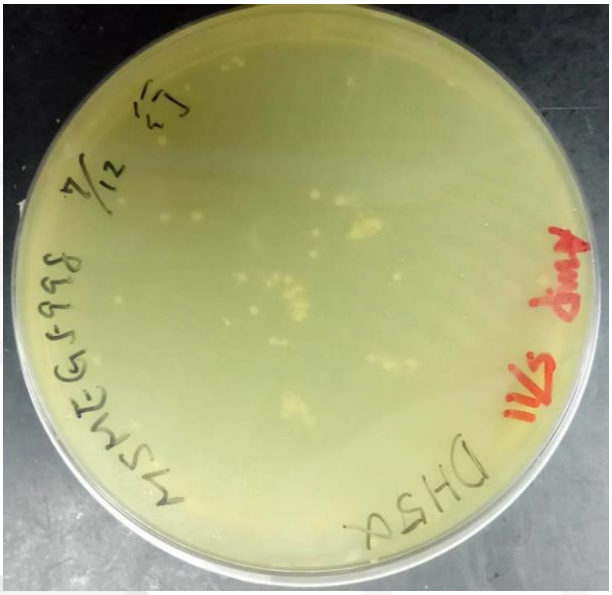
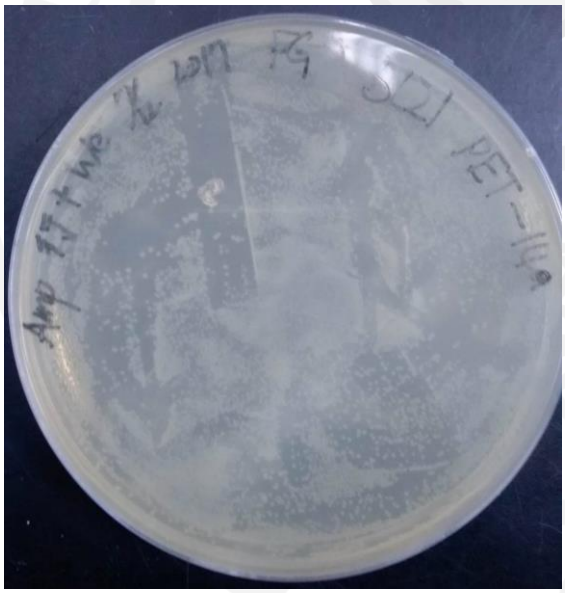
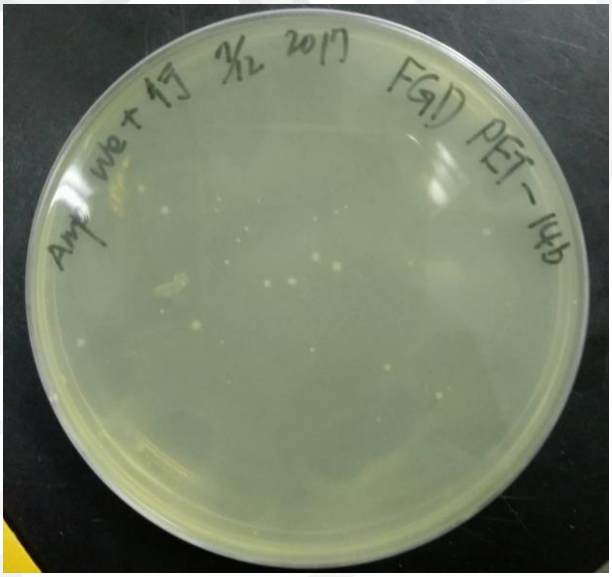
1. Transformation the plasmid MSMEG_5998(pDESTIN)、FGD(pET29a、pET14b) into E.coli DH5α.

   p.s.: Since E.coli is capable to produce mass DNA in a short time

2. Transform the plasmid (shaded above) in to the cell on site BL21-DE3

   p.s.: Since the e.coli can produce T7 polymerase, which means the cell can massively produce genes on plasmid pET, thus the target protein can be produced massively.

F. Results：

Colonies of MSMEG_5998 gene in cell BL21-DE3	Colonies of MSMEG_5998 gene in cell DH5α
	
Colonies of FGD (pET14b)gene in cell BL21-DE3	Colonies of FGD (pET14b) gene in DH5α
	

P.s.：FGD(pET29a) did not successfully transformed into the cell

G. Discussion：

1. The reason leading to transformation failure on FGD (pET29a) gene, after discussing with our advisor may be the plasmid carries a non-anti Ampicillin gen, while the plate we used contains ampicillin, therefore causing growth inhibition. However the speculation was not accurate because we add a different antibiotics and the results remains the same. Therefore, the team decided to run gel and examine the plasmid.

Lab notebook 20170717－20170723 (2)

A. Experiment: Plasmid extraction

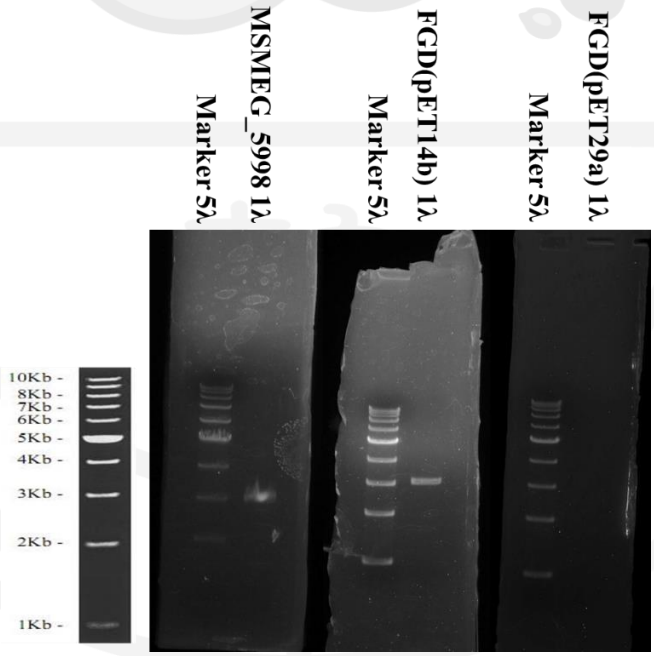
B. Schedule:
20170715—extract plasmid containing MSMEG_5998
20170720—extract plasmid containing FGD

C. Objectives：Extract the plasmid form the colonies after transformation, run Gel electrophoresis to confirm the results and store the plasmid.

D. Instruments and materials:
1. Colony: DH5α vector carrying MSMEG_5998、FGD(pET29a、pET14b)
2. Plasmid extraction related instruments
3. Gel electrophoresis related instruments

E. Methods:
1. Select a colony and proceed plasmid extraction as the protocol, as well as following the protocol of gel electrophoresis to proceed in order to confirm the size of the DNA by the band appearing in the gel. Store in -20℃.
2. Since plasmid pET29a (FGD gene) didn't grow in to colony on the plate, the team directly get the plasmid from previous lab works to run gel electrophoresis.

F. Results:



MSMEG_5998 and FGD(pET14b) appears to have a band on the site 3k, which means the transformation was success, leads to plasmid in the cell; however, FGD(pET29a) does not appear to have a band after gel electrophoresis.

G. Discussion:
1. There are no band presented in the FGD (pET29a), which means the transformation might not be successful, and maybe even the plasmid is not delivered to the filter paper, thus the gel electrophoresis results show no target plasmid band.

Lab Notebook 20170717－20170723 (3)

A. Experiment: IPTG induce E.coli to express and produce protein MSMEG_5998

B. Schedule:
20170718～20170721—express MSMEG_5998

C. Objective:
Since the transformed gene carries a T7 promoter, therefore, by adding IPTG into a bacteriophage-infected BL21 cell, it allows the cell to express the target DNA and produce the protein.

D. Instruments and materials：
1. IPTG solution
2. Cell lysis related instruments
3. Western blot、Coomassie blue related instruments (including Luminometer)
4. Primary antibody：6x Anti-His Tag (GeneTex)、6x Anti-His Tag clone H8
5. Secondary antibody：Anti-mouse HRP

E. Method:
1. IPTG induced cell BL21 (by following the protocol)
2. Cell lysis and detect the O.D. absorption (distribute some and dilute 50X), preserve in TE、sample buffer
3. Dye with Coomassie blue, to see whether IPTG can lead toBL21 different protein expression.
Loading gel order (12%gel ，10μl each)：

Marker	IPTG+	IPTG-	Dye						

4. Western blot detected by anti Histag to decide whether it went through IPTG or not, it can cause BL21 carries 6 Histidine amino acid.
a. Experiment details：

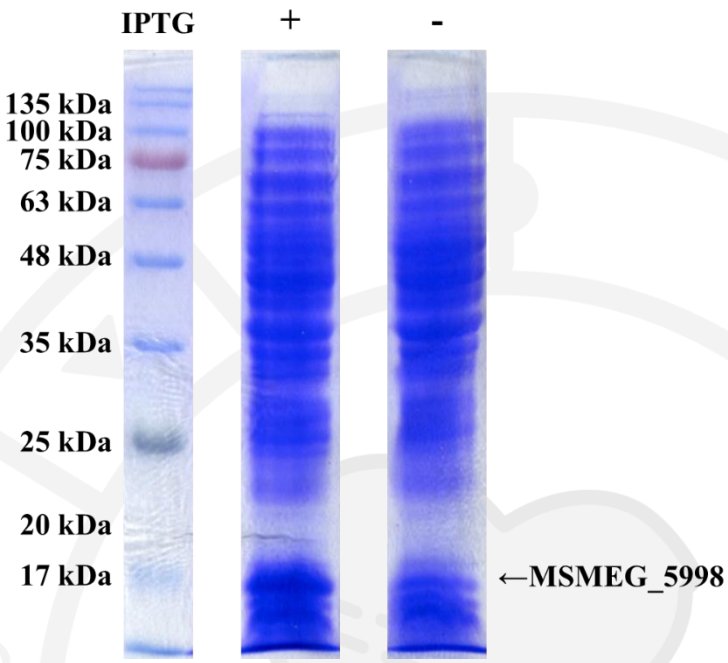
Gel (%)	12
Loading	各 10μl
Protein size	約 18kDa
Primary antibody	6x Anti-His Tag (genetex—1:2000(original)/1:1000(diluted)，milk 6x Anti-His Tag clone H8—1:2000(original)/1:1000(diluted)，milk
Secondary	Anti-mouse—1:5000，milk
Type of antibody	mouse

b. Loading well order：

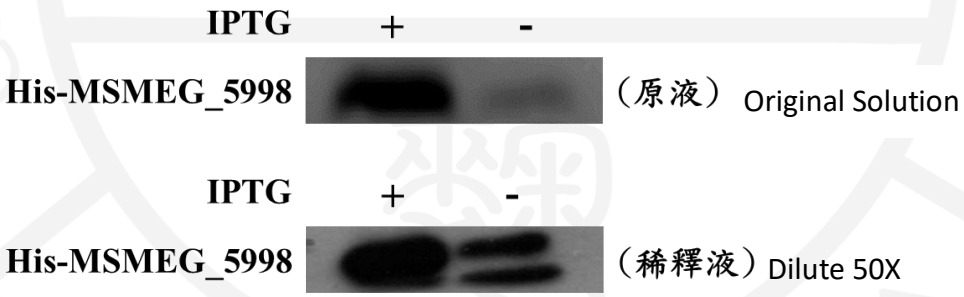
Marker	IPTG+	IPTG-	Dye	Marker	IPTG+	IPTG-	Dye		

F. Results:

1. Protein O.D.(Absorption): IPTG(+)—1.727，IPTG(-)—1.698
2. We can observe the target protein expressions increase in a large scale, after conducted the IPTG induction and dye with Coomassie blue



3. By using Western Blot technique, we can tell whether the original concentration or that diluted by 50 times can both produce target protein(MSMEG5998) via IPTG.



G. Discussion：

1. Since the experiment is to ensure whether the target protein is produced by the cell, thus the bacterial suspension isn't accurate while distribution, However, the two suspension (original and diluted 50X) shows very close numbers on O.D. , thus after Western blot we can still tell the one underwent IPDG induction obtains a higher productivity.
2. Should do further research on bacteria, to learn what house keeping genes do other researchers use to be the control of western blot.
3. After the experiment, the team ensure the two antibodies show high specificity, thus the experiments later on the two antibodies are ideal.

Lab Notebook 20170724－20170730 (1)

A. Experiment: Produce FGD protein by using IPTG induce method in E.coli.

B. Schedule:

20170720～20170723— FGD protein expression

C. Objective:

Since the transformed gene carries a T7 promoter, therefore, by adding IPTG into a bacteriophage-infected BL21 cell, it allows the cell to express the target DNA and produce the protein.

D. Instruments and materials:

1. IPTG solution
2. Cell lysis related instruments
3. Western blot、Coomassie blue related instruments (including Luminometer)
4. Primary antibody：6x Anti-His Tag (genetex)、6x Anti-His Tag clone H8
5. Secondary antibody：Anti-mouse

E. Method:

1. Use IPTG to induce BL21 cell express the interested protein
2. Lyse the cell and measure the OD. (Take some of them and dillute50X).Add TE and sample buffer then store at -20℃
3. Stain with Coomassie blue, and investigate whether IPTG would affect the protein expression in BL21cell.

   Loading method(12%Gel，10μl sample for each well)：

Marker	IPTG+	IPTG-	Dye						

4. Western blot detected by anti His-Tag to shows whether it went through IPTG or not.

   a. Experiment details：

Gel (%)	12
Loading	10μl for each well
Protein size	About 18kDa
Primary antibody	6x Anti-His Tag (GeneTex)─1:2000 in milk
Secondary	Anti-mouse—1:5000 in milk
Type of antibody	Mouse

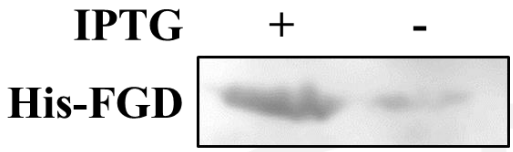
   b. Loading well order：

Marker	IPTG+	IPTG-	Dye	Marker	IPTG+	IPTG-	Dye		

F. Results:

A. Protein O.D.(absorption) Start—3.6, IPTG(+)—1.464 ,second IPTG(-)—1.559

B. By western blot we can see after IPTG induction, the productivity of the target protein is higher.



Lab Notebook 20170724－20170730 (2)

A. Experiment: IPTG induce and Cell lysis

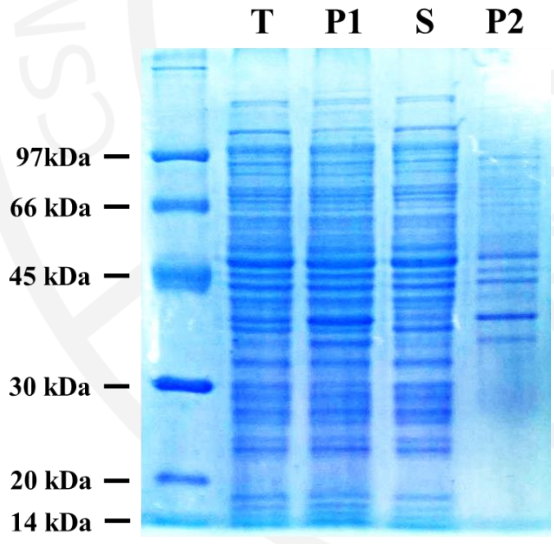
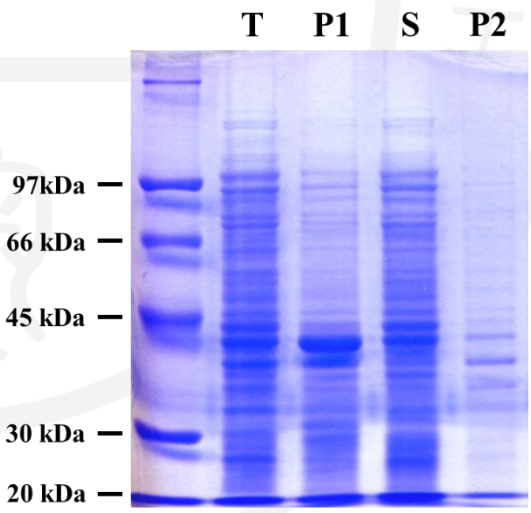
B. Schedule:
   20170725—MSMEG_5998
   20170726—FGD

C. Objectives:
   1. IPTG induce: acquire massive protein expressed by E.coli.
   2. Cell lysis: Obtain the pellet in order to evaluate the target's solubility in water.

D. Instruments and Materials:
   1. IPTG related instruments
   2. Ultrasonic Processor
   3. Centrifuge machine

E. Method:
   1. Refer to IPTG protocol
   2. Coomassie brilliant blue staining
   3. Western blot

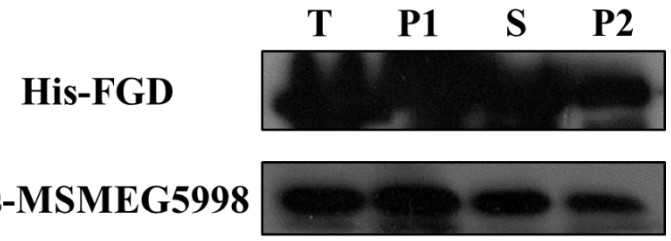
F. Result:
   1. Coomassie brilliant blue staining

MSMEG_5998	FGD
	

p.s.：T is referred to total cell, P1 is the pallet after 9500rpm, S is the supernatant after 12000rpm and P2 is the pallet after 20000rpm

Compare the two sites on S and P2 at the site 18kDa and 38kDa, notice that MSMEG_5998 on P2 can hardly see any band, however, the band on FGD is still visible, which means MSMEG5998 is mostly soluble in water however FGD does not have a high water solubility, which will create inclusion body.

2. Western blot



MSMEG_5998 on well P2have less protein, meaning that it is soluble, FGD band can be observed in well S and P2, Which means inclusion bodies may be produced.

G. Discussion:

A. Using Anti-His Tag (clone H8) as a primary antibody to process WB is prone to create background noise, thus the antibody toward FGD can alter in the next experiment and with a longer washing time.

B. When processing Coomassie brilliant blue staining, adding Methanol and glycerol can preserve the gel.

Lab Notebook 20170724－20170730 (3)

A. Experiment: Protein Purification

B. Schedule:
20170727—MSMEG_5998
20170728—FGD
20170729～20170731—Coomassie blue staining + Western Blot

C. Objective: Purify the suspension containing target protein to acquire a higher concentration of the protein expressed.

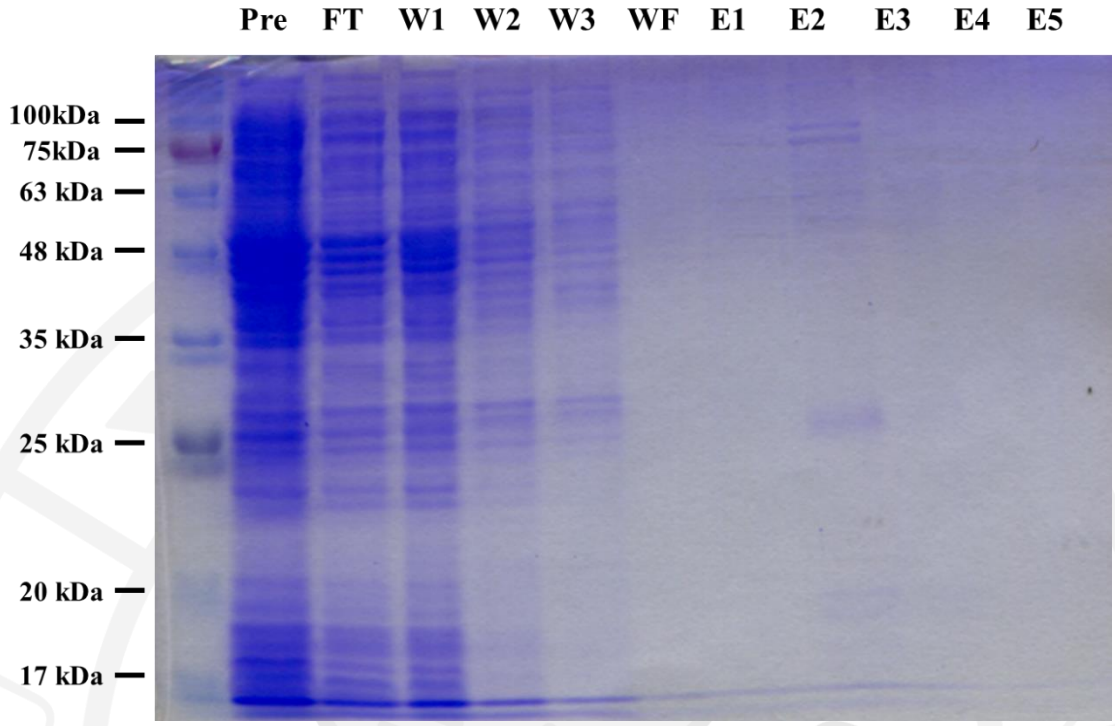
D. Instruments and Materials:
1. Nickel-resin column
2. Buffer：Equilibrium buffer、Washing buffer、Elution buffer
3. 30% ethanol
4. NaCl、Imidazole
5. Purification related Instruments

E. Method:
1. Protein purification protocol:
   - Pre: the supernatant before purification
   - FT(flow through): the liquid collected when adding Equilibrium buffer.
   - W1～WF: the suspension collected form the flow through of the washing buffer(F: final 1 c.c)
   - E1～E5: the suspension collected form the flow through of the Elution buffer.
2. WB
3. Coomassie blue staining

F. Results:

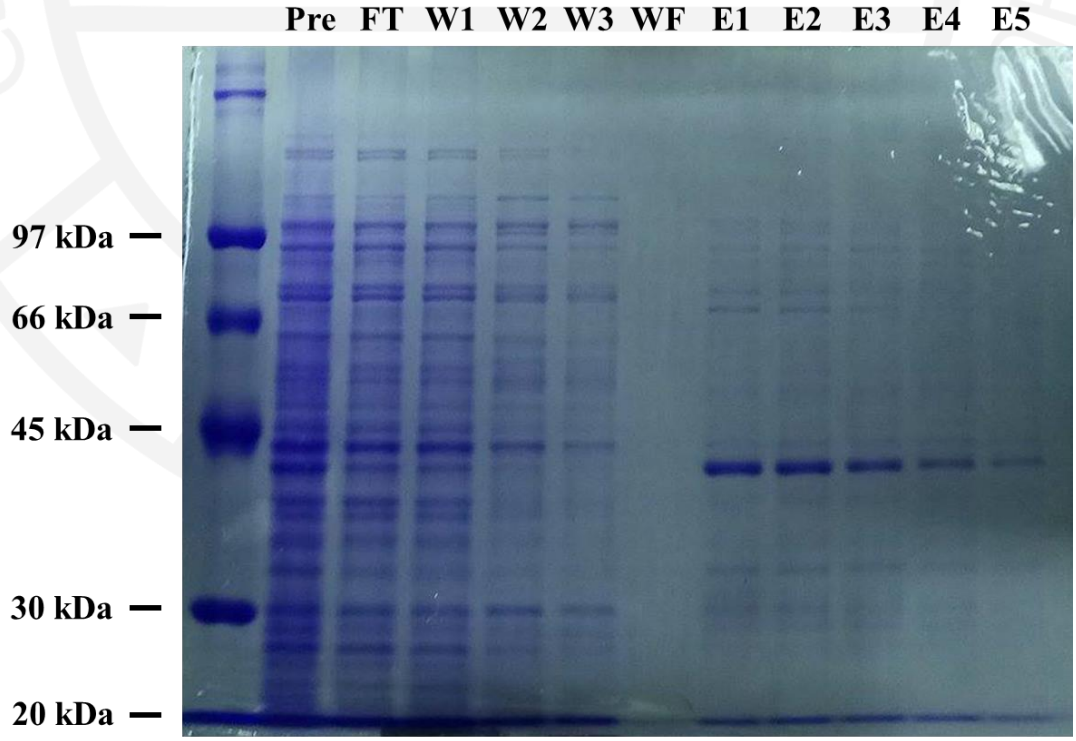
1. Coomassie brilliant blue staining

a. MSMEG_5998



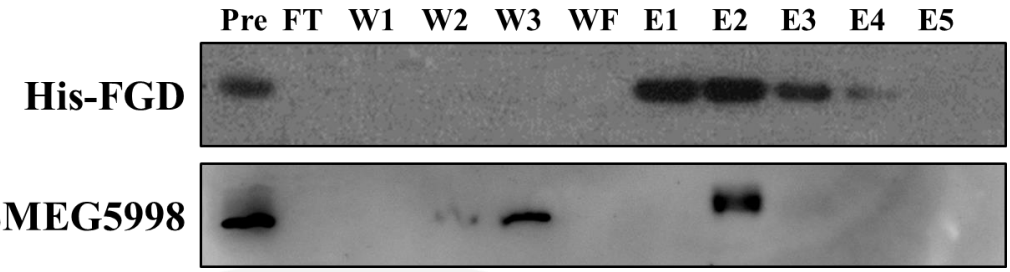
The target protein MSMEG5998 can be observed in tube E2

b. FGD



The target protein FGD can be observed in tube E1~3

2. Western blot



The target protein MSMEG5998 can be observed in tube E2, while FGD can be observed from E1~E3. The two protein can be observed is because the suspension is not yet purified, which contains all sorts of protein, including our target.

G. Discussion:

1. Using Anti-His Tag (clone H8) as a primary antibody to process WB is prone to create background noise, thus the antibody toward FGD can alter in the next experiment and with a longer washing time.
2. Purification is processed by suspension flowing through the Nickel-resin column, however the background noise is still observable, thus the target protein quantity can not be detected by O.D.(absorption) since the other protein is affecting the results. Thus the quantitative detection is conducted by gel electrophoresis with a standard control BSA, and try to measure the numbers by comparing with the target.

Lab Notebook 20170731－20170806 (1)

A. Experiment: Protein Dialysis

B. Schedule:
20170801—20170802

C. Objectives:
By processing protein dialysis, the imidazole in the suspension can be washed out and maintain the protein function by adding glycerol.

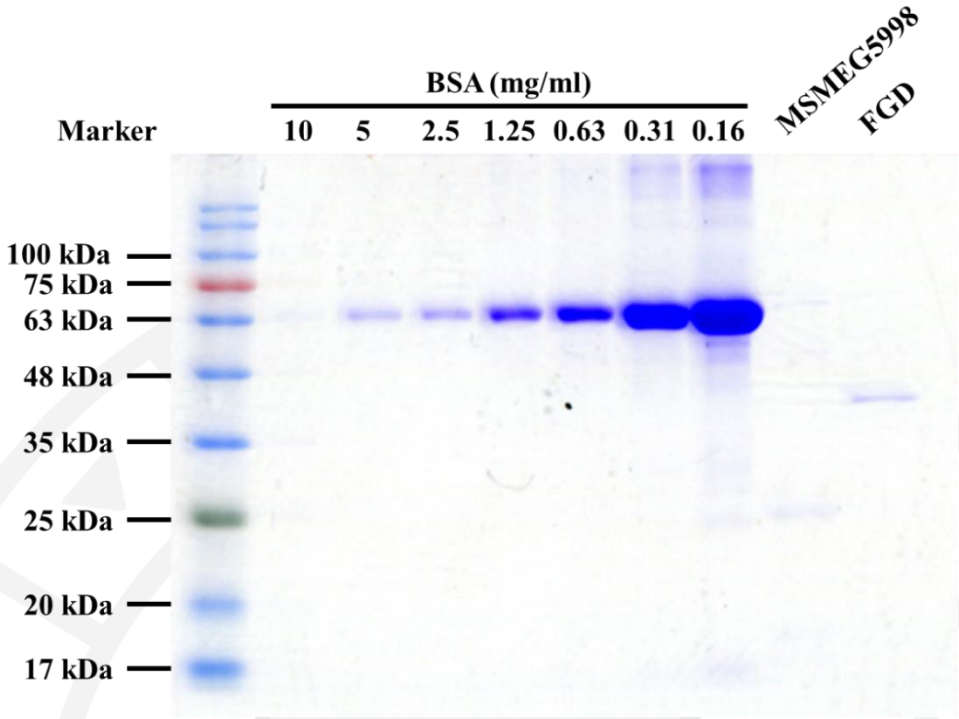
D. Instrument and Materials:
1. Dialysis related instruments
2. BSA 10 mg/ml、4x Sample buffer、TBS
3. Gel electrophoresis and Coomassie brilliant blue staining related instruments

E. Method:
1. Protein dialysis protocol
2. BSA suspension:
   - Concentration of each tube: 10、5、2.5、1.25、0.63、0.31、0.16 μg/μl
3. Loading well order:

marker	10 BSA 2 λ	5 BSA 2 λ	2.5 BSA 2 λ	1.25 BSA 2 λ	0.63 BSA 2 λ	0.31 BSA 2 λ	0.16 BSA 2 λ	5998 5 λ	FGD 5 λ
p.s.: take MSMEG5998 and FGD 5 λ each and mix with 5 λ of sample buffer, then load 5 λ to run electrophoresis.									

F. Results:

1. Coomassie brilliant blue staining:

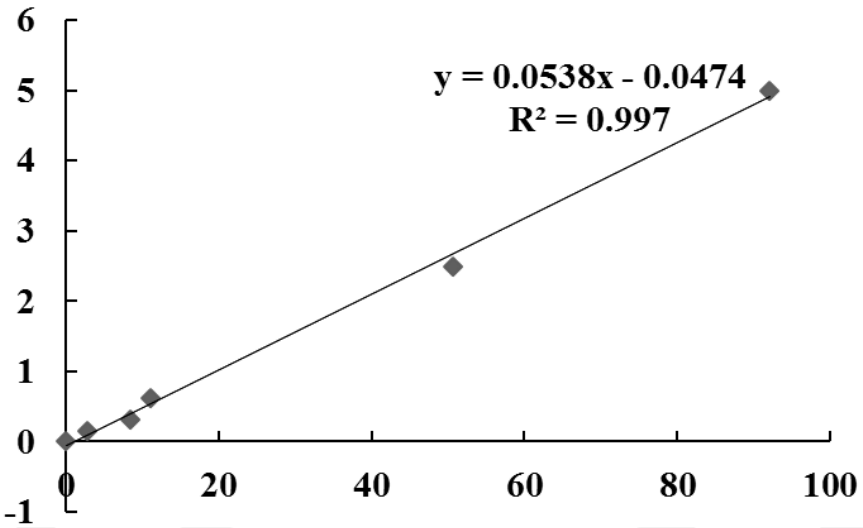


2. Standard curve

a. Data on reading the band:

Band color reading	Concentration (μg/μl)
0	0
2.892	0.15625
8.379	0.3125
11.093	0.625
32.853	1.25
50.609	2.5
92.137	5
122.739	10

b. The team drew a standard curve:



3. Protein quantity analysis:

a. MSMEG_5998 and FGD process by the formula:

	Reading figure	Theoretical concentration (μg/μl)	Actual concentration (μg/μl)
MSMEG5998(1)	2.634	0.094309	0.03772368
MSMEG5998(2)	0.666	-0.01157	0.014393154
FGD	4.53	0.196314	0.0785256

p.s.:

- Since we are uncertain to the location of the MSMEG5998 , it is either near to the 25kDa band or the 20kDa band, so when put in to calculation, two concentrations will all be processed.
- The concentration of Tmsmeg5998(2) is calculated as 0.666/2.892 = [MSMEG5998]（per 2.5μl）/0.15625，[MSMEG5998]＝0.035983，divided by 2.5＝0.014393 **(**μg/μl**)**

b. concentration (μM)：

MSMEG5998(1)＝0.03772368/18000×10^6＝2.09576 (μM)

MSMEG5998(2)＝0.014393154/18000×10^6＝0.79961964 (μM)

FGD＝0.0785256/37700×10^6＝2.082907162 (μM)

G. Discussion

1. The band of MSMEG5998 still needs to be examined.
2. The concentration of the protein is not very high, however it overpassed the limit, therefore the team proceed to inspections on the activity of the enzyme.

Lab Notebook 20170731－20170806 (2)

A. Experiment: resuspension of Aflatoxin B1、G6P

B. Schedule: 20170804, 20170807

C. Objective:

Produce suspension of AflatoxinB1 and G^P

D. Solutions and instruments:

1. Aflatoxin B1 100 μg
2. Methanol
3. D-Glucose 6-phosphate disodium salt hydrate (G7250 SIGMA)
4. Sterilized ddH_2O
5. 10 c.c. needle and filter

E. Method:

1. Aflatoxin B1 solution
   i. Concentration: 100 μg/ml
   ii. add 1 c.c. methanol and distribute into different tubes of 500、200、100、100、50、50 μl, Stock in -20℃.
2. G6P solution
   i. Concentration：25 mM

F. Results:

1. Aflatoxin B1: (Molecular weight＝312)

   100 μg/ml = 100 mg/L = 0.1 g/L = (0.1/312) mol/L = 0.00032 M =320 μM
2. G6P: (Molecular weight＝304.1)

   0.076 g/10 ml = 0.00025 mol/10 ml = 0.025 M = 25 mM

Lab Notebook 20170807-20170813(1)

A. Experiment : Prepare F_{420} solution

B. Date : 20170808

C. Objective:

Make F_{420} to target concentration

D. Results:

F_{420}：(molecular weight ＝778)

236 μM in 1 ml suspensions the powder contains 236×10^{-6} (M)$\times10^{-3}$ (L)$\times778=0.184$ mg

Lab Notebook 20170807-20170813 (2)

A. Experiment: ELISA on MSMEG_5998 activity

B. Date: 20170808

C. Objective:

Using the antibody of aflatoxin B1to detect the concentration of aflatoxin remaining after degrading by MSMEG5998.

D. Instruments and materials:

- Aflatoxin B1 100 μg/ml、G6P 25 mM、F_{420} 236 μM、MSMEG_5998 2.1 μM、FGD 2.1 μM
- ELISA related instruments
- Aflatoxin with standard concentration

E. Method:

1. Create reaction suspension (as listed), with the total volume of 0.5 ml for the ELISA

Name	Stock concentration	Ideal concentration	Volume needed for 500 μl suspension
Aflatoxin B1	100 μg/ml	1 μg/ml	500*1/100=5 μl
G6P	25 mM	2.5 mM	500*2.5/25=50 μl
F_{420}	236 μM	10 μM	500*10/236=21.2 μl
MSMEG_5998	2.1 μM	0.1 μM	500*0.1/2.1=23.8 μl
FGD	2.1 μ	0.45 μM	500*0.45/2.1=107.1 μl
Tris-HCl	1 M	50 mM	500*50/1000=25 μl
ddH_2O			500-5-50-21.2-23.8-107.1=292.9 μl

p.s.: Aflatoxin will be the last to add into the suspension(2.5 **μl** each)

- After adding Aflatoxin, the 0 min eppendorf directly heat 100℃,10 min; while the 10 min eppendorf should be in water bath on 37℃ for half an hour, then heat up to 100℃ for 10 min, both then proceed ELISA under room temperature.

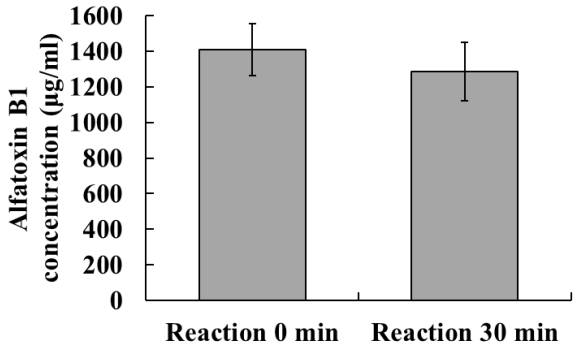
2. Refer to direct competitive ELISA protocol

F. Results:

1. Raw data

	Dilute to(X)	W data	raw data	SE	Concentration	Concentration	Ve. Concentr-ation	Original concentrat-ion
-standard	1	0.073	0.073	0				
	10	0.194	0.197	0.002				
	100	0.48	0.478	0.001				
	1000	0.695	0.733	0.027				
	2000	1.146	1.215	0.049				
	4000	1.601	1.737	0.096				
Reaction 0 min	1	0.007	0.007	0	42.478	42.478	42.48	42.478
	10	0.013	0.013	0	36.285	36.285	36.29	362.85
	100	0.068	0.069	0.000	13.583	13.404	13.49	1349.35
	1000	0.405	0.387	0.013	1.411	1.522	1.467	1466.5
	2000	0.53	0.617	0.062	0.872	0.646	0.759	1518
	4000	0.845	0.849	0.003	0.313	0.309	0.311	1244
Reaction 30 min	1	0.007	0.008	0.001	42.478	41.329	41.9	41.9035
	10	0.014	0.015	0.001	35.399	34.547	34.97	349.73
	100	0.066	0.075	0.006	13.953	12.406	13.18	1317.95
	1000	0.386	0.413	0.019	1.529	1.365	1.447	1447
	2000	0.605	0.628	0.016	0.672	0.622	0.647	1294
	4000	0.881	0.882	0.001	0.28	0.279	0.28	1118

2. Chart



G. Discussion:

1. The two main possible reasons to the failure:
   a. The enzyme is denatured
   b. The antibody still detects the product after degradation.
2. The team then decide try another method on detecting Aflatoxin B1, which is by detecting the OD absorption of Aflatoxin 362 nm.

Lab Notebook 20170807-20170813 (3)

A. Experiment: Measure the activity of MSMEG5998 by detecting the OD of Aflatoxin B1

B. Date: 20170811～20170812

C. Objectives:

Measure the Aflatoxin B1 concentration by detecting the OD on wavelength 362 nm

D. Instrument and materials:

- Aflatoxin B1 100 μg/ml、G6P 25 mM、F_{420} 236 μM、MSMEG_5998 2.1 μM、FGD 2.1 μM
- 96 well plate
- Methanol、ddH_2O

E. Method:

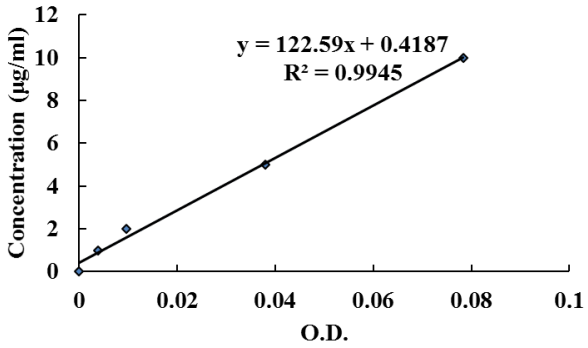
1. Prepare Aflatoxin B1 standard solution
2. Prepare Reaction suspension 250 μl (Condition: Reaction×5998, Reaction 0 h, 1 h, 24h, 50 μl each, at least 200 μl)

Name	Stock concentration	Ideal concentration	Volume for 250
Aflatoxin B1	100 μg/ml	15 μg/ml	250*15/100=37.5 μl
G6P	25 mM	2.5 mM	250*2.5/25=25 μl
F_{420}	236 μM	10 μM	250*10/236=10.6 μl
MSMEG_5998	2.1 μM	0.1 μM	250*0.1/2.1=11.9 μl
FGD	2.1 μ	0.45 μM	250*0.45/2.1=53.6 μl
Tris-HCl	1 M	50 mM	250*50/1000=12.5 μl
ddH_2O			250-37.5-25-10.6-11.9-53.6-12.5=98.9 μl

P.s. When making the reaction suspension, do not add MSMEG_5998until start to time the reaction time.

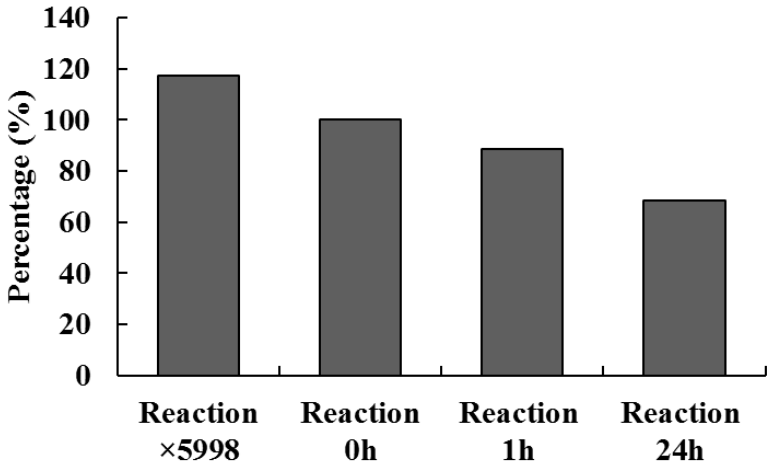
F. Results:

1. Aflatoxin B1 and OD standard line:



O.D.	concentration (μg/ml)
0	0
0.0039	1
0.00955	2
0.038	5
0.0783	10

2. Degradation percentage:



Condition	O.D.	(%)	Concentration (μg/ml)
Reaction ×5998	0.0355	117.1617162	4.778095
Reaction 0h	0.0303	100	4.143227
Reaction 1h	0.0268	88.44884488	3.715912
Reaction 24h	0.0207	68.31683168	2.971163

The experiment was not repeated multiple times due to the reason that this experiment is conducted for the purpose to see whether the enzyme will degrade Aflatoxin or not.

G. Discussion:

1. The data of O.D. this time is less than 0.1 after removing the background, thus the experiment should conduct again considering the sensitivity of the ELISA reader. The next time suspension loaded will be 100 μl/well order to increase the O.D. greater than 0.1.
2. Control group (without adding Aflatoxin B1) should be added into different period of time, in order to compare whether the toxic is self-degrading or degraded by the enzyme.
3. The experiment conducting next time should be multiple repeated to be more persuasive.

Lab Notebook 20170814－20170820 (1)

A. Experiment: Protein Purification

B. Date: 20170817

C. Objective: Purify the suspension containing target protein to acquire a higher concentration of the protein expressed.

D. Instrument and material:

1. BSA 10 mg/ml、4x Sample buffer、TBS
2. Gel electrophoresis and Coomassie brilliant blue staining related instruments

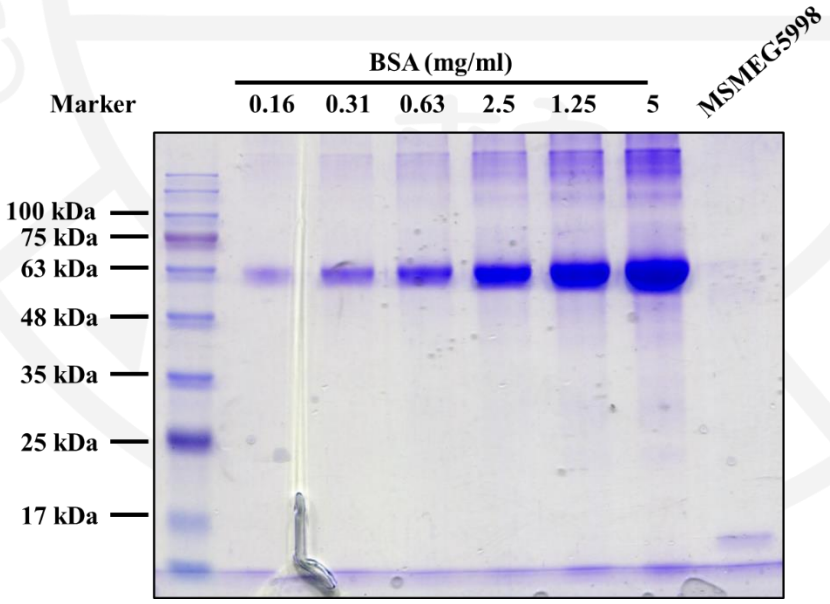
E. Method:

1. Protein dialysis protocol
2. BSA suspension:
   Concentration of each tube: 10、5、2.5、1.25、0.63、0.31、0.16 μg/μl
3. Loading well order:

marker	0.16 BSA 2 λ	0.31 BSA 2 λ	0.63 BSA 2 λ	1.25 BSA 2 λ	2.5 BSA 2 λ	5 BSA 2 λ	5998 5 λ	FGD 5 λ
p.s.: take MSMEG5998 and FGD 5 λ each and mix with 5 λ of sample buffer, then load 5 λ to run electrophoresis.								

F. Results:

1. Coomassie brilliant blue staining：

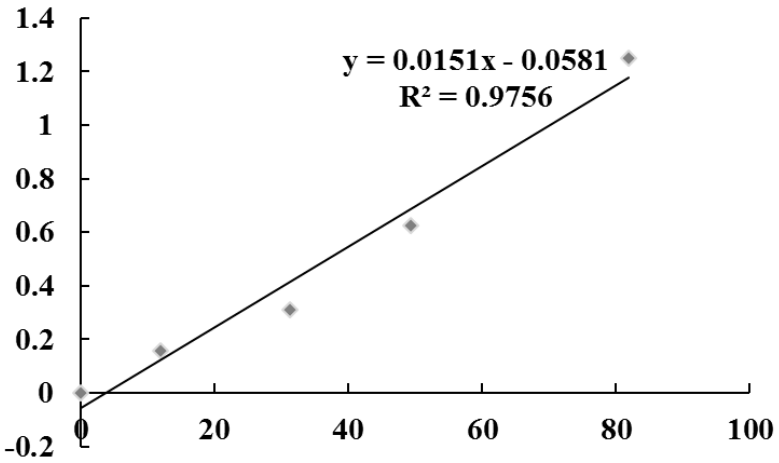


2. Standard curve:

Data on reading the band:

Reading data	Concentration (μg/μl)
0	0
11.91	0.15625
31.329	0.3125
49.299	0.625
81.944	1.25
104.627	2.5
125.223	5

The team drew a standard curve:



3. Protein quantity analysis:

a. MSMEG_5998 and FGD process by the formula:

	data	Theoretical concentration (μg/μl)	Actual con. (μg/μl)
MSMEG5998	3.158	-0.01041	0.016572208

b. concentration (μM)：

MSMEG5998＝0.016572208 /18000*10^6＝0.920678235 (μM)

G. Discussion

1. The affinity of nickel-resin is weak, leading the results not ideal.
2. IPTG induce change time to 4 hours

Lab Notebook 20170814－20170820 (2)

A. Experiment: Aflatoxin B1standard curve

B. Date: 20170814

C. Objective: draw a standard curve to process quantification of aflatoxin B1.

D. Instruments:

- Aflatoxin B1 100 μg/ml、methanol、ddH_2O

E. Methods:

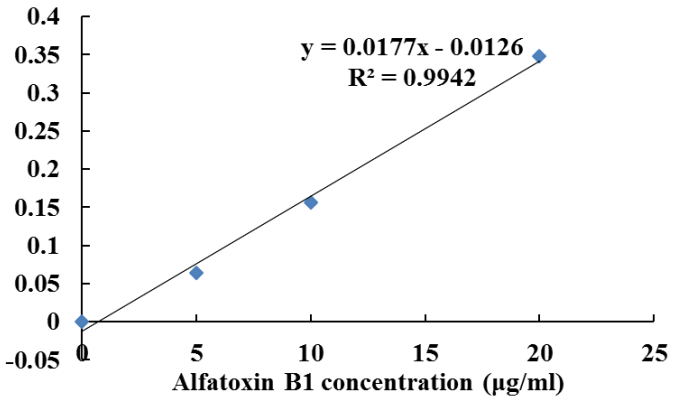
Prepare concentration of 5、10、20 μg/ml each have the volume of 100 μl add to 96 well plate, detect with ELISA reader 365 nm

Concentration	Stock usage (μl)	Methanol (μl)	ddH_2O (μl)
5	5	15	80
10	10	10	80
20	20	0	80

F. Results

A. data

Concentration (μg/ml)	O.D.
0	0
5	0.06415
10	0.1568
20	0.348



Equation

[Aflatoxin B1]= (O.D.+0.0126)/0.0177

Lab Notebook 20170814－20170820 (3)

A. Experiment: detection of enzyme activity MSMEG_5998 by using OD measurement

B. Schedule: 20170817～20170818

C. Objective:

Directly detect the Aflatoxin B1 concentration change by using O.D. (365nm)

D. Instruments and materials:

1. Aflatoxin B1 100 μg/ml, G6P 25 mM, F_{420} 236 μM, MSMEG_5998 2.1 μM, FGD 2.1 μM
2. 96 well plate
3. Methanol, ddH_2O

E. Method:

Prepare reaction solution 250 μl (Condition：Reaction ×5998 0h、1h、24h、48h, Reaction 0 h、1 h、24h、48hr repeat 3 times with the volume 100 μl each),total volume at least 800×3＝2400 μl)

Name	Stock Conc.	Target Conc.	Total volume 2700 μl
Aflatoxin B1	100 μg/ml	10 μg/ml	2700*10/100=270 μl
G6P	25 mM	2.5 mM	2700*2.5/25=270 μl
F_{420}	236 μM	10 μM	2700*10/236=114.48 μl
MSMEG_5998	2.1 μM	0.2 μM	1300*0.2/2.1=123.76 μl
FGD	2.1 μ	0.45 μM	2700*0.45/2.1=578.34 μl
Tris-HCl	1 M	50 mM	2700*50/1000=135 μl
ddH_2O			2700-270-270-114.48-578.34-135-0.2/2.1*2700=1075.14 μl

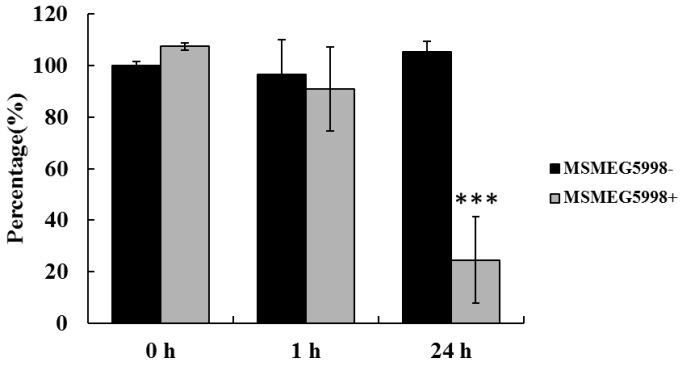
P.s.: After prepare two Eppendorf with the solution total of 123.76 μl, then add 123.76 μl MSMEG5998(or ddH2O for complement) last, distribute into four Eppendorf with the volume 300 μl each. After preparation there will be 8 Eppendorf, labeled as MSMEG5998(+) 0 h,1 h,24 h,48 h/MSMEG5998(-) 0 h,1 h,24 h,48 h. Immediately end the reaction of Eppendorf 0h, the rest 6 Eppendorf remains in 22℃ until the ending reaction time,. The groups that terminate the reaction will first stock in -20℃, until all 8 Eppendorf is ready to load 100μl each in the 96 well plate.

F. Results:

1. Raw data

MSMEG 5998(-)	0 h	1 h	24 h	48 h	MSMEG 5998(+)	0 h	1 h	24 h	48 h
	0.214	0.219	0.224	0.205		0.219	0.216	0.125	
	0.212	0.214	0.214	0.209		0.222	0.206	0.116	0.112
	0.21	0.19	0.217			0.222	0.181	0.117	0.124
Erase background	0.125	0.13	0.135	0.116	Erase background	0.13	0.127	0.036	
	0.123	0.125	0.124	0.12		0.133	0.116	0.026	0.022
	0.121	0.1	0.128			0.133	0.092	0.028	0.035
mean	0.123	0.118	0.129	0.118	mean	0.132	0.112	0.03	0.028
S.E.	0.002	0.016	0.005	0.003	S.E.	0.002	0.018	0.005	0.009
%	101.5	109.9	104.4	98.39	%	98.36	113.7	119	
	100	105.3	96.31	101.6		100.9	104.2	87.49	78.21
	98.45	84.74	99.25			100.8	82.1	93.47	121.8
mean%	100	96.47	105.2	95.85	mean%	107.4	90.85	24.53	23.18
S.E.%	1.548	13.41	4.118	2.284	S.E.%	1.424	16.21	16.76	30.82

2. Chart



a. The bar of 48 h has a excessive S.E thus it is not presented in the chart.

b. By Observing the chart, the Aflatoxin residues remains only 24%, comparing to the control group there is a obvious decrease in aflatoxin.（*** means $p < 0.001$）

G. Discussion:

1. Since the method needs a large volume of the sample, the team envision us to process by using HPLC in the future, which use far less sample by the method(10 to 20 μl each condition.)
2. Since the results of the 24 hours reaction is very significant, thus the experiment on enzyme activity can change the period of reaction time into 0,3h,6h,9h,12h,15h,18h,24h

Lab Notebook 20170904－20170910 (1)

A. Experiment: extract protein from Aflatoxin-treated $HepG_2$ cell

B. Schedule:
20170905～20170907

C. Objective:
Extract protein from Aflatoxin-treated cell, and exam the quantity in order to do further experiments like western blot.

D. Instruments and materials:
1. $HepG_2$ cell line
2. Aflatoxin B_1 1 mM、DMSO
3. Protein extraction related instruments
4. 12 well plate

E. Method:
1. 20170905：1×10^5 /well , a total of 8 wells
2. 20170906：incubate cell overnight, distribute 0、1、10 μM Aflatoxin B1into the medium (with one more only adding DMSO, each condition 2 wells, 1 ml per well.
   Details are listed below:
   - First centrifuge tube (15 c.c.)(labelled 0 μM）adding 2.2 ml medium
   - Second tube (labelled 1 μM) add 2.2 μl aflatoxin, 19.8 μl DMSO and 2178 μl medium
   - Third tube(labelled 10 μM)adding 22 μl of aflatoxin, and 2178 μl medium
   - Forth tube (labelled DMSO) adding 22 μl DMSO and 2178 μl medium
3. 20170928：After 24 hours ,proceed RIPA+PI to extract protein and stock in -20℃

F. Results:
   A. Details are listed in the chart (total volume: 250 μl)：

Name	O.D.	Theoretical concentration (μg/10 μl)	Measured concentration (μg/μl)	400 μg usage(μl)	10x sample buffer (μl)	Sum of former tow lane(μl)	ddH_2O (μl)
HepG2 0	1.518	20.625	2.06	193.94	25	218.94	31.06
HepG2 1	1.633	22.234	2.22	179.91	25	204.91	45.09
HepG2 10	1.497	20.331	2.03	196.74	25	221.74	28.26
HepG2 DMSO	1.605	21.842	2.18	183.13	25	208.13	41.87

Lab Notebook 20170911－20170917 (1)

A. Experiment: analysis on cell survival rate

B. Schedule:
20170913～20170916

C. Objective: examine the effect on different concentration of Aflatoxin B1 to cell survival be using MTT assay.

D. Instruments and materials:
   1. HepG2 cell line
   2. Aflatoxin B1 1 mM、DMSO
   3. 96 well plate*2
   4. MTT 5 mg/ml

E. Method:
   1. 20170913：incubate 1×10^4 per well, 30 wells each(two 96 well plate)
   2. 20170914：incubate overnight, observe the cell growth condition, if allowed the remove the previous medium, distribute aflatoxin into different well by 0、1.25、2.5、5、10 μM of Aflatoxin B1(with the last group only adding DMSO), every condition with 5 well each, load 100 μl per well,
   3. 20170915、16：after 24 h,48 h，move the previous medium, add MTT (dilute 10X)100 μl incubate for 3 hours, after reaction remove the solution and add 100 μl DMSO per well, use ELISA reader to measure O.D. absorption 570 nm

F. Results:

24 h	**0 μM**	**1.25μM**	**2.5μM**	**5μM**	**10 μM**	**DMSO**
Mean (%)	100	115.26967	111.42086	92.50330	90.34972	101.32479
S.E. (%)	8.96585	6.02536	3.47103	9.26151	6.04711	15.20990
48 h	**0 μM**	**1.25μM**	**2.5μM**	**5μM**	**10 μM**	**DMSO**
Mean (%)	100	92.56678	98.99271	99.49952	84.16851	81.52677
S.E. (%)	11.53478	10.07612	6.18407	12.48344	6.48459	3.20454

- The cell survival rate after 24 h or 48 h are both low, However, by observing the original data, the OD is too low.

G. Discussion:
   1. Repeat the experiment , and the conditions remains except the original cell number doubled to 2×10^4/well to see whether the OD will be higher than 0.5.

Lab Notebook 20170911－20170917 (2)

A. Experiment: Analysis of the toxicity of AflatoxinB1 on HepG2 cell

B. Schedule:
20170911～12（first WB）+20170914～20170915(second WB)

C. Objective:
By using western blot to see whether aflatoxin can cause the expression of p53 and p21

D. Instrument and material:
1. $HepG_2$ cell line
2. Aflatoxin B_1 1 mM、DMSO
3. Western blot related instrument

E. Method:
1. Use western blot and p-p53 antibody to determine the expression of phosphate p53 protein.

- information:

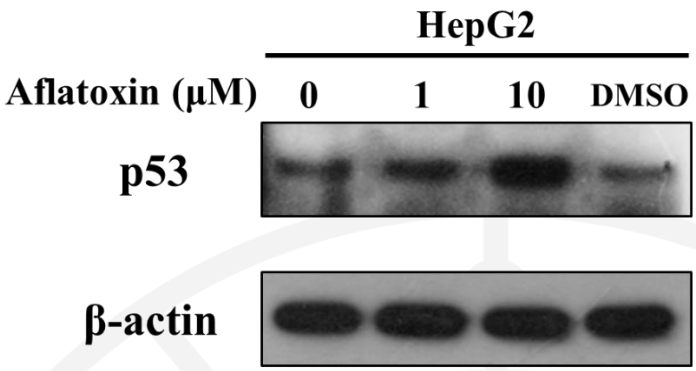
Gel (%)	12		
volume	50	50	25
Molecular weight	53	21	42
Primary antibody	p53 1:1000 milk	P21 1:1000 Milk	β-actin 1:1000 milk
Secondary antibody	Anti-rabbit 1:5000 milk	Anti-rabbit 1:5000 Milk	Anti-mouse 1:5000 milk
Antibody type	rabbit	rabbit	mouse

- Loading well order(12%Gel)：

Marker	0 μM 31.25 λ	1 μM 31.25 λ	10 μM 31.25 λ	DMSO 31.25 λ	0 μM 15.6 λ	1 μM 15.6 λ	10 μM 15.6 λ	DMSO 15.6 λ	

F. Results:

1. Western blot:



2. P53 protein is observable with aflatoxin concentration 10 μM, DMSO will not induce the expression of p53.
3. We can tell from the other WB result that besides the expression of p53 is visible, there is also expressions of p21.
4. There are no sharp increase when the aflatoxin concentration is at 1 μM.

G. Discussion:

The results can be referred to the DNA damage repair, which will active p53 to halt the cell cycle.

Lab Notebook 20170918－20170924 (1)

A. Experiment: the effects of aflatoxin on the p53 pathway of HepG2 and apoptosis.

B. Schedule:20170921～20170924

C. Objective:

Using Western Blot to examine whether there is p53 protein and apoptosis related protein produce by cell after treated with Aflatoxin.

D. Instruments and materials:

1. $HepG_2$ cell line
2. Aflatoxin B_1 1 mM、DMSO
3. Protein extraction related instruments
4. 6 cm dish*5
5. Western blot related instruments

E. Method:

1. 20170921：incubate cell 5×10^5 per dish, total of 5 dishes.
2. 20170922：incubate overnight, use microscope to observe, if allowed remove the previous medium, re-suspend into 5 tubes with the Aflatoxin B1 concentration of 0,2.5,5,10 μM(with the sixth tube only adding DMSO for Aflatoxin substitution.) each condition a dish, total 3 ml per dish.
3. 20170923: After 24 hours, use RIPA+PI+phos-stopto extaract the protein then process western blot.
4. Use western blot and p-p53 antibody to determine the expression of phosphate p53 protein.

- Related information:

Gel (%)	12	12	12	12	12	8	8
volume	50					30	30
Molecular weight	62	53	20	53	21	42	116、89
Primary antibody	p-Chk1 (Ser 345) 1:1000 BSA	p-p53 (Ser 20) 1:1000 BSA	Bax 1:1000 milk	p53 1:1000 milk	P21 1:1000 milk	β-actin 1:1000 milk	PARP 1:1000 milk
Secondary antibody	Anti-rabbit 1:5000 milk					Anti-mouse 1:5000 milk	Anti-rabbit 1:5000 milk
Type of antibody	rabbit					mouse	rabbit

- Loading well order (8%gel)

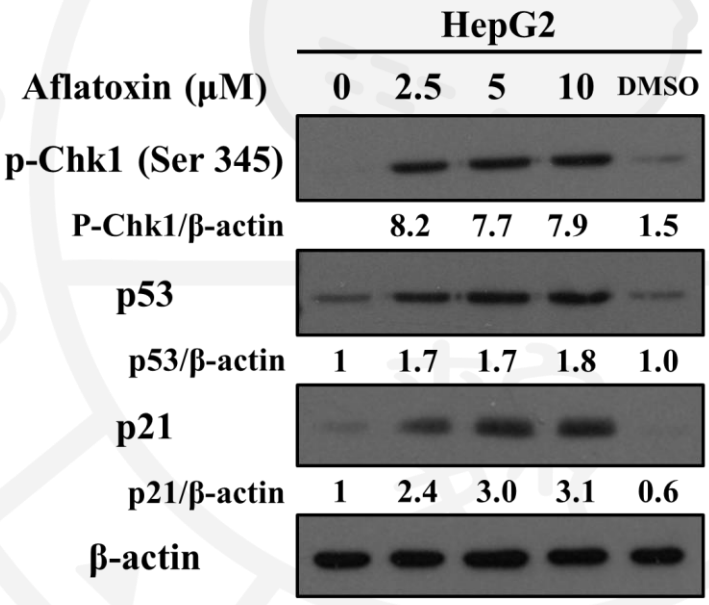
Marker	0 μM	2.5 μM	5 μM	10 μM	DMSO				

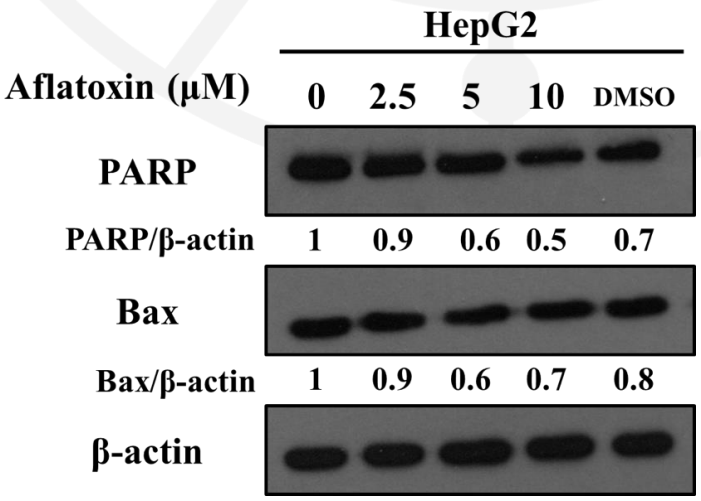
F. Results:

1. The proteins are listed below(total volume：260 μl):

tube	O.D.	Theoretical concentration (μg/10 μl)	Actual concentration (μg/μl)	200 μg usage (μl)	10x sample buffer (μl)	Sum of former two (μl)	ddH_2O (μl)
HepG2 0	0.942	12.56923	1.26	159.12	26	185.12	74.88
HepG2 2.5	0.874	11.61818	1.16	172.14	26	198.14	61.86
HepG2 5	0.661	8.639161	0.86	231.50	26	257.50	2.5
HepG2 10	0.799	10.56923	1.06	189.23	26	215.23	44.77
HepG2 DMSO	1.034	13.85594	1.39	144.34	26	170.34	89.66

2. Western blot results:





3. From the first figure, we can tell that Aflatoxin is able to induce p-Chk1, p53, p21. In the three protein, p21 is the easiest to observe since the expression increase with the concentration of Aflatoxin.
4. From the second figure, we can tell that the two apoptosis-related protein PARP and Bax have only minor changes, close to the control group. Therefore we speculate aflatoxin might not cause apoptosis immediately.

G. Discussion:
1. Since the DNA damage is more obvious than inducing apoptosis upon cells treated with Aflatoxin (proof our hypothesis), therefore, the experiments will be focusing on the DNA damage cause from Aflatoxin.
2. There is no band upon observing p-p53 (Ser 20), we speculate the reason is that the protein loading volume is not high enough, the ratio of the primary and secondary antibody is not accurate. Thus, we recommend process the experiment again under same conditions.

Lab Notebook20170918－20170924 (2)

A. Experiment: Purification and quantify the protein

B. Schedule: 20170923～20170924

C. Objectives: to ensure the four protein concentration(two from Australia and the team synthesis two)

D. Instrument and material:

1. BSA 10 mg/ml、4x Sample buffer、TBS
2. Coomassie brilliant blue staining reated instruments

E. Method:

1. BSA

   7suspension with different concentrations(10、5、2.5、1.25、0.63、0.31、0.16 μg/μl)

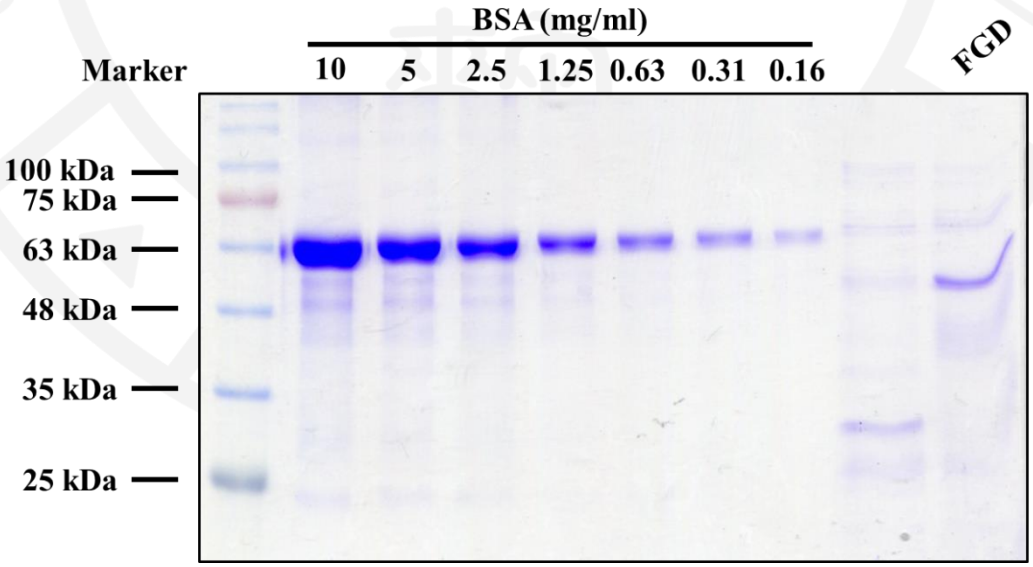
2. Loading well order (one for the two protein from Australia and one gel for the two we synthesis)：

marker	10 BSA 2 λ	5 BSA 2 λ	2.5 BSA 2 λ	1.25 BSA 2 λ	0.63 BSA 2 λ	0.31 BSA 2 λ	0.16 BSA 2 λ	5998 5 λ	FGD 5 λ
P.s.: Take MSMEG5998 and FGD 10 λ each, dilute with 10 λ sample buffer, take 10 λ to load in the well.									

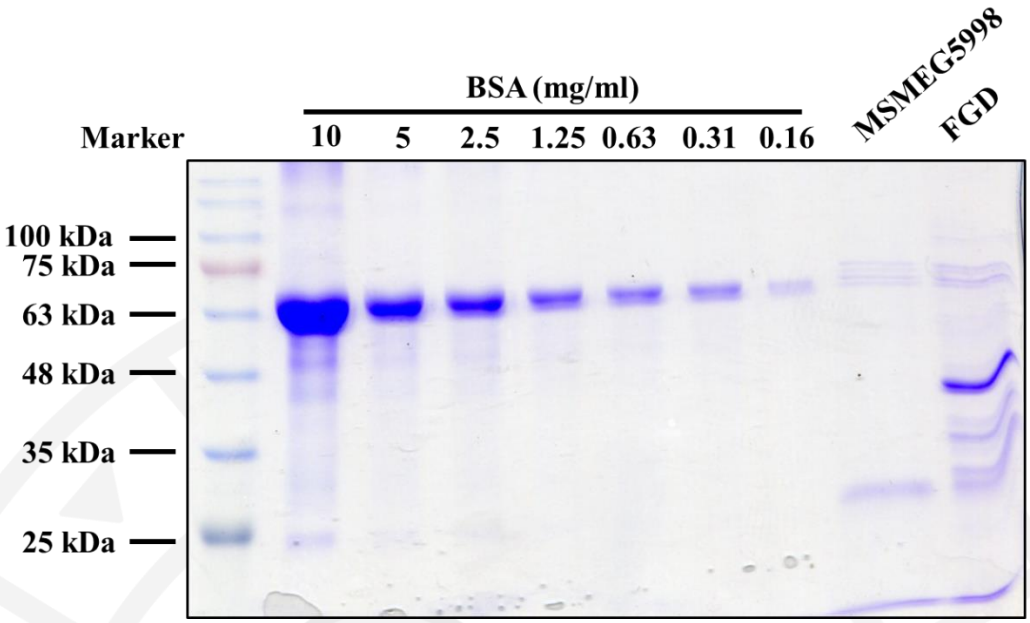
F. Results:

A. Coomassie brilliant blue staining：

a. Plasmid from Australia express protein as the figure below



b. Plasmid by synthesis express protein as the figure below

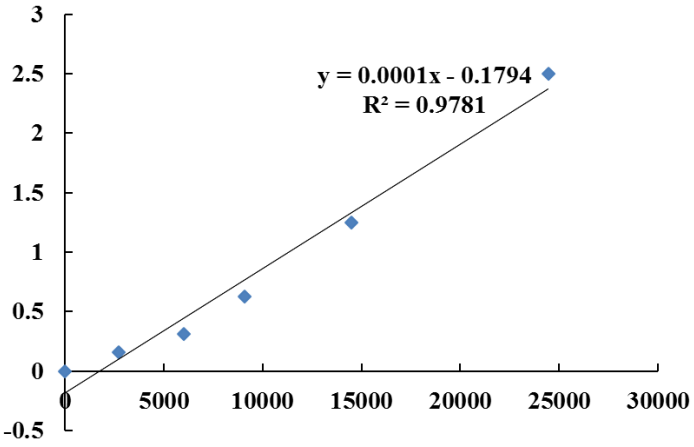


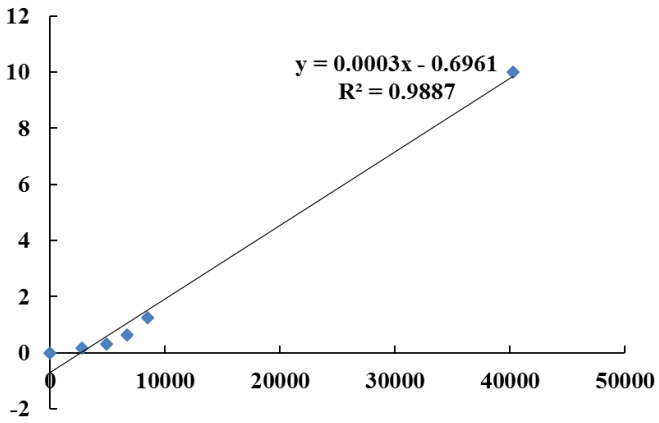
B. Standard curve

a.

Figure 1 figure	Figure 2 figure	Concentration (μg/μl)
0	0	0
2731.849	2761.799	0.15625
6004.163	4882.364	0.3125
9074.042	6725.213	0.625
14508.163	8463.97	1.25
24452.406	15240.21	2.5
30113.46	18633.09	5
41972.23	40264.53	10

b. The team draw a standard curve below:





C. Protein quantification

a. Take the concentration of MSMEG_5998 and FGD into the formula,

	Figure measured	theoretical (μg/μl)	Actual (μg/μl)
Aus MSMEG5998	5920.326	0.412633	0.082527
Syn MSMEG5998	4633.255	0.693877	0.138775
Aus FGD	8725.355	0.693136	0.138627
Syn FGD	12764.11	3.133134	0.626627

b. Concentration (μM)：

Aus MSMEG5998＝0.082527/18900*10^6＝4.36648254 (μM)

Syn MSMEG5998＝0.138775/32400*10^6＝4.283188272 (μM)

Aus FGD＝0.138627/37700*10^6＝3.677111406 (μM)

Syn FGD＝0.626627/51500*10^6＝12.16750913 (μM)

Lab Notebook 20171002－20171008 (1)

A. Experiment:

Use western blot to analyze the effect on cell when treated with MSMEG5998 and aflatoxin B1 simultaneously.

B. Schedule:

20170926～20170929

C. Objective:

To see whether MSMEG5998 can inhibit the p53 pathway when the cell is treated with Aflatoxin B1.

D. Instrument and materials:

- HepG2 cell line
- Aflatoxin B1 1 mM、DMSO
- G6P 1 mM、MSMEG5998、FGD、F_{420}、Tris-HCl (pH=7.5)
- Protein extraction related instruments
- 3.5 cm dish*5
- Western blot related instruments

E. Method:

1. 20170926：plant cell 6×10^5 /dis total of 5 dish
2. 20170927：ON, decant the medium, prepare solution of Control (only medium),AF(aflatoxin),A+R（aflatoxin+reagents）,A+R+MSMEG5998（aflatoxin+reagents+MSMEG5998）,5998（MSMEG5998）
3. the concentration is listed below

Name	Concentration
Aflatoxin B1	32 or 10 μM
MSMEG5998	0.1 μM
Reactants	
Glucose-6-phosphate (G6P)	2.5 mM
F_{420}	5 μM
F_{420}-dependent glucose-6-phosphate dehydrogenase (FGD)	0.225 μM
Tris-HCl (pH=7.5)	25 mM

4. 20170928：after 24 hours proceed western blot
5. Use western blot to locate the target protein

a. more information

Gel(%)	10					
loading	50	50	50	100	100	50
Molecular weight	53	21	62	53	62	42
1st antibody	p53 1:1000 milk	P21 1:1000 milk	p-Chk1 (Ser345) 1:1000 BSA	p-p53 (Ser 20) 1:500 BSA	p-Chk2 (Thr68) 1:500 BSA	β-actin 1:1000 milk
2nd antibody	Anti-rabbit 1:5000 milk	Anti-rabbit 1:5000 milk	Anti-rabbit 1:5000 milk	Anti-rabbit 1:2000 milk	Anti-rabbit 1:2000 milk	Anti-mouse 1:5000 milk
type	rabbit	rabbit	rabbit	rabbit	rabbit	mouse

b. Loading well order (10%del ，2 gel 36.4 μl, 1 gel 10.9 μl, 3 gel 片 18.2 μl):

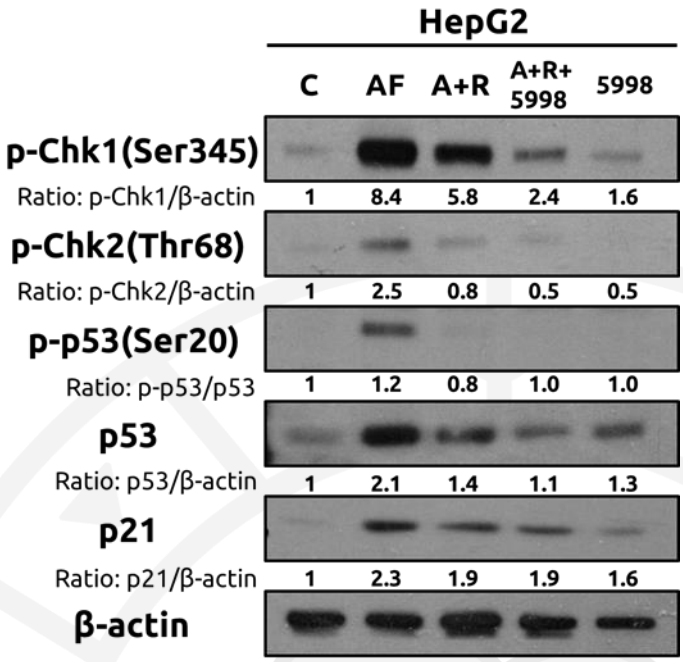
Marker	C	AF	A+R	A+R+5998	5998				

F. Results:

1. Protein concentration（total concentration：200 μl）:

Name	O.D.	Theoretical concentration (μg/10 μl)	Actual concentration (μg/μl)	300 μg 取量 (μl)	10x sample buffer (μl)	Sum (μl)	ddH_2O (μl)
C	0.868	11.5343	5.77	52.02	9	61.02	28.98
AF	0.843	11.1846	5.59	53.65	9	62.65	27.35
A+R	0.689	9.0308	4.52	66.44	9	75.44	14.56
A+R+5998	0.766	10.1077	5.05	59.36	9	68.36	21.64
5998	1.011	13.5343	6.77	44.33	9	53.33	36.67

2. Western blot results:



3. The protein expression is decreased with the addition of MSMEG5998 and reagents, showing the inhibiting function toward p53 pathway.
4. MSMEG5998 doe not cause cytotoxicity comparing to the control group.

G. Discussion:

1. The experiment confirms MSMEG5998 can inhibit the p53 pathway activated by Aflatoxin B1; indirectly prove that MSMEG5998 may prevent cell DNA damage.
2. To our surprise by adding simply reagents may cause inhibition to p53 pathway.

Lab Notebook 20171009－20171028 (1)

A. Experiment:

Analyze the DNA damage in HepG2 cell caused by Aflatoxin B2 with western blot.

B. Schedule: 20171012～20171015

C. Objective

γH2Ax is one of the subunit in histone, which can be phosphorylated when the DNA breaks, Therefore, the experiment is to examine whether there are Phospho-γH2AX presenting in the cell upon treated with Aflatoxin B1, which may due to DNA damage.

D. Instruments and materials

1. HepG2 cell line
2. Aflatoxin B1 1 mM、DMSO
3. Protein extraction related instruments.
4. 3.5 or 6 cm dish*5
5. Western blot related instruments

E. Method(the method listed below belongs to the second time we conduct the experiment, the first time is the same with the difference only on the dish, first time is the 6cm dish, cell number 1.2×10^6 /dish with the total volume of 4 ml)

1. 20171020: incubate cell, cell number 6×10^5 /dish, total of 5 dishes.
2. 20171021: incubate overnight if growing condition allowed, decant the previous medium, prepare different concentration of Aflatoxin B1: 0, 2.5, 5, 10 μMin the medium (with another group adding only DMSO), every condition a dish, and the volume per dish is 2.5 ml.
3. 20171022: After 24 hours, extract protein with RIPA + PI + phos-stopto conduct western blot.
4. Use antibody of p-p53 to see whether there is expression of p-p53 protein.
   a. Information are listed below:

Gel(%)	8		15
Adding volume	Ⅰ75、Ⅱ50	Ⅰ30、Ⅱ50	150
Molecular weight	90	42	17
1st antibody	MDM2 (SMP14) Ⅰ1:500、Ⅱ1:1000 milk	β-actin 1:1000 milk	γH2AX 1:500 BSA
2nd antibody	Anti-mouse Ⅰ1:2000、Ⅱ1:5000 milk	Anti-mouse 1:5000 milk	Anti-mouse 1:2000 milk
Antibody type	mouse	mouse	mouse

b. Loading well order (10% gel ，two gel 36.4 μl, one gel 10.9 μl, three gel 18.2 μl):

Marker	0 μM	2.5 μM	5 μM	10 μM	DMSO				

F. Results:

1. Protein are listed below

- First time (total volume: 150 μl)

Name	O.D.	Theoretical conc. (μg/10 μl)	Actual conc. (μg/μl)	500 μg usage (μl)	10x sample buffer (μl)	Sum of the fomer 2 (μl)	ddH_2O (μl)
HepG2 0	0.897	11.9394	3.98	125.63	15	145.63	9.37
HepG2 2.5	0.941	12.5552	4.19	119.47	15	139.47	15.53
HepG2 5	0.928	12.3734	4.12	121.23	15	141.23	13.77
HepG2 10	0.912	12.1497	4.05	123.46	15	143.46	11.54
HepG2 DMSO	1.155	15.5483	5.18	96.47	15	116.47	38.53

- Second time (total volume: 75 μl)

name	O.D.	Theoretical conc. (μg/10 μl)	Actual conc. (μg/μl)	300 μg usage (μl)	10x sample buffer (μl)	Sum of the former2(μl)	ddH_2O (μl)
HepG2 0	0.772	10.1916	5.10	52.98	7.5	66.37	14.52
HepG2 2.5	0.88	11.7021	5.85	51.27	7.5	58.77	16.23
HepG2 5	0.984	13.1566	6.58	45.60	7.5	53.10	21.90
HepG2 10	0.722	9.4923	4.75	63.21	7.5	70.71	4.29
HepG2 DMSO	1.025	13.7301	6.87	43.70	7.5	51.20	23.80

2. Western blot results:

- First time γH2AX can be detected in the HepG2 0 (control group), which is abnormal, the second time all group shows no sight of γH2AX.
- MDM2 is not detectable due to the background is too high, but the second time we can see the expression drops when the aflatoxin concentration increase.

G. Discussion:

1. The reaction time may be too short to cause DNA damage to a certain level which can detect phospho-γH2AX, the experiment will be redo and expand the time to 48 hours.
2. The MDM2 antibody lacks specificity, thus the next time can change an antibody.

Lab Notebook 20171009－20171028 (2)

A. Experiment: DNA agarose electrophoresis to analyze DNA damage in HepG2.

B. Schedule:
20171020～20171023、20171022～20171026

C. Objective:
When DNA is damaged, in the electrophoresis of agarose can detect fragments of DNA, therefore by using the technique, observations can be done to see whether Aflatoxin B1 can cause HepG2 cell damage.

D. Instruments and materials: s
- HepG2 cell line
- Aflatoxin B1 1 mM、DMSO
- Extraction of genome DNA related instruments.
- 3.5 cm dish*5
- DNA electrophoresis related instruments.

E. Method:
1. 20171020: incubate cell, cell number 6×10^5 /dish, total of 5 dishes.
2. 20171021: incubate overnight if growing condition allowed, decant the previous medium, prepare different concentration of Aflatoxin B1: 0, 2.5, 5, 10 μMin the medium (with another group adding only DMSO), every condition a dish, and the volume per dish is 2.5 ml.
3. 20171022: extact cell Genomic DNA after 24 hours, each group take 10 μg to DNA electrophoresis 15V,3 hours

F. Lab results:
1. DNA extraction concentration
   - First time

	230 nm	260 nm	280 nm	260/230	260/280	Concentration (μg/μl)
HepG2 0	0.121	0.103	0.054	0.85	1.91	0.257
HepG2 2.5	0.252	0.156	0.084	0.62	1.86	0.389
HepG2 5	0.220	0.109	0.057	0.49	1.91	0.273
HepG2 10	0.226	0.143	0.073	0.63	1.96	0.356
HepG2 DMSO	0.277	0.164	0.084	0.59	1.95	0.411

- Second time

	230 nm	260 nm	280 nm	260/230	260/280	Concentration (μg/μl)
HepG2 0	0.626	0.454	0.257	0.73	1.77	1.135
HepG2 2.5	0.557	0.247	0.144	0.44	1.71	0.617
HepG2 5	0.788	0.349	0.212	0.44	1.65	0.874
HepG2 10	0.458	0.093	0.054	0.20	1.70	0.232
HepG2 DMSO	1.361	0.763	0.463	0.56	1.65	1.907

- DNA electrophoresis results:
  The two experiment shows no significant difference, which is that the band is at the top part thus there is no DNA strand breaking.

G. Discussion:
We speculate the failure may due to the sensitivity is low or there is no DNA strand break.

Lab Notebook 20171009－20171028 (3)

A. Experiment: Observation of DNA damage by using immunocytochemistry.

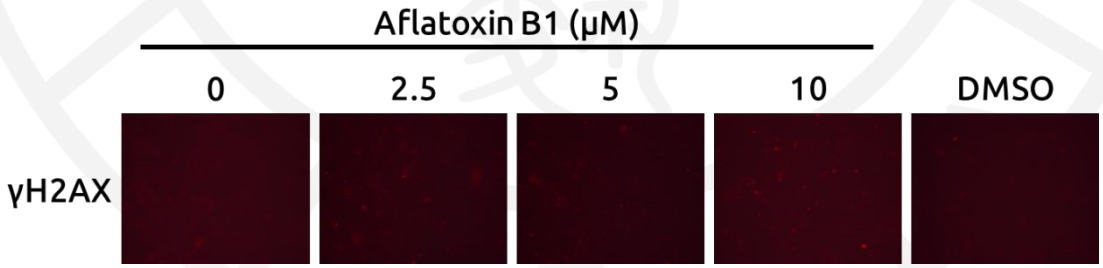
B. Schedule:
20171022～20171026

C. Objective: using antibody to recognize whether the nucleus express massive γH2AX, and by immunofluorescence we can observe under microscope.

D. Instrument and Material
- HepG2 cell line
- Aflatoxin B1 1 mM、DMSO
- Immunofluorescence related instruments
- γH2AX antibody、DAPI、Anti-mouse antibody
- 24-well plate*1
- Fluorescence Microscope

E. Method:
1. 20171022: incubate cell, cell number 1×10^5 /well, each condition 2 wells in total of 10 wells.
2. 20171023：Incubate overnight if growing condition allowed, decant the previous medium, prepare different concentration of Aflatoxin B1: 0, 2.5, 5, 10 μMin the medium (with another group adding only DMSO), every condition 2 wells each well load 500 μl.
3. 20171025: after reacting for 48 hours, follow the Immunocytochemistry protocol. Add diluted 50X γH2AXantibody to each well 40 μl.
4. 20171026: follow the IHC protocol the second day.

F. Results:
1. Pictures:



2. Under the concentration 10 μM, γH2AX expression is obvious, there are expressions in 2.5 and 5 μM but it is not obvious.
3. DMSO is same as the control group, thus is considered as negative control.
4. The error of the experiment cause low DAPI concentration. Therefore there are no obvious blue fluorescent.

G. Discussion:
1. Cell incubation should be treated with more caution next time to prevent err leads to the failure of the experiment.
2. DAPI can be used without dilute, which can be easier to locate the nucleus.