Experiment 0928

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◆Pick 2 colonies from each of the transformation plates and inoculate in 5mL

◆LB medium + Chloramphenicol. Grow the cells overnight (14 hours) at 37°C
and 220 rpm.

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◆**Cell growth, sampling, and measure**

1. Make a 1:10 dilution of each overnight culture in LB + Chloramphenicol (0.5 mL of culture into 4.5mL of LB + Chlor)
2. Measure Abs600 of these 1:10 diluted cultures
3. Record the data in notebook
4.

Abs600：

	1	2	3	4	5	6	N	P	LB
Colony1	0.155	0.151	0.096	0.111	0.102	0.111	0.122	0.110	0.048
Colony2	0.174	0.117	0.140	0.126	0.116	0.155	0.133	0.111	0.047

5. Dilute the cultures further to a target Abs600 of 0.02 in a final volume of 12 ml LB medium + Chloramphenicol in 50 mL Falcon tube (amber, or covered with foil to block light).

Culture (ml)：

	1	2	3	4	5	6	N	P
Colony1	2.24	2.33	5.00	3.80	4.44	3.81	3.24	3.87
Colony2	1.90	3.48	2.60	3.08	3.53	2.24	2.82	3.81

LB+Cm：

	1	2	3	4	5	6	N	P
Colony1	9.76	9.67	7.00	8.20	7.56	8.19	8.76	8.13
Colony2	10.10	8.52	9.40	8.92	8.47	9.76	9.18	8.19

6. Take 500 µl samples of the diluted cultures into 1.5 ml Eppendorf tubes at 0 hour, prior to incubation. (Take a sample from each of

the 8 devices, two colonies per device, for a total of 16 Eppendorf tubes with 500 µl samples at 0 hour and 6 hours, 32 samples total). Place the samples on ice.

7. Measure samples (Abs600 and Fluorescence measurement)

Abs600：(0hr)

Hour 0:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.074	0.202	0.065	0.267	0.090	0.099	0.083	0.242	0.115
Colony 1, Replicate 2	0.321	0.087	0.158	0.094	0.056	0.070	0.157	0.113	0.132
Colony 1, Replicate 3	0.108	0.073	0.296	0.076	0.070	0.197	0.241	0.089	0.107
Colony 1, Replicate 4	0.079	0.272	0.100	0.084	0.125	0.117	0.082	0.219	0.062
Colony 2, Replicate 1	0.074	0.063	0.046	0.055	0.043	0.039	0.109	0.080	0.188
Colony 2, Replicate 2	0.083	0.333	0.071	0.092	0.062	0.097	0.275	0.078	0.322
Colony 2, Replicate 3	0.102	0.285	0.060	0.164	0.077	0.327	0.077	0.091	0.328
Colony 2, Replicate 4	0.070	0.066	0.055	0.119	0.124	0.057	0.076	0.081	0.108

Abs600：(6hr)

Hour 6:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.701	0.518	0.170	0.620	0.666	0.823	0.382	0.447	0.065
Colony 1, Replicate 2	0.696	0.539	0.289	0.586	0.398	0.589	0.490	0.548	0.065
Colony 1, Replicate 3	0.651	0.634	0.160	0.456	0.513	0.471	0.443	0.439	0.065
Colony 1, Replicate 4	0.712	0.646	0.335	0.455	0.420	0.952	0.489	0.427	0.066
Colony 2, Replicate 1	0.735	0.534	0.385	0.354	0.632	0.883	0.493	0.696	0.065
Colony 2, Replicate 2	0.728	0.665	0.147	0.572	0.723	0.532	0.613	0.718	0.066
Colony 2, Replicate 3	0.687	0.589	0.270	0.508	0.640	0.669	0.495	0.732	0.063
Colony 2, Replicate 4	0.675	0.635	0.134	0.397	0.656	0.667	0.355	0.498	0.070

◆Digest pGAPZ A(X3)/ pUCIDT_Lac1/ pUCIDT_Px16/ pUCIDT_Px18 with AgeI, EcoRI

Step 1

Set up reaction for AgeI as follows:

	DNA(2µg)	AgeI	NEBuffer1.1	ddH_2O
pGAPZ A(X3)	5.6µl(X3)	1µl (X3)	1.5µl (X3)	6.9µl (X3)
pUCIDT_Lac1	6µl	1µl	1.5µl	6.5µl
pUCIDT_Px16	9µl	1µl	1.5µl	3.5µl
pUCIDT_Px18	11.4µl	1µl	1.5µl	1.1

Incubate at 37℃/ 4hr

Step 2

Heat inactive 65℃/ 20min

Step 3

Add the following for EcoRI reaction:

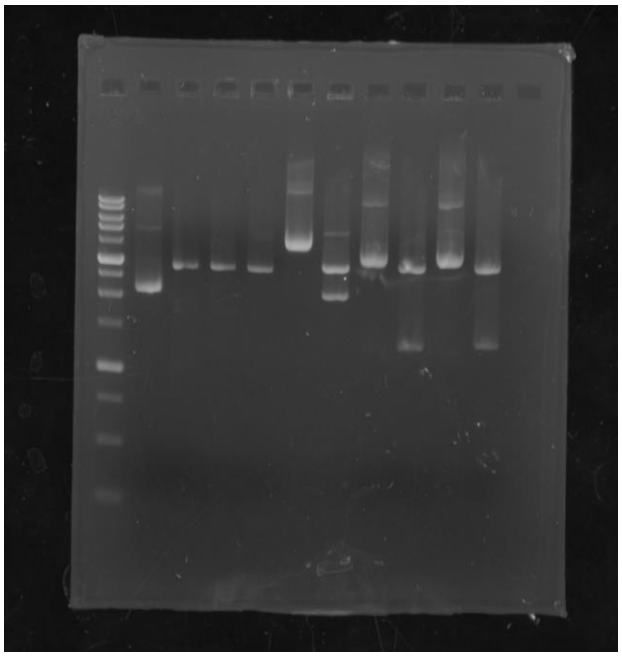
	1M NaCl	EcoRI	ddH20
pGAPZ A(X3)	1µl (X3)	0.5µl	3.5µl
pUCIDT_Lac1	1µl	0.5µl	3.5µl

pUCIDT_Px16	1μl	0.5μl	3.5μl
pUCIDT_Px18	1μl	0.5μl	3.5μl

Incubate at 37℃/ 1.5hr

Step 4

Check with gel electrophoresis



1-1 marker
1-2 pGAPZ A uncut
1-3 pGAPZ A(EcoR Ⅰ, Age Ⅰ) digest
1-4 pGAPZ A(EcoR Ⅰ, Age Ⅰ) digest
1-5 pGAPZ A(EcoR Ⅰ, Age Ⅰ) digest
1-6 pUCIDT_Lac1 uncut
1-7 pUCIDT_Lac1(EcoR Ⅰ, Age Ⅰ) digest
1-8 pUCIDT_Px16 uncut
1-9 pUCIDT_Px16 (EcoR Ⅰ, Age Ⅰ) digest
1-10 pUCIDT_Px18 uncut
1-11 pUCIDT_Px18 (EcoR Ⅰ, Age Ⅰ) digest

Step 5

Heat inactivate 65℃/ 20min

◆NanoDrop

	OD260/280	ng/μl
pGAPZ A cut (1)	1.52	12.5
pGAPZ A cut (2)	1.69	12.1
pGAPZ A cut (3)	1.77	19.1
Lac1 cut	1.56	16.6
Px16 cut	1.76	18.2
Px18 cut	1.69	15.1

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1. Fluorescence measurement

Fluorescence：(0hr)

Hour 0:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (
Colony 1, Replicate 1	155	233	294	274	150	348	166	253	19
Colony 1, Replicate 2	160	165	292	256	154	333	164	179	20
Colony 1, Replicate 3	161	166	296	259	148	326	167	189	19
Colony 1, Replicate 4	151	165	340	258	170	355	254	194	19
Colony 2, Replicate 1	154	167	297	195	159	232	186	164	18
Colony 2, Replicate 2	159	173	349	212	159	289	201	157	18
Colony 2, Replicate 3	154	178	361	198	181	268	247	181	20
Colony 2, Replicate 4	158	172	323	288	176	267	175	173	19

Fluorescence：(6hr)

Hour 6:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (
Colony 1, Replicate 1	202	875	1229	2564	218	5431	547	1014	105
Colony 1, Replicate 2	209	860	1268	2616	160	5376	509	869	117
Colony 1, Replicate 3	206	908	1256	2424	226	3951	511	918	99
Colony 1, Replicate 4	206	807	1276	2660	227	7369	508	872	117
Colony 2, Replicate 1	197	902	1394	1606	230	4239	563	682	108
Colony 2, Replicate 2	193	860	1348	1929	231	2939	583	677	164
Colony 2, Replicate 3	194	746	1384	1580	236	2798	644	686	141
Colony 2, Replicate 4	201	839	1364	1693	205	2772	666	673	160

◆Ligation

Set up the following reaction in a microcentrifuge tube on ice. (Insert: Vector = 3:1)

	T4 Ligase	Ligase buffer	Insert	Vector 50ng	ddH20
pGAPZA_Lac1	1μl	2μl	6.6μl 110ng	2.6μl	2.8μl
pGAPZA_Px16	1μl	2μl	3.8μl 70ng	2.6μl	5.6μl
pGAPZA_Px18	1μl	2μl	4.6μl 70ng	2.6μl	4.8μl

Incubate at 16℃/ 15hr

8/8

Pick 2 colonies from each of the transformation plates and inoculate in 5mL LB medium + Chloramphenicol. Grow the cells overnight (16 hours) at 37°C and 220 rpm.

◆**Transformation**

1. Add all pGAPZ A_Lac1/ pGAPZ A_Px16/ pGAPZ A_Px18 (15μl) into 20μl ECOS™ 101 Competent Cells [DH5α]
2. Incubate on ice 5min
3. Heat shock at 42℃ 45sec
4. Incubate on ice 5min
5.Add 140μl LB
6. Incubate at 37℃ 1hr
7. Spread on LB+ Zeocin plate

Ligation

Set up the following reaction in a microcentrifuge tube on ice. (Insert: Vector = 3: 1)

	T4 Ligase	Ligase buffer	Insert	Vector	ddH20
pGAPZ A_Lac1	1μl	2.5μl	13.2μl 170ng	5.2μl 75ng	3.1μl
pGAPZ A_Px16	1μl	2μl	7.6μl 144ng	5.2μl 100ng	4.2μl
pGAPZ A_Px18	1μl	2μl	9.2μl 144ng	5.2μl 100ng	2.6μl

And incubate at 16℃/ 15hr

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◆**Cell growth, sampling, and measure**

1. Make a 1:10 dilution of each overnight culture in LB + Chloramphenicol (0.5mL of culture into 4.5mL of LB + Chlor)
2. Measure Abs600 of these 1:10 diluted cultures
3. Record the data in notebook

Abs600：

	1	2	3	4	5	6	N	P	LB
Colony1	0.1494	0.1235	0.1169	0.1196	0.1275	0.1123	0.1088	0.1181	0.0445

Colony2	0.1282	0.1306	0.1333	0.1216	0.1438	0.1145	0.1312	0.1175	0.0432

4. Dilute the cultures to 0.02(Abs600) in a final volume of 12 ml LB medium + Chloramphenicol in 50 mL Falcon tube (amber, or covered with foil to block light).

Culture (ml)：

	1	2	3	4	5	6	N	P
Colony1	2.29	3.00	2.78	2.75	2.70	3.43	3.24	3.36
Colony2	2.87	2.79	2.70	3.11	2.42	3.43	2.77	3.29

LB+Cm：

	1	2	3	4	5	6	N	P
Colony1	9.71	9.00	9.22	9.25	9.30	8.57	8.76	8.64
Colony2	9.13	9.21	9.3	8.89	9.58	8.57	9.23	8.71

5. Take 500 µL samples of the diluted cultures at 0 hours into 1.5 ml Eppendorf tubes, prior to incubation. (At 0 hour and 6 hours, you will take a sample from each of the 8 devices, two colonies per device, for a total of 16 Eppendorf tubes with 500 µL samples per time point, 32 samples total). Place the samples on ice.
6. Measure samples (Abs600 and Fluorescence measurement)

Abs600：(0hr)

Hour 0:	Neg. Contro	Pos. Contro	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.346	0.060	0.223	0.360	0.277	0.242	0.285	0.355	0.106
Colony 1, Replicate 2	0.358	0.387	0.242	0.379	0.386	0.354	0.302	0.316	0.351
Colony 1, Replicate 3	0.291	0.374	0.154	0.378	0.246	0.348	0.388	0.054	0.346
Colony 1, Replicate 4	0.372	0.402	0.274	0.284	0.344	0.291	0.191	0.398	0.257
Colony 2, Replicate 1	0.332	0.404	0.400	0.375	0.336	0.386	0.170	0.358	0.333
Colony 2, Replicate 2	0.310	0.227	0.124	0.375	0.349	0.339	0.185	0.239	0.268
Colony 2, Replicate 3	0.181	0.367	0.381	0.406	0.385	0.298	0.417	0.281	0.229
Colony 2, Replicate 4	0.377	0.307	0.090	0.176	0.375	0.272	0.194	0.305	0.223

Abs600：(6hr)

Hour 6:	Neg. Contro	Pos. Contro	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.521	0.509	0.183	0.574	0.688	0.464	0.114	0.552	0.045
Colony 1, Replicate 2	0.531	0.668	0.181	0.618	0.717	0.458	0.115	0.593	0.033
Colony 1, Replicate 3	0.546	0.556	0.378	0.642	0.821	0.542	0.131	0.587	0.032
Colony 1, Replicate 4	0.522	0.535	0.311	0.685	0.770	0.515	0.189	0.619	0.046
Colony 2, Replicate 1	0.684	0.591	0.109	0.682	0.598	0.614	0.144	0.670	0.035
Colony 2, Replicate 2	0.746	0.556	0.236	0.700	0.426	0.609	0.131	0.638	0.218
Colony 2, Replicate 3	0.706	0.678	0.306	0.733	0.537	0.516	0.137	0.557	0.113
Colony 2, Replicate 4	0.597	0.552	0.148	0.646	0.418	0.506	0.092	0.555	0.030

◆Transformation

1. Add all pGAPZ A_Lac1/ pGAPZ A_Px16/ pGAPZ A_Px18 (20μl) into 20μl Competent Cells DH5a and pGAPZ A 1μl+19μl ddH_2O into 10μl Competent Cells DH5a as positive control.
2. Incubate on ice 15min
3. Heat shock at 42℃ 45sec
4. Incubate on ice 5min
5. Add 140μl LB
6. Incubate at 37℃ 1hr
7. Spread on LB+ Zeocin plate

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◆Digest pUCIDT_Lac1/ pUCIDT_Px16/ pUCIDT_Px18 with AgeI, EcoRI

Step 1

Set up reaction for AgeI as follows:

	DNA(5μg)	AgeI	NEBuffer1.1	ddH_2O
pUCIDT_Lac1	15.0μl	1.0μl	2.0μl	2.0μl
pUCIDT_Px16	24.2μl	2.0μl	3.0μl	1.8μl
pUCIDT_Px18	23.0μl	1.0μl	2.6μl	0

Incubate at 37℃/ 6hr

Step 2

Heat inactivate 65℃/ 20min

Step 3

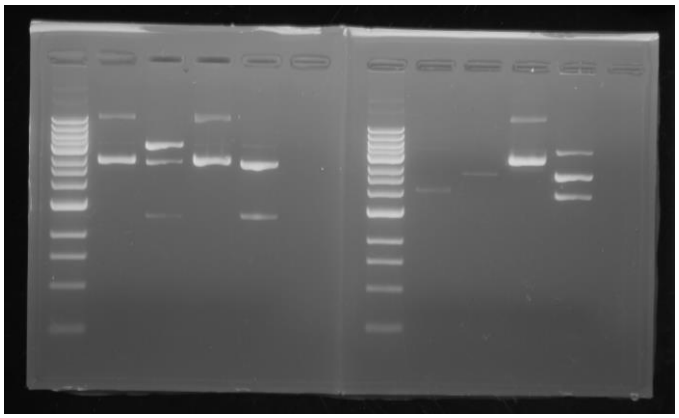
Add the following for EcoRI reaction:

	1M NaCl	EcoRI	ddH_2O
pUCIDT_Lac1	1.0μl	0.5μl	3.5μl
pUCIDT_Px16	1.0μl	0.5μl	3.5μl
pUCIDT_Px18	1.0μl	0.5μl	3.5μl

Incubate at 37℃/ 2.5hr

Step 4

Check with gel electrophoresis



1-1 2-1marker
1-2 pUCIDT_Px16 uncut
1-3 pUCIDT_Px16 cut
1-4 pUCIDT_Px18 uncut
1-5 pUCIDT_Px18 cut
2-2 pGAPZ A uncut
2-3 pGAPZ A cut
2-4 pUCIDT_Lac1 uncut
2-5 pUCIDT_Lac1 cut

Step 5

Heat inactivate 65℃/ 20min

◆NanoDrop

	OD260/280	ng/μl
Lac1 cut	1.72	33.8
Px16 cut	1.68	19.7
Px18 cut	1.81	30.4

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◆Ligation

Set up the following reaction in a microcentrifuge tube on ice. (Insert: Vector = 5: 1)

	T4 Ligase	Ligase buffer	Insert	Vector	ddH20
pGAPZ A_Lac1	1.0μl	1.5μl	5.0μl 170ng	5.2μl 80ng	2.3μl
pGAPZ A_Px16	1.0μl	1.5μl	7.3μl 144ng	5.2μl 80ng	0μl
pGAPZ	1.0μl	1.5μl	4.8μl	5.2μl 80ng	2.5μl

A_Px18			144ng		

Incubate at 16℃/ 18hr

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◆**Transformation**

pGAPZ A_Lac1/ pGAPZ A_Px16/ pGAPZ A_Px18

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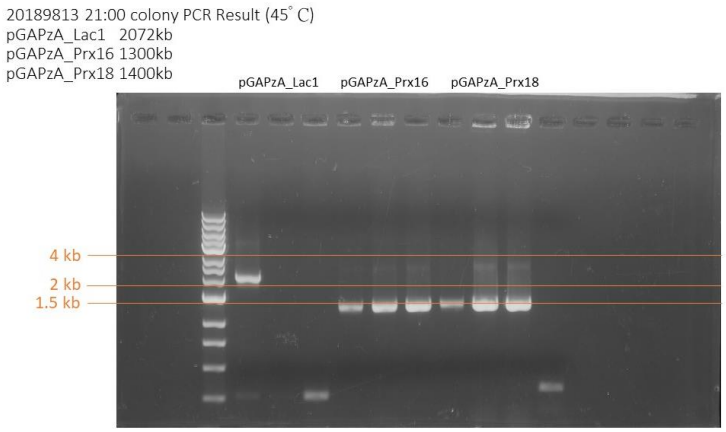
◆**pGAPZ A_Lac1-pGAPZ A_Px16-pGAPZ A_Px18-clone**

20180813 PCR (pGAPZ A_Lac1, pGAPZ A_Px16, pGAPZ-A_Px18)



Colony PCR Result

pGAPZ A_Lac1 2072kb

pGAPZ A _Px16 1300kb

pGAPZ A_Px18 1400kb

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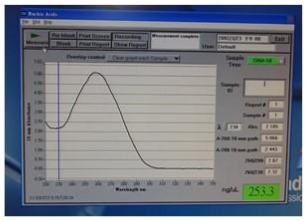
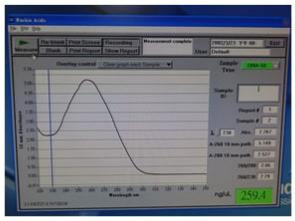
◆**Miniprep**

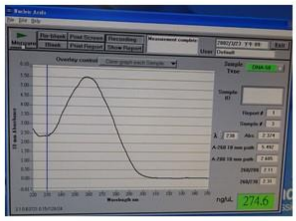
Result:



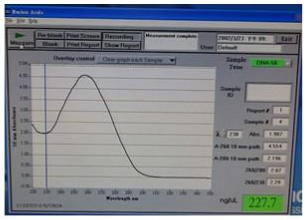
Lac1(1) $OD_{260/280}$=2.07



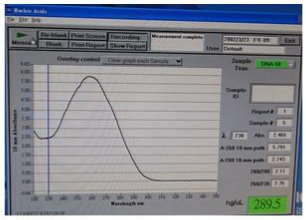
Lac1(2) $OD_{260/280}$=2.05



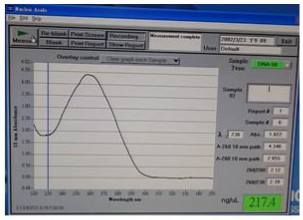
Lac1(3) $OD_{260/280}$=2.11



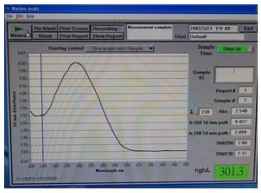
Prx16(1) $OD_{260/280}$=2.07



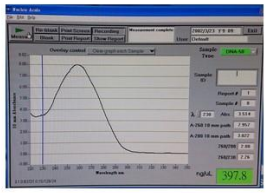
Prx16(2) $OD_{260/280}$=2.11



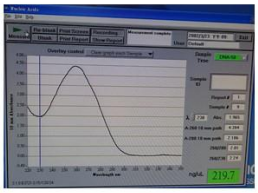
Prx16(3) $OD_{260/280}$=2.12



Prx18(1) $OD_{260/280}$=2.37



Prx18(2) $OD_{260/280}$=2.08



Prx16(3) $OD_{260/280}$=2.01

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◆Digest (use AgeI, EcoRI)

Protocol reference: https://nebcloner.neb.com/#!/protocol/re/sequential-heat/AgeI,EcoRI

	(1ng)	EcoRI	AgeI	10Xbuffer	ddH_2O	Total V
Lac1 1	4μl	0.5μl	0.4μl	2μl	13.1	20μl
2	4μl				13.1	
3	4μl				13.1	
Prx16 1	4.5μl				12.6	
2	4μl				13.1	
3	5μl				12.1	
Prx18 1	3.5μl				13.6	
2	3μl				14.1	

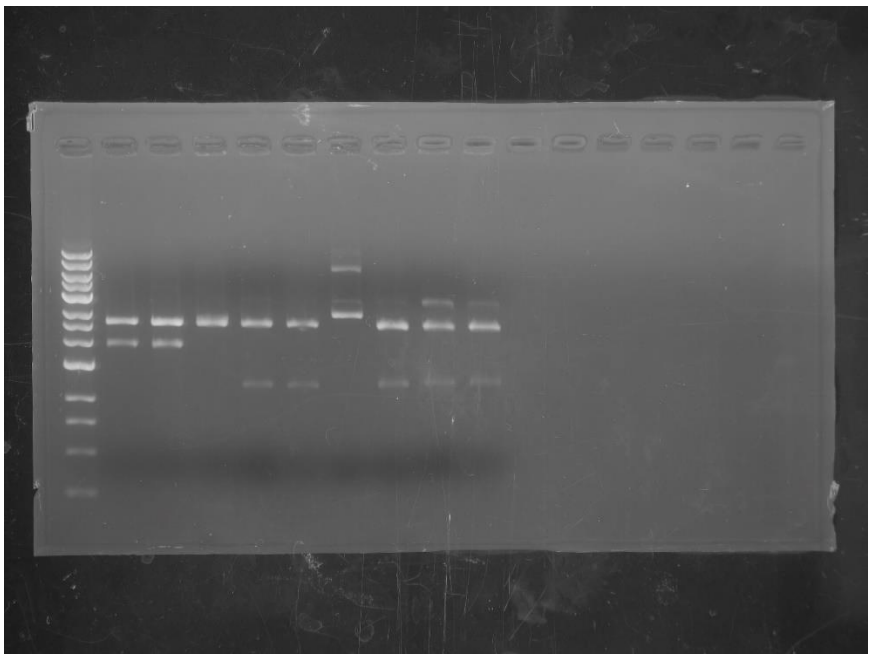
3	5μl				12.1	

Set up reaction for first restriction enzyme* as follows:

COMPONENT	20 µl REACTION
DNA	1 ng
10X NEBuffer 1.1	2µl (1X)
AgeI	0.4 µl (or 10 units)
Nuclease-free Water	to 20 µl

1. Incubate at 37°C for 2 hours.
2. Heat inactivate by incubating at 65°C for 20 minutes.
3. Add 50 mM NaCl(2μl) to bring the salt concentration of NEBuffer EcoRI. Add 0.5μl of EcoRI.
4. Incubate at 37°C for 2hr

◆Digest result

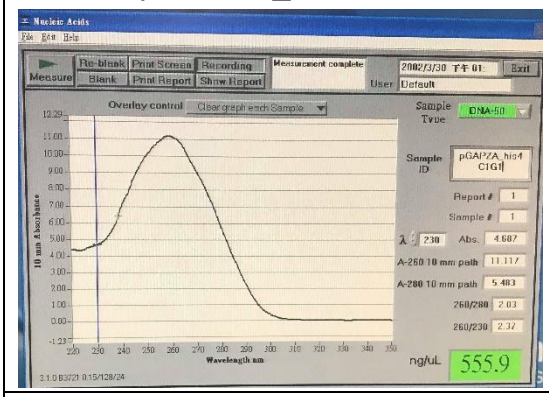


◆Digestion (pGAPZ A_HIS4 cut AgeI, EcoR)

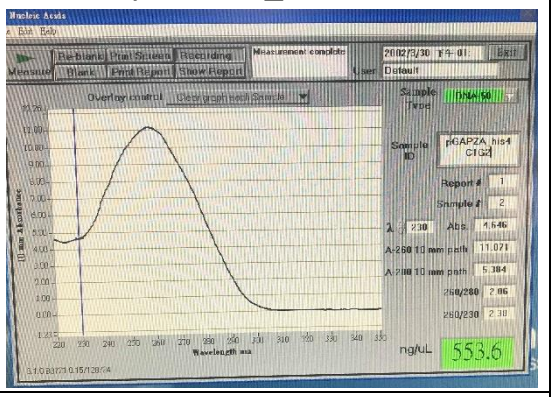
	DNA(3μl)	AgeI (5unit/μl)	EcoRI (20unit/μl)	10Xbuffer1.1	ddH_2O	Total V
1-1-1	5.4μl	0.6μl	0.4μl	2 μl	11.6 μl	20 μl
1-1-2	5.4μl					
1-2-1	5.4μl					
1-2-2	5.4μl					

◆DNA quality

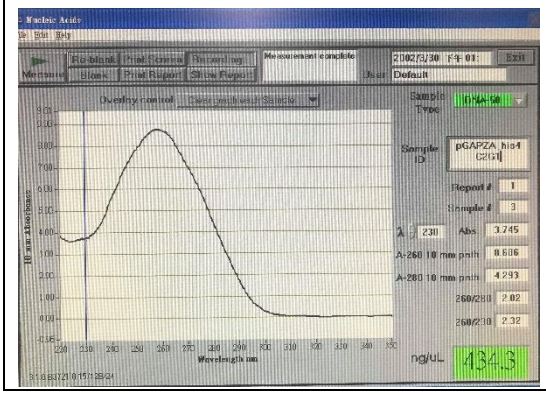
pGAPZ A_HIS4 1-1



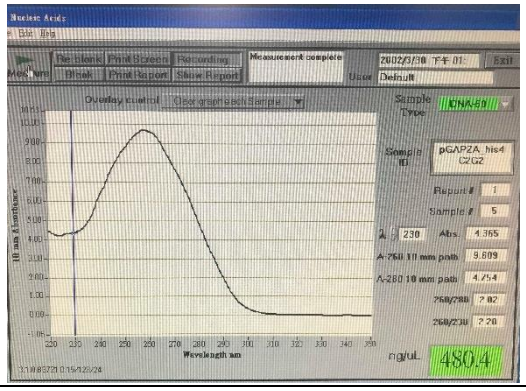
pGAPZ A_HIS4 1-2



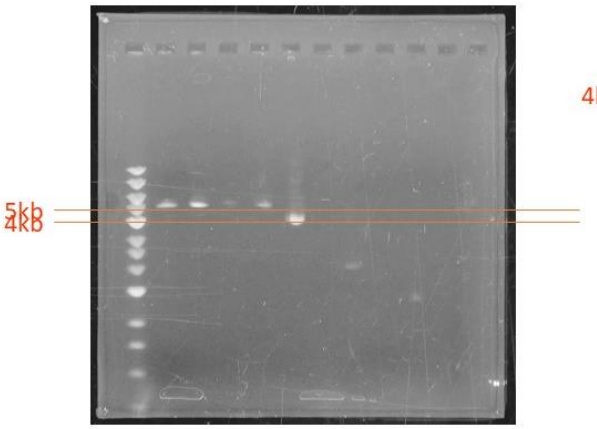
pGAPZ A_HIS4 2-1

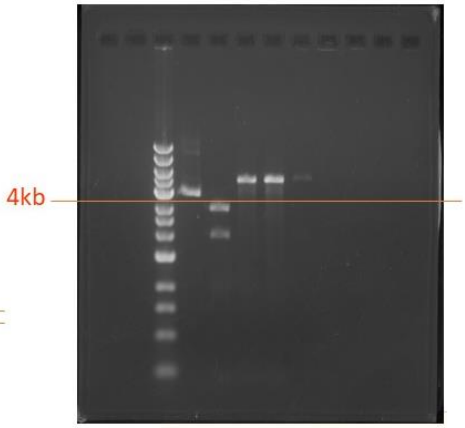


pGAPZ A_HIS4 2-2



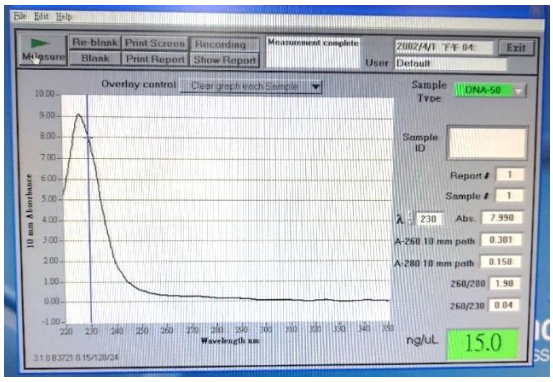
pGAPzA_His4 5183bp
EcoRI_AgeI 193bp
5183-193= 4990bp

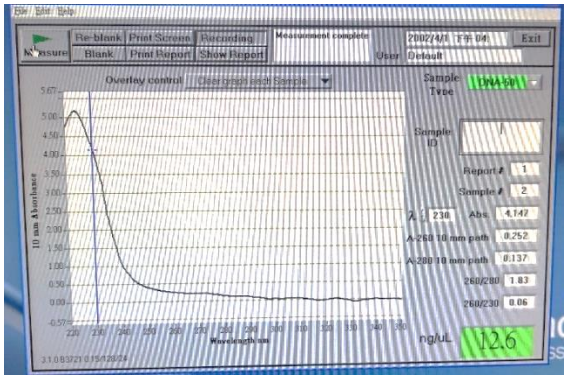




8/23

◆Result of gel extract





◆Ligation

insert	pGAPZA_HIS4 (vector)	(insert)	ligase	10Xligationn buffer	ddH_2O	Total V
Lac1	5.0μl	5μl	0.5μl	2.0μl	7.5μl	20.0μl
Prx16	5.0μl	5.5μl	0.5μl	2.0μl	7.0μl	
Prx18	5.0μl	3.5μl	0.5μl	2.0μl	9.0μl	

8/24

◆Transform over night

8/27

Digestion – pGAPZ A+HIS4 cut with AgeI and EcoRI (x3)

DNA 3μg	5.5 μl
AgeI (5 units/μl)	1 μl
NEbuffer 1.1	1.5 μl
ddH_2O	7 μl
Total	15 μl
T : 37 , 8hr	

NaCl	1 μl
EcoRI (20units/μl)	0.25 μl
DNA	15 μl
NEbuffer EcoRI	2 μl
ddH_2O	2 μl
Total	20 μl
T :37 , 4hr	

Ligation - vector: insert = 1:3

Px18 106.1ng(30.4ng/μl)	3.5 μl
DNA vector 75ng (15ng/μl)	5 μl
Ligase	0.5 μl
Ligation buffer	2 μl
ddH_2O	9 μl

Px16 107.3ng (19.7ng/μl)	5.5 μl
DNA vector 75ng (15ng/μl)	5 μl
Ligase	0.5 μl
Ligation buffer	2 μl
ddH_2O	7 μl

Lac1 169.8ng (33.8ng/μl)	5 μl
DNA vector 75ng (15ng/μl)	5 μl
Ligase	0.5 μl
Ligation buffer	2 μl
ddH_2O	7.5 μl

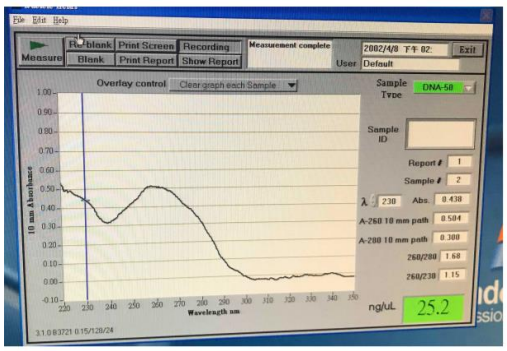
8/28

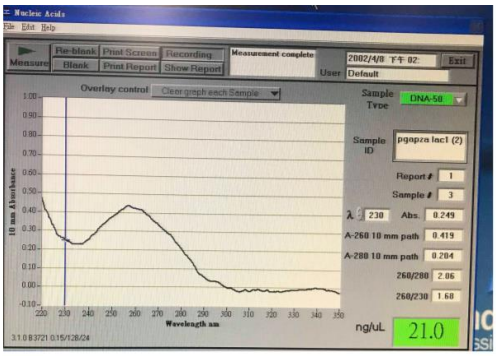
◆Transform (LB + zeocin)

1. pGAPZ A _HIS4_Px16
2. pGAPZ A_HIS4_Px18
3. pGAPZ A_HIS4_Lac1

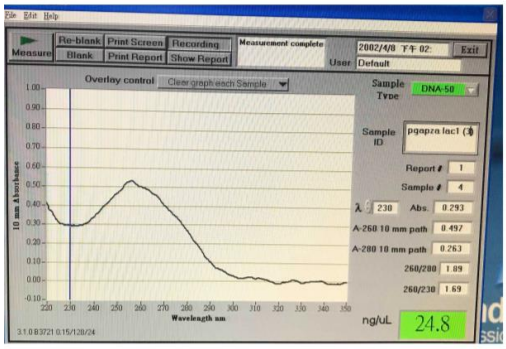
8/29

◆Nanodrop

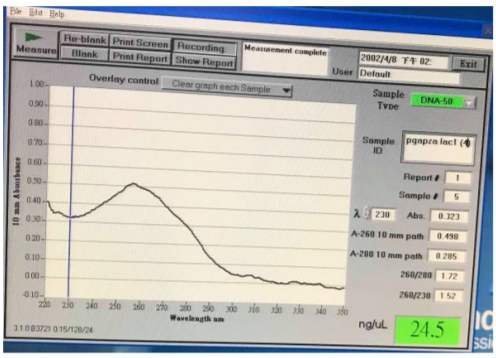




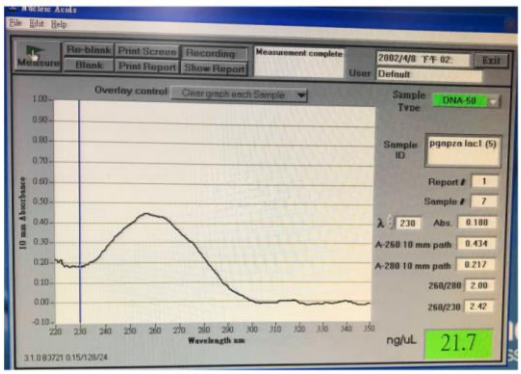
pGAPZ A + Lac1 (1)



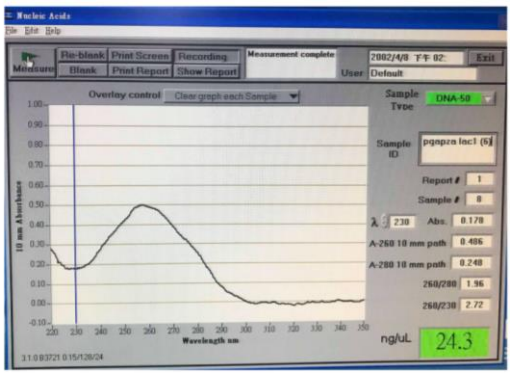
pGAPZ A + Lac1 (2)



pGAPZ A + Lac1 (3)



pGAPZ A + Lac1 (4)



pGAPZ A + Lac1 (5)

pGAPZ A + Lac1 (6)

8/30

◆Check by AgeI

pGAPZ A + HIS4

9/1

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

6x his	SOUP/PELLET	LOAD	ZEOCIN
1:20000	SOUP	LMH	o

◆Digest pGAPZ A HIS4(X2) with AgeI, EcoRI

Step 1

Set up reaction for AgeI as follows:

	DNA(3μg)	AgeI	NEBuffer1.1	ddH$_2$O
pGAPZ A(X2)	6.5μl(X3)	1.0μl (X3)	1.5μl (X3)	7.0μl (X3)

incubate at 37℃/ 6.5hr

Step 2
Add following for EcoRI reaction:

	1M NaCl	EcoRI	ddH$_2$O
pGAPZ A(X2)	1.0μl (X2)	0.25μl	2.0μl

incubate at 37℃/ 8hr

◆Ligation

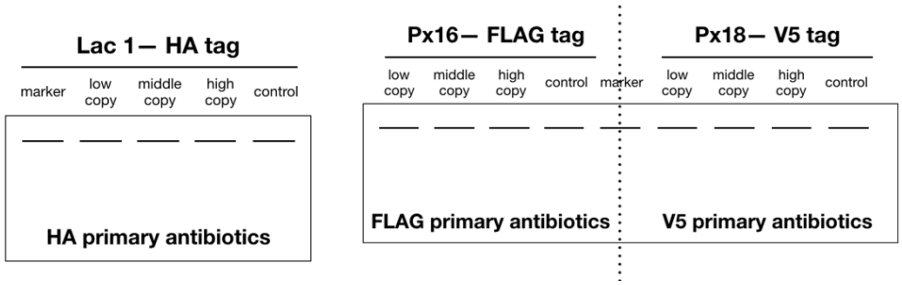
Set up the following reaction in a microcentrifuge tube on ice. (Insert: Vector = 3: 1)

	T4 Ligase	Ligase buffer	Insert	Vector 75ng	ddH$_2$O
pGAPZ A HIS4_Lac1	1.0μl	2.0μl	6.0μl	6.0μl	5.0μl
pGAPZ A HIS4_Px16	1.0μl	2.0μl	6.0μl	6.0μl	5.0μl
pGAPZ A HIS4_Px18	1.0μl	2.0μl	6.0μl	6.0μl	5.0μl

incubate at 16℃/ 18hr

9/2

◆Western blot: Antibody staining → Chemiluminescence detection



◆Transformation pGAPZ A HIS4_Lac1/ pGAPZ A HIS4_Px16/ pGAPZ A HIS4_Px18 (15μl) into 15μl ECOS™ 101 Competent Cells

9/3

◆Western blot: Sample preparation➔ SDS-PAGE➔ Transfer➔ Blocking➔ Antibody staining

6x his	SOUP/PELLET	LOAD	ZEOCIN
1:10000	SOUP	LMH	x

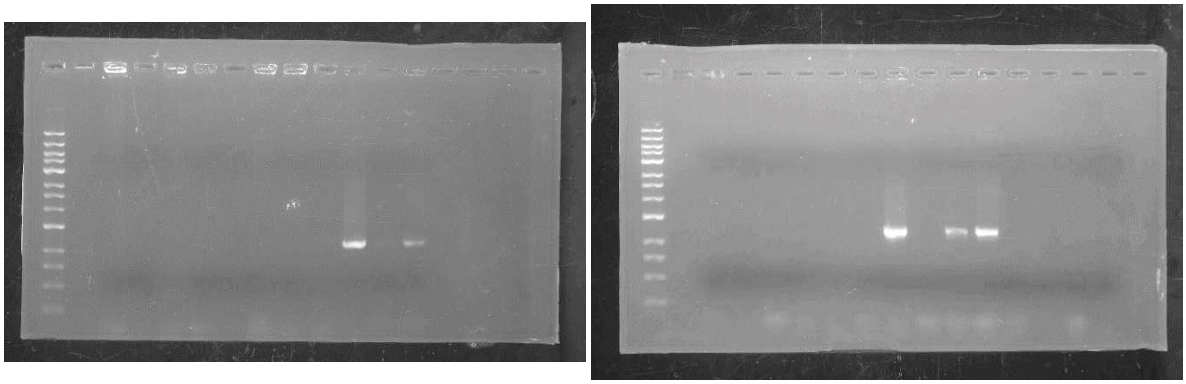
◆1.**PCR—pGAPZ A_HIS4 + Px16, Px18, Lac1**

Component	**Final(25**0µl)
10X Standard *Taq* Reaction Buffer	25µl
10 mM dNTPs	5µl
0.2 µM Primer suffix	5µl
0.2 µM Primer prefix	5µl
Template DNA	pGAPZ A_HIS4_Px16 pGAPZ A_HIS4_Px18 pGAPZ A_HIS4_Lac1
5 units/µl *Taq* DNA Polymerase	**3.8**µl
Nuclease-free water	**207.5**µl

Distribute to 25 eppendorf tubes, each of the tubes contains 10µl

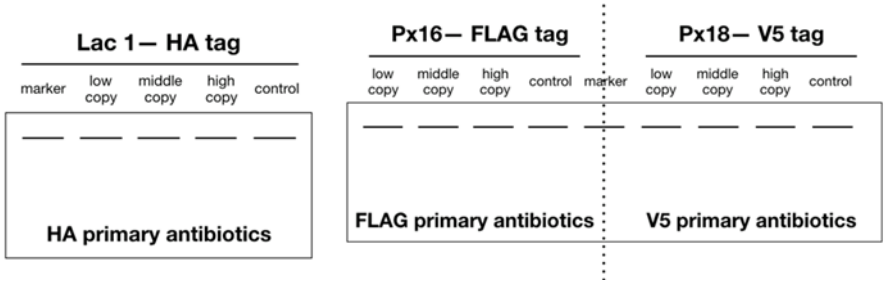
STEP	**TEMP**	**TIME**
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45°C 72°C	30 seconds 30 seconds 2.5minutes
Final Extension	72°C	10 minutes
Hold	4°C	

2.PCR Check



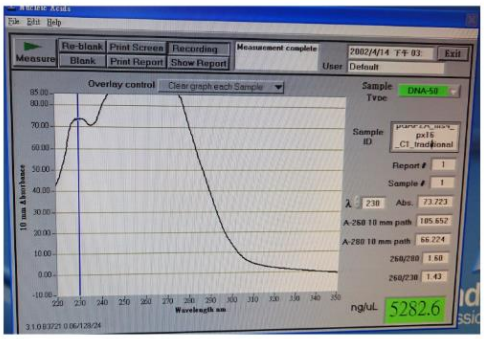
9/4

◆Western blot: Antibody staining → Chemiluminescence detection

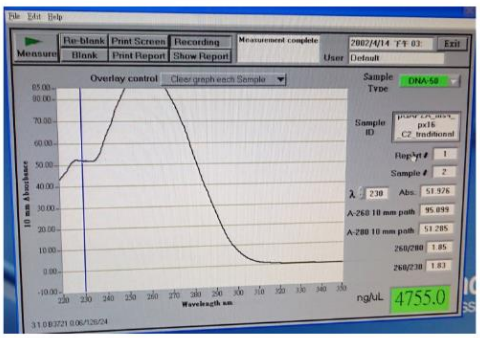


miniprep – pGAPZ A_HIS4 + Px16, Px18

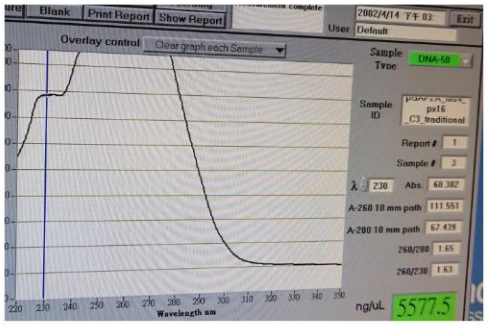
Traditional:



pGAPZ A_HIS4_Px16 (1)

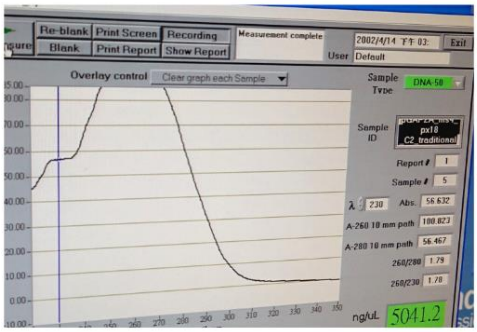


pGAPZ A-1_HIS4_Px16 (2)

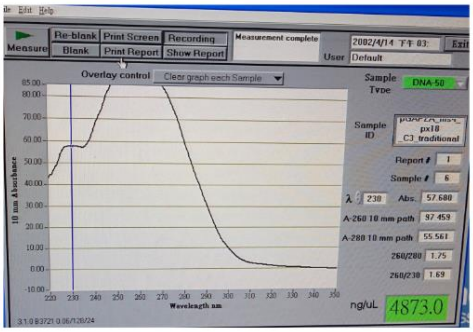


pGAPZ A _ HIS4_Px16 (3)

pGAPZ A_ HIS4_Px18 (1)

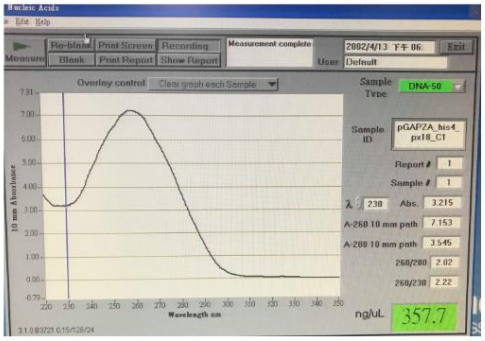


pGAPZ A_ HIS4_Px18 (2)

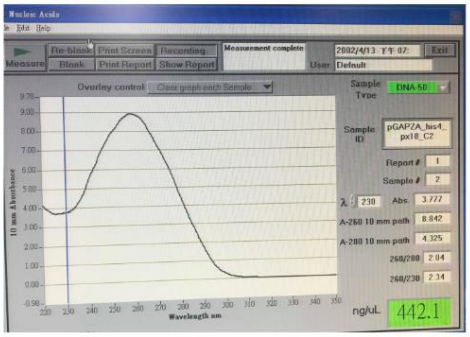


pGAPZ A_HIS4_Px18 (3)

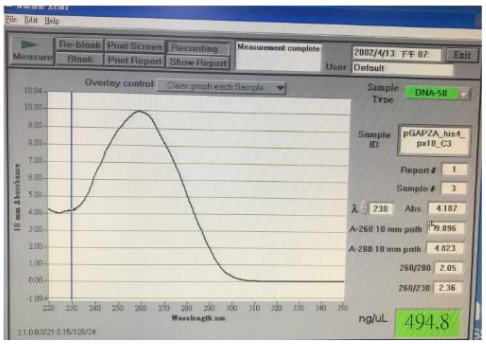
Miniprep:

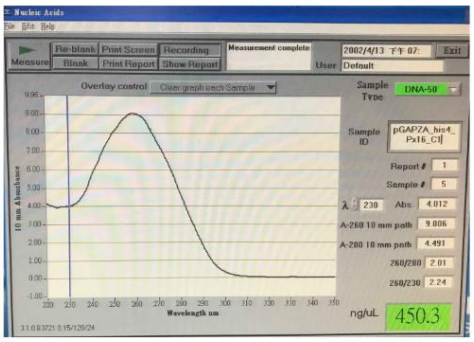


pGAPZ A _ HIS4_Px18 (1)



pGAPZ A1_HIS4_Px18 (2)





pGAPZ A_HIS4_Px18 (3)　　pGAPZ A_HIS4_Px16

9/5

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:5000	1:5000	1:5000	SOUP	LMH	x

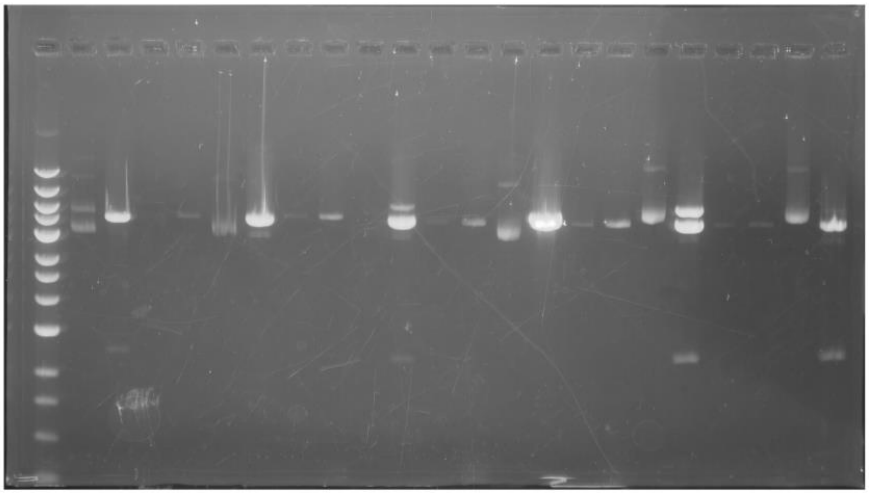
◆Transform – pGAPZ A_HIS4_Lac1(20μl competent cell, 20μl DNA)

DNA 3μg	5.5 μl
AgeI	0.6 μl
NEbuffer 1.1	1.5 μl
ddH_2O	7.4 μl
Total	15 μl
T : 37, 6hr	

NaCl	1 μl
EcoRI	0.25 μl
DNA	15 μl
ddH_2O	2 μl
NEbuffer EcoRI	2 μl
Total	20 μl
T :37, 10hr	

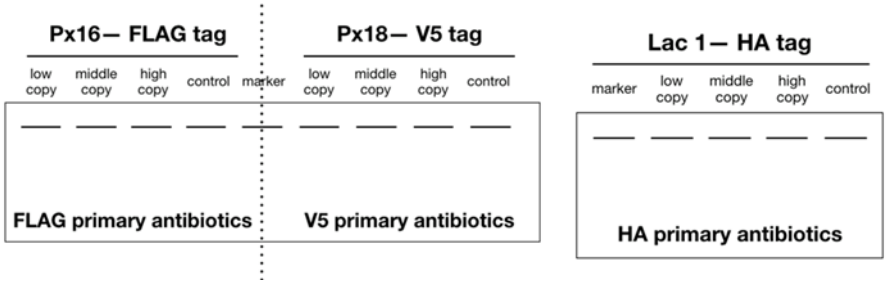
9/6

◆Digestion – pGAPZ A_HIS4_lac1



1-12. pGAPZ A_HIS4_Px16
1.5.9 miniprep uncut
2.6.10 miniprep cut
3.7.11 traditional uncut
4.8.12 traditional cut
13-22. pGAPZ A_HIS4_Px18
13.17.21 miniprep uncut
14.18.22 miniprep cut
15.19 traditional uncut
16.20 traditional cut

◆Western blot: Antibody staining → Chemiluminescence detection



9/7

◆Prepare yeast

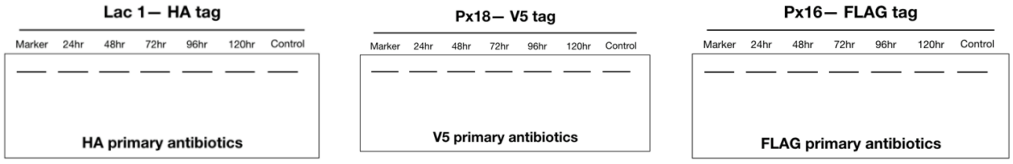
Pick up yeast(SD1168) from a colony + 50 ml YPD in erlenmeyer flask,
Breeding in incubator in 30℃ for days2.5

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:2000	1:2000	1:2000	SOUP	Time point	x

9/8

◆Western blot: Antibody staining → Chemiluminescence detection



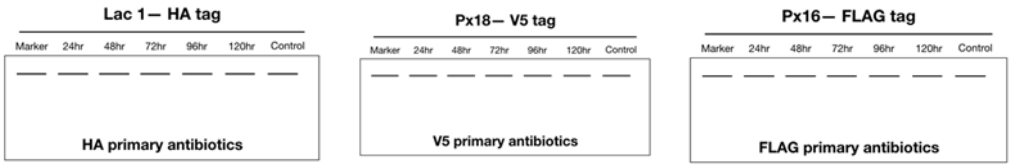
9/9

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:1000	1:1000	PELLET	Time point	x

9/10

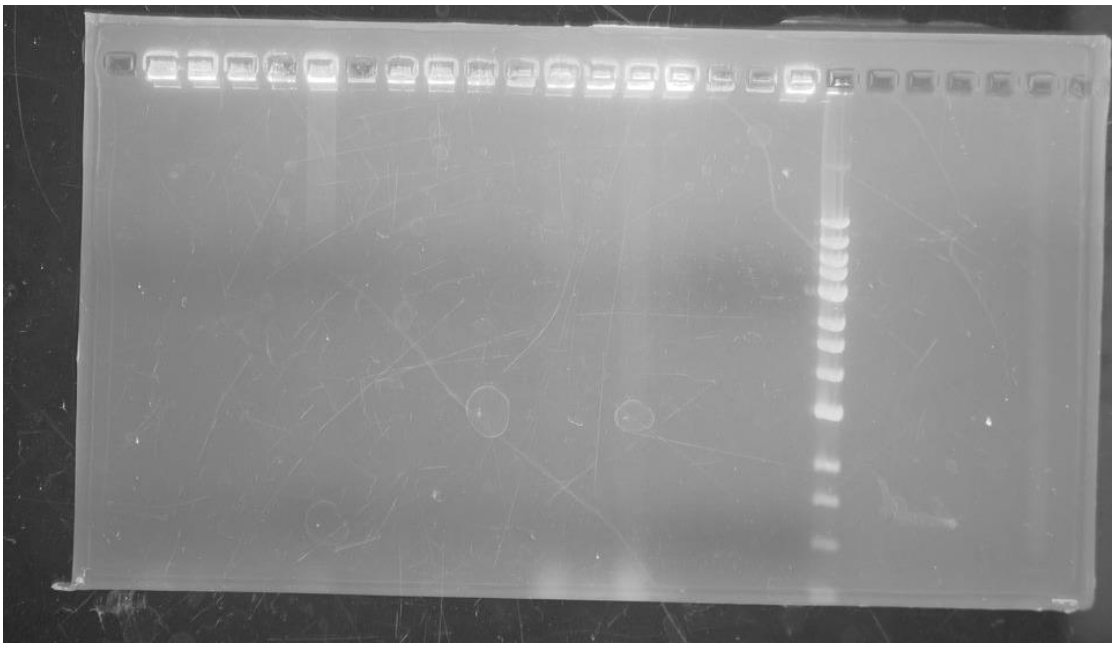
◆Western blot: Antibody staining → Chemiluminescence detection



1.Transform target genes into yeast → spread on SD plate

Plates:

1. Pichia pastoris
2. Pichia pastoris+ pGAPZ A_his4
3. Pichia pastoris+ pGAPZ A_his4_px18



>> FAIL ☹, cuz we pick too much bacteria

2.Minimal culturing of lac1

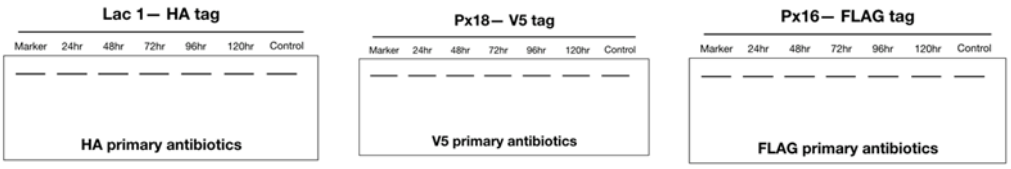
9/11

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:5000	1:5000	SOUP	Time point	o

9/12

◆Western blot: Antibody staining → Chemiluminescence detection



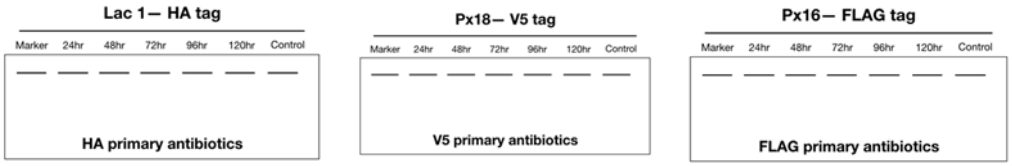
9/13

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	SOUP	Time point	o

9/14

◆Western blot: Antibody staining → Chemiluminescence detection

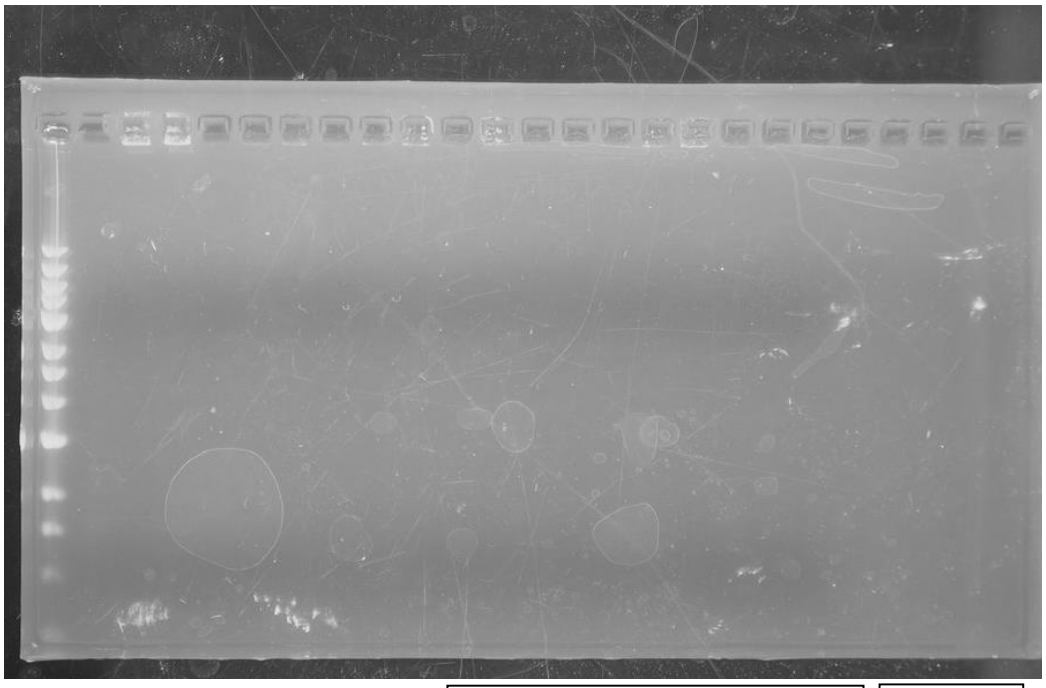


1.Check Px16, Px18

	DNA(3μl)	AgeI (5unit/μl)	10Xbuffer1.1	ddH_2O	Total V	time
Px16-1	2.5μl	0.40μl	2.00 μl	11.60 μl	15.00 μl	6hr
Px16-2	2.5μl					
Px18-1	2.0μl					
Px18-2	2.0μl					

		EcoRI (20unit/ μl)	10Xbuffer EcoR I	ddH_2O	Total V	time
Px16-1	DNA(15μl)	0.25μl	2.0 μl	2.0 μl	20.0 μl	4hr
Px16-2						
Px18-1						
Px18-2						

gel electrophoresis



Well 2 : miniprep cut px16 Well 3 : miniprep uncut px16 Well 4 : miniprep cut px18	Well 5 : miniprep uncut px18 Well 6 : traditon cut px18 Well 7 : traditon cut px18	Well 1 : marker

2.

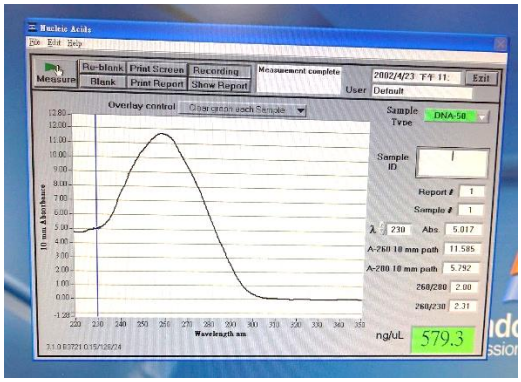
◆Miniprep Lac1 → store in -20ºC

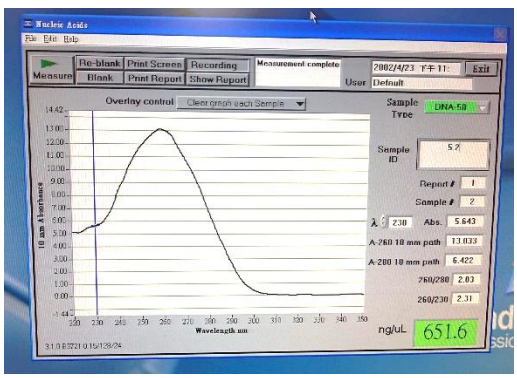
9/15

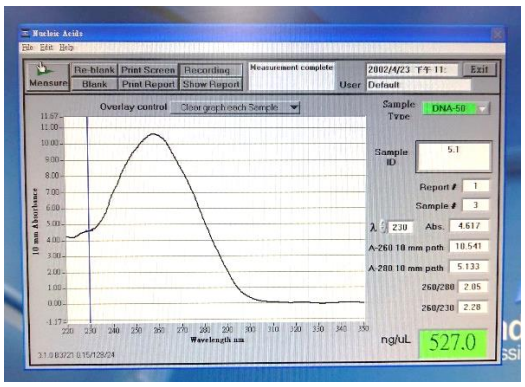
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

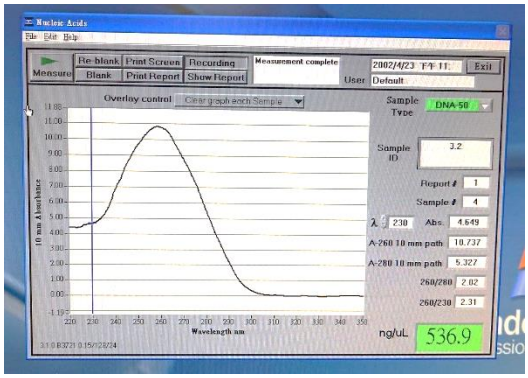
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	SOUP	Time point	o

◆Nanodrop miniprep Lac



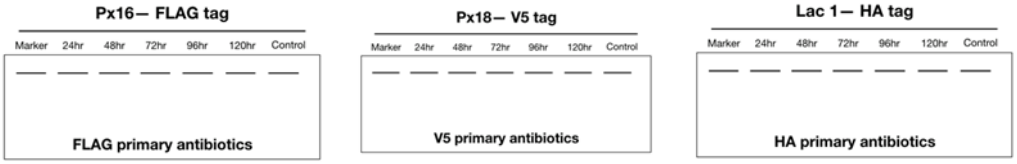




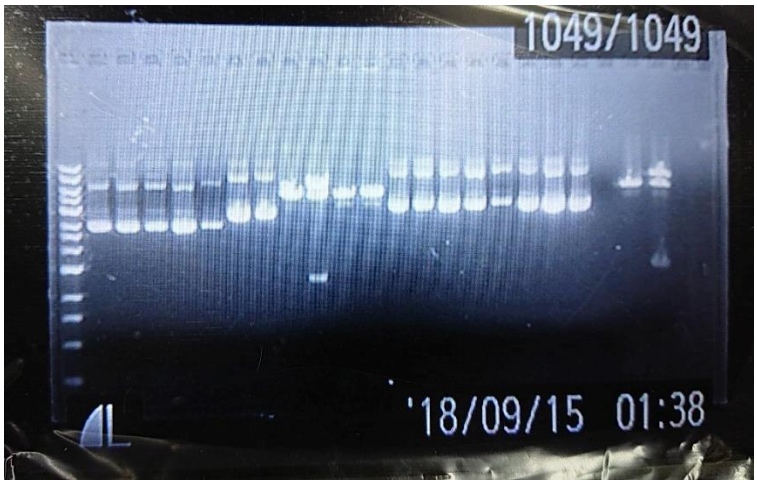


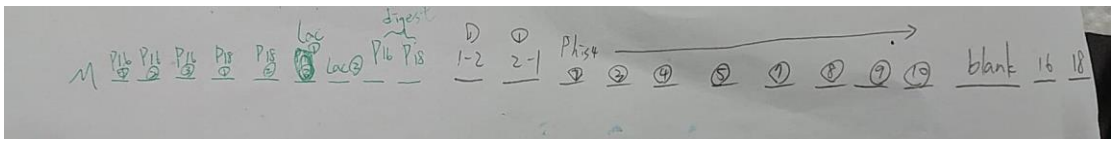
9/16

◆Western blot: Antibody staining → Chemiluminescence detection



◆Gel electrophoresis





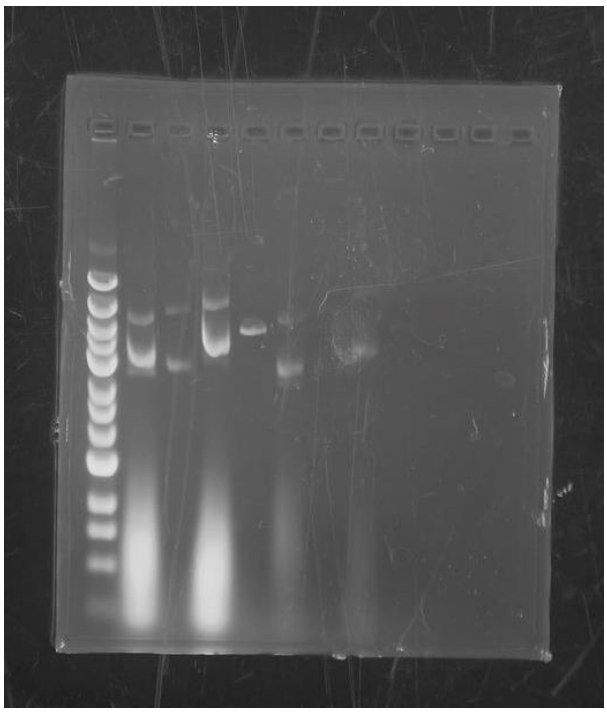
9/17

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN

1:1000	1:2000	1:5000	PELLET	Time point	o

◆Gel electrophoresis



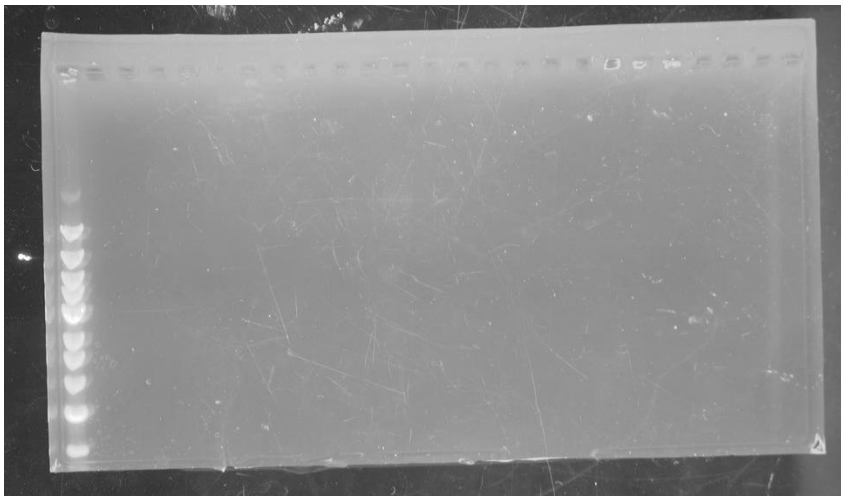
Well 1 : marker
Well 2 : uncut px16
Well 3 : cut px16
Well 4 : uncut px18
Well 5 : cut px18
Well 6 : uncut px16
Well 7 : cut px16
Well 8 : uncut px18
Well 9 : cut px18

◆PCR

Component	**Final(25**0µl)
10X Standard *Taq* Reaction Buffer	25µl
10 mM dNTPs	5µl
0.2 µM Primer suffix	5µl
0.2 µM Primer prefix	5µl
Template DNA	pGAPZ A_HIS4_Px16 pGAPZ A_HIS4_Px18 pGAPZ A_HIS4_Lac1
5 units/µl *Taq* DNA Polymerase	**3.8**µl
Nuclease-free water	**207.5**µl

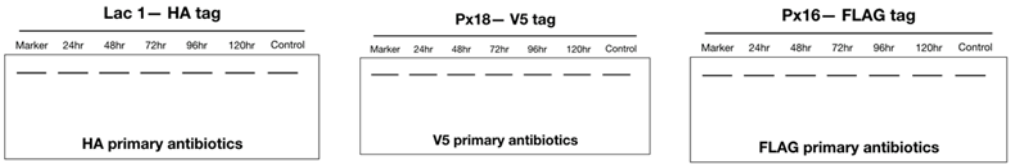
Distribute to 25 eppendorf tubes, each of the tubes contains 10µl

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45°C 72°C	30 seconds 30 seconds 2.5minutes
Final Extension	72°C	10 minutes
Hold	4°C	



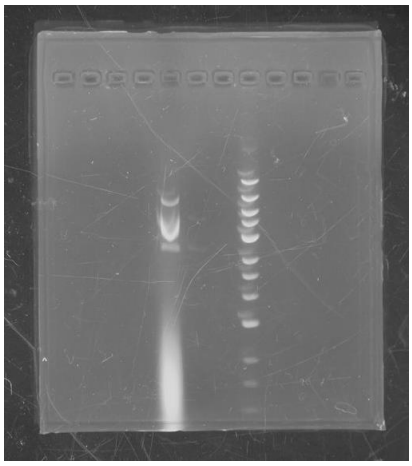
9/18

◆Western blot: Antibody staining ➔ Chemiluminescence detection



Cut to linear for yeast transform

	DNA(10μl)	Sal I (100unit/μl)	10Xbuffer3.1	ddH_2O	Total V	time
Px18	2.0μl	0.20μl	2.00 μl	16.00 μl	20.00 μl	6hr



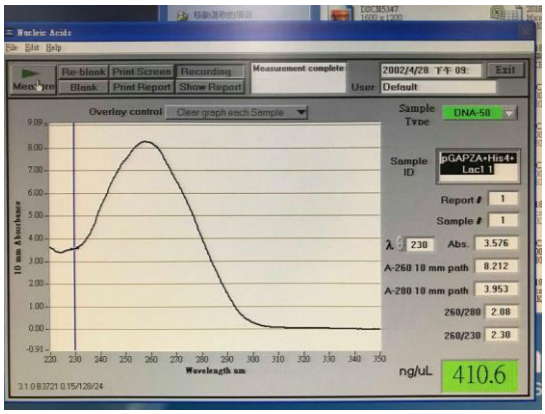
2. Miniprep Lac1 → store in -20°c

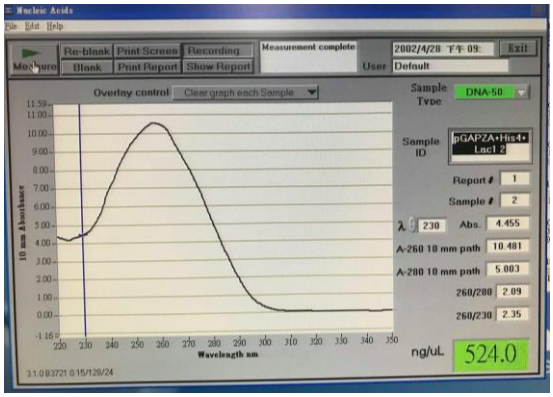
9/19

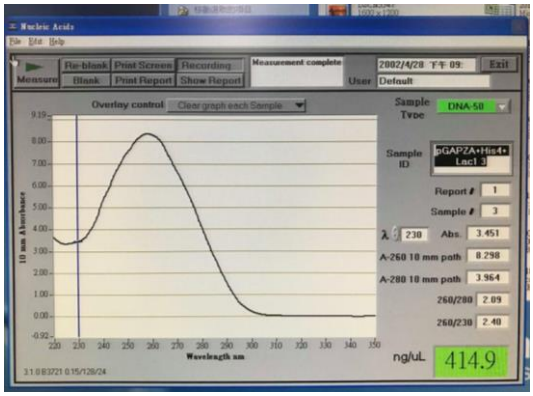
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

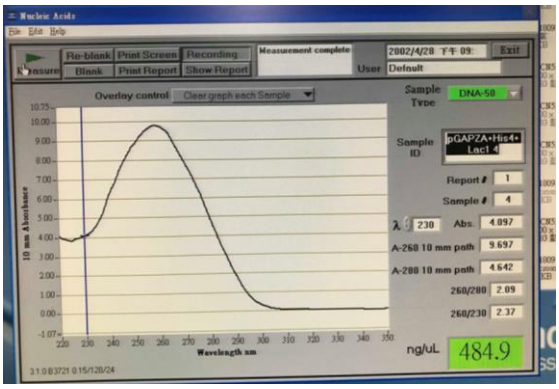
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	PELLET	Time point	o

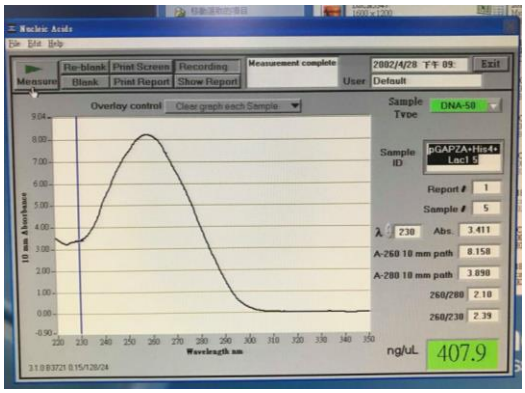
◆Nanodrop : Miniprep Lac1

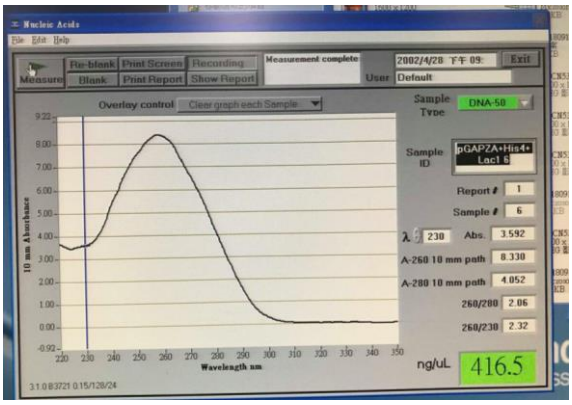


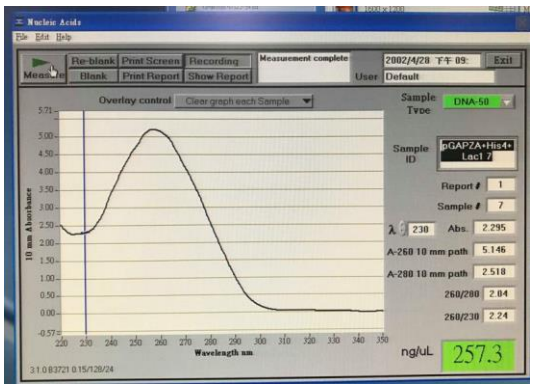


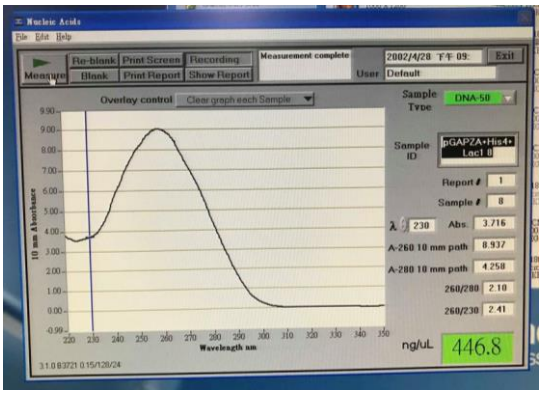


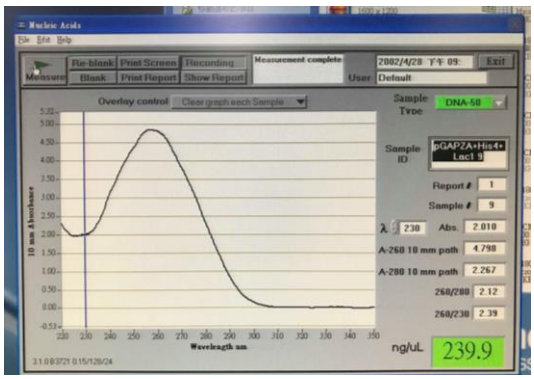


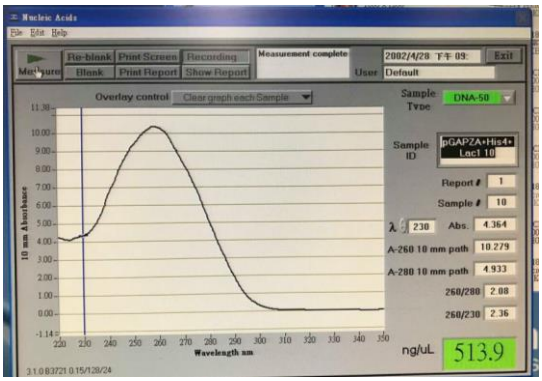








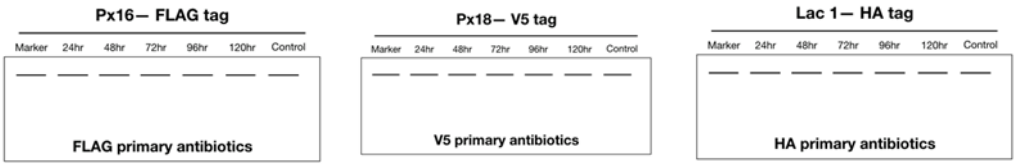




2.Minimal culturing of lac1

9/20

◆Western blot: Antibody staining → Chemiluminescence detection



1.Tradition miniprep Lac1 Day1

2.Prepare SD plate

9/21

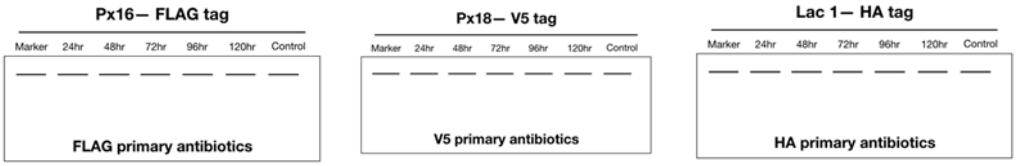
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	PELLET	Time point	o

◆Tradition miniprep Lac1 Day1

9/22

◆Western blot: Antibody staining → Chemiluminescence detection



◆1.Prepare yeast

Pick up yeast(SD1168) from a colony + 50 ml YPD in erlenmeyer flask, Breeding in incubator in 30℃ for days2.5

2.Tradition miniprep Lac1 Day2

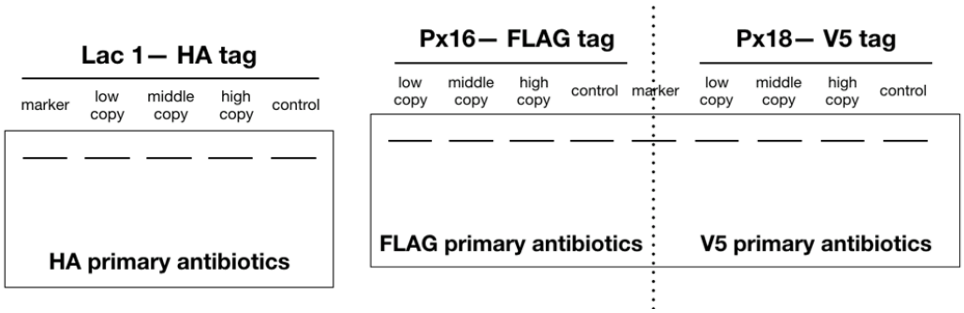
9/23

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

6x his	SOUP/PELLET	LOAD	ZEOCIN
1:20000	SOUP	LMH	x

9/24

◆Western blot: Antibody staining → Chemiluminescence detection



◆1.Minimal culturing of lac1
2.Store yeast in 4°c

9/25

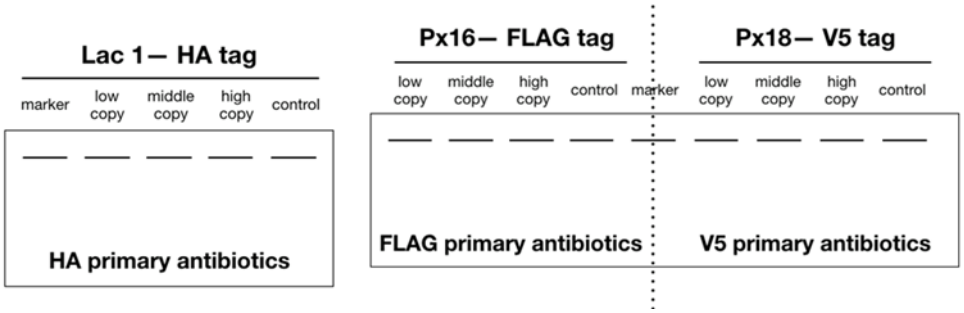
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

6x his	SOUP/PELLET	LOAD	ZEOCIN
1:10000	SOUP	LMH	X

◆Tradition miniprep Lac1 Day1

9/26

◆Western blot: Antibody staining → Chemiluminescence detection



◆1.Tradition miniprep Lac1 Day2

2.Transform px16&px18 into yeast(SD1168)

9/27

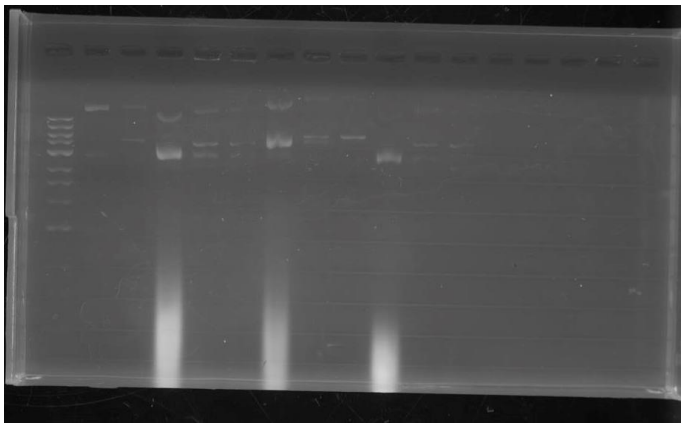
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:5000	1:5000	1:5000	SOUP	LMH	X

◆Cut to linear for yeast transform

	DNA(10μl)	Sal I (100unit/μl)	10Xbuffer3.1	ddH_2O	Total V	time
Px18	2.0μl	0.20μl	2.00 μl	16.00 μl	20.00 μl	6hr
Px16						

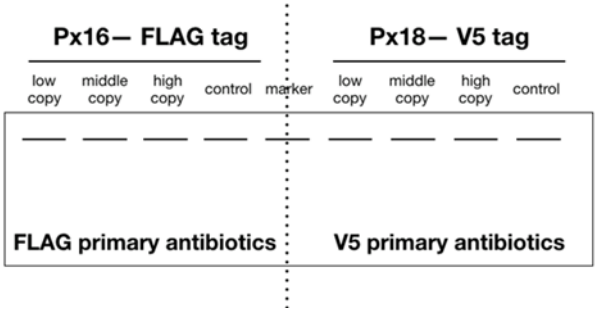
◆Gel electrophoresis

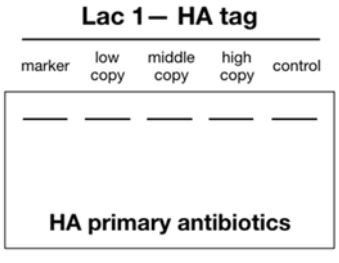


Well 1 :marker
Well 2 :pGAPZ A_his4 uncut
Well 3 : pGAPZ A_his4 cut
Well 4 : px16_his4 uncut
Well 5 : px16_his4 cut 1
Well 6 : px16_his4 cut 2
Well 7 : px18_his4 uncut
Well 8 : px18_his4 cut 1
Well 9 : px18_his4 cut 2
Well 10 : lac_his4 uncut
Well 11 : lac_his4 cut 1
Well 12 : lac_his4 cut 2

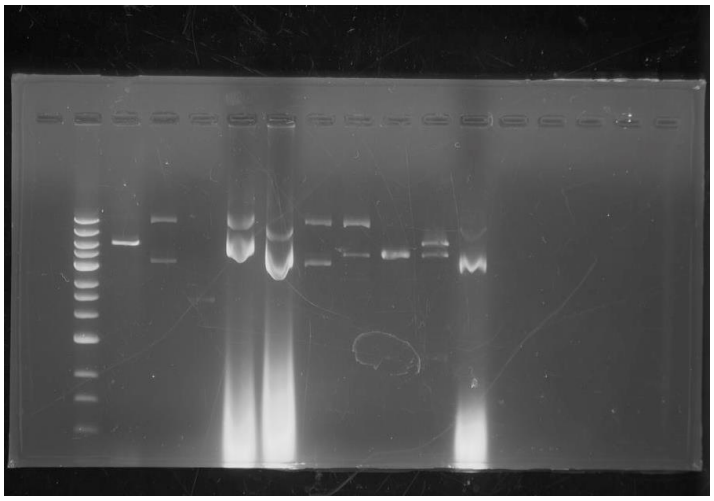
9/28

◆Western blot: Antibody staining → Chemiluminescence detection





1. **Transform px16&px18 into yeast(SD1168)**
2. **Gel electrophoresis**



Well 1.2 :marker
Well 3 : px18_his4 cut (sal I)
Well 4 : pGAPZ A_his4 2-2
Well 5 : ?
Well 6 : px18_his4 tradition uncut
Well 7 : px16_his4 tradition uncut
Well 8 : pGAPZ A_his4 1-2
Well 9 : 1-2
Well 10.11.12: ?

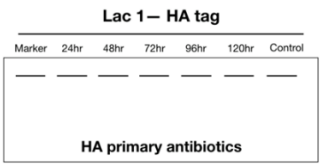
9/29

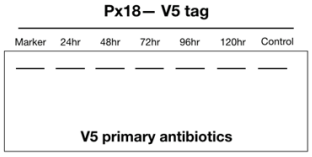
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

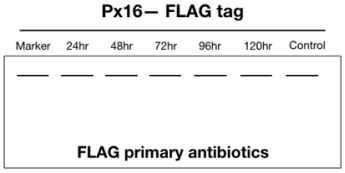
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:2000	1:2000	1:2000	SOUP	TIME POINT	X

9/30

◆Western blot: Antibody staining → Chemiluminescence detection





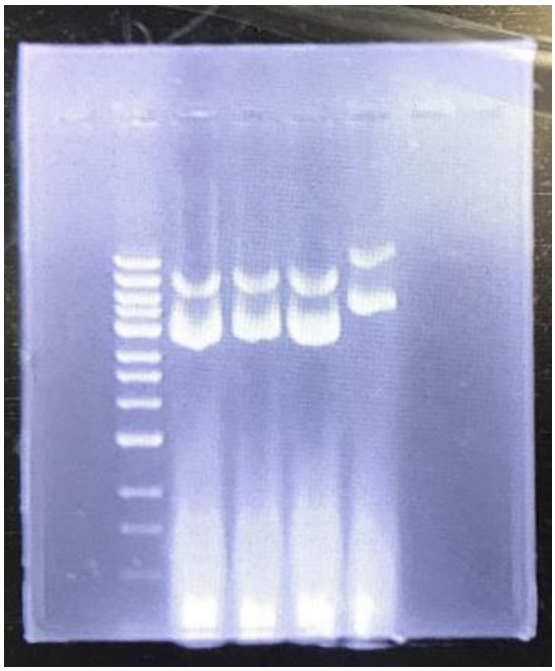


10/1

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:1000	1:1000	PELLET	TIME POINT	X

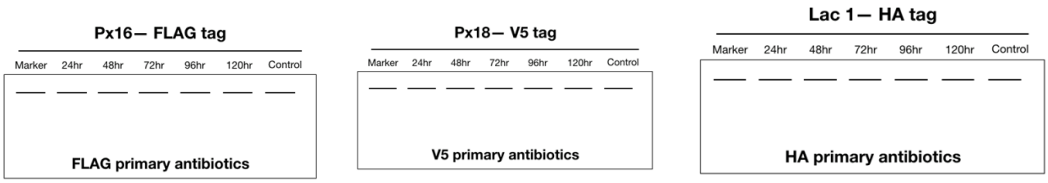
◆1.Minimal culturing of lac1
2.Gel electrophoresis



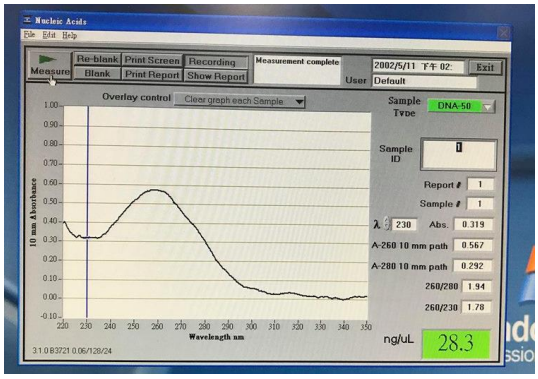
Well 1 :marker
Well 2.3.4 : px16_his4 cut (sal I)

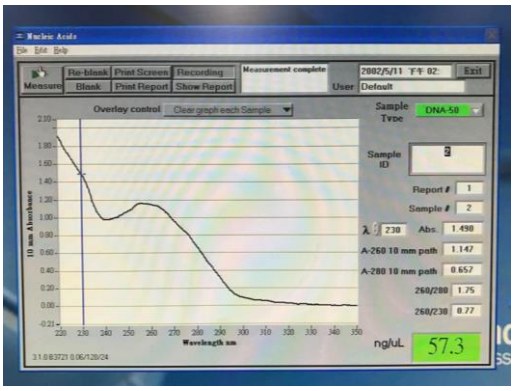
10/2

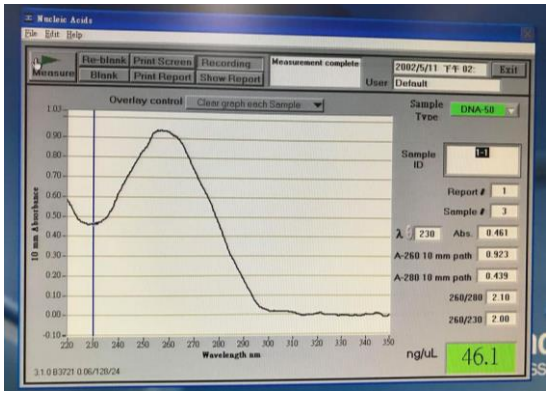
◆Western blot: Antibody staining → Chemiluminescence detection

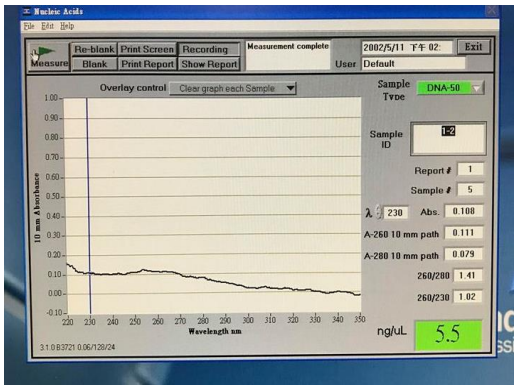


◆1.Nanodrop lac1(miniprep)









>> vector is out of the gene sequence ☹

2. **Transform px18 into yeast(SD1168)**
3. **Former SD plates are contaminated**
4. **Tradition DAY1 (Lac x1, Px16 x1)**

10/3

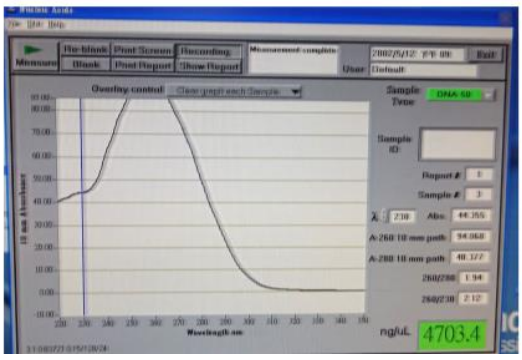
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

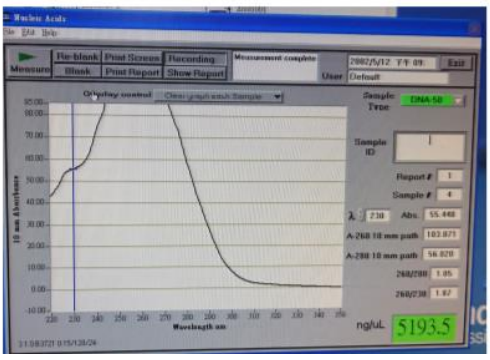
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:5000	1:5000	SOUP	TIME POINT	O

◆1.Safety part x3 (30°c、50°c、70°c)

2.Tradition DAY2 (Lac x1, Px16 x1)

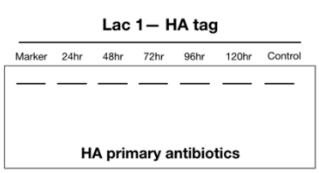
3. Colony PCR (Lac x6, Px16 x6)

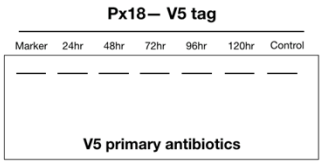


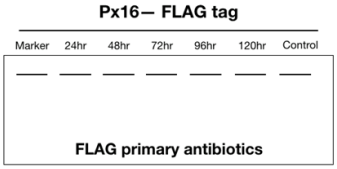


10/4

◆Western blot: Antibody staining → Chemiluminescence detection







◆Prepare new SD plate(the former are contaminated)

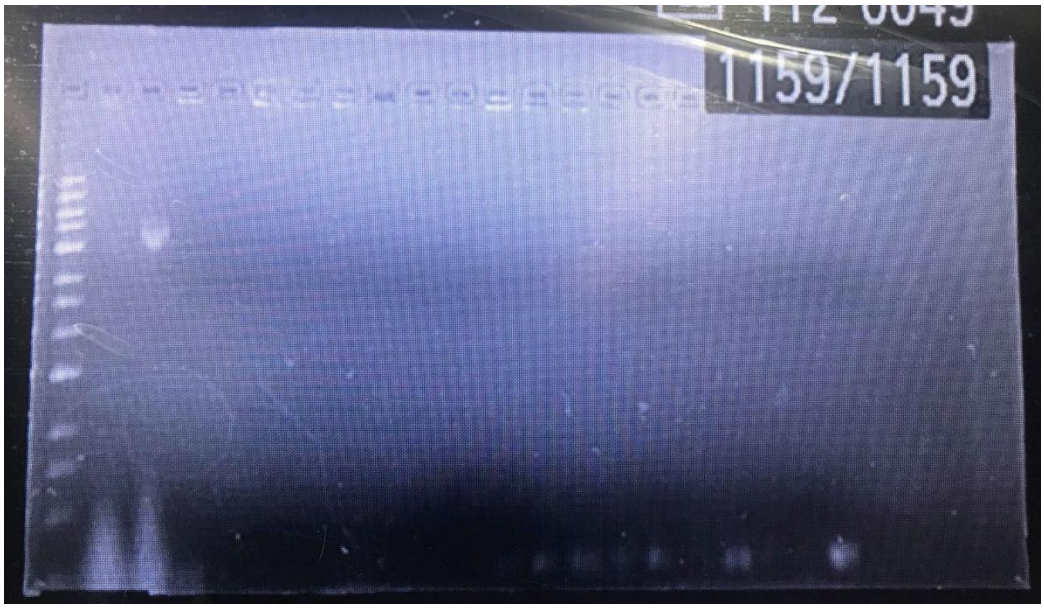
Yeast Nitrogen Base 6.7g + 100 ml H2O
Agar 20g + 750 ml H2O
50% Glucose 40 ml
Amino acids without Histidine 100 ml

10/5

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	SOUP	TIME POINT	O

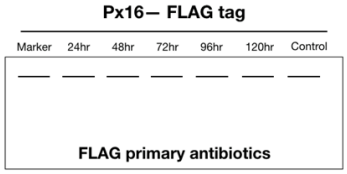
1.Gel electrophoresis (Tradition miniprep Lac x3 & PCR: Lac x6, Px16 x6)

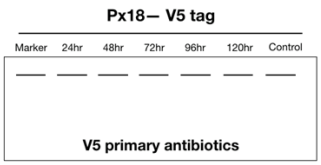


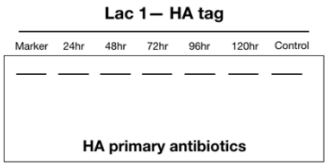
3. Transform px16&px18&pGAPZ A_his4 into yeast(SD1168)

10/6

◆Western blot: Antibody staining → Chemiluminescence detection







10/7

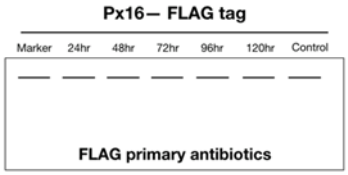
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

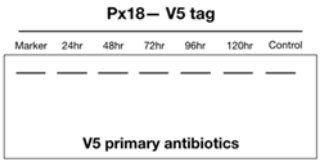
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	SOUP	TIME POINT	O

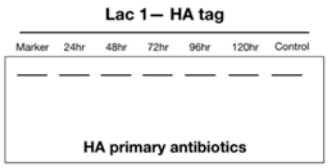
◆Minimal culturing of px16

10/8

◆Western blot: Antibody staining → Chemiluminescence detection







◆1.PCR Lac1 >>fail
2. Tradition Miniprep px16 day1

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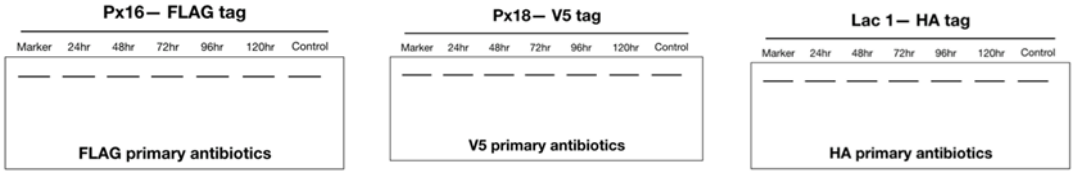
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	PELLET	TIME POINT	O

1. **Miniprep px16**
2. **Tradition Miniprep px16 day2**

10/10

◆Western blot: Antibody staining → Chemiluminescence detection



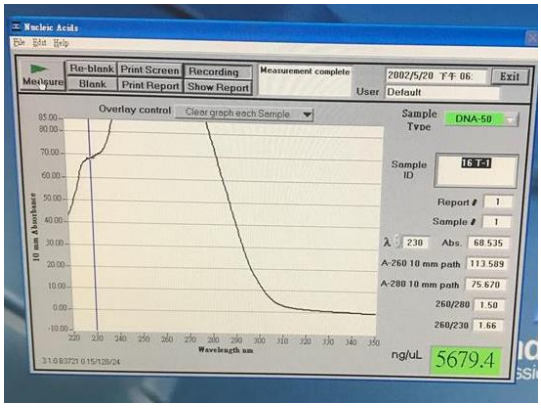
◆Tradition miniprep Lac1

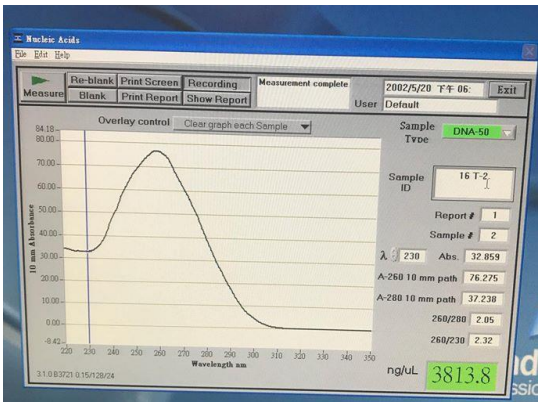
10/11

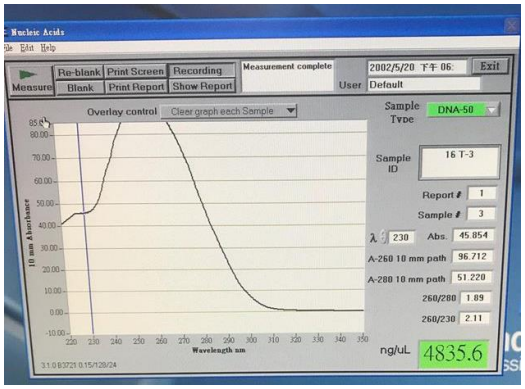
◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

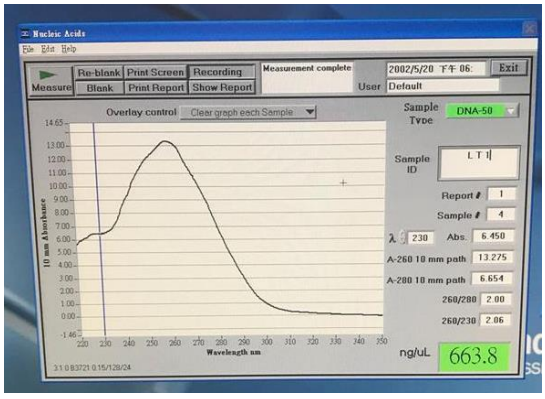
FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	PELLET	TIME POINT	O

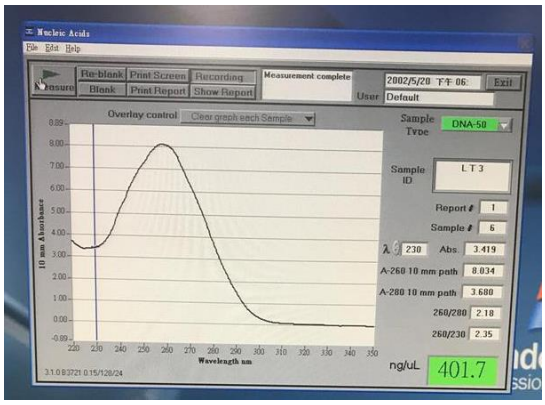
◆Nanodrop

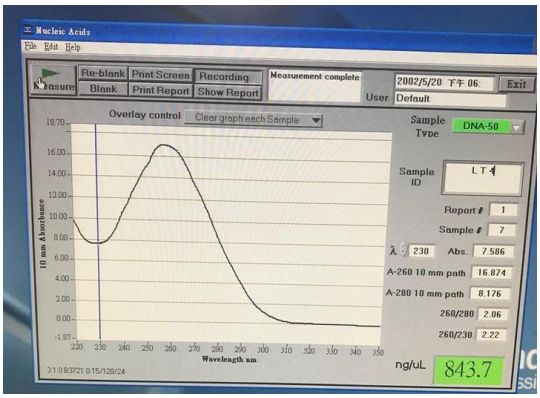












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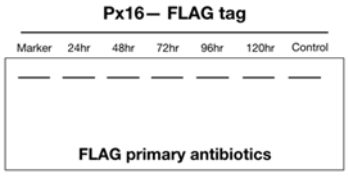
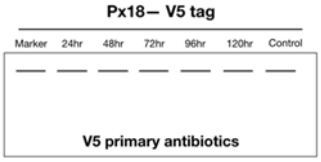
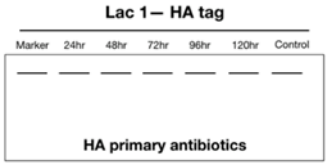
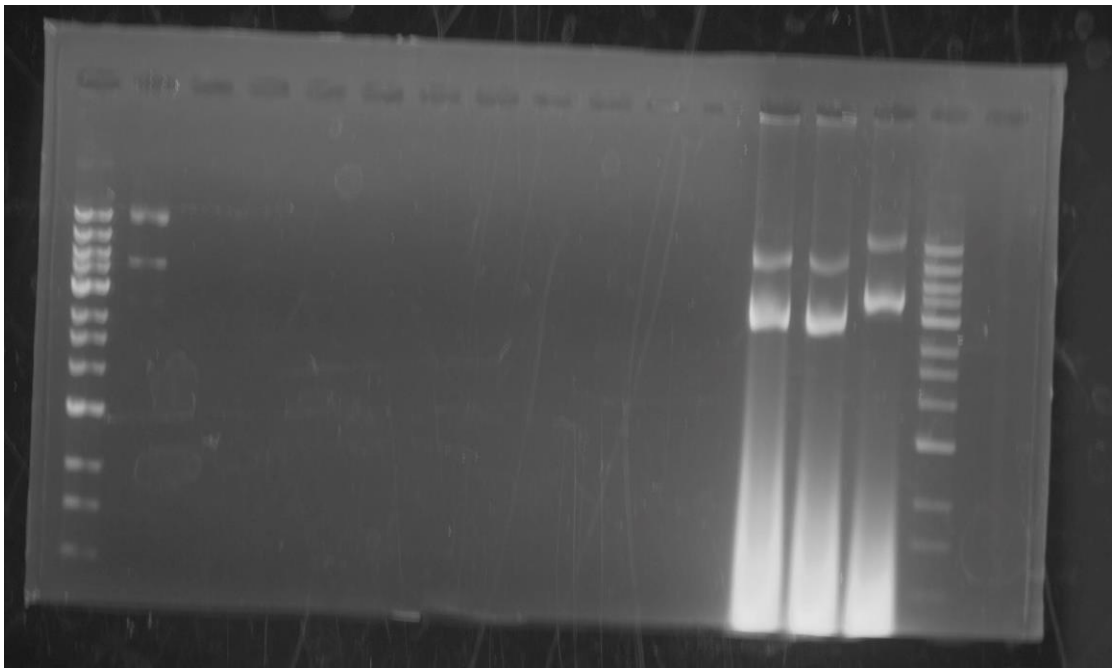
◆Western blot: Antibody staining → Chemiluminescence detection







◆Gel electrophoresis



marker/16-2 mini/ 16-3 cut/ 16-2cut/16-2cut/16-3 mini/16-3cut/lac1 mini/ lac1 cut/lac2 mini/lac2cut/lac2cut/lac3 mini/lac3cut/lac3cut/lac4 mini/lac4 cut

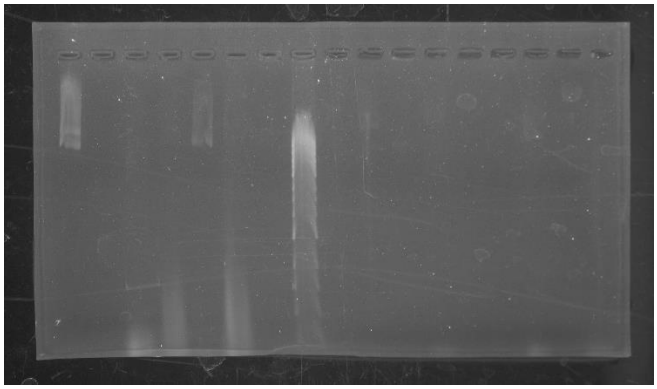
>> forget to dilute.

10/13

◆Western blot: Sample preparation→ SDS-PAGE→ Transfer→ Blocking→ Antibody staining

FLAG	HA	V5	SOUP/PELLET	LOAD	ZEOCIN
1:1000	1:2000	1:5000	PELLET	TIME POINT	O

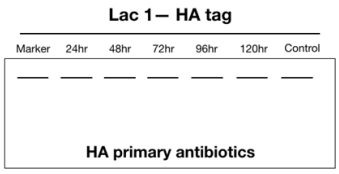
◆Gel electrophoresis

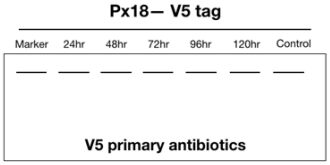


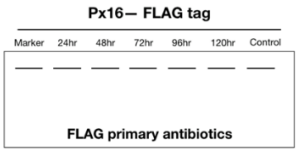
16-2 mini/ 16-3 cut/ 16-2cut/16-2cut/16-3 mini/16-3cut/lac1 mini/marker/lac1 cut/lac2 mini/lac2cut/lac2cut/lac3 mini/lac3cut/lac3cut/lac4 mini/lac4 cut

10/14

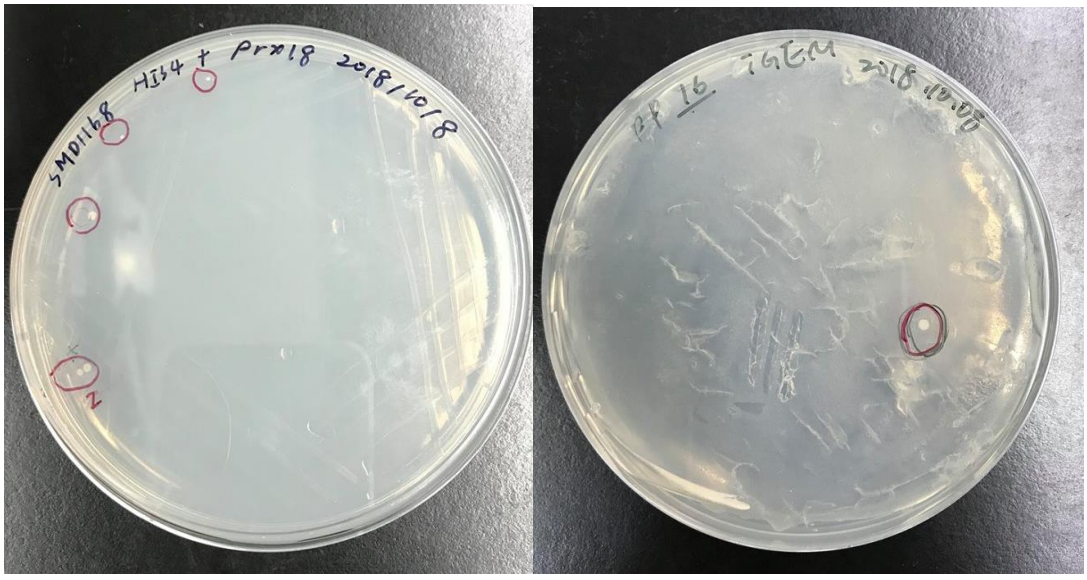
◆Western blot: Antibody staining → Chemiluminescence detection







◆PLATE

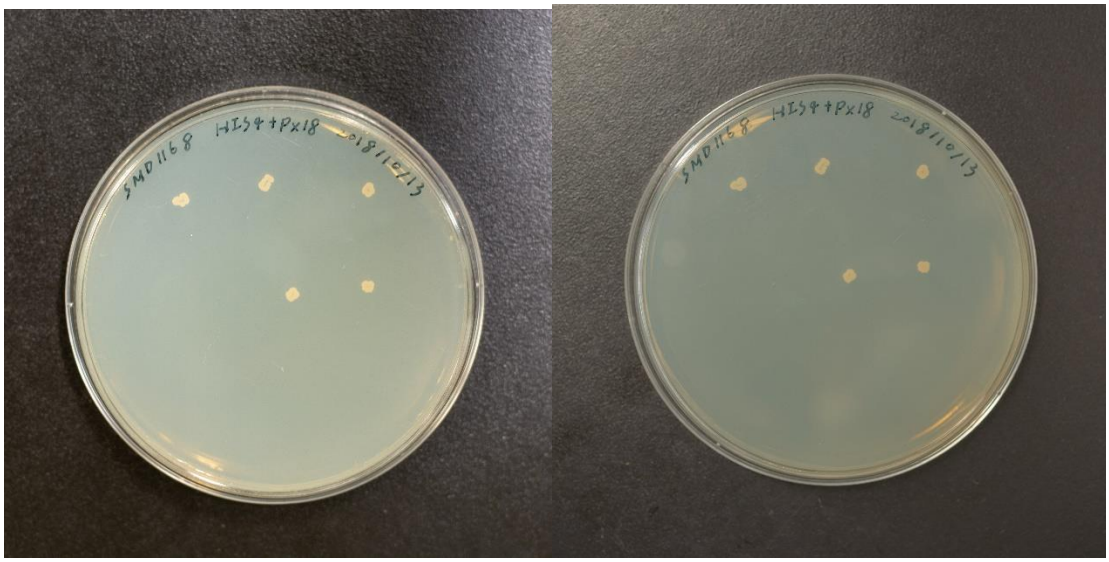


10/15

◆Western blot: Sample preparation➔ SDS-PAGE➔ Transfer➔ Blocking➔ Antibody staining

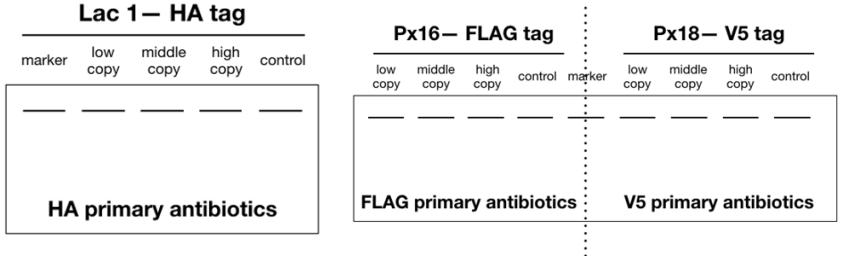
6x his	SOUP/PELLET	LOAD	ZEOCIN
1:20000	SOUP	LMH	X

◆Stick pellet to culture (SD)



10/16

◆Western blot: Antibody staining ➔ Chemiluminescence detection



Control

PLATE