Experimental Notes of Mixed Culture in 4.12

1. inoculate the G.xy and E.coli into culture media:

Glu/Gly： 6/4; 8ml/each.

$OD_{E.coli}$=1.689; OD_{G}=0.580.

E/G=1/3→VE=35ul; VG=300ul;

E/G=1/4→VE=28ul; VG=323ul;

E/G=1/5→VE=23ul; VG=336ul.

E/G=1/3	①	②	③+ethanol	④+ethanol
E/G=1/4	①	②	③+ethanol	④+ethanol
E/G=1/5	①	②	③+ethanol	④+ethanol

Experimental Notes of Mixed Culture in 5.21

1. Soak the BC membrane in NaoH solution
2. Prepared the culture media: Glu/Gly=3/2 *2000ml

   LB *500ml
3. PCR (4 kinds of primer)
4. Use gel electrophoresis method to check

Experimental Notes of Mixed Culture in 5.22

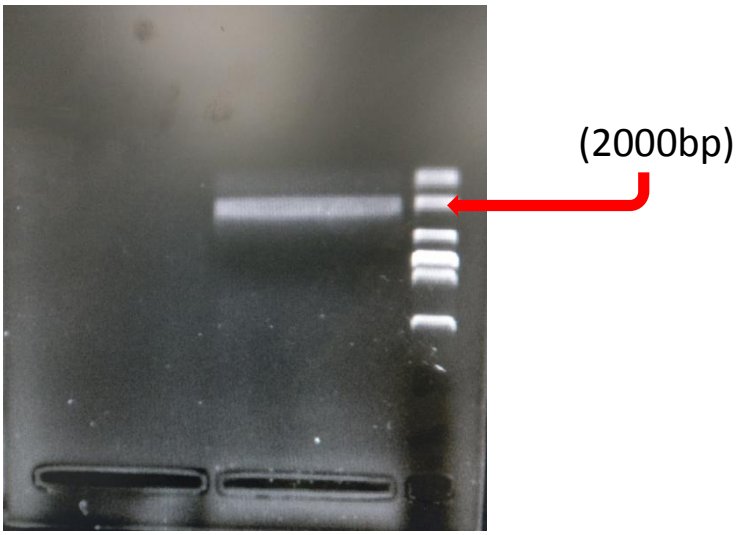
1. Gel extraction
2. A new PCR (the same result)
3. Gel electrophoresis
4. Prepared solution: 50*TAE 500ml
5. 25*TAE\1*TAE

Experimental Notes of Mixed Culture in 5.24

1. Gel extraction
2. Running the gel

Experimental Notes of Mixed Culture in 5.25

1. PCR to check Spy-tag sequence length again
2. Running the gel
3. Running gel result: (2000 Marker)



Experimental Notes of Mixed Culture in 5.26

1. Wash BC membrane by water

Experimental Notes of Mixed Culture in 5.27

1. Activate the Gxylinum in 3/2 culture medium
2. Activate the E.coli in LB culture medium
3. Activate the E.coli with spycathcer in LB with AMP culture medium

Experimental Notes of Mixed Culture in 5.28

1. Mix the bacterial into 3/2 culture medium：

Gxy ： E=4 : 1	x3
Gxy ： Es-c=4 : 1	X10
Blank control	x1

Purpose ： to testing and prepare the BC membrane with Spy-catcher.

Experimental Notes of Mixed Culture in 5.29

1. Prepared Culture Media (Glu/Gly=3/2)(5000ml)

Experimental Notes of Mixed Culture in 6.05

1. Activation of the Gxylinum in 3/2 culture medium
2. Washing BC membrane by NaOH solution
3. Checking pH

G/E_{SC} 4/1 3/2(Glu/Gly)	pH_1=4.48	pH_2=4.58	pH_3=4.57
G/E 4/1 3/2(Glu/Gly)	pH_1=4.50	pH_2=4.50	pH_3=4.60

4. Drawing three-zone in the solid plate with E.coli

Experimental Notes of Mixed Culture in 6.08

1. Prepare buffer solution (Phosphate buffer and Citrate

buffer)(200ml for each)

2. Activation of the Gxylinum in 3/2 culture medium with 4% cellulase (24h)

Experimental Notes of Mixed Culture in 6.09

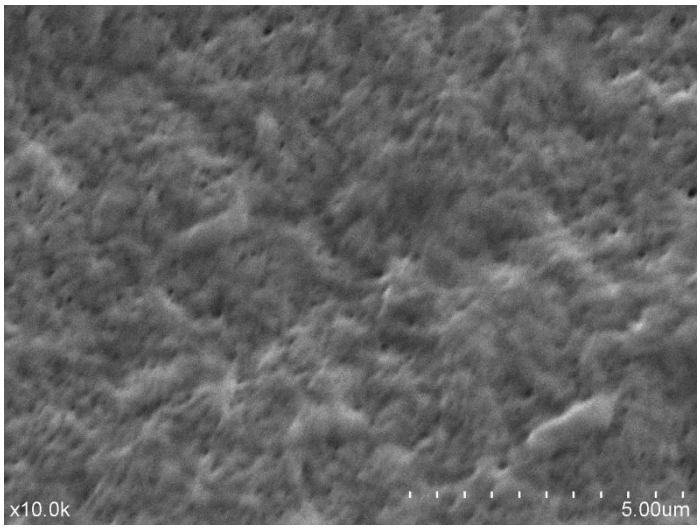
1. Culture the Gxylinum in 3/2 culture medium with buffer

Gxy	Citrate buffer	Number
3ml	+0.5%	*3
3ml	+1.0%	*3
3ml	+2.0%	*3
3ml	+3.0%	*3
3ml		*3

Experimental Notes of Mixed Culture in 6.15

1. observe the surface of samples by scanning electron microscope

Results：(BC membrane and Spy-catcher)



Experimental Notes of Mixed Culture in 7.11

1. Activate the chassis modules (including E1 and E2) in LB culture media(5 ml*3 each).
2. Activate the E.coli with AmpR in LB culture media(100ml).
3. Measuring the bacteria concentration

   OD_{Gxy}: 2.191

   OD_{E1}: 2.881

   OD_{E2}: 3.994
4. Incubate the Gxylinum and chassis modules respectively into 3/2 culture media
5. Transfer activated the Gxylinum in 3/2 culture media with cellulase
6. Transfer activated the E.coli (including Spy-catcher) with AmpR

Experimental Notes of Mixed Culture in 7.12

7. Inoculate the Gxylinum and Espycather respectively into 3/2 culture media
8. Measuring the pH and residual sugar in the co-culture at 22h

Experimental Notes of Mixed Culture in 7.14

1. Active the E.coli with AmpR in LB culture media(including AmpR)
2. Active the E.coli with Rubisco in LB culture media

3. Active the E.coli with PMV24 plasmid in LB culture media
4. Active the Gxylinum in 3/2 culture media
5. Confect 3/2 culture media

Experimental Notes of Mixed Culture in 7.15

1. Measuring the pH in each 3 hours

Results:

pH Groups / Time	G		Gxy/E.coli	
3h	5.70	5.70	5.68	5.70
6h	5.69	5.69	5.69	5.71
9h	5.61	5.61	5.65	5.62
12h	5.46	5.50	5.57	5.60
15h	5.29	5.27	5.33	5.38

2. Extracted plasmid from the E.coli(from peking)(Spy-catcher)

Experimental Notes of Mixed Culture in 7.16

1. convert the Spy-cathcer into E.coli by heat-shock transformation.
2. Activate the G.xy in the 3/2 culture media.

Experimental Notes of Mixed Culture in 7.17

1. Activating the E.coli(DH5α)(including Spy-catcher) in LB culture media with AmpR.

Purpose: Save the Glycerol tube.

2. Inoculate the Gxy. and E.coli/Gxy. into 3/2 cultule media.

Experimental Notes of Mixed Culture in 7.18

1. Monitoring the pH in different.

The result:

pH (Groups / Times)	G.xy		G.xy/E	
0h	5.91			
6h	5.81	5.83	5.79	5.84
12h	5.63	5.70	5.55	5.59
15h	5.37	5.43	5.47	5.49
18h	5.43	5.49	5.03	5.05
21h	5.20	5.20	4.87	4.90
24h	5.09	4.91	4.81	4.82
27h	5.07	4.63	4.81	4.95
29h	4.97		4.70	

Experimental Notes of Mixed Culture in 7.19

1. Activating the E.coli(including Spy-cathcer) in LB culture media with CMR(0.5ul/1ml).

2. Inocubate the G.xy and E.coli(including Spy-cathcer) into 3/2(Glu/Gly) culture media.

3. Inoculate the G.xy and E.coli into 3/2(Glu/Gly) culture

media.

4. Activating the G.xy in 3/2(Glu/Gly) culture media with 4% cellulase.

5. Activating the E.coli(including Spy-cathcer) in LB culture media with AmpR(1ul/ml).

Experimental Notes of Mixed Culture in 7.20

1. Activate E.coli(including chassis module 1) with KANR(1ul/ml) in LB culture media.

2. Restriction enzyme cutting the plasmid and verification

3. Incubate the G.xy/E.coli(including chassis module 1) into 3/2(Glu/Gly) culture media.

Experimental Notes of Mixed Culture in 7.21

1. Monitor the ph in mixed culture system.

Resluts:

Groups / pH / Times	G.xy/E.coli		
	Group 1	Group 2	Average
12h	5.61	5.71	5.66
15h	5.35	5.44	5.395
18h	5.05	5.08	5.065
21h	4.88	4.85	4.865
24h	4.83	4.74	4.775
36h	4.77	4.72	4.745

Experimental Notes of Mixed Culture in 7.22

1. Observe the green fluorescence content
2. Prepare for the competent cell

Experimental Notes of Mixed Culture in 7.23

1. Activate the E.coli(including P-asr+GlasA) in LB culture media with

   100ul kanR

2. Prepare the cellulase(200ml)

Experimental Notes of Mixed Culture in 7.24

1. inoculate the E.coli(including P-asr+GlasA) 0.1% into LB culture media to culture overnight
2. Activate the E.coli(including P-asr+GlasA) in LB culture media with 100ul kanR
3. Activate the E.coli(DH5α) in LB culture media
4. Deal with the BC membrane(including Spy-catcher) by UV light+Water

Experimental Notes of Mixed Culture in 7.25

1. make the competent cells(fail)
2. Activate the E.coli(DH5α) in LBculture media
3. Activate the G.xy in 3/2 culture media

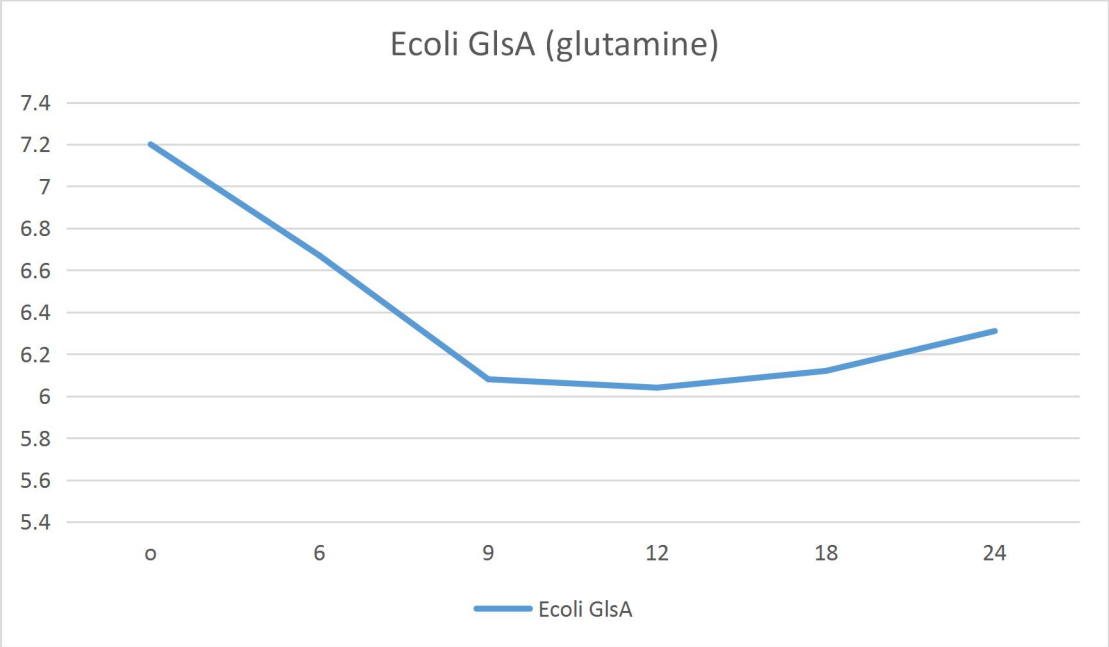
Experimental Notes of Mixed Culture in 7.26

1. make the competent cells

2. Activate the G.xy in 3/2culture media

3. Monitor the pH in single E.coli experiment

Results:



Experimental Notes of Mixed Culture in 7.27

1. Activate the E.coli(including the P-asr+GlasA) in LBculture media

2. Activate the E.coli in LBculture media

3. inoculate the G.xy and E.coli(including the P-asr+GlasA plasmid) into 3/2 culture media with sodium glutamate

Experimental Notes of Mixed Culture in 7.28

1. Inoculate the G.xy and E.coli into response surface culture media

Experimental Notes of Mixed Culture in 7.30

1. Activate the G.xy in 3/2 culture media with 4% cellulase

Experimental Notes of Mixed Culture in 7.31

1. Activate the E.coli in LBculture media
2. Activate the E.coli(including Spy-catcher)in LB culture media with AmpR
3. Activate the E.coli(including P-asr-GlasA)in LBculture media with KAN
4. Prepare different culture meidas for the response surface.

Experimental Notes of Mixed Culture in 8.1

1. Activate the G.xy into 3/2 culture media.(with 4% cellulase)
2. Activate the E.coli and E.coli (including the GlsA) into LB culture media.
3. Activate the G.xy into 3/2 culture media (with 4% cellulase).

Experimental Notes of Mixed Culture in 8.2

1. Prepare the LB and 3/2 culture medias
2. Activate the G.xy into 3/2 culture media (with 4% cellulase)*3

Experimental Notes of Mixed Culture in 8.3

1. Activate E.coli in the LB culture media*2
2. Activate E.coli (including P-asr and GlsA genes) in the LB culture media with KANR*1
3. Activate E.coli (including Spy-catcher genes) in the LB culture media with AmpR*1

4. Measure some basal datas:

G.xy: OD_{600}=0.828

E.coli(including GlsA gene) OD_{600}=2.92

5. Inoculate the G.xy and E.coli (including GlsA gene) onto the 3/2 culture media according to the original proportion

6. Inoculate the G.xy and E.coli (including Spy-catcher gene) onto the 3/2 culture media according to the original proportion

7. Inoculate the G.xy and E.coli onto the response surface culture media according to the original proportion.

Experimental Notes of Mixed Culture in 8.4-8.5

1. Measure some basal datas:

Times	pH	OD_{600}
24h	4.92	0.229
	4.84	0.373
48h	4.60	1.206
	4.57	1.136

Experimental Notes of Mixed Culture in 8.6

1. Measure some basal datas of the response surface experiments:

1	4.12	7	3.99	13	3.96
2	4.10	8	4.39	14	4.43

3	4.19	9	4.62	15	4.17
4	4.37	10	4.40	16	3.89
5	4.17	11	4.02	17	3.81
6	4.18	12	4.14		

2. Prepare the 3/2 and LB culture medias.

3. Activate G.xy in the 3/2 culture media with 4% cellulase

Experimental Notes of Mixed Culture in 8.7

1. Activate the E.coli (including Spy-catcher) in LB culture media with AmpR

2. Activate the E.coli (DH5α) in LB culture media.

3. Inoculate G.xy and E.coli (DH5α) in the 3/2 basal cullture media with different amounts of EtOH (0, 100ul, 300ul, 500ul, 700ul, 900ul, 1100ul, 1300ul, 1500ul, 2000ul)

Experimental Notes of Mixed Culture in 8.9

1. Prepare 3/2 and LB culture media*3400ml

2. Activate G.xy in the 3/2 culture meidia with 4% cellulase

Experimental Notes of Mixed Culture in 8.10

1. Activate E.coli (DH5α) in the LB culture media.

2. Activate E.coli (including Spy-catcher genes) in the LB culture media with AmpR.

Experimental Notes of Mixed Culture in 8.11

1. Inoculate G.xy and E.coli (DH5α) in the 3/2 basal cullture

media with different amounts of EtOH (0, 100ul, 300ul, 500ul, 700ul, 900ul, 1500ul, 2000ul)

Experimental Notes of Mixed Culture in 8.13

1. Prepare the LB culture media

Experimental Notes of Mixed Culture in 8.15

1. Activate E.coli (including P-asr-GlsA genes) in the LB culture media

2. Inoculate E.coli in 3/2 culture media into deep-well multiwell plate

Experimental Notes of Mixed Culture in 8.16

1. Measure some basal datas of the deep-well multiwell plate experiment

Results:

Times	pH			OD_{600}		
0h	5.86			0.016		
9h	5.88	5.88	5.88	0.072	0.082	0.101
9.5h	5.91	5.89	5.90	0.080	0.078	0.079
10h	5.88	5.87	5.85	0.078	0.094	0.094
10.5h	5.87	5.90	5.86	0.075	0.066	0.075
11h	5.87	5.87	5.87	0.088	0.110	0.094
11.5h	5.89	5.85	5.87	0.078	0.089	0.076
12h	5.88	5.86	5.85	0.110	0.111	0.098

12.5h	5.89	5.85	5.85	0.147	0.122	0.119
13h	5.85	5.83	5.82	0.144	0.128	0.118
14h	5.86	5.87	5.85	0.144	0.136	0.124
15.5h	5.80	5.84	5.83	0.122	0.122	0.136
17h	5.82	5.82	5.83	0.171	0.203	0.222
19h	5.83	5.80	5.79	0.194	0.175	0.154
21h	5.80	5.78	5.79	0.185	0.173	0.161
22h	5.77	5.79	5.79	0.210	0.204	0.246
25h	5.60	5.58	5.60	0.228	0.225	0.253

2. Activate G.xy in the 3/2 culture media with 4% cellulase.

Experimental Notes of Mixed Culture in 8.17-8.18

1. inoculate G.xy in the 3/2 culture media into deep-well multiwell plate

2. Measure some basal datas of the deep-well multiwell plate experiment

Results:

Times	pH			OD_{600}		
0h	5.84			0.024		
12h	5.60	5.63	5.59	0.053	0.047	0.039
13h	5.59	5.58	5.56	0.041	0.038	0.040
14h	5.50	5.47	5.48	0.080	0.046	0.052
15h	5.48	5.37	5.38	0.063	0.107	0.076

16.5h	5.40	5.41	5.41	0.070	0.052	0.055
17h	5.32	5.43	5.44	0.066	0.098	0.074
17.5h	5.32	5.36	5.32	0.110	0.072	0.116
18h	5.32	5.36	5.32	0.059	0.048	0.070
18.5h	5.30	5.29	5.19	0.053	0.049	0.062
19h	5.33	5.31	5.26	0.088	0.054	0.072
19.5h	5.30	5.31	5.33	0.044	0.046	0.039
20h	5.36	5.34	5.32	0.041	0.056	0.096
20.5h	5.34	5.38	5.44	0.046	0.054	0.073
21.5h	5.42	5.29	5.26	0.078	0.075	0.075
24h	5.28	5.28	5.24	0.098	0.064	0.064
35h	5.09	5.08	5.18	0.246	0.231	0.222

Experimental Notes of Mixed Culture in 8.19

1. Prepare 3/2 and LB culture media*1900ul.

Experimental Notes of Mixed Culture in 8.20

1. Activate G.xy in the 3/2 culture media with 4% cellolase.

Experimental Notes of Mixed Culture in 9.3

1. Activate G.xy in the 3/2 culture media with 4% cellolase.

Experimental Notes of Mixed Culture in 9.4

1. inoculate G.xy in the 3/2 culture media

2. Inoculate G.xylinum seed liquor into the solid culture media in the plate.

Experimental Notes of Mixed Culture in 9.5

1. Prepare enough 3/2 culture media

Experimental Notes of Mixed Culture in 9.7

1. Activate G.xy in the 3/2 culture media with 4% cellulase

Experimental Notes of Mixed Culture in 9.8

1. inoculate single G.xy into 3/2 culture media*12(one of flask with 4% cellulase)

Experimental Notes of Mixed Culture in 9.17

1. Activate G.xy in the 3/2 culture media with 4% cellulase

Experimental Notes of Mixed Culture in 9.18

1. Activate E.coli (Spy-catcher) in the LB culture media with AmpR.
2. Inoculate G.xy into 3/2 culture media
3. Mix G.xy and E.coli (including Spy-catcher) into 3/2 culture media.

Experimental Notes of Mixed Culture in 9.19-9.22

1. Measure some basal data of the co-culture experiments

Results:

Times	OD_{600}
0h	0.097
24h	0.978
72h	1.523/1.732

96h	2.123

Experimental Notes of Mixed Culture in 9.24

1. Prepare cellulase solution 200ml
2. Prepare LB and 3/2 culture media
3. Activate E.coli (DH5α) into the LB culture media
4. Deal with BC membrane with UV light.

Experimental Notes of Mixed Culture in 9.25

1. Inoculate E.coli (DH5α) into the deep-well multiwell plate.
2. Measure some basal datas of the deep-well multiwell plate experiment.

Results:

Times	pH		OD_{600}	
0h	5.93	5.93	0.103	0.103
12h	5.60	5.55	0.688	0.869
24h	5.32	5.33	0.807	0.786
48h	5.21	5.19	0.676	0.773

Experimental Notes of Mixed Culture in 9.26

1. Activate the G.xy into 3/2 culture media

Experimental Notes of Mixed Culture in 9.27

1. Activate the E.coli (including Spy-catcher) into LB culture media with AmpR
2. Inoculate the G.xy into 3/2 culture media

3. Mix the G.xy and E.coli into the 3/2 culture media according to the certain proportion.