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DNA 提取

◇ DNA

- (1) 2 cm 1.5 mL
- (2) 650 μ L 1.5 \times CTAB
- (3) 65 $^{\circ}$ C 30 min 5-10 min
- (4) 10 min 650 μ L
- (5) 15 min 2 min 12000 r/min 10 min
- (6) 400 μ L
400 μ L
- (7) 3 min 2 min 12000 r/min 8 min
- (8) 75% 800 μ L 3
- (9) 75% 800 μ L 3 7500 r/min 2 min
- (10) 10 μ L 75%
- (11) 50 μ L ddH₂O

◇ DNA

- (1) 0.1 g 600 μ L CTAB 1.5 mL
- (2) 65 $^{\circ}$ C 30 min 10 min 1
- (3) - v v/24 1 10 min
- (4) 12000 r/min 10 min
- (5) 10 min
- (6) 12000 r/min 10 min
- (7) 75%
- (8) 50 μ L TE -20 $^{\circ}$ C

◇ **DNA**

- (1) 0.5 g
- (2) 3 mL 65°C CTAB 65°C 30 min
- (3) 1 mL 5 mol/L KAc 20 min
24 1 1
- (4) 4°C 12000 r/min 5 min
- (5) 2/3 -20°C -20°C 30 min
- (6) 75% 3
1
- (7) 400 μL TE 1 μL RNaseA 10 mg/L 37°C 1 h
- (8) 25 24 1 24 1 1
4°C 12000 r/min 5 min
- (9) -20°C 30 min 12000 r/min 5
min
- (10) 75% 200 μL TE -20°C

RNA 提取

◇ **RNA**

- (1) 0.1 g 1.5 mL 500 μL
1 mL Trizol 3 min
- (2) 4°C 12000 r/min 10 min 0.2 mL
15 sec 3 min
- (3) 4°C 12000 r/min 10 min
10 min 2
30 min
- (4) 4°C 12000 r/min 10 min 70% DEPC
=7 3
- (5) RNA 30-50 μL DEPC RNA -80°C

(6)	DNase I	DNA	
	1.5 mL		50 μ L
			μ L
	RNA		20-50 μ g
	10 \times DNase I buffer		5.0
	DNase I RNase-free, 5 U/ μ L		2.0
	RNase Inhibitor 40 U/ μ L		0.5
	DEPC ddH ₂ O		Up to 50

37°C 30 min

(7) DEPC 400 μ L 15 sec

(8) 4°C 12000 r/min 10 min

10 min

(9) 4°C 12000 r/min 10 min 70% DEPC

= 7 3

(10) RNA 30-50 μ L DEPC RNA -80°C

✧ **RNA**

(1) 0.1 g 1 mL Trizol 1.5 mL

3 min

(2) 200 μ L 3 min

(3) 4 °C 12000 r/min 10 min

(4) 10 min

(5) 4 °C 12000 r/min 15 min

(6) 70% DEPC 2

(7) RNA 50 μ L 60°C 10 min -80°C

✧ **RNA**

CTAB-PVP

(1) -80°C 10 mL

0.2 0.5 g

-
- (2) 4 mL 65°C 80 μL β-
65°C 10 min 2 3
- (3) 4°C 12000 g 10 min 10 mL
24 1 2 10 min
4°C 12000 g 10 min
- (4) -20°C 0.5 h RNA 4°C
12000 g 10 min
- (5) 500 μL 70% 2
- (6) 44 μL DEPC
- (7) 600 μL DEPC
25 24 1 24 1 1
10 min 4°C 12000 g 10 min
- (8) -20°C 30 min
- (9) 4°C 12000 g 10 min 1 mL 70%
- (10) RNase RNA -80°C
- (1) 200 mmol/L pH 9.0 30 mmol/L EGTA 10 mmol/L
DTT 1% SDS 1% DOC 2% PVP-40 80°C 10 min
- (2) 1.0 g
- (3) 5 mL 50 μL 100 mmol/L DTT 2 min
- (4) 200 μL K 20 mg/mL 42°C 100 r/min
1.5 h
- (5) 2 mol/L KCl 160 mmol/L 1 h
- (6) 4°C 12000 r/min 20 min
- (7) 1/3 8 mol/L LiCl 2 mol/L 4°C 5 h
- (8) 4°C 12000 r/min 20 min
- (9) 4°C 2 mol/L LiCl 2-3 4°C 12000 r/min
12 min

-
- (10) 800 μ L DEPC
- (11) 4°C 13000 g 10 min
- (12) 2 mol/L KAc 200 mmol/L 15 min 4°C 13000 g 10 min
- (13) 2.5 -80°C 1 h
- (14) 4°C 12800 r/min 15 min 70% 2 13000 g 4 min 40 μ L 60°C

基因表达分析

◇

cDNA

Promega		50 μ L	
		μ L	
RNA		4 μ g	
Oligo dT primer	10 μ mol/L	5.0	
DEPC dd H ₂ O		Up to 25	
70 °C	5 min	3 min	
		μ L	
5×M-MLV Buffer		10.0	
dNTPs Mix	10 mmol/L	2.5	
RNase inhibitor	50 U/ μ L	1.0	
M-MLV	200 U/ μ L	1.0	
DEPC ddH ₂ O		25.5	
42 °C	1 h	cDNA	-20 °C

◇ **RT-PCR**

- (1) cDNA Ubiquitin
Ubi PCR
- (2) Image Lab
cDNA Ubi PCR

(3)	cDNA	PCR
PCR		
		μL
cDNA		8.0
Primer-F	10 μmol/L	1.0
Primer-R	10 μmol/L	1.0
2 × EasyTaq Mix		10.0
PCR	94 °C 5 min 94 °C 20 sec 58 °C 20 sec 72 °C 30 sec 30	72 °C 10 min 4 °C

✧ **qRT-PCR**

cDNA	SYBR Green qPCR
MyiQ2	BIO-RAD, USA
Ubiquitin	AK059011
SYBR Green1	3 20.0 μL
cDNA	8.0 μL
2×SYBR Green1 Mix	10.0 μL
Primer-F	1.0 μL
Primer-R	1.0 μL
40	95°C 2 min 95°C 15 sec 60°C 15 sec 72°C 25 sec 55°C-95°C 81

载体构建（常规）



- (1) NCBI
- (2) Primmer 5
 - (a) 15~30

(b)	GC	40%~60%	
(c)	3'	A	T
(d)			
(e)		,	BLAST

✧ PCR

KOD Plus		
		μL
Template DNA		2.0
10 × Buffer		2.0
2.5 mmol/L dNTPs		2.0
10 μmol/L Forward Primer		0.5
10 μmol/L Reverse Primer		0.5
KOD Plus 1 U/μL		1.0
25 mmol/L MgSO ₄		1.0
ddH ₂ O		up to 20
PCR		
	°C	cycles
	95	5 min 1
	95	30 s 30
	Tm-5	30 s 30
	68	1 kb/min 30
	68	10 min 1
	25	5 min 1

✧ DNA

◆	3'	A	
	KOD plus	PCR	3' A

TA	3'	A	
			μL
PCR			40
10×Easy Taq Buffer			5.0
dATP			1.0
Easy Taq DNA Polymerase	5 U/μL		1.0
ddH ₂ O			3.0
72°C 30 min 25°C 5 min			

◆ DNA

	DNA			
	11°C	30 min	75°C	20 min
μL				
	42.0			
10 mmol/L dNTPs	2.0			
T4 DNA polymerase	1.0			
10×BSA	5.0			

◆ DNA

(1) 1.5 mL

		μL
DNA Fragments		40.0
10×Buffer		5.0
CIAP	10-30 U/μL	1.0
ddH ₂ O		up to 50

(2) 37°C 50°C 30 min

(3) / / 25 24 1 2

(4) / 24 1 1

-
- (5) 5 μ L 3 mol/L NaAC
 - (6) 125 μ L 2.5 -20°C 30-60 min
 - (7) 200 μ L 70%
 - (8) 20 μ L ddH₂O

✧ **DNA**

- (1) DNA DNA
1.5 mL
- (2) RJ solution 100 mg 300 μ L 50-60°C 5-10 min
- (3) 1 min 7,000 r/min 1 min
- (4) 3 1
- (5) 500 μ L wash buffer 7,000 r/min 1 min
- (6) 5 1
- (7) 12,000 r/min 2 min wash buffer
- (8) 1.5 mL 30~50
 μ L 30 μ L ddH₂O 3 min
DNA
- (9) 12,000 r/min 3 min DNA
-20°C

✧ **TA**

- (1) pMD18-T 1 μ L 50 ng 3' A PCR
- (2) ATP 10 \times Buffer 1 μ L T4 DNA ligase 200 U/ μ L 0.3 μ L
ddH₂O 10 μ L
- (3) 4°C DH5 α

✧

- (1) *E.coli* DH5 α 5 mL LB
37°C 200 r/min 12 h

(2) 1 100 100 mL LB 37°C
 30 min OD₆₀₀ OD₆₀₀ ≤
 0.5

(3) 50 mL 30 min 4°C 4000 r/min
 10 min

(4) 10 mL 0.1 mol/L CaCl₂
 4°C 4000 r/min 10 min

(5) 2 mL 50 mL 2 mL 0.1 mol/L CaCl₂
 0.1 mol/L CaCl₂

(6) 15%
 50 μL 1.5 mL -80°C

✧

(1) -80°C

(2) DNA 1~2 μL 5~15 μL
 30 min

(3) 42°C 90 s

(4) 1~2 min

(5) 800 μL SOC LB
 37°C 90 r/min 45 min

(6) 7,000 r/min 1 min

(7) 600 μL 200 μL
 LB

(8)

(9) 37°C 12~16 h

✧ **PCR**

(1) PCR 10 μL ddH₂O

(2) PCR

(3)	10 μ L PCR	PCR	PCR
			μ L
	10 \times Easy Taq Buffer		2.0
	10 mmol/L dNTPs		0.3
	10 μ mol/L Forward Primer		0.3
	10 μ mol/L Reverse Primer		0.3
	Easy Taq DNA Polymerase	5 U/ μ L	0.1
	ddH ₂ O		up to 20
PCR			
			cycles
	95	5 min	1
	95	30 s	30
	Tm-5	30 s	30
	68	1 kb/min	30
	68	10 min	1
	25	5 min	1

✧

- | | | | | |
|-----|-------------|--------------|---------|-------------|
| (1) | PCR | | 5 mL | LB |
| | 37°C | 200 r/min | 10~12 h | |
| (2) | 2.0 mL | 2.0 mL | 4°C | 8,000 r/min |
| | 3 min | | | |
| (3) | 200 μ L | I | | 5 |
| | min | | | |
| (4) | 400 μ L | II | | |
| | 5 min | | | |
| (5) | 300 μ L | III | | 5~10 min |
| (6) | 4°C | 10,000 r/min | 3 min | 2.0 mL |

-
- (7) 12,000 r/min 5 min
1.5 mL
- (8) 10 min
- (9) 4°C 12,000 r/min 10 min
- (10) 1 mL 75% 2
5~10 min
- (11) 50 μ L ddH₂O DNA RNA 20 μ g/mL
37°C 30 min -20°C

✧

- (1)
- (2)
- DNA
- (3) DNA 3 1
DNA

	μ L
	6.0
DNA	2.0
10 \times T4 Buffer	1.0
T4 DNA	1.0

17°C

- (4) PCR
- (5) -20°C

Gateway 系列载体构建

	gateway	PCR
BP LR		PCR
attL		attR

(1) BP	5 μ L		
	PMD18-T-gene	1.0 μ L	
	pDONR vector	1.0 μ L	
	BP clonase	1.0 μ L	
	ddH ₂ O	2.0 μ L	
	25°C	2 h	
(2) LR	5 μ L		
	pDONR-gene	1.0 μ L	
	Expression vector	1.0 μ L	
	LR clonase	1.0 μ L	
	ddH ₂ O	2.0 μ L	
	25°C	2 h	
(3) BP LR			
	PCR product	1.0 μ L	
	pDONR vector	1.0 μ L	
	Expression vector	1.0 μ L	
	BP clonase	0.25 μ L	
	LR clonase	1.0 μ L	
	ddH ₂ O	0.75 μ L	
	25°C	12 h	
	2.5 μ L LR	DH5 α	Spe 50
	mg/L	PCR	

单位点 CRISPR 载体构建



CRISPR-P <http://cbi.hzau.edu.cn/cgi-bin/CRISPR#> **CRISPR-PLANT**

<http://www.genome.arizona.edu/crispr/CRISPRsearch.html>

✧

pRGEB32

(1) PAM 20 A
F 5'-GGCA NNNNNNNNNNNNNNNNNNNNNNNNNNNNN-3'
R 3'-NNNNNNNNNNNNNNNNNNNNNNNNNNN CAAA-5'

(2) PAM 20 A
F 5'-GGCA T/C NNNNNNNNNNNNNNNNNNNNNNNNNNNNN-3'
R 3'-NNNNNNNNNNNNNNNNNNNNNNNNNNN CAAA-5'

“NNNNNNNNNNNNNNNNNNNNNNNNNNNN”

✧

sgDNA

	ddH ₂ O	10 μmol/L	10 μL PCR	94°C
10 min	80 μL ddH ₂ O		sgDNA	

✧

(1)
pRGEB32 3.0 μL
Buffer 1.0 μL
*Bsa*I 0.5-1 μL
dH₂O 6.0 μL
37°C 4 h

(2)
pRGEB32 1 μL
T4 Buffer 2 μL
T4 DNA 0.5 μL
sgDNA 2 μL
25°C 30 min

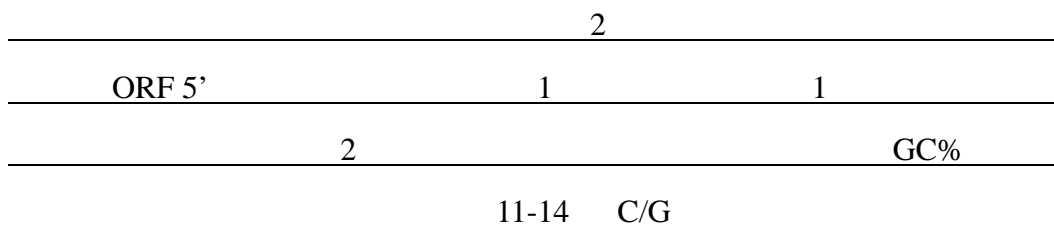
(3) PCR

多位点 CRISPR 载体构建

MKK1 CRISPR



CRISPR-P <http://cbi.hzau.edu.cn/cgi-bin/CRISPR#> **CRISPR-PLANT**
<http://www.genome.arizona.edu/crispr/CRISPRsearch.html>



(1)

L5AD5_F

CGGGTCTCAGGCAGGATGGGCAGTCTGGGCAACAAAGCACCAGT
GG

L3AD5_R

TAGGTCTCCAAACGGATGAGCGACAGCAAACAAAAAAAAAAGCA
CCGACTCG

S5AD5_F CGGGTCTCAGGCAGGATGGGCAGTCTGGGCA

S3AD5_R TAGGTCTCCAAACGGATG AGCGACAGCAAAC

(2)

1 AGCAAGGT**AGGG**CTCTGGAA

TTCCAGAG**CCCT**ACCTTGCT

gMKK1-1F

AC GGTCTC A **AGGGCTCTGGAA** GTTTTAGAGCTAGAA

gMKK1-1R

AC GGTCTC A **CCCTACCTTGCT** TGCACCAGCCGGGAA

2 GGGCCTC**GTTATGCT**AGAAAT

ATTCTAGCATA**ACGAGG**CCC

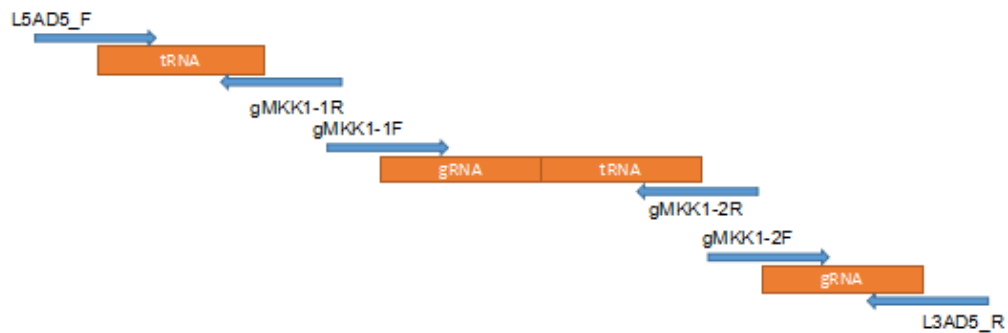
gMKK1-2F

TA GGTCTC A **GTTATGCT**AGAAAT GTTTTAGAGCTAGAA

gMKK1-2R

AT GGTCTC G **TAACGAGG**CCC TGCACCAGCCGGGAA

✧



(1)		pGTR
	3	3
(2) Golden Gate	3	
	1	1.5 µL
	2	1.5 µL
	3	2.0 µL
T7DNA buffer		10.0 µL
BSA		2.0 µL
<i>Bsa</i> I		0.5 µL
T7		0.5 µL
ddH ₂ O		2.0 µL
Total		20.0 µL

	Step 1	37°C	5 min	
	Step 2	20°C	10 min	
	Step 1 to Step 2		35	
	Step 3	20°C	60 min	
(3)	2		10	S5AD5_F
	S3AD5_R		3	DNA

		1.0 µL
S5AD5_F		1.0 µL
S3AD5_R		1.0 µL
2×Taq master mix		10.0 µL
ddH ₂ O		7.0 µL

PCR

Step 1	98°C	1 min
Step 2	98°C	10 s
Step 3	60°C	20 s
Step 4	72°C	30 s
Step 2 to Step 4		30
Step 5	72°C	5 min

(4)

pRGEB32		3	PCR
pRGEB32	8 µL		17 µL
<i>BsaI</i>	1 µL	<i>FokI</i>	1 µL
Smart Buffer	2 µL	Smart Buffer	2 µL
ddH ₂ O	9 µL		37°C 4 h
	37°C		

(5)

T4	pRGEB32	PCR
----	---------	-----

(6)

(7) PCR

jc-F AGTACCACCTCGGCTATCCACA

jc-R GGACCTGCAGGCATGCACGCGCTAAAAACGGACTAGC

PCR

Step 1 98°C 3 min

Step 2 98°C 20 s

Step 3 60°C 20 s

Step 4 72°C 35 s

Step 2 to Step 4 30

Step 5 72°C 5 min

(8) PCR

农杆菌操作

◇ DNA

- (1) 30 min
- (2) 5 μ L 2 30 min
- (3) 1 min 37°C 1 min 5 min
- (4) 750 μ L LB 37°C 150 r/min 90 min
- (5) 4,000 r/m 90 s
- (6) 100 μ L LB

◇

- (1) 1.5 mL
- (2) 100 μ L solution I 20 μ L 37°C 30 min
- (3) 200 μ L solution II 5 10 min
- (4) 150 μ L solution III solution III
5 min

-
- (5) 10000 r/min 3min
- (6) 12,000 r/min 5 min
- (7) 12,000 r/min 5 min
- 1.5 mL
- (8) 10 min
- (9) 4°C 12,000 r/min 10 min
- (10) 1 mL 75% 2
- 5~10 min
- (11) 50 μL ddH₂O DNA RNA 20 μg/mL
- (12) PCR

水稻转基因

✧

(1) 3 70%

1 min

(2) 3 30 s

(3) 0.1% 12 min

3

(4) 5 30 s

(5)

(6) 28°C 4

①

→ 75%

→ 75%

→

1

1

1

95%

→

20-30 min

5 min

②

①

②

③

④

⑤

2

⑥

2 d

7-10 d

2

✧

2

28°C

✧

4-14 d

2

28°C

✧

(1)

4°C -80°C

2

OD₆₀₀

0.8-1.0

(2)

(3)

30 min

(4)

1 h

(5)

20°C

3 d

1

95%

✧

(1)

(2)

(3) 400 mg/L

30 min

(4)

3h

(5) 50 mg/L

2

2

400 mg/L

250 mg/L

1

2

400 ppm

1

95%

1

✧

28 °C

✧

2

26 °C

1-2 d

1

95%

✧

10 cm

2 d

拟南芥转基因

- (1) 10 μ L 5 mL LB
+ 20 mg/mL Rif + 10 mg/mL
- 28 $^{\circ}$ C 200 r/min OD₆₀₀ 2.0
- (2) 1 100 1 500 μ L
50 mL 28 $^{\circ}$ C 200 r/min OD₆₀₀ 1.5 ~ 2.0
- (3) 4000 g 10 min
- (4) 5% w/v 1/2 MS
OD₆₀₀ 0.8 ~ 0.9
- (5) 0.02% v/v Silwet L-77 ,
- (6) 12 ~ 14 s
- (7) 1-2 d T1

洋葱表皮细胞瞬时转化

✧

1-2
4-7
2cm \times 2cm
MS 28 $^{\circ}$ C
4 h

✧

- (1) 60 mg 1.5 mL 1 mL 2 min
- (2) 5 min 10,000 r/min 1 min
- (3) 1 2 2

(4) 1 mL 2 min 5 min 10,000 r/min

1 min

(5) 4

(6) 1 mL 50 μ L 1.5 mL

✧ **DNA**

(1) 50 μ L 5 μ L DNA

1 μ g/ μ L 50 μ L 2.5 mol/L CaCl₂ 20 μ L 0.1 mol/L

(2) 3 min 10,000 r/min 10 sec

(3) 50 μ L 4

✧

9 cm 1350 psi 20 mmHg

28°C 8 h 488

nm EGFP

✧ **PCR**

PCR 20 μ L
10 \times buffer Mg²⁺ plus 2 μ L
dNTP 2.5 mmol/L 1 μ L
forward primer 10 μ mol/L 0.3 μ L
reverse primer 10 μ mol/L 0.3 μ L
DNA 100 nmol/L 1 μ L
EasyTaq DNA Polymerase 2.5 U/ μ L 0.2 μ L
ddH₂O 15 μ L

PCR

95°C 5 min 95°C 30 s 64°C 30 s 72°C 30 s 28-33 cycle 72°C 10 min

✧

(1) 3%

(2) 95%

(3) 5%

5-10 8 μ L 1.5 mL

95% 1 mL

5-10 min

(4)

(5) 55 mL 6% PAGE 400 μ L 10% AP 40 μ L

TEMED

(6) PAGE

45-60 min

✧

(1)

		30 min	2000 V	100 mA	90 W
(2)					
(3)	PCR			3 μ L	
(4)		500 V	100 mA	90 W	
✧					
(1)		1 min			
(2)	10-15 min				
(3)					10 s
(4)					
(5)					

蛋白质原核表达

✧

- (1) 10 μ L 5 mL LB 5 μ L
100 mg/mL
- (2) 1 50 1 mL 50 mL LB
50 μ L 100 mg/mL 3 h
- (3) 10 mL IPTG 40 mL
40 μ L IPTG 0.2 mol/L 37°C 200 r/min 3 h
- (4) 50 mL 8000 r/min 3 min
- (5) 40 mL dH₂O 1 12000 r/min 2 min
- (6) PBS buffer
- (7) 200 W 3 s 8 s 70
- (8) 4°C 12000 r/min 3 min
- (9) 250 μ L 1 \times SDS loading buffer 200 μ L 50 μ L 5 \times
SDS loading buffer 5 min
- (10) 12000 r/min 2 min
- (11) 40 μ L SDS-PAGE
SDS-PAGE

✧

- (1) 1 mmol/L PMSF 4°C
12000 r/min 20 min
- (2) GST 20%

-
- (3) 10 mL PBS buffer 3
 - (4) 5-10 mL GST
 - (5) 10 mL PBS buffer GST 3
OD₂₈₀ 5.0
 - (6) 5 mL 10 mmol/L Tris HCl pH 8.0
1.5 mL 0.5 mL 6-8
 - (7) PBS Buffer 3
 - (8) OD₂₈₀ 200 µg/mL
-20°C
 - (9) SDS-PAGE

抗体制备与纯化

✧

- (1) <http://imed.med.ucm.es/Tools/antigenic.pl>

- (2) cDNA
- (3)
- (4) 5 mg

✧

- (1) 1 mL Protein A-Agarose 1.5 mL 5 mL
20% 10 mL 1 × TBS
- (2) 4 mL 4°C 5000 r/min 10 min 4 mL
2 × TBS
- (3) 8 mL 1 mL/min
- (4) 20 mL 1 × TBS 80

-
- (5) $\mu\text{g/mL}$ IgG 3 mL 3 mL 10 3-5 min
 10 0.5 mL
- (6) 1 100 μL
- (7) IgG 10 μL 10
- (8) 7-8 IgG
 500 μL 4°C
- (9) IgG SDS-PAGE 5-10 μg A. B.
 C. IgG D. marker
- (10) Agarose 10 mL 10 mL 20% Agarose
 4°C
- (11) A. 20% 5 70% 10
 B. pH 1.9 50 mmol/L Glycine-HCl, pH 1.9 10

Brandford 法测定蛋白浓度

- (1) 1 mg/mL BSA 0 20
 40 60 80 $\mu\text{g/mL}$
- (2) BSA 1 mL 3 mL Brandford 10 min
- (3) 595 nm OD
- (4) Excel
- (5) 1 mL 3 mL Brandford 10 min
 595 nm

膜蛋白提取与分离

- (1) -80°C
- (2) 3
1 μmol/L Leupeptin 1 μmol/L Pepstatin A 1
mmol/L PMSF 50 mmol/L MOPS/NaOH buffer pH 7.5 5 min
15 mL
- (3) 4°C 2000 g 10 min
- (4) 4°C 45000 r/min
BECKMAN ptima LE-80K Ti 70 30 min
- (5) 3 50 mmol/L MOPS/NaOH buffer pH 7.5
0.05% Digitonin 4°C
30 min
- (6) 4°C 5000 g 15 min

免疫共沉淀 (CO-IP)

- (1) protein A-Agarose 40 μL 50% Protein A-Agarose
50 mmol/L Mops/NaOH buffer pH 7.5
1000 g 1 min 3 50 mmol/L
Mops/NaOH buffer pH 7.5 40 μL
- (2) 500 μL 5 μL 1 100 4°C
1 h 40 μL Protein A-Agarose 4°C 1 h
- (3) 10 min 2000 g 1 min
- (4) 50 mmol/L Mops Mops/NaOH buffer pH 7.5 3
- (5) 50 μL SDS-loading buffer 5% β- 70°C
5 min 12000 r/min 2 min

SDS-PAGE

(1)

	15%	12%	8%
ddH ₂ O	3.4 mL	4.9 mL	6.9 mL
30% Acr-Bis 29 1	7.5 mL	6.0 mL	4.0 mL
1.5 mol/L Tris-HCl pH 8.8	3.8 mL	3.8 mL	3.8 mL
10% SDS	150 μL	150 μL	150 μL
10%	150 μL	150 μL	150 μL
TEMED	20 μL	20 μL	20 μL
	15 mL	15 mL	15 mL
TEMED			
7 mL	1 mL ddH ₂ O	37°C	15 min

(2)

	5%
ddH ₂ O	3.4 mL
30% Acr-Bis 29 1	0.85 mL
1 mol/L Tris-HCl pH 6.8	0.63 mL
10% SDS	50 μL
10%	50 μL
TEMED	20 μL
	5 mL
2 mL	
	37°C 15 min

(3)

1 × Tris-Gly

-
- (4) 50 V 30 min 70 V 3 h
 - (5)
 - (6) 40 r/min 3 h
1 40 r/min
60 min

Western blot

- (1) SDS-PAGE
- (2) PAGE
- (3) 6 1 PVDF
- (4) 5 min
3 PVDF
3 3
- (5) 75 V 60 mA 4°C 10 h
- (6)
- (7) 15 mL 1 h
- (8) TTBS 3 3 min
- (9) 1 h
- (10) TTBS 3 5 min
- (11) 1 h
- (12) TTBS 3 5 min TBS 5 min
- (13) ECL 3 min

纤维素体外合成

(1)	380 μ L			
	UDP-glucose	100 mmol/L	2 mmol/L	10 μ L
	Cellobiose	100 mmol/L	10 mmol/L	50 μ L
	MgCl ₂	100 mmol/L	10 mmol/L	50 μ L
	CaCl ₂	200 mmol/L	2 mmol/L	5 μ L
	C ¹⁴ -UDPG	0.025 μ Ci/ μ L	--	2 μ L
	UDP-glucose	C ¹⁴ -UDP-glucose	Perkin NEC403050UC	
		7550		
(2)	25°C	2 h	37°C	2 h
(3)			10 min	
(4)				70%
	5 min	14000 r/min	10 min	150 μ L
	70%		5 min	14000 g 10min
	5-6	200 μ L		Perkin 1450-024
	C ¹⁴	60 cpm		
(5)	700 μ L	1 1	40°C	150 r/min 1 h
	3000 g	5 min	700 μ L	3000 g
	5 min	700 μ L	3000 g	5 min
(6)	500 μ L	0.1 mol/L	pH 4.5	2 μ L 20 U/mL
	Endo- β -1,3-glucanase	Megazyme	0.5 μ L	100 U/mL Exo- β -1,3-glucanase
	Megazyme		37°C	150 r/min 24 h
	10 min			5 min 14000
	r/min	10 min	150 μ L	200 μ L
	C ¹⁴	60 cpm		
(7)	800 μ L	0.1 mol/L	/	pH 4.8 200 μ L 0.32 mg/mL

				37°C	150
r/min	24 h	10 min			
	5 min	14000 r/min	10 min		150 μL
		200 μL	C ¹⁴	60 cpm	
(8)	150 μL	800 μL			
	C ¹⁴		β-1,4-glucan		
(9)	6 7	98°C		1 mL	2
			C ¹⁴		
	β-1,3-glucanase				

纤维素酶活性测定

✧

- (1) 0.1 g
- (2) 200 μL NaAC Buffer 100 mmol/L, pH 5.5
- (3) 4°C 13000 r/min Eppendorf Centrifuge 5417 R 10 min
- (4)
- (5) 96 Greiner Microlon 50 μL
50 μL NaAC Buffer 100 mmol/L, pH 5.5
- (6) 50 μL 0.5 mmol/L 4-β-Sigma 55°C
30 min
- (7) 30 μL 0.2 mol/L Na₂CO₃
- (8) TECAN Infinite M200 4-
365 nm 480 nm

✧

MU	0.5 mmol/L						
MU	μL	0	20	40	60	80	100

NaAc buffer	μL	100	80	60	40	20	0
◇							
	10	20 μL	96		Bradford		595 nm
BSA	ug/mL	0	20	40	60	80	100
BSA	μL	20	20	20	20	20	20
Bradford	μL	200	200	200	200	200	200

β-葡萄糖苷水解酶活性测定

- (1) 800 μL pH 5.0 1,2000
r/min 10 min
- (2)
- (3) 15 mL 1 mL 100 μL 400 μL
500 μL 8 mmol/L β-D
p-Nitrophenyl-β-D-glucopyranoside *p*NPG
- (4) 50 °C 100 r/min 30 min
- (5) 500 μL 1 mol/L Na₂CO₃
- (6) 3000 *g* 10 min 410 nm
5 0 mmol/L 0.04 mmol/L 0.08
mmol/L 0.16 mmol/L 0.24 mmol/L

酵母双杂交

◇

- (1) AH109/Y187 YPDA
20 mL YPDA 30 °C 200 r/min OD₆₀₀ 1-2 16-18 h

-
- (2) 2-5 mL 100 mL YPDA OD₆₀₀ 0.15-0.3
 30 °C 200 r/min OD₆₀₀ 0.4-0.6 3 h 1000 r/min
 5 min
- (3) 30 mL ddH₂O or 1 × TE 1000 r/min 5 min
 3 mL 1.1 × TE/LiAc 1.1 mL of 10 × TE with 1.1
 mL of 1 mol/L LiAc 10× Bring the total volume to 10 mL using
 sterile,deionized H₂O 2 1.5 mL 12000
 r/min 15 sec
- (4) 600 μL 1.1 × TE/LiAc

✧

- (1) 2.0 mL
- | | μL |
|------------------------------------|-----|
| ds DNA 25 ng/μL | 5 |
| pGADT7-Rec 500 ng/μL | 0.5 |
| Herring tests carrier DNA 10 μg/μL | 10 |
| AH109/Y187 | 100 |
- 50 μL carrier DNA 100 °C 5 min
 5 min 1
- (2) 500 μL PEG/LiAc 30 °C 30 min 10 min 1
- (3) 20 μL DMSO 42 °C 15 min 5 min 1
- (4) 7000 r/min 1 min 1 mL YPDA 30 °C
 120 r/min 90 min
- (5) 7000 r/min 1 min 1 mL 1 × TE 0.9%NaCl
- (6) SD/-Trp or SD/-Leu 100 mm 200 μL
 1/10 1/100 1/1000
- (7) 30 °C 3 d

✧

(1) 2.0 mL

	μL
ds DNA 25 ng/μL	0
pGADT7-Rec 500 ng/μL	20
Herring tests carrier DNA 10 μg/μL	10
AH109/Y187	200

50 μL carrier DNA 100 °C 5 min
5 min 1

(2) 500 μL PEG/LiAc 30 °C 30 min 10 min 1

(3) 70 μL DMSO 42 °C 15 min 5 min 1

(4) 1-2 min

(5) 7000 r/min 1 min 0.5 mL 1 × TE

(6) SD/-Trp or SD/-Leu 100 mm 200 μL

1/10 1/100 1/1000

(7) 30 °C 3 d

✧

(1) pGBKT7 pGADT7-Rec Y187 2-3 mm

500 μL SD/-Trp/Kan 20 μg/mL 1.5 mL

30°C 50 r/min 16-24 h

(2) 1

4 h

(3) 1000 g 10 min 0.5 mL 0.5 × YPDA/Kan 50 μg/mL

(4) 100 μL 200 μL

3-AT SD/-His/-Leu/-Trp 100 mm

30 °C 3-5 d

(5) 3-AT SD/-His/-Leu/-Trp/-Ade 30 °C

3-5 d

✧ **X-gal**

(1) 30°C 2-4 d 1-3 mm

8×8

(2) Z buffer/X-gal

(3) Z buffer/X-gal 2.5-5 mL

(4)

(5) 30 sec

(6)

(7)

30°C 8 h X-gal

8 h

染色质免疫共沉淀 (ChIP)

✧

(1) 1 2 3 1 2 3 2 g

50 mL 40 mL ddH₂O

1% 30 mL 15-30 min

(2) 0.125 mol/L 2 mol/L Glycine

5 min

(3) 40 mL ddH₂O 3

-70°C

-
- (4) 50 mL 30 mL Extraction Buffer 1
- (5) 50 mL 30 mL
Extraction Buffer 1
4 °C 30 min
- (6) miracloth 50 mL
- (7) Extraction Buffer 1 4°C 4000 g 20 min
- (8) 1 mL Extraction Buffer 2
1.5

- mL
- (9) 4°C 16000 g 10 min
- (10) 500 µL Extraction Buffer 3
- (11) 1.5 mL 500 µL Extraction Buffer 3
- (12) 4°C 16000 g 1 h

✧

- (1) 300 µL Nuclear Lysis Buffer
- (2) 2 µL
- | | | | | |
|--|---------|----------|-----------|-------------|
| | | | DNA | Biorupter H |
| | 30 s ON | 30 s OFF | 25 cycles | 100-500 bp |
- (3) 10 µL 2 µL 500 µL
ddH₂O 20 µL 5 mol/L NaCl 65 °C 4 h
-20 °C
- (4) 25 24 1
5 min 16000 g 10 min 480 µL
100 µL 3 mol/L NaAc 900 µL 2 µL -70°C
1 h -20°C

(5) 4 °C 16,000 g 20 min 1 mL 70%
 4 °C 16,000 g 5 min
 15-30 min 15 µL ddH₂O 0.5
 h 1.5%-2%

✧ **ChIP**

(1) 1.5 mL 1 mL ChIP Dilution Buffer 3
 protein A agarose beads 40 µL 4 °C 1000 g 30 s
 1 mL 2

(2) -20 °C 4 °C 16,000 g 5 min
 20 µL input -20 °C

(3) ChIP Dilution Buffer 3 mL
 1 3 protein A 1 mL
 4 °C 1 h

(4) 4 °C 12,000 g 2 min

(5) 3 beads

(6) 2 5 µg
 4 °C 6 h

(7) 50 µL protein A agarose beads 4 °C
 1 h

(8) ChIP Elution Buffer, Low Salt Wash Buffer, High Salt Wash Buffer, LiCl
 Wash Buffer, TE Buffer

(9) 8 2 1 mL
 4 °C 4 °C 5000 r/min 30 s

a. Low Salt Wash Buffer 150 mmol/L NaCl, 0.1% SDS, 1% TritonX-100, 2
 mmol/L EDTA, 20 mmol/L Tris-HCl pH 8.1 2
 4 °C 5 min

-
- b. High Salt Wash Buffer 500 mmol/L NaCl, 0.1% SDS, 1% TritonX-100, 2 mmol/L EDTA, 20 mmol/L Tris-HCl pH 8.1 2
4°C 5 min
- c. LiCl Wash Buffer 0.25 mmol/L LiCl, 1% NP40, 1% sodium deoxycholate, 1 mmol/L EDTA, 10 mmol/L Tris-HCl pH 8.1 2
4°C 5 min
- d. TE Buffer 10 mmol/L Tris-HCl pH 8.1 , 1 mmol/L EDTA 2
4 °C 5 min
- (10) 250 µL Elution Buffer 65 °C 15 min
5000 r/min 30s 1.5 mL
- (11) beads 2
- (12) input 500 µL ddH₂O input
20 µL 5 mol/L NaCl 65 °C 6 h
- (13) 10 µL 0.5 mol/L EDTA 20 µL Tris-HCl pH 6.5 2 µL
Proteinase K 20 mg/mL 1 µg RNase A 45 °C 1 h
- (14) 25 24 1 5 min
16,000 g 10 min 480 500 µL 100 µL 3
mol/L NaAc 900 µL 2 µL -70 °C 1 h
-20 °C
- (15) 4 °C 16,000 g 20 min 1 mL 70% 4 °C
16,000 g 5 min
15-30 min 20 µL ddH₂O
0.5 h
- (16) Picogreen assay Invitrogen Q-bit Agilent BioAnalyzer DNA 1000 chip
DNA -20 °C

琼脂糖包埋震动切片

- (1)
- (2) 4% 60°C
- (3)
- (4) 502
- (5) 50-100 μm

石蜡包埋半薄切片

- (1) 3-5 mm
- (2) 4%
10 min
- (3) 30% \rightarrow 40% \rightarrow 50% \rightarrow 60% \rightarrow 75% \rightarrow 80% \rightarrow 90% \rightarrow 95% \rightarrow
100% 40-60 min
- (4) 1/3 2/3 40-60 min
- (5) 70°C 50% 75%
2 h 100% 3 h
- (6)
- (7) RM2265 Leica
8 μm
- (8) 37°C 3
37°C
- (9) 30 min
5 min

-
- (10) 100% → 95% → 90% → 80% → 75% → 50% → 30%
1 min

树脂包埋超薄切片

- (1) 3-5 mm
- (2) /
30 min 2-3 h
- (3) 25 mM 3 5 min 2
- (4) 35% → 50% → 70% → 100% → 100%
25-30 min
- (5) LR White 100% 1 1 v/v 12-24 h 100%
LR White Medium grade, Ted Pella, 18181 12-24 h 3
- (6) Ted Pella, 130-14 100% LR
White
- (7) 4°C 48 h
- (8)
- (9) Lecic EM UC6 Diatome 250
nm
- (10) 6-8
3×2 4×2
- (11) 50°C
7-8 mm

叶片细胞形态学观察

- (1) 12.5% 10 mL + 70 mL
30 min

-
- (2) 15 min
 - (3) 50% v/v 15 min
 - (4) ddH₂O 15 min
 - (5) 1 mL 1 mol/L NaOH
 - (6)

纤维素染色观察

- (1) 10 μL Calcofluor White Stain Fluka 5 min
- (2) Calofluor
- (3) ddH₂O 3 Calcofluor
- (4) Olympus BX-61 UV

木质素染色观察

- (1) 95% 5% v/v] 2 min 1-2 5% [= Nikon 80i

多糖抗体免疫荧光观察

- (1) 3% 0.03 g/mL /PBS 1 h
- (2) 3% /PBS
2 h CCRC 5 JIM 10 10 μL 2 h
PBS 3 5 min
- (3) 2 h Alexa Fluor 488
anti-mouse IgG H + L Invitrogen, A11001 CCRC Alexa
Fluor 488 anti-rat IgG H + L Invitrogen, A-11006 JIM
FITC Fluorescein-isothiocyanate PBS

-
- (4) PBS 3 5 min
- (5) 10 μ L Calcofluor 5 min
- (6) PBS
- (7) Olympus BX-61 U-MWU2 330-385 nm
 Calcofluor U-MWB2 460-490 nm
 FITC
- 1.
- 2.
3. 4°C -20°C

多糖量子点免疫荧光观察

- (1) 3% 0.03 g/mL /PBS 1h
- (2) 3% /PBS
 2 h CCRC 5 10 μ L 2 h PBS
 3 5 min
- (3) 4 h Alexa Fluor 488
 anti-mouse IgG H + L , Invitrogen, A11001 CCRC
 Quantum dots CdSe/ZnS, 610 nm PBS 100
-
- (4) PBS 3 5 min
- (5) Olympus BX-61 U-MWU2 330-385 nm
- 1.
- 2.

3.

4°C

-20°C

拟南芥种子果胶质免疫荧光观察

- (1) 20 500 μ L ddH₂O 5 min
- (2) 470 μ L ddH₂O 100 μ L 5% BSA pH 7.0 PBS
30 min
- (3) 50 μ L 1 10 1% BSA pH 7.0 PBS
90 min
- (4) 300 μ L PBS 1-2 PBS
- (5) 4 4
- (6) 100 μ L 1 100 1% BSA pH 7.0 PBS
90 min
- (7) 300 μ L PBS 1-2 PBS
- (8) 4 4
- (9)

胍胍质测定与观察

✧

- (1) 0.1 g
- (2) 2.0 mL 1.0 mL 80% 5 min
- (3) 1200 r/min 5 min 5
- (4) 600 μ L 1 mol/L NaOH 80 30 min
- (5) 1200 r/min 5 min 50 μ L 600 μ L 40 mL 0.1%
21 mL 1 mol/L HCl 59 mL 1 mol/L /NaOH pH
9.5 50 30 min
- (6) 90 r/min 30 min
- (7) 1200 r/min 5 min

400 nm	510 nm	10	50
100	200	400	μg/mL

✧

(1)		24 h	2 cm	95%	
(2)	0.1%		pH 7.0		4 h
(3)		3			

GUS 染色观察

(1)		1.5 mL	90%	30
	min			
(2)				30 min
(3)		GUS	37	70%
(4)				

水稻原生质体分离与激光共聚焦观察

(1)		1/2 MS	9-12 d	9
(2)				
		9 cm	0.5-1 mm	
		10-20	5-10 mL	0.5-1×10 ⁶
		25		
(3)				30 min
	0.8 MPa			
(4)		80 r/min	3 h	

(5)

10 mL W5 solution 10 min

(6) 2 37 μm W5 solution

(7) 200 10-20 mL W5 solution

10 mL

(8) 100 g 8min

(9) 1-3 mL W5 solution

15 mL

$2 \times 10^5 \text{ mL}^{-1}$

30 min

24 h

(10)

扫描电镜观察

(1) 1-2 mm

2.5%

2 h 4°C

1

(2)

(3)

5.0 Pa

start

(4)

HT

SCAN3

透射电镜观察

◇

(1) 2 13 cm

7-8 cm

(2) 2-3 mm

(3)

(4) Hitachi H7500

◇

1 mm × 1 mm

原子力显微镜观察

◇

(1) 3 cm

50-100 μm

(2) 1% HCl 70%

poly-L-lysine solution P8920 Sigma 1 10 5 min

60 1 h

(3) AFM

(4) AFM Agilent 5500 AFM

10 nm 0.1 N/m Ni 250 Au 180

Top MAC I gain P gain = 1 3

2 V

1 ln/s

512 × 512

liquid cell

(5) AFM Agilent 5500 CBM3a

Recognition Signal

Tris-Cl buffer	10 mmol/L Tris-Cl	150 mmol/L NaCl, pH 7.5
CBM3a		
0.1 N/m	Ni 50	Au 150
HS-PEG2000-NTA cross linker	0.2 mg/mL, 1400 μ L	4 h
NiCl ₂	10 mmol/L, 20 μ L	1 h
400 μ L Tris-Cl buffer	10 mmol/L Tris-Cl and 150 mmol/L NaCl, pH 7.5	CBM3a 27 μ g /mL, 6 μ L
4	Tris-Cl buffer	3
4°	1-2	

✧

- | | | | | |
|-----|------------------------|-------|----------------|--------------------|
| (1) | 60 | 1 cm | 5 | buffer |
| | 1 | | | |
| (2) | 96% | 70 | 30 min | |
| (3) | | 2 3 | 2 | |
| (4) | 65% | 80% | 100% | |
| (5) | | AIR | | |
| | | | AFM | Bruker multimode |
| (6) | | 8 nm | 40 N/m | Sader |
| | | | | method |
| (7) | 10 \times 10 μ m | | 16 \times 16 | 10 |
| (8) | | Hertz | | NanoScope analysis |

机械力学性状考察

◇

(1) H cm

(2) W g

(3) YYD-1

F N 5 cm

(4)

$$= H \times W / F / 9.8 \times 1000 \times 5 \times 100$$

SPSS

◇

(1)

(2) 8 cm

RH-K300

W = 120

g/m²

D = 50 mm

V = 10 mm/min

S = 255 N

(3) SPSS

水稻考种

100

10

稻米品质测定

◇

- (1) 3 20 g
SY88-TH, BRIC, Korea
10 g Pearlest, Kett, Japan
JFQS-1320, China
- (2) Epson Expression 1680 Professional, Epson, America

◇

- (1) Udy corporation, Colorado, USA 100
- (2) 100 mg 95% 9 mL 1 mol/L NaOH
- (3) 37°C 16 h 50 mL
- (4) 2 mL 50 mL 1 mL 1 mol/L
HAc 2 mL
- (5) 20 min 620 nm
- (6)
- 100 mg 10 mL
2 mL 0.2 mol/L NaOH

2/3

cm

		ASV		6
	10 mL	1.7%	KOH	
		30°C		24 h
3	ASV		ASV	
			7 g	
	Infratec 1241, FOSS NIRSystems Inc, USA			

淀粉含量测定

	Megazyme			
(1)		0.5 mm		
(2)		100 mg		16 mm × 120 mm
(3)	0.2 mL	80%	v/v	
(4)	3 mL	α-	1 30	100 mmol/L pH 5.0
			6 min	2 min
				1
(5)		50°C		0.1 mL
		50°C	30 min	
(6)			100 mL	
		3,000 r/min	10 min	
(7)	2		0.1 mL	16 mm
	× 100 mm			

(8) 3.0 mL GOPOD

50°C 20 min 0.1 mL 1
 mg/mL + 3.0 mL GOPOD 0.1 mL 3.0 mL
 GOPOD

(9) 510 nm

$$\% = \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{w} \times \frac{162}{180} = \frac{\Delta A \times F \times FV \times 0.9}{W}$$

$\Delta A =$

$$F = \text{ } \mu\text{g} = \frac{100 \text{ (D-葡萄糖的重量 } \mu\text{g)}}{100 \mu\text{g D-葡萄糖的吸光值}}$$

FV =

0.1 =

$$\frac{1}{1000} = \text{ } \mu\text{g} \quad \text{mg}$$

$$\frac{100}{W} =$$

$$\frac{162}{180} = \text{ D- } \quad \text{ D-}$$

$$\% \text{ W/W} = \text{ } \% \text{ W/W} \times \frac{100}{100 - \text{含水量 (\% W/W)}}$$

mg/100 mL

$$= \Delta A \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{162}{180} \times 2 \times D = \Delta A \times F \times D \times 1.8$$

$\Delta A =$

$$F = \text{ } \mu\text{g} = \frac{100 \text{ (D-葡萄糖的重量 } \mu\text{g)}}{100 \mu\text{g D-葡萄糖的吸光值}}$$

100 = 100 mL

0.1 =

$\frac{1}{1000} = \mu\text{g} \quad \text{mg}$

$\frac{162}{180} = \quad \text{D-} \quad \text{D-}$

2 = AMG

D =

光合速率测定

LI-6400

11 00-13 00

稻瘟病抗性分析

◇

(1) 28°C 12 h /12 h

10 d

(2) 10^5

/mL 0.02%

◇

(1) 3

(2)

90% 28°C

(3) 24 h 12 h /12 h

5 d

◇

-
- (1) 3
 - (2) 1 cm 5 μ L 90% 28°C
 - (3) 24 h 12 h /12 h 3
10

白叶枯病抗性鉴定

✧

- (1) -80°C
- (2) 28°C 3 d
- (3) 2 28°C 2 d

✧

- (1) PBS 9×10^9 /mL
- (2)
- 2 cm 5
- (3) 20 d

✧

3 d 6 d 9 d 12 d 75% 30 min 2 mL
100 101 102 103 104 105 6
3 d

褐飞虱抗性鉴定

✧



3

50

4

3

3

10

$25 \pm 2^\circ\text{C}$

60%

10

3



3

100

3



24 h

2 h

CO_2

15 20 cm

20 μm

500 Hz 0.5 V

6 h

20

$25 \pm 2^\circ\text{C}$

$65\% \pm 5\%$

植物细胞壁成分分析

细胞壁多糖提取

◇ **buffer**

0.100 g 3 1 mL pH 7.0
 0.5 mol/L
 15 mL 3000 g 5 min
 5 mL 2 5 mL 2

◇ -

5 mL - 1 1 v/v 25 °C 150 r/min
 1 h 5 mL 1 5 mL 1
 5 mL 1

◇ **DMSO**

5 mL DMSO-H₂O 9 1 v/v 12 h
 5 mL DMSO-H₂O 2 5 mL 3 30.0
 mL 48 h 8000-14000 Da
 100 mL 70.0 mL
 DMSO Crude cell wall

◇

1
 5.0 mL 0.5% w/v , 1 h 10
 min 3000 g 5 min
 5 mL 0.5% 1 , 5 mL 2 ,

0.1 mol/L KOH 2

5.0 mL 0.1 mol/L KOH 1 mg/ml NaHB₄ 25°C

(5) 4000 r/min 5 min 500 μ L

(6)

比色法测戊糖、己糖和糖醛酸

✧

(1) 10 mL dH₂O 1.0 mL

(2) 134.0 μ L A 6.00 g 100.0 mL

(3) 2.0 mL B 0.100 g FeCl₃ 100.0 mL

(4) 20 min

(5) 660 nm

(6)

1.00 mg/mL 0.5 mL 1.0 mL 2.0 mL 3.0 mL 4.0 mL

100 mL 1.0 mL 10 mL

660 nm

✧

(1) 10 mL dH₂O 1.0 mL

(2) 2.0 mL 0.20% w/v

(3) 5 min

(4) 620 nm

(5)

1.00 mg/mL 2.0 mL 4.0 mL 6.0 mL 8.0 mL 10.0 mL

100 mL 1.0 mL 10 mL

620 nm

✧

(1) 10 mL dH₂O 1.0 mL

(2) 5.0 mL 0.50% w/v /

(3) 5 min

(4)		520 nm	A	
(5)	100.0 μ L	0.15% w/v		10 min
		520 nm	B	
(6)				
	1.00 mg/mL		2.0 mL	4.0 mL
			6.0 mL	8.0 mL
mL	100 mL		1.0 mL	10 mL
				520 nm

硅含量测定

(1)	80		100 mg	
		6 mL	2.0 mol/L NaOH	2.0 mL
				130°C
				30 min
(2)			9.0 mL	1.0 mol/L H ₂ SO ₄
				100 mL
		250 mL		
(3)		1.50 mL	50 mL	1.0 mol/L H ₂ SO ₄
				2.00
mL		5 min	20 mL	5.0%
				5.0 mL
		10 min	5.0 mL	5.0%
				2.0 mL
		20 min		0.5%
				810 nm

(1)		27.97 mg		3.59 g
		400°C	30 min	1 mol/L
		H ₂ SO ₄	1000 mL	
(2)			0.50	1.000
			1.50	2.00
			2.50	3.00
			3.50	
	4.00	4.50 mL		

提取粗纤维素

- (1) 0.3 g 10 mL 0.5 mol/L pH 7.0 50°C
150 r/min 2 h
- (2) 4000 r/min 5 min 10 mL dH₂O 3
- (3) 10 mL / 1 1 v/v 25°C 150 r/min 1 h
- (4) 4000 r/min 5 min 1
- (5) 10.0 mL 4.0 mol/L KOH 1.0 mg/mL NaHB₄ 25°C 150 r/min
2 h
- (6) 4000 r/min 5 min 10.0 mL dH₂O 5-6 pH
- (7) 10.0 mL 8.0% NaClO₂ w/v 1.5% 25°C 150 r/min
48 h 12 h 8.0% NaClO₂ w/v 1.5%
- (8) 4000 r/min 5 min 10.0 mL dH₂O 8-10 pH
- (9)

纤维素聚合度测定

◇

- (1) W1 0.05 g 15 mL
10.0 mL V 1.0 mol/L Sigma USA 25°C 150 r/min
12 h 10 mL
- (2) 3000 g 5 min 25
± 0.1 °C
- (3)
- (4)

T

-
- (5) T_0
- (6) W_2
- (7) $\eta = T/T_0$
- (8) $\eta = \frac{[\eta] \cdot C}{[\eta] \cdot C}$
- (9) $W = V \cdot C$
- (10) $\frac{W_1 - W_2}{V} = C$ g/100 mL
- DP = 190 × [η]

✧

- (1) 100 g 800 mL 1 L
- (2) 58 mL
20 min
- (3) 4
400 mL 10 min
10%
- (4) 340 mL 20% NaOH NaOH
- 13
- (5) 250 mL
40 g 98% 45 mL 1-2 d

OD 205 nm 2.88%

$$ASL\% = A \times D \times V / 1000 \times K \times W1 \times 100$$

A

D

V

K

110

W1

- (6) 4 dH₂O pH
- (7) 60°C W2
- +
- (8) 200°C 30 min 575 ± 25°C
- 4 h 30 min W3 +
- (9)

$$AIL\% = W2 - W3 \times 100 / W1$$

(10)

$$\text{Lignin \%} = AIL\% + ASL\% \times 100 / W1$$

W3-W4

W4

木质素单体分析

- (1) / 0.100 g 40
- / 67 6 h
- CWR
- (2) 50.0 mg CWR
- 25 mL 5.0 mL 2.0 mol/L NaOH 0.50 mL
- 1 170°C 3.5 h
- 15 r/min
- (3) 100 mL
- 2.0 mol/L NaOH

(4) 30.0 mL / 1 1/v
 3
 6.0 mol/L pH
 3-4 5 30.0 mL /
 1 1/v 3

(5)
 (6) 5.0 mL 0.22 μm

20.0 μL HPLC
 (7) HPLC
 universal C18 4.6 mm × 250 mm
 / / 25 1/v
 1.1 mL/min
 280 nm 28°C
 20.0 μL

(1) _____

(2) _____ 4 mg/mL 2.0 mol/L NaOH _____

(3) _____ 200 μL

_____, _____ 5 mL _____ 0.16

mg/mL _____ 0.16 mg/mL _____

(4) _____ 98% _____ 20% _____

(1) 200 mL 32.0 mg 200 mL 0.16
 mg/mL

(2) 5 0 100 200 500 1000 ug/mL 1000

ug/mL 50 mL H G S 50.0 mg

50 mL

键连接分析

- (1) /
- (2) 0.200 g CWR 10 mL 1 mol/L NaOH
1 mg/mL 30°C 250 r/min 18 h
10 mL 6 mol/L HCl pH 2
pH 2.0
40°C 1 mL 0.22
 μm HPLC
- (3) + 0.05 g
10 mL 4 mol/L NaOH 1 mg/mL ,170°C 2 h 5
mL 4 mol/L NaOH 15mL 6 mol/L HCl pH 2
pH 2.0
40°C 2.0 mL 0.22 μm
HPLC
- (4) HPLC

乙酰化分析单糖含量

✧

- (1) 800.0 μL dH₂O
- (2) 0.40 mL 10.0% NaBH₄ 40°C
30 min
- (3) 800.0 μL
- (4) 400.0 μL 600.0 μL 4.0 mL
10 min
- (5) 10.0 mL dH₂O

(6) 3.0 mL

(7) 20.0 mL dH₂O

3

(8) Na₂SO₄

(9) 2 mL GC-MS

✧

7	Rha	Fuc	Ara	Xyl
Man	Glc	Gal		

(1) 7 60°C

(2) 7 0.200 g dH₂O

50.0 mL 4.0 mg/mL

(3) 4.0 mg/mL 2.0 1.0

0.10 0.010 mg/mL

(4)

TFA

GCMS

✧ **GC-MS**

GCMS-QP2010 Plus

GCMS

GC

He

1 30

250°C

Rix-5ms 0.25 μm × 0.25 μm × 30 m

34.6 mL/min

1.02 mL/min

GC

155°C 23.0 min

3.0°C/min to 200°C 5.0 min

20.0°C/min to 300°C 2.0 min

MS

250°C

200°C

5 min

MS

SIM

IS SIM

	min	m/z	m/z
Rha	20.380	128.0	115.0 170.0
Fuc	21.197	115.0	128.0 170.0
Ara	21.683	115.0	145.0 103.0
Xyl	23.670	115.0	145.0 103.0
IS	34.433	168.0	126.0 115.0
Man	34.820	145.0	115.0 139.0
Glc	35.173	145.0	115.0 139.0
Gal	35.460	115.0	145.0 139.0

硅烷化分析

- (1) 0.010 g 500 µL dH₂O
- (2) 100 µL TFA 120°C 1 h
- (3)
- (4) 100 µL 20 mg/mL
- (5) 30°C 90 min
- (6) 100 µL N- -N -2 2 2 N-methyl-N-
trimethylsilyl trifluoroacetamide MSTFA
- (7) 37°C 60 min

(8) 800 μ L GC-MS

甲基化分析

(1)

(2) 200 μ L DMSO 45°C

(3) NaOH 25 mg 30°C 2 h

1 h

(4) 200 μ L 30°C 12 h

(5)

(6) 500 μ L 1 mL ddH₂O

(7) ddH₂O 40°C

(8) 500 μ L ddH₂O, 100 μ L TFA, 120°C 1 h

(9) 70°C

(10) 200 μ L ddH₂O 100 μ L NaBH₄ 40°C 1 h

(11) 200 μ L

(12) 200 μ L 600 μ L 4 mL 25°C 10 min

(13) 10 mL ddH₂O

(14) 3 mL

(15) ddH₂O 3

(16) GC-MS

2-AB 衍生化分析

(1) MixA 0.064 g + 0.041 g 2- 700
 μ L DMSO

(2) 10 μ g 50 μ L Mix 700 μ L MixA 300 μ L
37°C 24 h

(3) 85%

(4) 500 μ L ddH₂O, pH 10.0 500 μ L 8

(5)

(6) HyperSep C18 1ML

① 1 mL 1 mL HPLC ddH₂O 1 mL

1 mL HPLC ddH₂O

②

③ 1 mL HPLC ddH₂O

④ 1 mL HPLC 80% /ddH₂O 2

GCMS 測定可溶性糖

(1) 0.1 mL 0.1 mL 2 mg/ml

(2) N, O - N, N -

1 1 0.1 mL 75°C 60 min

(3) 1 mL GCMS

GC-MS

260°C

112 mL/min

1 μ L

20 1

150°C, 4°C/min 230°C 20°C/min 280°C

15 min

EI

280 °C

230 °C

碱预处理及酶解

- (1) 0.300 g 6 15 mL
- (2) 3 6 mL dH₂O 50°C 150 r/min 2 h 3000 g 5 min 3000 g 5 min 1 mL 10 mL dH₂O 5
- (3) 3 6 mL 1% w/v NaOH 50°C 150 r/min 2 h 3000 g 5 min 1 mL 10 mL 3000 g 5 min 6
- (4) 10 mL 0.2 mol/L pH 4.8 1
- (5) 3 mL 3.2 g/L 4.0 g/L 0.2 mol/L pH 4.8 6 mL 1.6 g/L 2.0 g/L 150 r/min 50°C 48 h
- (6) 3 0.2 mol/L pH 4.8 6 mL 150 r/min 50°C 48 h
- (7) 3000 g 5 min 1 mL
- (8) 10.0 mL 3 37 °C

酸预处理及酶解

- (1) 0.300 g 6 15 mL
- (2) 3 6 mL dH₂O 50°C 150 r/min 2 h 3000 g 5 min 1 mL 10 mL dH₂O 5
- (3) 3 6 mL 1% v/v H₂SO₄ 120°C 20 min

		50 °C	50°C	150 r/min	2 h
3000 g	5 min	1 mL			
10 mL		3000 g	5 min	6	
(4)		10 mL 0.2 mol/L pH 4.8		1	
(5)	3 mL 3.2 g/L	4.0 g/L		0.2 mol/L pH 4.8	
		6 mL	1.6 g/L	2.0 g/L	150
r/min	50°C	48 h			
(6)		0.2 mol/L pH 4.8		6 mL	150 r/min
	50°C	48 h			
(7)			3000 g	5 min	1 mL
(8)		10.0 mL	3	37 °C	

液体热水预处理及酶解

(1)		0.300 g	25 mL		2.4 mL dH ₂ O
			200°C	8 min	16 min
				32 min	64 min
(2)				dH ₂ O	15 mL
		3000 g	5 min	1 mL	
(3)			10 mL	3000 g	5 min
		6	pH		
(4)		10 mL 0.2 mol/L pH 4.8		1	3 mL
			pH 4.8	6 mL	2.0
	g/L	150 r/min	50°C	48 h	3000 g
	5 min				1 mL

CaO 预处理及酶解

- | | | | | | | | |
|-----|---------|-----------|--------|-------|-----------|-------|-----------|
| (1) | 0.300 g | 15 mL | | | | CaO | CaO/ |
| | = 1% | 5% | 10% | w/w | 6 mL | 50 °C | 150 r/min |
| | 3 | | | | | | 2 h |
| (2) | | 3000 g | 5 min | | | 1 mL | |
| (3) | | HCl | | 50°C | 150 r/min | | 2 h |
| | | | 5 min | | | | 3000 g |
| (4) | 10 mL | | 3000 g | 5 min | | | 6 |
| | | | | | | | pH |
| (5) | 10 mL | 0.2 mol/L | pH 4.8 | | | 1 | 3 mL |
| | | | pH 4.8 | | | 6 mL | 1.6 |
| | g/L | 150 r/min | 50 °C | 48 h | | | 3000 g |
| | 5 min | 1 mL | | | | | |

蒸汽爆破预处理

- | | | | | | | |
|-----|-------|--------|---------|-------|-------|---------|
| (1) | | 5~8 cm | | | | 50% |
| (2) | | | 5 L | | 225°C | 2.5 MPa |
| | 3 min | | 2.5 MPa | 3 min | | |
| (3) | | | | | 40 | 50°C |

表面活性剂的添加及酶解

- | | | | | | | |
|---|------|---------|---------|--|---------------------------|--------|
| | | | | | 10 mL 0.2 mol/L | pH 4.8 |
| 1 | 3 mL | 3.2 g/L | 4.0 g/L | | | |
| | | | | | 20% Tween-80, 10% PEG4000 | pH |

4.8		6 mL		1.6 g/L	2.0 g/L	Tween-80
		1% PEG4000		0.5%		
150 r/min	50°C	48 h		3000 g	5 min	
	1 mL					
	Tween-80				30 min	

Tween-80

PEG4000

乙醇发酵

✧

- (1) pH 4.8
- (2) 1.6 g/L 2.0 g/L
1% v/v -80 50°C 150 r/min 48 h
- (3) 120°C 20 min
- (4) 0.5 g/L 37°C
48 h
- (5) dH₂O
- (6) 96°C

✧

- (1) pH 4.8
- (2) 120°C 20 min
- (3) 1.6 g/L 2.0 g/L
1% v/v -80 50°C 150r/min
48 h
- (4) 0.5 g/L
37°C 48 h
- (5) dH₂O 96°C

乙醇含量测定

✧

- (1) 1.0 mL 2.0 mL 5% 100°C
10 min

(2) $\lambda = 600 \text{ nm}$

✧

(1) 20% v/v 1.0 2.0 3.0 4.0 5.0 6.0
8.0 mL 100 mL 100 mL % v/v
0.2 0.4 0.6 0.8 1.0 1.2 1.6

(2) 1.0 mL 2.0 2.0 mL 5%
100°C 10 min $\lambda = 600 \text{ nm}$

Simons' Stains 方法測定孔隙度

	Milli-Q	160	3000
g	2	3000 g	2
		50 °C	2 h

DY	
DY	100 KDa ultracentrifugation membrane

(1) 0.1 g 15 mL 1.0 mL 5 mmol/L KAl
SO₄ 2 + 1.5 mmol/L NaCl

(2) 0.25 mL 0.5 mL 0.75 mL 1.00 mL 1.50 mL 2.00 mL
10 mg/mL DB DY 1 1 10.0 mL

(3) 70 °C 200 r/min 9 h

(4) 8000 g 5 min 612.5 nm DY 410.5 nm DB
DB DY

1 2

3

$$A_{410.5 \text{ nm}} = \epsilon_{Y/410.5} LC_Y + \epsilon_{B/410.5} LC_B \quad 1$$

$$A_{612.5 \text{ nm}} = \epsilon_{Y/612.5} LC_Y + \epsilon_{B/612.5} LC_B \quad 2$$

$$Ae = \frac{C_i - C_e}{M} \times V \times 1000 \quad 3$$

A 410.5 nm or 612.5 nm

$$\epsilon_{DY/410.5} = 31.83 \text{ L g}^{-1} \text{ cm}^{-1},$$

$$\epsilon_{DB/410.5} = 3.418 \text{ L g}^{-1} \text{ cm}^{-1}, \epsilon_{DY/612.5} = 0.143 \text{ L g}^{-1} \text{ cm}^{-1}, \epsilon_{DB/612.5} = 23.96 \text{ L g}^{-1} \text{ cm}^{-1}.$$

L 1 cm
 C_Y C_B
 A_e mg/g A_Y A_B
 C_i mg/L
 C_e mg/L C_Y C_B
 M g , V mL

 Langmuir

刚果红染色测定纤维素孔隙度

- (1) 0.1 g 15 mL 1.0 mL 0.3 mol/L
 Na₃PO₄, 1.4 mmol/L NaCl, pH 6.8
- (2) 0.25 mL 0.5 mL 0.75 mL 1.00 mL 1.50 mL 2.00 mL 10
 mg/mL 10.0 mL
- (3) 60 °C 200 r/min 24 h
- (4) 8000 g 5 min 498 nm

$$A_e = (C_i - C_e) \times V / M \times 1000$$

A_e mg/g
 C_i mg/L
 C_e mg/L
 M g , V mL

 Langmuir

纤维素酶吸附法

- (1) 0.1 g 15 mL
- (2) 0.5 1.0 1.5 2.0 2.5 3.0 mg/mL 10 mL
- (3) 4 °C 15 h 3 h

(7) "L"

(8)

(9)

(1) 2460

(2) 2 "  " 

100

(3) 2

3-4

(1) 2460 →File→New Sample→Replace All→data→BJH→

Load→ Sample mass →Save as→Load→data→Save

(2) D →Micro→data→

(3) Unit 1→Start High throughput→Browse→Start

(4) Show Analysis Status

(5) Pre--- ---Terminal 3 h

(6)

report pdf excel

(1)

(2) "  " → "  "

(3) "gas"

“gas”

孢子液的制备

- (1) - PDA Rut-C30 114-2
30°C 5-7
- (2) 4-5 mL PDA
Rut-C30 2% Tween 80
PDA 114-2
- (3) 100 μL 2 2% Tween 80
M 10 2
- (4) 3 4 5
N N
2 C
 $C = N \cdot 4 / 5 / 16 \cdot M \cdot 10^6$
C N M
- (5) 4
 10^7 mL^{-1}

秸秆材料诱导里氏木霉或草酸青霉产纤维素酶

- (1) 100 mL
0.600 g 30 mL
- (2) 115°C 30 min
- (3) 500 μL
- (4) 30°C 200 r/min 7 d

(5) 50 mL 3000 g 5 min -20°C

诱导酶液活性测定

✧

- (1) Whatman No. 1 1 cm × 6 cm 50 mg
15 mL
- (2) 1 mL
3 mL pH 4.8 0.05 mol/L 50°C 150 r/min
1 h 1.9-2.1 mg
2 mg
- (3) 10 min 3000 g 5 min
- (4) DNS

✧ **DNS**

- (1) 0.2-0.8
- (2) 1 mL 10 mL 2 mL DNS
5 min
- (3) 540 nm 1 mL

$$\frac{1 \text{ min}}{\text{FPU}} = \frac{1 \text{ } \mu\text{mol}}{\text{mg/mL} \times \text{mL} \times 5.56 \times \frac{\text{mL}}{\text{min}}}$$

$$\frac{5.56 \text{ } \mu\text{mol}}{1 \text{ mg}} = \frac{1000/180 = 5.56 \text{ mg/mL}}{1 \text{ mL} / \text{min}}$$

1.0 mg/mL 10.0 20.0 30.0
40.0 50.0 mL 100.0 mL

0.1 0.2 0.3 0.4 0.5 mg/mL 1.0 mL
 10.0 mL 1.0 mL 2.0 mL DNS
 5 min 540 nm

DNS 3,5 6.3 g 50 mL
 NaOH NaOH 21 g 500 mL 185 g
 5 g 5 g
 1000 mL 7
 3,5
 50°C
pH 4.8 0.05 mol/L citrate buffer pH 4.8
 210 g 750 mL pH = 4.3 50-60 g
 1000 mL

酿酒酵母感受态细胞的制备及转化

- (1) 12
200 r/min
10 mL 2 × YPD 28°C
50 mL 2 × YPD 250 mL
- (2) 8
100 μL 1.5 mL
- (3) 900 μL ddH₂O 1.5 mL 10 10 μL
40 5 4 × 4
239 cells × 5 × 10
× 10,000 = 1.2 × 10⁸
- (4) 2.5 × 10⁸ cells 2 mL 50 mL
2 × YPD 250 mL 5 × 10⁶ cells/mL
28°C 200 r/min 3~5 h
3~4
- (5) 2 × 10⁷ cells/mL 4°C
4,000 r/min 5 min ddH₂O
1 mL ddH₂O 5 × 10⁶
cells/mL ddH₂O
- (6) 1 mL 1 1.5 mL Eppendorf 13,000 r/min
30 s ddH₂O 1 mL
- (7) 1.5 mL 100 μL 10⁸ 13,000
r/min 30 s
- (8) 100 μL
0.02~0.05 μg/μL

34 μL		0.68 μL		Carrier DNA		5 min	
		3~5				μL	
						1 5 6 \times 10 11 \times	
PEG3350	50% W/V					240	1440 2640
LiAc	1.0 mol/L					36	216 396
Boiled SS-carrier DNA	2 $\mu\text{g}/\mu\text{L}$					50	300 550
Plasmid DNA	0.1 to 1 μg	plus water				34	60 374
Total						360	2160 3960
(9)		42 $^{\circ}\text{C}$		0.5~1 h			
(10)		6,000~8,000 r/min		30 s			1 mL 2 \times YPD
(11)				1 mL			200 $\mu\text{L}/$
				10 μL	990 μL ddH ₂ O		100
		100 $\mu\text{L}/$		10 μL	90 μL ddH ₂ O		100 μL
		1,000					
(12)	28 $^{\circ}\text{C}$			2~3 d			

酿酒酵母基因组 DNA 提取

- (1) 5 mL YPD 28 $^{\circ}\text{C}$ 200 r/min
24 h
- (2) 2 mL 8,000 r/min 5 min
- (3) ddH₂O 2 8,000 r/min 5 min
- (4) 400 μL TE 30 μL 10% SDS 10 μL 20 mg/mL
K 50~55 $^{\circ}\text{C}$ 1 h 5 min
- (5) 1 h 180 μL 5 mol/L 10 min
- (6) 12,000 r/min 5 min
- (7) / Tris 2

(8)		1		
(9)		2		-20°C 20 min
(10)	12,000 r/min	5 min	DNA	
(11)	75%	DNA	2	
(12)	DNA	30~50 μ L ddH ₂ O	DNA	

吸附实验

Cd NO₃ 2 · 4H₂O HCl NaOH HNO₃
pH Cd²⁺

☆ --pH

- (1) 10.0 mL 100.0 mg/L Cd²⁺ 500 mL
 pH 4.0 5.0 6.0 7.0 8.0
 pH = 4.0 5.0 6.0 pH = 4.00 pH = 6.86 pH
 = 7.0 8.0 pH = 6.86 pH = 9.18
- (2) pH Cd²⁺ 25.0 mL Cd²⁺ 50 mL
- (3) 0.0250 g 1.0 g/L Cd²⁺
 150 r/min 25°C 4 h
- (4) 3000 g 5 min
 10 mL 50 μL Cd²⁺
 Cd²⁺
- (5) pH

☆ -- Cd²⁺

- (1) 100.0 mg/L Cd²⁺ Cd²⁺ 2.0 5.0 10.0 15.0 20.0
 30.0 40.0 mg/L pH 6.0
- (2) Cd²⁺ 25.0 mL Cd²⁺ 50 mL
- (3) 0.0250 g 1.0 g/L Cd²⁺
 150 r/min 25°C 4 h
- (4) 3000 g 5 min

			10 mL		50 μ L				
						Cd^{2+}			
(5)						Cd^{2+}			
◇			—						
(1)	100.0 mg/L	Cd^{2+}			Cd^{2+}	10.0 mg/L	pH		pH
(2)		pH	Cd^{2+}	25.0 mL	50 mL				
(3)		0.0050 g	0.0150 g	0.0250 g	0.0350 g	0.0450 g			
				0.2	0.6	1.0	1.4	1.8 g/L	
	150 r/min	25°C	4 h						
(4)			3000 g	5 min					
			10 mL		50 μ L				
						Cd^{2+}			
(5)									
◇			—						
(1)	100.0 mg/L	Cd^{2+}			Cd^{2+}	10.0 mg/L	pH	6.0	
(2)		pH	Cd^{2+}	25.0 mL	50 mL				
(3)		0.0250 g				1 g/L			Cd^{2+}
						15	25	35	45°C
							150 r/min	4	
									h
(4)			3000 g	5 min					
			10 mL		50 μ L				
						Cd^{2+}			
(5)									
◇			—						
(1)	100.0 mg/L	Cd^{2+}			Cd^{2+}	10.0 mg/L	pH	6.0	
(2)		pH	Cd^{2+}	25.0 mL	50 mL				
(3)		0.0250 g				1 g/L			Cd^{2+}
							1	5	10
									20
		25°C	150 rpm	9					

30 60 120 180 240 min 0.45 μm

(4) 10 mL 50 μL
Cd²⁺

(5)

✧

(1) 0.1 mol/L NaCl

(2) HCl NaOH NaCl pH 3.0 4.0 5.0 6.0
7.0 8.0 9.0

(3) pH NaCl 40.0 mL 50 mL

(4) 0.0800 g 1.0 g/L

(5) 150 r/min 25°C 48 h 48 h pH

✧

FTIR

Thermo scientific Nicolet iS50

160

KBr =50 1

4000-400 cm^{-1}

KBr

T

✧

Langmuir Freundlich

Langmuir
$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{bq_{max}}$$
 2-1

q_e mg/g q_{max} mg/g b

L/mg

C_e mg/L

F Freundlich
$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e$$
 2-2

W

g

秸秆中镉含量测定

	0.1-0.3 g	0.001g	200
0.5-1 h,	600	7 h	
1%		10 mL	25 mL
1%	3		1%

Agilent 240Z GF