ARAC total20µl, 1.5.ml Centrifuge tube

- ①ddH2O:6µl
- ②Buffer:2µl
- ③substrate:10µl

KpnI, SaII each 1µl

Flick to mix, instantaneous centrifuge

Preparation for sample addition 10min

TETRtotal20µl

- ①ddH2O:6µl
- ②Buffer: 2μl
- ③substrate: 10µl

PstI, SpeI each 1µl

37°C15 min 80°C20 min

Glue preparation:0.5g+5mlTae+melt→5μlDye 5μlTwo kinds of: 5000、15000+substrate20μl +loading buffer 4μl (10min) Power: voltage max+time26min

### 8.21

Results :tetr with two bands (more than 3000, more than 700 arac with one band 2000-3000)

Note: First turn on the gel imager next to the computer, and then start the software. (When the gel imager is turned on, there will be two yellow lights, "Power" and "Slow" lights.)

Cut glue: open the bottom cabinet of the imager, lay plastic wrap, insert the UV baffle, place the glue in the center, open the TRANS UV knife to cut the required strips, remove the empty glue as far as possible, chop it, transfer it into the empty centrifugal tube, turn off the UV and power supply, remove the plastic wrap and baffle, and wipe the table

The amount of tetr is too small and may not be recycled tetr plasmid was placed in two 50µl tubes at -20°C

arac 0.31g tetr 0.57g PN 310μl PN 570μl

PW plus anhydrous ethanol 60ml

(If the volume is too large, add a collection tube) (speed 12000rpm)

- 1. Column equilibrium, adsorption column 500 µ lBL, 1min
- 2. Sol, 310 μ 1/570 μ 1, 50°C
- 3. Transfer to the adsorption column, place at room temperature for 2min, then transfer for 1min
- 4. Add 600 µ lPW for 1min
- 5. Repeat 4
- 6. Idle for 1 minute, then remove the lid and leave to dry for a few minutes

 $7.>30 \mu$  IEB (be sure to drop on the white cylinder), replace the tube, and rotate for 2 minutes

After cutting and recovering, the concentration was: arac: 18ng/  $\mu$  1, tetr: 15.2ng/  $\mu$  1 14.4ng/  $\mu$  1

Mixed with 0.50g+50mlTAE+5 µ 1 dye

## digestion

Single enzyme digestion:

A1 arac 18ng/µl

arac 44.4μl——800ng

Buffer 1.6µl (2\*0.8)

Enzyme 0.8μ1 (1\*0.8)

Double digestion:

A2 Arac  $(250 \text{ng/}\mu\text{l}) 4\mu\text{l} - 1000 \text{ng}$ 

Buffer 2µl

Enzyme KpnI+SaII 2ul

H20 12ul

## Detection

A1:5.88µl 5.9µl

A2: 2μl

Run the glue (120V 400mA 30min):

Electrophoresis1 Marker

Electrophoresis2 plasmids

Electrophoresis3 A1

Electrophoresis4 A2

#### 8.22

Digestion tetr (T) 107ng/μl

Run the glue (120V 400mA 24min):

Electrophoresis1 Marker

Electrophoresis2 T

Electrophoresis3 A2(finalizing Single enzyme digestion)

Gum cutting: tetr 0.08g arac0.09g

PN 80µl 90µl Metallic bath 2min

# Rubber recovery

Measuring concentration:

tetr recovery concentration: 5.5ng/µl

Arac:  $10.9 \text{ng/}\mu\text{l}$  (A full ng takes  $91.74 \mu \text{l}$ )

Run the glue A1(120V 400mA 30min)

Loading buffer:Each hole has about 2µl

A1:Each hole has about 8µl

Electrophoresis 1 2µl loading buffer

Electrophoresis 28 µl A1

Electrophoresis3 marker GL5000

Second electrophoresis:

Electrophoresis 1 4µl loading buffer 10µlA1

Electrophoresis 22µl loadingbuffer 8µl A1

Electrophoresis3 marker GL5000

### 8.23

PN

A1 Cut glue (with about 30ml TAE liquid) and recycle glue

Empty pipe: 1.15g

 Pipe1
 pipe2
 pipe3
 pipe1
 pipe2
 pipe3

 0.72g
 0.57g
 0.54g
 0.37g
 0.64g
 0.57g

 720
 570
 540
 370
 640
 570

Glue recovery, eluent EB 50µl, centrifuge 3 times

Concentration 7.7ng/ $\mu$ l , n\*M=c\*v ,To get to 0.1pmol you need about 84  $\mu$  1 of solution [1057.1500]

Ptrc99a Double enzyme cutting, running glue

3.2g 100ml LB medium

### 8.24

Solid to liquid medium

The large vial of liquid culture has been spiked with ampicillin

One colony was selected from ① and ② and added to 7ml medium

### 8.25

Extract plasmid (3ml bacterial solution plus 70µlEB)

concentration

Digestion:

DNA 45µl

Buffer 2µl

Kpn1,Sa2 2µl

Glue 30ml

Run the glue

Electrophoresis1 Cutting plasmid

Electrophoresis2

Electrophoresis3 Cutting plasmid 4µl

Electrophoresis4 plasmids 4µl

Electrophoresis5 marker 1.5µl

Weighing after cutting glue: 0.26g

260µl PN

ptrc99a, enzyme digestion  $9.4 \text{ng/}\mu\text{l,need } 0.01 \text{pmol} = 0.01*10^(-12) \text{mol} = 10^(-14) \text{mol}$ 

4159\*650\*10^(-14)=2.7\*10^(-8)g=27ng

Volume:  $27 \div 9.4 = 2.9 \mu l$ 

8.26

The receptive + connected plasmid (arac) was added with 25ngDNA per 50µl cell

Yesterday: ptrc99a:27ng, arac:165ng

A total of 192ng(but the arac is increased, it should be greater than 192ng) is calculated at 200ng

For 100µ1 receptive cells (50µ1 cells :25ngDNA), 50ngDNA should be added

 $200 \text{ ng} / 2.9 + 84 + 1 + 1 \approx 90 \text{ }\mu\text{l}$ 

Take:22.5m1 and attach the plasmid

Sterilization: solid medium, liquid medium (solid medium,4.8g powder +150ml pure water, add 0.015gAmp after sterilization

Liquid medium, 2.5g powder +100ml pure water)

8.26 PM

100μl K12+22.5μl connected plasmid

Follow the K12 instructions

arabinose(inducer) was not added before the bacteria were attached to the plate, and arabinose was added after the bacteria were attached

arabinose is powder, 0.03g(150ml medium)

0.03g arabinose+1100ul pure water

Eleven plates were poured and 100µl arabinose solution was added to each plate

The green colonies were cultured and screened for successful connection

8.28 PM.

Enzyme digestion of tetr fragments:

t1 16.1ng/μl, t2 19.2ng/μl

Blp1 2µl Buffer

8.30 PM.

Run tetr cut glue cut waste, no strip

Start from the large fragment of tetr again

Arabinose dosage :1mM concentration, 100µl per plate

Maracbinose = 342.3 g/mol

Total 5 plates,500 UL solution,5\*10^(-4)mol aracbinose

Mass : $5*10^{-4}*342.3=0.17g$ That is,  $0.17g+500\mu$ l pure water

The bacteria cultured in 8.26 (placed in the refrigerator) were observed by fluorescence microscope, and the phenomenon was not obvious, and the colonies were in large pieces, which may have been caused by the lack of smooth coating during inoculation. Today, the colonies with slight bright spots in fluorescence observation were selected  $\rightarrow 200 \mu LB + Amp$  liquid medium dilution  $\rightarrow$  coated to LB plate (the incubator was not placed because it was closed).

In the next experiment, green colonies were found, and each observed one was marked at the bottom of the plate during fluorescence observation. The labeled colonies were picked into liquid medium, and the plasmid could be extracted after culture

#### 8.31

- 1. Column balance (1.08)
- 2. Weighing of rubber block:  $0.05g (0.1g \rightarrow 100 \mu l) \rightarrow Adding 50 \mu lPN$ , it is difficult to dissolve, and adding  $30 \mu lPN$  (weighing centrifugal tube problem)  $\rightarrow$  resulting in a volume of  $160 \mu l$
- 3. Add CA2,2min, then centrifuge
- 4. Add 600µlPW
- 5. Add EB Eluent about 35µl

(Repeat centrifugation twice during the collection phase)

Change 1.5ml centrifuge tube for collection (concentration to be measured)

Attempted digestion 6.9ng

Tetr 19μl DNA1μg 13μl Buffer 2μl Pst1 1μl Spe1 1μl

B1P1 1µl Buffer 2µl

37℃ 1h

The tetr gene has no band and cannot be recycled

~700bp glue recovery

PM: The tetr fragment was recovered and the concentration was measured at 8.6ng/µl

### 9.11

Enzyme digestion arac, tetr

Electrophoresis:

Electrophoresis1 maker

Electrophoresis2 arac

Electrophoresis3 tetr

Electrophoresis4 tetr

When the plasmid is used up, the plasmid is extracted from the bacterial solution, but the storage time is too long, the bacteria sink to the bottom, not the suspension of the fresh bacterial solution, which may affect the result

Plasmid concentration: a1a2 is the same, t1t2 is the same

```
A1 493.8ng/\mu l
A2 521.9ng/μl
T1 149.7ng/\mul
T2 119.1ng/µl
9.12
Digestion a1 t1
A1 substrate 2.02µl
                       t1 substrate 6.68µl
Buffer 2µl
                       buffer 2µl
Kpn1 Sa2 2µl
                       Pst1 Spe1 2µl
DdH2O 14µl
                        DdH2O 9.32μl
Electrophoresis:
Electrophoresis1 maker
Electrophoresis2 arac
Electrophoresis3 tetr
Rubber recovery:
Arac 0.16g tetr0.2g
    160µl
             200µl
    8.8 ng/\mu l 4 ng/\mu l
8.8 \text{ng/} \mu l = 0.5 \mu l
DNA
        6µl
Buffer 2µl
BLP1 1µl
DdH2O 11µl
37℃ 1.5h
Electrophoresis (30ml) 30min
Two target bands can be seen, but the amount is small and stored at 4°C
arac single enzyme cut, cut glue, stored in the upper layer of 4°C refrigerator
Glue recovery, concentration measurement, 5.8 ng/µl
9.14
Ptrc99a 53.3ng/µl
Arac Kpn1 Sa2 493.8ng/µl
Tetr
       Pst1
              Spe1 149.7ng/\mul
Ptrac99a Kpn1 Sal1 53.3ng/µl 18.8µl
Maker tetr arac ptrc
        2
              (3)
                   4
Empty pipe: 1.00g
Ptrc99a:
           0.16g 16.6 3µl
Tetr: 0.10g
Arac: 0.09g 15.1 12µl
Glue recovery, concentration not measured
```

```
9.17
```

Preparation: Gun tip, PCR tube, sterilization of medium, inverted plate

Connections:

arac 12µl

ptrc99a 3µl

Buffer 2µl

Ligase 1µl

H20 2µl

16:00~18:00, 25°C for 2h, 4°C storage

Tomorrow:

Open the ice machine, prepare the liquid medium, kanamycin, arabinose

Feelings of ice melting

Inducer is added before coating

The connecting system is refrigerator 4°C at the far gate of 307

9.20

50µl→25ngDNA

906.4ng/μl 100μl→50ngDNA

Dilution :0.5  $\mu$  l of the connection product is added to 4.5  $\mu$  lddH20

1  $\mu$ 1 connection product →100  $\mu$ 1K12

Coating plate 5:

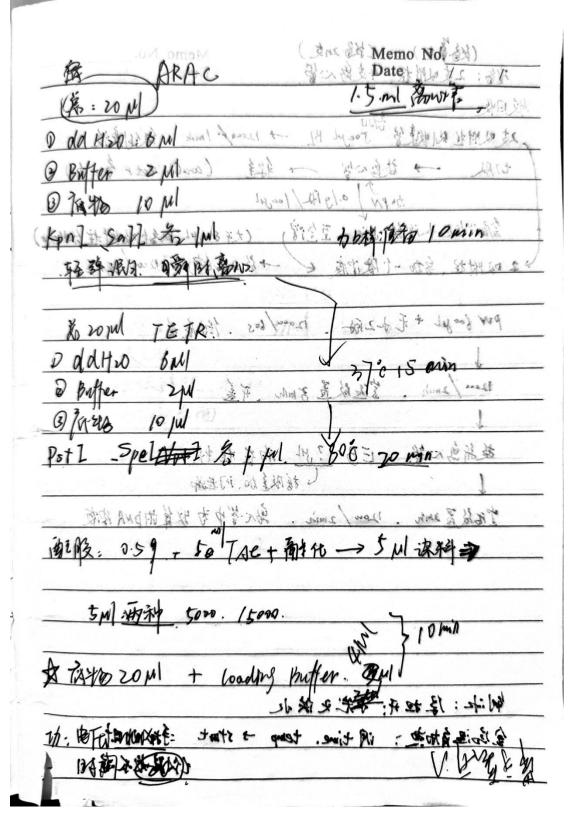
IPTG+ Arabinose :2 plates

IPTG:1 board Arabinose :1 plate None :1 board

(1mM arabinose :3.4g→10ml water

1mM IPTG 0.002383g/mol)

0.1M arabinose :0.15g→10m1 water 0.1M IPTG:0.238g→10ml water



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1.	林平约 三级附柱 400ml BL 11	nin ARRE
_ 2.	海底 10 pl / 570pl 50°C ~ 37	沙州
3.	纷转入吸附在宝温放2min后 /min	1
4.	600 pl PW . Noi	
٢.	》重复、旅游、别声min	*
6.	空转,后开盖晾几分钟 图 2 2mm	it
7.	>30HLEB 广定要滴到白色柱体上,整3min	
to his	国内下海度 arac: 18 ng/Min tetrings.	
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倒了十一个平板。每个平板加小的时	
接养、筛长出绿色角落为连接	水水水水
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MAN tetr Fig. Potal Potal	
ti 16-1 ng/ml t2-19.2	ng/ml.
BlpI. zur Buffer.	pro-

Memo No.	Memo No.  Date / /
8.30 晚.	5.26 보추.
THE TONTO	影美士连长的废料 (ame)
重新从tetr大分及确切开始。	BEXTE PERCOPA : 27 ng
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	- 3.192 ng (18 aras 203
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conic点面落排放 = (200以) LBH	이 없이 있는 그렇게 하는 이 없었다. 아이들이 얼마를 보고 있는 것이 되었다. 그 살아 있는 것이 없는 것이 없다고 있다.
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	[N] pa P.147 ap
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tetr 19ul	DNA 1 kig on 13 ml
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37°C 1h	数数 50 00 to
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		5:68 x	17-x	
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9.12	7.4			9.14 KK.
tA	成团以	Arac 0:169	THE MER IN	2.29
		160ml		popul
	73.80g/wl			mr. surva
8.8 ng/	me or orging	D . Isept	1339	Tota.
18.8/1	AND PONA. EL	I 18.68 pt	Epil.	Perac 990
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9.14 16克.	Date	Date	9.12 7.29
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Muker	Tetr In 1 bro	nc 14 Ptro	¥
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orae:	0.109	15. \ ×12	thr man
BENK	A STATE OF THE STA		conc fift
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tentiletet	ALLA TOTAL		4
V4.111/0	14		
15-16	Charles 1 to the		

ema No.			Memo Date	No.	7
-9717					9.10 B
龙头 PCR管	烂养其灭	南行	削桃		
	12	. 1	小加島	M. T.	藝
	· ANC	] par o2 <-	N (9)	MID	4.109
# to Albh	LATA L	nt light a	4 D At 37	W70.	Sisi
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	rc99a	3μ6	( ) ( ) ( )	W/ 1/2/ 1/2	
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	120	zul	WWW. CAL	: 1 7fg	al al
14 16:00~18		25°C 2h	4°0%		阿在伯
报天:			S. W.	7. No. 1	21.52.64
开制冰机.	准备消	纯培养基	牛爭	1/2主	阿拉伯
170077	Daniel	4			
地上海北	说后太.	101 ६ ६ भर	: 8 1 3		Mari
180	s ~		·M1.	()	0 -
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△涂布前-	生加油	= 45 PSECO	100	6 -1.7	
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连接华子在30	1:2001	16 534 en		MPOR	
TENENTALIZA	1 751 114	Charmal L. C.	) B	MIRI	1.0

Memo No.	Memo No.  Date
9.20 晚	7
1部 年1成	だ火 アロス党 は春花式面
SOME STAY DNA.	
906.4 ng lul 100 ml -> 50 n	DNA.
稀解: 05川 直接产物加到 4	M 45M) ad the + 12
1川连接产物 → 100川大江	
3 μ ζ	percena
海板扩:	Buffer
LPTG + ara阿拉伯糖:一次板	Ugase
IPTG: 1板 Jaya	Cut
阿拉伯糖 作板中 从 3	
表: 1板	: 2
	不到水中。 (社會)
·1mM 阿拉伯糖: 349→1	1 1 1
1976 0002383 mot 91	maj . Se mol
238.3 9 L = 1M. 10ML	
0272789	
The state of the s	白海布前先加港等
0.1 M P可拉伯雅: 0.159	lom1 74
01 M 19 TG : 0,53893	主義今年在如此的