8.19

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℃15 min
80℃20 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 l/570 μ l, 50℃
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 μ lEB (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/ μ l, tetr: 15.2ng/ μ l 14.4ng/ μ l
Mixed with 0.50g+50mlTAE+5 μ l dye

digestion
Single enzyme digestion:
A1 arac 18ng/μl
arac 44.4μl——800ng
Buffer 1.6μl（2*0.8）
Enzyme 0.8μl（1*0.8）
Double digestion:
A2 Arac（250ng/μl）4μl——1000ng
Buffer 2μl
Enzyme KpnI+SaII 2ul
H20 12μl

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: 10.9ng/μl　(A full ng takes 91.74 μl)

Run the glue A1(120V 400mA 30min)
Loading buffer:Each hole has about 2μl
A1:Each hole has about 8μl
Electrophoresis1 2μl loading buffer
Electrophoresis2 8μl A1
Electrophoresis3 marker GL5000
Second electrophoresis:
Electrophoresis1 4μl loading buffer 10μlA1
Electrophoresis2 2μl loadingbuffer 8μl A1
Electrophoresis3 marker GL5000

8.23
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
PN	720	570	540	370	640	570

Glue recovery, eluent EB 50μl, centrifuge 3 times
Concentration 7.7ng/μl， n*M=c*v ,To get to 0.1pmol you need about 84 μl 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 9.4ng/μl,need 0.01pmol=0.01*10^(-12)mol=10^(-14)mol
4159*650*10^(-14)=2.7*10^(-8)g=27ng
Volume：27÷9.4=2.9μl

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 (50μl cells :25ngDNA), 50ngDNA should be added
200 ng / 2.9 + 84 + 1 + 1≈90 μl
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+500μl 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 →200μlLB+Amp liquid medium dilution → 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→100μl) → Adding 50μlPN, it is difficult to dissolve, and adding 30μlPN (weighing centrifugal tube problem) → resulting in a volume of 160μl
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/μl
A2 521.9ng/μl
T1 149.7ng/μl
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
　　8.8ng/μl　4ng/μl
8.8ng/μl　0.5μl
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℃

arac single enzyme cut, cut glue, stored in the upper layer of 4℃ refrigerator
Glue recovery, concentration measurement,5.8ng/μl

9.14
Ptrc99a 53.3ng/μl
Arac　Kpn1　Sa2　493.8ng/μl
Tetr　　Pst1　　Spe1　149.7ng/μl
Ptrac99a　Kpn1　Sal1　53.3ng/μl　18.8μl

Maker　tetr　arac　ptrc
①　　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℃ for 2h, 4℃ 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℃ at the far gate of 307

9.20
50μl→25ngDNA
906.4ng/μl 100μl→50ngDNA
Dilution :0.5 μ l of the connection product is added to 4.5 μ lddH20
1 μ l connection product →100 μ lK12
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

Memo No.
Date

酶 ARAC
体系：20 μl

1.5 ml 离心管

① ddH2O 6 μl
② Buffer 2 μl
③ 产物 10 μl
KpnI SalI 各 1 μl
轻弹混匀，瞬时离心

加 6 样准备 10 min

总 20 μl TETR
① ddH2O 6 μl
② Buffer 2 μl
③ 产物 10 μl
PstI SpeI 各 1 μl

37°C 15 min

80°C 20 min

配胶：0.5 g + 50 ml TAE + 融化 → 5 μl 染料

5 μl 两种 5000. 15000.

☆ 产物 20 μl + loading buffer. 4 μl

} 10 min

点样：小心

功：电压，时间

(预备n支)　　　　(预备2n支)

准备：2支吸附柱、4支离心管。　　Memo No.______ Date　/　/

胶回收

2支吸附柱放入收集管，各加500μL BL → 12000/1min 倒废液

切胶 → 放离心管 → 称重（araC、tetr各一个）

加PN ↓ 0.1g胶/100μL

金属浴或水浴溶解50°C至全溶

（大于800μL 取多个吸附柱分批加）

→ 2吸附柱，各加一个胶溶液 → 放2min(室) → 12000/离心60s

PW 600μL + 无水乙醇，12000/60s，倒废液

↓

12000/2min，室温放置5min，开盖

↓

换新离心管，EB ?μL 滴在柱中央

↳ 按胶量加、问老师

↓

室温放置2min，12000/2min，离心管中为收集的DNA溶液

制冰：后拉开，水完又供水

金属浴加热：调time、temp → start 手机计时

(仪器也不准)

8.21 下午　　1　2　3.　Memo No.
5000 marker　tetr　arac　Date　/　/

酶切电泳图拍照，结果：tetr 两条带{3000多 700多　arac 一条带 2000~3000

√注意：先开电脑旁的凝胶成像仪，再启动软件，（凝胶成像仪开启会有两个黄个灯亮，Power 和 Slow 亮）

切胶　开成像仪底柜，铺保鲜膜 插紫外挡板
胶放置正中 开 TRANS UV 刀切所需条带
尽量剔除空胶 切碎 转移入空离心管
关 UV、电源
去保鲜膜、挡板
擦净台面

tetr量过少 可能无法回收

提 tetr 质粒 两管 50μL 置 30℃ -20℃

Memo No.
Date 8.31 1.51

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

△ PW 加无水乙醇 60mL

1个柱（太大的话 多加 （吸附柱→收集管） 12000 rpm

1. 柱平衡 吸附柱 500μL BL 1min
2. 溶胶 310μL / 570μL 50°C
3. 分别转入吸附柱室温放 2min 后 1min
4. 600μL PW 1min
5. 重复
6. 空转，后开盖晾几分钟 2min
7. >30μL EB 一定要滴到白色柱体上 换新管 2min

切胶回收后浓度：arac: 18 ng/μL tetr: 15.2 ng/μL 14.4 ng/μL

配胶 0.5g + 50 mL TAE + 5 μL 染料

· 酶切 a提 arac 18 ng/μL ~~体系 缩小 上法~~

arac 44.4 μL — 800 ng
buffer 1.6 μL (2×0.8)
酶 XhoI 0.8 μL (1×0.8)

} 单酶切

a头 arac (250 ng/μL) 4 μL — 1000 ng
buffer 2 μL
酶 ~~XhoI~~ KpnI + SalI 2 μL
水 12 μL

} 双酶切

Memo No.
Date / /

检测（跑胶）
a提：5.88 μl 5.9 μl
a头： 2 μl

跑胶：泳一 二 三 四
Marker 质粒 a头 a提
120V 400mA 30min

8.22 下午 107 ng/μl
酶切 tetr 提 最后（要做单酶切）
跑胶 泳一 二 三 四 的）
Marker t提 a来头
24min
切胶 tetr 0.08g arac 0.09g
PN 80 μl 90 μl 金属浴 2 min
切胶回收

测浓度：
tetr 回收浓度：5.5 ng/μl
arac： 10.9 ng/μl 大（满1μg要91.74 μl）

Memo No.
Date / /

8.22晚

① 对a提跑胶. ~~切胶. 胶回收.~~

loading buffer: 每个孔大的有2μl.

a提: 每个孔大的有8μl.

120V. 400mA. 30min.

泳道一 二 三 阳 英 文.

2μl loading buffer
8μl a提

Marker.
GL5000

第二次电泳.

泳道一 二 三

多有4μl loading buffer; 2μl L-buffer
10μl先与a提.

8μl a提

GL5000

Marker

1.92 1.15 Memo No.
Date / /

8.23 下午. →配30ml左右TAE液

a提 切胶 胶回收

空管. 1.15g. 管1 管2 管3 管① 管② 管③

~~0.89g~~ 0.72g ~~1.03g~~ | 0.57g ~~0.83g~~ ~~1.13g~~ | 0.54g ~~1.06g~~ | ~~0.78g~~ ~~0.87g~~ 0.37g | 0.64g | 0.57

PN 720 | 570 | 540 | 370 | 640 | 570

胶回收, 洗脱液EB 50ul. 离心3次.

浓度 7.7 ng/ul. n·M=c·V. 到0.1pmol 需84ul 左右溶液.

[1057. 1500]

ptrc99a 双酶切. 跑胶

8.23 晚上.

3.2g 100ml LB培养基.

8.24 下午

固→液.

大肠液体培养基中加菌苗

从1.2分别挑取一个菌落 加到7ml培养基

Memo No.
Date / /

~~8.22晚~~ 8.25 下午

提质粒 3ml 菌液 加70μl EB

浓度 22.2 ng/μl

酶切

DNA 45μl

buffer 2μl

Kpn I Sal I 2μl

胶 30ml

跑胶 泳一 二三 四五

质切 质切 原质粒 Marker.

≈25μl 20μl 4μl 1.5μl

8.25 晚上.

切胶后称得胶：0.26g

260μl pH

ptrc99a 酶切 9.4 ng/μl 需 0.01 pmol = 0.01 x 10^{-12} mol = 10^{-14} mol

4159 x 650 x 10^{-14} = 2.7 x 10^{-8} g

bp 运输损 = 27 ng.

1 bp 体积：27 / 9.4 = 2.9 μl.

g/mol

Memo No.
Date / /

8.26 上午.

感受态 + 连好的质粒 (arac)　　每50μl细胞加25ng DNA.

昨天算 ptrc 99a：27 ng　　arac：165 ng

共 192 ng (但arac加多了，应大于192 ng) 下按200 ng计算

100 μl 感受态细胞 (50μl细胞：25ng DNA)，应加 50ng DNA

→ 200ng / 2.9+84+1+1 ≈ 90 μl

取 22.5 μl 连好质粒.

灭菌：固体；液体 (固体培养基 4.8g粉末 + 150 ml纯水，灭菌后加 0.015g Amp ✓

液体培养基 2.5g粉末 + 100 ml纯水)

8.26 下午

100 μl KJX + 22.5 μl 连好质粒

按KJ说明书做.　　接完菌 ✓

☆ 菌接到平板前未加arabinose (诱导物)，之后补加的arabinose

arabinose 是粉末，取 0.03g (培养基150ml)

0.03g arabinose + 1100 μl 纯水

倒了十一个平板，每个平板加100 μl arabinose溶液

培养，筛长出绿色菌落为连接成功

8.28 下午.

酶切 tet 片段：~~P5+4 并 BlpI~~

t1　16.1 ng/μl　　t2　19.2 ng/μl.

BlpI.　2μl Buffer.

酶 1μl.

Memo No.
Date / /

8.30 晚.

跑 tetr 切胶. 无杂带.

重新从 tetr 大片段酶切开始.

阿拉伯糖加量: (1mM 浓度: 每平板 100 μl

$$\left(\frac{1\,mol/L}{\text{g/mol}}\right)$$

Maracbinose = 342.3 g/mol

共 5 个板 500 μl 溶液 $500 \cdot 5\times10^{-4}$ mol arabinose

质量: $5\times10^{-4}\times342.3 = 0.17$ g

即 0.17 g + 500 ml 纯水

荧光显微镜观察 8.26 培养的菌(放冰箱), 现象不明显.

菌落成大片, 可能是接种时未涂匀, 今天将荧光观察稍有亮点菌落挑取 → 200 μl LB+Amp 液体培养基稀释 → 涂至 LB 平板 (未放培养箱因关门了)

次下次实验, 找绿色菌落, 荧光观察时, 多观察几个, 在平板背部标记, 将有标记的菌落挑至液体培养基, 培养后可提质粒

8.31

Memo No.
Date / /

tetr ~~8~~781 bp 收回

1. ~~稀释液~~ ~~2.24 → 1.16 → 1.16/1.08~~ 1:08

2. 胶块称重：0.05g（0.1g → 100ul 1P ⇒ 加入 50ul PN

溶解后回收，再加 ~~50~~ 30ul pN（稀释离心管间歇）

⇒ 总数体积 160ul

3. 加入CA2. 静置 2min，离心.

4. 加 600ul PW.

~~5~~ …5. 加入 EB 洗脱液 约 35 ul

（收集滤液重复离心 2 次

→ 换 1.5ml 离心管收集（浓度待测）6.9 → 6.9

去试酶切 6.9ng

tetr 13ul

Buffer 2ul

BIP1 1ul

DNA 1ug 13uL

PstI 1uL SpeI 1uL

Buffer 2uL

37°C 1h

tetr 基因无条带

无法回收

~700bp 胶回收

下午：胶回收长 tetr 片段，测定浓度 8.6 ng/ul

Memo No. ___
Date / /

9.11 上午

酶切 arac, tetr. 体系同第1页 (arac 双酶切)

电泳 泳一 泳二 泳三 泳四

Marker arac tetr tetr

9.11 下午

质粒用完了，从菌液提质粒，但保藏时间过长，菌沉底，不是新鲜菌液的悬浊状，可能影响结果

质粒测浓度： a1 a2 是一个东西 t1 t2 是一个东西

a1 493.8 ng/ul

a2 521.9 ng/ul

t1 149.7 ng/ul

t2 119.1 ng/ul

9.12 上午

酶切 a1, t1.

a1 底物 2.02 ul t1 底物 6.68 ul

buffer 2 ul buffer 2 ul

KpnI SalI 2 ul PstI SpeI KpnI SalI 2 ul

ddH2O 14 ul ddH2O 9.32 ul

电泳 右→左 泳一 泳二 泳三

Marker arac tetr

5.68 x   18-x

8.8×10⁻⁵ x ÷ 20 = 1000

Memo No.
Date / /

9.12 下午

切胶回收 Arac 0.16g tetr 0.2g
160μL 200μL

8.8ng/μL I 4ng/μL

8.8ng/μL 0.5μg ~~8μL~~

~~24μL~~ DNA ~~5.68μL~~ 6μL

~~4μL~~ Buffer 2μL

~~2μL~~ BLPI 1μL

ddH₂O 11μL

37°C 1.5h

电泳 (30mL) 30min

可见两条目标条带但量较少 4℃保存

9.12 晚

arac单酶切 切胶 存4℃ 冰箱上层 蓝色盒 左下

胶回收 测浓度 5.8ng/μL

Memo No.
Date / /

9.14 晚.

pptrc99a 53.3 ng/μL.

arae. kpnI. SalI 493.8 ng/μL

Tetr. PstI SpeI. 149.7 ng/μL

ptrac99a. KpnI. SalI. 53.3 ng/μL 18.8μL

Muker Tetr Arac Ptrc

① ② ③ ④

总管: 1.00g

↓

ptrc99a: 0.16g

tetr: 0.10g

arae: 0.09g 15.1 12μL

Memo No.
Date / /

9.9日7

枪头 PCR管 培养基灭菌 倒平板

加卡那霉素备用

连接

arac	1.2μL
ptrc99a	3μL
Buffer	2μL
Ligase	1μL
H_2O	2μL

共 16:00~18:00 25°C 2h 4°C保存

明天：

开制冰机. 准备液体培养基、卡那霉素、阿拉伯糖

冰上融化感受态

△涂布前先加诱导物

连接体系在30℃恒温水浴锅4°C

Memo No.
Date / /

9.20 晚

K12
50 ml → 25ng DNA.

906.4 ng/ul 100 ul → 50 ng DNA.

稀释：0.5 ul 连接产物 加到 4ul 4.5 ul ddH2O 中

1 ul 连接产物 → 100 ul K12

涂板 5 个：

IPTG + ~~ara~~ 阿拉伯糖：2板

IPTG：1板

阿拉伯糖：1板

无：1板

· 1mM 阿拉伯糖：3.4 g → 10 ml 水

~~1mM~~ IPTG

238.3 g/L = 1M.

0.238 g/L = 1mM. 10mL.

0.0238g

0.1 M 阿拉伯糖：0.15g → 10ml 水

0.1 M IPTG：0.238g → 10ml 水