

# Pull plasmids from Library

11 Nov 2018

1	K118023	Cellulase	Amp <sup>R</sup>	2009 distribution
2	<del>E1033917</del>			
2	E1010	RFP	11N	Plate 3
3	K1033919	Purple	9K	6
4	K1033916	Yellow/Green	6M	4
5	K1033933	Pink	2K	6
6	K1323010	YFP	8P	5

## Transformation

Add 1µl (λ) plasmid to tube of cells

Ice 2 min

42°C 45 sec

Ice 2 min

Add 1ml LB broth  
37° overnight (O/N)

# Plasmid Prep

PA/4

<del>2</del> K118023 A	Cellulose	Plate 1
2 K118023 B	Cellulose	plate 1
2 K118023 C	cellulose	plate 1
E1010 A	RFP	plate 2
E1010 B	RFP	plate 2
E1010 C	RFP	plate 2

1/10/19

K118023 A

K118023 B

K118023 C

RFP: RA

RFP: RB

RFP: RC

Control: C and the frag colored bio LIC

Positive Control: P

L1: Long Ladders

L2: control C

L3: positive control ?

L4: R too small not sure

L5: T maybe

L6: A failed

L7: ~~Long Ladders~~ B failed

L8: B C failed

L9: C Long Ladders

L10: RA some possible bands

L11: RB failed

L12: RC failed

Plasmid Preps

1/17/19

K118023 D Cellulose  
K118023 E Cellulose

PCR

1/18/19

K118023 D → D  
K118023 E → E  
102 Old → O  
102 II → II  
Control → C

Gel for PCR

1/22/19

Black (-)

D E O II C

Red (+)

# Plasmid Preps

1/29/19

K118023 D

K118023 E

## PCR

1/31/19

Buffer	2.5 $\mu$ l X 3	7.5
Forward Primers	0.5 $\mu$ l X 3	1.5
Reverse Primers	0.5 $\mu$ l X 3	1.5
Tag	0.2 $\mu$ l X 3	0.6
dNTPs	1.0 $\mu$ l X 3	3.0
d water	13.3 $\mu$ l X 3	45.9
		<hr/>
		60 $\mu$ l

blacic

C = control

C 1 2

~~A~~

red

~~B~~

1 = K118023 D

2 = K118023 E

Look into the fridge for your gel. (your samples are in the same bag).

02/07/19

Did digest for K118023E

√ 24 μL of dH<sub>2</sub>O

√ 4 μL of Cwt smart

√ 1 μL of EcoRI

√ 1 μL of PstII

37°C more than 30min  
incubator

80°C for 10 min

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2/7/19

# PCR

Buffer	2.5 $\mu$ l	<del>10</del> $\times$ 3 = 15	15 $\mu$ l
Forward Primers	0.5 $\mu$ l	<del>2</del> $\times$ 3 = 6	6 $\mu$ l
Reverse Primers	0.5 $\mu$ l	<del>2</del> $\times$ 3 = 6	6 $\mu$ l
Tag	0.2 $\mu$ l	<del>1</del> $\times$ 6 = 6	6 $\mu$ l
dNTPs	1.0 $\mu$ l	<del>1</del> $\times$ 6 = 6	6 $\mu$ l
d water	15.3 $\mu$ l	<del>1</del> $\times$ 6 = 6	6 $\mu$ l

~~20  $\mu$ l  $\times$  3 = 60  $\mu$ l~~  
100  $\mu$ l = 20  $\mu$ l

- C = Control
- 1 = K118023 A
- 2 = K118023 B
- 3 = K118023 C
- 4 = K118023 D
- 5 = K118023 E

2/26

made competent cells

3/5

made 2 plain plates &  
17 chlor plates

3/12

Digest N&M

16  $\mu$ L  $\phi$  X 2  $\mu$ L cut smore buffer 2nd

4  $\mu$ L  $\phi$  X 0.5  $\mu$ L EcoRI } 3rd

4  $\mu$ L  $\phi$  X 0.5  $\mu$ L PstI }

96  $\mu$ L  $\phi$  X 12  $\mu$ L dH<sub>2</sub>O 1st

✓ 

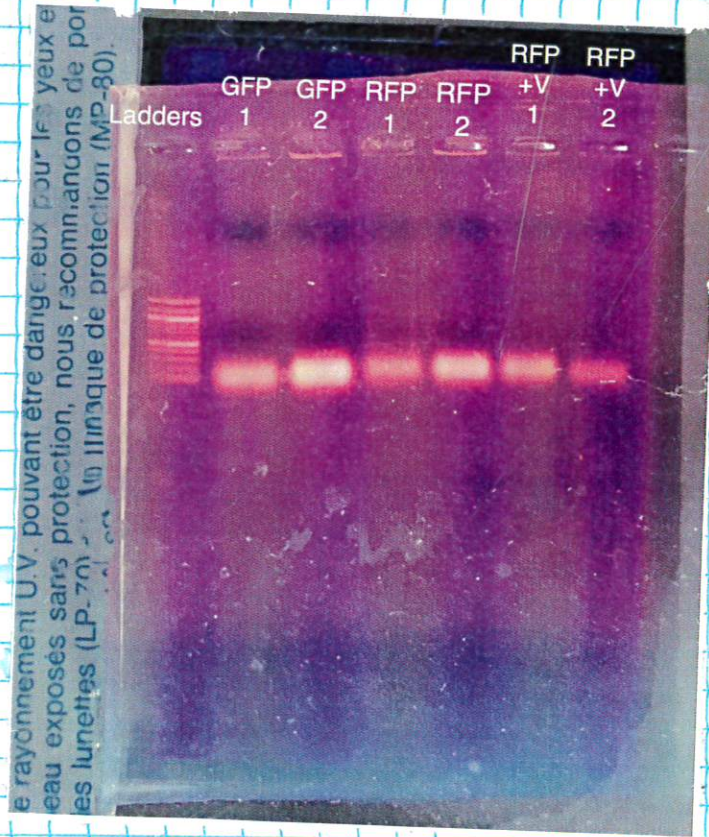
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 120  $\mu$ L  $\phi$  X 15  $\mu$ L  $\Sigma$



~~Digest~~ Digest Gel

3/13



Handwritten notes in French, including "Ladder", "GFP", "RFP", and "RFP +V".

3/14/19

Digest

~~Practitioner~~

MM

Cutsmart Buffer (10x) <del>10x</del>	2 ml	X 8	16 ml
Eco RI	.5 ml	X 8	4 ml
PST I	.5 ml	X 8	4 ml
dH <sub>2</sub> O	12 ml	X 8	96 ml
			120 ml
			↓
			15 ml each

RFP 1	} 5 ml each
RFP 2	
RFP v1	
RFP v2	
GFP 1	
GFP 2	

Pulled K592015 & K592012 one of  
registry ~~and per~~

Transformed K592015 and K592012.  
They were plated.