Lab Note

08/18(Wed)

nematode(Bx)&fungus(Ac) subculture (Day 1)

Bx:1 test tube(PDA slant) ； Ac:2 test tubes(PDA slant)

08/21(Sat)

MS medium + pine callus cultivation (之怡):

MS medium(500ml) contents:

MS	sucrose	inositol	6-BA	2,4-D
2.37g	15g	500mg	2mg	5mg

consume(30 *Pinus armandii* seeds):
(1)200ml: 14 embryo

(2)50ml: incomplete embryo

08/22(Sun)

	nanodrop(ng/μl)		nanodrop(ng/μl)
BxPel s	59.43	BxPel as	20
BxPel xs	34.89	BxPel x as	34.26
Prx s	21.42	Prx as	314.24
Prx x s	201.27	Prx x as	34.26

Fast-Run™ 2X Taq master mix

Tm: 57℃

PCR outcome:

	BxPel x	BxPel	Prx	Prx x
nanodrop(ng/μl)	17.91	45.5	25.6	24.4
reduce Tm	failed			55℃

#BxPel SDS-PAGE running failed

Troubleshooting:

(1) add the wrong primer
(2) put in Taq mixture for a too long period

08/23(Mon)

PCR (50μl)

	BxPel	BxPel x	Prx	Prx x
DNA(λ)	2	2	4	1
ddH_2O(λ)	21	21	19	22

Fast-Run™ 2X Taq master mix

Tm: 52℃

PCR outcome:

	nanodrop(ng/μl)
BxPel	39.9
BxPel x	52.5
Prx	48.7
Prx x	33.0

Digestion (100μl)

Xho1	1λ
Kpn1 HF	1λ
10x buffer (CuISmart)	10λ

	BxPel	BxPel x	Prx	Prx x	plasmid
DNA(μl)	40	40	40	40	40
ddH_2O(μl)	48	48	48	48	48

#digestion: the higher amount of DNA is better

dry bath 37℃ for 75min

clean with 30μl ddH_2O

placed at -20℃ refrigerator

08/25(Wed)

Digestion

	digestion conc.(ng/μl)
BxPel	17
BxPel x	15
Prx s	5
Prx x	15
plasmid	4-->11-->7

Bacteria cultivation
#raise plasmid concentration.

08/26(Thu)

miniprep

	(ng/μl)
1	44.87
2	54.49
3	65.42

Bacteria cultivation
(x3)5ml LB agar + 2μl E.coli

08/27(Fri)

nematode(Bx)&fungus(Ac) subculture (Day 9)

PDA 100ml sterilize and make 18 test tubes

Bx: 1 test tube(PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

miniprep (5ml LB overnight culture)

	(ng/μl)
1	112
2	68
3	58

Digestion(Kpn1-HF,Xho1)+cleanup

Nanodrop	(ng/μl)
1	36.4
2	44.22
3	51.24

MS medium + pine callus cultivation

5x MS medium(500ml) contents:

MS	surcose	6-BA	2,4-D
11.85g	75g	10mg	25mg

consume10 *Pinus armandii* seeds(total 40):
(1)150ml: 7 embryo

08/28(Sat)

Ligation

BxPel	5:1
BxPel x	3:1
Prx x	3:1

08/29(Sun)

PCR Fast-Run™ 2X Taq master mix

Tm: 55℃

	(ng/μl)
BxPel 1	17
BxPel 1 x	44.8
Prx	30.5
Prx x	25

reconstitution:50μl

*SDS-PAGE condition:
BxPel, Prx appear in multiple bands; but there is no this problem at Tm=52℃ and have better productivity at that temp.
→ PCR ™ reduce to 52~53℃

Add isopropanol or make more tubes next time to raise concentration.

Transformation:

BxPel , BxPel x , Prx x, NC(negative control)

(on LB ampicillin plate)

08/30(Mon)

Transform failed:
there is a colony in the NC plate, which suggests digestion may be the problem, but only one band appears in SDS-PAGE show it really digests.

-->**do miniprep and run SDS-PAGE again**, LB plate with ampicillin
SDS-PAGE result: digestion success (only one band) ； result of miniprep is not well(8/27)

though there are several bands, the band at 6kb is not obvious.

LB plate with Ampicillin(100μg/ml) x10

08/31(Tue)

because of the problem for concentration after digestion clean up, **do ligation again**

Ligation

vector:100ng (6μl)

NEBio Caculator

BxPel	60ng(3:1)	3.5μl
BxPel x	17.5ng(3:1)	1.2μl
Prx x	17.5ng(3:1)	1.2μl

total:20μl
10x buffer 2μl + T4 ligase 1μll

09/03(Fri)

nematode(Bx)&fungus(Ac) subculture (Day 16)

Bx: 3 test tubes (PDA slant with Ac) ； Ac: 6 test tubes(PDA slant)

09/04(Sat)

pine callus cultivation

1.passage#1 8/21 callus 200ml
(2.37g MS+5mg 2,4-D+2mg 6-BA+15g sucrose+0.5g inositol)

2.passage #2 8/27 callus 150ml(5x medium dilute)
(2.37g MS+5mg 2,4-D+2mg 6-BA+15g sucrose)

MS medium

1.2.37g MS+5mg 2,4-D+2mg 6-BA+15g sucrose+0.5g inositol-->500ml(1x)

2.2.37g MS+5mg 2,4-D+2mg 6-BA+15g sucrose+0.5g inositol+2mg kinetin

accumulation:50

09/10(Fri)

nematode(Bx)&fungus(Ac) subculture (Day 23)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 7 test tubes(PDA slant)

09/11(Sat)

PDA 100ml sterilize and make 20 test tubes

09/13(Mon)

pine callus cultivation

cultivation(add inositol+kinetin): 6 embryos, 200ml

accumulation:65-->80

09/17(Fri)

nematode(Bx)&fungus(Ac) subculture (Day 30)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

09/23(Thr)

pine callus cultivation

500ml MS medium + inositol

accumulation:130

09/26(Sun)

nematode(Bx)&fungus(Ac) subculture (Day 39)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

09/27(Mon)

pine callus cultivation

1.1000ml MS medium with inositol

2.passage the strange one (110ml MS+ inositol+kinetin)

3.cultivation
MS+ inositol

150ml	16
150ml	19
50ml	12
50ml	12
50ml	10

10/03(Sun)

nematode(Bx)&fungus(Ac) subculture (Day 46)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

10/04(Mon)

pine callus cultivation

1. Callus x1 (9 embryos)
50ml MS+ inositol

2. 1000ml MS medium with inositol

accumulation: 140 seeds

10/08(Fri)

callus subculture: 2x 150ml, 3x 50ml

10/10(Sun)

nematode(Bx)&fungus(Ac) subculture (Day 53)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

10/17(Sun)

nematode(Bx)&fungus(Ac) subculture (Day 46)

Bx: 3 test tubes (PDA slant with Ac) ; Ac: 6 test tubes(PDA slant)

10/18(Mon)

place the nematode on callus

15/ 10μl, 300μl in total

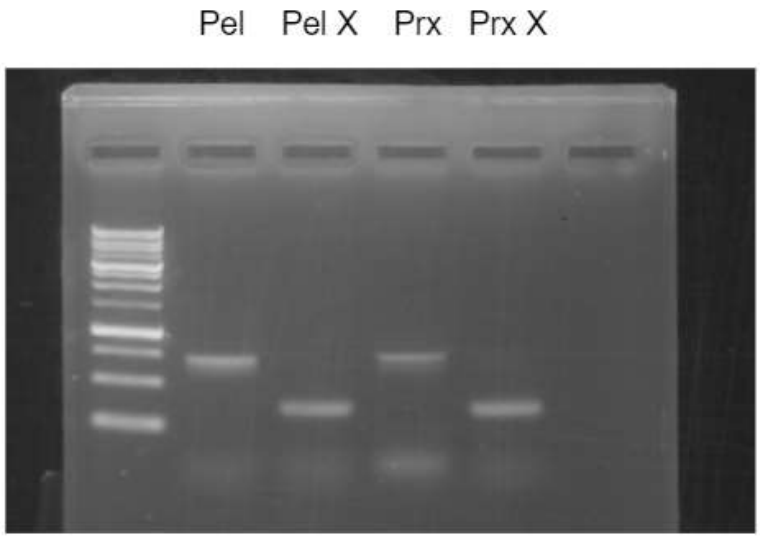
(1) 3 weeks callus(0.8g) : 150μl nematodes (about 225)

(2) 3 weeks callus(0.8g): 150μl ddH_2O

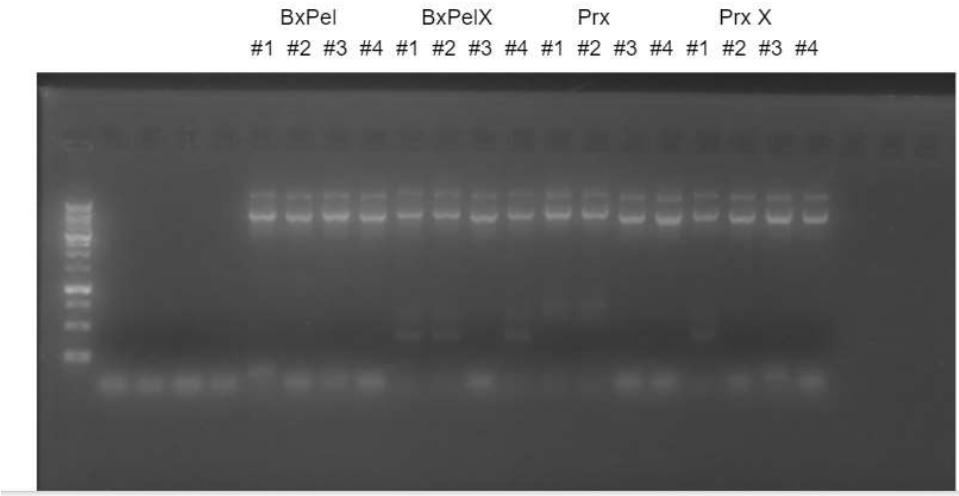
(3) space plate : 150μl nematodes (about 225)

***Cloning**

sense strand PCR

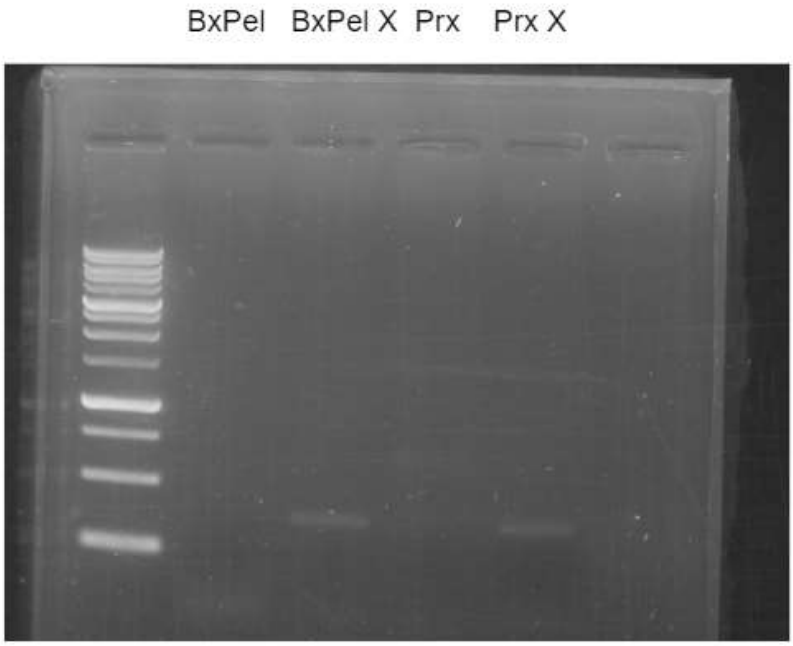


sense-pHANNIBAL PCR check

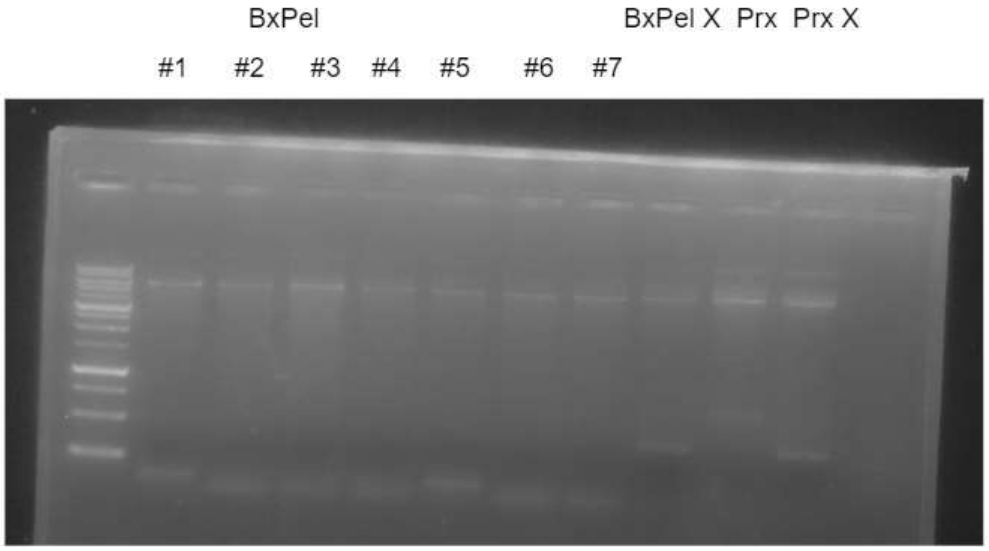


antisense PCR

Tm: 53 ℃, ET: 30s, AT: 50s

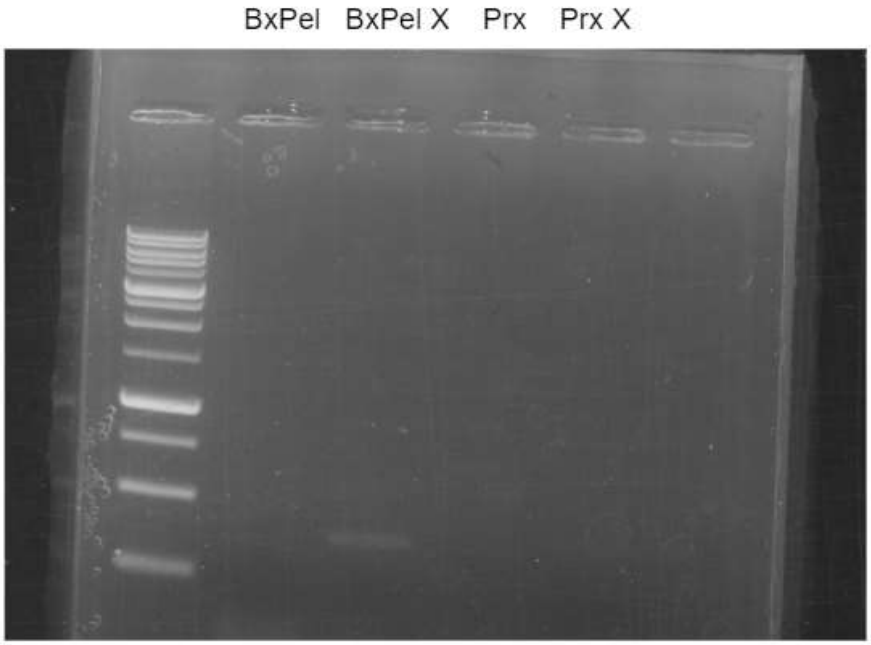


sense-pHANNIBAL PCR check



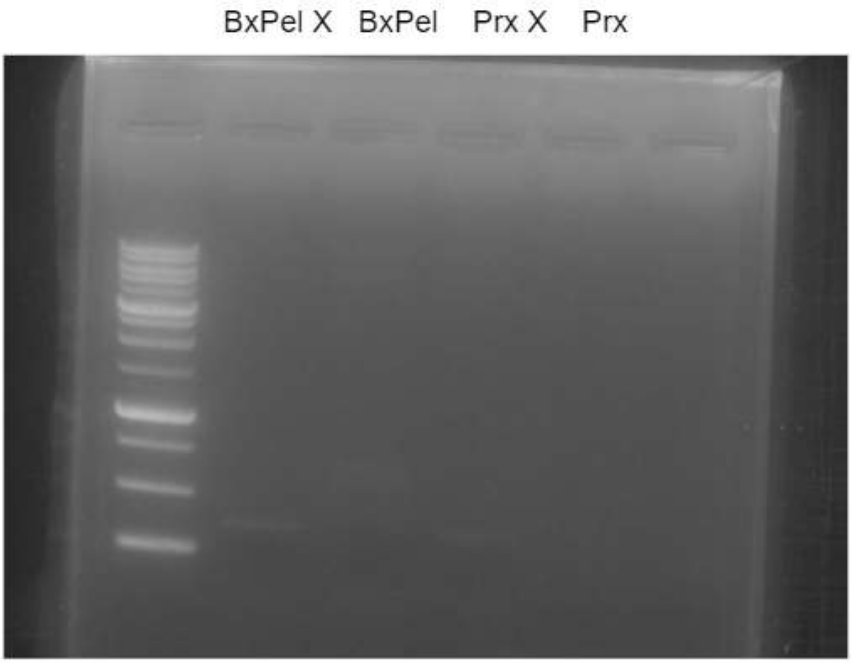
antisense PCR

DNA:PCR products
Tm: 52 ℃, ET: 60s, AT: 30s

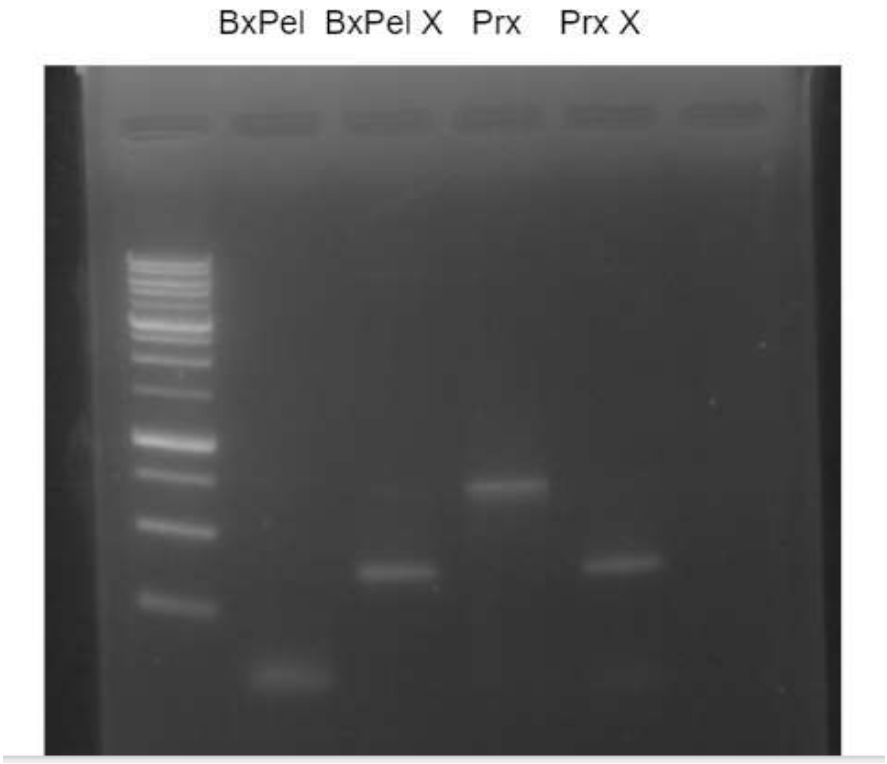


antisense PCR

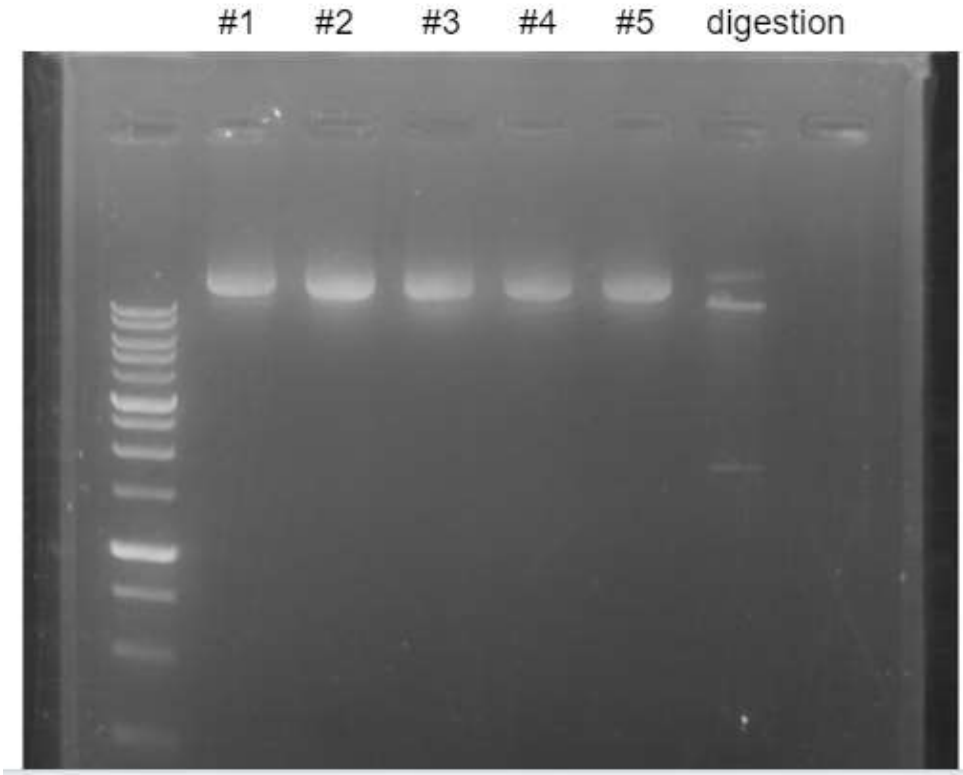
DNA:miniprep products
Tm: 52 ℃, ET: 60s, AT: 30s



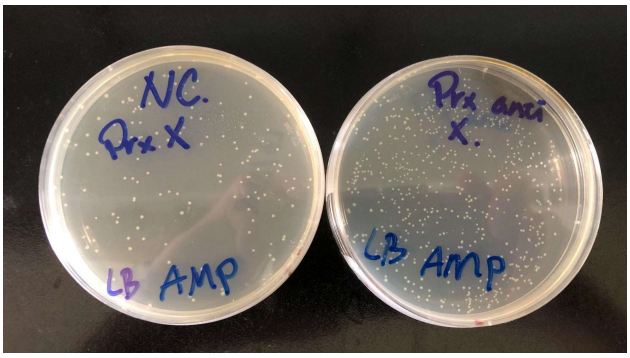
antisense digestion

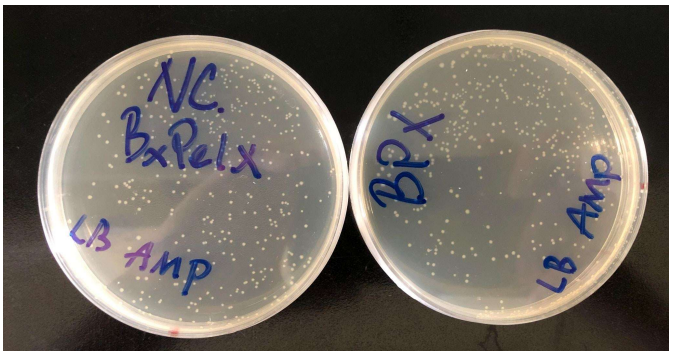


pBI121 & digestion



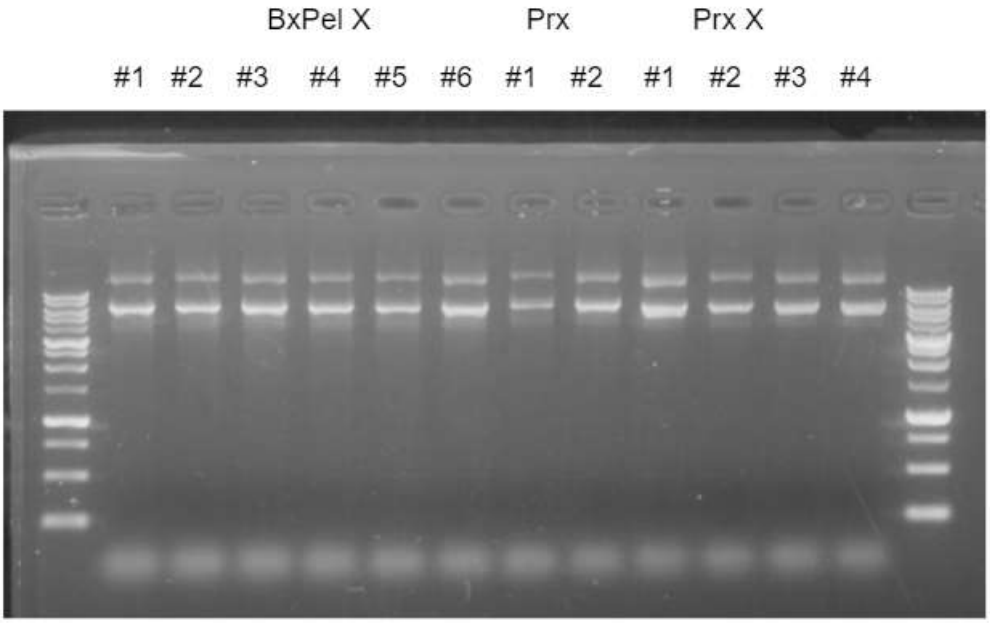
Antisense Tranformation





Antisense-pHANNIBAL PCR check

Tm: 53 ℃, ET: 60s, AT: 30s



Antisense-pHANNIBAL PCR check

Tm: 55 ℃, ET: 60s, AT: 30s