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***fae* Antisense
(*Brassica napus*)**

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(// : // :)

(C22:1)

β -ketoacyl-CoA synthase(KCS)
fae (Fatty Acid Elongase)

C22 C20 C18

(fae)
PCR CTAB DNA

(*HindIII EcoRV*)
pSK

fae

pBI121

pBI121

LBA4404

Agrobacterium tumefaciens

PCR

fae
B. napus

fae

KCS

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(C20:1) (C22:1) / % ()

) () % ()

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()

()

() C18

() () *Brassica napus* ()

() KCS ()

() *fae* ()

-
- 7. co-suppression
 - 8. β -ketoacyl-CoA synthase
 - 9. Fatty Acid Elongase

-
- 1. Cis-13-Decosenoic Acid
 - 2. High Erucic Acid Rapeseed (HEAR)
 - 3. Low Erucic Acid Rapeseed (LEAR)
 - 4. High-temperature lubricants
 - 5. Plasticizers
 - 6. Surface-active agents

... fae Antisense :

)

(

DNA

(HEAR)

PCR

)

(

DNA

fae

()

DNA

)

PCR

()

PCR

(B A

pBluescript II SK (pSK⁺)

fae

SacI

(pSK⁺)

PCR

Cfr9I

) *fae*

(AF274750

E. coli

(*fae*

)

MWG

(DH5α)

SacI Cfr9I

()

(

)

ZSJ7:5'- CACGAGCTC ATGACGTCCGTTAACGTA (27mer)

SacI

ZSJ9: 5'- CATCCCGGGTTAGGACCGACCGTTTTG (27 mer)

Cfr9I

) LB

(

PCR

BglI Cfr9I, SacI

Oligo

fae

%

fae

(Dideoxy-Chain Termination)

PCR

(

)

ZSJ9 ZSJ7

) DNA

PCR

(

/) Mg²⁺

(

3. Templet

4. *Taq* DNA polymerase

5. *pfu* polymerase

6. Proof reading

7. Ligation

8. Competent Cell

9. Sequencing

1. Dellaporta

2. CTAB

PCR

MWG

ZSJ9 ZSJ7

PCR

T7 T3

fae

()

(/ / /)

PCR

pBI121

fae

()

ZSJ9 ZSJ7

*Sma*I

*Cfr*9I

) *Cfr*9I

*Sac*I

LBA4404)

)

(C58pGV3101

pBI121

(

sticky

()

() GUS

fae

(B)

(*fae*)

HEAR

E. coli

)

/

PCR

(

MS

()

/ MS

LBA4404

)

BAP

(

) *npt*II

pBI121

(

)

rpm (pBIZSJ

3. Cotyledonary leaves
4. 6-Banzylaminopurine

1. Construct
2. Freeze and thaw

... fae Antisense :

DNA

DNA T₀

PCR

PCR DG Probe Synthesis Kit (Cat. No. 1 636

Roche 090)

Nos terminator

/ MS)

() Sambrook and Russell

(

)

(IBA MS)

(Southern Blotting)

)

()

T₀ DNA

(

EcoRV

/

DNA

PCR

DNA .

()

(fix)

(Gas chromatography)

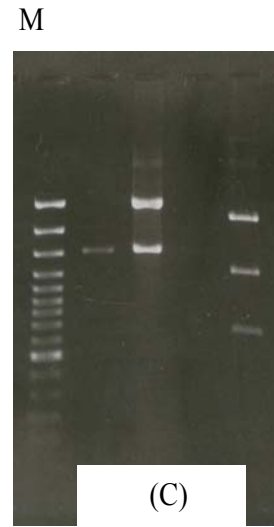
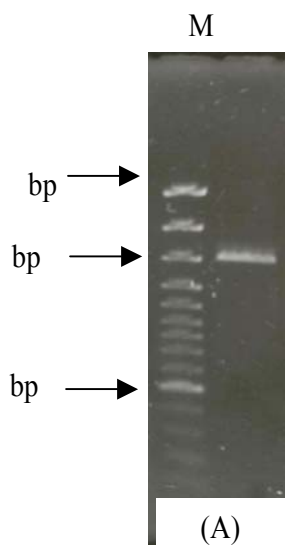
()

(Dot Blotting)

-
1. Infection
 2. Co-cultivation
 3. Shoot Induction Medium
 4. Dot blot
 5. Southern hybridization

(GC)

Hind III *EcoRV* / ()
EcoRV *fae* .
Hind III
 %
 pSK+ .(B)
 pSK+ *fae* .
) PCR
Bgl II *Sac* I *Cfr*9I (PCR
 .(C) Mg^{2+} .
fae / /
 ()
 A
 PCR



M ()
EcoRV *fae* : A .
 () *FAE* : B
*Hind*III ()
 () ZSJ9 ZSJ7 : C ()
 ()
 .() *Bgl* I *Sac* I *Cfr*9I *fae* pSK+
 ()

... fae Antisense :

pBI121 fae () fae
Hind III PCR
pBI121 () (AF274750
fae
HindIII ()
()

(BLAST and Alignment)

SacI Cfr9I

PCR

BAP

B.napus *B.napus*(AF 274750)
B. juncea (BJU 558197) (AF 490462)
B. oleracea (AF 490460) *B. rapa* (AF 490461)
Arabidopsis thaliana (NM- 119617)
% *Simmondsia chinensis* (SCU 37088)
% / % / % / % / % / % /
()

BAP

B. oleracea fae
Simmondsia *Arabidopsis thaliana* *B. juncea*
() % % % *chinensis*

BAP

/

Map

% /

% /

PF

Clustal

/

% /

%

()

(% / % /)

B. napus
(% /) % / (AF 274750)
Simmondsia chinensis (SCU 37088)

B. napus

-
1. *Brassica oleracea*
 2. *B. campestris*
 3. Partial

()

atgacgtccgtaaacgtaaagctccttaccattacgcataaccaacctttcaacctttgcttcttccgtaaacggc
 gatcgtcgcggaaaagcctatcggcttaccatagacgatcttaccacttatactattcctatctccaacacaacctcat
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 attactacgtccggatttcaacttgcattgaccattttgtatacatccggaggcagagccgtgattgatgtgctaga
 gaagaacctagccctagcaccgatcgtatgtagggcatcaagatcaacgttacatagatttggaaacacttcatctagc

fae

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	█	85.9	85.7	85.6	85.5	85.6	85.9	45.1	1	<i>Arabidopsis thaliana (NM)</i>
	2	15.5	█	99.7	99.7	98.6	99.7	100.0	46.4	2	<i>Brassica napus (askari)</i>
	3	15.8	0.3	█	99.9	98.6	99.6	99.7	46.4	3	<i>B. juncea</i>
	4	15.9	0.3	0.1	█	98.6	99.5	99.7	46.3	4	<i>B. rapa (AF)</i>
	5	16.0	1.5	1.4	1.5	█	98.6	98.6	45.8	5	<i>B. oleracea (AF)</i>
	6	15.9	0.3	0.4	0.5	1.5	█	99.7	46.2	6	<i>B. napus (westar)</i>
	7	15.5	0.0	0.3	0.3	1.5	0.3	█	46.4	7	<i>Gene complete1 (FAE)</i>
	8	64.0	61.2	61.2	61.4	60.9	61.8	61.2	█	8	<i>Simmondsia chinensis</i>
		1	2	3	4	5	6	7	8		

(Gene complete) fae (Homology)

. (DNA)

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	█	99.4	99.8	99.0	98.6	99.2	76.3	4.3	1	Gene Complete 1 (FAE)
	2	0.4	█	99.4	99.0	99.0	99.6	75.9	4.3	2	<i>B. juncea</i>
	3	0.0	0.4	█	99.0	98.6	99.2	76.3	4.3	3	<i>Brassica napus (askari)</i>
	4	0.8	0.8	0.8	█	98.6	98.8	75.5	4.3	4	<i>B. napus (westar)</i>
	5	1.2	0.8	1.2	1.2	█	98.8	75.5	4.3	5	<i>B. (oleracea) AF</i>
	6	0.6	0.2	0.6	1.0	1.0	█	75.7	4.3	6	<i>B. rapa AF</i>
	7	28.3	28.8	28.3	29.4	29.4	29.1	█	4.7	7	<i>Arabidopsis thaliana NM</i>
	8	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	█	8	<i>Simmondsia chinensis</i>
		1	2	3	4	5	6	7	8		

(Gene complete) fae (Homology)

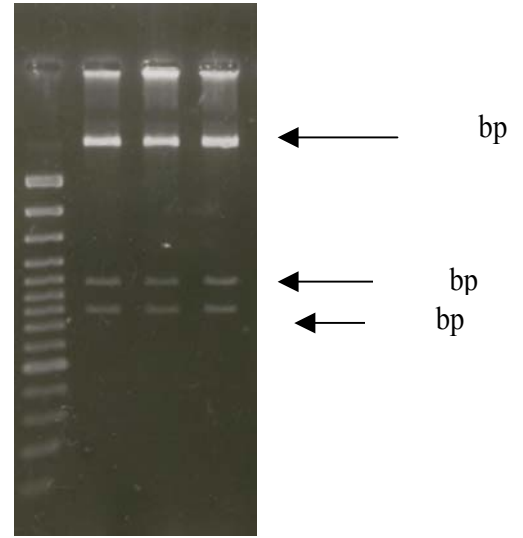
. (DNAstar)

... fae Antisense :

)
LBA4404) (C58pGV3101

% / Map
% / PF

(% / % /)
% / LBA4404
(% /) C58pGV3101



fae pBI121
HindIII

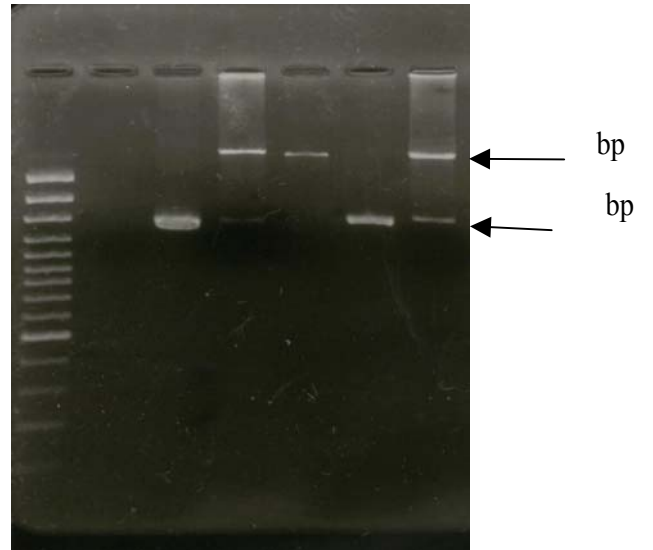
westar

RI25

.()

)

.(



BAP LBA4404 /

ZSJ7

PCR

ZSJ9

C22:1

T-DNA

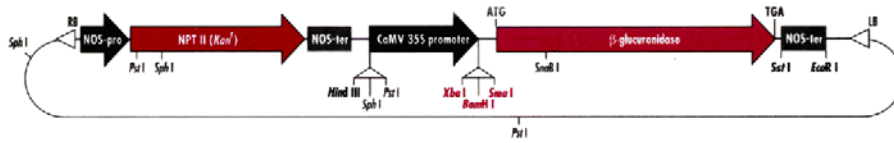
Hind III

pBI121

()

pBI121

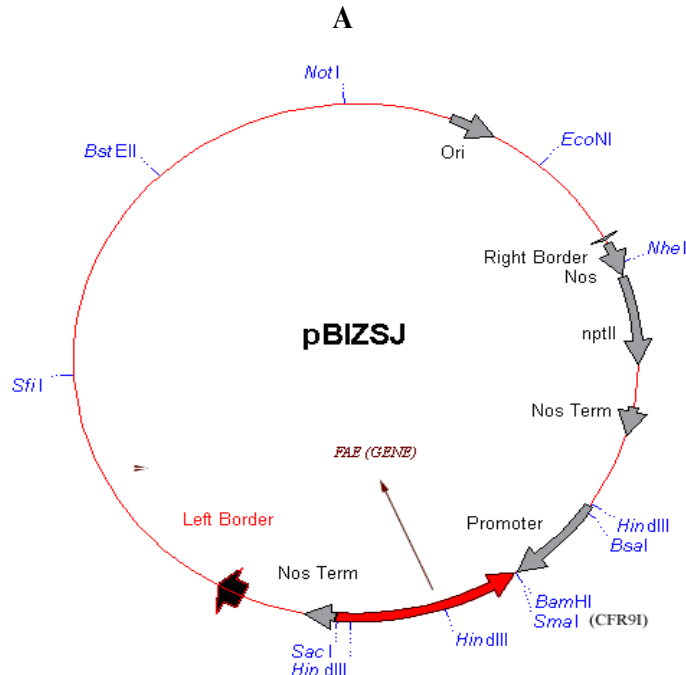
Cfr9 I Sac I



pBI121

Sma I (CFR91)
TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG
Xba I BamHI

from Clontech catalogue 1996 / 97

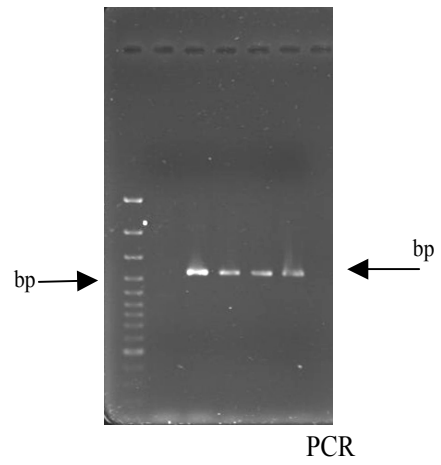
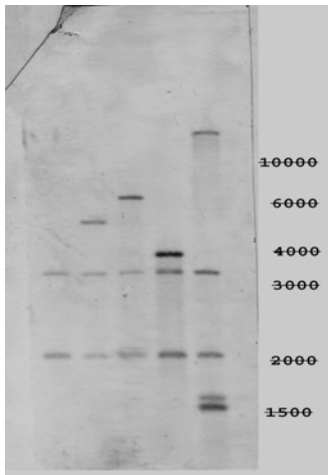


T-DNA pBI121 :A. pBI121
 pBI121 GUS :B.
 fae (pBIZSJ)



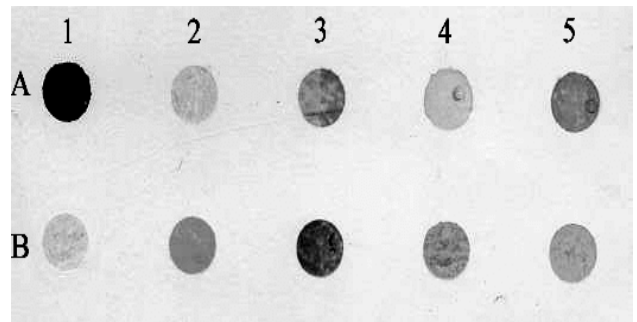
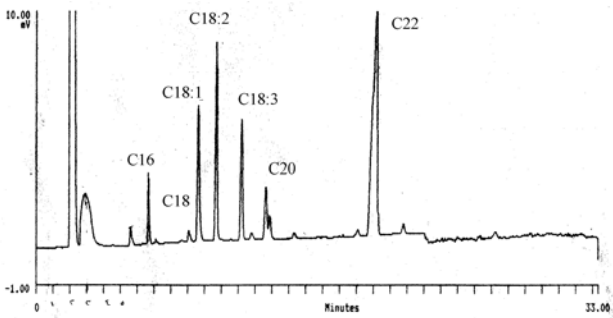
C D A B
 A.
 :C.
 :D ()
 :B.

... fae Antisense :

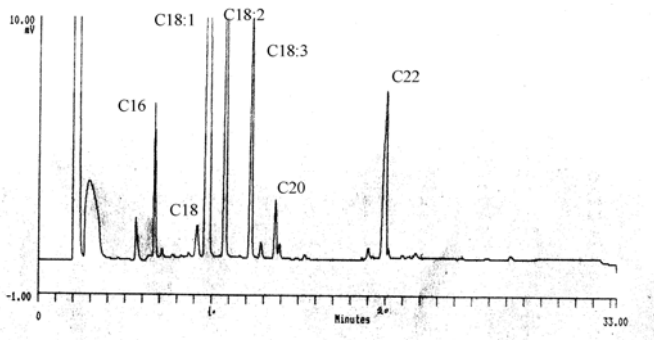


EcoRV DNA .Nos *fae*
() ()

pBI121) : (



fae
pBI121 : A₁
B₁ A₂



() GC
() (C22)

DNA PCR .
fae

()

(Dot blotting)

()

()

fae

(GC)

(0.1% SDS 2X SSC)

RNA

RNA

(0.1% SDS 0.5X SSC)

fae

()

)

()

(MapIus

()

fae

()

EcoRV

()

fae

(%)

(C18)

()

XbaI

% /

% /

(.)

(0.1% SDS 2X SSC)

) high stringency

(

()

fae

()

()

ω 3 ω 6 desaturase

()

α

1. High stringency

... fae Antisense :

() %

fae *B. napus*

()

%

Oleate desaturase

(*B. juncea*)

%

(*B. napus*)

()

GUS GFP

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- ()
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