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(54) **METHOD OF PRODUCING DOUBLE LOW RESTORER LINES OF BRASSICA NAPUS HAVING A GOOD AGRONOMIC VALUE**

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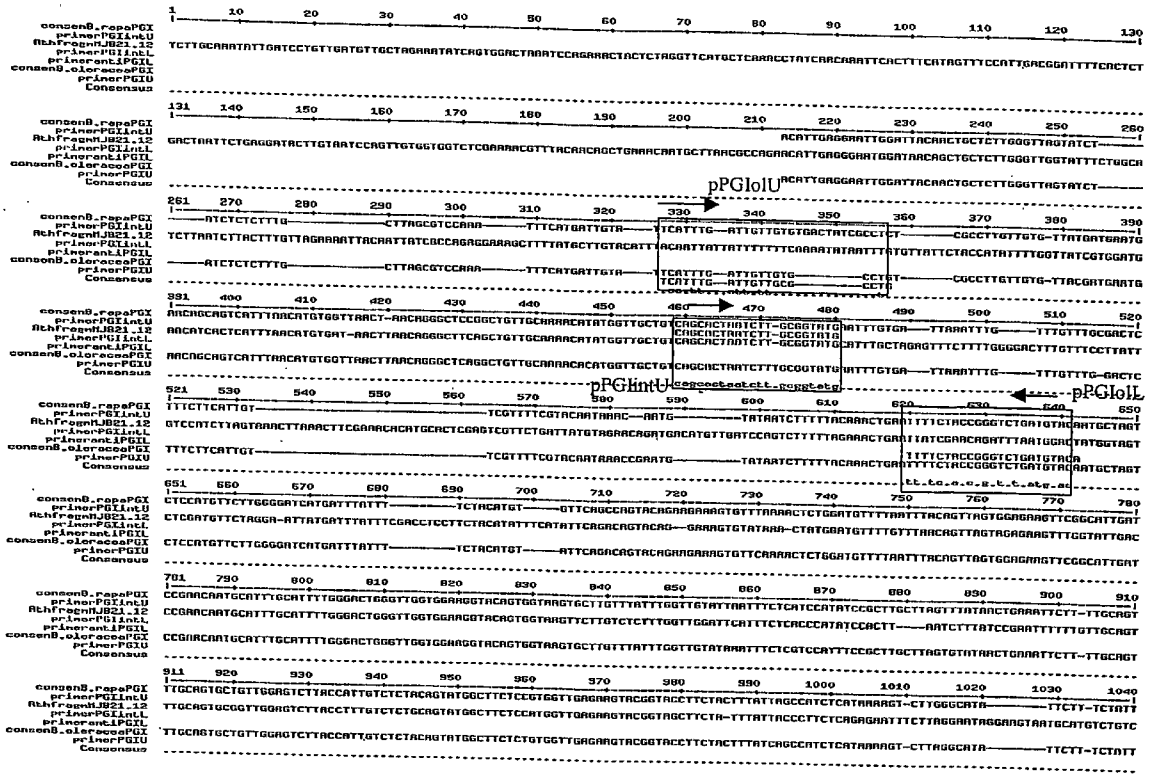
(57) **ABSTRACT**

A method of producing double low restorer line of *Brassica napus* for Ogura cytoplasmic male sterility (cms) pre-senting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. A method of forming *Brassica napus* hybrid seeds and progeny thereof. The seeds of *Brassica napus* and use of the combined markers PGIol, PGlunt, PGlint, BolJon and CP418 for characterising.



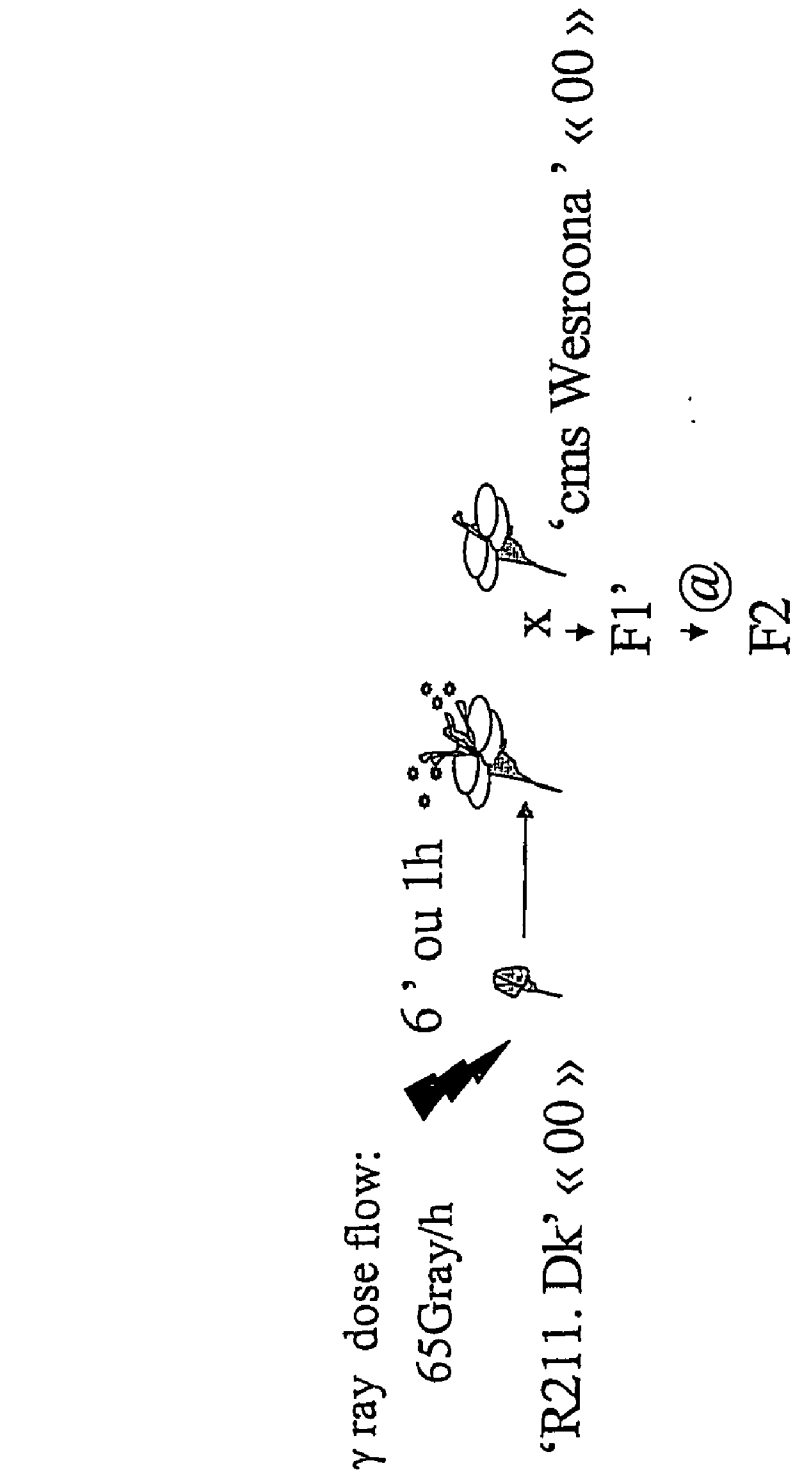

γ ray dose flow:
65Gray/h
6 ' ou 1h
'R211. Dk' « 00 »
x
'cms Wesroona ' « 00 »
F1'
@
F2

Fig 1

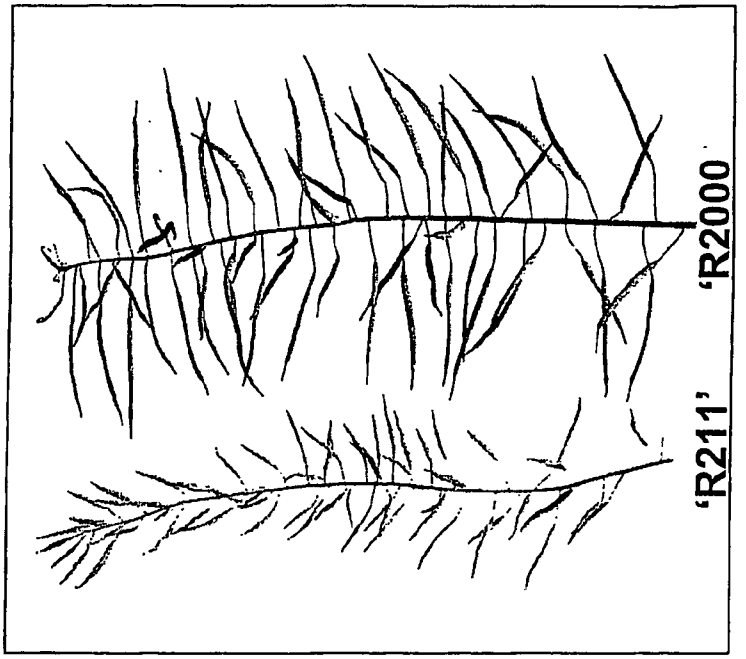


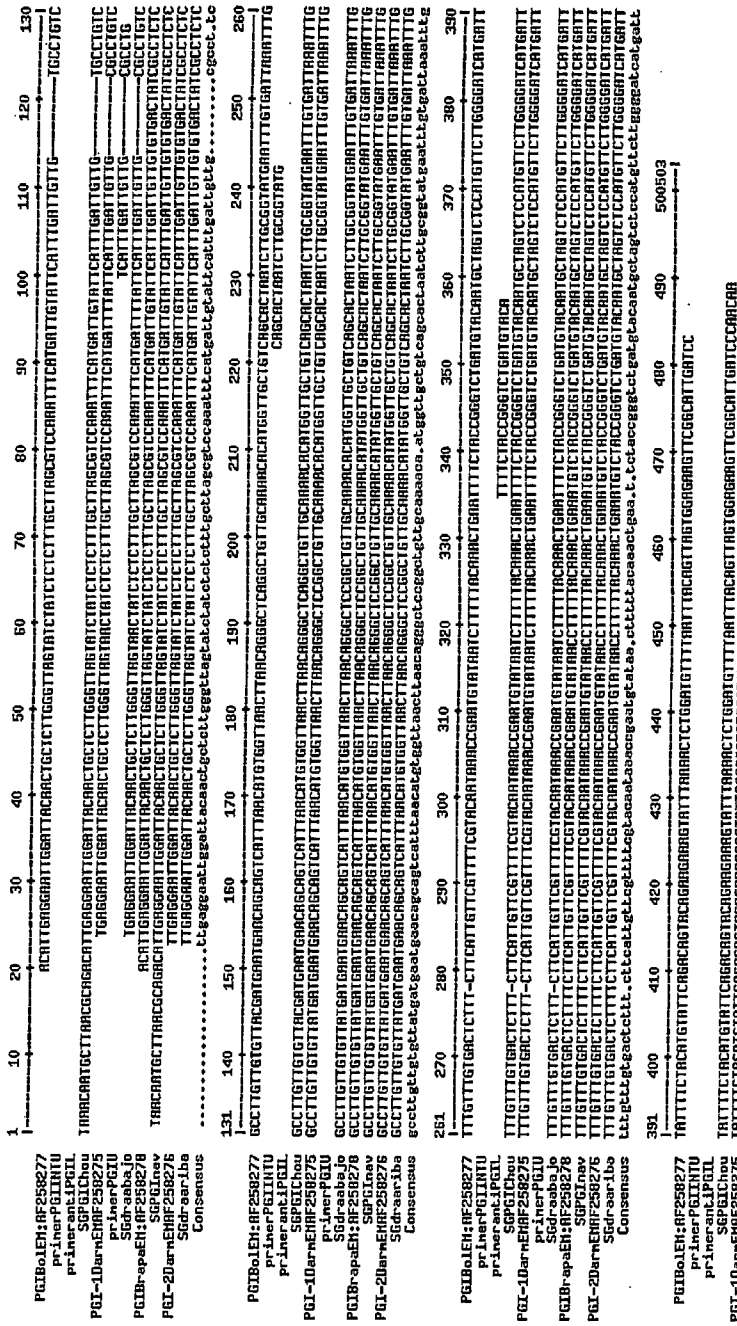
Fig. 1: Seed set on 'R211' and 'R2000'

Fig 2

Genotype	Selfings	Test Crosses
Drakkar	29.3	
Pactol	23.1	
R211	11.2	25.5
R2000	**26. 5** (24.0 – 31.1)	**27.0** (24.0 – 28.7)

Fig 3

Fig 4



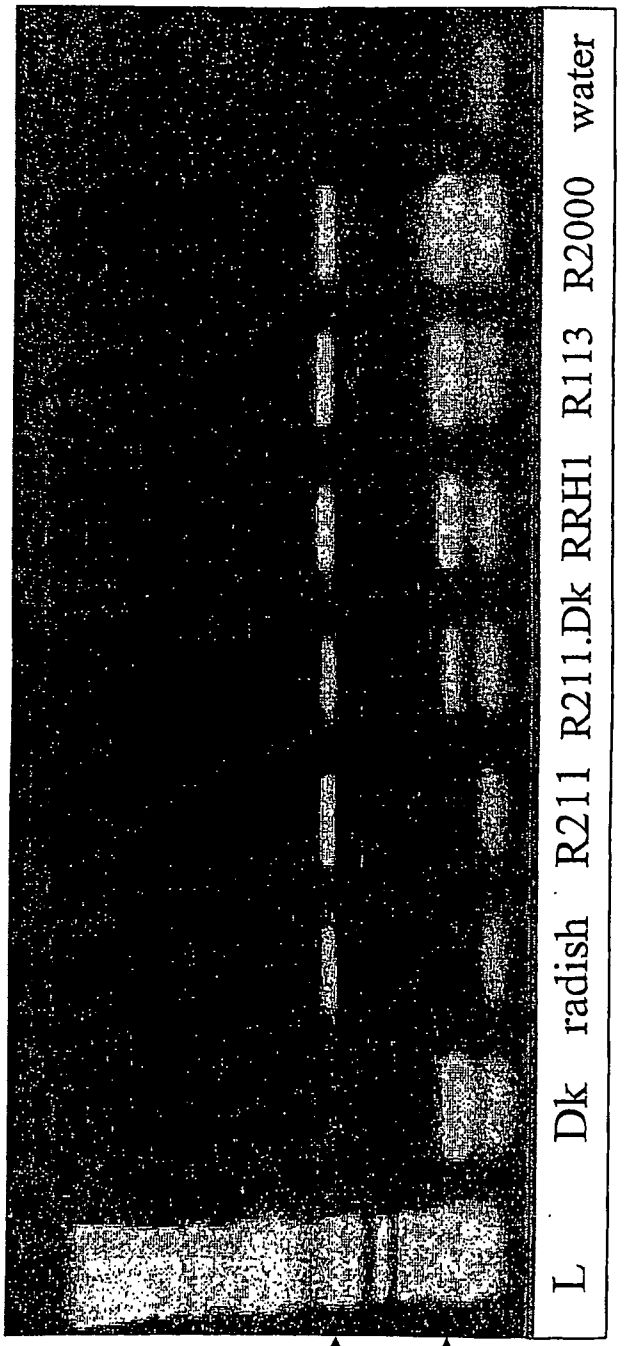
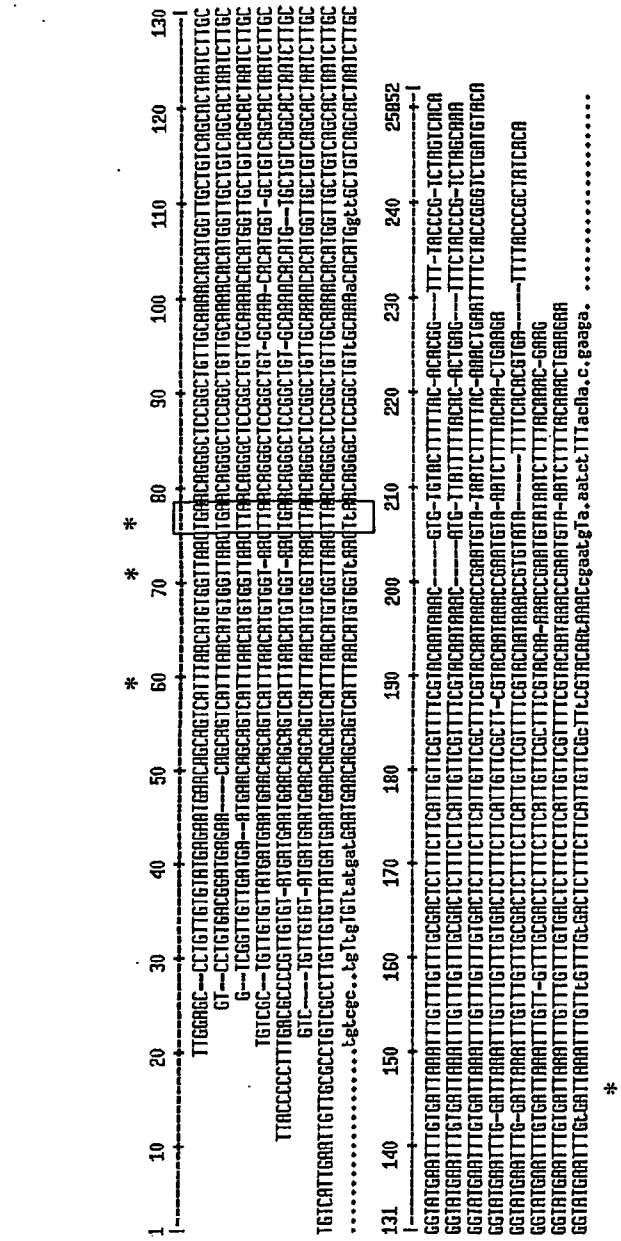

L
Dk
radish
R211
R211.Dk
RRH1
R113
R2000
water
SG91 1kb
PGIol 278bp

Fig 5

Fig 6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DraPGIUcor
HetepPGIUconsensus
Re1-15-1pPGIU
HeLantipPGIL
Consensus
SampPGIUconsensus
DraPGIUcor
HetepPGIUconsensus
Re1-15-1pPGIU
HeLantipPGIL
Consensus
MseI sites
35bp
11
5
70
105bp
T in Drakkar and R2000
MseI restriction enzyme cut DNA sequences at the T/TAA sites (*)

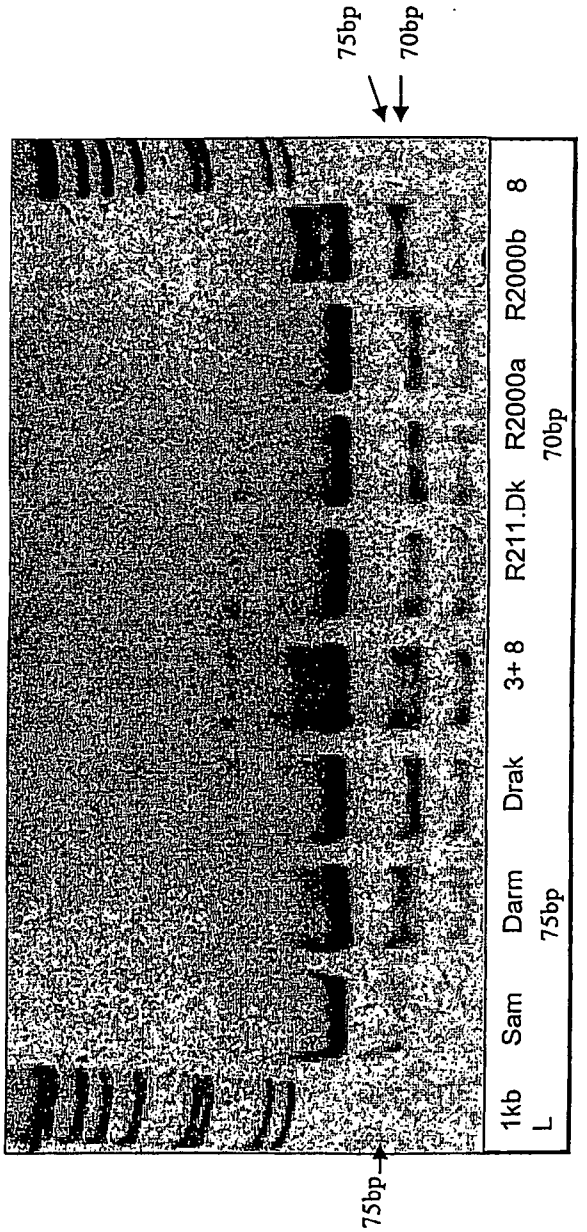

75bp
70bp
1kb L
Sam
Darm
75bp
Drak
3+ 8
R211.Dk
R2000a
70bp
R2000b
8
75bp

Fig 7

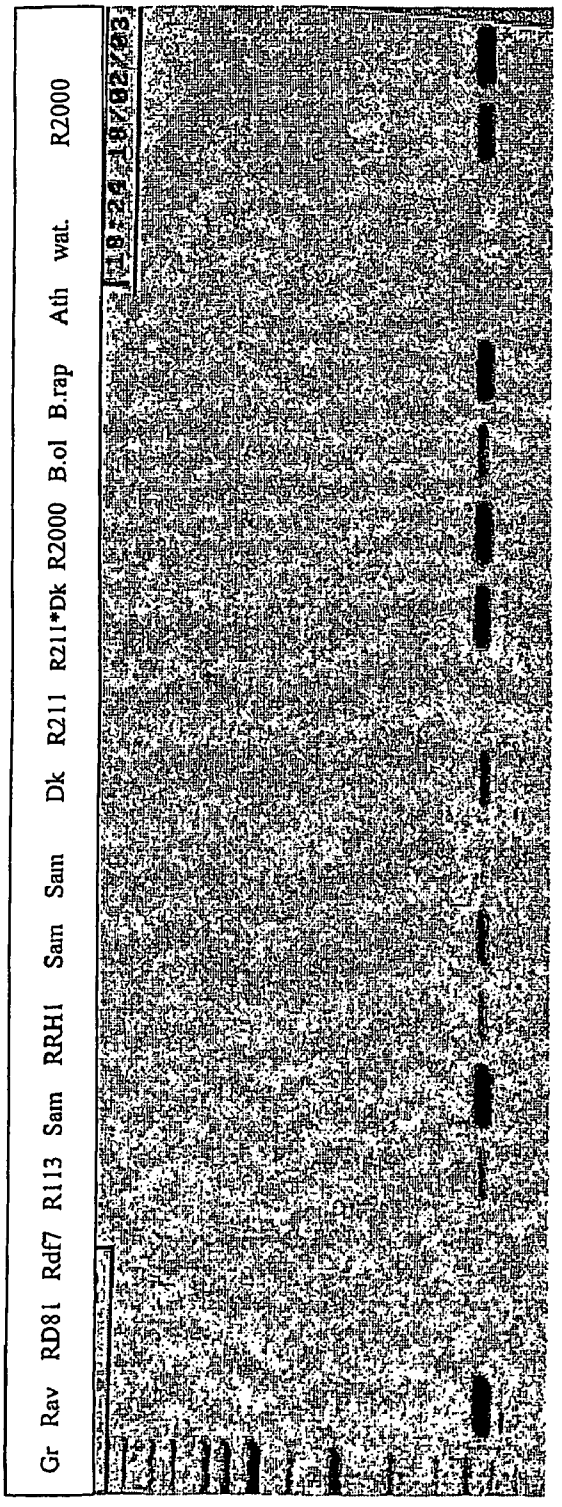

Gr Rav RD81 Rdf7 R113 Sam RRH1 Sam Sam Dk R211 R211*Dk R2000 B.ol B.rap Ath wat. R2000
950bp

Fig 8

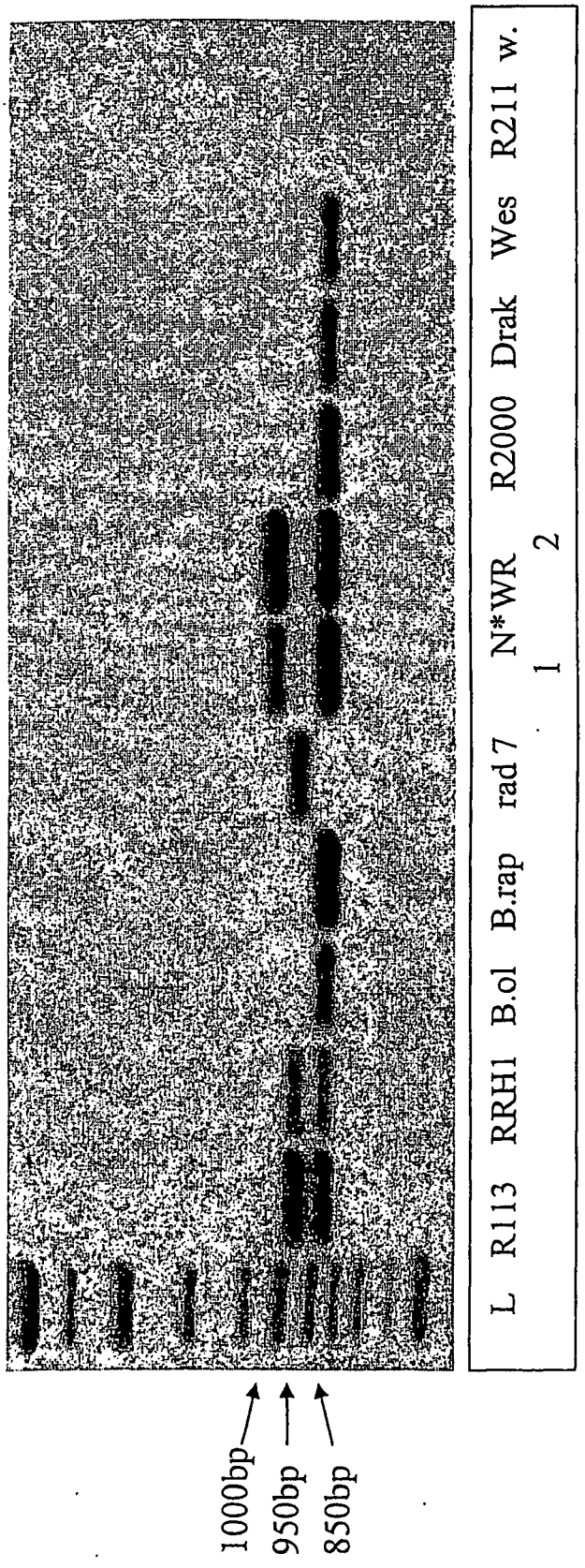
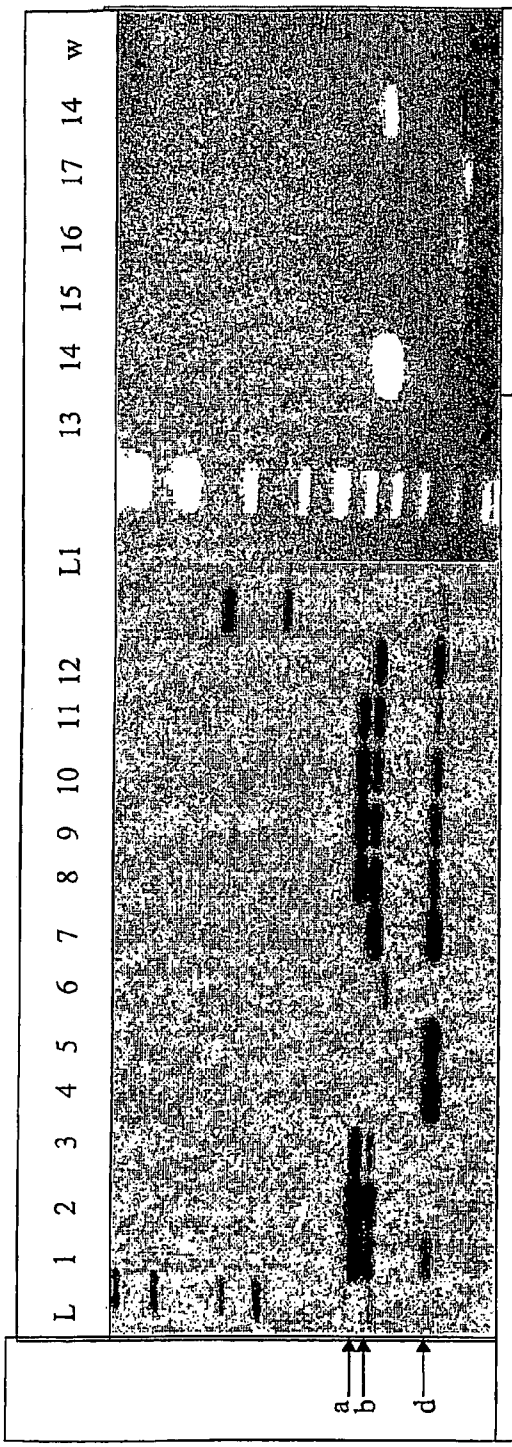
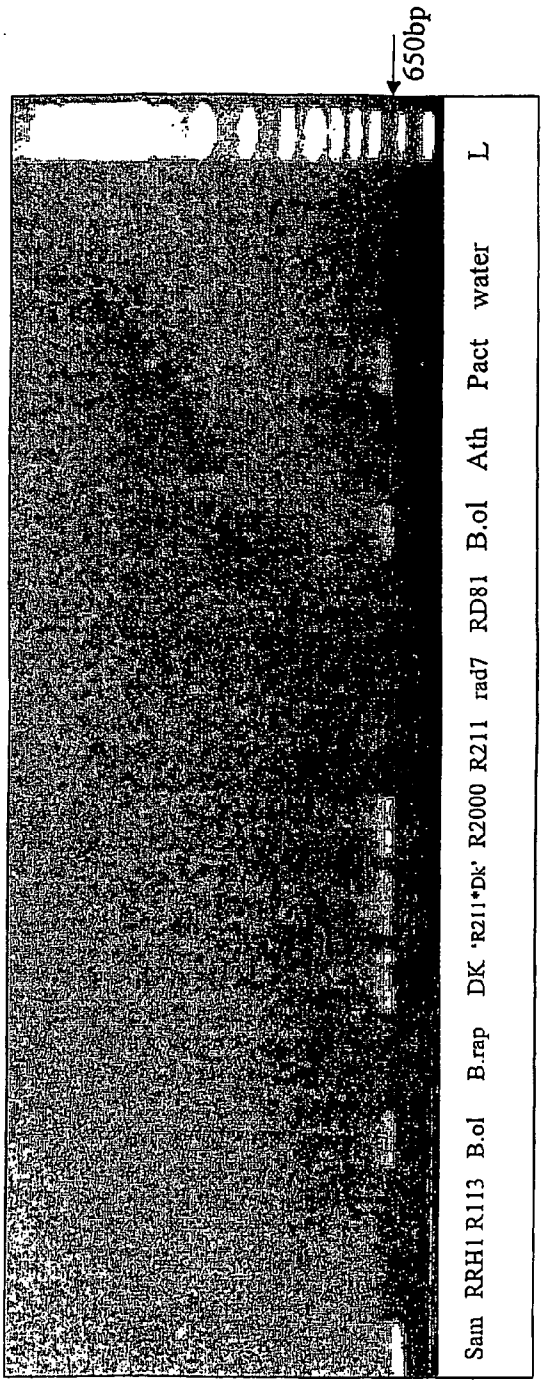

L R113 RRH1 B.ol B.rap rad7 N*WR R2000 Drak Wes R211 w.
1 2
1000bp
950bp
850bp

Fig 9

Fig 10



BolJon PCR bands (bp)	n°	a	b	c	d	e
4,5	radish species:line 7				600	
17	radish RD8i					590
14	B.rapa species		870			
13	B.oleracea species	950				
6	Arabidopsis thaliana (WS)			800		
2, 3	maintainer rapeseed: 2 Samourai; 3 Pactol	950	870			
7,12	homozygous restored rapeseed: 7 Samourai RRH1, 12 R211	0	870		600	
11	incomplete restored SRfi	950	870		600	
1,8	heterozygous restored rapeseed: R211.Dk	950	870		600	
9,10	homozygous restored R2000: 6 lineE38, 8,18,19 line R1.15	950	870		600	
L, L1	ladder1kb+, Ladder 100bp					



The CP418 band (about 650bp) specific to the B.oleracea genome.
It is present in B.ol, B.napus (Samourai, Drakkar, Pactol and the herterozygous R211*Dk)
It is absent from the restored rapeseed (RRH, R113 and R211)
It is present in the homozygous R2000.

Fig 11

Figure 12

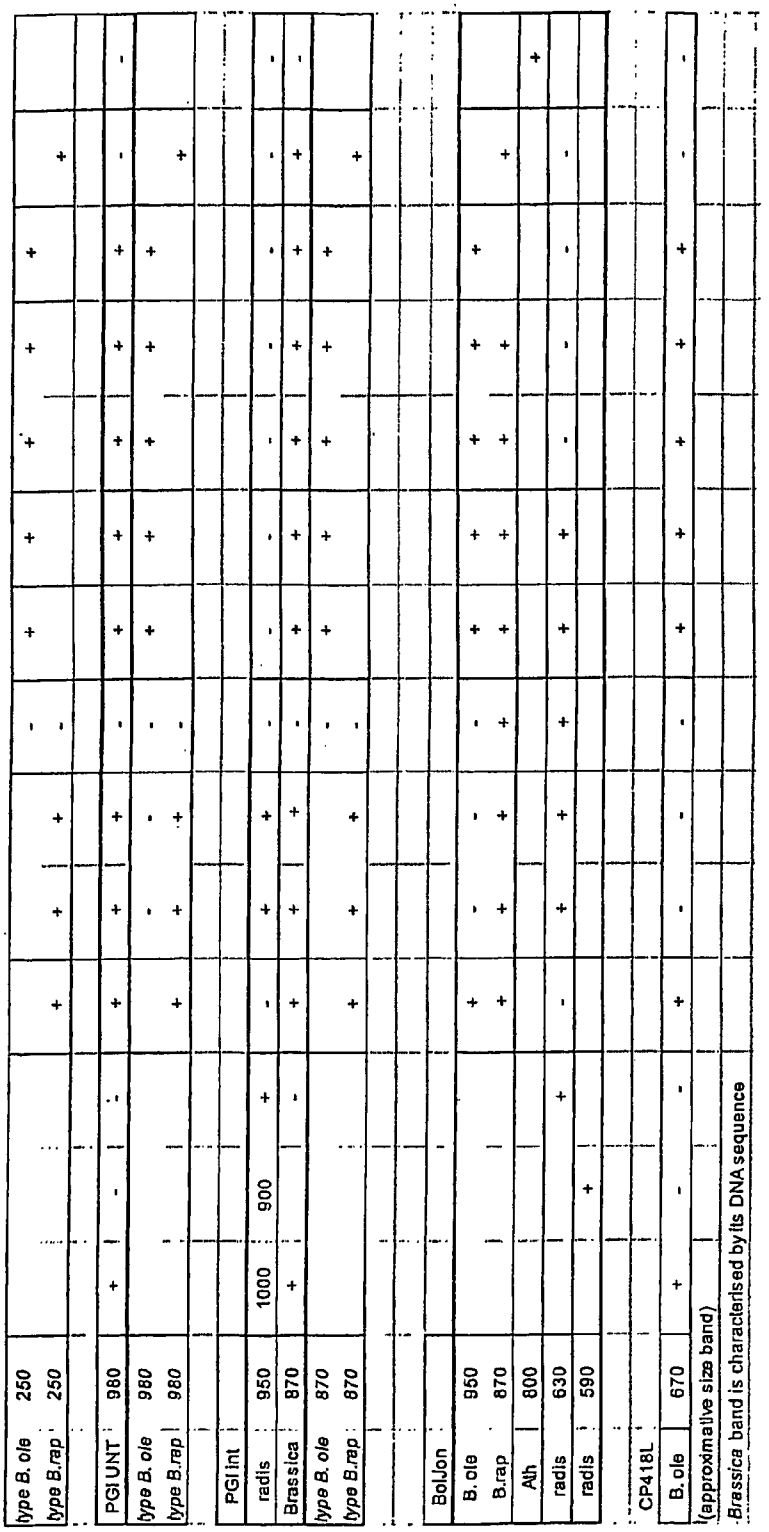
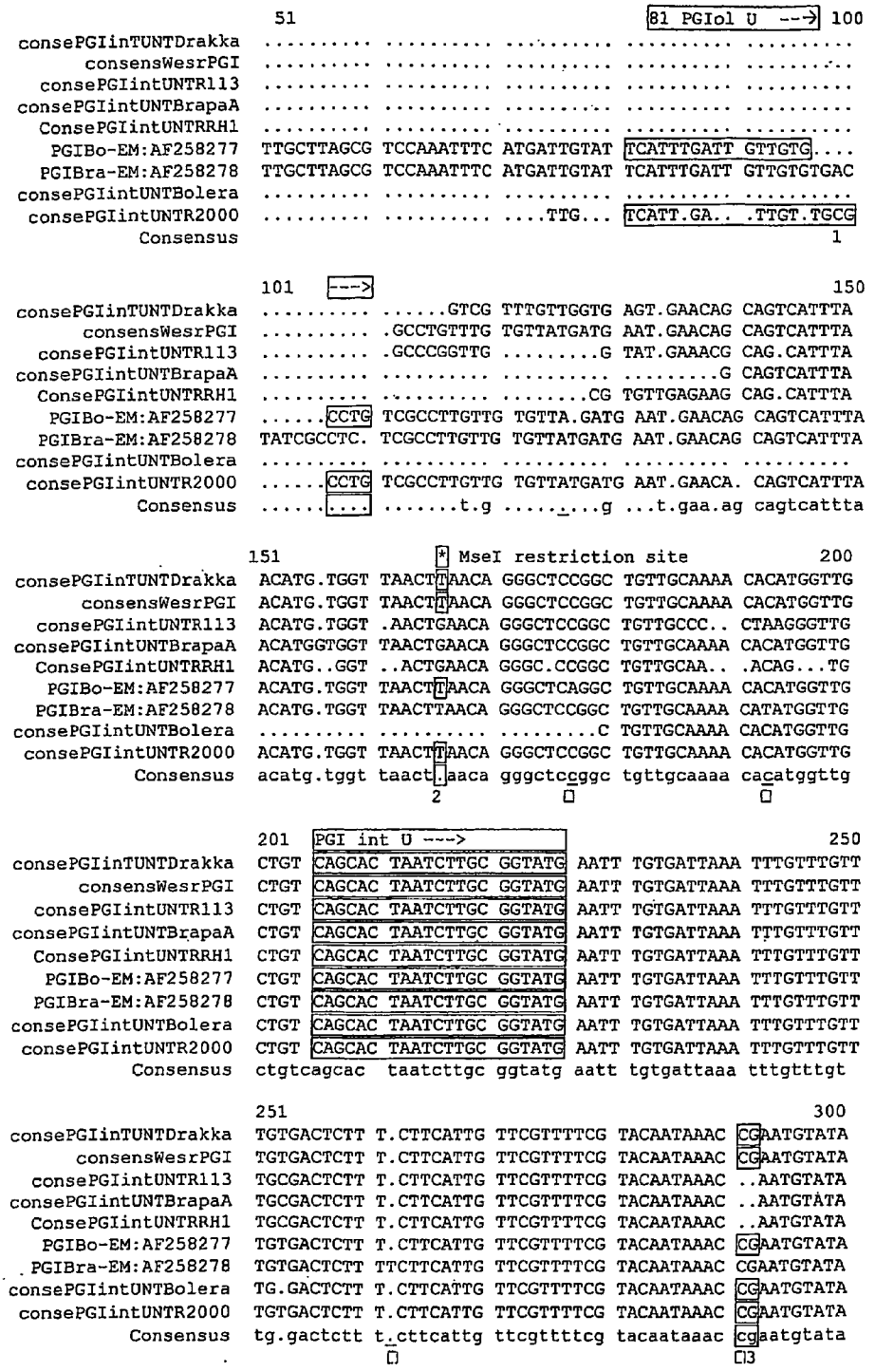

type B. ole 250
type B.rap 250
PGI UNT 980
type B. ole 980
type B.rap 980
PGI int
radis 950 1000 900
Brassica 870
type B. ole 870
type B.rap 870
BolJon
B. ole 950
B.rap 870
Ath 800
radis 630
radis 590
CP418L
B. ole 670
(approximative size band)
Brassica band is characterised by its DNA sequence

Figure 13 (a)


81 PGIol U --> 100
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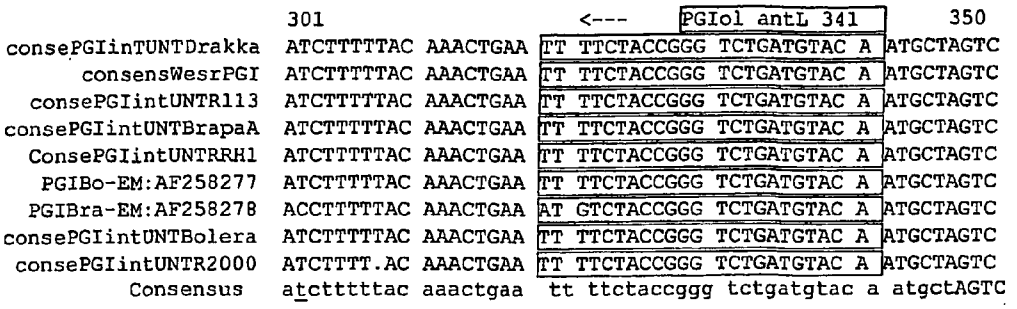
consensWesrPGI
consePGIintUNTR113
consePGIintUNTBrapaA
ConsePGIintUNTRRH1
PGIBo-EM:AF258277
PGIBra-EM:AF258278
consePGIintUNTBolera
consePGIintUNTR2000
Consensus
101 ---> 150
151 * MseI restriction site 200
201 PGI int U ---> 250
251 300

Figure 13 (b)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<---
PGIol antL 341
350
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consensWesrPGI ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTR113 ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTBrapaA ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
ConsePGIintUNTRRH1 ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
PGIBo-EM:AF258277 ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
PGIBra-EM:AF258278 ACCTTTTTAC AAACTGAA AT GTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTBolera ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTR2000 ATCTTTT.AC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
Consensus atcttttac aaactgaa tt ttctaccggg tctgatgtac a atgctAGTC

Figure 14 (a)


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consensWesrPGI CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
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consePGIintUNTBrapaA CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
ConsePGIintUNTRRH1 CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
PGIBo-EM:AF258277 CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
PGIBra-EM:AF258278 CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
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consePGIintUNTR2000 CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
Consensus ctgtcagcac taatcttgc ggtatg aatt tgtgattaaa tttgtttgt
251 300
consePGIinTUNTDrakka TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
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consePGIintUNTBrapaA TGCGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC ..AATGTATA
ConsePGIintUNTRRH1 TGCGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC ..AATGTATA
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consePGIintUNTR2000 TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
Consensus tg.gactctt t.cttcattg ttcgttttcg tacaataaac cgaatgtata
ε ε3
301 <--- PGIol antL 341 350
consePGIinTUNTDrakka ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consensWesrPGI ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTR113 ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTBrapaA ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
ConsePGIintUNTRRH1 ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
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PGIBra-EM:AF258278 ACCTTTTTAC AAACTGAA AT GTCTACCGGG TCTGATGTAC A ATGCTAGTC
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consePGIintUNTR2000 ATCTTTT.AC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
Consensus atcttttac aaactgaa tt ttctaccggg tctgatgtac a atgctAGTC
ε
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consePGIintUNTR2000 TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
Consensus TCCATGTTCT TGGGGATCAT GATTTATTTT CTaCATGTaT TCAGaCAGTA
ε5 ε6
401 450
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ConsePGIintUNTRRH1 CAGAAGAAAG TGTTTAAAAC TCTGGATGTT TTAATTTACA GTTAGTGGAG
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consePGIintUNTR2000 CAGAAGAAAG TGTTCAAAAC TCTGGATGTT TTAATTTACA GTTAGTGGAG
Consensus CAGAAGAAAg TgTTtAAAAC TCTGGATGTT TTaATTTACA GTTAGTGGAG
ε 7 ε

Figure 14 (b)

```
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consePGIintUNTBrapaA    AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
  ConsePGIintUNTRRH1    AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
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consePGIintUNTR2000     AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
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                        501                                                 550
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           Consensus    tggaaggtac agtggtaagt gcttgtttat ttggttgtat .aatttctc.
                                                                    8        9
                        551                                                 600
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                             10                  11
                        601                                                 650
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           Consensus    tgctgttgga gtcttaccat tgtctctaca gtatggcttc tc.gtggttg
                                                                   12
                        651                                                 700
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                                                13                  14
```

Figure 14 (c)


701 750
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consensWesrPGI CATATTCTTT CTATTTTATT TTCCTCTTAA TGATTTCTTC TTTTTTTTAT
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ConsePGIintUNTRRH1 CATATTCTTT CTATTTTATT TTCCTCTGAA TGATTTCTTC TCTTTTAT..
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PGIBra-EM:AF258278 ....
consePGIintUNTBolera CATATTCTTT CTATTTTATT TTCCTCTTAA TGATTTCTTC TTTTTTTA..
consePGIintUNTR2000 CATATTCTTT CTATTTTATT TCCCTCTTAA TGATTTCTTC TTTTTTTTAT
Consensus catattcttt ctattttatt ttcctct.aa tgatttcttc t.tttt.t..
15 16 17
751 800
consePGIinTUNTDrakka TGCATTCCCG TTTTATTTTC AAAAGTTGTT ACTGTCTCTA AATCAAGAAG
consensWesrPGI TGCATTCCCG TTTTATTTTC AAAAGTTGTT ACTGTCTCTA AATCAAGAAG
consePGIintUNTR113 TGCATTCCCG TTTTATTTTC AAAAGTTGTC ACTGTCTCTA AATCAAGAAG
consePGIintUNTBrapaA TGCATTCCCG TTTTATTTTC AAAAGTTGTC ACTGTCTCTA AATCAAGAAG
ConsePGIintUNTRRH1 TGCATTCCCG TTTTATTTTC AAAAGTTGTC ACTGTCTCTA AATCAAGAAG
PGIBo-EM:AF258277 ....
PGIBra-EM:AF258278 ....
consePGIintUNTBolera TGCATTCCCG TTTTATTT.C AAAAGTTGTC CGGCCCCCTA AACCAAGAAG
consePGIintUNTR2000 TGCATTCCCG TTTTATTTTC AAAAGTTGTT ACTGTCTCTA AATCAAGAAG
Consensus tgcattcccg ttttattttc aaaagttgt. actgtctcta aatcaagaag
801 850
consePGIinTUNTDrakka AAACCTTCTT AGTAGATCCA GCTGATATTC AGCCTTTTTT AAATTGGACT
consensWesrPGI AAACCTTCTT AGTAGATCCA GCTGATATTC AGCCTTTTTT AAATTGGACT
consePGIintUNTR113 AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT
consePGIintUNTBrapaA AAACCTTCTT AGTAGATCCA G.TGATATTC AGCCTTTTCT AAATTGGACT
ConsePGIintUNTRRH1 AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT
PGIBo-EM:AF258277 ....
PGIBra-EM:AF258278 ....
consePGIintUNTBolera AAACCTTTCT AGGA...CCA GA....CTCC ACCCTTTTTT AAATTGGACT
consePGIintUNTR2000 AAACCTTCTT AGTAGATCCA GCTGATATTC AGCCTTTTTT AAATTGGACT
Consensus aaaccttctt agtagatcca g.tgatattc agcctttt.t aaattggact
18 19
851 900
consePGIinTUNTDrakka GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATAAGCATT TCCAGTCCCC
consensWesrPGI GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATAAGCATT TCCAGTCCAC
consePGIintUNTR113 GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCC..
consePGIintUNTBrapaA GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCC..
ConsePGIintUNTRRH1 GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCAC
PGIBo-EM:AF258277 ....
PGIBra-EM:AF258278 ....
consePGIintUNTBolera GCAGGTTTTT AAA.GGGGGC TTCAAGCATT GATAAGCATT TCCAGTCCAC
consePGIintUNTR2000 GCAGGTTTTT AAACGGGAGC TTCAAGCATT GATAAGCATT TCCAGTCCAC
Consensus gcaggttttt aaa.gggagc ttcaagcatt gat.agcatt tccagtcc.c
20
901 950
consePGIinTUNTDrakka ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTTT GTGTGATTAT
consensWesrPGI ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT
consePGIintUNTR113 .CCCGTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. ..GTGATTAT
consePGIintUNTBrapaA .CCCGTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT
ConsePGIintUNTRRH1 ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT
PGIBo-EM:AF258277 ....
PGIBra-EM:AF258278 ....
consePGIintUNTBolera ACCCGTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT
consePGIintUNTR2000 ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT
Consensus acc.gtttga gaagaatata cccgtgagtt gcattagtt. gtgtgattat

Figure 14 (d)

```
                          951                                                1000
consePGIinTUNTDrakka      ACAGTTTTTC TTGTCTTTTT GCTATGCCCA TCAACACTAG AAGATTCGTG
   consensWesrPGI         ACAGTTTT.C TTGTCTTTT. GCTATGTCCA TCAACACTAG A.GATTCGTG
consePGIintUNTR113        ACAGTTTT.C TTGCCTTTTT GCTAT..AGG GCAAC.CTAG A.GATTCATG
consePGIintUNTBrapaA      ACAGTTTT.C TTGTCTTTT. GCTATG.TCA TCAAC.CTAG A.GATTCATG
ConsèPGIintUNTRRH1        ACAGTTTT.C TTGTCTTTTT GCTAT...AT GCAACCCTAG ..GATTCATG
 PGIBo-EM:AF258277        .......... .......... .......... .......... ..........
PGIBra-EM:AF258278        .......... .......... .......... .......... ..........
consePGIintUNTBolera      ACAGTTTT.C TTGTCTTTTT GCTAG..TGA TCAAC.CTAG A.GATTCGTG
consePGIintUNTR2000       ACAGTTTT.C TTGTCTTTTT GCTATGTCCA TCAACACTAG A.GATTCGTG
          Consensus       acagtttt.c ttgtcttttt gctat....a tcaac.ctag a.gattc.tg
                                                                             21

                          1001                                               1050
consePGIinTUNTDrakka      AAGTTATTAG TGTAGCCAAC GCCTAGGGGG AGGTTGGTTG GCTGTTTTGG
   consensWesrPGI         AAGTTATTAG TGTAGTCAAC GCA..... .......... ..........
consePGIintUNTR113        AAGTTATTAG TGTAGTCAAC GCAGAGGAGA G..TTCACTG ACGG......
consePGIintUNTBrapaA      AAGTTATTAG TGTAGTCAAC GCAGAGTGAG AGG.TGATTG ..........
ConsePGIintUNTRRH1        AAGTTATTAG TGTAGTCAAC GCAGAGGAGG AGATGGTT.. ..........
 PGIBo-EM:AF258277        .......... .......... .......... .......... ..........
PGIBra-EM:AF258278        .......... .......... .......... .......... ..........
consePGIintUNTBolera      AAGTTATTAG TGTAGTCAAC GCATAGGAGG AGC..... ..........
consePGIintUNTR2000       AAGTTATTAG TGTAGTCAAC GCATAGGGAG AGGTGAT.GG TGACTTTTGG
          Consensus       aagttattag tgtagtcaac gca.agg.g. .g........ ..........

                          1051                    1076
consePGIinTUNTDrakka      ACGTTTTCAC GTGCTCCGGG GGGTTTTTGG GGACCAAACC CCCAAC
   consensWesrPGI         .......... .......... .......... .......... ......
consePGIintUNTR113        .......... .......... .......... .......... ......
consePGIintUNTBrapaA      .......... .......... .......... .......... ......
ConsePGIintUNTRRH1        .......... .......... .......... .......... ......
 PGIBo-EM:AF258277        .......... .......... .......... .......... ......
PGIBra-EM:AF258278        .......... .......... .......... .......... ......
consePGIintUNTBolera      .......... .......... .......... .......... ......
consePGIintUNTR2000       ACGATTTCAG GTGCTTTAGG GTTATTG..... ..........
```

Figure 15 (a)

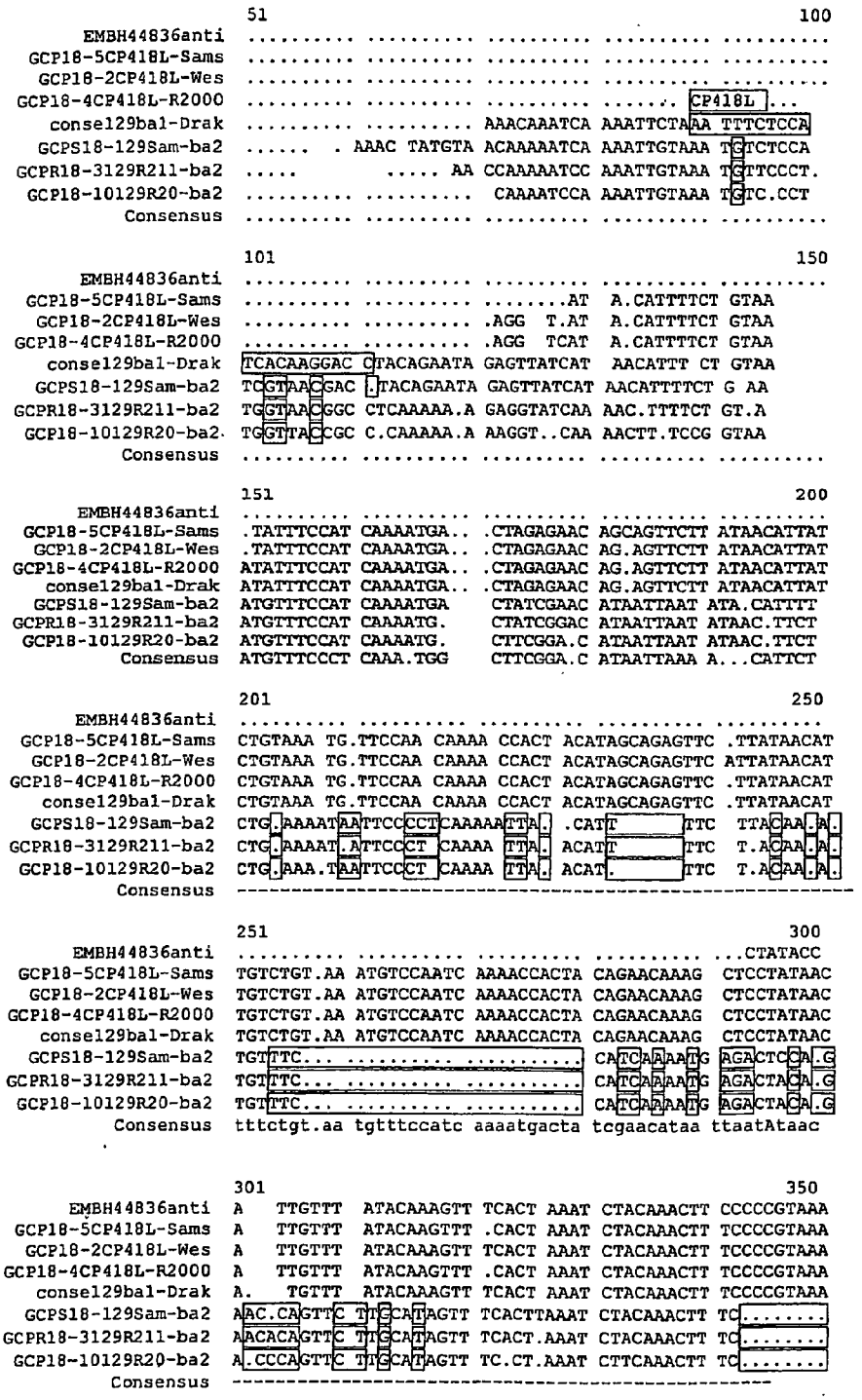

51 100
EMBH44836anti
GCP18-5CP418L-Sams
GCP18-2CP418L-Wes
GCP18-4CP418L-R2000
CP418L
consel29bal-Drak
GCPS18-129Sam-ba2
GCPR18-3129R211-ba2
GCP18-10129R20-ba2
Consensus
101 150
151 200
201 250
251 300
301 350

Figure 15 (b)

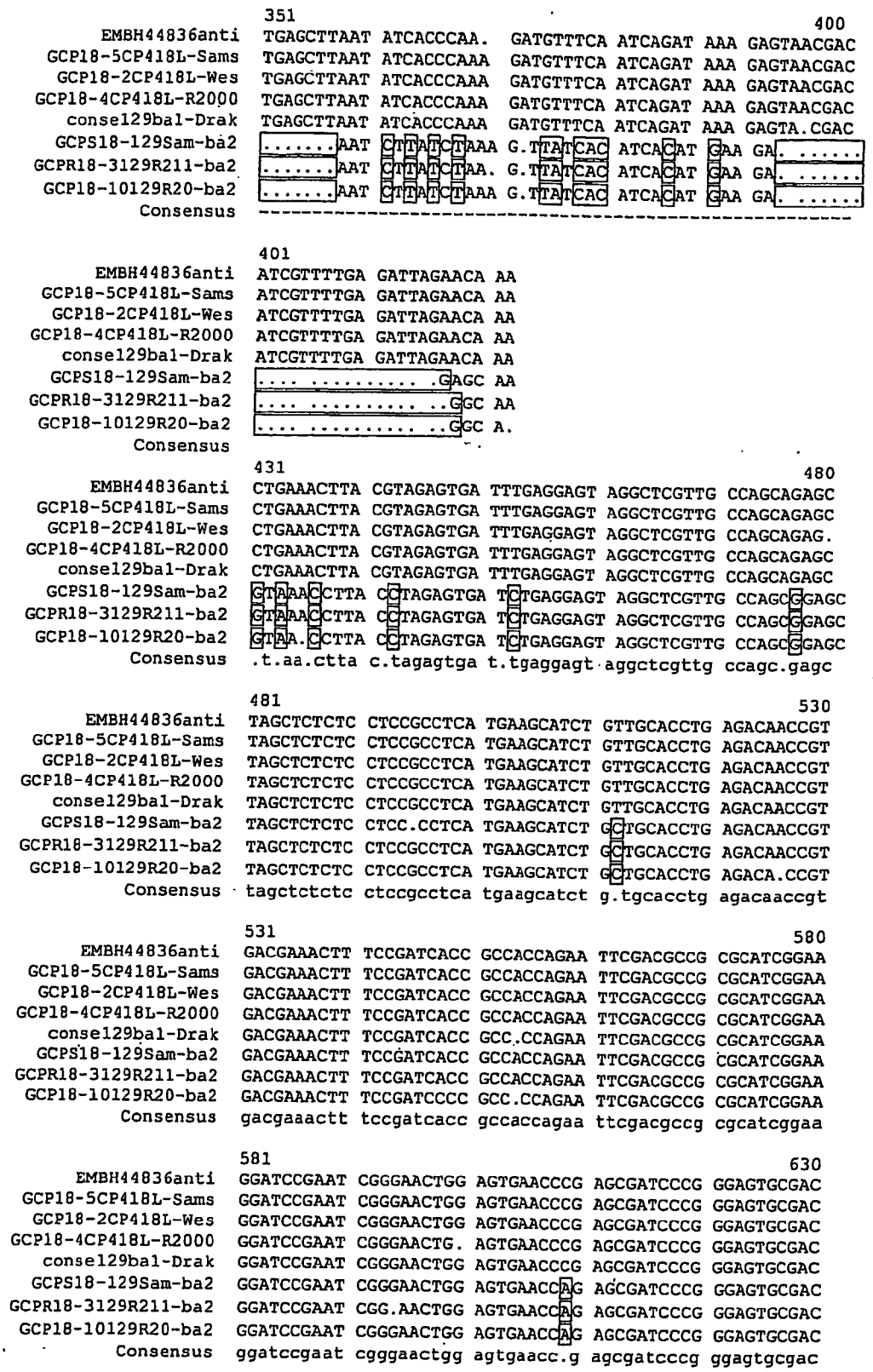

351
400
EMBH44836anti TGAGCTTAAT ATCACCCAA. GATGTTTCA ATCAGAT AAA GAGTAACGAC
GCP18-5CP418L-Sams TGAGCTTAAT ATCACCCAAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
GCP18-2CP418L-Wes TGAGCTTAAT ATCACCCAAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
GCP18-4CP418L-R2000 TGAGCTTAAT ATCACCCAAA GATGTTTCA ATCAGAT AAA GAGTAACGAC
consel29bal-Drak TGAGCTTAAT ATCACCCAAA GATGTTTCA ATCAGAT AAA GAGTA.CGAC
GCPS18-129Sam-ba2 .....AAT CTTATCTAAA G.TTATCAC ATCACAT GAA GA. .....
GCPR18-3129R211-ba2 .....AAT CTTATCTAA. G.TTATCAC ATCACAT GAA GA. .....
GCP18-10129R20-ba2 .....AAT CTTATCTAAA G.TTATCAC ATCACAT GAA GA. .....
Consensus
401
EMBH44836anti ATCGTTTTGA GATTAGAACA AA
GCP18-5CP418L-Sams ATCGTTTTGA GATTAGAACA AA
GCP18-2CP418L-Wes ATCGTTTTGA GATTAGAACA AA
GCP18-4CP418L-R2000 ATCGTTTTGA GATTAGAACA AA
consel29bal-Drak ATCGTTTTGA GATTAGAACA AA
GCPS18-129Sam-ba2 .... .GAGC AA
GCPR18-3129R211-ba2 ....GGC AA
GCP18-10129R20-ba2 ....GGC A.
Consensus
431
480
EMBH44836anti CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
GCP18-5CP418L-Sams CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
GCP18-2CP418L-Wes CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAG.
GCP18-4CP418L-R2000 CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
consel29bal-Drak CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC
GCPS18-129Sam-ba2 GTAAACCTTA CGTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGGAGC
GCPR18-3129R211-ba2 GTAAACCTTA CGTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGGAGC
GCP18-10129R20-ba2 GTAA.CCTTA CGTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGGAGC
Consensus .t.aa.ctta c.tagagtga t.tgaggagt aggctcgttg ccagc.gagc
481
530
EMBH44836anti TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT
GCP18-5CP418L-Sams TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT
GCP18-2CP418L-Wes TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT
GCP18-4CP418L-R2000 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT
consel29bal-Drak TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT
GCPS18-129Sam-ba2 TAGCTCTCTC CTCC.CCTCA TGAAGCATCT GCTGCACCTG AGACAACCGT
GCPR18-3129R211-ba2 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GCTGCACCTG AGACAACCGT
GCP18-10129R20-ba2 TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GCTGCACCTG AGACA.CCGT
Consensus tagctctctc ctccgcctca tgaagcatct g.tgcacctg agacaaccgt
531
580
EMBH44836anti GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
GCP18-5CP418L-Sams GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
GCP18-2CP418L-Wes GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
GCP18-4CP418L-R2000 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
consel29bal-Drak GACGAAACTT TCCGATCACC GCC.CCAGAA TTCGACGCCG CGCATCGGAA
GCPS18-129Sam-ba2 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
GCPR18-3129R211-ba2 GACGAAACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA
GCP18-10129R20-ba2 GACGAAACTT TCCGATCCCC GCC.CCAGAA TTCGACGCCG CGCATCGGAA
Consensus gacgaaactt tccgatcacc gccaccagaa ttcgacgccg cgcatcggaa
581
630
EMBH44836anti GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC
GCP18-5CP418L-Sams GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC
GCP18-2CP418L-Wes GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC
GCP18-4CP418L-R2000 GGATCCGAAT CGGGAACTG. AGTGAACCCG AGCGATCCCG GGAGTGCGAC
consel29bal-Drak GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC
GCPS18-129Sam-ba2 GGATCCGAAT CGGGAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC
GCPR18-3129R211-ba2 GGATCCGAAT CGG.AACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC
GCP18-10129R20-ba2 GGATCCGAAT CGGGAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC
Consensus ggatccgaat cgggaactgg agtgaacc.g agcgatcccg ggagtgcgac

Figure 15 (c)

```
                      631                                                690
          EMBH44836anti GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG
     GCP18-5CP418L-Sams GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG
      GCP18-2CP418L-Wes GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG
   GCP18-4CP418L-R2000 GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG
     consel29bal-Drak GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAA.A GTGGAAGAGG
     GCPS18-129Sam-ba2 GGAGCGTTGG AAAAAGAGAG TGGCACGATT TCGACGAAGA GAGGAAGAGG
   GCPR18-3129R211-ba2 GGAGCGTTGG AAAAAGAGAG TGGCACGATT TCGACGAAGA GAGGAAGAGG
     GCP18-10129R20-ba2 GGAGCGTTGG AAAAAGAGAG TGGCACGATT TCG.CGAAGA GAGGAAGAGG
               Consensus ggagcg.tgg .aaaagagag tggcacgatt tcgacgaaga g.ggaagagg

                      691                                                740
          EMBH44836anti AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
     GCP18-5CP418L-Sams AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
      GCP18-2CP418L-Wes AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
   GCP18-4CP418L-R2000 AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
     consel29bal-Drak AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
     GCPS18-129Sam-ba2 AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAT
   GCPR18-3129R211-ba2 AGAGG.TGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAA
     GCP18-10129R20-ba2 AGAGGGTGGT GGATAAACTC GCGTATGATC AAGTTCGTCA TCGTCCTGAA
               Consensus agagggtggt ggataaactc gcgtatgatc aagttcgtca tcgtcctga.

                      741                                  pSG129antiU  790        800
          EMBH44836anti TGCCGCCATT TTTTTTGTCA GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
     GCP18-5CP418L-Sams TGCCGCCATT TTTTTTGTCA GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
      GCP18-2CP418L-Wes TGCCGCCATT TTTTTTGTCA GGGCGCTCTG TGGCTTAGAA GTTTCCGATG
   GCP18-4CP418L-R2000 TGCCGCCATT TTTTTTGTCA GGGCGCTCTG TGGCTTAGAA GTTTCCGTG.
     consel29bal-Drak TGCCGCCATT TTTTTTGTCA GGGCGCTCTG .GGCTTAGAA GTTTCCGA..
     GCPS18-129Sam-ba2 TGCCGCCATT CTTGTTCAC. .GGCGCTCTG GGT..... ..........
   GCPR18-3129R211-ba2 TGCCGCC... .......... .......... .......... ..........
     GCP18-10129R20-ba2 TGCC..CAT. CTTGAGCTC. .GG.GCGCGG GCTCACAA.. ..........
               Consensus tgccgccat. .tt.....c. .gg.gc.c.g ..........  ..........

                      791
          EMBH44836anti TCAATGAAC A GTGACACGAC GAAATGC
     GCP18-5CP418L-Sams TCAATGAAAC AGAAT...TC CGGG...
      GCP18-2CP418L-Wes CCAATGAACA AGATTATTTC CGATG..
   GCP18-4CP418L-R2000 .......... .......... .......
     consel29bal-Drak .......... .......... .......
```

Figure 16

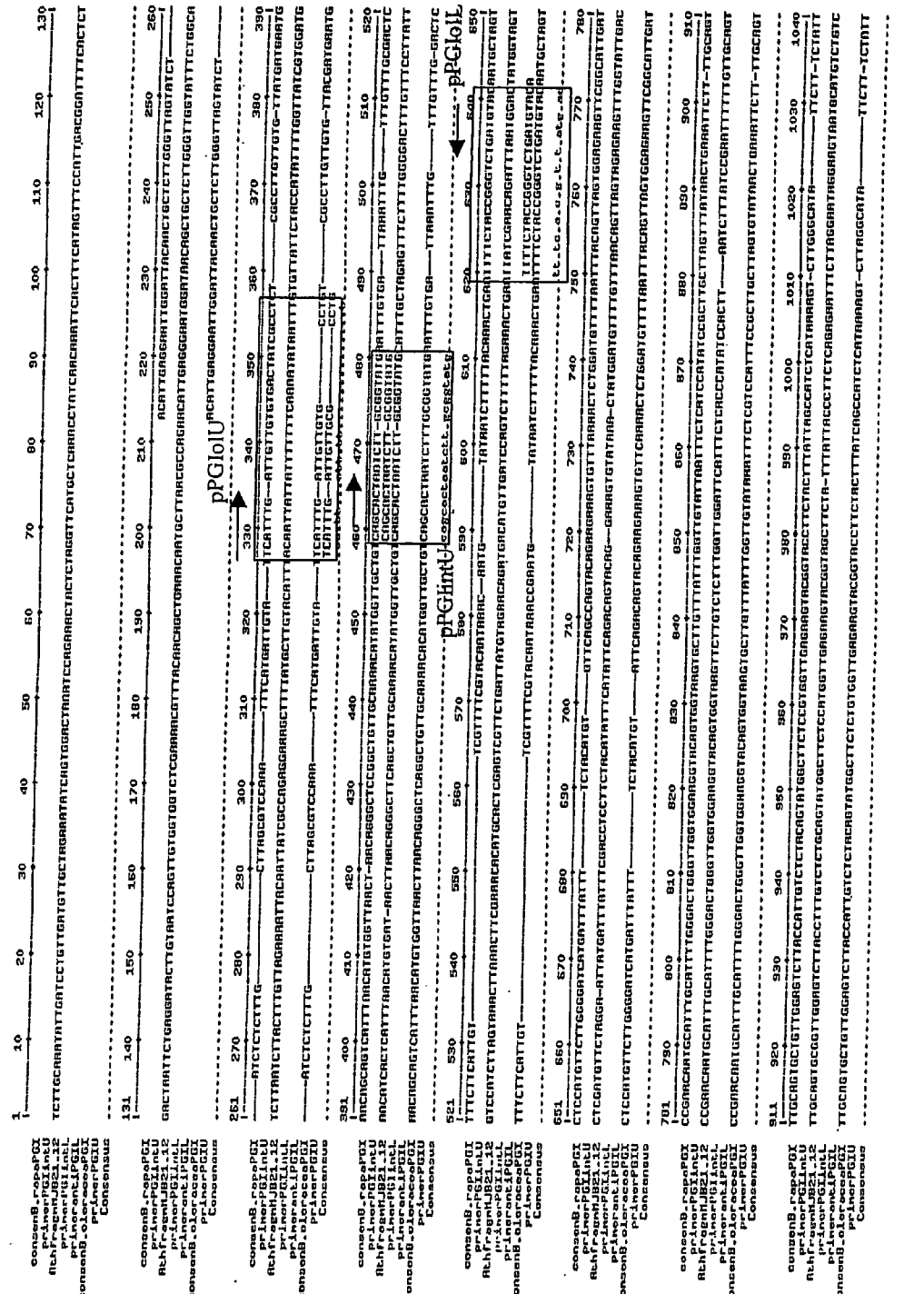


Figure 16 BIS

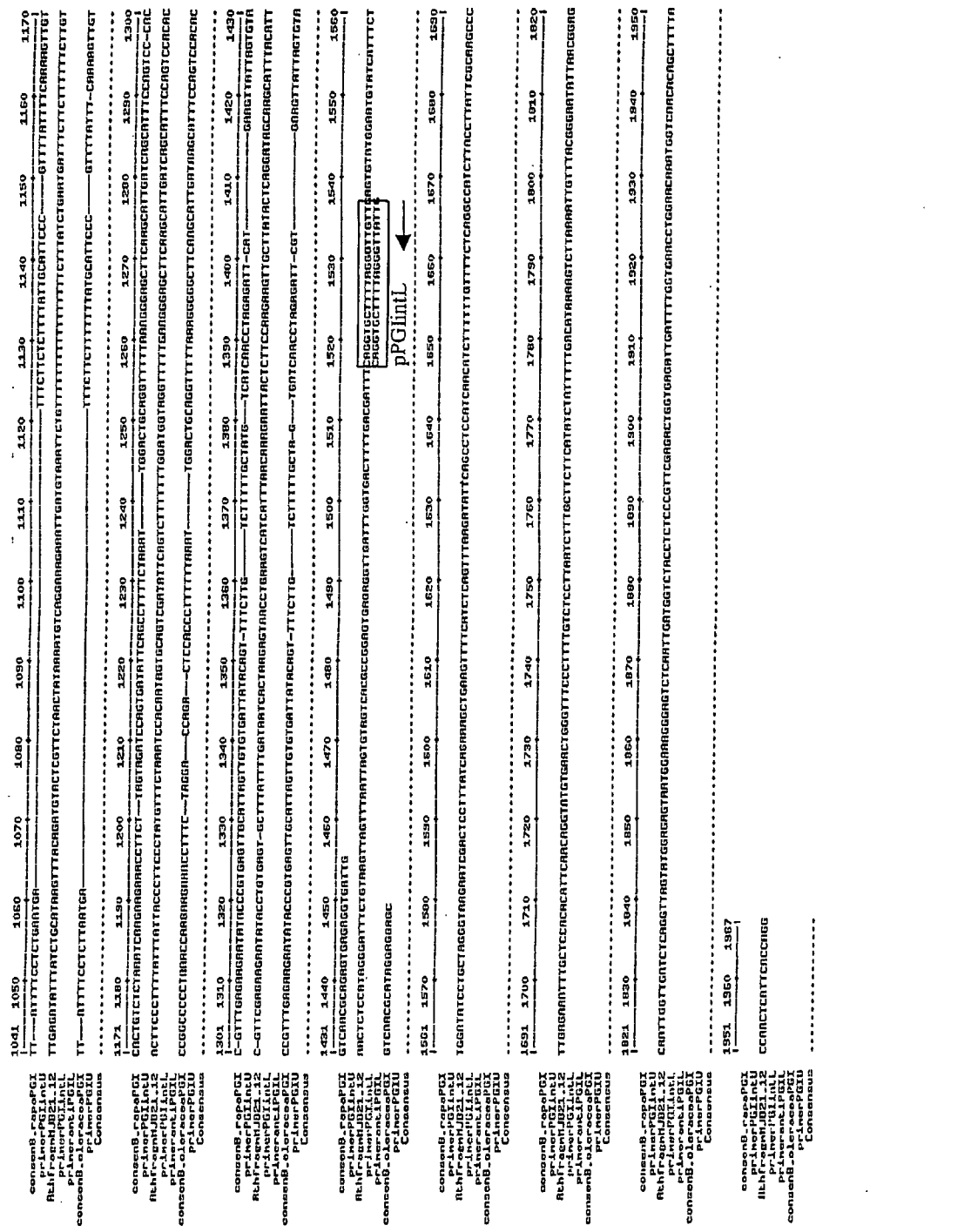


Figure 17

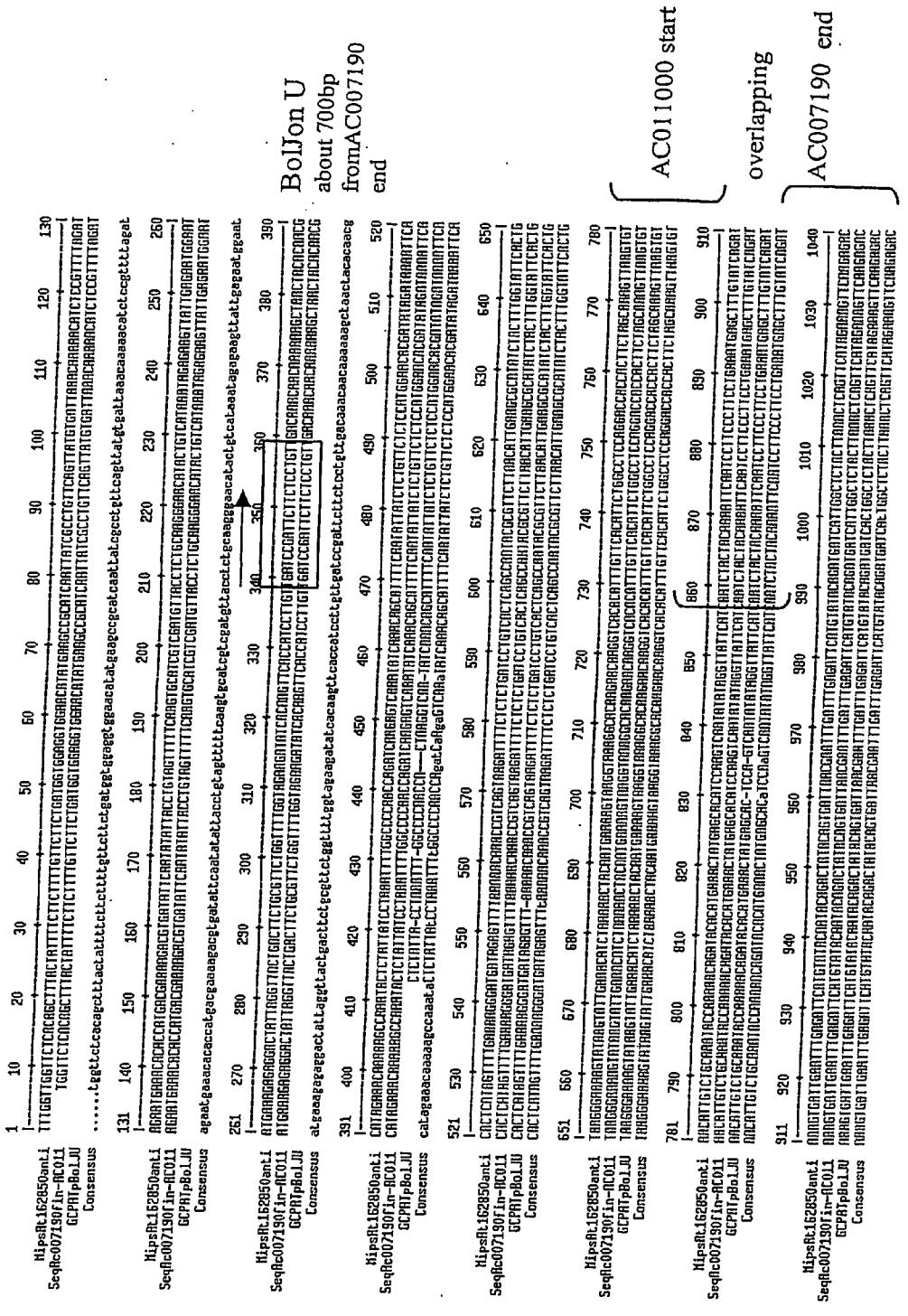
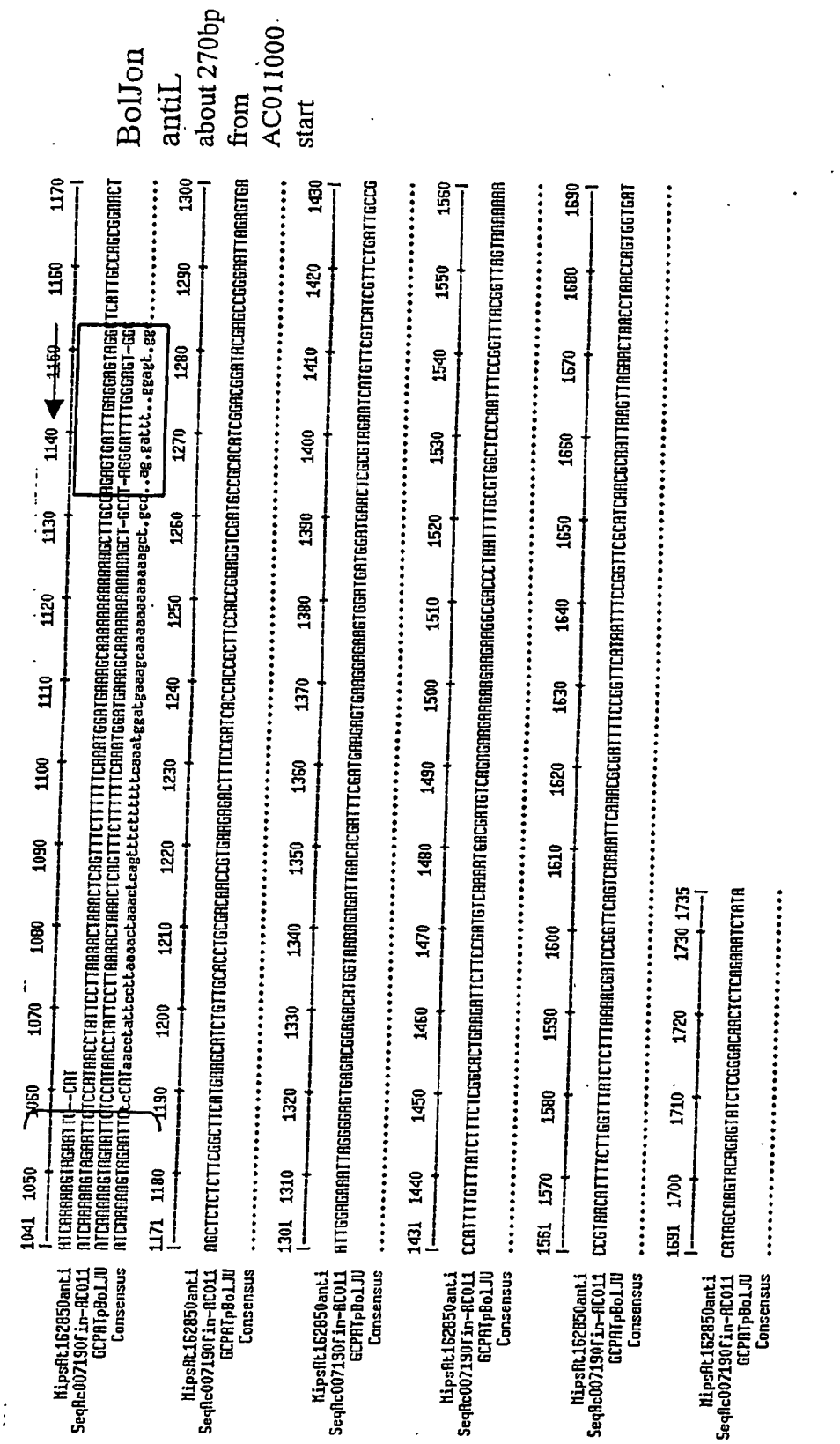

BolJon U
about 700bp
fromAC007190
end
AC011000 start
overlapping
AC007190 end

Figure 17 BIS



METHOD OF PRODUCING DOUBLE LOW RESTORER LINES OF BRASSICA NAPUS HAVING A GOOD AGRONOMIC VALUE

[0001] The invention relates to a method of producing a double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting a radish introgression carrying the Rfo restorer genes deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. The invention relates also to a method of forming *Brassica napus* hybrid seed and progeny thereof and to the use of markers for selection.

[0002] Breeding restorer lines for the Ogu-INRA Cytoplasmic Male Sterility (cms) system in rapeseed (*Brassica napus* L.) has been a major objective during the past few years. Extensive backcross and pedigree breeding were necessary to improve their female fertility and to get double low restorer lines. The so-called <<double low >> varieties are those low in erucic acid in the oil and low in glucosinolates in the solid meal remaining after oil extraction. However some difficulties can still be encountered in breeding these lines (introgression rearrangements, possible linkage with negative traits) due to the large size of the radish introgression.

[0003] The inventors thus assigned themselves the objective of providing a new improved double low restorer line with a good agronomic value.

[0004] This objective is obtained by a new method of producing a recombined double low restorer line for the Ogu-INRA cms in rapeseed.

[0005] A first object of the present invention relates to a method of producing double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting radis introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:

[0006] a) crossing double low cms lines of spring *Brassica napus* comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of *Brassica napus,*

[0007] b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,

[0008] c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,

[0009] d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,

[0010] e) selecting progeny lines.

[0011] In the present invention, the term "lines(s)" means a plant which is essentially homozygote and which is reproducible by auto-pollination.

[0012] A method according to claim **1**, wherein the irradiation dose in step b) is 65 Gray during 6 mn.

[0013] According to one advantageous form of embodiment of the method according to the present invention, the double low cms line of spring *Brassica napus* of step a) is R211.

[0014] R211 is an INRA spring restorer line.

[0015] Drakkar is a French spring registered variety.

[0016] Wesroona is an Australian spring registered variety.

[0017] According to one advantageous form of embodiment of the method according to the present invention, the testing is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.

[0018] Another object of the present invention relates to double low restorer lines of *Brassica napus* for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

[0019] According to one advantageous form of embodiment, the double low restorer lines present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.

[0020] Another object of the present invention relates to a method of forming *Brassica napus* hybrid plants and progeny thereof obtained though the steps of:

[0021] a) providing a restorer line produced according to claim **1** and bred to be homozygous,

[0022] b) using said restorer line in a hybrid production field as the pollinator,

[0023] c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and

[0024] d) harvesting the hybrid seed from the male sterile plant.

[0025] Another object of the present invention relates to seeds of *Brassica* plant obtained from the methods according to the present invention.

[0026] Still another object of the invention relates to seeds of *Brassica napus* deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on Jul. 4, 2003, under the reference number NCIMB41183.

[0027] Another object of the present invention relates to the use of at least four markers PGIol, PGInt, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of *Brassica napus* for Ogura ems presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

[0028] In a preferred embodiment, the combination is of five markers PGIol, PGIUNT, PGInt, BolJon and CP418.

[0029] In the present invention, the expression "any portion of them comprising at least one polymorphic site" means any part of the sequence showing at least a difference between the *B. oleracea* type sequence and *B. rapa* type

sequence. Such markers are represented in the following figures and sequence listing for the R2000 line.

[0030] According to one advantageous form of embodiment, the present invention relates to:

[0031] The marker PGIol which is amplified using the primers: PGIol U and PGIol L (PGIol U: 5'TCATTTGATTGTTGCGCCTG3'; PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')

[0032] The marker PGIint which is amplified using the primers: PGIint U and PGIint L (PGIint U: 5'CAGCACTAATCTTGCGGTATG3'; PGIint L: 5'CAATAACCCTAAAAGCACCTG3')

[0033] The marker PGIUNT which is amplified using the primers: PGIol U and PGIint L: (PGIol U:5'TCATTTGATTGTTGCGCCTG3'; PGIint L:5'CAATAACCCTAAAAGCACCTG3')

[0034] The marker BolJon which is amplified using the primers: BolJon U and BolJon L: (BolJon U:5'GATCCGATTCTTCTCCTGTTG3'; BolJon L:5'GCCTACTCCTCAAATCACTCT3')

[0035] The marker CP418 which is amplified using the primers: SG129 U and pCP418 L: (SG129 U:cf Giancola et al, 2003 *Theor Appl. Genet*. (in press) pCP418 L:5'AATTTCTCCATCACAAGGACC3')

[0036] Another object of the present invention relates to the PGIol, PGIUNT, PGIint, BolJon and CP418 markers whose sequences follow:

PGIol R2000 marker
TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60

ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120

AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGACTCTTTC TTCATTGTTC 180

GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTTACAAA CTGAATTTTC TACCGGGTCT 240

GATGTACA 248

PGIUNT R2000 marker:
TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60

ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120

AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGACTCTTTC TTCATTGTTC 180

GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTTACAAC TGAATTTTCT ACCGGGTCTG 240

ATGTACAATG CTAGTCTCCA TGTTCTTGGG GATCATGATT TATTTTCTAC ATGTATTCAG 300

ACAGTACAGA AGAAAGTGTT CAAAACTCTG GATGTTTTAA TTTACAGTTA GTGGAGAAGT 360

TCGGCATTGA TCCGAACAAT GCATTTGCAT TTTGGGACTG GGTTGGTGGA AGGTACAGTG 420

GTAAGTGCTT GTTTATTTGG TTGTATAAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480

AACTGAAATT CTTTTGCAGT TTGCAGTGCT GTTGGAGTCT TACCATTGTC TCTACAGTAT 540

GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAAATGT 600

CTTAGGCATA TTCTTTCTAT TTTATTTCCC TCTTAATGAT TTCTTCTTTT TTTTATTGCA 660

TTCCCGTTTT ATTTTCAAAA GTTGTTACTG TCTCTAAATC AAGAAGAAAC CTTCTTAGTA 720

GATCCAGCTG ATATTCAGCC TTTTTTAAAT TGGACTGCAG GTTTTTAAAG GGGAGCTTCA 780

AGCATTGATA AGCATTTCCA GTCCACACCG TTTGAGAAGA ATATACCCGT GAGTTGCATT 840

AGTTGTGTGA TTATACAGTT TTCTTGTCTT TTTGCTATGT CCATCAACAC TAGAGATTCG 900

TGAAGTTATT AGTGTAGTCA ACGCATAGGG AGAGGTGATT GGTGACTTTT GGACGATTTC 960

AGGTGCTTTA GGGTTATTG 979

PGIint R2000 marker:
CAGCACTAAT CTTGCGGTAT GAATTTGTGA TTAAATTTGT TTGTTTGTGA CTCTTTCTTC 60

ATTGTTCGTT TTCGTACAAT AAACCGAATG TATAATCTTT TACAAACTGA ATTTTCTACC 120

GGGTCTGATG TACAATGCTA GTCTCCATGT TCTTGGGGAT CATGATTTAT TTTCTACATG 180

TATTCAGACA GTACAGAAGA AAGTGTTCAA AACTCTGGAT GTTTTAATTT ACAGTTAGTG 240

GAGAAGTTCG GCATTGATCC GAACAATGCA TTTGCATTTT GGGACTGGGT TGGTGGAAGG 300

-continued

TACAGTGGTA AGTGCTTGTT TATTTGGTTG TATAAATTTC TCGTCCATTT CCGCTTGCTT 360

AGTGTATAAC TGAAATTCTT TTGCAGTTTG CAGTGCTGTT GGAGTCTTAC CATTGTCTCT 420

ACAGTATGGC TTCTCTGTGG TTGAGAAGTA CGGTACCTTC TACTTTATCA GCCATCTCAT 480

AAAATGTCTT AGGCATATTC TTTCTATTTT ATTTCCCTCT TAATGATTTC TTCTTTTTTT 540

TATTGCATTC CCGTTTTATT TTCAAAAGTT GTTACTGTCT CTAAATCAAG AAGAAACCTT 600

CTTAGTAGAT CCAGCTGATA TTCAGCCTTT TTTAAATTGG ACTGCAGGTT TTTAAAGGGG 660

AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTTT GAGAAGAATA TACCCGTGAG 720

TTGCATTAGT TGTGTGATTA TACAGTTTTC TTGTCTTTTT GCTATGTCCA TCAACACTAG 780

AGATTCGTGA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTTGGA 840

CGATTTCAGG TGCTTTAGGG TTATTG 866

BolJon R2000 marker:

GATCCGATTC TTCTCCTGTT GAGATCAGCT CCAAACATCA AACAACTTGT ACACAAATAT 60

CTTTACTTGC TAAATGGAAC ATGACAAGAG ATAGAAAATC TTGCTCATAG TATTGTACAA 120

GGGATAACAG TGTAGAAAAC AAACCGTCTG TAAGATTTTC TCCCTGATCC TCTCACTTAA 180

CCAGTAGGCG TTTTTCACAT TGAAGCGCAT ATCTACTTTG GTATTCACTG AATAAAAAAA 240

GAAAGCTGGT AACATGTGAA GGATATACAA GCATTGATAC ACCAAGTAGT CACAAACTAC 300

ATTATAAAGG TCAGACCTTT GTTCACATTC TGGCCTCCAG GACCACCGCT TCTAGCAAAG 360

TTAAGCGTAA CATGGTCTGC ACGTATACAA ATGAAAATGT TTCTATCAAA ATCCTATAAA 420

ATAGAGCTCT ATAACATTGT CGATACATAG TTTCACTAAC TCTGCAAGTA CTAAACACAT 480

ATACAAACAA AACTATGCGA ACAGATCAAA ACTACTACAG AACACAGTTC TATGACACTG 540

TCGATAGTAA CATCCTCTGC AAGTACCAAA GAGATAGCAA ATGAAACTAT GTAAACAAAT 600

CAAAATTCTA AATTTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTTCT 660

GTAAATATTT CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT 720

TCCAACAAAA CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAAA 780

ACCACTACAG AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAAA 840

CTTTCCCCGT AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAGAGTACGA 900

CATCGTTTTG AGATTAGAAC AAACTGAAAC TTACGTAGAG TGATTTGAGG AGTAGGC 957

CP418L R2000 marker:

AATTTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTTCT GTAAATATTT 60

CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT TCCAACAAAA 120

CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAAA ACCACTACAG 180

AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAAA CTTTCCCCGT 240

AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAGAGTAACG ACATCGTTTT 300

GAGATTAGAA CAAACTGAAA CTTACGTAGA GTGATTTGAG GAGTAGGCTC GTTGCCAGCA 360

GAGCTAGCTC TCTCCTCCGC CTCATGAAGC ATCTGTTGCA CCTGAGACAA CCGTGACGAA 420

ACTTTCCGAT CACCGCCACC AGAATTCGAC GCCGCGCATC GGAAGGATCC GAATCGGGAA 480

CTGAGTGAAC CCGAGCGATC CCGGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCACG 540

-continued

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ATTTCGACGA AGAGTGGAAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTCG 600

TCATCGTCCT GATTGCCGCC ATTTTTTTTG TCAGGGCGCT CTGTGGCTTA GAAGTTTCCG 660

ATGTCAATGA AC                                                      672
```

[0037] In the annexed drawing that follows, the following abbreviations are used:

[0038] Dra

[0039] Drakkar

[0040] Rel-15-1, E38, R15

[0041] R2000

[0042] Hete, He1, R211.Drakkar

[0043] heterozygous R211*Drakkar,

[0044] Darm

[0045] Darmor

[0046] Bol:

[0047] *Brassica oleracea*

[0048] Bra, *B. rap:*

[0049] *Brassica rapa*

[0050] GCPA18-A19, Wes, Aust:

[0051] Wesroona

[0052] Sam, Sam1PGIolSunt5

[0053] Samourai

[0054] RRH1, ba2c

[0055] RRH1

[0056] rav, N.WR

[0057] Hybrid *Brassica napus**wild Radish

[0058] FIG. **1** illustrates Gamma ray Iradiation and F2 production.

[0059] FIG. **2** illustrates seed set on 'R211' and 'R2000'.

[0060] FIG. **3** illustrates the number of seeds per pod of different lines.

[0061] FIG. **4** illustrates PGIol primer localisation on the segment of PGI sequence from Data Base. In that figure:

PGIol:	primer PGIol U (named in SGAP: BnPGIch 1 U) primer PGIol L (named in SGAP: Bn PGIch 1 L)
PGIint:	primer PGIint U primer PGIint L (is out side the sequence).

[0062] FIG. **5** illustrates electrophoresis gel of PGI-2 gene (PGIol), PCR marker and SG34, a PCR marker close to Rfo.

[0063] FIG. **6** illustrates Pgi-2 segment of DNA amplified by PCR with PGIol primers.

[0064] FIG. **7** illustrates digestion of the PCR product PGIol by Mse1.

[0065] In that figure:

[0066] Sam and Darm has a 75bp band.

[0067] Drak, R211.Dk and R2000 showed a 70pb one (Acrylamide 15%).

[0068] 8 was similar to Samourai (75bp); mix with Drakkar (70pb) it allowed the visualisation of the two bands.

[0069] FIG. **8** illustrates electrophoresis agarose gel of PGIUNT marker.

[0070] In that figure:

[0071] PGIUNT band (about 980bp) is present in *B. oleracea, B. rapa* cv Asko, maintainer and restored lines except in 'R211'.

[0072] There is no amplification in radish and *Arabidopsis.*

[0073] In various *Brassica* genotypes only one band was amplified. Size band are similar but sequences are different.

[0074] FIG. **9** illustrates electrophoresis gel of PGIint PCR marker.

[0075] In that figure PGIint of radish line 7 is of about 950bp. This band is the same as in the restored RRH1 and R113. It is not found in R211. It is not either in R2000.

[0076] However the PGIint band is of a similar size of about 870bp in the various *Brassica* species, but sequences are different.

[0077] FIG. **10** illustrates electrophoresis agarose gel of BolJon PCR marker.

[0078] FIG. **11** illustrates electrophoresis agarose gel of CP418 marker.

[0079] In that figure, the CP418 band (of about 670bp) is specific to the *B. oleracea* genome. It is present in *B. ol, B. napus* (Samourai, Drakkar, Pactol and the herterozygous R2111*Dk). It is absent from the restored rapeseed (RRH, R113 and R211). It is present in the homozygous R2000.

[0080] FIG. **12** illustrates summary markers table.

[0081] FIG. **13** (**13**(*a*), **13**(*b*)) illustrates PGIol marker sequence alignment between *Arabidopsis*, Radish, *B. rapa, B. oleracea* and R2000.

[0082] FIG. **14** (**14**(*a*), **14**(*b*), **14**(*c*), **14**(*d*)) illustrates the PGIint-UNT marker sequence alignment between *Arabidopsis*, Radish, *B. rapa, B. oleracea* and R2000.

[0083] FIG. **15** (**15**(*a*), **15**(*b*), **15**(*c*)) illustrates the CP418L marker sequence alignment between *Arabidopsis*, Radish, *B. rapa, B. oleracea* and R2000.

[0084] FIG. **16** (16 et 16bis) illustrates *Arabidopsis*, Radish and *B. rapa* BolJon markers.

[0085] There are aligned with DB sequences of *Arabidopsis* (AC007190end-AC011000beginning), the *B. oleracea* EMBH959102 end and EMBH448336 begining and representative consensus sequences of the SG129markers band 1 and 2 in *B. napus* (in Drakkar and Samourai respectively).

[0086] From the point 836bp, AC07190-AC11000 and GCPATpBOJ sequences are no longer closely homologous to the *Brassica* sequences.

[0087] The radish and *B. rapa* (GCPconsen RsRf BOJ and BR) sequences are still closely homologous to the *B. napus* one, from 858bp point to the 900bp and 981 points respectively.

[0088] In radish, only partial homology is found on the *Brassica* sequence further down.

[0089] In *B. rapa* species cv Asko, the left of its BolJon sequence can be aligned again, after a 78bp deletion, with those of *B. oleracea* and *B. rapa* in *B. napus* from the 1057bp point to the BolJon L primer.

[0090] FIG. **17** (17 et 17bis) illustrates the localisation of Pgi-2 primers on the *Arabidopsis* th MJB21.12 sequence.

[0091] FIG. **18** illustrates the BolJon primers localisation on the mipsAtl62850 gene and overlapping area of AC007190 and AC011000 *Arabidopsis* th clones.

[0092] Alignment with the *Arabidopsis* BolJon PCR product (740bp) is presented.

[0093] It should be understood, however, that the examples are given solely by way of illustration of the object of the invention, of which they in no way constitute a limitation.

Example I

[0094] method of producing a double low restorer line of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting a radish introgression, carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

Materials and Methods:

[0095] Genotypes: The 'R211' line with a deleted radish insertion was crossed to the spring low GLS rapeseed 'Drakkar' to produce a F1 progeny ('R211*Dk'). The spring low GLS cms line 'Wesroona' (australian origin) was used for following crosses. Were used as control in molecular analyses: Winter restored lines derived from 'Samourai' carrying the complete ('RRH1') or incomplete ('R113') introgression as well as European radish line7, Asiatic restored radish D81, hybrid *Brasica napus** wild radish, *Brassica oleracea*, and *B. rapa* cv Asko, *Arabidopsis thaliana*.

[0096] Gamma ray irradiation: Whole flowering plants were treated with gamma rays from a Co60 source in a controlled area. Subletal dose fo 65 Gray was applied before meioses.

[0097] Testcrosses and F2 production: Irradiated plants were transferred in an insectproof greenhouse after removing flower buds larger than 2 mm. The irradiated F1 progeny was used to handpollinate the cms 'Wesroona' line. The restored derived F1' plants were allowed to produce F2 families harvested individually and precisely sown in a field assay along with non irradiated controls (FIG. **1**).

[0098] Phenotypic selection: Three visual criteria were scored (on a 1 to 5 scale) over 2 years in field assays, on 1200 F2 offsprings plus 44 controls (82 330 quoted plants):

[0099] 1. Vegetative vigour,

[0100] 2. Normality of the ratio of fertile /sterile plants in the F2 segregation, and

[0101] 3. Female fertility (pod development and seed set).

[0102] Advanced selfed generations of the selected families were obtained either in field or greenhouse and produced homozygous lines (F4) for further analysis.

[0103] Isozyme analysis was performed as in (Delourme R. and Eber F. 1992. *Theor Appl Genet* 85: 222-228), marker development from (Fourmann M et al 2002. *Theor Appl. Genet.* 105:1196-1206.): PCR products are validated by sequencing. Alignments were made using Blast Ncbi and Uk Crop Net *Brassica* DB and the Multialin software INRA Toulouse.

Method:

[0104] We choose one low GLS spring homozygous restorer line, 'R211', already exhibiting deletions in the introgression (Delourme R. and Eber F. 1992. *Theor Appl Genet* 85: 222-228. Delourme R et al 1998. *Theor Appl Genet* 97: 129-134. Delourme R. et al 1999. 10th *Int. Rapeseed Congress, Canberra*). Several molecular markers are missing on either side of Rfo, such as spATCHIA (Fourmann M et al 2002. *Theor Appl. Genet.* 105:1196-1206), spSG91 (Giancola S et al 2003 *Theor Appl. Genet.* (in press)). 'R211' lost the isozyme expression of the Pgi-2 allele of the radish gene but also the one of Pgi-2 allele of *B. oleracea* genome (1,2). Moreover, the homozygous 'R211' shows linked negative traits such as low vigour and very poor seed set. We hypothesised that these plant lack a rapeseed chromosomal segment. The fertile ratio in F2 progenies derived from this material is lower than expected (64% instead of 75%). We initiated the program from this 'R211' line and tried to force recombination between the Rfo carrying introgression from this deleted line and the rapeseed homologous chromosome from a double low *B. napus* line.

[0105] Ionising irradiation is known to induce chromosomal rearrangements by double strand breaks followed by aberrant rejoining of the ends.Gamma-ray irradiation was used on a heterozygous F1 derived from the 'R211' line to induce chromosome breaks, just before meiosis, aiming at a recombination of the deleted radish introgression in the rapeseed genome.

Results:

[0106] Very few families were at the best score for the three criteria out of 1200 F2 families tested.

[0107] Only one, 'R2000', proved to produce a normal ratio of fertile plants per selfed progeny with a stable recovery of good agronomic traits such as a good female fertility, with a normal seed set compared to 'R211' (FIG. **2** and **3**). This family was obtained from a 6 mn irradiation treatment at a dose flow of 65 Gray per hour.

[0108] Glucosinolate analysis confirmed its low content.

[0109] In FIG. **2** (Seed set on 'R211' and 'R2000') R2000 showed normal inflorescences, with a normal looking architecture.

[0110] In FIG. **3** (Number of seeds per pod), we observe:

[0111] on the best 'R2000' F4 families in self pollination (Selfings) and in testcrosses

[0112] on 'Pactol' cms line on rapeseed and 'R211' controls.

Example II

Selection of Markers in the Pgi-2 Gene

[0113] PGI isoenzyme analysis: 'R2000' progeny expressed the rapeseed Pgi-2 allele from *B. oleracea* genome, originally lost in 'R211'.

[0114] Three PCR markers were defined to characterise the R2000 family compared to the known restorer rapeseed RRH1 and R113.

[0115] 1) PGIol marker was developed from the *Brassica* DB sequences to be specific to the *Brassica* genome. There is no amplification in radish nor in *Arabidopsis* th., but only in *Brassica*, with one 248 bp band.

[0116] 2) PGIint marker amplified a longer part of the Pgi-2 gene, allowing clear distinction between the various tested species *Brassica, Raphanus* and *Arabidopsis*. The species *B. rapa* and *B. oleracea* were not distinguished by the band size on agarose gel, but by their PGINT band sequence.

[0117] 3) PGIUnt marker, a combination of the PGI ol U and PGI int L primers. This marker had the specificity of the PGIol marker but amplifying a longer part as for PGIint one.

II.1 PGIol marker

[0118] With the PGIol primers, the 'R211' parental line showed no amplification, while the spring tested lines showed a 248bp band. Its DNA sequence is homologous to the PGI-2 sequences from the Crop Net UK DB in *Brassica* species and from previous work in our group (named SGAP sequences)(Localisation of the primers SG PGI chou, FIG. **4**).

[0119] It was ortholog of the clone MJB21-12, on the chromosome V, (34543bp) in *Arabidopsis* (NCBI DB).

[0120] PGIol plus SG34 to set an Homozygocity test:

[0121] The combined use of two sets of primers in a mix PCR, PGIol marling the Pgi-2 gene absent in the homozygote restored plant and SG34 (from S. Giancola et al, Giancola S et al 2003 *Theor Appl. Genet.* (in press)), a very close marker to the Rfo gene, was set up to discriminate homozygous from heterozygous plant among the fertile plants segregating in F2 progenies derived from 'R211'. In place of using SG34, it is possible to use any other marker close to or in the Rfo gene.

[0122] Only one family R2000 showed no difference between homozygote and heterozygote offsprings:

[0123] The Pgi-2 gene is present in the R2000 homozygote, which is not the case for the parental homozygous R211.

[0124] In FIG. **5** (PGIol and SG34 PCR markers):

[0125] The homozygous 'R2000' family has recovered the PGIol band.

[0126] DNA sequence of the band confirmed the homology with the known *Arabidopsis* and *Brassica* Pgi-2 sequence. Control genotypes (Drakkar, Pactol, and, Samourai, Darmor) had the same pattern on the gel. Sequence of this common band allowed to confirm their high homology as they were quasi similar except one base substitution.

[0127] The homozygous 'R2000' family has recovered the PGIol band of the *Brassica oleracea* type. It was distinct from the known restorer of the Samourai group.

[0128] This amplified part of the Pgi-2 is very conserved and hardly any differences were shown among the various genotypes. A longer part of Pgi-2 gene was investigated.

II.2 PGIUNT and PGIint Markers

[0129] Electrophoresis Patterns of PCR Products:

[0130] PGIUNT marker: A second reverse primer, PGIint L, was designed further down the Pgi-2 sequence, to amplify as well conserved and as variable regions of the gene. When used with the PGIol U primer, it amplifies a 980bp band only in *Brassica* genomes.

[0131] R211 didn't show any band, The homozygous 'R2000' showed the PGIUNT band as in the Drakkar parent.

[0132] In FIG. **8** (PGIUNT Marker):

[0133] PGIint marker amplified a segment of PGIUNT. The upper primer PGIint allows the amplification in all tested species, allowing a clear distinction between *Arabidopsis*, Radish and *Brassica. B. rapa* and *B. oleracea* were not distinguished by the band size on agarose gel, but by their PGIint sequence. All tested restored genotypes, but the 'R211' line, exhibited the European radish band and one *Brassica* band, homologous to the *B. rapa* one.

[0134] The homozygous 'R2000' didn't show the radish PGIint band, as in the deleted 'R211' parental line, but showed one *Brassica* band, homologous to the *B. oleracea* one.

[0135] Electrophoresis of PGIint marker is represented in FIG. **9**.

[0136] Sequence Analysis:

[0137] Comparison of the PGI sequences from the data bases.

[0138] A PGI segment of about 490bp is known.

[0139] Sequences of a segment of about 490bp from different genotypes (*B. oleracea, B. rapa, B. napus*) have been studied in our laboratory group and some sequences were given to *Brassica* Crop Net DB: EMAF25875 to 25788 by M.Fouramnn (4) These sequences are very conserved.

[0140] Comparison of the *B. rapa* et *B. oleracea* species PGI sequences (FIGS. **13** and **14**): Comparison between PGI

sequences we have obtained from the tested genotypes of *B. oleracea* and *B. rapa* species, showed that they were distinct by 21 base substitutions. Theses substitutions allowed to distinguish PGIint sequences from the other tested genotypes of rapeseed, homologous to either *B. rapa* cv Asko (RRH1 and R113) or *B. oleracea* (Drakkar, R211*DK but also R2000).

Example III

Selection of Marker in a Region Close to Rfo

[0141] Markers surrounding the Rfo gene in the radish insertion were determined in order to facilitate the Rfo gene cloning (Desloires S et al 2003 *EMBO reports* 4, 6:588-594). One of these, the SG129 PCR marker was located very close to Rfo (Giancola S et al 2003 *Theor Appl. Genet.* (in press)): it co-amplified distinct bands in *B. oleracea* and *B. rapa* genomes of *B. napus*, but the radish band was very difficult to see on an agarose gel.

[0142] The target SG129 sequence was ortholog of a clone (ACO11000, at the locus F16P17) in *Arabidopsis thaliana*. This clone overlapped an *Arabidopsis* adjacent contig clone (AC07190).

[0143] From the *Brassica* Crop Net DB, we found one *B. oleracea* clone, (EMBH448336, 764bp) blasting with the begining of the A011000, and a second *B. oleracea* clone (EMBH53971), distant from about 300bp on the *Arabidopsis* map, that blasted with the end of ACO7190.

[0144] We designed a new PCR marker, BolJon, between the two *B. oleracea* clones. We verified that it allowed amplification of a specific PCR bands in the different genotypes compared here.

[0145] In FIG. **16** (electrophoresis gel of BolJon PCR products):

[0146] In *Arabidopsis*, a BolJon 815bp band was amplified, homologue to the overlapping segment of the contigs.

[0147] In *Brassiceae diploid* species, BolJon marker showed distinct bands: one of 950bp in *B. oleracea* and one of 870bp in *B. rapa*. It showed that the two *B. oleracea* clones (EMBH53971 and EMBH448336) are in sequence continuity in *Brassica* genome as it is for the ortholog sequences in *Arabidopsis*.

[0148] In *B. napus*, these two bands are co-amplified in the maintainer lines, Samourai or Drakkar.

[0149] In radish line7, one BolJon band was amplified of about 630 bp long. The band of the restored radish cmsRd8l was slightly smaller.

[0150] In all the restored rapeseed lines, one of the BolJon bands was of the same size as the radish line7. BolJon is a marker of the radish introgression.

[0151] The homozygous restored rapeseed lines, 'RRH1', 'R113' and also 'R211', only showed the *B. rapa* band and the 630bp radish band bp suggesting the *B. oleracea* ortholog of the target gene is absent or has been modified when the radish segment of chromosome was inserted into the rapeseed *B. oleracea* constitutive genome.

[0152] 'R2000' homozygote plants showed radish PCR BolJon, plus the two *Brassica* BolJon bands, again having recovered the *B. oleracea* one, lost in 'R211' and other restorer lines.

[0153] We designed a primer, pCP418L, specific of the *B. oleracea* genome in the tested species. With the SG129U primer it amplified only one PCR band (670bp) in the *B. oleracea* species. (FIG. **17**).

[0154] There was no amplification in *B. rapa*, in radish, nor in *Arabidopsis*, but there was a clear CP418 band in *B. napus* maintainer lines. Its sequence was strictly homologous to the EMBH448336 sequence. This marker was in a very conserved DNA sequence allowing no polymorphism between genotypes except by presence/absence.

[0155] In RRH1, R113 and in R211 there was no CP418 band, indicating as previously that the *B. oleracea* ortholog of the target gene is absent or has been modified following the radish insertion.

[0156] 'R2000' homozygote plants showed CP418 band, again having recovered the specific *B. oleracea* one.

[0157] In the present invention, a new recombined low GLS restorer line has been selected with a good female fertility. The poor value of line 'R211' allowed selection in the field for a rare recombination event and characterisation the 'R2000' family.

[0158] The homozygous 'R2000' presents a unique combination of the PGIol, PGIUNT, PGIint and BolJon markers when compared with the rapeseed restorer analysed yet: PGIinT marker showed that the homozygous restored rapeseed lines, RRH1 and R113 presented the European radish band plus one *Brassica* band, homologous to *B. rapa* genome. 'R2000' shows no radish band, lost as in its parental deleted line R211, but showed one *Brassica* band homologous to *B. oleracea*. The ortholog PGIint sequence in its *B. rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

[0159] PGIol marker and PGIUNT marker sequences in restored lines RRH1 and R 113 were homologous to the *B. rapa* cv Asko one. In 'R2000', PGIUNT sequence is homologous to *B. oleracea*. The ortholog PGIUnt sequence in its *B. rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

[0160] BolJon marker showed that the homozygous restored rapeseed lines, including 'R211' presented the European radish band plus only the B.rapa one. 'R2000' shows the two bands of 'R211' plus the recovered *B. oleracea* BolJon band.

[0161] CP418 marker showed that 'R2000' recovered this conserved *B. oleracea* segment.

[0162] Our hypothesis is that a recombination event took place in the pollen mother cell which gave rise to 'R2000' plants. The deleted radish introgression was then integrated to the normal homologous chromosome segment, carrying the *B. oleracea* type Pgi-2 gene and BolJon target sequence, characterised by these markers, probably from the Drakkar '00' genome present in the irradiated heterozygous 'R211*DK'.

[0163] The pattern observed for BolJon suggests that the recombination event resulted in a particular duplicated region, one from radish and one *B. oleracea*, in the 'R2000' family.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 14

<210> SEQ ID NO 1
<211> LENGTH: 248
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: PGIo1 marker

<400> SEQUENCE: 1

tcatttgatt gttgcgcctg tcgccttgtt gtgttatgat gaatgaacag cagtcattta 60

acatgtggtt aacttaacag ggctccggct gttgcaaaac acatggttgc tgtcagcact 120

aatcttgcgg tatgaatttg tgattaaatt tgtttgtttg tgactctttc ttcattgttc 180

gttttcgtac aataaaccga atgtataatc tttttacaaa ctgaattttc taccgggtct 240

gatgtaca 248

<210> SEQ ID NO 2
<211> LENGTH: 979
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: PGI-UNT R2000 marker

<400> SEQUENCE: 2

tcatttgatt gttgcgcctg tcgccttgtt gtgttatgat gaatgaacag cagtcattta 60

acatgtggtt aacttaacag ggctccggct gttgcaaaac acatggttgc tgtcagcact 120

aatcttgcgg tatgaatttg tgattaaatt tgtttgtttg tgactctttc ttcattgttc 180

gttttcgtac aataaaccga atgtataatc ttttacaaac tgaattttct accgggtctg 240

atgtacaatg ctagtctcca tgttcttggg gatcatgatt tattttctac atgtattcag 300

acagtacaga agaaagtgtt caaaactctg gatgttttaa tttacagtta gtggagaagt 360

tcggcattga tccgaacaat gcatttgcat tttgggactg ggttggtgga aggtacagtg 420

gtaagtgctt gtttatttgg ttgtataaat ttctcgtcca tttccgcttg cttagtgtat 480

aactgaaatt cttttgcagt ttgcagtgct gttggagtct taccattgtc tctacagtat 540

ggcttctctg tggttgagaa gtacggtacc ttctacttta tcagccatct cataaaatgt 600

cttaggcata ttctttctat tttatttccc tcttaatgat ttcttctttt ttttattgca 660

ttcccgtttt attttcaaaa gttgttactg tctctaaatc aagaagaaac cttcttagta 720

gatccagctg atattcagcc ttttttaaat tggactgcag gtttttaaag gggagcttca 780

agcattgata agcatttcca gtccacaccg tttgagaaga atatacccgt gagttgcatt 840

agttgtgtga ttatacagtt ttcttgtctt tttgctatgt ccatcaacac tagagattcg 900

tgaagttatt agtgtagtca acgcataggg agaggtgatt ggtgactttt ggacgatttc 960

aggtgcttta gggttattg 979

<210> SEQ ID NO 3
<211> LENGTH: 866
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: PGI-int R2000 marker

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<400> SEQUENCE: 3

cagcactaat cttgcggtat gaatttgtga ttaaatttgt ttgtttgtga ctctttcttc 60

attgttcgtt ttcgtacaat aaaccgaatg tataatcttt tacaaactga attttctacc 120

gggtctgatg tacaatgcta gtctccatgt tcttggggat catgatttat tttctacatg 180

tattcagaca gtacagaaga aagtgttcaa aactctggat gttttaattt acagttagtg 240

gagaagttcg gcattgatcc gaacaatgca tttgcatttt gggactgggt tggtggaagg 300

tacagtggta agtgcttgtt tatttggttg tataaatttc tcgtccattt ccgcttgctt 360

agtgtataac tgaaattctt ttgcagtttg cagtgctgtt ggagtcttac cattgtctct 420

acagtatggc ttctctgtgg ttgagaagta cggtaccttc tactttatca gccatctcat 480

aaaatgtctt aggcatattc tttctatttt atttccctct taatgatttc ttctttttt 540

tattgcattc ccgttttatt ttcaaaagtt gttactgtct ctaaatcaag aagaaacctt 600

cttagtagat ccagctgata ttcagccttt tttaaattgg actgcaggtt tttaaagggg 660

agcttcaagc attgataagc atttccagtc cacaccgttt gagaagaata tacccgtgag 720

ttgcattagt tgtgtgatta tacagttttc ttgtcttttt gctatgtcca tcaacactag 780

agattcgtga agttattagt gtagtcaacg catagggaga ggtgattggt gacttttgga 840

cgatttcagg tgctttaggg ttattg 866

<210> SEQ ID NO 4
<211> LENGTH: 957
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: BolJon marker R2000

<400> SEQUENCE: 4

gatccgattc ttctcctgtt gagatcagct ccaaacatca aacaacttgt acacaaatat 60

ctttacttgc taaatggaac atgacaagag atagaaaatc ttgctcatag tattgtacaa 120

gggataacag tgtagaaaac aaaccgtctg taagattttc tccctgatcc tctcacttaa 180

ccagtaggcg tttttcacat tgaagcgcat atctactttg gtattcactg aataaaaaaa 240

gaaagctggt aacatgtgaa ggatatacaa gcattgatac accaagtagt cacaaactac 300

attataaagg tcagaccttt gttcacattc tggcctccag gaccaccgct tctagcaaag 360

ttaagcgtaa catggtctgc acgtatacaa atgaaaatgt ttctatcaaa atcctataaa 420

atagagctct ataacattgt cgatacatag tttcactaac tctgcaagta ctaaacacat 480

atacaaacaa aactatgcga acagatcaaa actactacag aacacagttc tatgacactg 540

tcgatagtaa catcctctgc aagtaccaaa gagatagcaa atgaaactat gtaaacaaat 600

caaaattcta aatttctcca tcacaaggac ctacagaata gagttatcat aacattttct 660

gtaaatattt ccatcaaaat gactagagaa cagagttctt ataacattat ctgtaaatgt 720

tccaacaaaa ccactacata gcagagttct tataacattg tctgtaaatg tccaatcaaa 780

accactacag aacaaagctc ctataacatt gtttatacaa agtttcacta aatctacaaa 840

ctttccccgt aaatgagctt aatatcaccc aaagatgttt caatcagata aagagtacga 900

catcgttttg agattagaac aaactgaaac ttacgtagag tgatttgagg agtaggc 957

<210> SEQ ID NO 5

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<211> LENGTH: 672
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: CP418L marker R2000

<400> SEQUENCE: 5

aatttctcca tcacaaggac ctacagaata gagttatcat aacattttct gtaaatattt 60

ccatcaaaat gactagagaa cagagttctt ataacattat ctgtaaatgt tccaacaaaa 120

ccactacata gcagagttct tataacattg tctgtaaatg tccaatcaaa accactacag 180

aacaaagctc ctataacatt gtttatacaa agtttcacta aatctacaaa ctttccccgt 240

aaatgagctt aatatcaccc aaagatgttt caatcagata aagagtaacg acatcgtttt 300

gagattagaa caaactgaaa cttacgtaga gtgatttgag gagtaggctc gttgccagca 360

gagctagctc tctcctccgc ctcatgaagc atctgttgca cctgagacaa ccgtgacgaa 420

actttccgat caccgccacc agaattcgac gccgcgcatc ggaaggatcc gaatcgggaa 480

ctgagtgaac ccgagcgatc ccgggagtgc gacggagcga tgggaaaaga gagtggcacg 540

atttcgacga agagtggaag aggagagggt ggtggataaa ctcgcgtatg atcaagttcg 600

tcatcgtcct gattgccgcc attttttttg tcagggcgct ctgtggctta gaagtttccg 660

atgtcaatga ac 672

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIol U primer

<400> SEQUENCE: 6

tcatttgatt gttgcgcctg 20

<210> SEQ ID NO 7
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIol L primer

<400> SEQUENCE: 7

tgtacatcag acccggtaga aaa 23

<210> SEQ ID NO 8
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIint U primer

<400> SEQUENCE: 8

cagcactaat cttgcggtat g 21

<210> SEQ ID NO 9
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIint L primer

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<400> SEQUENCE: 9

caataaccct aaaagcacct g 21

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIol U primer

<400> SEQUENCE: 10

tcatttgatt gttgcgcctg 20

<210> SEQ ID NO 11
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PGIint L primer

<400> SEQUENCE: 11

caataaccct aaaagcacct g 21

<210> SEQ ID NO 12
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BolJon U primer

<400> SEQUENCE: 12

gatccgattc ttctcctgtt g 21

<210> SEQ ID NO 13
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BolJon L primer

<400> SEQUENCE: 13

gcctactcct caaatcactc t 21

<210> SEQ ID NO 14
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pCP418 L primer

<400> SEQUENCE: 14

aatttctcca tcacaaggac c 21

1-18. (canceled)

19. A method of producing double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, comprising:

a) crossing double low cms lines of spring *Brassica napus* comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of *Brassica napus;*

b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation;

c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line;

d) testing the progeny for vigour, female fertility and transmission rate of the cms gene; and

e) selecting progeny lines.

20. The method of claim 19, wherein said irradiation dose in step b) is 65 Gray during 6 mn.

21. The method of claim 19 wherein the double low cms line of spring *Brassica napus* of step a) is R211.

22. The method of claim 19 wherein said testing is performed with the combination of five markers selected from the group consisting of PGIol, PGIUNT, PGIint, BolJon and CP418.

23. Double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting an Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

24. The double low restorer lines of claim 23, wherein said lines present a unique combination of five markers selected from the group consisting of PGIol, PGIUNT, PGIint, BolJon and CP418.

25. A method of producing *Brassica napus* hybrid plants and progeny thereof, comprising:

a) providing a restorer line produced by the method of claim 19 and bred to be homozygous;

b) using said restorer line in a hybrid production field as the pollinator;

c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant; and

d) harvesting the hybrid seed from the male sterile plant.

26. Seeds of *Brassica* plant developed from the *Brassica* line obtained by the method of claim 19.

27. Seeds of *Brassica napus* obtained by the method of claim 25.

28. Seeds of *Brassica napus* obtained by the method of claim 19 deposited in NCIMB Limited, under reference number NCIMB41183.

29. A method for characterising recombined restorer lines of *Brassica napus* for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, comprising the use of at least four markers, PGIol, PGIint, BolJon and CP418, or any portion of them containing at least one polymorphic site.

30. The method of claim 29 wherein the combination of markers further comprises PGIUNT.

31. The method of claim 30, wherein:

the marker PGIol is amplified using the primers: PGIol U, comprising SEQ ID NO:6 and PGIol L, comprising SEQ ID NO:7;

the marker PGIint is amplified using the primers: PGIint U, comprising SEQ ID NO:8 and PGIint L, comprising SEQ ID NO:9;

the marker BolJon is amplified using the primers: BolJon U, comprising SEQ ID NO:12 and BolJon L, comprising SEQ ID NO:13;

the marker CP418 is amplified using the primers: SG129 U, and pCP418 L, comprising SEQ ID NO: 14;

the marker PGIUNT is amplified using the primers: PGIol U, comprising SEQ ID NO:6 and PGIint L, comprising SEQ ID NO:9.

32. A PGIol marker comprising SEQ ID NO:1.

33. A PGIUNT marker comprising SEQ ID NO:2.

34. A PGIint marker comprising SEQ ID NO:3.

35. A BolJon marker comprising SEQ ID NO:4.

36. A CP418 marker comprising SEQ ID NO:5.

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