



(11) **EP 2 298 066 A9**

(12) **CORRECTED EUROPEAN PATENT APPLICATION**

(15) Correction information:
Corrected version no 1 (W1 A1)
Corrections, see
Drawings 8

(51) Int Cl.:
A01H 1/02 (2006.01) **A01H 1/06** (2006.01)
A01H 5/00 (2006.01) **A01H 5/10** (2006.01)
C12N 15/11 (2006.01) **C12Q 1/68** (2006.01)

(48) Corrigendum issued on:
26.10.2011 Bulletin 2011/43

(43) Date of publication:
23.03.2011 Bulletin 2011/12

(21) Application number: **10183322.6**

(22) Date of filing: **05.07.2004**

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IT LI LU MC NL PL PT RO SE SI SK TR

(30) Priority: **04.07.2003 EP 03291677**
08.12.2003 EP 03293057

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
09175998.5 / 2 179 643
04744142.3 / 1 641 336

(71) Applicant: **INSTITUT NATIONAL DE LA**
RECHERCHE AGRONOMIQUE
75007 Paris (FR)

(72) Inventors:
• **Primard-Brisset, Catherine**
78470 Saint Remy Les Chevreuse (FR)
• **Delourme, Régine**
35590 L'Hermitage (FR)

- **Poupard, Jean-Pierre**
78026 Versailles (FR)
- **Horvais, Raymonde**
35440 MontreuilL-sur-Ille (FR)
- **Budar, Françoise**
91470 Les Molières (FR)
- **Pelletier, Georges**
91440 Bures Sur Yvette (FR)
- **Renard, Michel**
35650 Le Rheu (FR)

(74) Representative: **Faivre Petit, Frédérique et al**
Cabinet Regimbeau
20, rue de Chazelles
75847 Paris Cedex 17 (FR)

Remarks:
This application was filed on 30-09-2010 as a
divisional application to the application mentioned
under INID code 62.

(54) **Method of producing double low restorer lines of Brassica napus having a good agronomic value**

(57) A method of producing double low restorer line 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 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, PGIunt, PGlint, BolJon and CP418 for characterising.

EP 2 298 066 A9

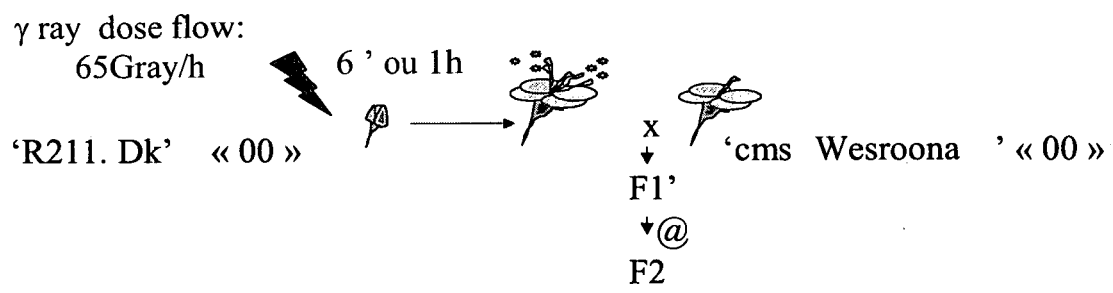


Figure 1

Description

[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 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, said method including the step of:

- 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,
- e) selecting progeny lines.

[0006] According to one advantageous form of embodiment of the method of the present invention, the irradiation dose in step b) is 65 Gray during 6 mn.

[0007] 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.

[0008] R211 is an INRA spring restorer line.

[0009] Drakkar is a French spring registered variety.

[0010] Wesroona is an Australian spring registered variety.

[0011] According to one advantageous form of embodiment of the method according to the present invention, the testing in step d) is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.

[0012] 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.

[0013] According to one advantageous form of embodiment, the double low restorer lines present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, Boron and CP418.

[0014] Another object of the present invention relates to Brassica napus hybrid plants and progeny thereof obtained through the steps of

- a) providing a restorer line produced according to the previous disclosed method of the invention 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.

[0015] Another object of the present invention relates to seeds of Brassica plant obtained from the methods according to the present invention.

[0016] 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 July 4, 2003, under the reference number NCIMB41183.

[0017] Another object of the present invention relates to the use of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombinant 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.

[0018] In a preferred embodiment, the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

[0019] 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.

[0020] Such markers are represented in the following figures and sequence listing for the R2000 line, a double low restorer lines of Brassica napus for cms according to the present invention.

[0021] According to one advantageous form of embodiment, the present invention relates to:

- The marker PGIol which is amplified using the primers: PGIol U and PGIol L
(PGIol U: 5'TCATTGATTGTTGCGCCTG3';
PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
- The marker PGIint which is amplified using the primers: PGIint U and PGIint L
(PGIint U: 5'CAGCACTAATCTTGCGGTATG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
- The marker PGIUNT which is amplified using the primers: PGIol U and PGIint L:

(PGIol U: 5'TCATTGATTGTTGCGCCTG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')

- The marker BolJon which is amplified using the primers: BolJon U and BolJon L:

(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';
BolJon L: 5'GCCTACTCCTCAAATCACTCT3')

- 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')

[0022] Another object of the present invention relates to the PGIol, PGIUNT, PGIint, BolJon and CP418 markers whose sequences follow:

PGIol R2000 marker:

[0023]

```
TCATTGATT GTTGCCTG TCGCCTTGT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGCGG TATGAATTTG TGATTAAATT TGTGTTTGTG TGACTCTTTC TTCATTGTTT 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTTC TACCGGGTCT 240
GATGTACA 248
```

PGIUNT R2000 marker:

[0024]

EP 2 298 066 A9

```

5      TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
      ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
      AATCTTGCGG TATGAATTTG TGATTAAATT TGTTCGTTTG TGAATTTTCT ACCGGGTCTG 180
      GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAAC TGAATTTTCT ACCGGGTCTG 240
      ATGTACAATG CTAGTCTCCA TGTTCTTGGG GATCATGATT TATTTTCTAC ATGTATTCAG 300
      ACAGTACAGA AGAAAGTGTT CAAAACCTCTG GATGTTTTTAA TTTACAGTTA GTGGAGAAGT 360
      TCGGCATTGA TCCGAACAAT GCATTTGCAT TTTGGGACTG GGTTGGTGGA AGGTACAGTG 420
      GTAAGTGCTT GTTTATTTGG TTGTATAAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480
      AACTGAAATT CTTTTGCAGT TTGCAGTGCT GTTGGAGTCT TACCATTGTC TCTACAGTAT 540
10     GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAAATGT 600
      CTTAGGCATA TTCTTCTAT TTTATTTCCC TCTTAATGAT TTCTTCTTTT TTTTATTGCA 660
      TTCCCGTTTT ATTTTCAAAA GTTGTTACTG TCTCTAAATC AAGAAGAAAC CTTCTTAGTA 720
      GATCCAGCTG ATATTCAGCC TTTTTTAAAT TGGACTGCAG GTTTTTTAAAG GGGAGCTTCA 780
      AGCATTGATA AGCATTTCCA GTCCACACCG TTTGAGAAGA ATATACCCGT GAGTTGCATT 840
15     AGTTGTGTGA TTATACAGTT TTCTTGTCCT TTTGCTATGT CCATCAACAC TAGAGATTCG 900
      TGAAGTTATT AGTGTAGTCA ACGCATAGGG AGAGGTGATT GGTGACTTTT GGACGATTC 960
      AGGTGCTTTA GGGTTATTG

```

20 PGIint R2000 marker:

[0025]

```

25     CAGCACTAAT CTTGCGGTAT GAATTTGTGA TTAAATTTGT TTGTTTGTGA CTCTTTCTTC 60
      ATTGTTCTGT 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
30     TACAGTGGTA AGTGCTTGTT TATTTGGTTG TATAAATTTT TCGTCCATTT CCGCTTGCTT 360
      AGTGATATAAC TGAAATTCCT TTGCAGTTTG CAGTGCTGTT GGAGTCTTAC CATTGTCTCT 420
      ACAGTATGGC TTCTCTGTGG TTGAGAAGTA CGGTACCTTC TACTTTATCA GCCATCTCAT 480
      AAAATGTCTT AGGCATATTC TTTCTATTTT ATTTCCCTCT TAATGATTTT TTCTTTTTTT 540
      TATTGCATTC CCGTTTTATT TTCAAAAGTT GTTACTGTCT CTAAATCAAG AAGAAACCTT 600
      CTTAGTAGAT CCAGCTGATA TTCAGCCTTT TTTAAATTGG ACTGCAGGTT TTTAAAGGGG 660
35     AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTTT GAGAAGAATA TACCCGTGAG 720

```

```

40     TTGCATTAGT TGTGTGATTA TACAGTTTTT TTGTCTTTTT GCTATGTCCA TCAACACTAG 780
      AGATTCGTGA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTTGGG 840
      CGATTCAGG TGCTTTAGGG TTATTG

```

BolJon R2000 marker:

45 [0026]

50

55

EP 2 298 066 A9

5 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
 10 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
 15 CTTTCCCCGT AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAGAGTACGA 900
 CATCGTTTTG AGATTAGAAC AAACGTAAAC TTACGTAGAG TGATTTGAGG AGTAGGC 957

CP418L R2000 marker:

20

[0027]

25 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
 30 GAGCTAGCTC TCTCCTCCGC CTCATGAAGC ATCTGTTGCA CCTGAGACAA CCGTGACGAA 420
 ACTTTCCGAT CACCGCCACC AGAATTCGAC GCCGCGCATC GGAAGGATCC GAATCGGGAA 480
 CTGAGTGAAC CCGAGCGATC CCGGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCACG 540
 ATTTTCGACGA AGAGTGGAAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTTCG 600
 TCATCGTCCT GATTGCCGCC ATTTTTTTTTG TCAGGGCGCT CTGTGGCTTA GAAGTTTCCG 660
 35 ATGTCAATGA AC 672

[0028] In the annexed drawing that follows, the following abbreviations are used:

40	Dra	Drakkar
	Rel-15-1, E38, R15	R2000
	Hete, Hel, R211.Drakkar	heterozygous R211 *Drakkar
45	Darm	Darmor
	Bol:	Brassica oleracea
	Bra, B.rap:	Brassica rapa
50	GCPA18-A19, Wes, Aust:	Wesroona
	Sam, SamIPGIolSunt5	Samourai
55	RRH1, ba2c	RRH1
	rav, N.WR	Hybrid Brassica napus*wild Radish

- Figure 1 illustrates Gamma ray Irradiation and F2 production.
- Figure 2 illustrates seed set on 'R211' and 'R2000'.
- Figure 3 illustrates the number of seeds per pod of different lines.
- Figure 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)

PGlint: - primer PGIint U

- primer PGIint L (is out side the sequence).

- Figure 5 illustrates electrophoresis gel of PGI-2 gene (PGIol), PCR marker and SG34, a PCR marker close to Rfo.
- Figure 6 illustrates Pgi-2 segment of DNA amplified by PCR with PGIol primers.
- Figure 7 illustrates digestion of the PCR product PGIol by Mse1.

[0029] In that figure:

Sam and Darm has a 75bp band.

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

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

- Figure 8 illustrates electrophoresis agarose gel of PGIUNT marker.

[0030] In that figure:

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

[0031] There is no amplification in radish and Arabidopsis.

[0032] In various Brassica genotypes only one band was amplified. Size band are similar but sequences are different. - Figure 9 illustrates electrophoresis gel of PGIint PCR marker.

[0033] 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. However the PGIint band is of a similar size of about 870bp in the various Brassica species, but sequences are different.

- Figure 10 illustrates electrophoresis agarose gel of BolJon PCR marker.
- Figure 11 illustrates electrophoresis agarose gel of CP418 marker.

[0034] 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 R211 *Dk). It is absent from the restored rapeseed (RRH, R113 and R211). It is present in the homozygous R2000.

- Figure 12 illustrates summary markers table.
- Figures 13(a), 13(b) illustrate PGIol marker sequence alignment between Arabidopsis, Radish, B. rapa, B. oleracea and R2000.
- Figures 14(a), 14(b), 14(c), 14(d) illustrate the PGIint-UNT marker sequence alignment between Arabidopsis, Radish, B. rapa, B. oleracea and R2000.
- Figures 15(a), 15(b), 15(c) illustrate the CP418L marker sequence alignment between Arabidopsis, Radish, B. rapa, B. oleracea and R2000.
- Figures 16 and 16bis illustrate Arabidopsis, Radish and B. rapa BolJon markers. There are aligned with DB sequences of Arabidopsis (AC007190end-AC01 1000beginning), the B. oleracea EMBH959102 end and EMBH448336 beginning and representative consensus sequences of the SG129markers band 1 and 2 in B. napus (in Drakkar and Samourai respectively).

[0035] From the point 836bp, AC07190-AC11000 and GCPATpBOJ sequences are no longer closely homologous to the Brassica sequences.

[0036] 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.

[0037] In radish, only partial homology is found on the Brassica sequence further down.

[0038] 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.

- Figures 17 and 17bis illustrate the localisation of Pgi-2 primers on the Arabidopsis th MJB21.12 sequence.
- Figure 18 illustrates the BolJon primers localisation on the mipsAt162850 gene and overlapping area of AC007190 and ACO11000 Arabidopsis th clones. Alignment with the Arabidopsis BolJon PCR product (740bp) is presented.

[0039] 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: Method of producing a double low restorer line of *Brassica napus* for Ogura 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:

[0040] Genotypes: The 'R211' line with a deleted radish insertion was crossed to the spring low glucosinolates (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 *Brassica napus** wild radish, *Brassica oleracea*, and *B. rapa* cv Asko, *Arabidopsis thaliana*.

[0041] Gamma ray irradiation: Whole flowering plants were treated with gamma rays from a Co60 source in a controlled area. Sublethal dose of 65 Gray was applied before meioses.

[0042] 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).

[0043] 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): 1-Vegetative vigour, 2- Normality of the ratio of fertile /sterile plants in the F2 segregation, and 3- Female fertility (pod development and seed set).

[0044] Advanced selfed generations of the selected families were obtained either in field or greenhouse and produced homozygous lines (F4) for further analysis.

[0045] 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:

[0046] 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.

[0047] 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:

[0048] Very few families were at the best score for the three criteria out of 1200 F2 families tested.

[0049] 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' (Figs. 2 and 3). This family was obtained from a 6 mn irradiation treatment at a dose flow of 65 Gray per hour.

[0050] Glucosinolate analysis confirmed its low content.

[0051] In figure 2 (Seed set on 'R211' and 'R2000') R2000 showed normal inflorescences, with a normal looking architecture.

[0052] In figure 3 (Number of seeds per pod), we observe:

- on the best 'R2000' F4 families in self pollination (Selfings) and in testcrosses,
- on 'Pactol' cms line on rapeseed and 'R211' controls.

Example II: Selection of markers in the Pgi-2 gene

[0053] PGI isoenzyme analysis: 'R2000' progeny expressed the rapeseed Pgi-2 allele from *B. oleracea* genome, originally lost in 'R211'.

[0054] Three PCR markers were defined to characterise the R2000 family compared to the known restorer rapeseed RRH1 and R113.

1) PGIol marker was developed from the BrassicaDB 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.

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.

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

[0055] 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).

[0056] It was ortholog of the clone MJB21-12, on the chromosome V, (34543bp) in *Arabidopsis* (NCBI DB).

[0057] PGIol plus SG34 to set an Homozygosity test:

[0058] The combined use of two sets of primers in a mix PCR, PGIol marking 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.

[0059] Only one family R2000 showed no difference between homozygote and heterozygote offsprings:

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

[0060] In figure 5 (PGIol and SG34 PCR markers):

The homozygous 'R2000' family has recovered the PGIol band.

[0061] 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.

[0062] 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.

[0063] 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

[0064] Electrophoresis Patterns of PCR products:

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

[0065] In figure 8 (PGIUNT marker):

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.

[0066] 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.

[0067] Electrophoresis of PGIint marker is represented in figure 9.

[0068] Sequence analysis:

Comparison of the PGI sequences from the data bases.

[0069] A PGI segment of about 490bp is known.

[0070] 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: EMBL25875 to 25788 by M. Fouramnn (4) These sequences are very conserved.

[0071] Comparison of the B. rapa et B. oleracea species PGI sequences (figures 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. These 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

[0072] 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.

[0073] The target SG129 sequence was ortholog of a clone (AC011000, at the locus F16P17) in Arabidopsis thaliana. This clone overlapped an Arabidopsis adjacent contig clone (AC07190).

[0074] From the Brassica Crop Net DB, we found one B. oleracea clone, (EMBL448336, 764bp) blasting with the beginning of the A011000, and a second B. oleracea clone (EMBL53971), distant from about 300bp on the Arabidopsis map, that blasted with the end of AC07190.

[0075] 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.

[0076] In figures 16 (electrophoresis gel of BolJon PCR products):

- In Arabidopsis, a BolJon 815bp band was amplified, homologue to the overlapping segment of the contigs.
- 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 (EMBL53971 and EMBL448336) are in sequence continuity in Brassica genome as it is for the ortholog sequences in Arabidopsis.
- In B. napus, these two bands are co-amplified in the maintainer lines, Samourai or Drakkar.
- In radish line7, one BolJon band was amplified of about 630 bp long. The band of the restored radish cmsRd81 was slightly smaller.
- 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.

- The homozygous restored rapeseed lines, 'RRH1', 'R113' and also 'R211', only showed the *B. rapa* band and the 630bp radish band 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.

[0077] '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.

[0078] 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 (Figs. 17).

[0079] 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.

[0080] 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.

[0081] 'R2000' homozygote plants showed CP418 band, again having recovered the specific *B. oleracea* one.

[0082] 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.

[0083] The homozygous 'R2000' presents a unique combination of the PGIol, PGIUNT, PGlint and BolJon markers when compared with the rapeseed restorer analysed yet:

PGlinT 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 PGlint sequence in its *B. rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

[0084] PGIol marker and PGIUNT marker sequences in restored lines RRH1 and R113 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.

[0085] 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.

[0086] CP418 marker showed that 'R2000' recovered this conserved *B. oleracea* segment.

[0087] 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'.

[0088] 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.

EP 2 298 066 A9

SEQUENCE LISTING

<110> Institut National de la Recherche Agronomique (INRA)

5 <120> Method of producing double low restorer lines of Brassica napus having a good agronomic value

<130> D21413

10 <140> EP 04744142.3
<141> 2004-07-05

<150> EP 03291677.7
<151> 2003-07-04

15 <150> EP 03293057.0
<151> 2003-12-08

<150> EP 09175998.5
<151> 2009-11-13

20 <160> 5

<170> PatentIn version 3.2

25 <210> 1
<211> 248
<212> DNA
<213> Brassica napus

<220>
30 <223> PGIo1 marker

<400> 1
tcatttgatt gttgcgcctg tcgccttggt gtgttatgat gaatgaacag cagtcattta 60
acatgtgggt aacttaacag ggctccggct gttgcaaaac acatggttgc tgtcagcact 120
aatcttgcgg tatgaatttg tgattaaatt tgtttgtttg tgactctttc ttcattgttc 180
35 gttttcgtac aataaaccga atgtataatc tttttacaaa ctgaattttc taccgggtct 240
gatgtaca 248

<210> 2
40 <211> 979
<212> DNA
<213> Brassica napus

<220>
45 <223> PGI-UNT marker

<400> 2
tcatttgatt gttgcgcctg tcgccttggt gtgttatgat gaatgaacag cagtcattta 60
acatgtgggt aacttaacag ggctccggct gttgcaaaac acatggttgc tgtcagcact 120
aatcttgcgg tatgaatttg tgattaaatt tgtttgtttg tgactctttc ttcattgttc 180
50 gttttcgtac aataaaccga atgtataatc tttttacaaa tgaattttct accgggtctg 240
atgtacaatg ctagtctcca tgttcttggg gatcatgatt tattttctac atgtattcag 300
acagtacaga agaaagtgtt caaaactctg gatgttttaa tttacagtta gtggagaagt 360
tcggcattga tccgaacaat gcatttgcac tttgggactg ggttggtgga aggtacagtg 420
gtaagtgtct gtttatttgg ttgtataaat ttctcgtcca tttccgcttg cttagtgtat 480
aactgaaatt cttttgcagt ttgcagtgcg gttggagtct taccattgtc tctacagtat 540
55 ggcttctctg tggttgagaa gtacggtacc ttctacttta tcagccatct cataaaatgt 600

EP 2 298 066 A9

5	cttaggcata	ttctttctat	tttatttccc	tcttaaatgat	ttctttctttt	ttttattgca	660
	ttcccgtttt	attttcaaaa	gttggtactg	tctctaaatc	aagaagaaac	cttcttagta	720
	gatccagctg	atattcagcc	ttttttaa	tggactgcag	gtttttaaag	gggagcttca	780
	agcattgata	agcatttcca	gtccacaccg	tttgagaaga	atatacccg	gagttgcatt	840
	agttgtgtga	ttatacagtt	ttcttgtctt	tttgctatgt	ccatcaacac	tagagattcg	900
	tgaagttatt	agtgtagtca	acgcataagg	agaggtgatt	ggtgactttt	ggacgatttc	960
	aggtgcttta	gggttattg					979
10	<210> 3						
	<211> 866						
	<212> DNA						
	<213> Brassica napus						
15	<220>						
	<223> PGI-int marker						
20	<400> 3						
	cagcactaat	cttgoggtat	gaatttgtga	ttaaatttgt	ttgtttgtga	ctctttcttc	60
	attgttcggt	ttcgtacaat	aaaccgaatg	tataatcttt	tacaaactga	atctttctacc	120
	gggtctgatg	tacaatgcta	gtctccatgt	tcttggggat	catgatttat	tttctacatg	180
	tattcagaca	gtacagaaga	aagtgttcaa	aactctggat	gttttaattt	acagttagt	240
	gagaagttcg	gcattgatcc	gaacaatgca	tttgcatttt	gggactgggt	tgggtggaagg	300
	tacagtggta	agtgcctgtt	tatttgggtt	tataaatttc	tcgtccattt	ccgcttgctt	360
	agtgtataac	tgaattctct	ttgcagtttg	cagtgcctgt	ggagtcttac	cattgtctct	420
	acagtatggc	ttctctgtgg	ttgagaagta	cggtagcttc	tactttatca	gccatctcat	480
	aaaatgtctt	agccatattc	tttctatttt	atttccctct	taatgatttc	ttcttttttt	540
	tattgcattc	ccgtttttatt	ttcaaaagt	gttactgtct	ctaaatcaag	aagaaacctt	600
	cttagtagat	ccagctgata	ttcagccttt	tttaaattgg	actgcaggtt	tttaaagggg	660
	agcttcaagc	tttgataagc	atttccagtc	cacaccgttt	gagaagaata	taccgtgag	720
	ttgcattagt	tgtgtgatta	tacagttttc	ttgtcttttt	gctatgtcca	tcaaacactag	780
	agattcgtga	agttattagt	gtagtcaacg	catagggaga	ggtgattggg	gacttttggg	840
	cgatttcagg	tgcttttaggg	ttattg				866
35	<210> 4						
	<211> 957						
	<212> DNA						
	<213> Brassica napus						
40	<220>						
	<223> BolJon marker						
45	<400> 4						
	gatccgattc	ttctctctgtt	gagatcagct	ccaaacatca	aacaacttgt	acacaaatat	60
	ctttacttgc	taaatggaac	atgacaagag	atagaaaatc	ttgctcatag	tattgtacaa	120
	gggataacag	tgtagaaaac	aaaccgtctg	taagattttc	tccctgatcc	tctcacttaa	180
	ccagttagcg	tttttccat	tgaagcgcat	atctactttg	gtattcactg	aataaaaaaa	240
	gaaagctggg	aacatgtgaa	ggatatacaa	gcattgatac	accaagtagt	cacaaactac	300
	attataaagg	tcagaccttt	gttcacattc	tggcctccag	gaccaccgct	tctagcaaag	360
	ttaagcgtaa	catggtctgc	acgtatacaa	atgaaaatgt	ttctatcaaa	atcctataaa	420
	atagagctct	ataacattgt	cgatacatag	tttactaac	tctgcaagta	ctaaacacat	480
	atacaaaaca	aactatgcga	acagatcaaa	actactacag	aacacagttc	tatgacactg	540
	tcgatagtaa	catcctctgc	aagtacaaaa	gagatagcaa	atgaaactat	gtaaacaaat	600
	caaaattcta	aatttctcca	tcacaaggac	ctacagaata	gagttatcat	aacattttct	660
	gtaaaatttt	ccatcaaaat	gactagagaa	cagagttctt	ataacattat	ctgtaaatgt	720
	tccaacaaaa	ccactacata	gcagagttct	tataacattg	tctgtaaatg	tccaatcaaa	780
	accactacag	aacaaagctc	ctataacatt	gtttatacaa	agtttacta	aatctacaaa	840
	ctttcccggt	aaatgagctt	aatatcacc	aaagatgttt	caatcagata	aagagtacga	900
	catcgttttg	agattagaac	aaactgaaac	ttacgtagag	tgatttgagg	agtaggc	957

<210> 5
 <211> 672
 <212> DNA
 <213> Brassica napus

<220>
 <223> CP418L marker

<400> 5
 aattttctcca tcacaaggac ctacagaata gagttatcat aacatttttct gtaaataattt 60
 ccatacaaat gactagagaa cagagttctt ataacattat ctgtaaatgt tccaacaaaa 120
 ccactacata gcagagttct tataacattg tctgtaaatg tccaatcaaa accactacag 180
 aacaaagctc ctataacatt gtttatacaa agtttctact aatctacaaa ctttccccgt 240
 aaatgagctt aatatcacc aaagatgttt caatcagata aagagtaacg acatcgtttt 300
 gagattagaa caaactgaaa cttacgtaga gtgatttgag gagtaggctc gttgccagca 360
 gagctagctc tctcctccgc ctcatgaagc atctgttgca cctgagacaa ccgtgacgaa 420
 actttccgat caccgccacc agaattcgac gccgcgcac ggaaggatcc gaatcgggaa 480
 ctgagtgaac ccgagcgcac ccgggagtgac gacggagcga tgggaaaaga gagtggcacg 540
 atttcgacga agagtggaa aggagaggggt ggtggataaa ctgcgctatg atcaagtctg 600
 tcatcgctct gattgccgcc attttttttg tcagggcgct ctgtggctta gaagtttccg 660
 atgtcaatga ac 672

Claims

1. Combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon, and CP418, wherein the PGIol marker has the sequence of SEQ ID NO: 1, the PGIUNT marker has the sequence of SEQ ID NO: 2, the PGIint marker has the sequence of SEQ ID NO: 3, the BolJon marker has the sequence of SEQ ID NO: 4, and the CP418 marker has the sequence of SEQ ID NO: 5.
2. Use of the combination of five marker of claim 1 for characterising recombined double low restorer lines of Brassica napus for Ogura cms presenting a radish introgression carrying the male fertility Rfo restorer gene deleted of the radish isozyme Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value of female fertility, a good transmission rate of Rfo and a high vegetative vigour.
3. The use of claim 2, wherein the recombined double low restorer lines progeny of Brassica napus for Ogura cms presenting a radish introgression carrying the male fertility Rfo restorer gene deleted of the radish isozyme Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value of female fertility, a good transmission rate of Rfo and a high vegetative vigour, is obtained by a method comprising the steps of:
 - 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 the progeny.
4. The use of claim 3, wherein step d) comprises amplifying the said five markers of claim 1.
5. The use of claim 4, wherein step e) comprises the selecting the progeny wherein a 248 bp band corresponding to PGIol is amplified in step d).
6. The use of claim 4, wherein step e) comprises the selecting the progeny wherein a 866 bp band corresponding to PGIint is amplified in step d).

EP 2 298 066 A9

7. The use of claim 4, wherein step e) comprises the selecting the progeny wherein a 979 bp band corresponding to PGIUNT is amplified in step d).

5 8. The use of claim 4, wherein step e) comprises the selecting the progeny wherein a 957 bp band corresponding to BolJon is amplified in step d).

9. The use of claim 4, wherein step e) comprises the selecting the progeny wherein a 672 bp band corresponding to CP418 is amplified in step d).

10 10. The use of any of claims 4 to 9, wherein step e) comprises the selecting the progeny wherein a 248 bp band corresponding to PGIol and a 866 bp band corresponding to PGIint and a 979 bp band corresponding to PGIUNT and a 957 bp band corresponding to BolJon and a 672 bp band corresponding to CP418 are amplified in step d).

15

20

25

30

35

40

45

50

55

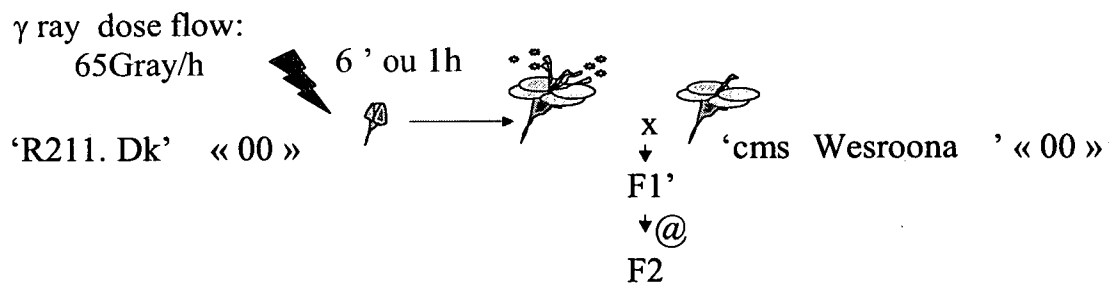


Figure 1

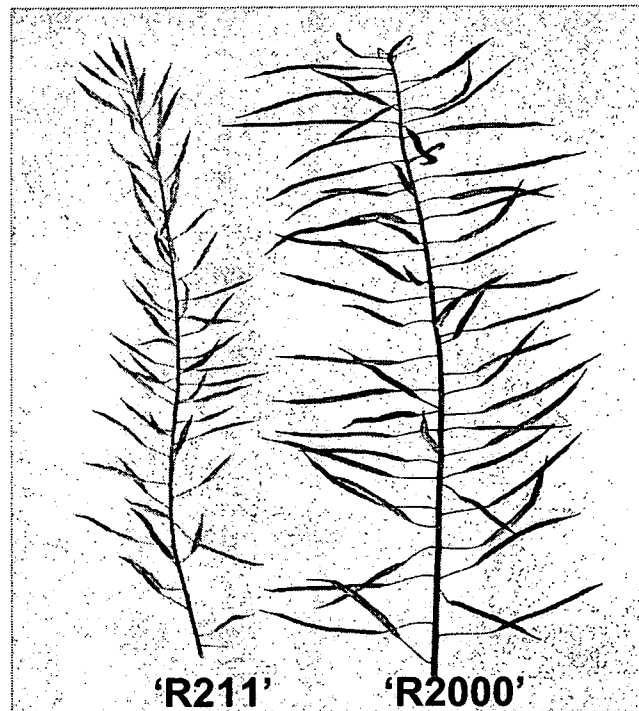


Fig. 1: Seed set on 'R211' and 'R2000'

Figure 2

Genotype	Selfings	Test Crosses
Drakkar	29.3	25.5
Pactol	23.1	
R211	11.2	
R2000	26.5 (24.0 – 31.1)	27.0 (24.0 – 28.7)

Figure 3

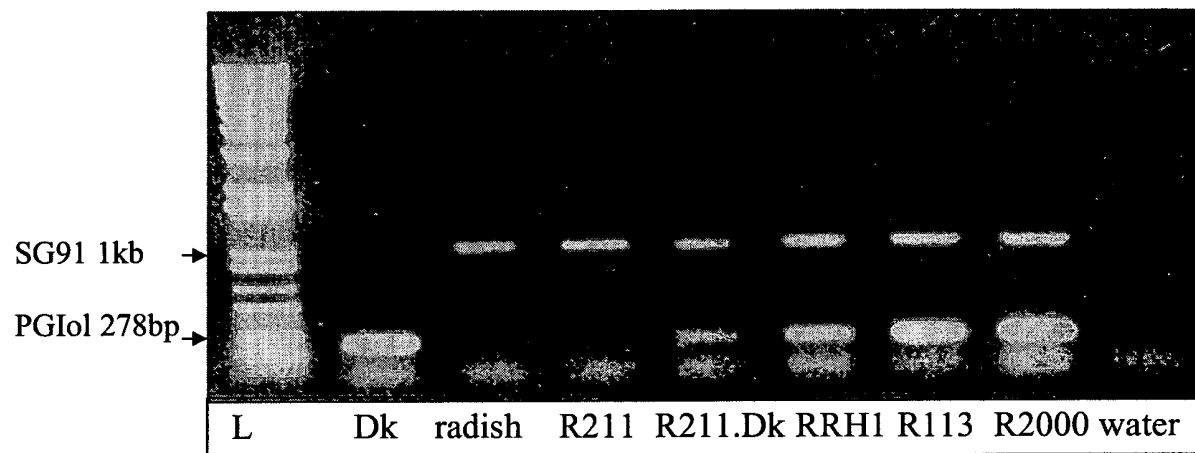


Figure 5



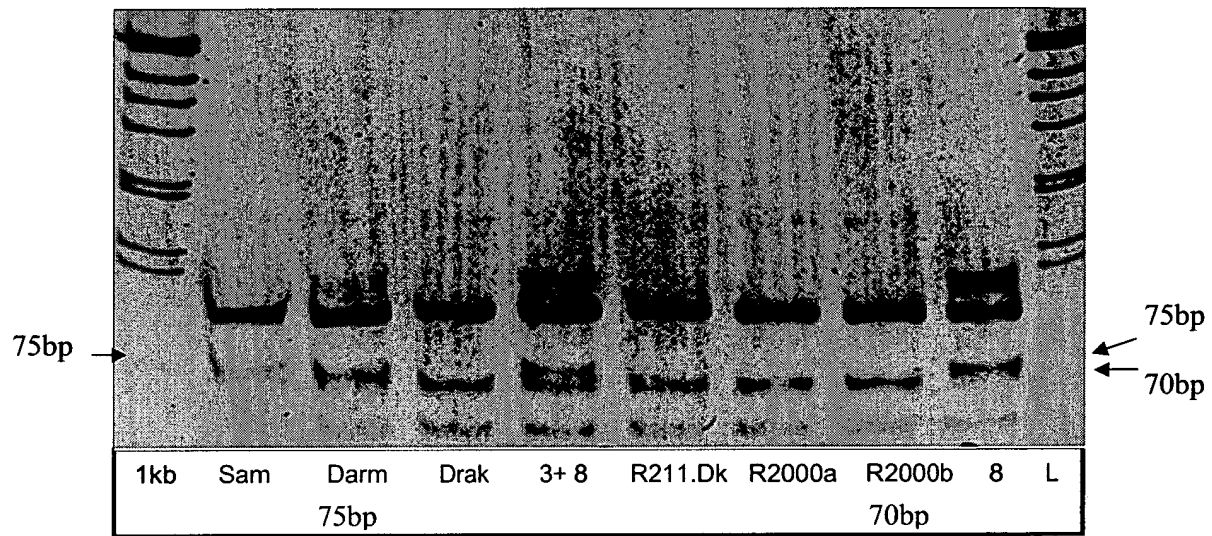


Figure 7

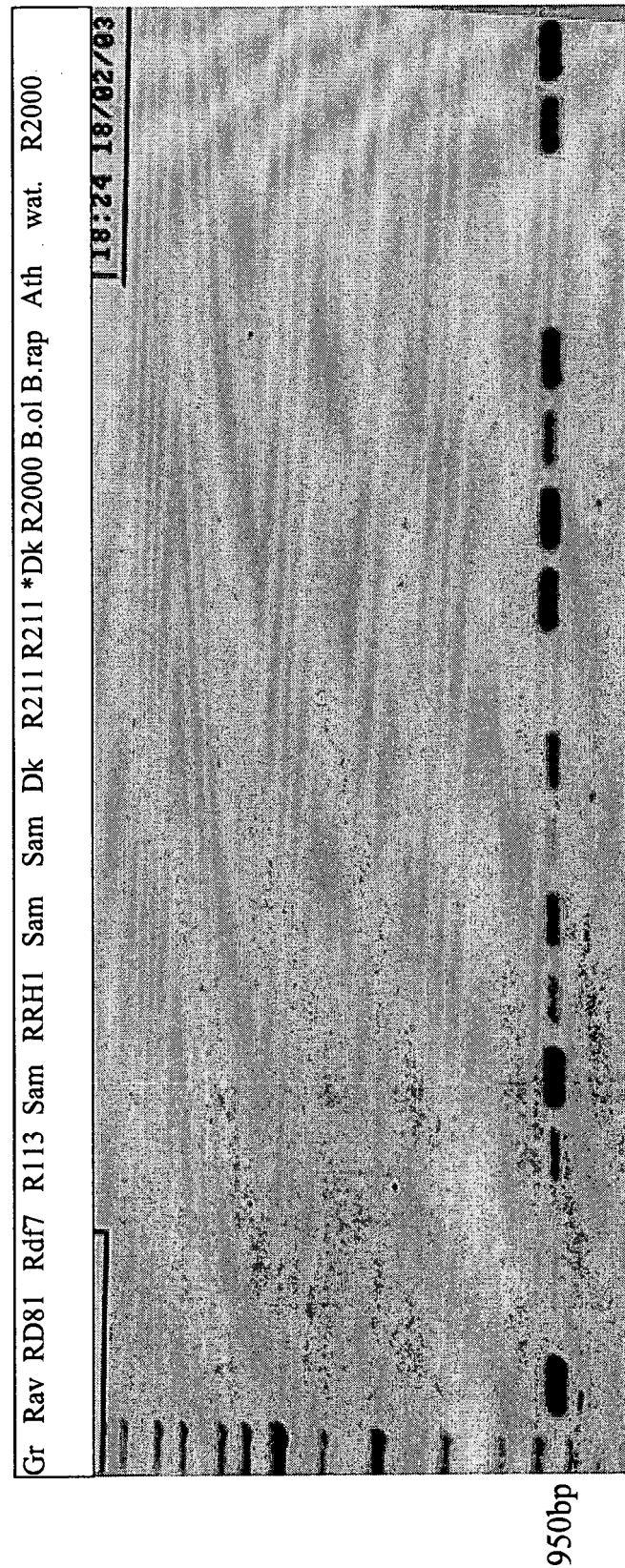


Figure 8

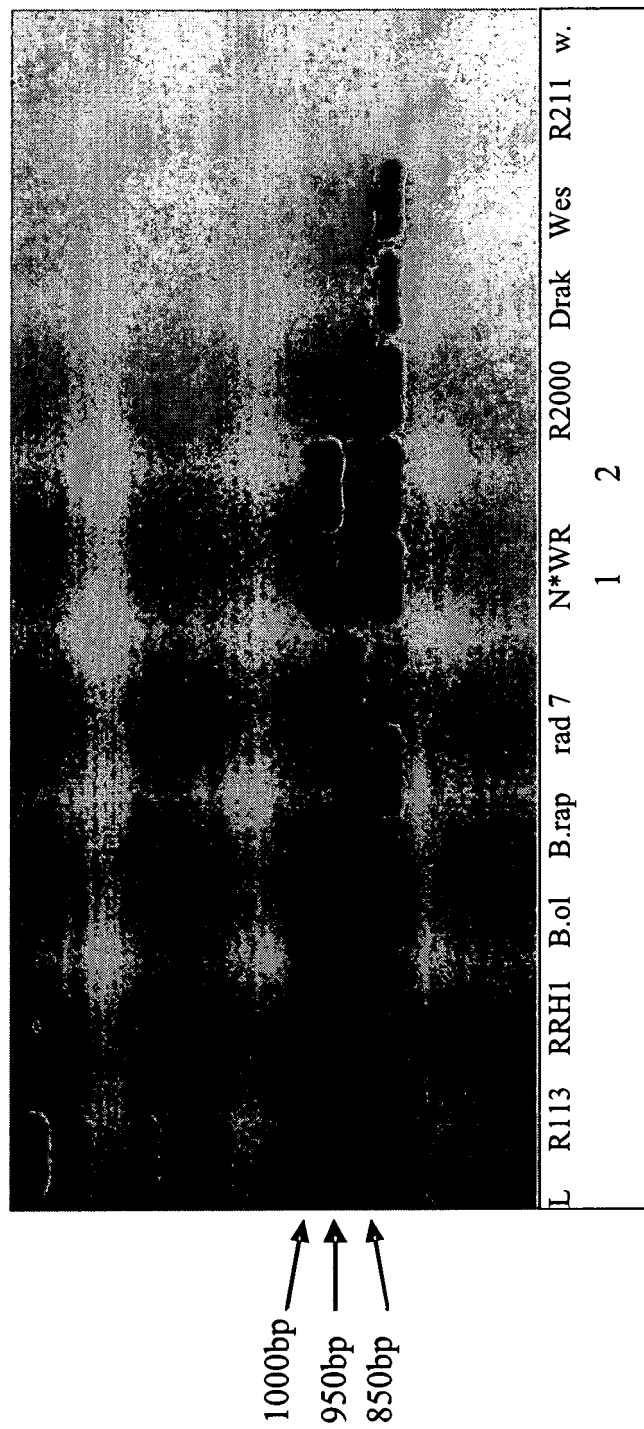


Figure 9

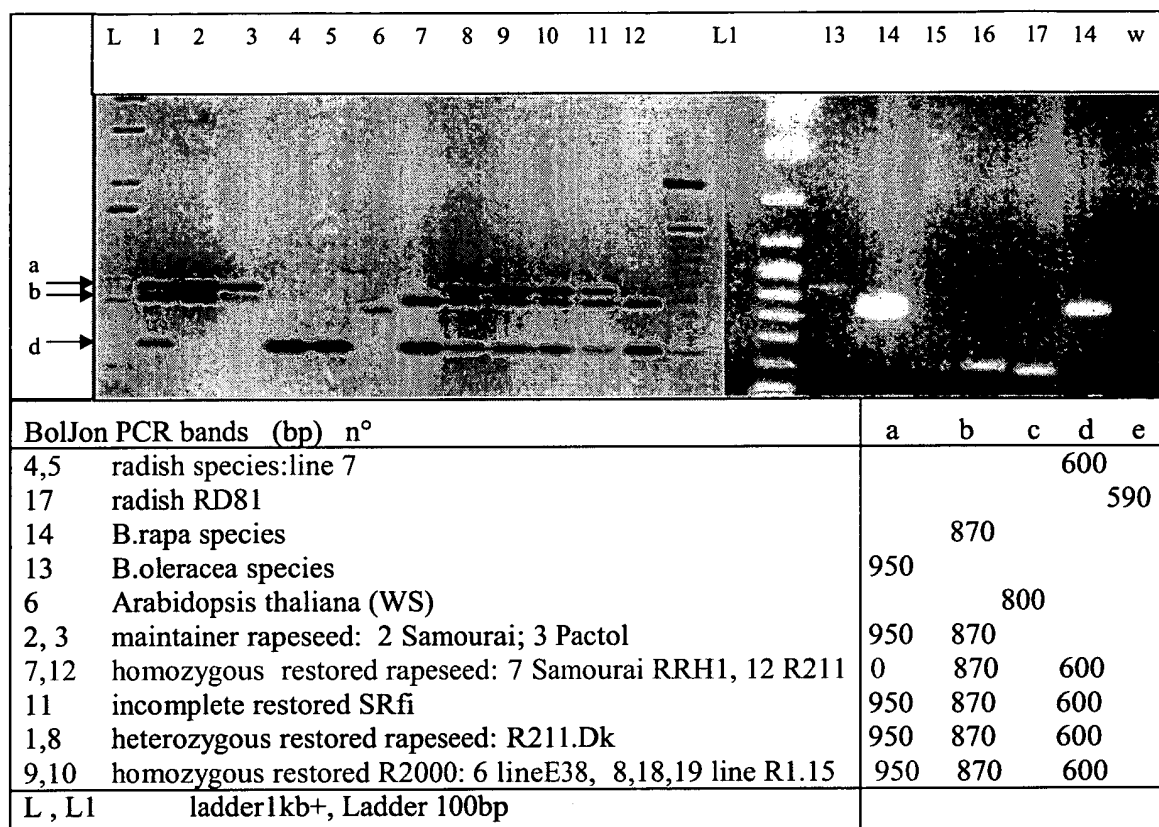
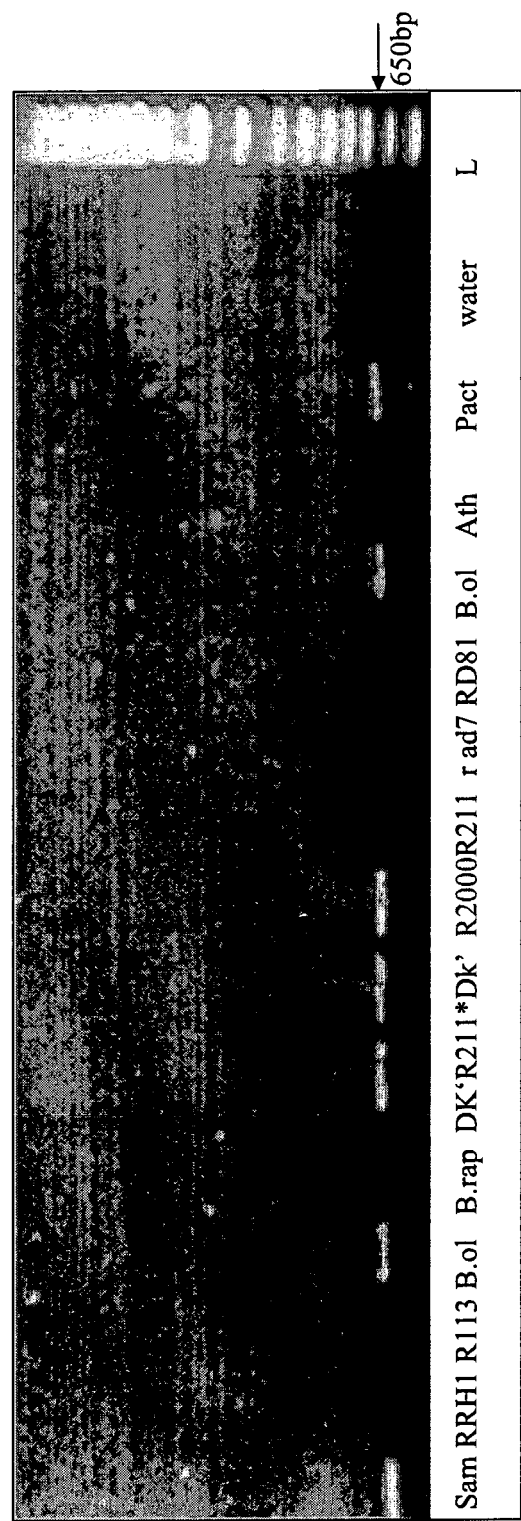


Figure 10



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

Figure 11

[illegible]

Figure 12

	51		81 PGIol U -->	100
consePGIintUNTDrakka				
consensWesrPGI				
consePGIintUNTR113				
consePGIintUNTBrapaA				
ConsePGIintUNTRRH1				
PGIBo-EM:AF258277	TTGCTTAGCG	TCCAAATTC	ATGATTGTAT	TCATTTGATT GTTGTG....
PGIBra-EM:AF258278	TTGCTTAGCG	TCCAAATTC	ATGATTGTAT	TCATTTGATT GTTGTGTGAC
consePGIintUNTBolera				
consePGIintUNTR2000			TTG...	TCATT.GA...TTGT.TGCC
Consensus				1
	101	----		150
consePGIintUNTDrakka		GTCG	TTTGTGGTG	AGT.GAACAG CAGTCATTTA
consensWesrPGI		..GCCTGTTTG	TGTTATGATG	AAT.GAACAG CAGTCATTTA
consePGIintUNTR113		..GCCCAGTTG	G	TAT.GAAACG CAG.CATTTA
consePGIintUNTBrapaA			G	CAGTCATTTA
ConsePGIintUNTRRH1			CG	TGTTGAGAAG CAG.CATTTA
PGIBo-EM:AF258277		CCTG	TCGCCTTGTG	TGTTA.GATG AAT.GAACAG CAGTCATTTA
PGIBra-EM:AF258278	TATCGCTC	TCGCCTTGTG	TGTTATGATG	AAT.GAACAG CAGTCATTTA
consePGIintUNTBolera				
consePGIintUNTR2000		CCTG	TCGCCTTGTG	TGTTATGATG AAT.GAACA. CAGTCATTTA
Consensus			t.g	g ...t.gaa.ag cagtcattta
	151		* MseI restriction site	200
consePGIintUNTDrakka	ACATG.TGGT	TAACCTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
consensWesrPGI	ACATG.TGGT	TAACCTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
consePGIintUNTR113	ACATG.TGGT	..AACTGAACA	GGGCTCCGGC	TGTTGCCC.. CTAAGGGTTG
consePGIintUNTBrapaA	ACATGGTGGT	TAACCTGAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
ConsePGIintUNTRRH1	ACATG..GGT	..AACTGAACA	GGGC.CCGGC	TGTTGCAA.. .ACAG...TG
PGIBo-EM:AF258277	ACATG.TGGT	TAACCTAACA	GGGCTCAGGC	TGTTGCAAAA CACATGGTTG
PGIBra-EM:AF258278	ACATG.TGGT	TAACCTAACA	GGGCTCCGGC	TGTTGCAAAA CATATGGTTG
consePGIintUNTBolera			C	TGTTGCAAAA CACATGGTTG
consePGIintUNTR2000	ACATG.TGGT	TAACCTAACA	GGGCTCCGGC	TGTTGCAAAA CACATGGTTG
Consensus	acatg.tggt	taact..aaca	gggctccggc	tgttgcaaaa cacatggttg
	201		PGI int U ---->	250
consePGIintUNTDrakka	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consensWesrPGI	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR113	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBrapaA	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
ConsePGIintUNTRRH1	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBo-EM:AF258277	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
PGIBra-EM:AF258278	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTBolera	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
consePGIintUNTR2000	CTGT	CAGCAC TAATCTTGC	GGTATG	AATT TGTGATTAAA TTTGTTTGT
Consensus	ctgtcagcac	taatcttgc	ggtatg	aatt tgtgattaaa tttgtttgt
	251			300
consePGIintUNTDrakka	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
consensWesrPGI	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTR113	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC ..AATGTATA
consePGIintUNTBrapaA	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC ..AATGTATA
ConsePGIintUNTRRH1	TGCGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC ..AATGTATA
PGIBo-EM:AF258277	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
PGIBra-EM:AF258278	TGTGACTCTT	TTCTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTBolera	TG.GACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
consePGIintUNTR2000	TGTGACTCTT	T.CTTTCATTG	TTCGTTTTCG	TACAATAAAC CGAATGTATA
Consensus	tg.gactctt	t.cttctattg	ttcgttttcg	tacaataaac cgaatgtata

Figure 13 (a)

	301		<---	PGIol antL 341	350
consePGIintUNTDrakka	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consensWesrPGI	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTR113	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTBrapaA	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
ConsePGIintUNTRRH1	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
PGIBo-EM:AF258277	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
PGIBra-EM:AF258278	ACCTTTTAC AAACGAA	AT GTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTBolera	ATCTTTTAC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
consePGIintUNTR2000	ATCTTTT.AC AAACGAA	TT TTCTACCGGG TCTGATGTAC A		ATGCTAGTC	
Consensus	a <u>t</u> cttttttac aaactgaa	tt ttctaccggg tctgatgtac a		atgctAGTC	

Figure 13 (b)

201	PGI int U ---->			250
consePGIintUNTDrakka	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
consensWesrPGI	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
consePGIintUNTR113	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
consePGIintUNTBrapaA	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
ConsePGIintUNTRRH1	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
PGIBo-EM:AF258277	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
PGIBra-EM:AF258278	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
consePGIintUNTBolera	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
consePGIintUNTR2000	CTGT	CAGCAC TAATCTTGC GGTATG	AATT	TGTGATTAAA TTTGTTTGT
Consensus	ctgtcagcac	taatcttgc ggtatg	aatt	tgtgattaaa tttgtttgt
	251			300
consePGIintUNTDrakka	TGTGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
consensWesrPGI	TGTGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
consePGIintUNTR113	TGCGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	..AATGTATA	
consePGIintUNTBrapaA	TGCGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	..AATGTATA	
ConsePGIintUNTRRH1	TGCGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	..AATGTATA	
PGIBo-EM:AF258277	TGTGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
PGIBra-EM:AF258278	TGTGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
consePGIintUNTBolera	TG.GACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
consePGIintUNTR2000	TGTGACTCTT	T.CTTTCATTG TTCGTTTTTCG TACAATAAAC	CGAATGTATA	
Consensus	tg.gactctt	t.cttcattg ttcgttttcg tacaataaac	CGAATGTATA	
		ε	ε3	
	301	<---	PGIol antL 341	350
consePGIintUNTDrakka	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
consensWesrPGI	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
consePGIintUNTR113	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
consePGIintUNTBrapaA	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
ConsePGIintUNTRRH1	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
PGIBo-EM:AF258277	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
PGIBra-EM:AF258278	ACCTTTTTTAC AAAC TGAA	AT GTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
consePGIintUNTBolera	ATCTTTTTTAC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
consePGIintUNTR2000	ATCTTTTT.AC AAAC TGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC	
Consensus	atcttttttac aaactgaa	tt ttctaccggg tctgatgtac a	atgctAGTC	
	ε			
	351			400
consePGIintUNTDrakka	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTAT	TCAGACAGTA	
consensWesrPGI	TCCATGTTCT	TGGGGATCAT GATTATTTTT CT.CATGTAT	TCAGACAGTA	
consePGIintUNTR113	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTGT	TCAGCCAGTA	
consePGIintUNTBrapaA	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTGT	TCAGCCAGTA	
ConsePGIintUNTRRH1	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTGT	TCAGCCAGTA	
PGIBo-EM:AF258277	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTAT	TCAGACAGTA	
PGIBra-EM:AF258278	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTAT	TCAGACAGTA	
consePGIintUNTBolera	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTAT	TCAGACAGTA	
consePGIintUNTR2000	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTACATGTAT	TCAGACAGTA	
Consensus	TCCATGTTCT	TGGGGATCAT GATTATTTTT CTaCATGTAT	TCAGACAGTA	
		ε 5	ε 6	
	401			450
consePGIintUNTDrakka	CAGAAGAAAG TGTTCAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
consensWesrPGI	CAGAAGAAAG TGTTCAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
consePGIintUNTR113	CAGAAGAAAG TGTTTAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
consePGIintUNTBrapaA	CAGAAGAAAG TGTTTAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
ConsePGIintUNTRRH1	CAGAAGAAAG TGTTTAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
PGIBo-EM:AF258277	CAGAAGAAAG TATTTAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
PGIBra-EM:AF258278	CAGAAGAAAA TGTTTAAAAC	TCTGGATGTT TTGATTTACA	GTTAGTGGAG	
consePGIintUNTBolera	CAGAAGAAAG TGTTCAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
consePGIintUNTR2000	CAGAAGAAAG TGTTCAAAAC	TCTGGATGTT TTAATTTACA	GTTAGTGGAG	
Consensus	CAGAAGAAAG TgTTCAAAAC	TCTGGATGTT TTaATTTACA	GTTAGTGGAG	
	ε 7	ε		

Figure 14 (a)

	451	end of Data Base PGI sequences				500
consePGIintUNTDrakka	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consensWesrPGI	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTR113	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTR113	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
ConsePGIintUNTRRH1	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
PGIBo-EM:AF258277	AAGTTCGGCA	TTGATCC...				
PGIBra-EM:AF258278	AAGTTCGGCA	TTGATCCGAA	CAA.....			
consePGIintUNTBolera	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
consePGIintUNTR2000	AAGTTCGGCA	TTGATCCGAA	CAATGCATT	GCATTTTGGG	ACTGGGTTGG	
Consensus	AAGTTCGGCA	TTGATCCgaa	caatgcattt	gcattttggg	actggggttg	
	501					550
consePGIintUNTDrakka	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consensWesrPGI	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consePGIintUNTR113	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
consePGIintUNTR113	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
ConsePGIintUNTRRH1	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	TAATTTCTCA	
PGIBo-EM:AF258277						
PGIBra-EM:AF258278						
consePGIintUNTBolera	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
consePGIintUNTR2000	TGGAAGGTAC	AGTGGTAAAGT	GCTTGTTTAT	TTGGTTGTAT	AAATTTCTCG	
Consensus	tggaaggtac	agtggtaagt	gcttgtttat	ttggttgtat	.aatttctc.	
					8	9
	551					600
consePGIintUNTDrakka	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consensWesrPGI	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTR113	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTR113	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
ConsePGIintUNTRRH1	TCCATATCCG	CTTGCTTAGT	TTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
PGIBo-EM:AF258277						
PGIBra-EM:AF258278						
consePGIintUNTBolera	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
consePGIintUNTR2000	TCCATTTCCG	CTTGCTTAGT	GTATAACTGA	AATTCTTTTG	CAGTTTGCAG	
Consensus	tccatTTccg	cttgcttagt	GTataactga	aattcttttg	cagtttgcag	
	10		11			
	601					650
consePGIintUNTDrakka	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consensWesrPGI	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consePGIintUNTR113	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
consePGIintUNTR113	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
ConsePGIintUNTRRH1	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCCGTGGTTG	
PGIBo-EM:AF258277						
PGIBra-EM:AF258278						
consePGIintUNTBolera	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
consePGIintUNTR2000	TGCTGTTGGA	GTCTTACCAT	TGTCTCTACA	GTATGGCTTC	TCTGTGGTTG	
Consensus	tgctgttgga	gtcttaccat	tgtctctaca	gtatggcttc	tcTgtggttg	
					12	
	651					700
consePGIintUNTDrakka	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
consensWesrPGI	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
consePGIintUNTR113	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
consePGIintUNTR113	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
ConsePGIintUNTRRH1	AGAAGTACGG	TACCTTCTAC	TTTATTAGCC	ATCTCATAAA	ATGTCTTGGG	
PGIBo-EM:AF258277						
PGIBra-EM:AF258278						
consePGIintUNTBolera	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	A.GTCTTAGG	
consePGIintUNTR2000	AGAAGTACGG	TACCTTCTAC	TTTATCAGCC	ATCTCATAAA	ATGTCTTAGG	
Consensus	agaagtacgg	taccttctac	tttatCagcc	atctcataaa	atgtcttAgg	
			13		14	

Figure 14 (b)

	701				750
consePGIintUNTDrakka	CATATTCTTT CTATTTTATT TTCCCTCTTAA TGATTTCTTC TTTTTTTTAT				
consensWesrPGI	CATATTCTTT CTATTTTATT TTCCCTCTTAA TGATTTCTTC TTTTTTTTAT				
consePGIintUNTR113	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTTCTTC TCTTTTAT..				
consePGIintUNTR113	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTTCTTC TCTTTTAT..				
ConsePGIintUNTRRH1	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTTCTTC TCTTTTAT..				
PGIBo-EM:AF258277					
PGIBra-EM:AF258278					
consePGIintUNTBolera	CATATTCTTT CTATTTTATT TTCCCTCTTAA TGATTTCTTC TTTTTTTA..				
consePGIintUNTR2000	CATATTCTTT CTATTTTATT TCCCTCTTAA TGATTTCTTC TTTTTTTTAT				
Consensus	catattcttt ctatttttatt ttccctct.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				
consePGIintUNTR113	TGCATTCCCG TTTTATTTTC AAAAGTTGTC ACTGTCTCTA AATCAAGAAG				
ConsePGIintUNTRRH1	TGCATTCCCG TTTTATTTTC AAAAGTTGTC ACTGTCTCTA AATCAAGAAG				
PGIBo-EM:AF258277					
PGIBra-EM:AF258278					
consePGIintUNTBolera	TGCATTCCCG TTTTATTTTC AAAAGTTGTC CGGCCCCCTA AACCAAGAAG				
consePGIintUNTR2000	TGCATTCCCG TTTTATTTTC AAAAGTTGTT ACTGTCTCTA AATCAAGAAG				
Consensus	tgcattcccg ttttattttc aaaagttgt. actgtctcta aatcaagaag				
	801				850
consePGIintUNTDrakka	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
consensWesrPGI	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
consePGIintUNTR113	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
consePGIintUNTR113	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
ConsePGIintUNTRRH1	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
PGIBo-EM:AF258277					
PGIBra-EM:AF258278					
consePGIintUNTBolera	AAACCTTCTT AGGA...CCA GA...CTCC ACCCTTTTCT AAATTGGACT				
consePGIintUNTR2000	AAACCTTCTT AGTAGATCCA GTTGATATTC AGCCTTTTCT AAATTGGACT				
Consensus	aaaccttctt agtagatcca g. tgatattc agcctttt. t aaattggact	18	19		
	851				900
consePGIintUNTDrakka	GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCCC				
consensWesrPGI	GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCAC				
consePGIintUNTR113	GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCC..				
consePGIintUNTR113	GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCC..				
ConsePGIintUNTRRH1	GCAGGTTTTT AAA.GGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCAC				
PGIBo-EM:AF258277					
PGIBra-EM:AF258278					
consePGIintUNTBolera	GCAGGTTTTT AAA.GGGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCAC				
consePGIintUNTR2000	GCAGGTTTTT AAACGGGAGC TTCAAGCATT GATCAGCATT TCCAGTCCAC				
Consensus	gcaggttttt aaa.gggagc ttcaagcatt gat. agcatt tccagtcc.c	20			
	901				950
consePGIintUNTDrakka	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT GTGTGATTAT				
consensWesrPGI	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT				
consePGIintUNTR113	.CCCGTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. .GTGATTAT				
consePGIintUNTR113	.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 (c)

```

                                     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 TTGTCTTTTT. GCTATG.TCA TCAAC.CTAG A.GATTCATG
ConsePGIintUNTRRH1 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 acagttttt.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..TTCAC TG AC GG.....
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 ACGTTTTT CAC GTGCTCCGGG GGGTTTTTGG GGACCAAACC CCCAAC
consensWesrPGI .....
consePGIintUNTR113 .....
consePGIintUNTBrapaA .....
ConsePGIintUNTRRH1 .....
PGIBo-EM:AF258277 .....
PGIBra-EM:AF258278 .....
consePGIintUNTBolera .....
consePGIintUNTR2000 ACGATTTT CAG GTGCTTTAGG GTTATTG

```

Figure 14 (d)

	51		100
EMBH44836anti			
GCP18-5CP418L-Sams			
GCP18-2CP418L-Wes			
GCP18-4CP418L-R2000			CP418L....
conse129ba1-Drak		AAACAAATCA	AAATTCTAA TTTCTCCA
GCP18-129Sam-ba2		AAAC TATGTA	ACAAAAATCA AAATTGTAAA TGTCTCCA
GCPR18-3129R211-ba2			AA CCAAAAATCC AAATTGTAAA TGTTCCT.
GCP18-10129R20-ba2			CAAAATCCA AAATTGTAAA TGTTCCT
Consensus			
	101		150
EMBH44836anti			
GCP18-5CP418L-Sams			AT A.CATTTTCT GTAA
GCP18-2CP418L-Wes			AGG T.AT A.CATTTTCT GTAA
GCP18-4CP418L-R2000			AGG TCAT A.CATTTTCT GTAA
conse129ba1-Drak	TCACAAGGAC	CTACAGAATA	GAGTTATCAT AACATTT CT GTAA
GCP18-129Sam-ba2	TCGTAAAGGAC	CTACAGAATA	GAGTTATCAT AACATTTTCT G AA
GCPR18-3129R211-ba2	TGGTAAAGGC	CTCAAAAA.A	GAGGTATCAA AAC.TTTTCT GT.A
GCP18-10129R20-ba2	TGGTAAAGGC	C.CAAAAA.A	AAGGT..CAA AACTT.TCCG GTAA
Consensus			
	151		200
EMBH44836anti			
GCP18-5CP418L-Sams	.TATTTCCAT	CAAAATGA..	.CTAGAGAAC AGCAGTTCTT ATAACATTAT
GCP18-2CP418L-Wes	.TATTTCCAT	CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
GCP18-4CP418L-R2000	ATATTTCCAT	CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
conse129ba1-Drak	ATATTTCCAT	CAAAATGA..	.CTAGAGAAC AG.AGTTCCTT ATAACATTAT
GCP18-129Sam-ba2	ATGTTTCCAT	CAAAATGA	CTATCGAAC ATAATTAAT ATA.CATTTT
GCPR18-3129R211-ba2	ATGTTTCCAT	CAAAATG.	CTATCGGAC ATAATTAAT ATAAC.TTCT
GCP18-10129R20-ba2	ATGTTTCCAT	CAAAATG.	CTTCGGA.C ATAATTAAT ATAAC.TTCT
Consensus	ATGTTTCCCT	CAAA.TGG	CTTCGGA.C ATAATTAAT A...CATTCT
	201		250
EMBH44836anti			
GCP18-5CP418L-Sams	CTGTAAA TG.TTCCAA	CAAAA CCACT	ACATAGCAGAGTTC .TTATAACAT
GCP18-2CP418L-Wes	CTGTAAA TG.TTCCAA	CAAAA CCACT	ACATAGCAGAGTTC ATTATAACAT
GCP18-4CP418L-R2000	CTGTAAA TG.TTCCAA	CAAAA CCACT	ACATAGCAGAGTTC .TTATAACAT
conse129ba1-Drak	CTGTAAA TG.TTCCAA	CAAAA CCACT	ACATAGCAGAGTTC .TTATAACAT
GCP18-129Sam-ba2	CTG.AAAAT.AATTCCT	CAAAA TTA.	.CATT TTC TTACAA.A
GCPR18-3129R211-ba2	CTG.AAAAT.AATTCCT	CAAAA TTA.	.CATT TTC T.ACAA.A
GCP18-10129R20-ba2	CTG.AAA.TAATTCCT	CAAAA TTA.	.CATT TTC T.ACAA.A
Consensus			
	251		300
EMBH44836anti			...CTATACC
GCP18-5CP418L-Sams	TGTCTGT.AA	ATGTCCAATC	AAAACCACTA CAGAACAAAG CTCCTATAAC
GCP18-2CP418L-Wes	TGTCTGT.AA	ATGTCCAATC	AAAACCACTA CAGAACAAAG CTCCTATAAC
GCP18-4CP418L-R2000	TGTCTGT.AA	ATGTCCAATC	AAAACCACTA CAGAACAAAG CTCCTATAAC
conse129ba1-Drak	TGTCTGT.AA	ATGTCCAATC	AAAACCACTA CAGAACAAAG CTCCTATAAC
GCP18-129Sam-ba2	TGTTC...		CATCAAAATG AGACTCA.G
GCPR18-3129R211-ba2	TGTTC...		CATCAAAATG AGACTCA.G
GCP18-10129R20-ba2	TGTTC...		CATCAAAATG AGACTCA.G
Consensus	tttctgt.aa	tggttccatc	aaaatgacta tcgaacataa ttaatAtaac
	301		350
EMBH44836anti	A	TTGTTT	ATACAAAGTT TCACT AAAT CTACAAACTT CCCCCGTAAA
GCP18-5CP418L-Sams	A	TTGTTT	ATACAAAGTT .CACT AAAT CTACAAACTT TCCCCGTAAA
GCP18-2CP418L-Wes	A	TTGTTT	ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA
GCP18-4CP418L-R2000	A	TTGTTT	ATACAAAGTT .CACT AAAT CTACAAACTT TCCCCGTAAA
conse129ba1-Drak	A.	TGTTT	ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA
GCP18-129Sam-ba2	AAC.CAGTTC	TTTCATAGTT	TCACTTAAAT CTACAAACTT TC.....
GCPR18-3129R211-ba2	AACACAGTTC	TTTCATAGTT	TCACT.AAAT CTACAAACTT TC.....
GCP18-10129R20-ba2	A.CCCAGTTC	TTTCATAGTT	TC.CT.AAAT CTTCAAACTT TC.....
Consensus			

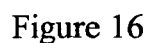
Figure 15 (a)

	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
conse129ba1-Drak	TGAGCTTAAT ATCACCCAAA	GATGTTTCA ATCAGAT AAA	GAGTA.CGAC
GCPS18-129Sam-ba2	AAT CTATATCTAAA	G.TTATCAC ATCACAT	GAA GA.....
GCPR18-3129R211-ba2	AAT CTATATCTAAA	G.TTATCAC ATCACAT	GAA GA.....
GCP18-10129R20-ba2	AAT CTATATCTAAA	G.TTATCAC ATCACAT	GAA GA.....
Consensus	-----	-----	-----
	401		
EMBH44836anti	ATCGTTTGA GATTAGAACA AA		
GCP18-5CP418L-Sams	ATCGTTTGA GATTAGAACA AA		
GCP18-2CP418L-Wes	ATCGTTTGA GATTAGAACA AA		
GCP18-4CP418L-R2000	ATCGTTTGA GATTAGAACA AA		
conse129ba1-Drak	ATCGTTTGA GATTAGAACA AA		
GCPS18-129Sam-ba2	GAGC AA		
GCPR18-3129R211-ba2	GAGC AA		
GCP18-10129R20-ba2	GAGC 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 CCAGCAGAGC	
GCP18-4CP418L-R2000	CTGAAACTTA CGTAGAGTGA TTTGAGGAGT	AGGCTCGTTG CCAGCAGAGC	
conse129ba1-Drak	CTGAAACTTA CGTAGAGTGA TTTGAGGAGT	AGGCTCGTTG CCAGCAGAGC	
GCPS18-129Sam-ba2	GTAAACCTTA CTTAGAGTGA TTTGAGGAGT	AGGCTCGTTG CCAGCAGAGC	
GCPR18-3129R211-ba2	GTAAACCTTA CTTAGAGTGA TTTGAGGAGT	AGGCTCGTTG CCAGCAGAGC	
GCP18-10129R20-ba2	GTAA.CCTTA CTTAGAGTGA TTTGAGGAGT	AGGCTCGTTG CCAGCAGAGC	
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	
conse129ba1-Drak	TAGCTCTCTC CTCCGCCTCA TGAAGCATCT	GTTGCACCTG AGACAACCGT	
GCPS18-129Sam-ba2	TAGCTCTCTC CTCC.CCTCA TGAAGCATCT	GTTGCACCTG AGACAACCGT	
GCPR18-3129R211-ba2	TAGCTCTCTC CTCCGCCTCA TGAAGCATCT	GTTGCACCTG AGACAACCGT	
GCP18-10129R20-ba2	TAGCTCTCTC CTCCGCCTCA TGAAGCATCT	GTTGCACCTG 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	
conse129ba1-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 cgcacccgaa	
	581		630
EMBH44836anti	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCP18-5CP418L-Sams	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCP18-2CP418L-Wes	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCP18-4CP418L-R2000	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
conse129ba1-Drak	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCPS18-129Sam-ba2	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCPR18-3129R211-ba2	GGATCCGAAT CGG.AACTGG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
GCP18-10129R20-ba2	GGATCCGAAT CGGGAACCTG AGTGAACCCG	AGCGATCCCG GGAGTGCAC	
Consensus	ggatccgaat cgggaactgg agtgaaccg	agcgatcccg ggagtgcgac	

Figure 15 (b)

	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
conse129ba1-Drak	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAA.A GTGGAAGAGG
GCP18-129Sam-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
conse129ba1-Drak	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA TCGTCCTGAT
GCP18-129Sam-ba2	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA TCGTCCTGAT
GCPR18-3129R211-ba2	AGAGG.TGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA TCGTCCTGAA
GCP18-10129R20-ba2	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA TCGTCCTGAA
Consensus	agaggggtggt	ggataaaactc	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 GTTTCCGATG
conse129ba1-Drak	TGCCGCCATT	TTTTTTGTCA	GGGCGCTCTG	.GGCTTAGAA GTTTCCGA..
GCP18-129Sam-ba2	TGCCGCCATT	CTTGTTTCAC.	.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	TCAATGAAC	AGAAT...TC	CGGG...	
GCP18-2CP418L-Wes	CCAATGAACA	AGATTATTTC	CGATG..	
GCP18-4CP418L-R2000				
conse129ba1-Drak				

Figure 15 (c)





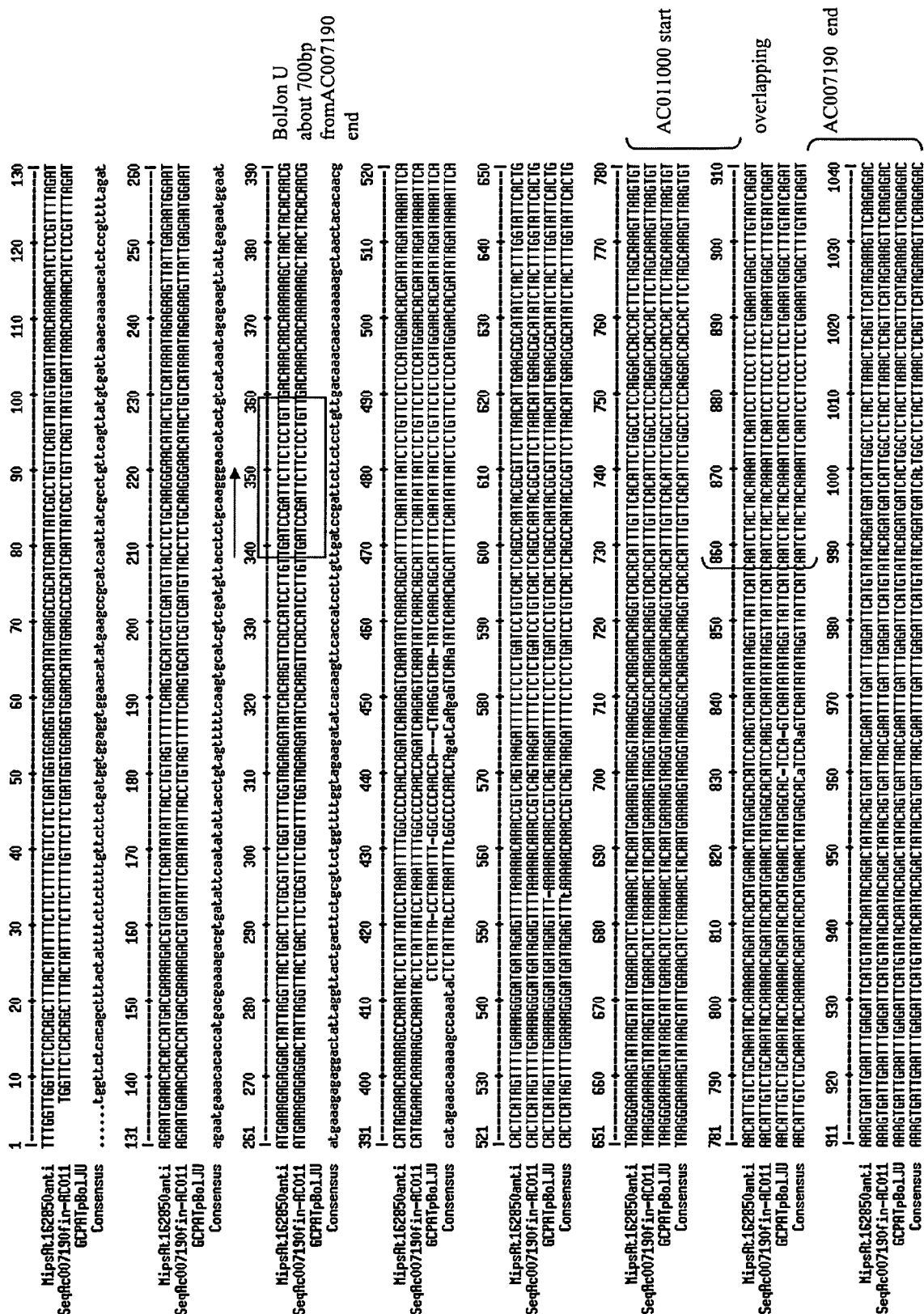


Figure 17

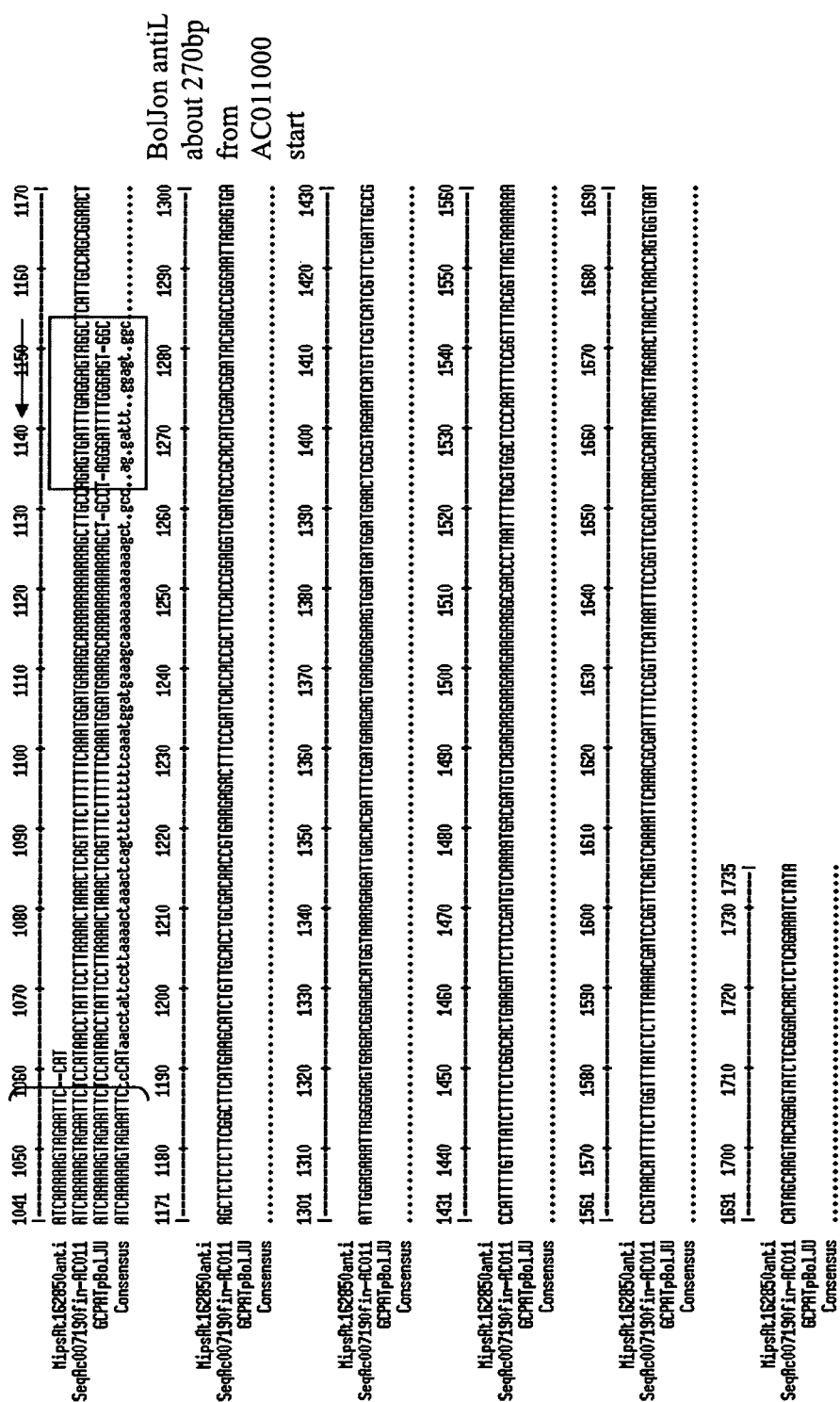


Figure 17BIS



EUROPEAN SEARCH REPORT

Application Number
EP 10 18 3322

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	DATABASE EMBL [Online] 2 May 2001 (2001-05-02), "Brassica napus cultivar Darmor glucose-6-phosphate isomerase (PGI-BN-1) gene, partial cds.", XP002619740, retrieved from EBI accession no. EMBL:AF258275 Database accession no. AF258275 * For SEQ IDs 1-3 - sequence * -----	1-10	INV. A01H1/02 A01H1/06 A01H5/00 A01H5/10 C12N15/11 C12Q1/68
A	DATABASE EMBL [Online] 14 February 2001 (2001-02-14), "Leavenworthia stylosa cultivar ST44a-S glucose-6-phosphate isomerase (PgiC) gene, partial cds.", XP002619741, retrieved from EBI accession no. EMBL:AF293475 Database accession no. AF293475 * For SEQ ID 3 - sequence * -----	1-10	TECHNICAL FIELDS SEARCHED (IPC) A01H C12N C12Q
A	DATABASE EMBL [Online] 5 October 2002 (2002-10-05), "odh44h09.b1 B.oleracea002 Brassica oleracea genomic, DNA sequence.", XP002619742, retrieved from EBI accession no. EMBL:BH959102 Database accession no. BH959102 * For SEQ ID 4 - sequence * ----- -/--	1-10	
2 The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 8 February 2011	Examiner Chakravarty, Ashok
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03.82 (P04C01)



EUROPEAN SEARCH REPORT

 Application Number
 EP 10 18 3322

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	DATABASE EMBL [Online] 15 December 2001 (2001-12-15), "BOHCZ75TR BOHC Brassica oleracea genomic clone BOHCZ75, genomic survey sequence.", XP002619743, retrieved from EBI accession no. EMBL:BH448336 Database accession no. BH448336 * For SEQ ID 5 - sequence * -----	1-10	
Y	DELOURME R ET AL: "DOUBLE LOW RESTORED F1 HYBRIDS CAN BE PRODUCED WITH THE Ogu-INRA CMS IN RAPESEED", PROCEEDINGS 10TH INTERNATIONAL RAPESEED CONGRESS., 1999, pages 6-9, XP002575754, * the whole document * -----	1-10	
Y	DELOURME R ET AL: "IDENTIFICATION OF RAPD MARKERS LINKED TO A FERTILITY RESTORER GENE FOR THE OGURA RADISH CYTOPLASMIC MALE STERILITY OF RAPESEED (BRASSICA NAPUS L.)", THEORETICAL AND APPLIED GENETICS, SPRINGER, BERLIN, DE, vol. 88, no. 6/7, 1994, pages 741-748, XP002043620, ISSN: 0040-5752 * the whole document * ----- -/--	1-10	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC)
Place of search Munich		Date of completion of the search 8 February 2011	Examiner Chakravarty, Ashok
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

 2
 EPO FORM 1503 03.82 (P04C01)



EUROPEAN SEARCH REPORT

Application Number
EP 10 18 3322

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Y	DELOURME R ET AL: "BREEDING DOUBLE LOW RESTORER LINES IN RADISH CYTOPLASMIC MALE STERILITY OF RAPESEED (BRASSICA NAPUS L)", PROCEEDINGS 9TH INTERNATIONAL RAPESEED CONGRESS, CAMBRIDGE, UK, 4 July 1995 (1995-07-04), - 7 July 1995 (1995-07-07), pages 6-08, XP002575753, * the whole document *	1-10	
Y,D	DELOURME R ET AL: "Characterisation of the radish introgression carrying the Rfo restorer gene for the Ogu-INRA cytoplasmic male sterility in rapeseed (Brassica napus L.)", THEORETICAL AND APPLIED GENETICS, vol. 97, no. 1-2, July 1998 (1998-07), pages 129-134, XP002283532, ISSN: 0040-5752 * the whole document *	1-10	
Y	DESLOIRE SOPHIE ET AL: "Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family.", EMBO REPORTS, vol. 4, no. 6, June 2003 (2003-06), pages 588-594, XP9013642, * the whole document *	1-10	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC)
Place of search Munich		Date of completion of the search 8 February 2011	Examiner Chakravarty, Ashok
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

2
EPO FORM 1503 03.82 (P04C01)



EUROPEAN SEARCH REPORT

Application Number
EP 10 18 3322

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X,P	GIANCOLA SANDRA ET AL: "Characterization of a radish introgression carrying the Ogura fertility restorer gene Rfo in rapeseed, using the Arabidopsis genome sequence and radish genetic mapping.", TAG. THEORETICAL AND APPLIED GENETICS. THEORETISCHE UND ANGEWANDTE GENETIK. GERMANY NOV 2003, vol. 107, no. 8, 27 August 2003 (2003-08-27), pages 1442-1451, XP002283533, ISSN: 0040-5752 * the whole document * -----	1-10	
A	WO 98/27806 A1 (PIONEER HI BRED INT [US]; CHARNE DAVID G [CA]; GRANT IAN [CA]; KRALING) 2 July 1998 (1998-07-02) * the whole document * -----	1-10	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC)
Place of search Munich		Date of completion of the search 8 February 2011	Examiner Chakravarty, Ashok
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

 2
EPO FORM 1503 03.02 (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 10 18 3322

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

08-02-2011

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9827806 A1	02-07-1998	AU 731564 B2	05-04-2001
		AU 5544798 A	17-07-1998
		CZ 9803026 A3	17-03-1999
		DE 891130 T1	16-09-1999
		JP 2000505654 T	16-05-2000
		NO 983855 A	23-10-1998
		NZ 331276 A	26-05-2000
		PL 328612 A1	01-02-1999
		US 2002032916 A1	14-03-2002

EPO FORM P/459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **GIANCOLA et al.** *Theor Appl. Genet.*, 2003 [0021]
- **DELOURME R. ; EBER F.** *Theor Appl Genet*, 1992, vol. 85, 222-228 [0045] [0046]
- **FOURMANN M. et al.** *Theor Appl. Genet.*, 2002, vol. 105, 1196-1206 [0045] [0046]
- **DELOURME R. et al.** *Theor Appl Genet*, 1998, vol. 97, 129-134 [0046]
- **DELOURME R. et al.** *10th Int. Rapeseed Congress*, 1999 [0046]
- **GIANCOLA S. et al.** *Theor Appl. Genet.*, 2003 [0046] [0058] [0072]
- **DESLOIRES S. et al.** *EMBO reports*, 2003, vol. 4 (6), 588-594 [0072]