

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
13 January 2005 (13.01.2005)

PCT

(10) International Publication Number
WO 2005/002324 A2

(51) International Patent Classification⁷: **A01H** Michel [FR/FR]; 9, avenue du Stade, F-35650 Le Rheu (FR).

(21) International Application Number:
PCT/IB2004/002491

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(22) International Filing Date: 5 July 2004 (05.07.2004)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(25) Filing Language: English
(26) Publication Language: English

(30) Priority Data:
03291677.7 4 July 2003 (04.07.2003) EP
03293057.0 8 December 2003 (08.12.2003) EP

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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/002324 A2

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

(57) Abstract: 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, PGIint, BolJon and CP418 for characterising.

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

The invention relates to a method of producing a double low restorer lines of
5 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
10 Brassica napus hybrid seed and progeny thereof and to the use of markers for
selection.

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
15 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.

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

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

A first object of the present invention relates to a method of producing double low
25 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:

30 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,
- 5 d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
- e) selecting progeny lines.

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

- 10 10 A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.

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.

- 15 15 R211 is an INRA spring restorer line.

Drakkar is a French spring registered variety.

Wesroona is an Australian spring registered variety.

- 20 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, PGInt, BolJon and CP418.

- 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.

- 25 According to one advantageous form of embodiment, the double low restorer lines present a unique combination of five markers selected from PGIol, PGIUNT, PGInt, BolJon and CP418.

- Another object of the present invention relates to a method of forming Brassica napus hybrid plants and progeny thereof obtained through the steps of:

- 30 a) providing a restorer line produced according to claim 1 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.
- 5 Another object of the present invention relates to seeds of Brassica plant obtained from the methods according to the present invention.
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.
- 10 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 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
- 15 agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.
- In a preferred embodiment, the combination is of five markers PGiol, PGIUNT, PGIint, BolJon and CP418.
- In the present invention, the expression " any portion of them comprising at least 20 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.
- According to one advantageous form of embodiment, the present invention relates 25 to:
- The marker PGiol which is amplified using the primers: PGiol U and PGiol L
(PGiol U: 5'TCATTTGATTGTTGCGCCTG3';
PGiol L: 5'TGTACATCAGACCCGGTAGAAAA3')
 - The marker PGIint which is amplified using the primers: PGIint U and PGIint L
(PGIint U: 5'CAGCACTAATCTTGCAGGTATG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
 - The marker PGIUNT which is amplified using the primers: PGiol U and PGIint L:

(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';

PGIint L: 5'CAATAACCCCTAAAAGCACCTG3')

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

(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';

5 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'AATTCTCCATCACAAAGGACC3')

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

PGIol R2000 marker:

TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATT	60
ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCA	120
AATCTTGC	GGGG	TATGAATT	TGATTAATT	TGTTTGT	TGACTCTTC	180
15 GTTTCTG	TAC	AACT	TTTG	TGACTCTTC	TTCATTGTT	240
TGAC	ATG	AAAC	ACAA	CTGAATTTC	TACCGGGTCT	248
GATGTACA						

PGIUNT R2000 marker:

TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATT	60
ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCA	120
AATCTTGC	GGGG	TATGAATT	TGATTAATT	TGTTTGT	TGACTCTTC	180
25 GTTTCTG	TAC	AACT	TTTG	TGACTCTTC	TTCATTGTT	240
TGAC	ATG	AAAC	ACAA	CTGAATTTC	TACCGGGTCT	248
AGAGTACAGA	AGAAAGTGT	CAAAACTCTG	GATGTTTAA	TTTACAGTTA	GTGGAGAA	300
20 TCGGCATTGA	TCCGAACAAT	GCATTG	TTGGGACTG	GGTGGTGG	AGGTACAGT	360
GTAAGTGC	TTT	TTT	TTG	TTT	CTTAGTGT	420
TTG	TTT	TTT	TTG	TTT	CTTAGTGT	480
AACTGAAATT	CTTTGCA	GTG	GGAG	TAC	TCTACAGT	540
25 GGCTTCTCTG	TGGT	GAGA	GTAC	TTCT	TCAGCCAT	600
CTT	GG	AA	GG	ACTT	TCATAAA	660
30 TTCCCG	TTT	TTT	TTT	TTT	TTCTCT	720
TTT	TTT	TTT	TTT	TTT	TTCTCT	780
GATCCAGCTG	ATATT	CAGCC	TTT	TTA	TTCTCT	840
AGCATTGATA	AGC	ATT	CC	AA	TTCTCT	900
35 AGTTGTGA	TTA	TACAGT	TT	TTG	TTCTCT	960
TGAAGTTATT	AGT	GTAGTCA	ACG	CATAG	TTCTCT	979
AGGTGCTT	TTA	GGG	TTA	GGG	GGG	

PGIint R2000 marker:

CAGCACTAAT	CTTGC	GGTAT	GAATT	TGTGA	TTA	AAATT	TGT	TTG	CT	CTT	CTTC	60
ATTGTTCGTT	TTC	GTACA	AAAC	CGAAT	TAT	AAAT	CTT	TAC	AA	TTT	CTACC	120
40 GGGTCTGATG	TAC	AATG	GT	CTCC	CAT	TCT	GGG	GAT	TTT	CTAC	ATG	180
TATT	CAG	A	CA	AA	AA	CT	GG	AT	TTT	AC	AGT	240
GAGAAGTTCG	GT	ACAGA	AG	TG	TT	AA	CT	GG	TT	AG	TG	300
TACAGTGGTA	AG	TG	CT	TT	TT	AA	AT	GG	CT	CC	GT	360
45 AGTGTATAAC	TG	AA	TT	TC	GT	TT	GG	AG	CT	TAC	TT	420
ACAGTATGGC	TT	CT	CT	GT	GT	TT	GA	AG	CT	TC	TC	480
AAAATGTCTT	AG	GC	AT	AT	CT	TT	TA	AT	TT	TT	TT	540
TATTGCA	TT	CA	AA	AG	GT	TT	TA	AT	TT	TT	TT	600

5 CTTAGTAGAT CCAGCTGATA TTCAGCCTT TTTAAATTGG ACTGCAGGTT TTTAAAGGGG 660
 AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTT GAGAAGAATA TACCCGTGAG 720
 TTGCATTAGT TGTGTGATTA TACAGTTTC TTGTCTTTT GCTATGTCCA TCAACACTAG 780
 AGATTCGTGA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTGGA 840
 CGATTTCAAGG TGCTTTAGGG TTATTG 866

BolJon R2000 marker:

10 GATCCGATTC TTCTCCTGTT GAGATCAGCT CCAAACATCA AACAACTTGT ACACAAATAT 60
 CTTTACTTGC TAAATGGAAC ATGACAAGAG ATAGAAAATC TTGCTCATAG TATTGTACAA 120
 GGGATAACAG TGTAGAAAAC AAACCGCTG TAAGATTTTC TCCCTGATCC TCTCACTTAA 180
 CCAGTAGGCG TTTTCACAT TGAAGCGCAT ATCTACTTTG GTATTCACTG AATAAAAAAA 240
 GAAAGCTGGT AACATGTGAA GGATATACAA GCATTGATAC ACCAAGTAGT CACAAACTAC 300
 ATTATAAAGG TCAGACCTT GTTACATTC TGGCCTCCAG GACCACCGCT TCTAGCAAAG 360
 TTAAGCGTAA CATGGTCTGC ACGTATACAA ATGAAAATGT TTCTATCAAATCCTATAAA 420
 15 ATAGAGCTCT ATAACATTGT CGATACATAG TTTCACTAAC TCTGCAAGTA CAAACACAT 480
 ATACAAACAA AACTATGCGA ACAGATCAA ACTACTACAG AACACAGTTC TATGACACTG 540
 TCGATAGTAA CATCCTCTGC AAGTACAAA GAGATAGCAA ATGAAACTAT GTAAACAAAT 600
 CAAAATTCTA AATTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTCT 660
 GTAAATATT CCATCAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGAAATGT 720
 20 TCCAACAAA CCACTACATA GCAGAGTCT TATAACATT TCTGAAATG TCCAATCAA 780
 ACCACTACAG AACAAAGCTC CTATAACATT GTTATACAA AGTTCACTA AATCTACAAA 840
 CTTCCCCGT AAATGAGCTT AAATCACCC AAAGATGTT CAATCAGATA AAGAGTACGA 900
 CATCGTTTG AGATTAGAAC AAACTGAAAC TTACGTAGAG TGATTGAGG AGTAGGC 957

CP418L R2000 marker:

25 AATTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTCT GTAAATATTT 60
 CCATCAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGAAATGT TCCAACAAA 120
 CCACTACATA GCAGAGTCT TATAACATT TCTGAAATG TCCAATCAA ACCACTACAG 180
 AACAAAGCTC CTATAACATT GTTATACAA AGTTCACTA AATCTACAAA CTTCCCCGT 240
 30 AAATGAGCTT AAATCACCC AAAGATGTT CAATCAGATA AAGAGTAACG ACATCGTTT 300
 GAGATTAGAA CAAACTGAAA CTTACGTAGA GTGATTGAG GAGTAGGCTC GTGCCAGCA 360
 GAGCTAGCTC TCTCTCCGC CTACATGAAGC ATCTGTTGCA CCTGAGACAA CCGTGACGAA 420
 ACTTCCGAT CACCGCCACC AGAATTGAC GCCGCGCATC GGAAGGATCC GAATCGGGAA 480
 CTGAGTGAAC CCGAGCGATC CGGGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCAGC 540
 35 ATTTCGACGA AGAGTGGAAAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTCG 600
 TCATCGTCT GATTGCCGCC ATTTCCTTG TCAGGGCGCT CTGTTGCTTA GAAGTTCCG 660
 ATGTCAATGA AC 672

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,
	Darm	Darmor
	Bol:	Brassica oleracea
45	Bra, B.rap:	Brassica rapa
	GCPA18-A19, Wes, Aust:	Wesroona
	Sam, SamlPGIolSunt5	Samourai
	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

5 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).

- 10 - 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.

In that figure:

- 15 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.

- 20 In that figure:

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

There is no amplification in radish and Arabidopsis.

In various Brassica genotypes only one band was amplified. Size band are similar

- 25 but sequences are different.

- Figure 9 illustrates electrophoresis gel of PGIint PCR marker.

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

- 30 species, but sequences are different.

- Figure 10 illustrates electrophoresis agarose gel of Bo1Jon PCR marker.

- Figure 11 illustrates electrophoresis agarose gel of CP418 marker.

In that figure, the CP418 band (of about 670bp) is specific to the B.oleraea 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.

- 5 - Figure 12 illustrates summary markers table.
 - Figure 13 (13(a),13(b)) illustrates PGIol marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleraea and R2000.
 - Figure 14 (14(a), 14(b), 14(c), 14(d)) illustrates the PGInt-UNT marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleraea and R2000.
- 10 - Figure 15 (15(a), 15(b), 15(c)) illustrates the CP418L marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleraea and R2000.
 - Figure 16 (16 et 16bis) illustrates Arabidopsis, Radish and B.rapa BolJon markers. There are aligned with DB sequences of Arabidopsis (AC007190end-AC011000beginning), the B.oleraea EMBH959102 end and EMBH448336 begining and representative consensus sequences of the SG129markers band 1 and 2 in B.napus (in Drakkar and Samourai respectively).

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

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.

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

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

 - Figure 17 (17 et 17bis) illustrates the localisation of Pgi-2 primers on the Arabidopsis th MJB21.12 sequence.
 - Figure 18 illustrates the BolJon primers localisation on the mipsAtl62850 gene and overlapping area of AC007190 and AC011000 Arabidopsis th clones.
- 30 Alignment with the Arabidopsis BolJon PCR product (740bp) is presented.

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.

- 5 **Example I: 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**
- 10 **Rfo and a high vegetative vigour.**

Materials and methods :

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 15 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.

Gamma ray irradiation: Whole flowering plants were treated with gamma rays from 20 a Co60 source in a controlled area. Sublethal dose fo 65 Gray was applied before meioses.

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 25 were allowed to produce F2 families harvested individually and precisely sown in a field assay along with non irradiated controls (Fig 1).

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,
30 2- Normality of the ratio of fertile /sterile plants in the F2 segregation, and
3- Female fertility (pod development and seed set).

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

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.

- 5 *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 Multalin software INRA Toulouse.

Method :

- We choose one low GLS spring homozygous restorer line, 'R211', already
10 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 15 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 Boleracea 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
20 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.

- Ionising irradiation is known to induce chromosomal rearrangements by double
25 strand breaks followed by aberrant rejoicing 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 :

- 30 Very few families were at the best score for the three criteria out of 1200 F2 families tested.

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.

5 Glucosinolate analysis confirmed its low content.

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

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

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

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

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

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

1) PGIdl 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.

20 2) PGInt 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.

25 3) PGInt marker, a combination of the PGI ol U and PGI int L primers. This marker had the specificity of the PGIdl marker but amplifying a longer part as for PGInt one.

II.1 PGIdl marker

With the PGIdl primers, the 'R211' parental line showed no amplification, while the spring tested lines showed a 248bp band. Its DNA sequence is homologous to 30 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).

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

PGIol plus SG34 to set an Homozygocity test:

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.

10 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.

In figure 5 (PGIol and SG34 PCR markers):

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

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 20 substitution.

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.

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.

25 II.2 PGIUNT and PGIint markers

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.

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

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 Boleracea were not distinguished by the band size
5 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.

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

Electrophoresis of PGIint marker is represented in figure 9.

Sequence analysis:

Comparison of the PGI sequences from the data bases.

A PGI segment of about 490bp is known.

15 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.

Comparison of the B. rapa et B.oleracea species PGI sequences (figures 13 and 14):

20 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).

25

Example III: selection of marker in a region close to Rfo

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
30 (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.

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).

From the Brassica Crop Net DB, we found one *Boleracea* clone, (EMBH448336, 5 764bp) blasting with the begining of the A011000, and a second *Boleracea* clone (EMBH53971), distant from about 300bp on the *Arabidopsis* map, that blasted with the end of ACO7190.

We designed a new PCR marker, BolJon, between the two *Boleracea* clones. We verified that it allowed amplification of a specific PCR bands in the different 10 genotypes compared here.

In figure 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 15 950bp in *Boleracea* and one of 870bp in *B.rapa*. It showed that the two *Boleracea* clones (EMBH53971 and EMBH448336) 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.
 - 20 - 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', 25 only showed the *B.rapa* band and the 630bp radish band bp suggesting the *Boleracea* ortholog of the target gene is absent or has been modified when the radish segment of chromosome was inserted into the rapeseed *Boleracea* constitutive genome.
- 'R2000' homozygote plants showed radish PCR BolJon, plus the two *Brassica* BolJon bands, again having recovered the *Boleracea* one, lost in 'R211' and other restorer lines.

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)

There was no amplification in *B.rapa*, in radish, nor in *Arabidopsis*, but there was a 5 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.

In RRH1, R113 and in R211 there was no CP418 band, indicating as previously that 10 the *B.oleracea* ortholog of the target gene is absent or has been modified following the radish insertion.

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

In the present invention, a new recombined low GLS restorer line has been selected 15 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.

The homozygous 'R2000' presents a unique combination of the PGIol, PGIUNT, PGInT and BolJon markers when compared with the rapeseed restorer analysed yet: 20 PGInT 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 PGInT sequence in its *B.rapa* genome is not amplified with this marker in R211 and Drakkar genetic background.

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

BolJon marker showed that the homozygous restored rapeseed lines, including 30 '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.

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

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 *Boleracea* type *Pgi-2* gene and BolJon target sequence, characterised by these markers,
5 probably from the Drakkar '00' genome present in the irradiated heterozygous 'R211*DK'.

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

CLAIMS

1. 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, 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.
- 20 2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
3. A method according to claim 1 wherein the double low cms line of spring *Brassica napus* of step a) is R211.
- 25 4. A method according to claim 1 wherein the testing in step d) is performed with the combination of five markers selected from PG1ol, PG1UNT, PG1int, BolJon and CP418.
- 30 5. Double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (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.

6. Double low restorer lines of Brassica napus according to claim 5, wherein
5 they present a unique combination of five markers selected from PGIol,
PGIUNT, PGIint, BolJon and CP418.
7. Brassica napus hybrid plants and progeny thereof obtained through the
steps of:
 - 10 a) providing a restorer line produced according to claim 1 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
 - 15 d) harvesting the hybrid seed from the male sterile plant.
8. The seeds of Brassica plant developed from the Brassica line obtained in
claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.
10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4 , 2003, under the reference number NCIMB41183.
25
11. Use of the combination of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined
30 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.

12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

5 13. Use according to claim 12, wherein:

 - The marker PGIol is amplified using the primers: PGIol U and PGIol L
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';
PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
 - The marker PGIint is amplified using the primers: PGIint U and PGIint L
(PGIint U: 5'CAGCACTAATCTTGCCTG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
 - The marker PGIUNT is amplified using the primers: PGIol U and PGIint L:
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
 - The marker BolJon is amplified using the primers: BolJon U and BolJon L:
(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';
BolJon L: 5'GCCTACTCCTCAAATCACTCT3')
 - The marker CP418 is amplified using the primers: SG129 U and pCP418 L:
(SG129 U: cf Giancola et al (5)
pCP418 L : 5'AATTTCTCCATCACAAAGGACC3')

14. PGI₁ marker whose sequence follows:

25	TCATTTGATT	GTTGCGCCTG	TCGCCCTTGT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
	ACATGTGGTT	AACTAACAG	GGCTCCGGCT	GTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
	AATCTTGC GG	TATGAATTG	TGATTAAATT	TGTTTGT TTTG	TGACTCTTTC	TTCATGTTC	180
	GT T T C G T A C	AATAAACCGA	ATGTATAATC	TTTTTACAAA	CTGAATTTTC	TACCGGGTCT	240
	GATGTACA						248

15. PGIUNT marker whose sequence follows:

30	TCATTTGATT	GTTGCGCCGT	TCGCCTTGT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
	ACATGTGGTT	AACTAACAG	GGCTCCGGCT	GTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
	AATCTTGC GG	TATGAATTG	TGATTAAATT	TGTTTGT	TGACTCTTTC	TTCA TTGTC	180
	GT T T C G T A C	AATAAACCGA	ATGTATAAATC	TTTTACAAAC	TGAATTTCT	ACCGGGTCTG	240
35	ATGTACAATG	CTAGTCTCCA	TGTTCTGGG	GATCATGATT	TATTTTCTAC	ATGTATTCA G	300
	ACAGTACAGA	AGAAAAGTGT	CAAAACTCTG	GATGTTTAA	TTTACAGTTA	GTGGAGAAGT	360
	TCGGCATTGA	TCCGAAACAAT	GCATTTGCAT	TTTGGGACTG	GGTTGGTGG	AGGTACAGTG	420
	GTAAGTGC TT	GT T T A T T G G	TTGTATAAAAT	TTCTCGTCCA	TTCCGCTTG	CTTAGTGTAT	480
	AACTGAAATT	CTTTTGCAGT	TTGCAGTGC	GTG GGAGTCT	TACCATTGTC	TCTACAGTAT	540
	GGCTTCTCTG	TGGTTGAGAA	GTACGGTACC	TTCTACTTTA	TCAGGCCATCT	CATAAAATGT	600

	CTTAGGCATA	TTCTTTCTAT	TTTATTCccc	TCTTAATGAT	TTCTTCTTT	TTTATTGCA	660
	TCGCCGTTT	ATTTCAAAA	GTTGTTACTG	TCTCTAAATC	AAGAAGAAC	CTTCTTAGTA	720
	GATCCAGCTG	ATATTCAGCC	TTTTTAAAT	TGGACTGCAG	GTTTTAAAG	GGGAGCTTC	780
5	AGCATTGATA	AGCATTCCA	GTCCACACCG	TTTGAGAAGA	ATATACCGT	GAGTCATT	840
	AGTTGTGTA	TTATACAGTT	TTCTGTCTT	TTTGCTATGT	CCATCAACAC	TAGAGATTG	900
	TGAAGTTATT	AGTGTAGTCA	ACGCATAGGG	AGAGGTGATT	GGTGA	GGACGATTTC	960
	AGGTGCTTA	GGGTTATTG					979

16. PGLint marker whose sequence follows:

10	CAGCACTAAT	CTTGGGGTAT	GAATTGTGA	TTAAATTGTT	TTGTTGTGA	CTCTTCTTC	60
	ATTGTTCGTT	TTCGTACAAT	AAACCGAATG	TATAATCTTT	TACAAACTGA	ATTTTCTACC	120
	GGGTCTGATG	TACAATGCTA	GTCTCCATGT	TCTTGGGGAT	CATGATTAT	TTTCTACATG	180
	TATTCAAGACA	GTACAGAAGA	AAGTGTCAA	AACTCTGGAT	GTTTTAATT	ACAGTTAGTG	240
15	GAGAAGTTCG	GCATTGATCC	GAACAATGCA	TTTGCA	GGGACTGGGT	TGGTGGAAAGG	300
	TACAGTGGTA	AGTGTGTT	TATTGGTTG	TATAAAATTTC	TCGTCCATT	CCGCTTGCTT	360
	AGTGTATAAC	TGAAATTCTT	TTGCAGTTG	CAGTGTGTT	GGAGTCTTAC	CATTGTCCT	420
	ACAGTATGGC	TTCTCTGTGG	TTGAGAAGTA	CGGTACCTTC	TACTTTATCA	GCCATCTCAT	480
	AAAATGTCTT	AGGCATATTTC	TTTCTATT	ATTTCCCTCT	TAATGATTC	TTCTTTTTT	540
20	TATTGCATTC	CCGTTTATT	TTCAAAAGTT	GTTACTGTCT	CTAAATCAAG	AAGAAACCTT	600
	CTTAGTAGAT	CCAGCTGATA	TTCAGCCTT	TTAAATTGG	ACTGCAGGTT	TTAAAGGGG	660
	AGCTTCAAGC	ATTGATAAGC	ATTCCAGTC	CACACCGTTT	GAGAAGAATA	TACCCGTGAG	720
	TTGCATTAGT	TGTGTGATTA	TACAGTTTC	TTGTCTTTT	GCTATGTCCA	TCAACACTAG	780
	AGATTCTGTA	AGTTATTAGT	GTAGTCAAACG	CATAGGGAGA	GGTGATTGGT	GACTTTGGA	840
	CGATTCAGG	TGCTTAGGG	TTATTG				866
25							

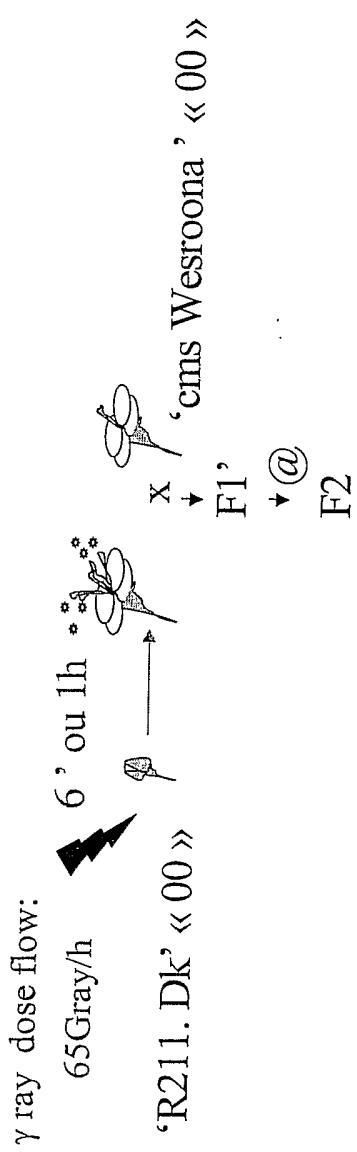
17. BolJon marker whose sequence follows:

	GATCCGATTC	TTCTCCTGTT	GAGATCAGCT	CCAAACATCA	AACAACTGT	ACACAAATAT	60
	CTTTACTTGC	TAATGGAAC	ATGACAAGAG	ATAGAAAATC	TTGCTCATAG	TATTGTACAA	120
	GGGATAACAG	TGTAGAAAAC	AAACCGCTG	TAAGATTTTC	TCCCCTGATCC	TCTCACTTAA	180
30	CCAGTAGGCG	TTTTCACAT	TGAAGCGCAT	ATCTACTTTG	GTATTCACTG	AATAAAAAAA	240
	GAAAGCTGGT	AACATGTGAA	GGATATACAA	GCATTGATAC	ACCAAGTAGT	CACAAACTAC	300
	ATTATAAAGG	TCAGACCTT	GTTCACATT	TGGCCTCCAG	GACCACCGCT	TCTAGCAAAG	360
	TTAAGCGTAA	CATGGTCTGC	ACGTATACAA	ATGAAAATGT	TTCTATCAA	ATCCTATAAA	420
	ATAGAGCTCT	ATAACATTGT	CGATAACATAG	TTTCACTAAC	TCTGCAAGTA	CTAAACACAT	480
35	ATACAAACAA	AACTATGCGA	ACAGATCAAA	ACTACTACAG	AACACAGTTC	TATGACACTG	540
	TCGATAGTAA	CATCCTCTGC	AAAGTACAAA	GAGATAGCAA	ATGAAACTAT	GTAAACAAAT	600
	CAAAATTCTA	AATTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTCT	660
	GTAAATATT	CCATCAAAAT	GAATAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	720
40	TCCAACAAA	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAATG	TCCAATCAA	780
	ACCACTACAG	AAACAAAGCTC	CTATAACATT	GTAAATACAA	AGTTTCACTA	AATCTACAAA	840
	CTTCCCCGCT	AAATGAGCTT	AAATACACCC	AAAGATGTTT	CAATCAGATA	CAATCAGATA	900
	CATCGTTTG	AGATTAGAAC	AAACTGAAAC	TTACGTAGAG	TGATTTGAGG	AGTAGGC	957

18. CP418 marker whose sequence follows:

45	AATTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTCCT	GTAAATATT	60
	CCATCAAAAT	GAATAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	TCCAACAAA	120
	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAATG	TCCAATCAA	ACCACTACAG	180
	AAACAAAGCTC	CTATAACATT	GTAAATACAA	AGTTTCACTA	AATCTACAAA	CTTCCCCGT	240
	AAATGAGCTT	AAATACACCC	AAAGATGTTT	CAATCAGATA	AAGAGTAACG	ACATCGTTT	300
50	GAGATTAGAA	CAAACGTAAA	CTTACGTAGA	GTGATTTGAG	GAGTAGGCTC	GTTGCCAGCA	360
	GAGCTAGCTC	TCTCTCCGC	CTCATGAAGC	ATCTGTTGCA	CCTGAGACAA	CCGTGACGAA	420
	ACTTCCGAT	CACGCCACC	AGAATTGAC	GCCGCGCATC	GGAAGGATCC	GAATCGGGAA	480
	CTGAGTGAAC	CCGAGCGATC	CCGGGAGTGC	GACGGAGCGA	TGGGAAAAGA	GAGTGGCAGC	540
	ATTCGACGA	AGAGTGGAAAG	AGGAGAGGGT	GGTGGATAAA	CTCGCGTATG	ATCAAGTTCG	600
55	TCATCGTCT	GATTGCCGCC	ATTTTTTTG	TCAGGGCGCT	CTGTGGCTTA	GAAGTTCCG	660
	atgtcaatga	ac					672

Fig 1



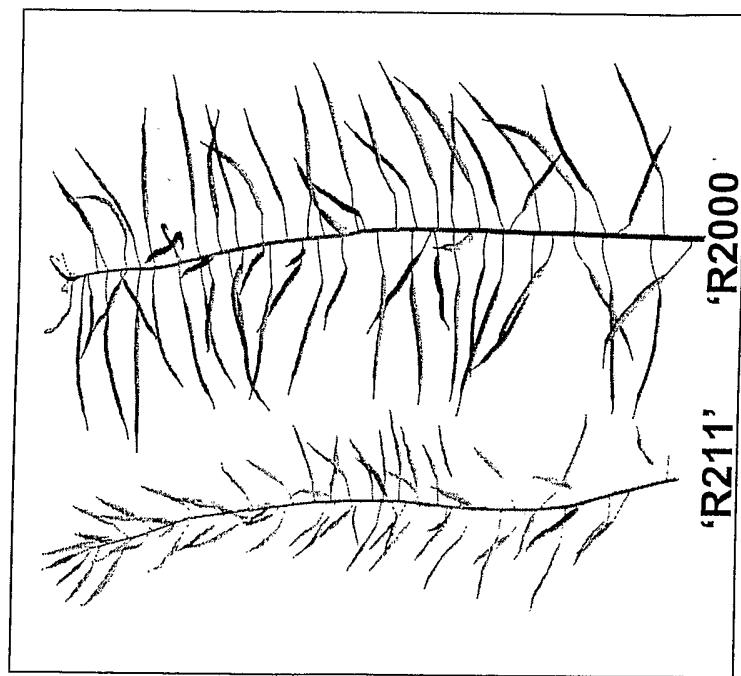


Fig. 1: Seed set on 'R211' and 'R2000'

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

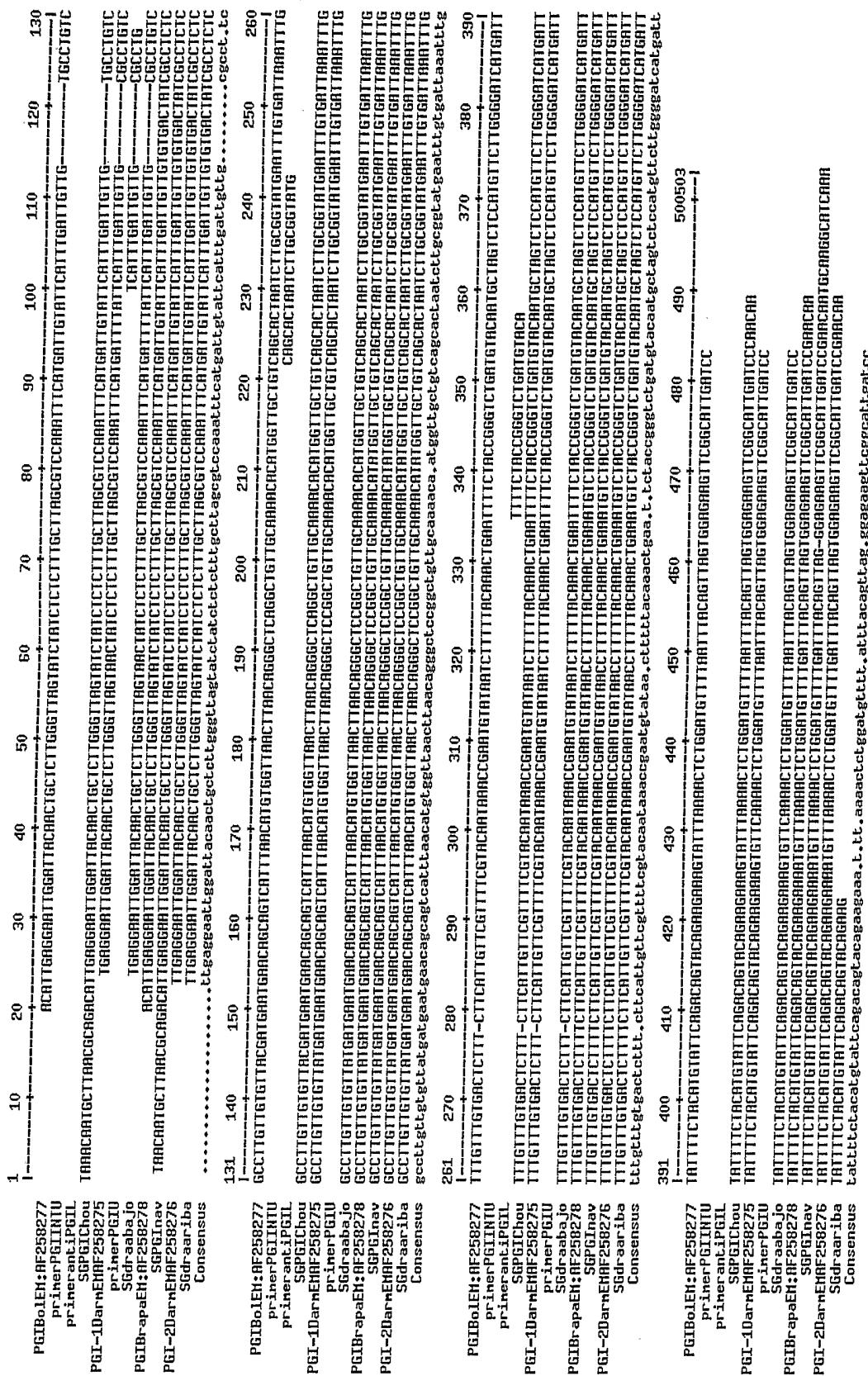


Fig 5

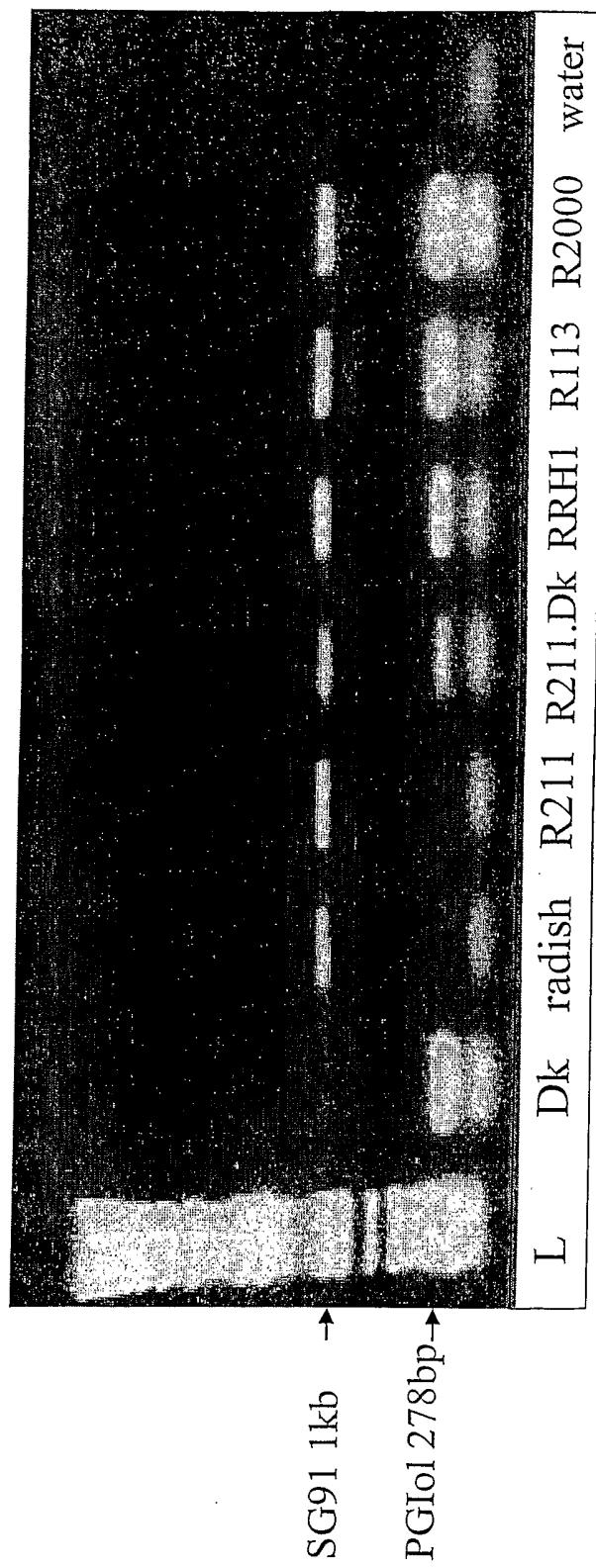
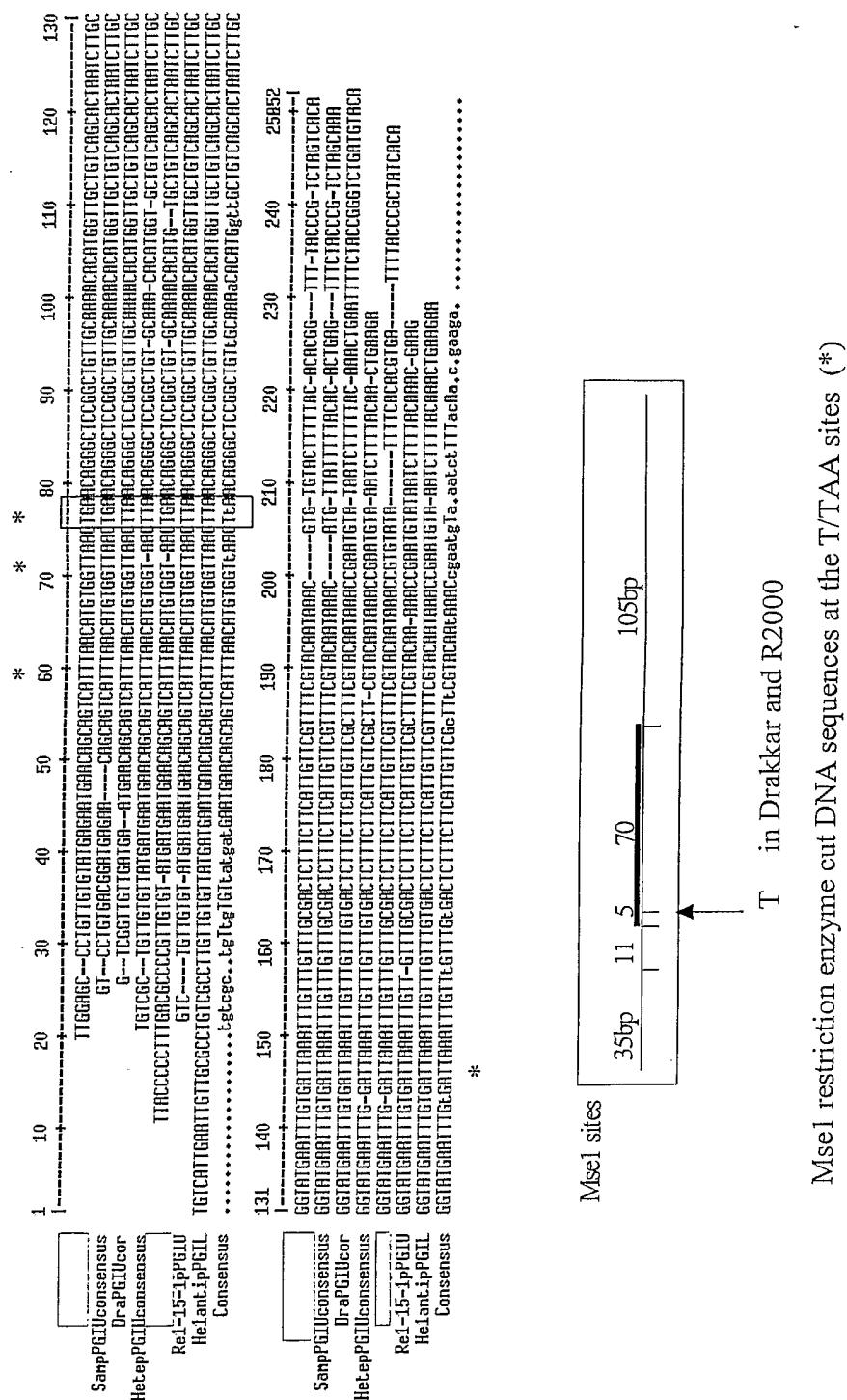


Fig 6



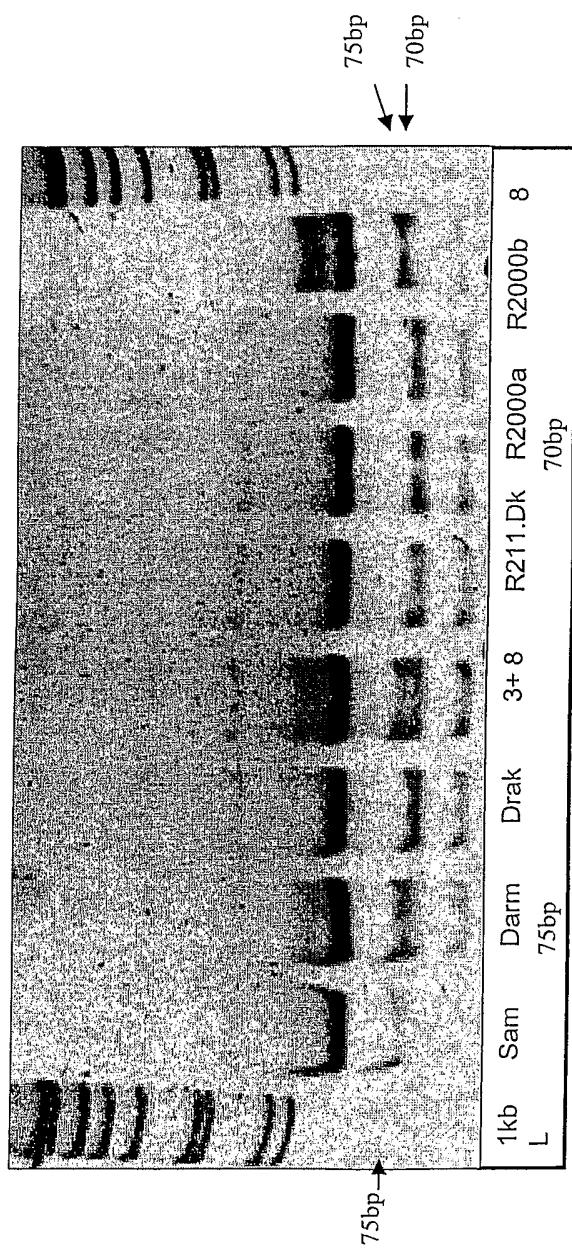


Fig 7

Fig 8

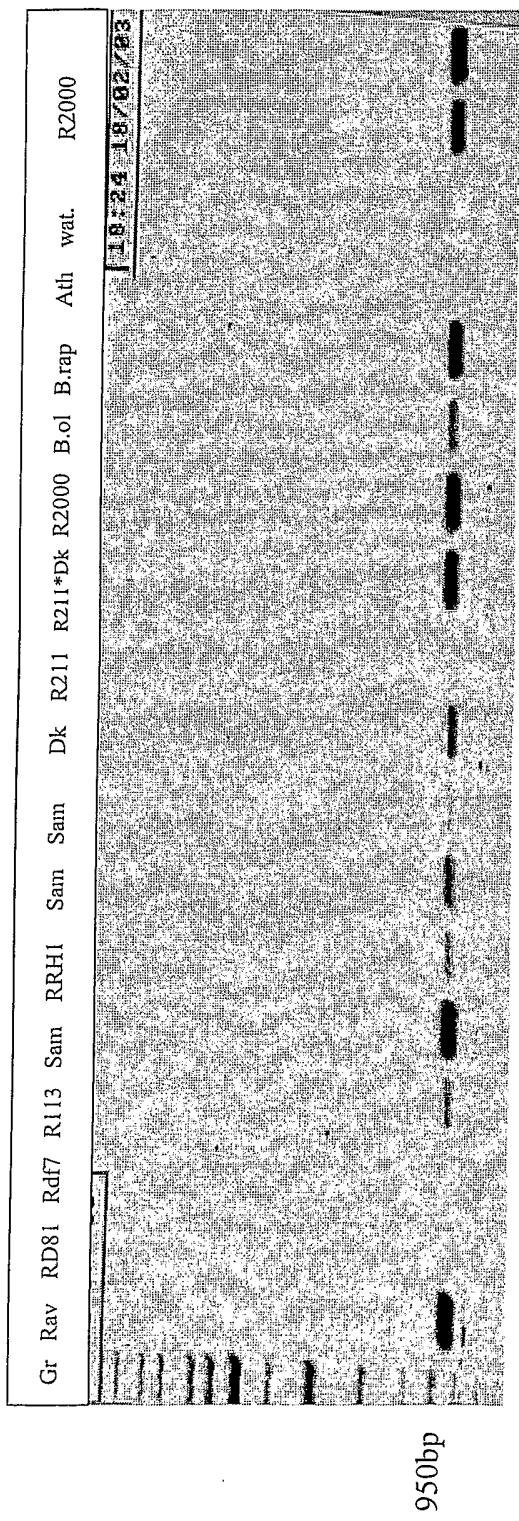


Fig 9

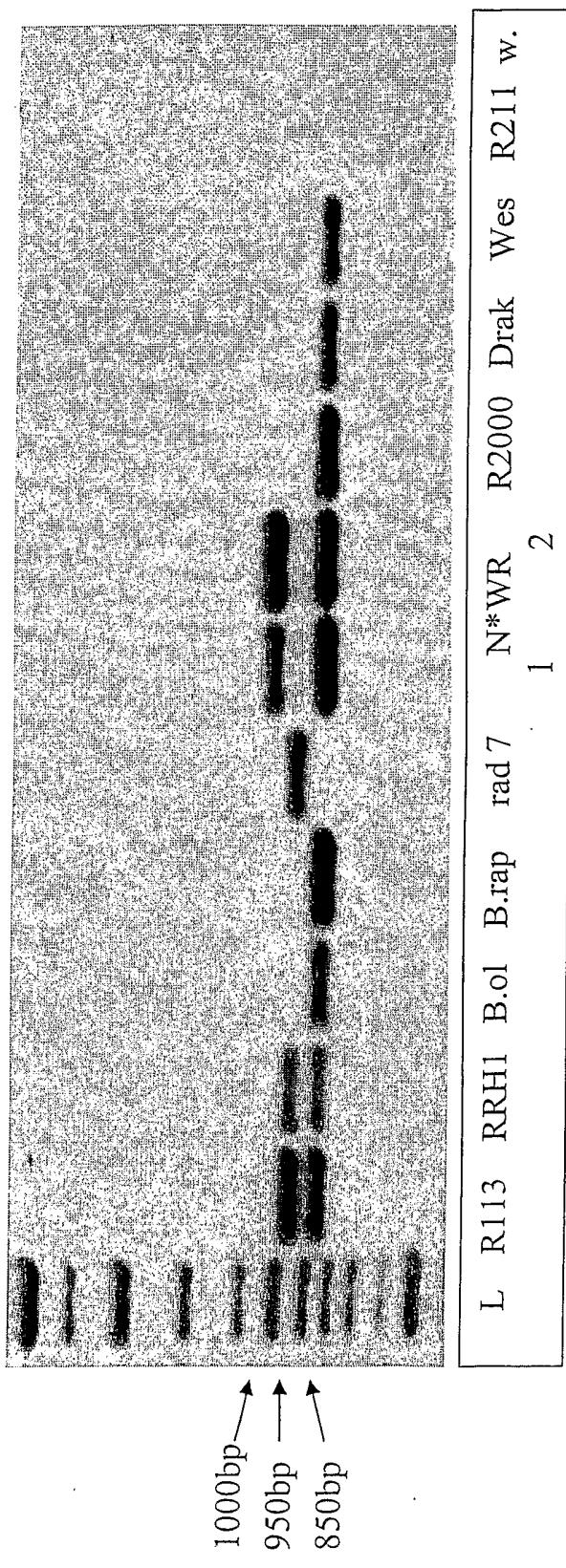
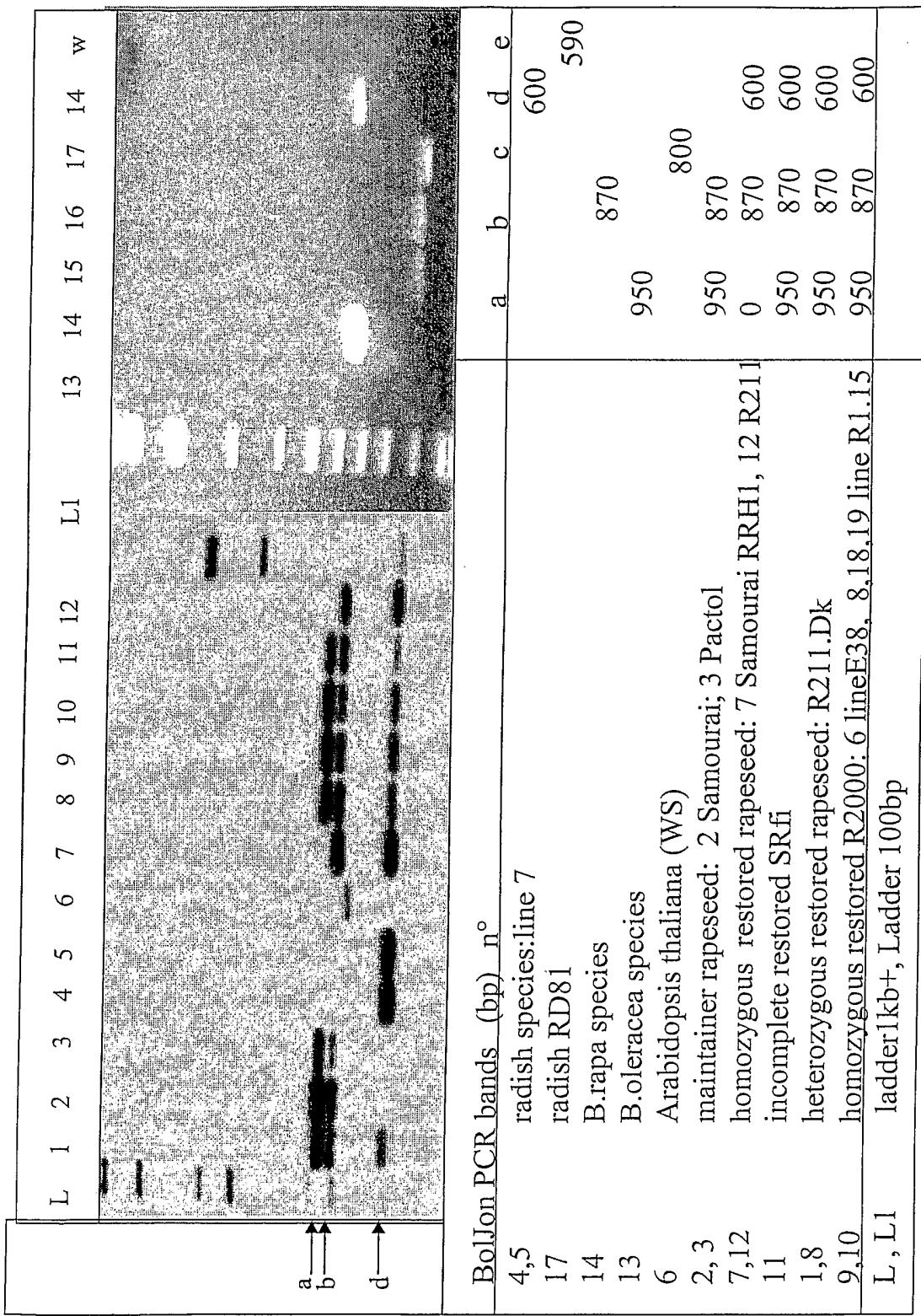


Fig 10



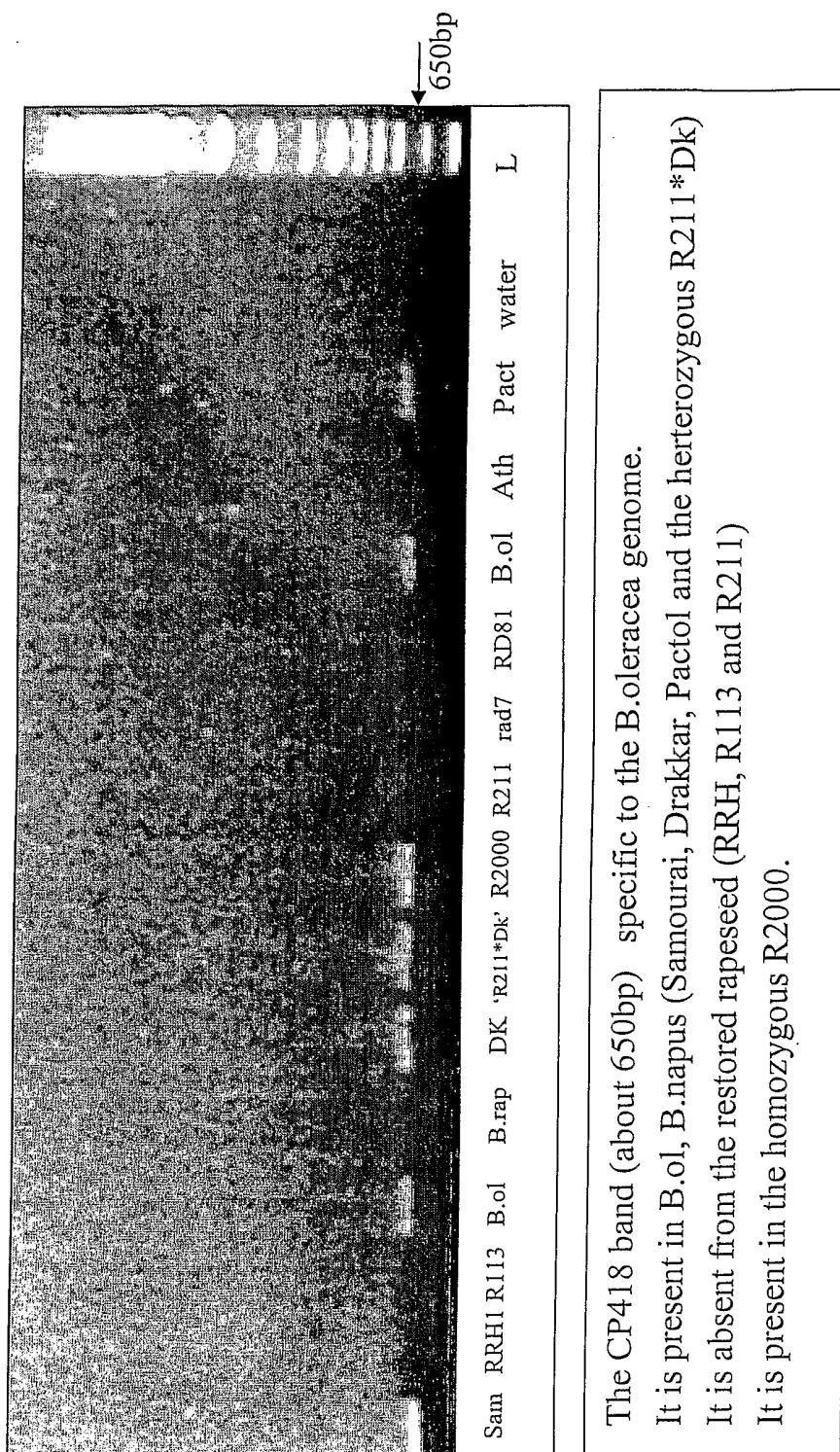


Fig 11

Figure 12

Figure 13 (a)

	51	81 PGIol U --> 100
consePGIintTUNDrakka	.	.
consensWesrPGI	.	.
consePGIintUNTR113	.	.
consePGIintUNTBrapaA	.	.
ConsePGIintUNTRRH1	.	.
PGIBo-EM:AF258277	TTGCTTAGCG TCCAAATTTC ATGATTGTAT	TCATTTGATT GTTGTG...
PGIBra-EM:AF258278	TTGCTTAGCG TCCAAATTTC ATGATTGTAT	TCATTTGATT GTTGTGTGAC
consePGIintUNTBolera	.	.
consePGIintUNTR2000	.	TTG... TCATT.GA... TTGT.TGCG
Consensus		1
	101	150
consePGIintTUNDrakka	.	.
consensWesrPGI	.	.
consePGIintUNTR113	.	.
consePGIintUNTBrapaA	.	.
ConsePGIintUNTRRH1	.	.
PGIBo-EM:AF258277	CGCTGTTG TGTATGATG AAT.GAACAG CAGTCATTTA	CG TGTTGAGAAG CAG.CATTAA
PGIBra-EM:AF258278	TATCGCCTC. TCGCCTGTTG TGTATGATG AAT.GAACAG CAGTCATTTA	CCTG TCGCCTGTTG TGTTA.GATG AAT.GAACAG CAGTCATTTA
consePGIintUNTBolera	.	.
consePGIintUNTR2000	.	.
Consensus	CCTG TCGCCTGTTG TGTTATGATG AAT.GAACAG CAGTCATTTAt.g_g ...t.gaa.ag cagtcattta
	151	200
consePGIintTUNDrakka	.	.
consensWesrPGI	.	.
consePGIintUNTR113	.	.
consePGIintUNTBrapaA	.	.
ConsePGIintUNTRRH1	.	.
PGIBo-EM:AF258277	ACATG.TGGT TAACTAAACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG	ACATG..GGT ..ACTGAACA GGGCTCCGGC TGTTGCAAA.. ACAG...TG
PGIBra-EM:AF258278	ACATG.TGGT TAACTAAACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG	ACATG.TGGT TAACTAAACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG
consePGIintUNTBolera	.	.
consePGIintUNTR2000	.	.
Consensus	ACATG.TGGT TAACTAAACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG	acatg.tggtaactaaaca gggctccggc tgttgcaaaa cacatggttg
	201	250
consePGIintTUNDrakka	.	.
consensWesrPGI	.	.
consePGIintUNTR113	.	.
consePGIintUNTBrapaA	.	.
ConsePGIintUNTRRH1	.	.
PGIBo-EM:AF258277	CTGT CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTGTTGTTGTT
PGIBra-EM:AF258278	CTGT CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTGTTGTTGTT
consePGIintUNTBolera	.	.
consePGIintUNTR2000	.	.
Consensus	CTGT CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTGTTGTTGTT
	251	300
consePGIintTUNDrakka	.	.
consensWesrPGI	.	.
consePGIintUNTR113	.	.
consePGIintUNTBrapaA	.	.
ConsePGIintUNTRRH1	.	.
PGIBo-EM:AF258277	TGTGACTCTT T.CTTCATTG TTCGTTTCG TACAATAAAC CCAATGTATA	TGTGACTCTT T.CTTCATTG TTCGTTTCG TACAATAAAC CCAATGTATA
PGIBra-EM:AF258278	TGCAGACTCTT T.CTTCATTG TTCGTTTCG TACAATAAAC ..AATGTATA	TGCAGACTCTT T.CTTCATTG TTCGTTTCG TACAATAAAC ..AATGTATA
consePGIintUNTBolera	.	.
consePGIintUNTR2000	.	.
Consensus	TG.GACTCTT T.CTTCATTG TTCGTTTCG TACAATAAAC CCAATGTATA	tg.gactctt t._cttcattg ttccgtttcg tacaataaac cgaatgtata

Figure 13 (b)

301	<---	PGIol antL 341	350	
consePGIintTUNDdrakka	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
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	atctttttac	aaactgaa	tt ttctacccggg tctgatgtac a atgctAGTC	

Figure 14 (a)

201 PGI int U --->		250	
consePGIintTUNDrakka	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
consensWesrPGI	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
consePGIintUNTR113	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
consePGIintUNTBrapaA	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
ConsePGIintUNTRRH1	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
PGIBo-EM:AF258277	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
PGIBra-EM:AF258278	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
consePGIintUNTBolera	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
consePGIintUNTR2000	CTGT	CAGCAC TAATCTTGC GGTATG	AATT TGTGATTAAA TTTGTTGTT
Consensus		ctgtcagcac taatcttgc ggtatg aatt tgtgattaaa ttgttttgt	
251			
consePGIintTUNDrakka	TGTGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
consensWesrPGI	TGTGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTR113	TGCGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	..AATGTATA
consePGIintUNTBrapaA	TGCGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	..AATGTATA
ConsePGIintUNTRRH1	TGCGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	..AATGTATA
PGIBo-EM:AF258277	TGTGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
PGIBra-EM:AF258278	TGTGACTCTT	TTCTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTBolera	TG.GACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
consePGIintUNTR2000	TGTGACTCTT	T.CTTCATTG TTCGTTTCG TACAATAAAC	CGAATGTATA
Consensus	tg.gactctt t_cttcattg ttctgtttcg tacaataaac	cgaatgtata	
ε			
300			
ε3			
301 <---- PGIol antL 341 350			
consePGIintTUNDrakka	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consensWesrPGI	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTR113	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTBrapaA	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
ConsePGIintUNTRRH1	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
PGIBo-EM:AF258277	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
PGIBra-EM:AF258278	ACCTTTTAC AAACTGAA	AT GTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTBolera	ATCTTTTAC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
consePGIintUNTR2000	ATCTTT.AC AAACTGAA	TT TTCTACCGGG TCTGATGTAC A	ATGCTAGTC
Consensus	atcttttac aaactgaa	tt ttctaccggg tctgatgtac a	atgctAGTC
ε			
351 400			
consePGIintTUNDrakka	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTAT TCAGA CAGTA		
consensWesrPGI	TCCATGTTCT TGGGGATCAT GATTTATTT CT.CATGTAT TCAGA CAGTA		
consePGIintUNTR113	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTGT TCAGCCAGTA		
consePGIintUNTBrapaA	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTGT TCAGCCAGTA		
ConsePGIintUNTRRH1	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTGT TCAGCCAGTA		
PGIBo-EM:AF258277	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTAT TCAGA CAGTA		
PGIBra-EM:AF258278	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTAT TCAGACAGTA		
consePGIintUNTBolera	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTAT TCAGA CAGTA		
consePGIintUNTR2000	TCCATGTTCT TGGGGATCAT GATTTATTT CTACATGTAT TCAGA CAGTA		
Consensus	TCCATGTTCT TGGGGATCAT GATTTATTT CTaCATGTAT TCAGA CAGTA		
ε 5 ε 6			
401 450			
consePGIintTUNDrakka	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
consensWesrPGI	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
consePGIintUNTR113	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
consePGIintUNTBrapaA	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
ConsePGIintUNTRRH1	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
PGIBo-EM:AF258277	CAGAAGAAAG TATT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
PGIBra-EM:AF258278	CAGAAGAAA TGTT CAAAAC TCTGGATGTT TTGATTAC A GTTAGTGGAG		
consePGIintUNTBolera	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
consePGIintUNTR2000	CAGAAGAAAG TGTT CAAAAC TCTGGATGTT TTAATTAC A GTTAGTGGAG		
Consensus	CAGAAGAAA TgTT CAAAAC TCTGGATGTT TTaATTAC A GTTAGTGGAG		
ε 7 ε			

Figure 14 (b)

	451	end of Data Base PGI sequences	500
consePGIinTUNDrakka	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
consensWesrPGI	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
consePGIintUNTR113	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
consePGIintUNTBrapaA	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
ConsePGIintUNTRRH1	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
PGIBo-EM:AF258277	AAGTTCGGCA	TTGATCCGAA CAA.....	
PGIBra-EM:AF258278	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
consePGIintUNTBolera	AAGTTCGGCA	TTGATCCGAA CAATGCATT GCATTTGGG ACTGGGTG	
consePGIintUNTR2000	AAGTTCGGCA	TTGATCCGAA caatgcattt gcattttggg actgggttg	
Consensus	AAGTTCGGCA	TTGATCCGAA caatgcattt gcattttggg actgggttg	
	551		550
consePGIinTUNDrakka	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCG	
consensWesrPGI	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCG	
consePGIintUNTR113	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCA	
consePGIintUNTBrapaA	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCA	
ConsePGIintUNTRRH1	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCA	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCG	
consePGIintUNTR2000	TGGAAGGTAC	AGTGGTAAGT GCTTGTAT TTGGTTGTAT AAATTCCTCG	
Consensus	tggaaaggtac	agtggtaagt gcttgttat ttgggttat aaatttcctc.	
	551		550
consePGIinTUNDrakka	TCCATTCGG	CTTGCTTAGT GTATAACTGA AATTCTTTG CAGTTGCAG	
consensWesrPGI	TCCATTCGG	CTTGCTTAGT GTATAACTGA AATTCTTTG CAGTTGCAG	
consePGIintUNTR113	TCCATATCCG	CTTGCTTAGT TTATAACTGA AATTCTTTG CAGTTGCAG	
consePGIintUNTBrapaA	TCCATATCCG	CTTGCTTAGT TTATAACTGA AATTCTTTG CAGTTGCAG	
ConsePGIintUNTRRH1	TCCATATCCG	CTTGCTTAGT TTATAACTGA AATTCTTTG CAGTTGCAG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TCCATTCGG	CTTGCTTAGT GTATAACTGA AATTCTTTG CAGTTGCAG	
consePGIintUNTR2000	TCCATTCGG	CTTGCTTAGT GTATAACTGA AATTCTTTG CAGTTGCAG	
Consensus	tccat[tcgg	tttgcttagt [tataactga aattctttg cagttgcag	
	10	11	
	601		600
consePGIinTUNDrakka	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TC[G]GTGGTTG	
consensWesrPGI	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TC[G]GTGGTTG	
consePGIintUNTR113	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TCCGTGGTTG	
consePGIintUNTBrapaA	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TCCGTGGTTG	
ConsePGIintUNTRRH1	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TCCGTGGTTG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TC[G]GTGGTTG	
consePGIintUNTR2000	TGCTGTTGGA	GCTTACCAT TGTCTCTACA GTATGGCTTC TC[G]GTGGTTG	
Consensus	tgctgttggaa	gtcttaccat tgtctctaca gtatggcttc tc[g]gtggttg	
	12		
	651		700
consePGIinTUNDrakka	AGAAGTACGG	TACCTTCTAC TTTAT[CAGCC ATCTCATAAA ATGTCTT[AGG	
consensWesrPGI	AGAAGTACGG	TACCTTCTAC TTTAT[CAGCC ATCTCATAAA ATGTCTT[AGG	
consePGIintUNTR113	AGAAGTACGG	TACCTTCTAC TTTATTAGCC ATCTCATAAA ATGTCTTGGG	
consePGIintUNTBrapaA	AGAAGTACGG	TACCTTCTAC TTTATTAGCC ATCTCATAAA ATGTCTTGGG	
ConsePGIintUNTRRH1	AGAAGTACGG	TACCTTCTAC TTTATTAGCC ATCTCATAAA ATGTCTTGGG	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	AGAAGTACGG	TACCTTCTAC TTTAT[CAGCC ATCTCATAAA A.GTCTT[AGG	
consePGIintUNTR2000	AGAAGTACGG	TACCTTCTAC TTTAT[CAGCC ATCTCATAAA ATGTCTT[AGG	
Consensus	agaagtacgg	taccttctac tttat[cagcc atctcataaaa atgtctt[gg	

Figure 14 (c)

	701		750
consePGIintTUNDrakka	CATATTCTTT CTATTTTATT TTCCCTCTAA TGATTCTTC TTTTTTTAT		
consensWesrPGI	CATATTCTTT CTATTTTATT TTCCCTCTAA TGATTCTTC TTTTTTTAT		
consePGIintUNTR113	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTCTTC TCTTTAT..		
consePGIintUNTBrapaA	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTCTTC TCTTTAT..		
ConsePGIintUNTRRH1	CATATTCTTT CTATTTTATT TTCCCTCTGAA TGATTCTTC TCTTTAT..		
PGIBo-EM:AF258277		
PGIBra-EM:AF258278		
consePGIintUNTBolera	CATATTCTTT CTATTTTATT TTCCCTCTAA TGATTCTTC TTTTTTA..		
consePGIintUNTR2000	CATATTCTTT CTATTTTATT TTCCCTCTAA TGATTCTTC TTTTTTAT		
Consensus	catattcttt ctattttatt ttccctctaa tgatttcttc ttttt.t..		
	15		16 17
	751		800
consePGIintTUNDrakka	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 TTTTATTTTC AAAAGTTGTC CGGCCCTCTA AACCAAGAAG		
consePGIintUNTR2000	TGCATTCCCG TTTTATTTTC AAAAGTTGTT ACTGTCTCTA AATCAAGAAG		
Consensus	tgcattcccg ttttattttc aaaagtgt. actgtctcta aatcaagaag		
	801		850
consePGIintTUNDrakka	AAACCTTCTT AGTAGATCCA GCTGATATT AGCCTTTTT AAATTGGACT		
consensWesrPGI	AAACCTTCTT AGTAGATCCA GCTGATATT AGCCTTTTT AAATTGGACT		
consePGIintUNTR113	AAACCTTCTT AGTAGATCCA GTTGATATT AGCCTTTCT AAATTGGACT		
consePGIintUNTBrapaA	AAACCTTCTT AGTAGATCCA G.T.GTATATT AGCCTTTCT AAATTGGACT		
ConsePGIintUNTRRH1	AAACCTTCTT AGTAGATCCA GTTGATATT AGCCTTTCT AAATTGGACT		
PGIBo-EM:AF258277		
PGIBra-EM:AF258278		
consePGIintUNTBolera	AAACCTTCTT AGGA...CCA GA...CTCC ACCCTTTTT AAATTGGACT		
consePGIintUNTR2000	AAACCTTCTT AGTAGATCCA GCTGATATT AGCCTTTTT AAATTGGACT		
Consensus	aaaccttctt agtagatcca g.tgatattc agcctttt.aaattggact		
	18		19
	851		900
consePGIintTUNDrakka	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	gcaggtttt aaa.gggagc ttcaagcatt gat.agcatt tccagtcc.c		
	20		
	901		950
consePGIintTUNDrakka	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT GTGTGATTAT		
consensWesrPGI	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT		
consePGIintUNTR113	.CCCGTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. .GTGTGATTAT		
consePGIintUNTBrapaA	.CCCGTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT		
ConsePGIintUNTRRH1	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT		
PGIBo-EM:AF258277		
PGIBra-EM:AF258278		
consePGIintUNTBolera	ACCCGTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT		
consePGIintUNTR2000	ACC.GTTTGA GAAGAATATA CCCGTGAGTT GCATTAGTT. GTGTGATTAT		
Consensus	acc.gtttga gaagaatata cccgtgagtt gcattagtt. gtgtgattat		

Figure 14 (d)

	951	1000
consePGIintTUNTDrakka	ACAGTTTTTC TTGTCTTTTT GCTATGCCCA TCAACACTAG AAGATTCCGTG	
consensWesrPGI	ACAGTTTT.C TTGTCTTTT. GCTATGTCCA TCAACACTAG A.GATTCCGTG	
consePGIintUNTR113	ACAGTTTT.C TTGCCCTTTTT GCTAT. AGG GCAAC.CTAG A.GATTCATG	
consePGIintUNTBrapaA	ACAGTTTT.C TTGTCTTTT. 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.GATTCCGTG	
consePGIintUNTR2000	ACAGTTTT.C TTGTCTTTTT GCTATGTCCA TCAACACTAG A.GATTCCGTG	
Consensus	acagtttt.c ttgtctttt gctat....a tcaac.ctag a.gattc.tg	
		21
	1001	1050
consePGIintTUNTDrakka	AAGTTATTAG TGTAGCCAAC GCCTAGGGGG AGGTTGGTTG GCTGTTTGG	
consensWesrPGI	AAGTTATTAG TGTAGTCAAC GCA.....	
consePGIintUNTR113	AAGTTATTAG TGTAGTCAAC GCAGAGGAGA G..TTCACTG ACGG.....	
consePGIintUNTBrapaA	AAGTTATTAG TGTAGTCAAC GCAGAGTGAG AGG.TGATTG	
ConsePGIintUNTRRH1	AAGTTATTAG TGTAGTCAAC GCAGAGGGAGG AGATGGTT	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	AAGTTATTAG TGTAGTCAAC GCATAGGAGG AGC.....	
consePGIintUNTR2000	AAGTTATTAG TGTAGTCAAC GCATAGGGAG AGGTGAT.GG TGACTTTGG	
Consensus	aagttattag tgtagtcaac gca.agg.g. .g.....	
	1051	1076
consePGIintTUNTDrakka	ACGTTTTCAC GTGCTCCGGG GGGTTTTGG GGACCAAACC CCCAAC	
consensWesrPGI	
consePGIintUNTR113	
consePGIintUNTBrapaA	
ConsePGIintUNTRRH1	
PGIBo-EM:AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	
consePGIintUNTR2000	ACGATTTCAG GTGCTTAGG GTTATTG	

Figure 15 (a)

EMBH44836anti	51	100
GCP18-5CP418L-Sams		
GCP18-2CP418L-Wes		
GCP18-4CP418L-R2000		
conse129ba1-Drak		
GCPS18-129Sam-ba2		
GCPR18-3129R211-ba2		
GCP18-10129R20-ba2		
Consensus		
EMBH44836anti	101	150
GCP18-5CP418L-Sams		
GCP18-2CP418L-Wes		
GCP18-4CP418L-R2000		
conse129ba1-Drak	TCACAAAGGAC C		
GCPS18-129Sam-ba2	TACAGAATA GAGTTATCAT		
GCPR18-3129R211-ba2	AACATTTCT G AA		
GCP18-10129R20-ba2.	TGGTAAACCGC CTCAAAAAA.A GAGGTATCAA AAC. TTTCT GT.A		
Consensus	TGGTAAACCGC C.CAAAAAA.A AAGGT..CAA AACTT.TCCG GTAA		
EMBH44836anti	151	200
GCP18-5CP418L-Sams	.TATTTCCAT CAAAATGA..		
GCP18-2CP418L-Wes	.TATTTCCAT CAAAATGA..		
GCP18-4CP418L-R2000	ATATTTCAT CAAAATGA..		
conse129ba1-Drak	ATATTTCAT CAAAATGA..		
GCPS18-129Sam-ba2	ATGTTTCCAT CAAAATGA		
GCPR18-3129R211-ba2	ATGTTTCCAT CAAAATG.		
GCP18-10129R20-ba2	ATGTTTCCCT CAAA.TGG		
Consensus	CTAGAGAAC AG.AGTTCTT ATAACATTAT		
EMBH44836anti	201	250
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	CTG AAAATATTCCCT CAAAAAFTA		
GCPS18-129Sam-ba2	CTG AAAATATTCCCT CAAAAA TTA		
GCPR18-3129R211-ba2	CTG AAA.TAATTCCCT CAAAAA TTA		
GCP18-10129R20-ba2	CTG AAA.TAATTCCCT CAAAAA TTA		
Consensus	CTAGAACAGTTC TTACAAATG TTC TTACAA.A.		
EMBH44836anti	251	300
GCP18-5CP418L-Sams	TGTCTGT.AA ATGTCCTAAC AAAACCCTA CAGAACAAAG CTCCTATAAC		
GCP18-2CP418L-Wes	TGTCTGT.AA ATGTCCTAAC AAAACCCTA CAGAACAAAG CTCCTATAAC		
GCP18-4CP418L-R2000	TGTCTGT.AA ATGTCCTAAC AAAACCCTA CAGAACAAAG CTCCTATAAC		
conse129ba1-Drak	TGTCTGT.AA ATGTCCTAAC AAAACCCTA CAGAACAAAG CTCCTATAAC		
GCPS18-129Sam-ba2	TGTCTTC.....		
GCPR18-3129R211-ba2	TGTCTTC.....		
GCP18-10129R20-ba2	TGTCTTC.....		
Consensus	tttctgt.aa tgTTTccatc aaaatgacta tcgaacataa ttaatAtaac		
EMBH44836anti	301	350
GCP18-5CP418L-Sams	A TTGTTT ATACAAAGTT TCACT AAAT CTACAAACTT CCCCCGTAAA		
GCP18-2CP418L-Wes	A TTGTTT ATACAAAGTT .CACT AAAT CTACAAACTT TCCCCGTAAA		
GCP18-4CP418L-R2000	A TTGTTT ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA		
conse129ba1-Drak	A. TTGTTT ATACAAAGTT TCACT AAAT CTACAAACTT TCCCCGTAAA		
GCPS18-129Sam-ba2	A.C.CAGTTTC TTGCTAGTT TCACCTAAAT CTACAAACTT TC.....		
GCPR18-3129R211-ba2	A.CACAGTTTC TTGCTAGTT TCAC.TTAAAT CTACAAACTT TC.....		
GCP18-10129R20-ba2	A.CCCAGTTTC TTGCTAGTT TC.CT.AAAT CTTACAAACTT TC.....		
Consensus			

Figure 15 (b)

EMBH44836anti	351	TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
GCP18-5CP418L-Sams		TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
GCP18-2CP418L-Wes		TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
GCP18-4CP418L-R2000		TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
conse129bal-Drak		TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
GCPS18-129Sam-ba2		TGAGCTTAAT ATCACCCAA.	GATGTTCA ATCAGAT AAA GAGTAACGAC
GCPR18-3129R211-ba2		AAT CTTATCTAAA G.TTATCAC	ATCACCAT GAA GA.
GCP18-10129R20-ba2		AAT CTTATCTAAA G.TTATCAC	ATCACCAT GAA GA.
Consensus		AAT CTTATCTAAA G.TTATCAC	ATCACCAT GAA GA.
EMBH44836anti	401	ATCGTTTG AATTAGAAC AA	
GCP18-5CP418L-Sams		ATCGTTTG AATTAGAAC AA	
GCP18-2CP418L-Wes		ATCGTTTG AATTAGAAC AA	
GCP18-4CP418L-R2000		ATCGTTTG AATTAGAAC AA	
conse129bal-Drak		ATCGTTTG AATTAGAAC AA	
GCPS18-129Sam-ba2	 GAGC AA	
GCPR18-3129R211-ba2	 GCC AA	
GCP18-10129R20-ba2	 GCC A.	
Consensus	 GAGC AA	
EMBH44836anti	431	CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCGAGC	480
GCP18-5CP418L-Sams		CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCGAGC	
GCP18-2CP418L-Wes		CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCGAGC.	
GCP18-4CP418L-R2000		CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCGAGC	
conse129bal-Drak		CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCGAGC	
GCPS18-129Sam-ba2		GTAAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGAGC	
GCPR18-3129R211-ba2		GTAAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGAGC	
GCP18-10129R20-ba2		GTAA.CCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCGAGC	
Consensus		.t.aa.ctta c.tagagtga t.tgaggagt aggctcggt ccagc.gagc	
EMBH44836anti	481	TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	530
GCP18-5CP418L-Sams		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
GCP18-2CP418L-Wes		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
GCP18-4CP418L-R2000		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
conse129bal-Drak		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
GCPS18-129Sam-ba2		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
GCPR18-3129R211-ba2		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
GCP18-10129R20-ba2		TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT	
Consensus		tagctctctc ctccgcctca tgaagcatct g.tgcacctg agacaacccgt	
EMBH44836anti	531	GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	580
GCP18-5CP418L-Sams		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
GCP18-2CP418L-Wes		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
GCP18-4CP418L-R2000		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
conse129bal-Drak		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
GCPS18-129Sam-ba2		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
GCPR18-3129R211-ba2		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
GCP18-10129R20-ba2		GACGAAACTT TCCGATCACC GCCACCAGAA TTGACGCC CGCATCGGAA	
Consensus		gacgaaactt tccgatcacc gccaccagaa ttgacgcc cgcatcgaa	
EMBH44836anti	581	GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	630
GCP18-5CP418L-Sams		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
GCP18-2CP418L-Wes		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
GCP18-4CP418L-R2000		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
conse129bal-Drak		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
GCPS18-129Sam-ba2		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
GCPR18-3129R211-ba2		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
GCP18-10129R20-ba2		GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGGCAG	
Consensus		ggatccgaat cgggaaactgg agtgaacc.g agcgatcccg qgagtgqgac	

Figure 15 (c)

631	EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG GGAGCGATGG GAAAAGAGAG TGGCACGATT TCGACGAAGA GTGGAAGAGG ggagcg.tgg .aaaagagag tggcacgatt tcgacgaaga g.ggaagagg	690
691	EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	AGAGGGTGGT GGATAAAACTC GCGTATGATC AAGTCGTCA TCGTCCTGAT AGAGGGTGGT GGATAAAACTC GCGTATGATC AAGTCGTCA TCGTCCTGAA AGAGGGTGGT GGATAAAACTC GCGTATGATC AAGTCGTCA TCGTCCTGAA agaggggtggt ggataaaactc gcgtatgatc aagttcgtca tcgtctcta.	740
741	EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	TGCCGCCATT TTTTTTGTC A GGGCGCTCTG TGGCTTAGAA GTTTCCGATG TGCCGCCATT CTTGTTCAC. .GGCGCTCTG GGT..... TGCCGCCATT TGCC..CAT. CTTGAGCTC. .GG.GGCCGG GCTCACAA.. tgccgcatt. .tt.....c. .gg.gc.c.g	pSG129antiU 790 800
791	EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129bal-Drak	[TCAATGAAAC] A GTGACACGAC GAAATGC TCAATGAAAC AGAAT...TC CGGG... CCAATGAACA AGATTATTTC CGATG..	

Figure 16

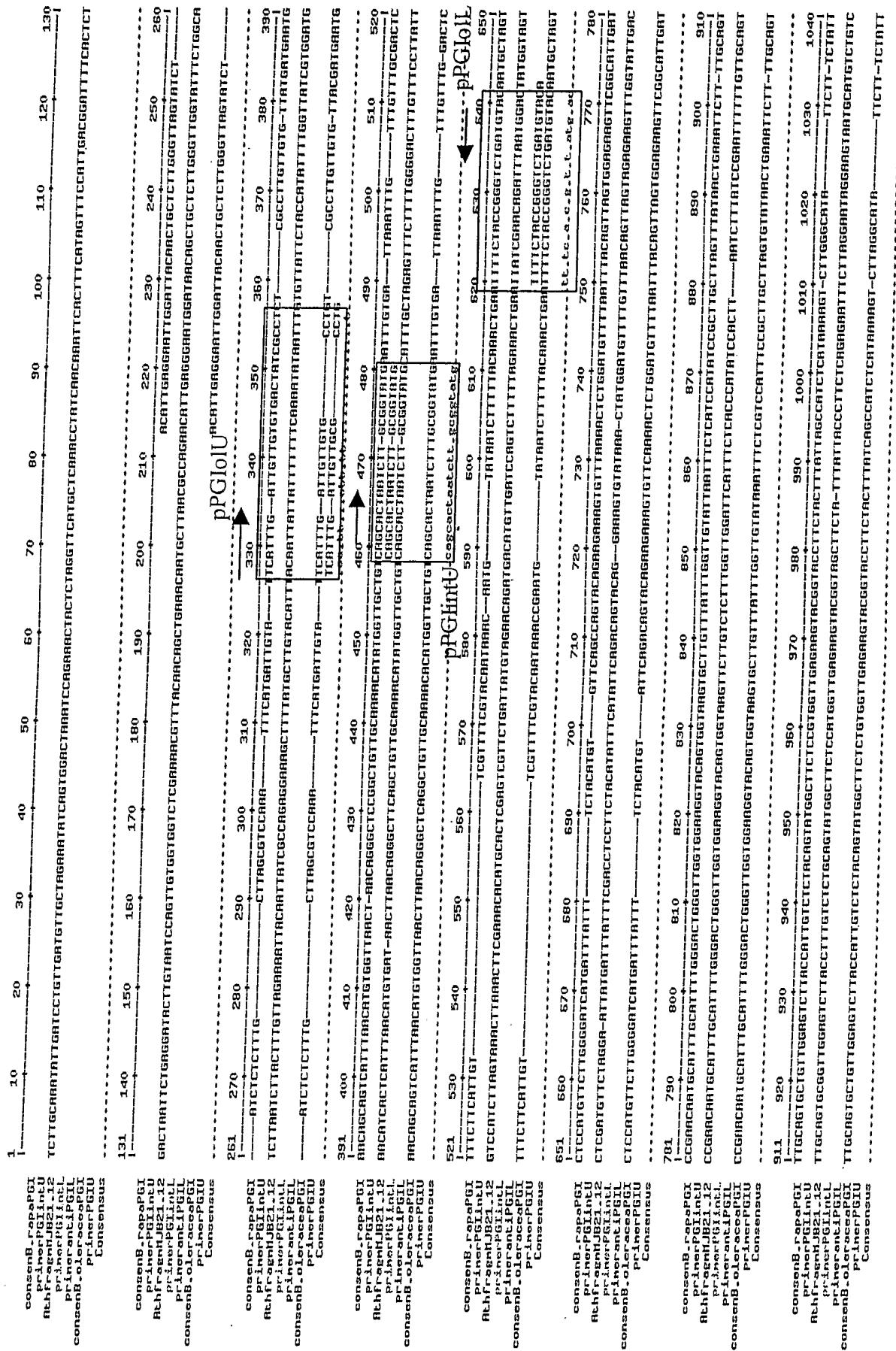


Figure 16 BIS

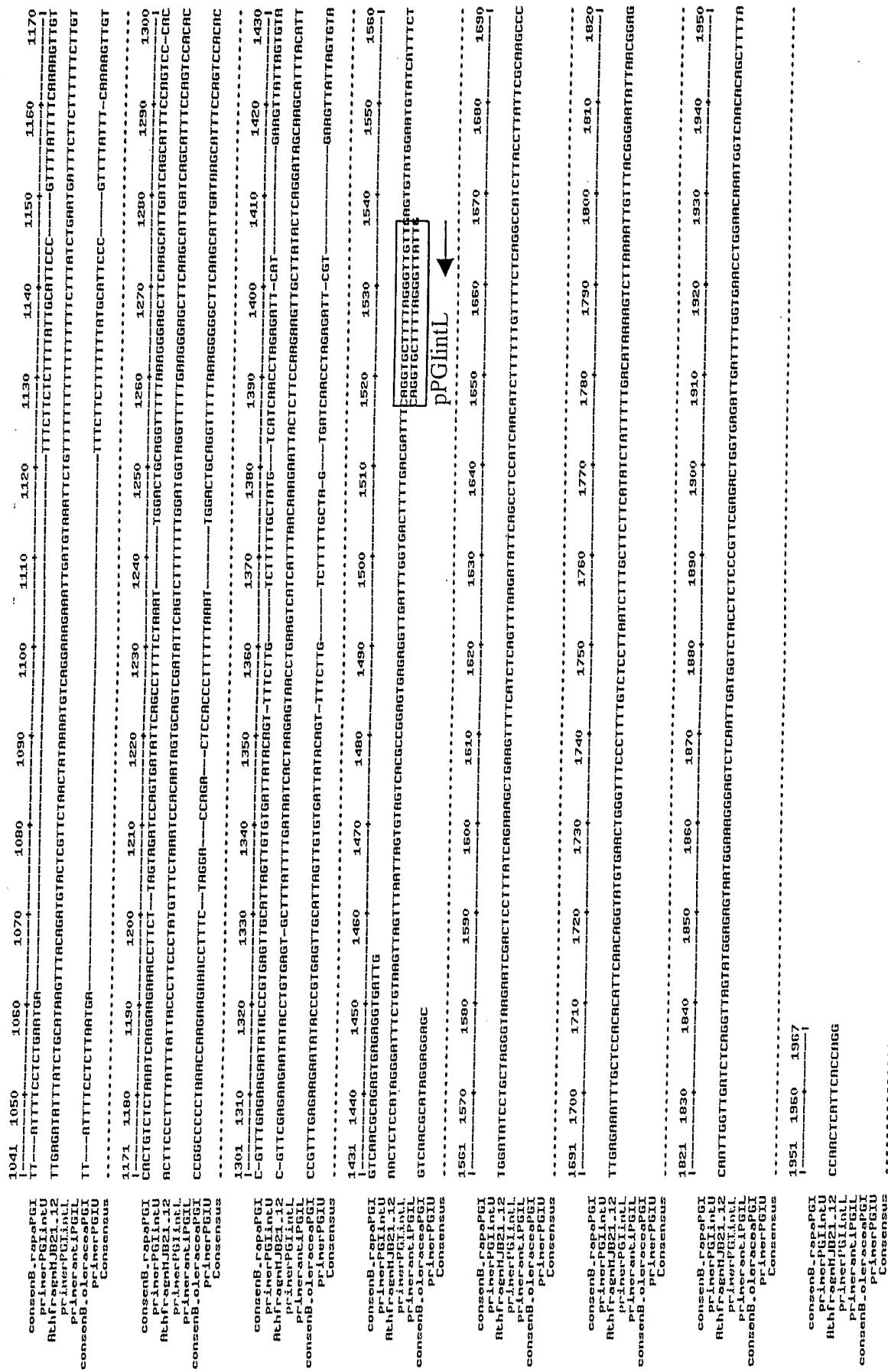


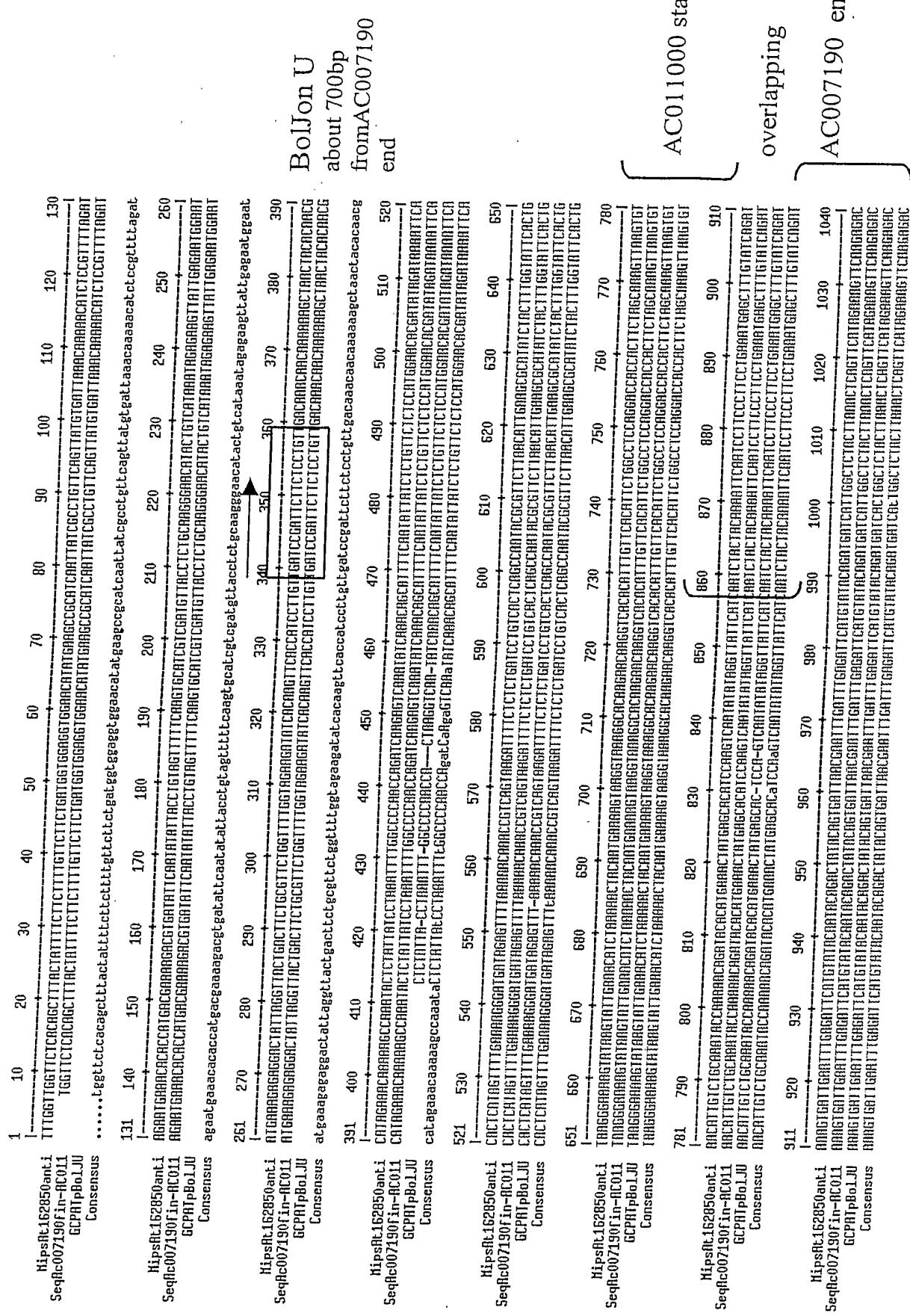
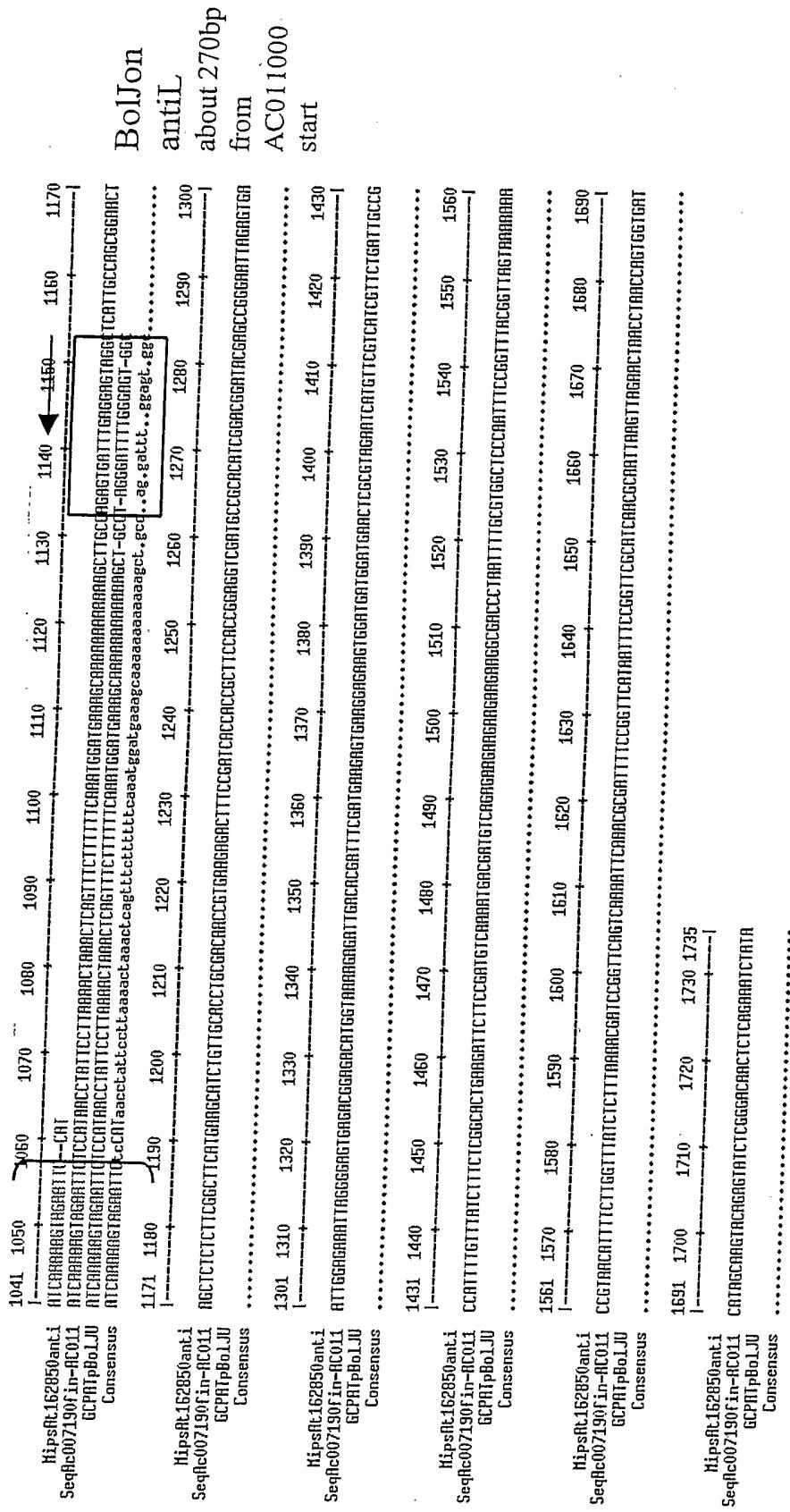
Figure 17

Figure 17 BIS



SEQUENCE LISTING

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<170> PatentIn version 3.2

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