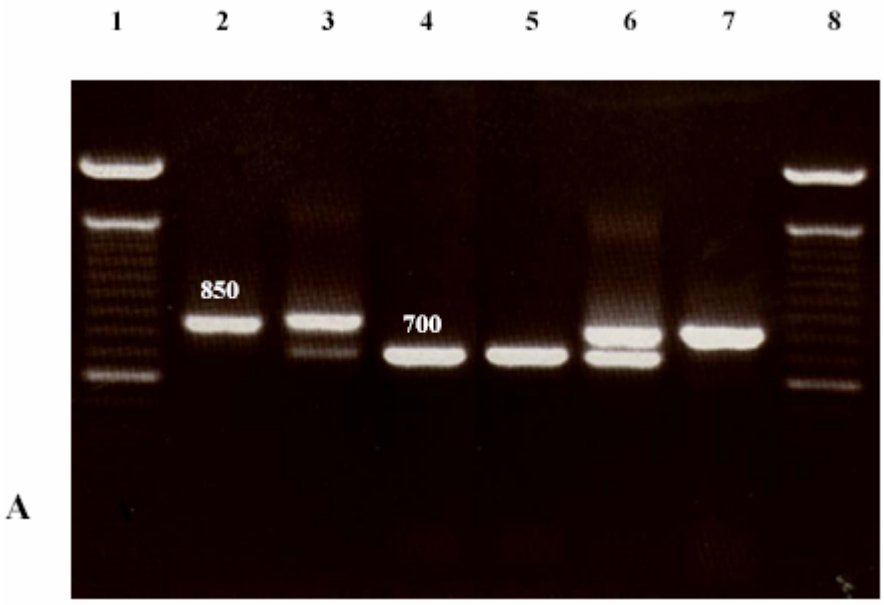
MK000003=Q1/850

Marker: Q1/850
Type: Codominant
Description: STS primers (derived from a subset of RAPD markers) that were tested for polymorphisms at the *Mj-1* locus in carrot. Linked to *Mj-1*.
Reference: Leonardo Ph.D. Thesis page 201, 213
Primers: Q1/800 Forward .....5'-GGA CGA TGG CCA GGG AAA GC-3'
Q1/800 Reverse.....5'-AAC CAA GTC ACG CCA ACA GTA AAT-3'
PCR Reaction: "DNA amplification reaction was done with 23µl master mix reaction + 2µl DNA template. The master mix consisted of 11.32µl of milliQ water, 2.5µl of 10x DNA polymerase buffer, 2.3µl of MgCl2 (25mM), 5.7µl of deoxynucleotide triphosphates (0.5mM of each), 1µl of primer (5mM), and 0.18µl (5 units/µl) of thermostable DNA polymerase (Perkin Elmer). PCR was done with either Perkin Elmer Cetus 9600 or 9700 thermocyclers."
PCR Program: 94°C 2:00; 40 cycles of {94°C 0:30; 59°C 1:00; 72°C 1:30}; 68°C 10:00
Screening Method: Agarose Gel, size difference
Product Sizes: Susceptible: 700 b.p.
Resistant: 850 b.p.
Example: Leonardo Ph.D. Thesis page 204=Figure 4.2. Agarose gel electrophoresis of the codominant sequence-tagged site (STS) marker SQ1850 / SQ1700. Lanes 1 and 8 = 100bp ladder; Lane 2 = 'Brasília-1252'; Lane 3 = 'HCM'; Lane 4 ='USDA 6274B'; Lane 5 = homozygous susceptible; Lane 6 = heterozygous and Lane 7 = homozygous resistant F2 individual from the cross 'Brasilia-1252' x '6274B'.



Sequence Information:
The sequence of the Q1 (OPQ-01) RAPD primer is GGGACGATGG
Leonardo's Ph.D. thesis has two relevant sequences, these have been placed in our database as VC000025 (Original title OP-Q1/800) and as VC000026 (Original title SQ1/700 US)

>VC000025 OP-Q1/800 BR Sequenced RAPDs from Leonardo Ph.D. Thesis Table 8.25
GAATTCGCCCTTAACCAAGTCACGCCAACAGTAAATTTTATGGATTTGCAAATATTGTGTTTATGACCATCAGCAATTTGGTATATGTTTATGTGTG
GTTTGTGGAAGACAATGTTAATCAGCCCATTGTTAGCAATTTGTGCTTTATAGTGCAAAAGTTCTGTTGTACATGATGGTCTTTTCCCTAATTTCAG
CAACTATAGGAAACCAACAACAAAATTTACAGTCAGAGTAGTATGGTATGCCGTTCGTGTGTAGTCTGGGATGTATAGCTAGCACTGCATAGCATGA
ATATATAGAAGTTTGTCTCTAATCATGAAGCTGTACATGTCTTAGGTTATGATTAAATACTTATTGCTGTGACAAAGTTCTGATGGCAAGTTGCCGA
GTATCACCCTCTTTGCATATGATCGCTTTTTTGTAGGCCTCGGAGCCTCTAAATGGTGACACAGAACAAAATTTGGATACTGCACCAATAGGTAGTA
TGGAGCCTCCAAATAATGCCGAACTGCCTATTCCTGACATCATTGAAAATGCACCAAATAATGACAATACAGGTACACTATAAGAATTATCATCTCG
TTTAAGAAAAATAAGTTGAGTTTATGCCTCAGCCAATATTTGTATGATACAATGTTATTGACTTTATGGTGAAACAATGTTATTACATAAAGGTCAG
GATAGCAAAATTTTGAATTGTAGAATGTAATATGATATTGGTCACCACACACATAGAATGATGAGAATGTTGTTCTCCATGGTGAAGGTATGGAAGA

TTCCCGTGGACGTGATGTCAACTTCGGTAATACTAACACCGAGCTTTCCCTGGCCATCGTCCAAGGGCGAATTC

>VC000026 SQ1/700 US Sequenced RAPDs from Leonardo Ph.D. Thesis Table 8.25
GAATTCGCCCTTAACCAAGTCACGCCAACAGTAAATTTTACAGATTTGTAAATATTGTGTTTATGACCATTAGCAATATGGTATATGTTTATGTGTA
GTTTGTGGAAGACAATGTTAATCAGCCCATTGTTAGCAATTTGTGCTTTATAGTGCAAAAGTTCTGTTGTACATGATGGTCTTTTCCCTAATTTCAG
CAACTATAGGAAACCAACAACAAAATTTACAGTCAGAGTAGTATGGTATGCTGTTCGTGTGTAGTCTGGGATGTATAGCTAGCACTGCATAGCATGA
ATATATAGAAGTTTGTCTCTAATCATGAAGCTGTACATGTCTTAGGTTATGATTAAATACTTATTGCTGTGACAAAGTTCTGATGGCAAGTTGCCGA
GTATCACCCTCTTTGCATATGATCGTTTTTTTGTAGGCCTCGGAGCCTCTAAATGGTGACACGGAACAAAATTTGGATACTGCACCAATAGGTAGTA
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ACTAACACCgAGCTTTCCCTGGCCATCGTCCAAGGGCGAATTC

Q1/800 US nema from file Q1_800.doc 5/9/2000 = sequence of original RAPD band. Green marks the RAPD primer location:

Q1/800 US nema

>VC012706 The original full sequence of the Q1-800 RAPD band, from file Q1_800.doc 5/9/2000; has been reverse complemented to match Leonardo Thesis orientation
GAATTCGCCCTTGGGACGATGGTACAATATAATGTCCTTTATAAATCTGTGAAGTATAAATTTTTGATTTTGCTGATAATATATATATTTGAATGCT
TAAATAGATGATTTAGAGAACGATTATTGTTTTATATAACCTTTATTATTTACAGGGATTAGTCATTAGTTCAATAGTTTATTTCTGAGGTGTTGCA
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CAAAAGTTCTGtTGtACATGATGGTCTTTTcCCTAATTtCAGCAACTATAGGAAACCaACaACaAAAtTTACAGtCAGAGTAGTATGGTATGCTGTT
CGTGTGTAGTCTGGgATGTATAGCTAGCACTGCATAGCATGAATATATAGAAGTTTGTCTCTAATCATGAAGCTGTACATGTCTTAGGTTATGATTA
AATACTTATTGCTGTGACAAAGTTCTGATGGCAAGTTGCCGAGTATCACCCTCTTTGCATATGATCGCTTTTTTGTAGGCCTCGGAGCCTCTAAATG
GTGACACGGAACAAAATTTGGATACTGCACCAATAGGTAGTATGGAGCCTCCAAATAATGCCGAACTGCCTATTCCTGACATCATTGAAAATGCACC
AAATAATGACAATACAGGTAAACTATAAGAATTATCATCTCGTTTAAGAAAAATAAGTTGAGTTTATGCCTCAGCCAATATTTGTATGATACAATGT
TATTGACTTTATGGTGAAACAATGTTATTACATAAAGGTAATACTAACACCGAGCTTTCCCTGGCCATCGTCCCAAGGGCGAATTC
698 bp primer to primer

SQ1/800 BR = Resistant
GAATTCGCCCTTAACCAAGTCACGCCAACAGTAAATTTTATGGATTTGCAAATATTGTGTTTATGACCATCAGCAATTTGGTATATGTTTATGTGTG
GTTTGTGGAAGACAATGTTAATCAGCCCATTGTTAGCAATTTGTGCTTTATAGTGCAAAAGTTCTGTTGTACATGATGGTCTTTTCCCTAATTTCAG
CAACTATAGGAAACCAACAACAAAATTTACAGTCAGAGTAGTATGGTATGCCGTTCGTGTGTAGTCTGGGATGTATAGCTAGCACTGCATAGCATGA
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GATAGCAAAATTTTGAATTGTAGAATGTAATATGATATTGGTCACCACACACATAGAATGATGAGAATGTTGTTCTCCATGGTGAAGGTATGGAAGA
TTCCCGTGGACGTGATGTCAACTTCGGTAATACTAACACCGAGCTTTCCCTGGCCATCGTCCAAGGGCGAATTC
826 bp primer to primer

SQ1/700 US = Susceptible
GAATTCGCCCTTAACCAAGTCACGCCAACAGTAAATTTTACAGATTTGTAAATATTGTGTTTATGACCATTAGCAATATGGTATATGTTTATGTGTA
GTTTGTGGAAGACAATGTTAATCAGCCCATTGTTAGCAATTTGTGCTTTATAGTGCAAAAGTTCTGTTGTACATGATGGTCTTTTCCCTAATTTCAG
CAACTATAGGAAACCAACAACAAAATTTACAGTCAGAGTAGTATGGTATGCTGTTCGTGTGTAGTCTGGGATGTATAGCTAGCACTGCATAGCATGA
ATATATAGAAGTTTGTCTCTAATCATGAAGCTGTACATGTCTTAGGTTATGATTAAATACTTATTGCTGTGACAAAGTTCTGATGGCAAGTTGCCGA
GTATCACCCTCTTTGCATATGATCGTTTTTTTGTAGGCCTCGGAGCCTCTAAATGGTGACACGGAACAAAATTTGGATACTGCACCAATAGGTAGTA
TGGAGCCTCCAAATAATGCCGAACTGCCTATTCCTGACATCATTGAAAATGCACCAAATAATGACAATACAGGTAACTATAAGAATTATCATCTCG
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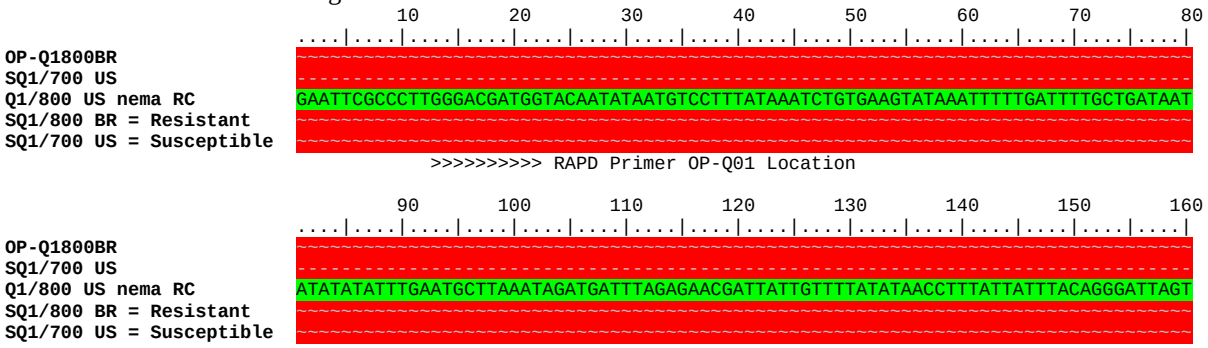
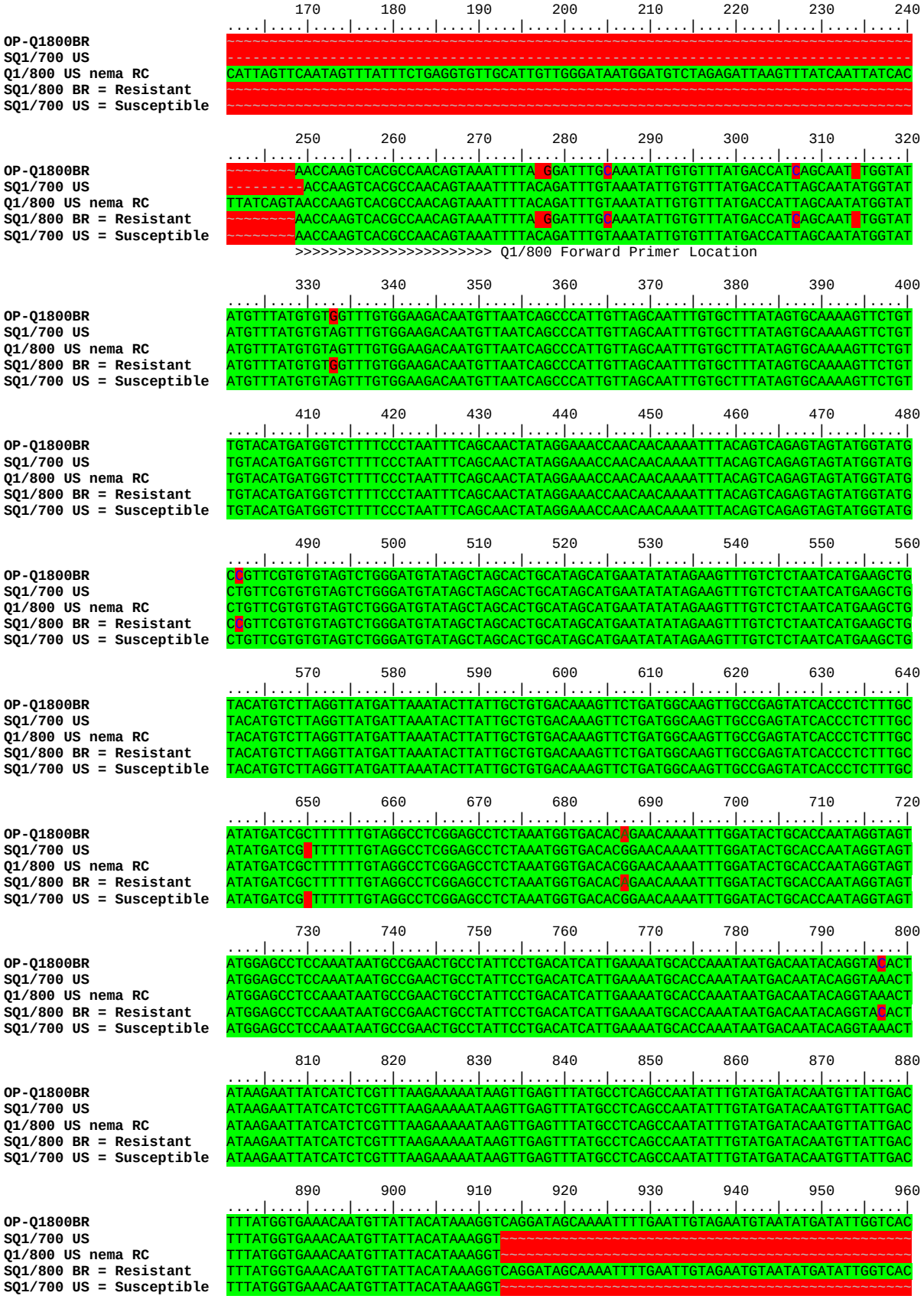
ACTAACACCgAGCTTTCCCTGGCCATCGTCCAAGGGCGAATTC
698 bp primer to primer

Here all of these have been aligned

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OP-Q1800BR
SQ1/700 US
Q1/800 US nema RC
SQ1/800 BR = Resistant
SQ1/700 US = Susceptible
CATTAGTTCAATAGTTTATTTCTGAGGTGTTGCATTGTTGGGATAATGGATGTCTAGAGATTAAGTTTATCAATTATCAC
250 260 270 280 290 300 310 320
AACCAAGTCACGCCAACAGTAAATTTTA GGATTTG AAATATTGTGTTTATGACCAT AGCAAT TGGTAT
ACCAAGTCACGCCAACAGTAAATTTTACAGATTTGTAAATATTGTGTTTATGACCATTAGCAATATGGTAT
TTATCAGTAACCAAGTCACGCCAACAGTAAATTTTACAGATTTGTAAATATTGTGTTTATGACCATTAGCAATATGGTAT
>>>>>>>>>>>>>>>>>>>>>> Q1/800 Forward Primer Location
330 340 350 360 370 380 390 400
ATGTTTATGTGTGGTTTGTGGAAGACAATGTTAATCAGCCCATTGTTAGCAATTTGTGCTTTATAGTGCAAAAGTTCTGT
410 420 430 440 450 460 470 480
TGTACATGATGGTCTTTTCCCTAATTTCAGCAACTATAGGAAACCAACAACAAAATTTACAGTCAGAGTAGTATGGTATG
490 500 510 520 530 540 550 560
CGGTTCGTGTGTAGTCTGGGATGTATAGCTAGCACTGCATAGCATGAATATATAGAAGTTTGTCTCTAATCATGAAGCTG
570 580 590 600 610 620 630 640
TACATGTCTTAGGTTATGATTAAATACTTATTGCTGTGACAAAGTTCTGATGGCAAGTTGCCGAGTATCACCCTCTTTGC
650 660 670 680 690 700 710 720
ATATGATCGCTTTTTTGTAGGCCTCGGAGCCTCTAAATGGTGACACAGAACAAAATTTGGATACTGCACCAATAGGTAGT
730 740 750 760 770 780 790 800
ATGGAGCCTCCAAATAATGCCGAACTGCCTATTCCTGACATCATTGAAAATGCACCAAATAATGACAATACAGGTACACT
810 820 830 840 850 860 870 880
ATAAGAATTATCATCTCGTTTAAGAAAAATAAGTTGAGTTTATGCCTCAGCCAATATTTGTATGATACAATGTTATTGAC
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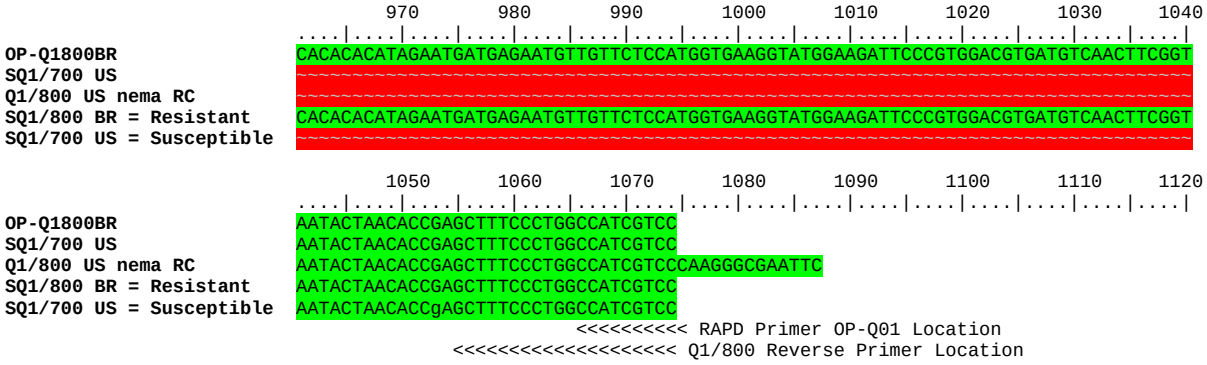
970 980 990 1000 1010 1020 1030 1040
OP-Q1800BR
SQ1/700 US
Q1/800 US nema RC
SQ1/800 BR = Resistant
SQ1/700 US = Susceptible
CACACACATAGAATGATGAGAATGTTGTTCTCCATGGTGAAGGTATGGAAGATTCCCGTGGACGTGATGTCAACTTCGGT
1050 1060 1070 1080 1090 1100 1110 1120
OP-Q1800BR
SQ1/700 US
Q1/800 US nema RC
SQ1/800 BR = Resistant
SQ1/700 US = Susceptible
AATACTAACACCGAGCTTTCCCTGGCCATCGTCC
AATACTAACACCGAGCTTTCCCTGGCCATCGTCCCAAGGGCGAATTC
AATACTAACACCgAGCTTTCCCTGGCCATCGTCC
<<<<<<<<<< RAPD Primer OP-Q01 Location
<<<<<<<<<<<<<<<<<<<< Q1/800 Reverse Primer Location