Primer3 Output

```
PRIMER PICKING RESULTS FOR Puccinia recondita consensus consensus group 1
Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
OLIGO
                                       gc% any th 3' th hairpin seq
                 start len
                                 tm
LEFT PRIMER
                   751
                         20
                              58.95
                                      55.00
                                               0.00
                                                      0.00
                                                              0.00 cgggaattggtatcggagga
                                                              0.00 tgtcactgctaccgatcctc
RIGHT PRIMER
                   926
                         20
                              58.90
                                      55.00
                                               0.00
                                                      0.00
                   821
                        20
                              60.39
                                      55.00
                                               0.00
                                                      0.00
                                                              0.00 gggaatgacatgctctggga
INTERNAL OLIGO
SEOUENCE SIZE: 1335
INCLUDED REGION SIZE: 1335
PRODUCT SIZE: 176, PAIR ANY TH COMPL: 3.86, PAIR 3' TH COMPL: 0.00
   1 ttttaattcatcttgtaatcgttctaatcgcttttcggcccggtgcctttaataataaaa
   61 ttatacaaatcatcaaaaagactattcacaNctatttacacctatttacatttattata
  121 caaattaaatcaataacaagattacaatcaatatgcagtaNtcatcggctattctcattg
 181 ctgctttcgcagtNactagtgtctttacccatggagtcgttgattctgtcgaaggcgcca
  241 atggagtcaccatgccgggtctctcagttgccgacggcaccccgcgtgattgcgccagcc
  301 ctcgctgcggatctgaggctgatacttctatcatccggaaacgtgagatgggaaccaaca
  361 aggccactgcacttggtcgcactcagggcggtggcccggtcgatgccactaagatgatgg
  421 ccatgttcatgggaggtggcggcaatgctacagcgacaaaggaagcacgggaagttcacg
  481 cagccaacgtccttcgtcgaagtcttggagagcgtgcagccaatggaggaaccaagacgc
  541 ctaagggcaccttggagactggtgtcaaggctgcagcNggagctggagcNNccagNggca
  601 tgccgacttgctcggacgacggcaaggtcaagatgacctaccatcaaatcaaccaagatg
```

```
661 gagccgggccattgacggccatggttgacgccacttcgggtggaaccgatccagcagcat
  721 tcaaatcggccgaagtaacacaaaatgtgccgggaattggtatcggaggactgtcagccg
                                   >>>>>>>>>>>>>
  781 cggccqtgatggacttcccggtagaagtacagatgccagcgggaatgacatgctctggga
  841 cagtaggcgacgctcagaacgtatgcattgttcgcatgcaaaatgcagcagcagcaggcc
  901 catttggaggatcggtagcagtgacacaaagcccagctgccaagaaacgtgccattgagt
           <<<<<<<<
  961 acaacttatcgaagcgtcactttgcccgagctttggccaagaNNaNNgcNgNagatgaNg
 1021 cNgcagcagcNgcagcNatcgcagaNcttgaNaaNatNggatcNgcNaccaaggaNgNNg
 1081 aagccgaaaaggaggatgccgNcgNagcacctttgaccaagaagagtgctgaagatgatg
 1141 cagcagcagccgcagctatcgcagagcttgaaaaaattggatcagcgaccaaggatgaag
 1201 aagcttccactgcagacgttgtgactaagagagacactgactcggataacacccagaaag
 1261 cagaaNttgaagctttagacgcagagaccgaagcggaagacaaagaataggctaattaga
 1321 tttggataattcaga
KEYS (in order of precedence):
>>>>> left primer
<<<<< right primer
^^^^^ internal oligo
ADDITIONAL OLIGOS
                   start len tm gc% any th 3' th hairpin seq
 1 LEFT PRIMER
                     307
                           20
                                59.03
                                        55.00
                                                 5.48
                                                        0.00
                                                                0.00 gcggatctgaggctgatact
                                                               0.00 cttcccgtgcttcctttgtc
   RIGHT PRIMER
                     474
                           20
                                59.12
                                        55.00
                                                 0.00
                                                        0.00
                                                               42.44 tgatggccatgttcatggga
   INTERNAL OLIGO
                     415
                           20
                                60.32
                                        50.00
                                                27.29
                                                        0.00
  PRODUCT SIZE: 168, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
```

58.90

59.26

55.00

55.00

0.00

0.00

0.00

0.00

0.00 gaggatcggtagcagtgaca

0.00 gcatcctccttttcggcttc

2 LEFT PRIMER

RIGHT PRIMER

907

1099

20

	INTERNAL OLIGO PRODUCT SIZE: 193,	967 20 59.54 PAIR ANY_TH COMPL:	50.00 0.00,	0.00 0.00 4 PAIR 3'_TH COMPL:	0.03 0.00	tatcgaagcgtcactttgcc
3	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 192,	742 20 58.65 933 20 59.12 821 20 60.39 PAIR ANY_TH COMPL:	45.00 55.00 55.00 0.00,	2.18 0.00 4.27 0.00 0.00 0.00 PAIR 3'_TH COMPL:	0.00	aaaatgtgccgggaattggt ggctttgtgtcactgctacc gggaatgacatgctctggga
4	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 199,	527 20 59.39 725 20 58.90 548 20 60.17 PAIR ANY_TH COMPL:	55.00 50.00 55.00 0.00,	0.00 0.00 0.00 0.00 0.00 0.00 4 PAIR 3'_TH COMPL:	0.00 5.23	aggaaccaagacgcctaagg tttgaatgctgctggatcgg caccttggagactggtgtca
5	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 164,	760 20 59.61 923 20 59.18 821 20 60.39 PAIR ANY_TH COMPL:	60.00 55.00 55.00 16.72,	10.38 8.90 0.00 0.00 0.00 0.00 PAIR 3'_TH COMPL:	0.00 0.00	gtatcggaggactgtcagcc cactgctaccgatcctccaa gggaatgacatgctctggga 7
6	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 171,	670 20 59.83 840 20 59.45 751 20 59.88 PAIR ANY_TH COMPL:	55.00 55.00 55.00 7.83,	14.67 7.21 0.00 0.00 0.00 0.00 PAIR 3'_TH COMPL:	0.00	cattgacggccatggttgac tcccagagcatgtcattccc cgggaattggtatcggagga
7	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 196,	494 20 59.48 689 20 59.83 548 20 60.17 PAIR ANY_TH COMPL:	55.00 55.00 55.00 0.00,	0.00 0.00 13.04 0.00 0.00 0.00 4 PAIR 3'_TH COMPL:	0.00 5.23	tcgtcgaagtcttggagagc gtcaaccatggccgtcaatg caccttggagactggtgtca
8	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 162,	710 20 58.90 871 21 59.34 751 20 59.88 PAIR ANY_TH COMPL:	50.00 47.62 55.00 0.00,	0.00 0.00 12.42 12.42 0.00 0.00 PAIR 3'_TH COMPL:	0.00 0.00	tccagcagcattcaaatcgg acaatgcatacgttctgagcg cgggaattggtatcggagga
9	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 164,	305 19 59.25 468 20 58.49 415 20 60.32 PAIR ANY_TH COMPL:	57.89 50.00 50.00 0.00,	5.48 0.00 0.00 0.00 27.29 0.00 4 PAIR 3'_TH COMPL:	0.00 2.44	ctgcggatctgaggctgat gtgcttcctttgtcgctgta tgatggccatgttcatggga
10	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 194,	726 20 58.05 919 20 58.12 751 20 59.88 PAIR ANY_TH COMPL:	55.00	28.99 0.00 0.00 0.00 0.00 0.00 PAIR 3'_TH COMPL:	0.00	tcggccgaagtaacacaaaa gctaccgatcctccaaatgg cgggaattggtatcggagga
11	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 165,	252 19 59.40 416 20 58.51 305 20 59.96 PAIR ANY_TH COMPL:	57.89 55.00 55.00 0.00,	2.18 0.00 0.00 0.00 5.99 0.00 PAIR 3'_TH COMPL:	0.00	atgccgggtctctcagttg catcttagtggcatcgaccg ctgcggatctgaggctgata
12	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 186,	171 20 57.44 356 20 58.55 305 20 59.96 PAIR ANY_TH COMPL:	45.00 55.00 55.00 0.00,	0.00 0.00 0.00 0.00 5.99 0.00 PAIR 3'_TH COMPL:	0.00	attctcattgctgctttcgc ggttcccatctcacgtttcc ctgcggatctgaggctgata

13 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 172,	558 18 59.08 729 20 58.90 618 20 59.82 PAIR ANY_TH COMPL:	50.00 0.00 0.00 0.00 c	ctggtgtcaaggctgca cgatttgaatgctgctgga acggcaaggtcaagatgac
14 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 192,	745 20 58.02 936 19 59.34 821 20 60.39 PAIR ANY_TH COMPL:	57.89 4.27 6.14 0.00 c	tgtgccgggaattggtatc tgggctttgtgtcactgc ggaatgacatgctctggga
15 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 164,	796 21 58.97 959 20 57.66 915 20 60.03 PAIR ANY_TH COMPL:	50.00 0.00 0.00 0.00 c	cccggtagaagtacagatgc tcaatggcacgtttcttgg tagcagtgacacaaagccc
16 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 179,	397 20 58.51 575 18 59.08 455 20 60.03 PAIR ANY_TH COMPL:	55.56 0.00 0.00 0.00 to	ggtcgatgccactaagatg gcagccttgacaccagt acaaaggaagcacgggaag
17 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 173,	757 20 57.77 929 20 57.64 821 20 60.39 PAIR ANY_TH COMPL:	50.00 4.27 0.00 0.00 t	tggtatcggaggactgtca tgtgtcactgctaccgatc ggaatgacatgctctggga
18 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 178,	369 18 58.75 546 20 59.39 455 20 60.03 PAIR ANY_TH COMPL:	55.00 0.00 0.00 0.00 c	cacttggtcgcactcag cttaggcgtcttggttcct acaaaggaagcacgggaag
19 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 171,	308 22 59.11 478 20 59.53 415 20 60.32 PAIR ANY_TH COMPL:	50.00 0.00 0.00 0.00 to	ggatctgaggctgatacttct gaacttcccgtgcttcctt gatggccatgttcatggga
20 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 186,	915 20 59.12 1100 18 58.49 967 20 59.54 PAIR ANY_TH COMPL:	61.11 0.00 0.00 0.00 gg	tagcagtgacacaaagccc gcatcctccttttcggc atcgaagcgtcactttgcc
21 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 182,	455 20 59.12 636 19 57.47 548 20 60.17 PAIR ANY_TH COMPL:	52.63 0.00 0.00 0.00 to	acaaaggaagcacgggaag catcttgaccttgccgtc accttggagactggtgtca
22 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 198,	937 20 59.69 1134 21 57.88 1080 20 60.17 PAIR ANY_TH COMPL:	47.62 0.00 0.00 0.00 c	tgccaagaaacgtgccatt ttcagcactcttcttggtca aagccgaaaaggaggatgc
23 LEFT PRIMER	803 20 59.82	55.00 0.00 0.00 0.00 ag	gaagtacagatgccagcgg

	RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 161,	963 915 PAIR	21 57.88 20 60.03 ANY_TH COMPL:	42.86 55.00 0.00,	9.05 0.27 0.00 gtagcagtgacacaaagccc
24	RIGHT PRIMER	1114 1291 1177 PAIR	21 57.88 20 59.83 20 60.02 ANY_TH COMPL:	47.62 55.00 50.00 0.00,	0.00 0.00 0.00 tcggtctctgcgtctaaagc
25	RIGHT PRIMER : INTERNAL OLIGO ::	1121 1294 1177 PAIR	22 58.73 20 59.83 20 60.02 ANY_TH COMPL:	45.45 55.00 50.00 13.64	0.00 0.00 0.00 gcttcggtctctgcgtctaa
26	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 180,	207 386 305 PAIR	21 59.09 18 58.75 20 59.96 ANY_TH COMPL:	47.62 61.11 55.00 0.00,	0.00 0.00 0.00 ctgagtgcgaccaagtgc 5.99 0.00 0.00 ctgcggatctgaggctgata
27	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 170,	821 990 915 PAIR	20 59.45 18 60.36 20 60.03 ANY_TH COMPL:	55.00 61.11 55.00 0.00,	0.00 0.00 0.00 ctcgggcaaagtgacgct 9.05 0.27 0.00 gtagcagtgacacaaagccc
28	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 199,	753 951 904 PAIR	21 57.89 19 59.72 20 60.10 ANY_TH COMPL:	52.38 57.89 55.00 0.00,	17.12 0.00 0.00 cacgtttcttggcagctgg
29	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 180,	301 480 415 PAIR	18 59.97 19 59.14 20 60.32 ANY_TH COMPL:	66.67 57.89 50.00 1.27,	0.00 0.00 0.00 cgtgaacttcccgtgcttc 27.29 0.00 42.44 tgatggccatgttcatggga
30	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 182,	260 441 305 PAIR	18 59.04 19 60.08 20 59.96 ANY_TH COMPL:	61.11 57.89 55.00 0.00,	0.00 0.00 0.00 cgccacctcccatgaacat
31	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 194,	497 690 548 PAIR	20 59.76 18 60.36 20 60.17 ANY_TH COMPL:	61.11 55.00	13.04 0.00 0.00 cgtcaaccatggccgtca
32	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 197,	459 655 548 PAIR	20 59.53 22 57.35 20 60.17 ANY_TH COMPL:	50.00 40.91 55.00 0.00,	0.00 0.00 0.00 tggttgatttgatggtaggtca
33	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO	634 822 751	22 57.35 20 59.82 20 59.88	40.91 55.00 55.00	0.00 0.00 0.00 ccgctggcatctgtacttct

```
PRODUCT SIZE: 189, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00
34 LEFT PRIMER
                             18
                                           55.56
                                                           0.00
                       669
                                  57.98
                                                   14.67
                                                                    0.00 ccattgacggccatggtt
   RIGHT PRIMER
                       860
                             19
                                  59.49
                                          57.89
                                                   14.86
                                                           0.00
                                                                    0.00 gttctgagcgtcgcctact
   INTERNAL OLIGO
                       751
                             20
                                  59.88
                                          55.00
                                                    0.00
                                                           0.00
                                                                    0.00 cgggaattggtatcggagga
  PRODUCT SIZE: 192, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
35 LEFT PRIMER
                       257
                             18
                                  58.62
                                           61.11
                                                    0.00
                                                           0.00
                                                                    0.00 gggtctctcagttgccga
                       453
                             18
                                          61.11
   RIGHT PRIMER
                                  59.21
                                                    4.70
                                                           0.00
                                                                    0.00 ctgtagcattgccgccac
                       305
                             20
                                  59.96
                                          55.00
                                                    5.99
   INTERNAL OLIGO
                                                           0.00
                                                                    0.00 ctgcggatctgaggctgata
  PRODUCT SIZE: 197, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
36 LEFT PRIMER
                       398
                             22
                                  58.94
                                           50.00
                                                    0.00
                                                           0.00
                                                                    0.00 ggtcgatgccactaagatgatg
  RIGHT PRIMER
                       562
                             18
                                  59.65
                                          66.67
                                                    0.00
                                                           0.00
                                                                    0.00 ccaqtctccaaqqtqccc
                       455
                             20
                                  60.03
                                           55.00
                                                           0.00
   INTERNAL OLIGO
                                                    0.00
                                                                    0.00 gacaaaggaagcacgggaag
  PRODUCT SIZE: 165, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
37 LEFT PRIMER
                       372
                             18
                                  60.74
                                           66.67
                                                           0.00
                                                    0.00
                                                                    0.00 cttggtcgcactcagggc
                       532
                             19
                                  59.11
                                           57.89
   RIGHT PRIMER
                                                    0.50
                                                           0.00
                                                                    0.00 gttcctccattggctgcac
   INTERNAL OLIGO
                       455
                             20
                                  60.03
                                          55.00
                                                    0.00
                                                           0.00
                                                                    0.00 gacaaaggaagcacgggaag
  PRODUCT SIZE: 161, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
38 LEFT PRIMER
                       212
                             21
                                  59.12
                                          47.62
                                                    0.00
                                                           0.00
                                                                    0.00 tggagtcgttgattctgtcga
                       389
                             18
                                  60.74
                                           66.67
   RIGHT PRIMER
                                                    0.00
                                                           0.00
                                                                    0.00 gccctgagtgcgaccaag
                       305
                             20
                                  59.96
                                           55.00
   INTERNAL OLIGO
                                                    5.99
                                                           0.00
                                                                    0.00 ctgcggatctgaggctgata
   PRODUCT SIZE: 178, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
                       250
39 LEFT PRIMER
                             18
                                  59.50
                                           66.67
                                                    2.18
                                                           0.00
                                                                    0.00 ccatqccqqqtctctcaq
                                  57.61
   RIGHT PRIMER
                       420
                             21
                                           47.62
                                                    0.00
                                                           0.00
                                                                    0.00 ccatcatcttagtggcatcga
                                  59.96
                       305
                             20
                                          55.00
   INTERNAL OLIGO
                                                    5.99
                                                           0.00
                                                                    0.00 ctgcggatctgaggctgata
   PRODUCT SIZE: 171, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 1.92
                                           40.91
40 LEFT PRIMER
                        38
                             22
                                  57.08
                                                    0.00
                                                           0.00
                                                                    0.00 gcccggtgcctttaataataaa
                       227
                             21
                                  59.09
                                           47.62
   RIGHT PRIMER
                                                   13.02
                                                           8.85
                                                                    0.00 agaatcaacgactccatgggt
   INTERNAL OLIGO
                       172
                             20
                                  59.67
                                          45.00
                                                    0.00
                                                           0.00
                                                                   45.94 ttctcattgctgctttcgca
  PRODUCT SIZE: 190, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
41 LEFT PRIMER
                       636
                             23
                                  57.74
                                           39.13
                                                    0.00
                                                           0.00
                                                                    0.00 acctaccatcaaatcaaccaaga
                       816
                             21
                                  58.97
   RIGHT PRIMER
                                           52.38
                                                    6.32
                                                           0.00
                                                                    0.00 gcatctgtacttctaccggga
                       751
                             20
                                  59.88
                                           55.00
   INTERNAL OLIGO
                                                    0.00
                                                           0.00
                                                                    0.00 cgggaattggtatcggagga
  PRODUCT SIZE: 181, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
42 LEFT PRIMER
                       231
                             18
                                  58.50
                                           61.11
                                                   31.54
                                                           0.00
                                                                    0.00 gaaggcgccaatggagtc
                                                    6.32
   RIGHT PRIMER
                       411
                             18
                                  61.85
                                          66.67
                                                           0.00
                                                                    0.00 tagtggcatcgaccgggc
   INTERNAL OLIGO
                       305
                             20
                                  59.96
                                          55.00
                                                    5.99
                                                           0.00
                                                                    0.00 ctgcggatctgaggctgata
  PRODUCT SIZE: 181, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
Statistics
         con
               too
                      in
                             in
                                  not
                                                no
                                                      tm
                                                            tm
                                                                  high high high
                                                                                           high
                                   ok
                                        bad
                                                GC
                                                     too
                                                           too any_th 3'_th hair-
         sid
              many
                      tar
                          excl
                                                                                    poly
                                                                                            end
                                        GC% clamp
                                                     low
                                                          high
                                                                 compl compl
                                                                                       Χ
                                                                                           stab
        ered
                Ns
                      get
                            reg
                                  reg
                                                                               pin
                                                                                                   ok
        5882
               235
                              0
                                    0
                                        862
                                                    1082
                                                                     0
                                                                                       22
                                                                                              0
Left
                        0
                                                 0
                                                          1948
                                                                           0
                                                                               641
                                                                                                 1087
```

Right 5889 235 0 444 0 1262 1954 0 557 235 20 0 1411 282 524 0 1536 5590 0 2540 Intl 10729 0 0 22 0 Pair Stats:

considered 848287, unacceptable product size 848236, primer in pair overlaps a primer in a better pair 461481, ok 51 libprimer3 release 2.4.0

(primer3_results.cgi release 4.1.0)