Primer3 Output

```
PRIMER PICKING RESULTS FOR Puccinia recondita elongation factor 1 alpha elongation factor 1 consensus group 2
Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
                                       gc% any th 3' th hairpin seq
0LIG0
                 start len
                                 tm
LEFT PRIMER
                   577
                              59.24
                                      50.00
                                               0.00
                                                      0.00
                                                              0.00 tctggcttcaacggagacaa
                                                              0.00 ggagggtttatcggtaggg
RIGHT PRIMER
                   736
                         20
                              58.95
                                      60.00
                                               0.00
                                                      0.00
                         20
                                               9.84
                                                      0.00
INTERNAL OLIGO
                   667
                              59.59
                                      50.00
                                                              0.00 accggaaaaactctcctcga
SEOUENCE SIZE: 811
INCLUDED REGION SIZE: 811
PRODUCT SIZE: 160, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00
   1 gtNaattacaataaaNacctcNtaatNatccctcttttcgtctttNtttttcaatccgtt
   61 NctctqaaNctNataaactqccacaqqaaqcaqcaqaactcqqaaaaqqatctttcaaat
 121 acgcctgggtcctcNacaagctaaaNgccgaacgtgaacgtggtattacNattgacattg
  181 ctctatggaaNtttgagactcctagatactacgtcaccgtcattggtaagaaatNNtNtc
  241 aaattttNttactacaNtNctNacccgNtttcagacgctcctggacacagagatttNatc
  301 aagaacatgattacNggaacctctcaagctgactgtgccattctcatcatcgcggccggt
  361 accggtgagttNgaggcaggtatttcaaaggatggtcaaaccagagaacatgcaNtgctt
  421 gcgttcacattaggtgtcaaacaattgattgttgccattaaNaagatggacacggccaaa
  481 tgggctgaagcNagataccttgaaatNatcaaggagacgaccaacttNataaagaaagtc
  541 ggatttaaccccaaggttgttccatttgtgccaatttctggcttcaacggagacaatatg
                                          >>>>>>>>>>>>>
```

601 attgataattccgNNaactgccctggtacaaaggctgggaaaaagaaaaNaagactgga

721 accgataaacccctccgcctaccactacaagatgtttacaaaattggtggcatNggcacg <

781 gttcctgttggacgtgtNgagaccggaatca

```
KEYS (in order of precedence):
>>>>> left primer
<<<<< right primer
^^^^ internal oligo</pre>
```

ADDITIONAL OLIGOS

| <u>S</u> | tart <u>len</u> <u>tm</u> | gc% any th 3' th hairpin | seq |
|---|--|--------------------------|---|
| 1 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 180, | 416 20 58.11 595 20 59.24 463 20 59.74 PAIR ANY_TH COMPL: | 50.00 0.00 0.00 0.00 | tgcttgcgttcacattaggt tgtctccgttgaagccagaa aagatggacacggccaaatg |
| 2 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 179, | 574 20 57.14 752 20 58.53 712 20 60.17 PAIR ANY_TH COMPL: | 55.00 0.00 0.00 0.00 | atttctggcttcaacggaga tcttgtagtggtaggcggag agtcgccctaccgataaacc |
| 3 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 165, | 579 22 58.73 743 20 59.60 667 20 59.59 PAIR ANY_TH COMPL: | 60.00 0.00 0.00 0.00 | tggcttcaacggagacaatatg ggtaggcggaggggtttatc accggaaaaactctcctcga |
| 4 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 199, | 533 23 58.67 731 20 59.25 577 20 60.17 PAIR ANY_TH COMPL: | 55.00 0.00 0.00 0.00 | agaaagtcggatttaaccccaag ggtttatcggtagggcgact tctggcttcaacggagacaa |
| 5 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 163, | 322 20 59.31 484 18 60.28 416 20 59.02 PAIR ANY_TH COMPL: | 61.11 0.00 0.00 0.00 | tctcaagctgactgtgccat cccatttggccgtgtcca tgcttgcgttcacattaggt |
| 6 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 182, | 375 22 58.63 556 20 57.20 463 20 59.74 PAIR ANY_TH COMPL: | 50.00 0.00 0.00 0.00 | ggcaggtatttcaaaggatggt ccttggggttaaatccgact aagatggacacggccaaatg |
| 7 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 181, | 558 23 58.62 738 19 58.87 667 20 59.59 PAIR ANY_TH COMPL: | 57.89 0.00 0.00 0.00 | tgttccatttgtgccaatttctg gcggaggggtttatcggta accggaaaaactctcctcga |

```
47.83
 8 LEFT PRIMER
                      196
                            23
                                 58.04
                                                   0.00
                                                          0.00
                                                                  0.00 agactcctagatactacgtcacc
                            19
                                 59.14
                                          57.89
   RIGHT PRIMER
                      355
                                                   8.23
                                                          0.00
                                                                  0.00 ccgcgatgatgagaatggc
                      269
                            20
                                 60.88
                                          55.00
   INTERNAL OLIGO
                                                   0.00
                                                          0.00
                                                                  35.47 tttcagacgctcctggacac
   PRODUCT SIZE: 160, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
 9 LEFT PRIMER
                                 57.26
                                          39.13
                                                          0.00
                      581
                            23
                                                   0.00
                                                                  0.00 gcttcaacggagacaatatgatt
   RIGHT PRIMER
                      755
                            20
                                 57.04
                                                   0.00
                                                                  0.00 acatcttgtagtggtaggcg
                                          50.00
                                                          0.00
   INTERNAL OLIGO
                      712
                            20
                                 60.17
                                          55.00
                                                                 40.22 agtcgccctaccgataaacc
                                                   0.00
                                                          0.00
   PRODUCT SIZE: 175, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
Statistics
                            in
                                                                high high high
               too
                      in
                                                                                         high
         con
                                 not
                                               no
                                                     tm
                                       bad
                                               GC
                                                          too any_th 3'_th hair-
                     tar
                          excl
         sid
              many
                                  ok
                                                    too
                                                                                   poly
                                                                                          end
                     get
                                       GC% clamp
                                                         high
                                                                                      Χ
                           reg
                                 reg
                                                    low
                                                               compl compl
                                                                                         stab
        ered
                Ns
                                                                              pin
        1940
               427
                       0
                                        77
                                                    710
                                                          210
                                                                              258
                                                                                      6
                                                                                            0
                                                                                                252
Left
                                                    763
```

0

0

108

1

0

0

3693 Intl Pair Stats:

2074

Right

considered 81657, unacceptable product size 81641, primer in pair overlaps a primer in a better pair 30003, ok 16 libprimer3 release 2.4.0

916

236

948

0

0

ok

463

1181

0

0

89

114

0

0

30

0

(primer3 results.cgi release 4.1.0)

385

533

0

0

0

0