## **Primer3 Output**

```
PRIMER PICKING RESULTS FOR Puccinia recondita beta-tubulin consensus group 5
Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
0LIG0
                start len
                                       gc% any th 3' th hairpin seg
                                tm
LEFT PRIMER
                  344
                        21
                             58.93
                                     52.38
                                              4.00
                                                     0.00
                                                            0.00 ctgtacgagctggtccttttg
RIGHT PRIMER
                  508
                        20
                             59.10
                                     55.00
                                              0.00
                                                     0.00
                                                            0.00 gacagtcacatccctctgct
                  396
                        20
                                     55.00
                                              0.00
                                                     0.00
                                                           32.08 cggacaatctggagcaggaa
INTERNAL OLIGO
                             60.67
SEOUENCE SIZE: 514
INCLUDED REGION SIZE: 514
PRODUCT SIZE: 165, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00
   1 aaaccaaatcggtgccgcttctgttcgtaNaatctaaagcttctgNctcaNctcgctca
   61 cacccactttacagggcaaacaatttctggtgaacatggactNgatggttcaggagtgta
 121 agttccctcgcaNaacgaggtatccgcaNccatcaacaaattataatgactctattagct
  181 aNaatggtacatcagatttacaacttgagaggatgaacgtatactttaaNgaggtctgat
  241 gaatctctcaaaggagacgttatagctaaccctctNaaggcttctggcaacaaNtatgtt
  301 cctcgtgctgttctcgtNgaNttagagccaggtaccatggatgctgtacgagctggtcct
                                                >>>>>>>>>
  361 tttggccagctattcagaccagacaactttgttttcggacaatctggagcaggaaataat
                                        ^^^^^
     >>>>
  421 tgggcaaaaggtcactatactgagggagcggagcttgtcgaccaagtactagatgtggtg
  481 cgaagagaagcagagggatgtgactgtcttcagg
             <<<<<<<<
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 296 20 57.91 50.00 0.00 0.00 0.00 atattcctcqtqctqttctc RIGHT PRIMER 491 20 59.28 55.00 0.00 0.00 0.00 gcttctcttcgcaccacatc 59.95 INTERNAL OLIGO 362 20 50.00 5.67 0.00 0.00 ttggccagctattcagacca PRODUCT SIZE: 196, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00 52.38 2 LEFT PRIMER 332 21 58.26 21.47 0.00 0.00 gtaccatggatgctgtacgag 20 RIGHT PRIMER 499 59.54 55.00 0.00 0.00 0.00 atccctctqcttctcttcqc INTERNAL OLIGO 362 20 59.95 50.00 5.67 0.00 0.00 ttggccagctattcagacca PRODUCT SIZE: 168, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00 3 LEFT PRIMER 55 20 58.85 55.00 0.00 0.00 0.00 cgctcacacccactttacag 57.25 220 21 42.86 RIGHT PRIMER 2.11 0.00 0.00 acgttcatcctctcaagttgt 112 20 60.03 55.00 0.00 INTERNAL OLIGO 0.00 34.41 aggagtgtaagttccctcgc PRODUCT SIZE: 166, PAIR ANY\_TH COMPL: 0.00, PAIR 3'\_TH COMPL: 0.00 57.25 4 LEFT PRIMER 200 42.86 21 0.00 0.00 0.00 acaacttgagaggatgaacgt RIGHT PRIMER 373 20 59.30 50.00 7.01 0.00 0.00 atagctggccaaaaggacca 59.80 INTERNAL OLIGO 324 20 55.00 26.45 11.61 41.14 agagccaggtaccatggatg PRODUCT SIZE: 174, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00 **Statistics** in high high high con too in not no tm high GC too any\_th 3'\_th hairtar excl ok bad too poly end sid many GC% clamp high Χ ered Ns get reg reg low compl compl pin stab 0 0 0 82 470 133 102 0 Left 1140 219 0 0 134 82 1580 125 0 0 0 0 760 156 0 0 18 0 0 439 Right

0

15

0

3020 Intl Pair Stats:

considered 33512, unacceptable product size 33505, primer in pair overlaps a primer in a better pair 19709, ok 7 libprimer3 release 2.4.0

821

884

0

0

48

0

0

ok

1008

(primer3 results.cgi release 4.1.0)

244

0