## **Primer3 Output**

```
PRIMER PICKING RESULTS FOR Puccinia recondita beta-tubulin consensus group 0
Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
0LIG0
                start len
                                tm
                                      gc% any th 3' th hairpin seg
LEFT PRIMER
                  255
                        20
                             58.56
                                     50.00
                                             0.00
                                                    0.00
                                                            0.00 tgagccatacaacgcaactc
RIGHT PRIMER
                  420
                        20
                             59.03
                                     50.00
                                             0.00
                                                    0.00
                                                            0.00 acctgacattacggcagaca
                  297
                        20
                                             0.00
                                                    0.00
                                                           42.13 aaactccgacgagacgttct
INTERNAL OLIGO
                             59.96
                                     50.00
SEQUENCE SIZE: 449
INCLUDED REGION SIZE: 449
PRODUCT SIZE: 166, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00
   1 ggagcaggaaataattgggcaaaaggtcactatactgagggagcggagcttgtcgaccaa
  61 qtactaqatqtqqqaaqaaqaaqcaqaqqqatqtqactqtcttcaqqqattccaaata
 121 acacattctcttgggggtggtacaggtgccggtatgggtacattattaatttcaaaaatc
 181 cgggaagaNtttcctgatcgaatgatggcaaccttctcggttgtgccatccccNaaggtt
 241 tcagacactgttgttgagccatacaacgcaactctttcagtccatcagctggttgaaaac
                   >>>>>>>>>>
  301 tccgacgagacgttctgtatcgacaatgaggctctNtacgatatctgtatgaggacgtta
     ^^^^^
 361 aagctttctaacccttcatacggtgatctcaatcacttggtgtctgccgtaatgtcaggt
                                            <<<<<<<<
 421 gtaactacctgtcttcgattccctggtca
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	75	20 59.54	55.00	0.00 0.00 0.00 gcgaagagaagcagagggat
	RIGHT PRIMER	274	20 58.56	50.00	0.00 0.00 0.00 gagttgcgttgtatggctca
	INTERNAL OLIGO	126	20 59.58	55.00	0.00 0.00 42.52 ttctcttgggggtggtacag
	PRODUCT SIZE: 200,	PAIR	ANY_TH COMPL:	0.00,	PAIR 3'_TH COMPL: 0.00
2	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 161,	257 417 297 PAIR	21 58.50 20 59.32 20 59.96 ANY_TH COMPL:	42.86 50.00 50.00 0.00,	0.00 0.00 0.00 agccatacaacgcaactcttt 0.00 0.00 0.00 tgacattacggcagacacca 0.00 0.00 42.13 aaactccgacgagacgttct PAIR 3'_TH COMPL: 0.00
3	LEFT PRIMER	1	21 58.61	47.62	0.00 0.00 0.00 ggagcaggaaataattgggca
	RIGHT PRIMER	164	20 59.82	55.00	0.00 0.00 0.00 aatgtacccataccggcacc
	INTERNAL OLIGO	85	20 60.03	55.00	9.94 0.00 33.99 gcagagggatgtgactgtct
	PRODUCT SIZE: 164,	PAIR	ANY_TH COMPL:	0.86,	PAIR 3'_TH COMPL: 0.37
4	LEFT PRIMER	28	20 58.02	55.00	0.00 0.00 0.00 cactatactgagggagcgga
	RIGHT PRIMER	213	21 59.39	52.38	0.00 0.00 0.00 ggttgccatcattcgatcagg
	INTERNAL OLIGO	85	20 60.03	55.00	9.94 0.00 33.99 gcagagggatgtgactgtct
	PRODUCT SIZE: 186,	PAIR	ANY_TH COMPL:	0.00,	PAIR 3'_TH COMPL: 0.00
5	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 198,	81 278 126 PAIR	20 58.05 21 58.15 20 59.58 ANY_TH COMPL:	50.00 47.62 55.00 0.00,	0.00 0.00 0.00 agaagcagagggatgtgact 0.00 0.00 0.00 gaaagagttgcgttgtatggc 0.00 0.00 42.52 ttctcttgggggtggtacag PAIR 3'_TH COMPL: 0.00
6	LEFT PRIMER	57	21 58.46	52.38	0.00 0.00 0.00 ccaagtactagatgtggtgcg
	RIGHT PRIMER	216	21 57.38	47.62	0.00 0.00 0.00 gaaggttgccatcattcgatc
	INTERNAL OLIGO	85	20 60.03	55.00	9.94 0.00 33.99 gcagagggatgtgactgtct
	PRODUCT SIZE: 160,	PAIR	ANY_TH COMPL:	0.00,	PAIR 3'_TH COMPL: 0.00
7	LEFT PRIMER	139	18 57.15	61.11	1.90 0.00 0.00 ggtacaggtgccggtatg
	RIGHT PRIMER	304	20 58.41	50.00	17.39 0.00 0.00 cggagttttcaaccagctga
	INTERNAL OLIGO	255	20 59.47	50.00	0.00 0.00 0.00 tgagccatacaacgcaactc
	PRODUCT SIZE: 166,	PAIR	ANY_TH COMPL:	1.68,	PAIR 3'_TH COMPL: 0.00
8	LEFT PRIMER	259	22 57.93	45.45	0.00 0.00 0.00 ccatacaacgcaactctttcag
	RIGHT PRIMER	423	20 57.33	50.00	0.00 0.00 0.00 tacacctgacattacggcag
	INTERNAL OLIGO	297	20 59.96	50.00	0.00 0.00 42.13 aaactccgacgagacgttct
	PRODUCT SIZE: 165,	PAIR	ANY_TH COMPL:	0.00,	PAIR 3'_TH COMPL: 0.00
9	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 186,	198 383 297 PAIR	21 58.37 22 57.86 20 59.96 ANY_TH COMPL:	47.62 45.45 50.00 5.04,	10.15 0.00 0.00 tcgaatgatggcaaccttctc 0.00 0.00 0.00 ccgtatgaagggttagaaagct 0.00 0.00 42.13 aaactccgacgagacgttct PAIR 3'_TH COMPL: 0.00
10	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 194,	114 307 255 PAIR	23 57.67 20 59.77 20 59.47 ANY_TH COMPL:	43.48 55.00 50.00 0.00,	0.00 0.00 0.00 ccaaataacacattctcttgggg 0.00 0.00 0.00 cgtcggagttttcaaccagc 0.00 0.00 0.00 tgagccatacaacgcaactc PAIR 3'_TH COMPL: 0.00
11	LEFT PRIMER RIGHT PRIMER	16 210	22 58.05 21 57.72	45.45 42.86	<pre>0.00 0.00 0.00 tgggcaaaaggtcactatactg 0.00 0.00 0.00 tgccatcattcgatcaggaaa</pre>

```
PRODUCT SIZE: 195, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
12 LEFT PRIMER
                                  58.17
                         2
                            23
                                          39.13
                                                    0.00
                                                           0.00
                                                                   0.00 gagcaggaaataattgggcaaaa
                             20
                                  61.62
                      161
                                          60.00
                                                    0.00
   RIGHT PRIMER
                                                           0.00
                                                                   0.00 gtacccataccggcacctgt
   INTERNAL OLIGO
                                                                  33.99 gcagagggatgtgactgtct
                        85
                             20
                                  60.03
                                          55.00
                                                    9.94
                                                           0.00
   PRODUCT SIZE: 160, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
Statistics
                                                                 high high high
                                                                                          high
                             in
         con
               too
                      in
                                  not
                                               no
                                                      tm
                                                            tm
                                        bad
                                                           too any_th 3'_th hair-
                          excl
         sid
              many
                      tar
                                   ok
                                               GC
                                                     too
                                                                                    poly
                                                                                           end
                                        GC% clamp
                                                          high
                                                                compl compl
                            reg
                                  reg
                                                     low
                                                                                       Χ
                                                                                          stab
        ered
                Ns
                      get
                                                                               pin
                                                                                                  ok
                                                     584
                                                                              279
                                                                                      24
        1440
                        0
                                         94
                                                           158
                                                                                                 255
Left
                46
                                         83
                                                     657
                                                            75
                                                                              173
                                                                                                 359
                        0
                                                                    0
Right
        1418
                64
                              0
                                    0
                                                                           0
                                                                                       7
                                                                                             0
                81
                                                          1075
                                                                                59
                                                                                                1432
        3681
                        0
                              0
                                    0
                                         34
                                                 0
                                                    1000
                                                                    0
                                                                           0
                                                                                       0
                                                                                             0
Intl
Pair Stats:
considered 44584, unacceptable product size 44568, primer in pair overlaps a primer in a better pair 29852, ok 16
libprimer3 release 2.4.0
```

9.94

0.00

33.99 gcagaggatgtgactgtct

(primer3 results.cgi release 4.1.0)

INTERNAL OLIGO

85

20

60.03

55.00