Primer3 Output

```
PRIMER PICKING RESULTS FOR Puccinia recondita beta-tubulin consensus group 3
Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
                 start len
                                       qc% any th 3' th hairpin seq
0LIG0
                                tm
LEFT PRIMER
                   478
                        20
                              59.40
                                     55.00
                                              0.00
                                                     0.00
                                                             0.00 aggtcactacactgaaggcg
RIGHT PRIMER
                  664
                        20
                             58.87
                                     50.00
                                              0.00
                                                     0.00
                                                             0.00 tgccatcattcgatcaggga
                   539
                        20
                                     55.00
                                              9.94
                                                     0.00
                                                            33.99 gcagaggatgtgactgtct
INTERNAL OLIGO
                             60.03
SEOUENCE SIZE: 688
INCLUDED REGION SIZE: 688
PRODUCT SIZE: 187, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00
   1 tacqttaatatttaqtttqccttqtaactctactaataqaqttttaqqqaaaccaaatcq
   61 gagccgcattctgttcgtagaatctcaagcttctagctcaactcgctcacacccactcta
 121 cagggcaaacaatttctggtgagcatggacttgatggttcaggagtgtaagttccctcgc
  181 atNattaggtatccgcattcatcaacgaaNtataatgactccatNagctacaatggtaca
  241 tcagatttacaacttgagaggatgaacgtatactttaacgaggtctggtgaagctctcaa
  301 aagagaagttatcgctaaccctcttaaggcttctggtaacaaatatgttcctcgtgctgt
  361 tctcgtcgacttggagccaggtacNatggatgctgtacgagctggtccttttggccagct
  421 attcagaccagacaacttcgtcttcggacaatcNggagccggaaataattgggcaaaagg
  481 tcactacactgaaggcgcggagcttgtcgaccaagtNctagatgtagtgcgaagagaagc
     >>>>>>>>>
  541 agagggatgtgactgtcttcaggggtttcaaataacacattctcttgggggtggtacagg
      ^^^^^
  601 tgccggtatgggtacgttattaatttcaaaaatccgggaagaattccctgatcgaatgat
```

<<<<<<<

661 ggcaaccttctcagttgtgccgtcccct <<<<

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

ADDITIONAL OLIGOS

ADDITIONAL ULIGOS	start <u>len</u> <u>tm</u> _	gc% <u>any th</u> <u>3' th hairpin</u> <u>seq</u>
1 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 165	135 20 58.65 299 20 58.74 161 20 60.03 PAIR ANY_TH COMPL:	50.00 0.00 0.00 0.00 tctggtgagcatggacttga 55.00 0.00 0.00 0.00 tgagagcttcaccagacctc 55.00 0.00 0.00 34.41 aggagtgtaagttccctcgc 22.36, PAIR 3'_TH COMPL: 5.30
2 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 195	354 20 59.22 548 20 59.54 411 20 59.95 PAIR ANY_TH COMPL:	55.00 14.70 6.17 0.00 gtgctgttctcgtcgacttg 55.00 0.00 0.00 0.00 atccctctgcttctcttcgc 50.00 5.67 0.00 0.00 ttggccagctattcagacca 0.00, PAIR 3'_TH COMPL: 0.00
3 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 199	411 20 59.01 609 19 59.10 539 20 60.03 PAIR ANY_TH COMPL:	50.00 5.11 0.00 0.00 ttggccagctattcagacca 57.89 0.00 0.00 0.00 ataccggcacctgtaccac 55.00 9.94 0.00 33.99 gcagagggatgtgactgtct 0.00, PAIR 3'_TH COMPL: 0.00
4 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 200	358 20 60.04 557 20 59.10 411 20 59.95 PAIR ANY_TH COMPL:	55.00 14.70 10.79 0.00 tgttctcgtcgacttggagc 55.00 0.00 0.00 0.00 gacagtcacatccctctgct 50.00 5.67 0.00 0.00 ttggccagctattcagacca 0.00, PAIR 3'_TH COMPL: 0.00
5 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 191	105 20 59.75 295 20 58.37 161 20 60.03 PAIR ANY_TH COMPL:	60.00 0.00 0.00 0.00 gctcacacccactctacagg 50.00 0.00 0.00 0.00 agcttcaccagacctcgtta 55.00 0.00 0.00 34.41 aggagtgtaagttccctcgc 0.00, PAIR 3'_TH COMPL: 0.00
6 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 186	188 21 58.52 373 20 59.22 280 20 59.66 PAIR ANY_TH COMPL:	47.62 0.00 0.00 0.00 ggtatccgcattcatcaacga 55.00 10.46 0.00 0.00 caagtcgacgagaacagcac 55.00 0.00 0.00 38.66 gaggtctggtgaagctctca 0.00, PAIR 3'_TH COMPL: 0.00
7 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 162	393 21 58.93 554 20 58.05 455 20 60.24 PAIR ANY_TH COMPL:	52.38 4.00 0.00 0.00 ctgtacgagctggtccttttg 50.00 0.00 0.00 0.00 agtcacatccctctgcttct 55.00 5.43 4.11 44.34 ggagccggaaataattgggc 0.00, PAIR 3'_TH COMPL: 0.00
8 LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 199	470 20 58.02 668 21 58.43 539 20 60.03 PAIR ANY_TH COMPL:	50.00 0.00 0.00 0.00 tgggcaaaaggtcactacac 47.62 0.00 0.00 0.00 aggttgccatcattcgatcag 55.00 9.94 0.00 33.99 gcagagggatgtgactgtct 1.08, PAIR 3'_TH COMPL: 0.00

9 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 433	21 58.24 20 58.05 20 60.10 ANY_TH COMPL:	47.62 55.00 55.00 0.00,	0.00 15.15	0.00 0.0 6.64 0.0	0 tttaacgaggtctggtgaagc 0 gtctggtctgaatagctggc 0 gtgctgttctcgtcgacttg 0
10 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 612	20 58.05 19 59.85 20 60.03 ANY_TH COMPL:	55.00 63.16 55.00 0.00,	0.00 9.94	0.00 0.0 0.00 33.9	0 gccagctattcagaccagac 0 cccataccggcacctgtac 9 gcagagggatgtgactgtct 0
11 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 620	22 58.47 20 58.59 20 60.03 ANY_TH COMPL:	45.45 50.00 55.00 0.65,	0.00 9.94	0.00 0.0 0.00 33.9	0 ccggaaataattgggcaaaagg 10 ataacgtacccataccggca 19 gcagagggatgtgactgtct 18
12 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 541	20 57.91 21 58.12 20 59.95 ANY_TH COMPL:	50.00 47.62 50.00 0.00,	0.00 5.67	0.00 0.0 0.00 0.0	0 atgttcctcgtgctgttctc 0 tgcttctcttcgcactacatc 0 ttggccagctattcagacca
13 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 683	21 57.84 20 59.82 20 59.58 ANY_TH COMPL:	47.62 50.00 55.00 0.00,	0.00 0.00	0.00 0.0 0.00 42.5	0 agatgtagtgcgaagagaagc 0 acggcacaactgagaaggtt 2 ttctcttgggggtggtacag 0
14 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 422	21 57.25 20 59.30 20 60.10 ANY_TH COMPL:	42.86 50.00 55.00 0.00,	7.01 15.15	0.00 0.0 6.64 0.0	0 acaacttgagaggatgaacgt 0 atagctggccaaaaggacca 0 gtgctgttctcgtcgacttg 0
15 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 294	20 59.83 22 59.26 20 60.03 ANY_TH COMPL:	60.00 50.00 55.00 0.00,	0.00 0.00	0.00 0.0 0.00 34.4	0 ctcgctcacacccactctac 0 gcttcaccagacctcgttaaag 1 aggagtgtaagttccctcgc
16 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 552	18 59.04 21 58.46 20 59.95 ANY_TH COMPL:	61.11 47.62 50.00 0.00,	0.00 5.67	0.00 0.0 0.00 0.0	0 tcgtcgacttggagccag 0 tcacatccctctgcttctctt 0 ttggccagctattcagacca 0
17 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 361	20 59.39 22 57.31 20 59.66 ANY_TH COMPL:	55.00 40.91 55.00 8.11,	0.00 0.00	0.00 0.0 0.00 38.6	0 ggagtgtaagttccctcgca 0 aacagcacgaggaacatatttg 6 gaggtctggtgaagctctca 0
18 LEFT PRIM RIGHT PRI INTERNAL PRODUCT S	MER 688	20 59.54 18 60.85 20 59.81 ANY_TH COMPL:	55.00 61.11 50.00 0.00,	0.00 0.00	0.00 0.0 0.00 34.8	0 gcgaagagaagcagagggat 0 aggggacggcacaactga 0 tccctgatcgaatgatggca 0
19 LEFT PRIM RIGHT PRI		19 59.63 21 57.25	57.89 42.86			0 aactcgctcacacccactc 0 acgttcatcctctcaagttgt

	INTERNAL OLIGO PRODUCT SIZE: 170,	161 20 60.03 PAIR ANY_TH COMPL:	55.00 0.00 0.00 34.41 0.00, PAIR 3'_TH COMPL: 0.00	aggagtgtaagttccctcgc
20	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 192,	485 18 59.43 676 20 57.04 539 20 60.03 PAIR ANY_TH COMPL:	45.00 0.00 0.00 0.00	tacactgaaggcgcggag aactgagaaggttgccatca gcagagggatgtgactgtct
21	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 195,	421 21 57.25 615 19 59.85 539 20 60.03 PAIR ANY_TH COMPL:	63.16 0.00 0.00 0.00	attcagaccagacaacttcgt gtacccataccggcacctg gcagagggatgtgactgtct
22	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 200,	281 19 57.54 480 22 58.47 411 20 59.95 PAIR ANY_TH COMPL:	45.45 0.00 0.00 0.00	aggtctggtgaagctctca ccttttgcccaattatttccgg ttggccagctattcagacca
23	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 185,	138 20 58.61 322 23 57.32 161 20 60.03 PAIR ANY_TH COMPL:	39.13 21.66 0.00 0.00	ggtgagcatggacttgatgg agggttagcgataacttctcttt aggagtgtaagttccctcgc
24	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 188,	353 18 58.56 540 23 58.71 411 20 59.95 PAIR ANY_TH COMPL:	47.83 0.00 0.00 0.00	cgtgctgttctcgtcgac gcttctcttcgcactacatctag ttggccagctattcagacca
25	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 160,	340 22 57.31 499 19 60.08 411 20 59.95 PAIR ANY_TH COMPL:	57.89 0.00 0.00 0.00	caaatatgttcctcgtgctgtt cgcgccttcagtgtagtga ttggccagctattcagacca
26	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 180,	113 20 57.73 292 23 57.27 161 20 60.03 PAIR ANY_TH COMPL:	39.13 0.00 0.00 0.00	ccactctacagggcaaacaa ttcaccagacctcgttaaagtat aggagtgtaagttccctcgc
27	LEFT PRIMER RIGHT PRIMER INTERNAL OLIGO PRODUCT SIZE: 173,	407 21 58.27 579 23 57.22 455 20 60.24 PAIR ANY_TH COMPL:	39.13 0.00 0.00 0.00	ccttttggccagctattcaga tgtgttatttgaaacccctgaag ggagccggaaataattgggc
Sta	atistics con too	in in not	no tm tm high	high high high
In	sid many ered Ns ft 2599 130 ght 2476 130	tar excl ok b get reg reg G 0 0 0 1 0 0 0 1	no tm tm high lad GC too too any_th 3 C% clamp low high compl compl compl compl compl compl comple do not be seen to seen the seen to see the	'_th hair- poly end

Pair Stats: considered 91287, unacceptable product size 91257, primer in pair overlaps a primer in a better pair 81231, ok 30

libprimer3 release 2.4.0

(primer3_results.cgi release 4.1.0)