WP3F-WP1F之间的序列

TCAATGCAACCTTCCATTACCTTAAAAAA**AT**CATTTGCCATATGTACTTCCATCTAGAACACTCCTGCTTCTTTTTACCTTCAGCCTGGTCA**T**AGTGATTTTCTATGTAACGTGATCAGAGG**A**CAAACTAGCTTTGTCCCCCCGCCAATATTTAACATTTTAGTTCAAATAAAACAACCCACCCACCAAATCCAAATTATTAAGAATGCCTCTTCCCCCTCTAAAAACAGCATTCATCCTCG**T**GCAACATATGCAAATTAATTCGTGATTTCTATATAATTCTTTCGTTATACCCATAACTTTGATTTGATCATGCAAGAACGTACTGTGTGTCAATAAAACAAATGGCACGGGGGTAGCTTAGAGCATTATTGGAGTACTCTTATATAGGAGTTAGTAGTGAGTGATATATCTGTTCTAATTAAGCTC**CA**TCTTATTGGGTTAAGGGTTTTTATTTTTGGTAAACTGGTCACTAAAATACACTGATACAGACTAGTCCTAATAATTCATAAGCACATTGATGATGCAGAGGAGGCCATATGTAGGGGCATGGGCAAAAGTGCTTTGACAGTGGCAGAGCAGTTAAGAAAGTTAAAGTCTAGGAGAACTTGTGAACGAATGCTGAGGCCCAATCAGGCTGAAGATCGATGTGTGTTCTGACTTTGGCTAGTTTCTGCTTCCCCTTTGCAGACAGCTTATTAATCAGATCCCCCACAGTCTCATAGTTGCATAGGGTCGCTAGTGGTGGGCTTGATCTGCCTCTGCCTCTGGAAACAA**C**GGAGGAGTAGATCCGATGTGTTTTATGGAACATGATCGATTGATCATATATCAAAGGCGGCTTGGATATGGGTGTGCAAGGCAATCAGAGATCC**C**GTACTATGGTTCACGTATCAGTGGCACAATTGCAAACTATACAAAATGA**T**AATCAGATAAGCCACATCCACAAACG**CAATGCTTCGAATTTTGCAG**AGGATGGCGATTTTTCAAGTGCGGTCGTTTAGA**A**GGGCCGCTGATAGAAAT**C**TATTTTTCACCGGGG**G**TACTTT

**>WP2 Se, 以WP2为模板扩增**

AGCACATTGATGATGCAGAGGAGGCCATATGTAGGGGCATGGGCAAAAGTGCTTTGACAGTGGCAGAGCAGTTAAGAAAGTTAAAGTCTAGGAGAACTTGTGAACGAATGCTGAGGCCCAATCAGGCTGAAGATCGATGTGTGTTCTGACTTTGGCTAGTTTCTGCTTCCCCTTTGCAGACAGCTTATTAATCAGATCCCCCACAGTCTCATAGTTGCATAGGGTCGCTAGTGGTGGGCTTGATCTGCCTCTGCCTCTGGAAACAA**C**GGAGGAGTAGATCCGATGTGTTTTATGGAACATGATCGATTGATCATATATCAAAGGCGGCTTGGATATGGGTGTGCAAGGCAATCAGAGATCC**C**GTACTATGGTTCACGTATCAGTGGCACAATTGCAAACTATACAAAATGA**T**AATCAGATAAGCCACATCCACAAACG**CAATGCTTCGAATTTTGCAG**AGGATGGCGATTTTTCAAGTGCGGTCGTTTAGA**A**GGGCCGCTGATAGAAAT**C**TATTTTTCACCGGGG**G**TACTTTGAAAGGGCCGTCTGTTTAAAAATCAATTTCCCTTAACCTATTTTGCTGATGTATTTTCACATTCTTAACCAGACTATCCCGACGTATTTACATATATAGACGTGTATGTTTTAATCCGCCTCTAAAAATACAAAGGGTGTCACTAAAATTGATTTTTTTAACAGTGTTGGCCACTAATACTAGTAAGTACATGTTAATTTATATAAGCACATTGATGTAGAGAGGGACCATAAACAAAAGGGGCTTTGGCAACGGAGGGTTGCGGCGTGGACTGTAGCGTAGCAACAAAGTTACTAAGTCTAGGCCAAACTTGTGAAAGGGCGAGGCCCAACTAGGCTAAAGATTGGCCTTTTTGCTTACGCGTTTGTTGTGTTCCTCTTTGTATTTAGCTTAATTAGATCCCATGCATATGGTTCAATTGTCAGCGATCTCCCAATAGTTGCGCAGGGTTGCTGCTGGTGGCTCTGCCTCTGCGTCAGGAAAGAGTGGAAAAGAGAACATCGGGACATGCAACTTCACTGAAAATAAATATCAAATATGGATATGTAAAATAAAAAAACTACTAATTAAGTTTTAATAATTTCAAACTTGAAAAATATTTTATCTGATATTTTAAAGCATCTTCTATTTATATATAAAGTTTTCGCATAAAATACACTATTTAACAATTTAAAAAACATGCTAACGGAAACCGATGTAAGATCTATACGTTAAACAGAACGAACAAGGTATTAAAAATGAGTAGATATGATGTGCATGTACGAGTGAGAATCATATAAAGATGCGAAGAAATGTGACAGTGACCTGCACTAATAGAAAAAACATTTTTGTGACGTTGGGTATTTATTTTTGCAGGCAGACATGATTTTTGGAGCTAAATAGCAACCTAAAAAATGCAAACCGGGGTGAAGCTCGCTGTCCACTTGTAAAAATCTATTTTCACAGGCGGACCACTTAAGCGGTCCGCCTGCAAAAGTACCCTTTATTTTTGCAGGCAAATCTCTTATGTGCCCCGTCTACAAAAATAAACTTGGAATATAAATAGAGGCGACATAGGGGAGATTTTTTCTAAGTCCTCTCTCTTTCTCCCTCACTCCTCTCCTGCGTCCCTCACCTTCGGCCCTTCCCCCTCCCCTCCCCTCCCCTCCCCGGCGTGCTCCCGGCAGCGGGCGGCGGCGACTCCCGGCCCCTTCCTCCCAGATCTGGCTAGAGGGGGCAGGGGGAGGGCGGTGGCTGGCAGTGCTCCCCTCCCTCGCGACATGGCGGCGGCGGCCTCCGGCAGCTGGGCGGTGGTGGCGGCCGCATGCGGAGGCGGCGTGGAGGCGGGAGGTCGGCGGCAGTGGCGGCGTCCCCACCTCCCACATCCGGCAGCTCCCCTCTCCTCCCTCTCTCTCTCCAAAAACACTAACCGAGAGTGGAGGGCAGCGGTGGGTAGGGCTAGGGCACCGAATTTTTTTTTACGCTTAAAAATATTTTTGCAGGCGGGTGGATTTAGCCGCCTGTAAAAGTCATTAATTTTCGCAGGCGTTTGGTTGCAGGCGGAGGCTATGGACGCCTGCAAAAATGTGTTTTAGCCGCCAGTAAAAACGTGTTTTCTAGTAGTGCTAGAAGGACATTACAGACCATCAAAGATAATTTAGATAAGAGTGAGAATAGGATCGAGGTGTGTTTGTGAAGACTTTGTTTGGTTAATCCCATCAAAAGGGAATTAATTTCATACCTATCTTTCTTAGAGATTAACTGAACAAGGTCTAATGGAAGATGATAGATGATCAAAGGCATATATAAATACTACGCCTCATACATAGATCTTGAGCAAGGGTGAGAGAGAAGTAACTAAGGACATAACGCTGATATTAATGTCAATATAAATACTACTCCACATGCATAGATGTCGTTGTATTAGCAAAACAATGTACACAGTTATCCGGTTTCATAATTGGAGGCCAAACTTTAGACCATGTTAATAGACTGGACATGTAAAAGATAAATATAACCGCCGCATACAATTTTTAACATTTCATATCCTTTTCTCTGGTTGATTTTCGACTATGGTGCGACATCAACACTATTGTAGCAAAACCACAACCAACTTAACTCGATCAACATTTTAACTTGCACAGTGAATAAGAGATACTCAAATTAGAGACCATAGAAATTATACATAATTATTATCAAATCAACATGTATATAAGAGTAAACCAGGATACCCTTCAAAGAAACCAAAAGAAATAAAGAAGAATGTCCGTCGTGATTTCAAAGGAAAAAGAGAGAGGGGAAGGTTGGGTGCGATCCTTCACCTCTCTTTTTAAAAGAAAGTTCATCGCTCTCCACGTGCACGCTTCTTAAACTGATAAACTGTATTTTTTTTAAACAAGAAGTTATATAGAAAATTATTTTGAAAATCATATTAATTTATTTTTATATCTAAAATAATTATATCTAATTAATTGTACGCAAGTGGCTCATCTCATTTTCCTTTTATCTACTCGTTTCTCCTTGCGTCAATCACACACTTACAAAAAAAAACTTGGGGGTATCTGTGGTTTCCATTTTACGTTTTACCCACTAGAAAGCGTATTCCACCGCGCTGTCCATCCACTGACTCCTATATATATTTTCTTGCGCGTTATTTACCGTGCTCGTGCCGGCATCTCTTCCCACCCACCAATACGGTTACTTCCCTCCCCCTTTCCCAAACCATTTCTTATATAACGGTCAACCACCGCCTCCTCCCCTCCCCTCCCCTCTCCTCTCTCTCTCTCTTCTCTCCTCCTCCACTCTCCCACGCCGCCGACGACGACGATGGCCGACGAGCACTTCTTCCCCGCCGGCGACTACTTCTCCACCTCCTCGTCCGGCGCCGGCACGGGCGGTGCGGGCGCGTTGCTGCCCGCCGCGGCGTACGGGACGATGACGATGATGCCGCCGTGGGCAGTGGCGGCCGCCGAGCAGATGATGATGATGGCGCCGGCGGCGGCCGCGGCGGAGTTCGACTCCGCGCTCAGCTCCCTCGTGTCGTCTCCGCAGGGCGGCGGCGGCGGCGACGAGATGGCGGCCATCGGCGACCTCATCGGCCGGCTTGGGAGCATCTGCAGCCACGGCGGCGCCAGCGCCAACAACTCCTGCTACAGCACGCCGCTCAGCTCCCCGCCGCGCGCCGCCCCGCCGCCGCCGTTCCGTGGCTACGCCGCCGCCGGCGGCAGGCTGTCCCGTGTCTCCAGCAGCAAGTCCCTCGGCGGCGCCGCCGCGGCATTGGACAGCTCCGAGGCCGACATGAACCCCACCACCGCCGCCGCCGCCGACCAACCATCCAAGCCCTCCGCCGCCGCCGCCCGGAAGCGCAAGTCGTCGGCGAAGCCCAAGGCCTCGTCCTCATCCTTACCCACGGTACGACACCATACTCCGACCACCGCTCTCCATCACTTCTCCGATCTCCACTGATCACCAACCCCATGCACAATGCAGGCCACGGCGACGACGAACGCGAGCCCGAAGCGGTCCAAGGTCGCCGCCGGCGCCGGAGACGACGGCGACGGCGACGCCGACGCGGCGGAGGAGAAGCCGGAGCCAGCCAAAGACTACATCCATGTGAGGGCGAGGCGGGGGCAAGCCACCGATAGCCATAGCCTCGCCGAGAGGGTAATTAATCACCTAATTAATTAAATCCTAATTAGCTTTTTTTGCAAGTACTACACTAATCCCAAAATTAATCACAATGCGAGGGCACCCCCTAATTGCGCACCGTGTTACTAATTGCTCTACTTATTGAGTTGCAAGGACGCTTAATTATTATTAAAATTTTAATTAGCTTTGTGTTTTGTTTAATATTGGTAGGTGAGGAGGGAGAGGATAAGCGAGAGGATGAAGCTTCTGCAGTCGCTCGTGCCAGGCTGCAACAAGGTAGTATGAACACAAACACACTCCAGCGGCTAATCTCATTCTTAAATTTCCTCCAAATTCATGTATCCAAACAAGTGGCTTCTTTTTTTTCCCGCTCCAAATTACACTCAAAATTTAAAATTCTAGTTTTAGAGTTTAATTAATATCAAAAGCAGTTGTACTAATTTGTAATATTA

**EcoRI**

**GAATTC**CGTTATACGGGGCAGTACTGAAAATTAGTGGTCAAAAATGTATTTTCGAGGACGCGCATTAAGGGGGAATAGTAACCTAACGTACAAAACGGACTTGCCTGTGTGGCAATAATTAAGTTTAATATATATACAATTACTTTAGTTTCAGTATTGCCTAGAGCTAGGAGTTGGACTTTTTGCTACTGGGATAGATAAAATTAAGTAAAAAAATAATAATCTTTTTTGTACCGAAAGTGTGAGCACTAACTGCGGTGGTTAGTCTATGCAGCCAGTAGTAATTTTGACCGTAATGCATGTAAGCTACTAGTAGTACTCCTAAGTTAAGCTCCCAGTGGTCCAAGAGGATATAATAATAATTGATTAGTTAATAATCTGAGAGGCTCTTGCTCTGTACTTAAACTAATCAAACCGGCTAATCAATTGCAAGGTCCTGGTGGTTTAGGTGGTGCGCACGATTATTTTAGTTGCCCCAATTATTGCCACGGTAGTGATGCCACCGCCGGTGGTCAAATTTGGGTTGAATTTTAAATTTGGTTGATAACTTTTCTTACCTTTTTAAAATTTATTGCTGAGTTTTTTTCTAGATGATTGTTTATTGTTTTTGTTTTGTTAATTAGTTAGCAAATAAATGTGAATGTTGCGGTGTGCAGATCACCGGCAAGGCTCTCATGCTGGACGAGATCATCAACTATGTGCAGTCGCTGCAGCGTCAGGTCGAGGTACCAATGCAAGCATTTGCTTTAATAGTGTGCTAAATGAACTTGTTTTAATGACAATAAATATGTTGGAGAAAAATGTTGTTGTGTTCTCCTTCTGTCGTCCTAGCTAGCTCTTATTTGAAGTTATGTATGAAATTAAGCCCAAAAGCTAAATTTTGACATTGGTGTGTCTCACAGTTTTTGTCCATGAAGTTGGCGACCATGAATCCTCAGCTGGACTTTGACAGCCATTACATGCCTTCCAAAGATGTAAGTATAGCATCTGAAAACACTTTTATCTGATCTAGAGAGACAGTTGACACAGAGTACTATTACGATATTGTCCCTCAATTTGCAAATGTTATATTCGCCGCACTCGGGGTATCATTTTCAGATGAGCCATATGCCAGTACCCGCATACCCGTCAAGCGATCCGACCACCACCACCGCGTTCTCCTACACCGGCTCACCCGCCACTGCTGATCCATTCACCGTCTACAACTGCTGGGAGCTCGACCTCCACACCGCTATGCAAATGGGAGCCACCACCGGACTCAGCCAAGACGGTCCAATCGCAACGATGGCACCCTCTCCCTCGCCATTGCCGCACCATCCTCCTCTTCACGGCTTCTACGGTAAGTGAAATCGAACCACCACATCTCCTTACATCCCTAACAAATTATACATGAATTTTTAAAAATACTCAATTTTTTTTTAAAAAAAAAGTTCAAAAATAAGATTATTTTTTTTACTGGACTACAGTAGTGGAGTGGTGGTGGTCATACTACTCCAGTACGGTAGGTTTGTTTGTCCAAGTTTGTTGAGTTTCGCTGTTGGTGGTAATTGGGCGCAGGGGGGCAGCAGCAGCAGGGGACGACAGTAAACCACATGAAGGCCGAGCCATAATAAATGCGGCGACCTCTCCTTCTGTACATACGCCCAGCCGCCGTACGTGTACTCCGCTTTTCTGCTCCCATCCTGCAGCATCAGCATCACCAGCAGCAGCAAACCCAAGCTCACAATCATTGCCATCAAAAGAAGAAAGAAGAGGATGTTGTGTGTGACTGTGTGTGTGGCTTTGATCATGGCTTTGGCTTGATCCTACAAAAGCCACTATCTTTTTTTCCGCTTTTCTACCCCCTCTGCCCTCTGTCACTGCAGGTGGGGCCCAGCTGCACAGGGAAGGAGAAGGTTTTCCAAGGCTTAACTTTTTCCAGATGCAGTGTCATATTGTTTAGGCAAAGAACTTGTTTTGATGTTGTGATGCTTGTAGGACAGAGGAGTATATGTGTAGTATGTCTGGAAATGGCAAGGCAGGGGAGCTGTGTGACCTTTGTGTGTGCTGGTTGCATGCCCTAACTGTAGAAAAAAAAAAGAGGAGGATTGTGCTAGTGATGTTAGTAGTGGTAGTGGCTTTGTAGGATTTGGCATGCATGGGGATGTATGTACTATGTATGTAGAGATTGGAGAGGATTTTGAAAAACACCTTCCTTTTTTGTGGACATTGTTTTGTATCAAGAGTACTGGAATCATCATGCATGCAAGAAATGTTCTTTGAACAATAATCGACGATGTCGTTAGTATTCTTGATGTGGTTGTTTATGTTAGTTTAGAATCCTTTGGTTGTCAAAAGTTATCCGCATTATAATCTGGATGGATTTAGAATCTTGGGGACAATCAGAGGATGAGGGACAATATTACTATGGTTATATATATCCCATTCACTTCATCTTTCACCAGTTTTTGCCATAGATTTTTGTGTTGATCCTTGTCACTGGA

以上是单酶切位点。

AGCACATTGATGATGCAGAGGAGGCCATATGTAGGGGCATGGGCAAAAGTGCTTTGACAGTGGCAGAGCAGTTAAGAAAGTTAAAGTCTAGGAGAACTTGTGAACGAATGCTGAGGCCCAATCAGGCTGAAGATCGATGTGTGTTCTGACTTTGGCTAGTTTCTGCTTCCCCTTTGCAGACAGCTTATTAATCAGATCCCCCACAGTCTCATAGTTGCATAGGGTCGCTAGTGGTGGGCTTGATCTGCCTCTGCCTCTGGAAACAA**C**GGAGGAGTAGATCCGATGTGTTTTATGGAACATGATCGATTGATCATATATCAAAGGCGGCTTGGATATGGGTGTGCAAGGCAATCAGAGATCC**C**GTACTATGGTTCACGTATCAGTGGCACAATTGCAAACTATACAAAATGA**T**AATCAGATAAGCCACATCCACAAACG**CAATGCTTCGAATTTTGCAG**AGGATGGCGATTTTTCAAGTGCGGTCGTTTAGA**A**GGGCCGCTGATAGAAAT**C**TATTTTTCACCGGGG**G**TACTTTGAAAGGGCCGTCTGTTTAAAAATCAATTTCCCTTAACCTATTTTGCTGATGTATTTTCACATTCTTAACCAGACTATCCCGACGTATTTACATATATAGACGTGTATGTTTTAATCCGCCTCTAAAAATACAAAGGGTGTCACTAAAATTGATTTTTTTAACAGTGTTGGCCACTAATACTAGTAAGTACATGTTAATTTATATAAGCACATTGATGTAGAGAGGGACCATAAACAAAAGGGGCTTTGGCAACGGAGGGTTGCGGCGTGGACTGTAGCGTAGCAACAAAGTTACTAAGTCTAGGCCAAACTTGTGAAAGGGCGAGGCCCAACTAGGCTAAAGATTGGCCTTTTTGCTTACGCGTTTGTTGTGTTCCTCTTTGTATTTAGCT

用EcoRI单酶切且保留该位点：

Vector seqEcoRI位点

5’cacaggaaacagctatgaccatgattac**g aattc**gagctcggtacccggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgtcgt

An1 基因中EcoRI位点

5’  **G AATTC**CGTTATACGGGGCAGTACTGAAAATTAGTGGTC

Revse strand 5’GACCACTAATTTTCAGTACTGCCCCGTATAACG**G AATTC**

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An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR7 4634 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4634, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 2.00

***Site1:***  GCAAACTATACAAAATGA**T**AATCAGATAAGCCACATC**(C)**

GATGTGGCTTATCTGATT**A**TCATTTTGTATAGTTTGC**(G)**

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An1WP2-MF1 394 35 67.03 31.43 7.00 0.00 GCAAACTATACAAAATGA**C**AATCAGATAAGCCACATC

An1WP2- MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4242, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 0.00

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An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR1 394 35 50.00 6.00 6.00 GATGTGGCTTATCTGATT**G**TCATTTTGTATAGTTTGC

***Site2*** :缺失引入：TCAGATAAGCCACATCCACAAACG**CAATGCTTCGAATTTTGCAG**aggatggcgatttttcaagtgcg

缺失引入：TCAGATAAGCCACATCCACAAACGaggatggcgatttttcaagtgcg

cgcacttgaaaaatcgccatcctCGTTTGTGGATGTGGCTTATCTGA

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An1WP2-MF2 459 23 68.32 47.83 4.00 2.00 TCAGATAAGCCACATCCACAAACGaggatggcgatttttcaagtgcg

An1WP2-MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4177, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

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An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR2 438 24 65.36 45.83 3.00 3.00 cgcacttgaaaaatcgccatcctCGTTTGTGGATGTGGCTTATCTGA

PRODUCT SIZE: 438, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

**Site3:** CAAGTGCGGTCGTTTAGA**A**GGGCCGCTGATAGAAATC **(T)**

GATTTCTATCAGCGGCCC**T**TCTAAACGACCGCACTTG **(A)**

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An1WP2-MF3 CAAGTGCGGTCGTTTAGA**T**GGGCCGCTGATAGAAATC

An1WP2-MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4177, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

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An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR3 GATTTCTATCAGCGGCCC**A**TCTAAACGACCGCACTTG

PRODUCT SIZE: 438, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

***Site4:*** AGGGCCGCTGATAGAAAT**C**TATTTTTCACCGGGGGTA **(A)**

TACCCCCGGTGAAAAATA**G**ATTTCTATCAGCGGCCC **(T)**

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An1WP2-MF4 AGGGCCGCTGATAGAAAT**A**TATTTTTCACCGGGGGTA

An1WP2-MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4177, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html" \l "PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR4 TACCCCCGGTGAAAAATA**T**ATTTCTATCAGCGGCCC

PRODUCT SIZE: 438, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

***Site5:*** AAATCTATTTTTCACCGGGG**G**TACTTTGAAAGGGCCGTCT **(A)**

AGACGGCCCTTTCAAAGTA**C**CCCCGGTGAAAAATAGATTT **(T)**

OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

An1WP2-MF5 AAATCTATTTTTCACCGGGG**A**TACTTTGAAAGGGCCGTCT

An1WP2-MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4177, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html" \l "PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR5 AGACGGCCCTTTCAAAGTA**T**CCCCGGTGAAAAATAGATTT

PRODUCT SIZE: 438, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

***Site6:*** TTTACATATATAGACGTG**T**ATGTTTTAATCCGCCTCT **(C)**

AGAGGCGGATTAAAACAT**A**CACGTCTATATATGTAAA **(G)**

OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

An1WP2-MF6 TTTACATATATAGACGTG**C**ATGTTTTAATCCGCCTCT

An1WP2-MR7 4635 24 63.80 50.00 6.00 6.00 GTACTGCCCCGTATAACGGAATTC

PRODUCT SIZE: 4177, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html" \l "PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

An1WP2-MF7 1 22 63.06 45.45 6.00 0.00 AGCACATTGATGATGCAGAGGA

An1WP2-MR6 AGAGGCGGATTAAAACAT**G**CACGTCTATATATGTAAA

PRODUCT SIZE: 438, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

An1WP2-MF7: **tatgaccatgattacgaattc**AGCACATTGATGATGCAGAGGA

An1WP2-MR7: GTACTGCCCCGTATAACGGAATTC

**载体制备：EcoRI 酶切pW6-2或者pW3-2，回收大片段。**

EcoRI

EcoRI

pW6-2/pW3-2

**An1**

**插入片段制备：重叠PCR，例如：第1个突变如下图。**

EcoRI

MF7

MR1

MF1

MR7

MF7

MR7

(1)

(2)

(3)

**第2-6个，只是分别用MF2-3/MR2-3代替。**

**步骤：**

1. **做（1）和（2）PCR，柱纯化PCR产物，将（1）和（2）的产物1:1混合，稀释20-50倍做模板。**
2. **再做步骤（3），用引物MF7/MR7。纯化（3），**
3. **将纯化的（3）和酶切回收的载体进行重组。**

|  |
| --- |
| >W1943 An1 PROMOTER / NIP blast  Query: 1 TCAATGCAACCTTCCATTACCTTaaaaaaa-TCATTTGCCATATGTACTTCCATCTAGAA 59  |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||  Sbjct: 16743506 TCAATGCAACCTTCCATTACCTTAAAAAAAATCATTTGCCATATGTACTTCCATCTAGAA 16743447  Query: 60 CACTCCTGCTTCTTTTTACCTTCAGCCTGGTCATAGTGATTTTCTATGTAACGTGATCAG 119  ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||  Sbjct: 16743446 CACTCCTGCTTCTTTTTACCTTCAGCCTGGTCAGAGTGATTTTCTATGTAACGTGATCAG 16743387  Query: 120 AGGACAAACTAGCTTTGTCCCCCCGCCAATATTTAACATTTTAGTTCAAATAAAACAACC 179  ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743386 AGGCCAAACTAGCTTTGTCCCCCCGCCAATATTTAACATTTTAGTTCAAATAAAACAACC 16743327  Query: 180 CACCCACCAAATCCAAATTATTAAGAATGCCTCTTCCCCCTCTAAAAACAGCATTCATCC 239  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743326 CACCCACCAAATCCAAATTATTAAGAATGCCTCTTCCCCCTCTAAAAACAGCATTCATCC 16743267  Query: 240 TCGTGCAACATATGCAAATTAATTCGTGATTTCTATATAATTCTTTCGTTATACCCATAA 299  ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743266 TCGGGCAACATATGCAAATTAATTCGTGATTTCTATATAATTCTTTCGTTATACCCATAA 16743207  Query: 300 CTTTGATTTGATCATGCAAGAACGTACTGTGTGTCAATAAAACAAATGGCACGGGGGTAG 359  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743206 CTTTGATTTGATCATGCAAGAACGTACTGTGTGTCAATAAAACAAATGGCACGGGGGTAG 16743147  Query: 360 CTTAGAGCATTATTGGAGTACTCTTATATAGGAGTTAGTAGTGAGTGATATATCTGTTCT 419  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743146 CTTAGAGCATTATTGGAGTACTCTTATATAGGAGTTAGTAGTGAGTGATATATCTGTTCT 16743087  Query: 420 AATTAAGCTCC---ATCTTATTGGGTTAAGGGTTTTTATTTTTGGTAAACTGGTCACTAA 476  ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743086 AATTAAGCTCCTTCATCTTATTGGGTTAAGGGTTTTTATTTTTGGTAAACTGGTCACTAA 16743027  Query: 477 AATACACTGATACAGACTAGTCCTAATAATTCATAAGCACATTGATGATGCAGAGGAGGC 536  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16743026 AATACACTGATACAGACTAGTCCTAATAATTCATAAGCACATTGATGATGCAGAGGAGGC 16742967  Query: 537 CATATGTAGGGGCATGGGCAAAAGTGCTTTGACAGTGGCAGAGCAGTTAAGAAAGTTAAA 596  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16742966 CATATGTAGGGGCATGGGCAAAAGTGCTTTGACAGTGGCAGAGCAGTTAAGAAAGTTAAA 16742907  Query: 597 GTCTAGGAGAACTTGTGAACGAATGCTGAGGCCCAATCAGGCTGAAGATCGATGTGTGTT 656  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16742906 GTCTAGGAGAACTTGTGAACGAATGCTGAGGCCCAATCAGGCTGAAGATCGATGTGTGTT 16742847  Query: 657 CTGACTTTGGCTAGTTTCTGCTTCCCCTTTGCAGACAGCTTATTAATCAGATCCCCCACA 716  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16742846 CTGACTTTGGCTAGTTTCTGCTTCCCCTTTGCAGACAGCTTATTAATCAGATCCCCCACA 16742787  Query: 717 GTCTCATAGTTGCATAGGGTCGCTAGTGGTGGGCTTGATCTGCCTCTGCCTCTGGAAACA 776  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16742786 GTCTCATAGTTGCATAGGGTCGCTAGTGGTGGGCTTGATCTGCCTCTGCCTCTGGAAACA 16742727  Query: 777 ACGGAGGAGTAGATCCGATGTGTTTTATGGAACATGATCGATTGATCATATATCAAAGGC 836  | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  Sbjct: 16742726 ATGGAGGAGTAGATCCGATGTGTTTTATGGAACATGATCGATTGATCATATATCAAAGGC 16742667  Query: 837 GGCTTGGATATGGGTGTGCAAGGCAATCAGAGATCCCGTACTATGGTTCACGTATCAGTG 896  |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||  Sbjct: 16742666 GGCTTGGATATGGGTGTGCAAGGCAATCAGAGATCCTGTACTATGGTTCACGTATCAGTG 16742607  Query: 897 GCACAATTGCAAACTATACAAAATGATAATCAGATAAGCCACATCCACAAACGCAATGCT 956  |||||||||||||||||||||||||| |||||||||||||||||||||||||| |  Sbjct: 16742606 GCACAATTGCAAACTATACAAAATGACAATCAGATAAGCCACATCCACAAACGTA----- 16742552  Query: 957 TCGAATTTTGCAGAGGATGGCGATTTTTCAAGTGCGGTCGTTTAGAAGGGCCGCTGATAG 1016  ||||||||||||||||||||||||||||||||| |||||||||||||  Sbjct: 16742551 -------------AGGATGGCGATTTTTCAAGTGCGGTCGTTTAGATGGGCCGCTGATAG 16742505  Query: 1017 AAATCTATTTTTCACCGGGGGTACTTT 1043  |||| ||||||||||||||| ||||||  Sbjct: 16742504 AAATATATTTTTCACCGGGGATACTTT 16742478 |