

Friday 9/18/09

Primer Design for Sequencing of Pu GFP leu

Primers created using DNA star primer select

Characteristics

Length between 400 and 1500 bp

Tm 60-65C

The sequence used for primer design was the part sequence plus the plasmid backbone
500bp upstream and downstream

Pu GFP leu landing pad sequence

Pu-Red

Pu primer overlap-dark red

GFP-Green

GFP backbone-dark green

Landing pad-black

EcoRI cut sight-Purple

PCR primer-Blue

XbaI cut sight-orange

SpeI cut sight-yellow

Scar teal

PstI cut sight-pink

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GTTCGAGTGGCAGAAAGTAACGGTATGCATTAAGGCCTACTCGTAAGTAGTCCGCCCGTTCTTACACTTATT
TCCGGCCTATTTTGAACACGAATAAAAAAGAAATGCCAGAAATTTTTCCGGCATTATAGGTGCGACTTGCCAG
ACCAATATCCATGTAACCTCGTTGACTGACTTTACGGAGTTTTACAAGAAATGCTACGGTAACCCCTATATAG
TTGCCACCATATAGGTCACTAAAAAAGAGGTAAAATCGAAGGAATCGAGGACTTTTAGAGCTGTTGAGTT
TTTTATGCGGGCCATCACTAGAATAAAGTAATACCACTTTCAACCTTGGAGAATGCACGGCTAGTTGCAGA
GTAAAAGCGGTTTTCAACCGGTTCCCGAAGGGCCATAGTTGTCCCTGTGGTCCTAAATAAATAAGACGCTT
CACTAGAAGGCAGTGTCCATCCGCGCGGCTTCAAGGATATGAAAGACCTTTATCCTTGAAGCCTTATCCT
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TTATGAGGTTAACCCTACCGGGACAGGAAAAATGGTCTGTTGGTAATGGACAGGTGTGTTAGACGGGAAAG
CTTTCTAGGGTTGCTTTTCTCTCTGGTGTACCAGGAAGAAGTCAAACATTGTGACGACCCCTAATGTGTAC
CGTACCTACTTGATATGTTTATTATTATGATCTCGGTCCGTAGTTTATTTTGGCTTTCCGAGTCAGCTTTCT
GACCCGAAAAGCAAAATAGACAACAAACAGCCACTTGCAGAGAGATGATCTCAGTGTGACCGAGTGAAGCC
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CACCCGGAAAGACGCAAATATATGATCATCGCCGGCGACGTCGCCAGTGGTCGCCAGTGCACGAGCTCGGA
 ACGGGCCAGTTTACTGGCCCGAAAGGCGATAGCAGGTGCAGTAGTTTGCAGAAATTGGAAACGCCAATTTT
 TATTACGCTGTTGTCTTTATTGGCGTTAATGGGTCACTGTCTGTTTACGGGGGTTGAAACACGTTAACGA
 GGGACTTATTGCGGTTCGGTCTTAATGGGGCCATCTTTATGACAATCATTTATTCCACCATCGACGGGGGCG
 AGAAGGTCCGTAATTTAGGACTTTGGTATCGTAGGGTTATGGGCGTTGTTACGGCTATTTATGTGCAATT
 TTTCAACGTGCGAGTAGTTGCGGTATGCCGATAATTTCTTTTAGTTAGGCCACATTTTGTTCATGAGGACGG
 CGGTGACGATACTGGTAGTAAGCGGCGAATGGTATTGCAAATGTATCATCGGTTCGGTCTTAGTAACGACC
 ATAAAGGTCAGGTCGACGGCAGGGGTAGTCCTTTGGGCGGTCAA

Primer Set 1 (first part of Pu GFP leu sequence)

| | | |
|-------------------------------|--------------|---------------------------------|
| | | |
| Upper Primer: | 24-mer | 5' GGTCCCGAAGGGCCATAGTTGTCC 3' |
| Lower Primer: | 25-mer | 5' CTTTCGTGATCCGGTCGCAGTGTCT 3' |
| DNA 250 pM | Salt 50 mM | |
| | Upper Primer | Lower Primer |
| Primer Tm | 64.3 °C | 64.2 °C |
| Primer Overall Stability | -50.1 kc/m | -49.1 kc/m |
| Primer Location | 376..399 | 910..886 |
| Product Tm - Primer Tm | 13.2 °C | |
| Primers Tm Difference | 0.1 °C | |
| Optimal Annealing Temperature | 58.5 °C | |
| Product Length | 535 bp | |
| Product Tm (%GC Method) | 77.3 °C | |
| Product GC Content | 45.6% | |
| Product Tm at 6xSSC | 98.9 °C | |

Product Melting Temperature (%GC Method)

| Salt | | | Formamide | | | |
|------|-------|-------|-----------|--------------------------|------|------|
| nM | xSSC | xSSPE | 0% | 10% | 20% | 50% |
| 1 | 0.005 | 0.006 | 49.1 | 42.6 | 36.1 | 16.6 |
| 10 | 0.051 | 0.062 | 65.7 | 59.2 | 52.7 | 33.2 |
| 50 | 0.256 | 0.312 | 77.3 | 70.8 | 64.3 | 44.8 |
| 165 | 0.846 | 1.031 | 85.9 | 79.4 | 72.9 | 53.4 |
| 330 | 1.692 | 2.062 | 90.9 | 84.4 | 77.9 | 58.4 |
| 500 | 2.564 | 3.125 | 93.9 | 87.4 | 80.9 | 61.4 |
| 1000 | 5.128 | 6.250 | 98.9 | 92.4 | 85.9 | 66.4 |
| 195 | 1.000 | 1.219 | + 0.0 | % formamide = Tm 87.2 °C | | |

Upstream Primer set 1 Pu GFP leu
 5' to 3' 376 to 399 bp
 GGTCCCGAAGGGCCATAGTTGTCC

Downstream Primer set 1 Pu GFP leu
 5' to 3' 910 to 866 bp
 CTTTCGTGATCCGGTTCGCAGTGTCT

Primer Set 2 (second part of Pu GFP leu sequence)

| | | |
|-------------------------------|--------------|---------------------------------|
| | | |
| Upper Primer: | 25-mer | 5' CCGCCAACGATCGATATGCTCTGAA 3' |
| Lower Primer: | 24-mer | 5' CCCCCGTCGATGGTGAATAAATG 3' |
| DNA 250 pM | Salt 50 mM | |
| | Upper Primer | Lower Primer |
| Primer Tm | 64.7 °C | 63.7 °C |
| Primer Overall Stability | -50.5 kc/m | -49.9 kc/m |
| Primer Location | 747..771 | 2128..2105 |
| Product Tm - Primer Tm | 13.8 °C | |
| Primers Tm Difference | 1.0 °C | |
| Optimal Annealing Temperature | 58.4 °C | |
| Product Length | 1382 bp | |
| Product Tm (%GC Method) | 77.5 °C | |
| Product GC Content | 44.0% | |
| Product Tm at 6xSSC | 99.0 °C | |

Product Melting Temperature (%GC Method)

| Salt | | | Formamide | | | |
|------|-------|-------|-----------|-----------------------------|------|------|
| mM | xSSC | xSSPE | 0% | 10% | 20% | 50% |
| 1 | 0.005 | 0.006 | 49.2 | 42.7 | 36.2 | 16.7 |
| 10 | 0.051 | 0.062 | 65.8 | 59.3 | 52.8 | 33.3 |
| 50 | 0.256 | 0.312 | 77.5 | 71.0 | 64.5 | 45.0 |
| 165 | 0.846 | 1.031 | 86.1 | 79.6 | 73.1 | 53.6 |
| 330 | 1.692 | 2.062 | 91.1 | 84.6 | 78.1 | 58.6 |
| 500 | 2.564 | 3.125 | 94.1 | 87.6 | 81.1 | 61.6 |
| 1000 | 5.128 | 6.250 | 99.0 | 92.5 | 86.0 | 66.5 |
| 195 | 1.000 | 1.219 | + 0.0 | % or formamide = Tm 87.3 °C | | |

Upstream Primer set 2 Pu GFP leu
 5' to 3' 747 to 770 bp
 CCGCCAACGATCGATATGCTCTGA

Downstream Primer set 2 Pu GFP leu
 5' to 3' 2128 to 2105 bp
 CCCCCGTCGATGGTGAATAAATG