

**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**

**DO NOT USE! ORIENTED IN THE WRONG  
DIRECTION!!!!!!** (See notes on 9/22/09)

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GTCGAGTGGCAGAAAGTAACGGTATGCATTAAGGCCTACTCGTAAAGTAGTCCGCCCGTTCTTACACTTATT
TCCGGCCTATTTTTGAACACGAATAAAAAGAAATGCCAGAAATTTTTCCGGCATTATAGGTTCGACTTGCCAG
ACCAATATCCATGTAACCTCGTTGACTGACTTTACGGAGTTTTACAAGAAATGCTACGGTAACCCCTATATAG
TTGCCACCATATAGGTCACTAAAAAAGAGGTAAAATCGAAGGAATCGAGGACTTTTAGAGCTGTTGAGTT
TTTTATGCGGGCCATCACTAGAATAAAGTAATACCACTTTCAACCTTGGAGAATGCACGGCTAGTTGCAGA
GTAAAAGCGGTTTTCAACCGGGTCCCGAAGGGCCATAGTTGTCCCTGTGGTCTAAATAAATAAGACGCTT
CACTAGAAGGCAGTGTCCATCCGCGCGGCTTCAAGGATATGAAAGACCTCTTATCCTTGAAGCCTTATCCT
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CGCGCTACTTGGAAAAAATAGCGACGGAAGTCTTTAGCTGTCCACCAATACGCGCTAACTACTAAACGAG
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TTTTGCCCGGTTCGGCATCGGCAATCGGACCTCAGACACTCGCACCAGGATCACGAAAGACTCCCGCTCGGG
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TCTCCTCTTTATGATCTACGCATTTCTCTTCTTGAAGAGTGACCTCAACAGGGTTAAGAACAACCTTAATC
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TTCCAATACATGTCTTTCTTGATATAAAAAGTTTTCTACTGCCCTTGATGTTCTGTGCACGACTTCAGTTC
AAACTTCCACTATGGGAACAATTATCTTAGCTCAATTTTCCATAACTAAAATTTCTTCTACCTTTGTAAGA
ACCTGTGTTTAAACCTTATGTTGATATTGAGTGTGTTACATATGTAGTACCGTCTGTTTGTCTTACCTT
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AGTTTCAATTGAAGTTTTAATCTGTGTTGTAACCTTCTACCTTCGCAAGTTGATCGTCTGGTAATAGTTGTT  
 TTATGAGGTTAACCGCTACCGGGACAGGAAAATGGTCTGTTGGTAATGGACAGGTGTGTTAGACGGGAAAG  
 CTTTCTAGGGTTGCTTTTCTCTCTGGTGTACCAGGAAGAAGCTCAAACATTGTCGACGACCCTAATGTGTAC  
 CGTACCTACTTGATATGTTTATTATTATGATCTCGGTCCGTAGTTTATTTTGGCTTTCCGAGTCAGCTTTCT  
 GACCCGGAAAGCAAATAGACAACAACAGCCACTTGCAGAGATGATCTCAGTGTGACCGAGTGGAAGCC  
 CACCCGGAAAGACGCAAATATATGATCATCGCCGGCGACGTCGCCAGTGGTCGCCAGTGCACGAGCTCGGA  
 ACGGGCCAGTTTACTGGCCCGAAAGGCGATAGCAGGTGCAGTAGTTTGCAGAAATTGGAAACGCCAATTTT  
 TATTACGCTGTTGTCTTTATTGGCGTTAATGGGTGCATCTGTCTGTTTACGGGGGTTGAAACACGTTAACGA  
 GGGACTTATTGCGGTCCGTCTTAATGGGGCCATCTTTATGACAATCATTATTCCACCATCGACGGGGGCG  
 AGAAGGTCCGTAATTTAGGACTTTGGTATCGTAGGGTTATGGGCGTTGTTACGGCTATTTATGTGCGCAATT  
 TTTCAACGTGCGCAGTAGTTGCGGTATGCCGATAATTTCTTTTAGTTAGGCCACATTTTGTGATGAGGACGG  
 CGGTGACGATACTGGTAGTAAGCGGCGAATGGTATTGCAAAGTATCATCGGTCCGTCTTAGTAACGACC  
 ATAAAGGTCAGGTCGACGGCAGGGGTAGTCCTTTGGGCGGTCAA

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

Upper Primer:	24-mer	5' GGTCCCGAAGGGCCATAGTTGTCC 3'
Lower Primer:	25-mer	5' CTTTCGTGATCCGGTCGAGTGTCT 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	
Primer s 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			
mM	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 % or formamide = Tm 87.2 °C			

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

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' CCCCCGTGATGGTGAATAAATG 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

CCCCGTCGATGGTGAATAAATG