

Supplemental Table 1. PCR primers for generation of lacritin hydrophobic or charge mutants.

Mutant	Primer sequence (5'-3')	Tem-plate
L65S <i>f</i> <i>r</i>	CAGGAACTAAACCCAGCAAATCCATAGTGGAGAAAAGT ACTTTTCTCCACTATGGATTTGCTGGGGTTTAGTTCTG	pLAC
K66S <i>f</i> <i>r</i>	CAGGAACTAAACCCCTGAGCTCCATAGTGGAGAAAAGTATC GATACTTTTCTCCACTATGGAGCTCAGGGGGTTTAGTTCTG	pLAC
K66S/E70S <i>f</i> <i>r</i>	CCCCTGAGCTCCATAGTGTGCAAAAAGTATCTTACTAACAGAACAAG CTTGTTCTGTTAGTAAGATACTTTTCGACACTATGGAGCTCAGGGG	K66S
K66S/E70S/ K95S <i>f</i> <i>r</i> /E99S <i>f</i> <i>r</i>	CACGGAGGCGTGCCAGGTGGAAGCCAATTCATCGAAAATGGAAGTG CACTTCCATTTTCGATGAATTGGCTTCCACCTGGCACGCCTCCGTG GGTGGAAGCCAATTCATCTCAAATGGAAGTGAATTTGCAC GTGCAAATTCACTTCCATTTGAGATGAATTGGCTTCCACC	K66S/ E70S
K66S/E70S/ E103S <i>f</i> <i>r</i> /K107S <i>f</i> <i>r</i>	CAATTCATCGAAAATGGAAGTTCATTTGCACAAAATTACTGAAG CTTCAGTAATTTTTGTGCAAATGAACTTCCATTTTCGATGAATTG AATGGAAGTTCATTTGCACAAAGCTTACTGAAGAAATTCAGTCTA TAGACTGAATTTCTTCAGTAAGCTTTGTGCAAATGAACTTCCATT	K66S/ E70S/K
I68S <i>f</i> <i>r</i>	AACCCCTGAAATCCAGCGTGGAGAAAAGTATCTTA TAAGATACTTTTCTCCACGCTGGATTTCAGGGGGTT	pLAC
V69S <i>f</i> <i>r</i>	CCCCTGAAATCCATAAGCGAGAAAAGTATCTTACTAACAG CTGTTAGTAAGATACTTTTCTCGCTTATGGATTTTCAGGGG	pLAC
E70S/E103S <i>f</i> <i>r</i>	CACGGAGGCGTGCCAGGTGGAAGCCAATTCATCGAAAATGGAAGTG CACTTCCATTTTCGATGAATTGGCTTCCACCTGGCACGCCTCCGTG CAATTCATCGAAAATGGAAGTTCATTTGCACAAAATTACTGAAG CTTCAGTAATTTTTGTGCAAATGAACTTCCATTTTCGATGAATTG	pLac
I73S <i>f</i> <i>r</i>	ATAGTGGAGAAAAGTAGCTTACTAACAGAACAAGCC GGCTTGTCTGTTAGTAAGCTACTTTTCTCCACTAT	pLAC
V91S <i>f</i> <i>r</i>	GGAATGCACGGAGGCTCGCCAGGTGGAAAACAA TTGTTTTCCACCTGGCGAGCCTCCGTGCATTCC	pLAC
V91S/L109S <i>f</i> <i>r</i>	AGTGAATTTGCACAAAATTATCGAAGAAATTCAGTCTATTA TTTTAATAGACTGAATTTCTTCGATAATTTTTGTGCAAATTCCT	V91S
K95S <i>f</i> <i>r</i>	CACGGAGGCGTGCCAGGTGGAAGCCAATTCATCGAAAATGGAAGTG CACTTCCATTTTCGATGAATTGGCTTCCACCTGGCACGCCTCCGTG	pLAC
K95S/E99S <i>f</i> <i>r</i>	CACGGAGGCGTGCCAGGTGGAAGCCAATTCATCGAAAATGGAAGTG CACTTCCATTTTCGATGAATTGGCTTCCACCTGGCACGCCTCCGTG	K95S
K95S/E99S/E103 S /K107S <i>f</i> <i>r</i>	AATGGAAGTTCATTTGCACAAAGCTTACTGAAGAAATTCAGTCTA TAGACTGAATTTCTTCAGTAAGCTTTGTGCAAATGAACTTCCATT CAATTCATCGAAAATGGAAGTTCATTTGCACAAAATTACTGAAG CTTCAGTAATTTTTGTGCAAATGAACTTCCATTTTCGATGAATTG	K95S/E 99S
I98S <i>f</i> <i>r</i>	CCAGGTGAAAACAATTCTCCGAAAATGGAAGTGAATT AATTCACTTCCATTTTCGGAGAATTGTTTTCCACCTGG	pLAC
E99S <i>f</i> <i>r</i>	GGTGGAAGCCAATTCATCTCAAATGGAAGTGAATTTGCAC GTGCAAATTCACTTCCATTTGAGATGAATTGGCTTCCACC	pLAC
E103S <i>f</i> <i>r</i>	CAATTCATCGAAAATGGAAGTTCATTTGCACAAAATTACTGAAG CTTCAGTAATTTTTGTGCAAATGAACTTCCATTTTCGATGAATTG	pLAC
E103S/K107S <i>f</i> <i>r</i>	AATGGAAGTTCATTTGCACAAAGCTTACTGAAGAAATTCAGTCTA TAGACTGAATTTCTTCAGTAAGCTTTGTGCAAATGAACTTCCATT	E103S
F104S <i>f</i>	GAAAATGGAAGTGAATCTGCACAAAATTACTGAAG	pLAC

<i>r</i>	CTTCAGTAATTTTTGTGCAGATTCACTTCCATTTTC	
A105S <i>f</i> <i>r</i>	ATCGAAAATGGAAGTGAATTTTCACAAAATTACTGAAGAAATTC GAATTTCTTCAGTAATTTTTGTGAAAATTCACTTCCATTTTCGAT	pLAC
L108S <i>f</i> <i>r</i>	GAATTTGCACAAAATCACTGAAGAAATTCAGTCTA TAGACTGAATTTCTTCAGTGATTTTTGTGCAAATTC	pLAC
L108S/L109S <i>f</i> <i>r</i> /F112S <i>f</i> <i>r</i>	AGTGAATTTGCACAAAATTATCGAAGAAATTCAGTCTATTA TTTTAATAGACTGAATTTCTTCGATAATTTTTGTGCAAATTC AAATCATGCAAGAAATCCAGTCTATTA CCATGGTTTTAATAGACTGGATTTCTTGCATGATT	L108S
L109S <i>f</i> <i>r</i>	AGTGAATTTGCACAAAATTATCGAAGAAATTCAGTCTATTA TTTTAATAGACTGAATTTCTTCGATAATTTTTGTGCAAATTC	pLAC
F112S <i>f</i> <i>r</i>	AAATCATGCAAGAAATCCAGTCTATTA CCATGGTTTTAATAGACTGGATTTCTTGCATGATT	pLAC

Supplemental Table 2. PCR primers for pLAC codon mutagenesis

Primer	Primer sequence (5'-3')	Template
747576f 747576r	C ATA GTG GAG AAA AGT ATC CTG CTG ACC GAA CAA GCC CTT GCA AAA GCA G C TGC TTT TGC AAG GGC TTG TTC GGT CAG CAG GAT ACT TTT CTC CAC TAT G	pLAC
6264f 6264r	CG ACC TCA AGC AGG CAG GAA CTG AAC CCG CTG AAA TCC ATA GTG GAG AA TT CTC CAC TAT GGA TTT CAG CGG GTT CAG TTC CTG CCT GCT TGA GGT CG	lacrt-RC01
3456f 3456r	GT CCA GCA GAA CCA GCT TCA CCG CCG GAA ACA ACC ACA ACA GCC CAG GAG CAC CTG GGC TGT TGT GGT GTG TTC CCG CCG TGA AGC TGG TTC TGC TGG AC	lacrt-RC02
5154f 5154r	G GCG GCA GCA GTT CAG GGC ACA GCC AAA GTA ACC TCA AGC AGG CAG GAA G TTC CTG CCT GCT TGA GGT GAC TTT GGC TGT GCC CTG AAC TGC TGC CGC C	lacrt-RC03
114115f 114115r	A TTA CTG AAG AAA TTC AGT CTG CTG AAA CCA TGG GCA TGC TTT GCC AAG G C CTT GGC AAA GCA TGC CCA TGG TTT CAG CAG ACT GAA TTT CTT CAG TAA	lacrt-RC04
37940f 37940r	AA CCA GCT TCA CCG CCG GAA ACC ACC ACC ACC GCC CAG GAG ACT TCG GCG CGC CGA AGT CTC CTG GGC GGT GGT GGT GGT TTC CGG CGG TGA AGC TGG TT	lacrt-RC05
8486f 8486r	CAA GCC CTT GAC AAA GAC GGC AAA GGC ATG CAC GGA GGC GTG CCA TGG CAC GCC TCC GTG CAT GCC TTT GCC GTC TTT GTC AAG GGC TTG	lacrt-RC06
108110f 108110r	GGA AGT GAA TTT GCA CAA AAA CTG CTG AAA AAA TTC AGT CTG CTG AAA TTT CAG CAG ACT GAA TTT TTT CAG CAG TTT TTG TGC AAA TTC ACT TCC	lacrt-RC06
59f 59r	GCC AAA GTC ACC TCA AGC CGT CAG GAA CTG AAC CCG CTG AAA TTT CAG CGG GTT CAG TTC CTG ACG GCT TGA GGT GAC TTT GGC	lacrt-RC06
6870f 6870r	CTG AAC CCG CTG AAA ATT GTG GAA AAA AGT ATC CTG CTG ACC GGT CAG CAG GAT ACT TTT TTC CAC AAT TTT CAG CGG GTT CAG	lacrt-RC07
4344f 4344r	ACC ACA ACA GCC CAG GAA ACC TCG GCG GCA GCA GTT CAG GGG CCC CTG AAC TGC TGC CGC CGA GGT TTC CTG GGC TGT TGT GGT	lacrt-RC09
9294f 9294r	GGC ATG CAC GGA GGC GTG CCG GGT GGC AAA CAA TTC ATC GAA AAT GGA AG CT TCC ATT TTC GAT GAA TTG TTT GCC ACC CGG CAC GCC TCC GTG CAT GCC	lacrt-RC10
1720f 1720r	GAT CCT GCC CAG GAA GCT GGC ACC TCT AAA CCT AAT GAA GAG AC TCA GG CC TGA GAT CTC TTC ATT AGG TTT AGA GGT GCC AGC TTG CTG GGC AGG ATC	lacrt-RC11
101f 101r	CAA TTC ATC GAA AAT GGC AGT GAA TTT GCA CAA AAA CTG CTG CAG CAG TTT TTG TGC AAA TTC ACT GCC ATT TTC GAT GAA TTG	lacrt-RC12

Supplemental Table 3. Progressive pLAC coding sequence mutagenesis

Construct	Codons changed	Unwanted mutations (codon #)	Total rare to common changes
<i>pLAC-RC01</i>	74, 75, 76	0	3
<i>pLAC-RC02</i>	62, 64	0	5
<i>pLAC-RC03</i>	34, 35, 36	0	8
<i>pLAC-RC04</i>	51, 54	0	10
<i>pLAC-RC05</i>	114, 115	0	12
<i>pLAC-RC06</i>	37, 39, 40	0	15
<i>pLAC-RC07</i>	59, 84, 86, 108, 110	2 (81, 83)	20
<i>pLAC-RC08</i>	68, 70	1 (83)	22
<i>pLAC-RC09</i>	None	0	22
<i>pLAC-RC10</i>	43, 44	0	24
<i>pLAC-RC11</i>	92, 94	0	26
<i>pLAC-RC12</i>	17, 20	0	28
<i>pLAC-RC13</i>	101	0	29