6/26/2008

Part Design

Prefixes: gaattcgcggccgcttctag
gaattcgcggccgcttctagag
Suffixes: tactagtagcggccgctgcag

1. (Prefix) TetR promoter → RBS → λcI gene → Terminator (Suffix)

***Individual Parts:**

Promoter:
gaattcgcggccgcttctagagtccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac
tactagtagcggccgctgcag

RBS:
gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:
gaattcgcggccgcttctagatgagcacaaaaaagaaaccattaacacaagagcagcttgaggacgcacgtcgcctt
aaagcaatttatgaaaaaaagaaaaatgaacttggcttatcccaggaatctgtcgcagacaagatggggatggggca
gtcaggcgttggtgctttatttaatggcatcaatgcattaaatgcttataacgccgcattgcttgcaaaaattctca
aagttagcgttgaagaatttagcccttcaatcgccagagaaatctacgagatgtatgaagcggttagtatgcagccg
tcacttagaagtgagtatgagtaccctgttttttctcatgttcaggcagggatgttctcacctgagcttagaacctt
taccaaaggtgatgcggagagatgggtaagcacaaccaaaaaagccagtgattctgcattctggcttgaggttgaag
gtaattccatgaccgcaccaacaggctccaagccaagctttcctgacggaatgttaattctcgttgaccctgagcag
gctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcagggatag
cggtcaggtgtttttacaaccactaaacccacagtacccaatgatcccatgcaatgagagttgttccgttgtgggga
aagttatcgctagtcagtggcctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagcttaataa
tactagtagcggccgctgcag

Terminator (Ah-nuld):
gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgc
tgcag

***Composition (921):**

gaattcgcggccgcttctagagtccctatcagtgatagagattgacatccctatcagtgatagagatactgagcact
actagagtcacacaggaaagtactagatgagcacaaaaaagaaaccattaacacaagagcagcttgaggacgcacgt
cgccttaaagcaatttatgaaaaaaagaaaaatgaacttggcttatcccaggaatctgtcgcagacaagatggggat
ggggcagtcaggcgttggtgctttatttaatggcatcaatgcattaaatgcttataacgccgcattgcttgcaaaaa
ttctcaaagttagcgttgaagaatttagcccttcaatcgccagagaaatctacgagatgtatgaagcggttagtatg
cagccgtcacttagaagtgagtatgagtaccctgttttttctcatgttcaggcagggatgttctcacctgagcttag
aacctttaccaaaggtgatgcggagagatgggtaagcacaaccaaaaaagccagtgattctgcattctggcttgagg
ttgaaggtaattccatgaccgcaccaacaggctccaagccaagctttcctgacggaatgttaattctcgttgaccct
gagcaggctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcag
ggatagcggtcaggtgtttttacaaccactaaacccacagtacccaatgatcccatgcaatgagagttgttccgttg
tggggaaagttatcgctagtcagtggcctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagct
taataatactagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgctgcag

2. (Prefix) LacI Promoter → RBS → p22 mnt gene → Terminator (Suffix)

***Individual Parts:**

Promoter:

gaattcgcggccgcttctagagcaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggca
cgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacccc
aggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacatactagtag
cggccgctgcag

RBS:

gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:

gaattcgcggccgcttctagatggcccgggatgatcctcacttcaattttcgtatgccaatggaagtaagagagaaa
ttgaaatttagagcagaggcaaacggacggagcatgaactctgagcttttgcaaatcgtacaagatgccctaagcaa
accgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcgagttagtgaagaagatgg
tcttcgatacactgaaggatctttataaaaaaaccaccgctgcaaacgacgaaaactacgctttagtagcttaataa
tactagtagcggccgctgcag

Terminator (Ah-nuld):

gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgc
tgcag

***Composition Design for Synthesis (605):**

gaattcgcggccgcttctagagcaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggca
cgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacccc
aggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacatactagagt
cacacaggaaagtactagatggcccgggatgatcctcacttcaattttcgtatgccaatggaagtaagagagaaatt
gaaatttagagcagaggcaaacggacggagcatgaactctgagcttttgcaaatcgtacaagatgccctaagcaaac
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcgagttagtgaagaagatggtc
ttcgatacactgaaggatctttataaaaaaaccaccgctgcaaacgacgaaaactacgctttagtagcttaataata
ctagag aaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgctgcag

3. (Prefix) LacI/λcI Promoter → RBS → RFP → Terminator

***Individual Parts**

P1,LacI:caatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccg
actggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacacttt
atgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacaca

P2,λcI: taacaccgtgcgtgttgactatttacctctggcggtgataatggttgc

RBS:
gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:
gaattcgcggccgcttctagatggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttc
aaggtgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgaggg
cacccagaccgccaagctgaaggtgaccaagggtggcccccctgcccttcgcctgggacatcctgtcccctcagttca
tgtacggctccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttc
aagtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccttgcaggacggcga
gttcatctacaaggtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaagaagaccatgggct
gggaggcctcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcagaggctgaagctgaag
gacggcggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaa
cgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggcc
gccactccaccggcggcatggacgagctgtacaagtaataatactagtagcggccgctgcag

Terminator (Ah-nuld):
Gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggtttttttttt
actagtagcggccgctgcag

***Promoter Composition (160):**

gaattcgcggccgcttctagaggcgcaacgcaattaatgtgagttagctcactcattaggcataacaccgtgcgtgt
tgactatttacctctggcggtgataatgtgtggaattgtgagcggataacaatttcacacatactagtagcggccg
ctgcag

*Notes: Prefix → λcI O1 → λcI -35 → λcI O2 → λcI -10 → LacI O1 → Suffix
**Questions: Does the CAP binding site need to be present?

***Overall Composition (948):**

gaattcgcggccgcttctagaggcgcaacgcaattaatgtgagttagctcactcattaggcataacaccgtgcgtgt
tgactatttacctctggcggtgataatgtgtggaattgtgagcggataacaatttcacacatactagagtcacaca
ggaaagtactagatggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttcaaggtgca
catggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcacccaga
ccgccaagctgaaggtgaccaagggtggcccccctgcccttcgcctgggacatcctgtcccctcagttcatgtacggc
tccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtggga
gcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccttgcaggacggcgagttcatct
acaaggtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaagaagaccatgggctgggaggcc
tcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcagaggctgaagctgaaggacggcgg
ccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaaca
tcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccactcc
accggcggcatggacgagctgtacaagtaataatactagagaaaaaaaaaccccgcccctgacagggcggggttttt
tttactagtagcggccgctgcag

4. (Prefix) TetR/p22 mnt Promoter → RBS → GFP → Terminator

*Individual Parts

P1,TetR tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac

P2,p22: ctcgaggtgagtgcacagtactaggtccacggtgacctagatctcctatagtgagtcgtattaattt

RBS:
gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:
Gaattcgcggccgcttctagatgcgtaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagat
ggtgatgttaatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttacccttaa
atttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttcaatgct
ttgcgagatacccagatcatatgaaacagcatgacttttcaagagtgccatgcccgaaggttatgtacaggaaaga
actatatttttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgttaatag
aatcgagttaaaaggtattgattttaaagaagatggaaacattcttggacacaaattggaatacaactataactcac
acaatgtatacatcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaagat
ggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaacca
ttacctgtccacacaatctgccctttcgaaagatcccaacgaaaagagagaccacatggtccttcttgagtttgtaa
cagctgctgggattacacatggcatggatgaactatacaaataataatactagtagcggccgctgcag

Terminator (Ah-nuld):
gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgc
tgcag

*Promoter Composition (97 or 104):

gaattcgcggccgcttctagagtccctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagtagcggccgctgcag

or

gaattcgcggccgcttctagagtccctatcagtgatagagattgacaaggtccacggtgacctagatctccgatact
gagcactactagtagcggccgctgcag

*Notes: Prefix → TetR O1 → TetR -35 → p22 mnt O1 → TetR -10 → Suffix

*Overall Composition (891):

gaattcgcggccgcttctagagtccctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagagtcacacaggaaagtactagatgcgtaaaggagaagaacttttcactggagttgtcccaattcttgttgaa
ttagatggtgatgttaatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttac
ccttaaatttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttc
aatgctttgcgagatacccagatcatatgaaacagcatgacttttcaagagtgccatgcccgaaggttatgtacag
gaaagaactatatttttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgt
taatagaatcgagttaaaaggtattgattttaaagaagatggaaacattcttggacacaaattggaatacaactata
actcacacaatgtatacatcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacatt
gaagatggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccaga
caaccattacctgtccacacaatctgccctttcgaaagatcccaacgaaaagagagaccacatggtccttcttgagt
ttgtaacagctgctgggattacacatggcatggatgaactatacaaataataatactagagaaaaaaaaaccccgcc
cctgacagggcggggttttttttactagtagcggccgctgcag

5. (Prefix) TetR/p22 cII promoter → RBS → GFP → Terminator

***Individual Parts**

P1,TetR tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac

P2,p22 cII: aataaacttgactaaagattcctttagtagataatttaagtgttctttaatttc

RBS:
gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:
Gaattcgcggccgcttctagatgcgtaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagat
ggtgatgttaatgggcacaaatttttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttacccttaa
atttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttcaatgct
ttgcgagatacccagatcatatgaaacagcatgacttttcaagagtgccatgcccgaaggttatgtacaggaaaga
actatatttttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgataccttgttaatag
aatcgagttaaaaggtattgattttaaagaagatggaaacattcttggacacaaattggaatacaactataactcac
acaatgtatacatcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaagat
ggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaacca
ttacctgtccacacaatctgccctttcgaaagatcccaacgaaaagagagaccacatggtccttcttgagtttgtaa
cagctgctgggattacacatggcatggatgaactatacaaataataatactagtagcggccgctgcag

Terminator (Ah-nuld):
gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgc
tgcag

***Promoter Composition (109):**

gaattcgcggccgcttctagagtccctatcagtgatagagattgactaaagattcctttagtagataatttaagtgt
tctttaatttctactagtagcggccgctgcag

*Notes: Prefix → TetR O1 → p22 cII -35 → p22 cII O1 → p22 cII -10 → p22 cII O2 → Suffix

***Overall Composition:**

gaattcgcggccgcttctagagtccctatcagtgatagagattgactaaagattcctttagtagataatttaagtgt
tctttaatttctactagagtcacacaggaaagtactagatgcgtaaaggagaagaacttttcactggagttgtccca
attcttgttgaattagatggtgatgttaatgggcacaaatttttctgtcagtggagagggtgaaggtgatgcaacata
cggaaaacttacccttaaatttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttcg
gttatggtgttcaatgctttgcgagatacccagatcatatgaaacagcatgacttttcaagagtgccatgcccgaa
ggttatgtacaggaaagaactatatttttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaagg
tgataccttgttaatagaatcgagttaaaaggtattgattttaaagaagatggaaacattcttggacacaaattgg
aatacaactataactcacacaatgtatacatcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaatt
agacacaacattgaagatggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgt
ccttttaccagacaaccattacctgtccacacaatctgccctttcgaaagatcccaacgaaaagagagaccacatgg
tccttcttgagtttgtaacagctgctgggattacacatggcatggatgaactatacaaataataatactagagaaaa
aaaaaccccgcccctgacagggcggggttttttttactagtagcggccgctgcag

Sequences to synthesize

1. (Prefix) LacI Promoter → RBS → p22 mnt gene → Terminator (Suffix)

gaattcgcggccgcttctagagcaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggca
cgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacccc
aggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacatactagagt
cacacaggaaagtactagatggcccgggatgatcctcacttcaattttcgtatgccaatggaagtaagagagaaatt
gaaatttagagcagaggcaaacggacggagcatgaactctgagcttttgcaaatcgtacaagatgccctaagcaaac
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcgagttagtgaagaagatggtc
ttcgatacactgaaggatctttataaaaaaaccaccgctgcaaacgacgaaaactacgctttagtagcttaataata
ctagag aaaaaaaaaccccgcccctgacagggcggggttttttttttactagtagcggccgctgcag

2. LacI/λcI Promoter → RBS

gaattcgcggccgcttctagaggcgcaacgcaattaatgtgagttagctcactcattaggcataacaccgtgcgtgt
tgactatttacctctggcggtgataatgtgtggaattgtgagcggataacaatttcacacatactagagtcacaca
ggaaagtactagtagcggccgctgcag

3. TetR/p22 mnt Promoter → RBS

gaattcgcggccgcttctagagtccctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagagtcacacaggaaagtactagtagcggccgctgcag

4. TetR/p22 cII Promoter → RBS

gaattcgcggccgcttctagagtccctatcagtgatagagattgactaaagattcctttagtagataatttaagtgt
tctttaatttctactagagtcacacaggaaagtactagtagcggccgctgcag

Primer Set 1:

P22 cII c
5’ – TTTCTTGGTTCGACTTCGGG – 3’
For sequencing from ~450 across to 1
5’ – CTATCATAGTAGGCATGAGCC – 3’
For sequencing from ~250 to the end (687)

Lambda cI c
5’ – CTTGGAGCCTGTTGGTTCGG – 3’
475 - 1
5’ – AAGCGGTTAGTATGCAGCCG – 3’
269 – end (750)

p22 MNT c
5’ – TCGTCGTTTGCAGCGGTGG – 3’
264-1
5’ – AGAGCAGAGGCAAACGGACGG – 3’
67-end (288)

EYFP, YFP
5’ – CGTTCTTCTGCTTGTGGCC – 3’
482-1
5’ – CTTCAAGTCCGCCATGCCCG – 3’
252-end (723)

pSB 2K3 (p22 MNT promoter)
5’ – ACCGTCATGTTCCTGTAGGC – 3’
1199……..

pSB 1A2 (RBS, Lambda cI promoter, TetR promoter, p22 cII promoter)
5’ – CCATAGTTGCCTGACTCCCCG – 3’
1013……

pSB 1AK3 (terminator)
5’ – CAAGTCAGCGTAATGCTCTGCC – 3’
1047…….

GFP
5’ – GCCATGATGTATACATTGTGTGAG -3’
461 -1
5’ – GCCCGAAGGTTATGTACAGG – 3’
265-end (720)

mCherry
5’ – TTCTGCATTACGGGGCCGTC – 3’
429 -1
5’ – TTGAAGCTGTCCTTCCCCGAGG – 3’
262-end

LacI Promoter
5’ – CCACACAACATACGAGCCGG – 3’
173-1
5’ – CGTTGGCCGATTCATTAATG – 3’
27-end

Primer Set 2:

λcI gene(lcIgene) (with RBS, prefix tails):

Reverse: CTGCAGCGGCCGCTACTAGTATTATTAAGC

Forward 1: TCACACAGGAAAGTACTAGATGAGCACAAAAAAGAAACC
Forward 2: GAATTCGCGGCCGCTTCTAGAGTCACACAGGAAAGTACTAGATGAGC

LacI/λcI Promoter (LaclcIDP):

Reverse: CTGCAGCGGCCGCTACTAGTATGTGTGAAATTGTTATCCGC
Forward: GAATTCGCGGCCGCTTCTAGAGGC

TetR/p22 mnt Promoter (Tetp22DP):

Reverse: CTGCAGCGGCCGCTACTAGTAGTGCTCAGTATCTAGGTCACCG
Forward: GAATTCGCGGCCGCTTCTAGAGTCCC

Plasmid Primers (plasmid):

Reverse: TGCCACCTGACGTCTAAGAA
Forward: GCTCACTCAAAGGCGGTAAT

Primer set 3:

Terminator:

AAAAAAAACCCCGCCCTGTCA

TetRpromoter:

GTGCTCAGTATCTCTATCACTGATAGGG