

Part Design

Prefixes: gaattcgcggccgcttctag
 gaattcgcggccgcttctagag
 Suffixes: tactagtagcggccgctgcag

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

*Individual Parts:

Promoter:

gaattcgcggccgcttctagagtcacctatcagtgatagagattgacatccctatcagtgatagagatactgagcac
 tactagtagcggccgctgcag

RBS:

gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:

gaattcgcggccgcttctagatgagcacaaaaaagaaaccattaacacaagagcagcttgaggacgcacgtcgcctt
 aaagcaatattatgaaaaaaagaaaaatgaacttggcttatcccaggaatctgtcgcagacaagatggggatggggca
 gtcagggcgttgggtgctttatttaaatggcatcaatgcattaaatgcttataacgccgcattgcttgcaaaaaattctca
 aagtttagcgttgaagaattagcccttcaatcgccagagaaaatctacgagatgtatgaagcgggttagtatgcagccg
 tcacttagaagtgagtatgagtaccctgttttttctcatgttcaggcagggatgttctcacctgagcttagaacctt
 taccaaaggtgatgaggagagatgggtaagcacacaaaaaagccagtgattctgcattctggcttgagggtgaag
 gtaattccatgaccgcaccaacaggtccaagccaagctttcctgacggaatgttaattctcgttgaccctgagcag
 gctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcagggatag
 cggtcaggtgtttttacaaccactaaaccacagtagccaatgatcccatgcaatgagagttgttccggttggtgggga
 aagttatcgctagtcagtgccctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagcttaataa
 tactagtagcggccgctgcag

Terminator (Ah-nuld):

gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttttactagtagcggccgc
 tcag

*Composition (921):

gaattcgcggccgcttctagagtcacctatcagtgatagagattgacatccctatcagtgatagagatactgagcact
 actagagtcacacaggaaagtactagatgagcacaaaaaagaaaccattaacacaagagcagcttgaggacgcacgt
 cgcttaaagcaatattatgaaaaaaagaaaaatgaacttggcttatcccaggaatctgtcgcagacaagatggggat
 ggggcagtcagggcgttgggtgctttatttaaatggcatcaatgcattaaatgcttataacgccgcattgcttgcaaaaa
 ttctcaaagtttagcgttgaagaattagcccttcaatcgccagagaaaatctacgagatgtatgaagcgggttagtatg
 cagccgtcacttagaagtgagtatgagtaccctgttttttctcatgttcaggcagggatgttctcacctgagcttag
 aacctttaccaaaggtgatgaggagagatgggtaagcacacaaaaaagccagtgattctgcattctggcttgagg
 ttgaaggttaattccatgaccgcaccaacaggtccaagccaagctttcctgacggaatgttaattctcgttgaccct
 gagcaggtctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcag
 ggatagcggtcaggtgtttttacaaccactaaaccacagtagccaatgatcccatgcaatgagagttgttccggtg
 tggggaaagttatcgctagtcagtgccctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagct
 taataataactagtagagaaaaaaaaccccgcccctgacagggcggggttttttttttactagtagcggccgctgcag

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

*Individual Parts:

Promoter:

gaattcgggcccgtttctagagcaatacgcgaaaccgcctctccccgcgcggttgccgattcattaatgcagctggca
cgacagggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacc
aggctttacactttatgcttccggctcgtatggtgtgtggaattgtgagcggataacaatttcacacatactagtag
cggccgctgcag

RBS:

gaattcgggcccgtttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:

gaattcgggcccgtttctagatggcccgggatgatcctcacttcaattttcgtagtgcgaatggaagtaagagagaaa
ttgaaattagagcagaggcaaaccggacggagcatgaactctgagcttttgcaaactcgtacaagatgccctaagcaa
accgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcaggttagtgaagaagatgg
tcttcgatacactgaaggatctttataaaaaaacaccgctgcaaacgacgaaaactacgcttttagtagcttaataa
tactagtagcggccgctgcag

Terminator (Ah-nuld):

gaattcgggcccgtttctagagaaaaaaaaaccccgcccctgacagggcgggggttttttttttactagtagcggccgc
tgcag

*Composition Design for Synthesis (605):

gaattcgggcccgtttctagagcaatacgcgaaaccgcctctccccgcgcggttgccgattcattaatgcagctggca
cgacagggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacc
aggctttacactttatgcttccggctcgtatggtgtgtggaattgtgagcggataacaatttcacacatactagtagt
cacacaggaaagtactagatggcccgggatgatcctcacttcaattttcgtagtgcgaatggaagtaagagagaaatt
gaaattagagcagaggcaaaccggacggagcatgaactctgagcttttgcaaactcgtacaagatgccctaagcaa
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcaggttagtgaagaagatggc
ttcgatacactgaaggatctttataaaaaaacaccgctgcaaacgacgaaaactacgcttttagtagcttaataa
ctagag aaaaaaaaaaccccgcccctgacagggcgggggttttttttttactagtagcggccgctgcag

3. (Prefix) LacI/ λ cI Promoter → RBS → GFP → Terminator

*Individual Parts

P1, LacI: caatacgc^{aa}accgcctctccccgcgcg^{tt}ggccgattcattaatgcagctggcagcagaggtttcccgactggaaagcggg^{cag}tga^gcgcaacgca^{atta}atgtgag^{tt}agctcactcattag^{gc}accccaggc^{tt}taca^{ct}ttatgcttccggctcg^{at}atg^{tt}gtgtgga^{att}gtgagcggataaca^{att}ttcacaca

P2, λ cI: taacaccgtgcgtgttgactat^{ttt}tac^{ct}ctggcgg^{tg}ata^{at}ggttgc

RBS:

gaattcgcggccgcttctagag^tcacacaggaaag^tactagtagcggccgctgcag

Protein:

Gaattcgcggccgcttctagatg^cgtaaaggagaagaact^{ttt}tactggagttgtcccaattctt^gttgaattagatgg^tgatg^{tt}aatgggcacaaa^{ttt}tctgtcag^tggagaggg^tgaaggtgatgcaacatacggaaaact^tacccttaa^{at}ttat^{tt}g^cactactg^gaaaactac^{ct}gttccatggccaacact^tgtcactact^{tt}cgg^{tt}atgg^tgttcaatg^cttg^cgagatacc^cagatcatatgaaacagcatgact^{ttt}tcaagagtgccatgcccgaagg^{tt}atgtacaggaaaga^aactat^{ttt}tcaaagatgacgggaactacaagacacgtgctgaag^tcaag^{ttt}gaaggtgatacc^{ct}tgt^{ta}atagaatcgag^{tt}aaaaggtattgatt^{tt}taagaagatg^gaaacattcttggacacaaa^{tt}tggaatacaactataactcac^aaatgtatacatcatggcagacaaa^caaaagaatggaatcaaag^{tt}aacttcaa^{aa}attagacacaacattgaagatggaagcgttcaactagcagaccattatcaacaaa^aactccaattggc^gatggccctgtc^{ct}tttaccagacaacca^ttac^{ct}gtccacacaatctgcc^{ct}tttcgaaagatcccaacgaaaagagagaccacatgg^tccttctt^gagttt^gtaacagctgctgggattacacatggc^{at}ggatg^aactatacaaa^aataata^atactagtagcggccgctgcag

Terminator (Ah-nuld):

Gaattcgcggccgcttctagag^aaaaaaaaaaccccgc^{cc}ctgacagggcggggt^{tt}ttttttt^tactagtagcggccgctgcag

*Promoter Composition (160):

gaattcgcggccgcttctagag^gcgcaacgca^{atta}atgtgag^{tt}agctcactcattag^{gc}ataacaccgtgcgtgttgactat^{ttt}tac^{ct}ctggcgg^{tg}ata^{at}gtgtgga^{att}gtgagcggataaca^{att}ttcacacatactagag^tcacacagaaag^tactagatgcg^ttaaaggagaagaact^{ttt}tactggagttgtcccaattctt^gttgaattagatgg^tgatg^ttaatgggcacaaa^{ttt}tctgtcag^tggagaggg^tgaaggtgatgcaacatacggaaaact^tacccttaa^{att}tatttgcactactg^gaaaactac^{ct}gttccatggccaacact^tgtcactact^{tt}cgg^{tt}atgg^tgttcaatg^ctttgcgagatacc^cagatcatatgaaacagcatgact^{ttt}tcaagagtgccatgcccgaagg^{tt}atgtacaggaaagaactatatt^ttcaaagatgacgggaactacaagacacgtgctgaag^tcaag^{ttt}gaaggtgatacc^{ct}tgt^{ta}atagaatcgag^ttaaaaggtattgatt^{tt}taagaagatg^gaaacattcttggacacaaa^{tt}tggaatacaactataactcacacaatg^{ta}tacatcatggcagacaaa^caaaagaatggaatcaaag^{tt}aacttcaa^{aa}attagacacaacattgaagatggaagcgttcaactagcagaccattatcaacaaa^aactccaattggc^gatggccctgtc^{ct}tttaccagacaaccattac^{ct}gtccacacaatctgcc^{ct}tttcgaaagatcccaacgaaaagagagaccacatgg^tccttctt^gagttt^gtaacagctgctgggattacacatggc^{at}ggatg^aactatacaaa^aataata^atactagag^aaaaaaaaaaccccgc^{cc}ctgacagggcggggt^{tt}ttttttt^ttactagtagcggccgctgcag

*Notes: Prefix → λ cI O1 → λ cI -35 → λ cI O2 → λ cI -10 → LacI O1 → Suffix

**Questions: Does the CAP binding site need to be present?

*Overall Composition (954):

gaattcgcggccgcttctagag^gcgcaacgca^{atta}atgtgag^{tt}agctcactcattag^{gc}ataacaccgtgcgtgttgactat^{ttt}tac^{ct}ctggcgg^{tg}ata^{at}gtgtgga^{att}gtgagcggataaca^{att}ttcacacatactagag^tcacacagaaag^tactagatgcg^ttaaaggagaagaact^{ttt}tactggagttgtcccaattctt^gttgaattagatgg^tgatg^ttaatgggcacaaa^{ttt}tctgtcag^tggagaggg^tgaaggtgatgcaacatacggaaaact^tacccttaa^{att}tatttgcactactg^gaaaactac^{ct}gttccatggccaacact^tgtcactact^{tt}cgg^{tt}atgg^tgttcaatg^ctttgcgagatacc^cagatcatatgaaacagcatgact^{ttt}tcaagagtgccatgcccgaagg^{tt}atgtacaggaaagaactatatt^ttcaaagatgacgggaactacaagacacgtgctgaag^tcaag^{ttt}gaaggtgatacc^{ct}tgt^{ta}atagaatcgag^ttaaaaggtattgatt^{tt}taagaagatg^gaaacattcttggacacaaa^{tt}tggaatacaactataactcacacaatg^{ta}tacatcatggcagacaaa^caaaagaatggaatcaaag^{tt}aacttcaa^{aa}attagacacaacattgaagatggaagcgttcaactagcagaccattatcaacaaa^aactccaattggc^gatggccctgtc^{ct}tttaccagacaaccattac^{ct}gtccacacaatctgcc^{ct}tttcgaaagatcccaacgaaaagagagaccacatgg^tccttctt^gagttt^gtaacagctgctgggattacacatggc^{at}ggatg^aactatacaaa^aataata^atactagag^aaaaaaaaaaccccgc^{cc}ctgacagggcggggt^{tt}ttttttt^ttactagtagcggccgctgcag

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

*Individual Parts

P1, TetR `tcctatcagtgatagagattgacatccctatcagtgatagagatactgagcac`

P2, p22: `ctcgaggtgagtgacacagtactaggtccacggtgacctagatctcctatagtgagtcgtattaattt`

RBS:

`gaattcgcggccgcttctagagtcacacaggaaagtactagtagcggccgctgcag`

Protein:

`Gaattcgcggccgcttctagatgggtgagcaagggcgaggaggataaacatggccatcatcaaggagttcatgcgcttc
aaggtgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgaggg
caccagaccgccaagctgaaggtgaccaaggggtggccccctgcccttcgcctgggacatcctgtccccctcagttca
tgtacggctccaaggcctacgtgaagcaccgccgacatccccgactacttgaagctgtccttccccgagggcttc
aagtgaggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgaccaggactcctccttgaggacggcga
gttcatctacaaggtgaagctgcgcggcaccacttccccctccgacggccccgtaatgcagaagaagaccatgggct
gggagggcctcctccgagcggatgtaccccgaggacggcgcctgaagggcgagatcaagcagaggctgaagctgaag
gacggcggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgccggcgcctaaa
cgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtagcgaacgcgcccagggcc
gccactccaccggcggcatggacgagctgtacaagtaataataactagtagcggccgctgcag`

Terminator (Ah-nuld):

`gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttactagtagcggccgc
tcag`

*Promoter Composition (97 or 104):

`gaattcgcggccgcttctagagtcctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagtagcggccgctgcag`

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

*Overall Composition (885):

`gaattcgcggccgcttctagagtcctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagagtcacacaggaaagtactagatgggtgagcaagggcgaggaggataaacatggccatcatcaaggagttcatg
cgcttcaaggtgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgccccta
cgagggcaccagaccgccaagctgaaggtgaccaaggggtggccccctgcccttcgcctgggacatcctgtccccctc
agttcatgtacggctccaaggcctacgtgaagcaccgccgacatccccgactacttgaagctgtccttccccgag
ggcttcaagtgaggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgaccaggactcctccttgaggac
cggcgagttcatctacaaggtgaagctgcgcggcaccacttccccctccgacggccccgtaatgcagaagaagacca
tgggctgggagggcctcctccgagcggatgtaccccgaggacggcgcctgaagggcgagatcaagcagaggctgaag
ctgaaggacggcggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgccggcgc
ctacaacgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtagcgaacgcgccc
agggcggccactccaccggcggcatggacgagctgtacaagtaataataactagagaaaaaaaaaccccgcccctgac
agggcgggggttttttttactagtagcggccgctgcag`

Sequences to synthesize

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

```
gaattcgcggccgcttctagagcaatacgcgaaaccgcctctccccgcgcgcttgccgattcattaatgcagctggca  
cgacagggtttcccgactggaaagcggggcagtgagcgcgcaacgcaattaatgtgagttagctcactcattaggcacccc  
aggctttacactttatgcttccggctcgtatggtgtgtggaattgtgagcggataacaatttcacacatactagagt  
cacacaggaaagtactagatggcccgggatgatcctcacttcaattttcgtatgccaatggaagtaagagagaaatt  
gaaatttagagcagaggcacaacggacggagcatgaactctgagcttttgcaaatcgtacaagatgccctaagcaaac  
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcaggttagtgaagaagatggct  
ttcgatacactgaaggatctttataaaaaaacaccgctgcaaacgacgaaaactacgcttttagtagcttaataata  
ctagag aaaaaaaaaaccccgcccctgacagggcgggttttttttactagtagcggccgctgcag
```

2. LacI/λCI Promoter → RBS

```
gaattcgcggccgcttctagaggcgcgcaacgcaattaatgtgagttagctcactcattaggcataacaccgtgcgtgt  
tgactattttacctctggcggtgataatgtgtggaattgtgagcggataacaatttcacacatactagagtcacaca  
ggaaagtactagtagcggccgctgcag
```

3. TetR/p22 mnt Promoter → RBS

```
gaattcgcggccgcttctagagtcctatcagtgatagagattgacaagggtccacgggtgacctagatactgagcact  
actagagtcacacaggaaagtactagtagcggccgctgcag
```