

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
 aagtttagcgttgaagaatattagcccttcaatcgccagagaaaatctacgagatgtatgaagcgggttagtatgcagccg
 tcacttagaagtgagtgatgagtagaccctgttttttctcatgttcaggcagggatgttctcacctgagcttagaacctt
 taccaaaggtgatgaggagagatgggtaagcacacaaaaaagccagtgattctgcattctggcttgagggtgaag
 gtaattccatgaccgcaccaacaggtccaagccaagctttcctgacggaatgttaattctcgttgaccctgagcag
 gctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcagggatag
 cggtcaggtgtttttacaaccactaaaccacagtagccaatgatcccatgcaatgagagttgttccggttggtgggga
 aagttatcgctagtcagtgccctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagcttaataa
 tactagtagcggccgctgcag

Terminator (Ah-nuld):

gaattcgcggccgcttctagagaaaaaaaaaccccgcccctgacagggcggggttttttttttactagtagcggccgc
 tcgag

*Composition (921):

gaattcgcggccgcttctagagtcacctatcagtgatagagattgacatccctatcagtgatagagatactgagcact
 actagagtcacacaggaaagtactagatgagcacaaaaaagaaaccattaacacaagagcagcttgaggacgcacgt
 cgcttaaagcaatattatgaaaaaaagaaaaatgaacttggcttatcccaggaatctgtcgcagacaagatggggat
 ggggcagtcagggcgttgggtgctttatttaaatggcatcaatgcattaaatgcttataacgccgcattgcttgcaaaaa
 ttctcaaagtttagcgttgaagaatattagcccttcaatcgccagagaaaatctacgagatgtatgaagcgggttagtatg
 cagccgtcacttagaagtgagtgatgagtagaccctgttttttctcatgttcaggcagggatgttctcacctgagcttag
 aacctttaccaaaggtgatgaggagagatgggtaagcacacaaaaaagccagtgattctgcattctggcttgagg
 ttgaaggttaattccatgaccgcaccaacaggtccaagccaagctttcctgacggaatgttaattctcgttgaccct
 gagcaggtctgttgagccaggtgatttctgcatagccagacttgggggtgatgagtttaccttcaagaaactgatcag
 ggatagcggtcaggtgtttttacaaccactaaaccacagtagccaatgatcccatgcaatgagagttgttccggttg
 tggggaaagttatcgctagtcagtgccctgaagagacgtttggcgctgcaaacgacgaaaactacgctttagtagct
 taataataactagtagagaaaaaaaaccccgcccctgacagggcggggttttttttttactagtagcggccgctgcag

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

*Individual Parts:

Promoter:

gaattcgggcccgtttctagagcaatacgcgaaaccgcctctccccgcgcggttgccgattcattaatgcagctggca
cgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacccc
aggctttacactttatgcttccggctcgtatggtgtgtggaattgtgagcggataacaatttcacacatactagtag
cggccgctgcag

RBS:

gaattcgggcccgtttctagagtcacacaggaaagtactagtagcggccgctgcag

Protein:

gaattcgggcccgtttctagatggcccgggatgatcctcacttcaatcttctgtagcctttgcaaactcgtacaagatgccctaagcaa
ttgaaatttagagcagaggcaaaccggacggagcatgaactctgagcttttgcaaactcgtacaagatgccctaagcaa
accgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcagagttagtgaagaagatgg
tcttcgatacactgaaggatctttataaaaaaacaccgctgcaaacgacgaaaactacgcttttagtagcttaataa
tactagtagcggccgctgcag

Terminator (Ah-nuld):

gaattcgggcccgtttctagagaaaaaaaaaccccgcccctgacagggcgggggttttttttttactagtagcggccgc
tgcag

*Composition Design for Synthesis (605):

gaattcgggcccgtttctagagcaatacgcgaaaccgcctctccccgcgcggttgccgattcattaatgcagctggca
cgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacccc
aggctttacactttatgcttccggctcgtatggtgtgtggaattgtgagcggataacaatttcacacatactagtagt
cacacaggaaagtactagatggcccgggatgatcctcacttcaatcttctgtagcctttgcaaactcgtacaagatgccctaagcaa
gaaatttagagcagaggcaaaccggacggagcatgaactctgagcttttgcaaactcgtacaagatgccctaagcaa
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcagagttagtgaagaagatggc
ttcgatacactgaaggatctttataaaaaaacaccgctgcaaacgacgaaaactacgcttttagtagcttaataa
ctagag aaaaaaaaaaccccgcccctgacagggcgggggttttttttttactagtagcggccgctgcag

3. (Prefix) LacI/ λ CI Promoter \rightarrow RBS \rightarrow RFP \rightarrow Terminator

*Individual Parts

P1, LacI: caatacgc^{aa}accg^{cctctccccgcgcg}ttggccgattcattaatgcagctggcagcagaggtttcccgactggaaagcggg^{cagtgagcgcaacgcaattaatgtgagttagctcactcattaggc}accccaggc^{tttaca}ctttatgcttccggctcg^{atgtgtgtgtggaattgtgagcggataacaatttcacaca}

P2, λ CI: taacaccgtgcgtgttgactat^{tttacctctggcgg}tgataatggttgc

RBS:

gaattcgcggccgcttctagag^tcacacaggaaag^{tactagtagcggccgctgcag}

Protein:

gaattcgcggccgcttctagatgg^tgagcaagggcgaggaggataacatggccatcatcaaggagttcatg^cgcttc aaggtgcacatggagggctccg^tgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgaggg caccagaccgccaagctgaaggtgaccaaggg^tggccccctgccccttcgcctgggacatcctgtcccctcagttca tgtacggctccaaggcctacgtgaagcacc^{cc}gcgcgacatccccgactacttgaagctgtccttccccgagggcttc aagtgaggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgaccaggactcctccttgcaggacggcga gttcatctacaaggtgaagctg^cgcggcaccaacttccccctccgacggccccgtaatgcagaagaagaccatgggct gggagggcctcctccgagcggatgtac^{cc}cgaggacggcgcctgaagggcgagatcaagcagaggctgaagctgaag gacggcggccactacgacgctgaggtcaagaccac^{ct}acaaggccaagaagcccgtgcagctgcccggcgcctacaac g^tcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtagcgaacgcgcccgagggcc gccactccaccggcggcatggacgagctgtacaagtaata^atactagtagcggccgctgcag

Terminator (Ah-nuld):

Gaattcgcggccgcttctagag^aaaaaaaaaaccccgcccctgacagggcggggtttttttttt actagtagcggccgctgcag

*Promoter Composition (160):

gaattcgcggccgcttctagag^gcgcaacgcaattaatgtgagttagctcactcattaggc^ataacaccgtgcgtgt tgactat^{tttacctctggcgg}tgataatgtgtggaattgtgagcggataacaatttcacacatactagtagcggccg ctgcag

*Notes: Prefix \rightarrow λ CI O1 \rightarrow λ CI -35 \rightarrow λ CI O2 \rightarrow λ CI -10 \rightarrow LacI O1 \rightarrow Suffix

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

*Overall Composition (948):

gaattcgcggccgcttctagag^gcgcaacgcaattaatgtgagttagctcactcattaggc^ataacaccgtgcgtgt tgactat^{tttacctctggcgg}tgataatgtgtggaattgtgagcggataacaatttcacacatactagagtcacaca ggaaagtactagatgg^tgagcaagggcgaggaggataacatggccatcatcaaggagttcatg^cgcttcaaggtgca catggagggctccg^tgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcaccaga ccgccaagctgaaggtgaccaaggg^tggccccctgccccttcgcctgggacatcctgtcccctcagttcatgtacggc tccaaggcctacgtgaagcacc^{cc}gcgcgacatccccgactacttgaagctgtccttccccgagggcttcaagtggga g^cgcgctgatgaacttcgaggacggcggcgtggtgaccgtgaccaggactcctccttgcaggacggcgcgagttcatct acaaggtgaagctg^cgcggcaccaacttccccctccgacggccccgtaatgcagaagaagaccatgggctgggagggc tcctccgagcggatgtac^{cc}cgaggacggcgcctgaagggcgagatcaagcagaggctgaagctgaaggacggcgg ccactacgacgctgaggtcaagaccac^{ct}acaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaaca tcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtagcgaacgcgcccgagggccgcccactcc accggcggcagtgacgagctgtacaagtaata^atactagag^aaaaaaaaaaccccgcccctgacagggcggggttttt ttttactagtagcggccgctgcag

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

*Individual Parts

P1, TetR `tcctatcagtgatagagattgacatccctatcagtgatagagatactgagcac`

P2, p22: `ctcgaggtgagtgacacagtactaggtccacggtgacctagatctccctatagtgagtcgtattaattt`

RBS:

`gaattcgcggccgcttctagagtcacacaggaagtaactagtagcggccgctgcag`

Protein:

`Gaattcgcggccgcttctagatgcgtaaaggagaagaacttttactggagttgtcccaattcttgttgaattagat
ggtgatgttaatgggcacaaatcttctgtcagtgaggaggggtgaaggtgatgcaacatacggaaaacttacccctaa
atattttgactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttcaatgct
ttgcgagataccagatcatatgaaacagcatgactttttcaagagtgccatgcccgaaggttatgtacaggaaga
actatattttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgttaatag
aatcgagttaaaaggtattgatttttaagaagatggaacattcttggacacaaattggaatacaactataactcac
acaatgtatacatcatggcagacaaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaagat
ggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaacca
ttacctgtccacacaatctgccctttcgaagatcccaacgaaaagagagaccacatggtccttcttgagttttaa
cagctgctgggattacacatggcatggatgaactatacaaaataataataactagtagcggccgctgcag`

Terminator (Ah-nuld):

`gaattcgcggccgcttctagagaaaaaaaaaccccgccctgacagggcggggttttttttactagtagcggccg
ctgcag`

*Promoter Composition (97 or 104):

`gaattcgcggccgcttctagagtcctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagtagcggccgctgcag`

or

`gaattcgcggccgcttctagagtcctatcagtgatagagattgacaaggtccacggtgacctagatctccgatact
gagcactactagtagcggccgctgcag`

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

*Overall Composition (891):

`gaattcgcggccgcttctagagtcctatcagtgatagagattgacaaggtccacggtgacctagatactgagcact
actagagtcacacaggaagtaactagatgcgtaaaggagaagaacttttactggagttgtcccaattcttgtttaa
ttagatggtgatgttaatgggcacaaatcttctgtcagtgaggaggggtgaaggtgatgcaacatacggaaaacttac
ccttaaattttatgtcactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttc
aatgctttgcgagataccagatcatatgaaacagcatgactttttcaagagtgccatgcccgaaggttatgtacag
gaaagaactatattttcaaagatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgt
taatagaatcgagttaaaaggtattgatttttaagaagatggaacattcttggacacaaattggaatacaactata
actcacacaatgtatacatcatggcagacaaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacatt
gaagatggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccaga
caaccattacctgtccacacaatctgccctttcgaagatcccaacgaaaagagagaccacatggtccttcttgagt
ttgtaacagctgctgggattacacatggcatggatgaactatacaaaataataataactagtagaaaaaaaaaaccccgcc
cctgacagggcggggttttttttactagtagcggccgctgcag`

Sequences to synthesize

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

```
gaattcgcggccgcttctagagcaatacgcgcaaacgcctctccccgcgcggttgccgattcattaatgcagctggca  
cgacagggtttcccgactggaaagcgggagtgagcgcgcaacgcaattaatgtgagttagctcactcattaggcacccc  
aggctttacactttatgcttccggctcgatgttgtgtggaattgtgagcggataacaatttcacacatactagagtg  
cacacaggaaagtactagatggccccgggatgatcctcacttcaattttcgtatgccaatggaagtaagagagaaatt  
gaaatttagagcagaggcaaacggacggagcatgaactctgagcttttgcaaatcgtacaagatgccctaagcaaac  
cgtcaccagtcactgggtaccgcaatgatgcggaacgactcgccgatgagcagagcaggttagtgaagaagatggtc  
ttcgatacactgaaggatctttataaaaaaacccgctgcaaacgacgaaaactacgcttttagtagcttaataata  
ctagag aaaaaaaaaaccccgccttgacagggcgggttttttttactagtagcggccgctgcag
```

2. LacI/λcI Promoter → RBS

```
gaattcgcggccgcttctagaggcgcgaacgcaattaatgtgagttagctcactcattaggcataacaccgtgcgtgt  
tgactattttacctctggcgggtgataatgtgtggaattgtgagcggataacaatttcacacatactagagtcacaca  
ggaaagtactagtagcggccgctgcag
```

3. TetR/p22 mnt Promoter → RBS

```
gaattcgcggccgcttctagagtcctatcagtgatagagattgacaagggtccacgggtgacctagatactgagcact  
actagagtcacacaggaaagtactagtagcggccgctgcag
```

Primers to order

λcI gene (lcI gene):

Reverse: CTGCAGCGGCCGCTACTAGTATTATTAAGC

Forward 1: TCACACAGGAAAGTACTAGATGAGCACAAAAAAGAAACC

Forward 2: GAATTCGCGGCCGCTTCTAGAGTCACACAGGAAAGTACTAGATGAGC

LacI/λcI Promoter (LacIcIDP):

Reverse: CTGCAGCGGCCGCTACTAGTATGTGTGAAATTGTTATCCGC

Forward: GAATTCGCGGCCGCTTCTAGAGGC

TetR/p22 mnt Promoter (Tetp22DP):

Reverse: CTGCAGCGGCCGCTACTAGTAGTGCTCAGTATCTAGGTCACCG

Forward: GAATTCGCGGCCGCTTCTAGAGTCCC

Plasmid Primers (plasmid):

Reverse: tgccacctgacgtctaagaa

Forward: gctcactcaaaggcggtaat