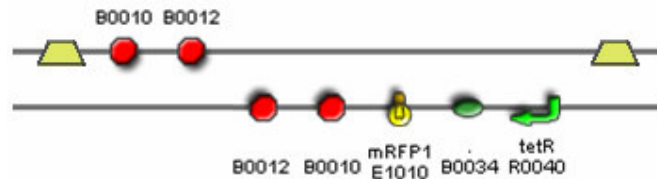


Cre Lox Part Design

John Chattaway



This part is designed to be placed downstream of a promoter and prevent any Pops from the Promoter passing through this part. It will do this until an accompanying Cre Recombinase plasmid becomes activated. Once the Cre recombinase is activated the enzyme produced will permanently cut a section of DNA from the plasmid containing this part. This short section of DNA contains stop codons so once these are removed the polymerase can pass through this part and transcribe downstream genes. This short section of DNA is degraded.

This is useful if a component of the system must be grown up but not activated until a certain external stimulus is added such as a positive feedback loop.

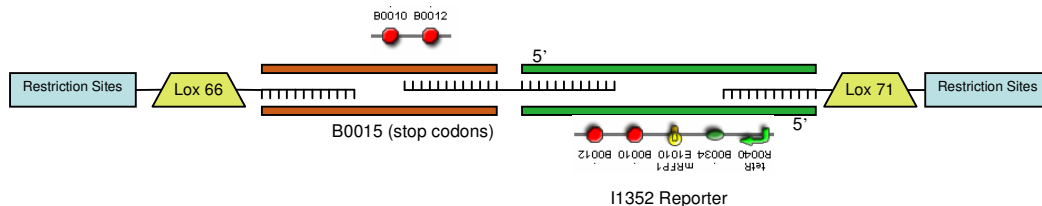
This part should be very efficient at preventing Pops passing through it.

The Part also contains a RFP reporter which is transcribed in the 3'-5' direction. This means that unactivated parts will fluoresce red and activated parts will not fluoresce. This allows you to see that the part is working in your system. It also allows you to observe the efficiency of activation of the part in your system. The part runs the other way to the rest of the system to prevent any fluorescence due to external Pops passing into the device

Because of the way Cre recombinase works the excised reporter will remain in a small plasmid and continue to be transcribed for a short time however this plasmid will not have an origin of replication so will not be copied and the fluorescent protein should stop being produced after around 15min

The design uses mutated lox sites, lox66 and lox71, which will make the excision irreversible.(1)

We will make



References;

1. **Zuwen Zhang and Beat Lutz** (2002) Cre Recombinase-mediated inversion using lox66 and lox71: method to introduce conditional point mutations into the CREB-binding protein
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Sequence

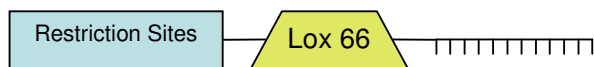
From Primers

From B0015

From I13521

5'CGATAACTTGGTATAGCATACATTATACGAACGGTACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTCTCGCTTATAGATATAAAACGCAGAAAGGCCACCCGAAGGTGAGCCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCAGTCTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTAGCGATCTACACTAGCACTATCAGCGTTATTAAGCACCGTGGAGTGACGACCTTCAGCACGTTCTGACTGTTCAACGATGGTGTAGTCTTCGTTGTGGGAGGTGATGTCCAGTTTGATGTCGGTTTTGTAAGCACCCGGCAGCTGAACCGTTTTTTAGCCATGTAGGTGGTTTTAACTTCAGCGTCGTAGTGACCACCGCTTTCAGTTTTCAGACGCATTTTTGATTTACCTTTTCAGAGCACCGTCTCCGGGTACATACGTTTCGGTGAAGCTTCCCAACCCATGGTTTTTTCTGCATAACCGACCGTCGGACGGGAAGTTGGTACCACGCAGTTTAACTTTGTAGATGAACTCACCGTCTGCAGGGAAGAGTCCGGTAACGGTAACAACACCACCGTCTTCGAAGTTCATAACACGTTCCCATTTGAAACCTTCCGGGAAGGACAGTTTCAGGTAGTCCGGGATGTCAGCCGGGTGTTAACGTAAGCTTTGGAACCGTACTGGAAGTGCAGGACAGGATGTCCCAAGCGAAGCGCAGCGACACCTTTGGTAACTTTAGCTTTAGCGGTCTGGTACCTTCGTACGGACGACCTTCACCTTCACCTTCGATTCGAACTCGTGACCGTTAACGGAACTTCCATACGAACTTTGAAACGCATGAACTCTTTGATAACGTCCTTCGGAGGAAGCCATCTAGTATTTCTCCTCTTCTCTAGTAGTGCTCAGTATCTCTATCACTGATAGGGATGTCAATCTCTATCACTGATAGGGAAGTTACCGTTCGTATACGATACATTATACGAAGTTAT3'

Primer 1



Restriction sites
 ATCTAGAG 2 random bases
 CG
 Lox 66
 ATAACTTGGTATAGCATACATTATACGAACGGTAC
 Complementary (38% AG)
 GGTCCTAGTTTATTTTG

ATCTAGAGCGATAAAGTGGTATAGCATACATTATACGAACGGTAGGTCCGTAGTTTATTTTG

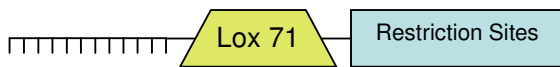
Primer 2



Comp B0015 +
 GGTCCTAGTTTATTTTG Link
 GAT comp I13521 5' -strand
 ggcctttctgcgtttata

GGTCCTAGTTTATTTTGATggcctttctgcgtttata

Primer 3



Comp +coding strand 5' end I13521
 CTCTATCACTGATAGGGA 2 random bases
 GT
 Lox 71
 TACCGTTCGTATACGATACATTATACGAAGTTAT
 Restriction Sites
 TACTAGTA

CTCTATCACTGATAGGGAAGTTACCGTTCGTATACGATACATTATACGAAGTTATTAAGTACTAGTA