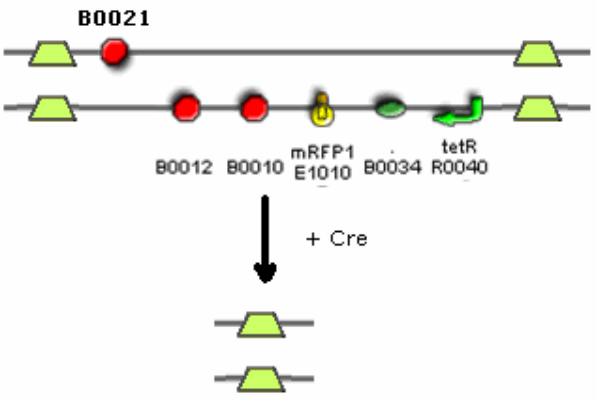
Cre - Lox Part Design



Overview

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 excised section of DNA is degraded. This short section of DNA contains stop codons and terminator sequences so once these are removed the polymerase can pass through this part and transcribe downstream genes.

Reporter Gene

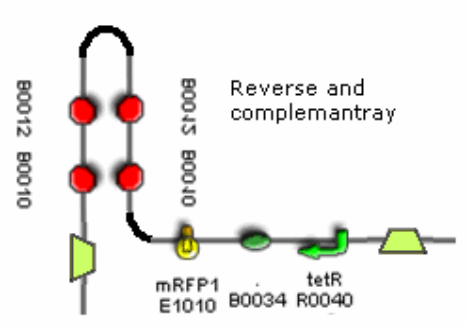
The Part also contains a RFP reporter which is transcribed in the 3'-5' direction. This means that un-activated parts will fluoresce red and activated parts will not fluoresce. This allows you to see that the part is working in your system. It also allows you to observe the efficiency of activation of 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

Lox Sites

The Cre lox system is very complex and I will not describe it fully. There are multiple Lox site which exist I have chosen lox 66 and 71. They are both placed in the forward direction on the sense strand of the DNA. This causes the DNA between the lox sites to be excised rather than reversed. Lox 66 and 71 have been mutated so that the reversed strand of DNA cannot be re-inserted by cre. Lox 66 should be upstream of lox 77. The DNA between the lox sites is seen as a plasmid in the cells once it has been excised however it has no origin of replication so will be quickly degraded inside the cell.

Terminator Sequences

There are two main terminator sequences in the registry, B0015 which consists of two separate sequences B0010 and B0012. The second terminator sequence is B0012 which is a single terminator sequence. B0015 is much more efficient than B0021, 98.4% and 60% respectively. The two terminator sequences in this part cannot be the same as when you try to PCR them they will bind together and form a 131 base pair hairpin loop which the polymerase cannot pass through. Therefore the two terminator sequences must be different. We just have to accept that the stopping will not be as efficant. We cannot have multiple copies of B0021 as this would require several ligations and we don't have time.



Stop Codons

I Have included a stop codon between lox66 and B0021 to prevent the e-coli cells from translating past the first lox site. This reduces the load that this part will place on the E.coli cell so increases it's stability in vivo. I checked the E-coli codon usage database on NCBI and found that TGA was by far the most commonly used in nature.

Frameshift

The Lox sites are 34bp long therefore if this part is placed after an RBS it will induce a frameshift in the protein produced, therefore creating a mangled protein. I added two random bases to the start of the lox site to prevent a frameshift.

PCR

The plan is to do 2 PCR reactions in parallel

one with Primer 1, reversed and complementary primer 2 and part B0025
The Second contains primer 3 and non-complanentary primer 2

The products of these PCRs should be purified on a gel then PCR'd together with primers one and three giving a full length construct.

Final Sequence (coding Strand)

Lox 66 Stop

CGATAACTTGGTATAGCATACATTATACGAACGGTATGAAAATAATAAAAAAGCCGGATTAATAATCTGGCTTTTTATATTCTCTTAT
AAACGCAGAAAGGCCCACCCGAAGGTGAGCCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCA
GTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTAGCGATCTACACTAGCACTATCAGCGTTATTAAGCACCGGTGG
AGTGACGACCTTCAGCACGTTCGTACTGTTCAACGATGGTGTAGTCTTCGTTGTGGGAGGTGATGTCCAGTTTGATGTCGGTTTTGTA
AGCACCCGGCAGCTGAACCGGTTTTTTAGCCATGTAGGTGGTTTTAACTTCAGCGTCGTAGTGACCACCGTCTTTCAGTTTCAGACGC
ATTTTGATTTCACCTTTCAGAGCACCGTCTTCCGGGTACATACGTTCGGTGGAAGCTTCCCAACCCATGGTTTTTTTCTGCATAACCG
GACCGTCGGACGGGAAGTTGGTACCACGCAGTTTAACTTTGTAGATGAACTCACCGTCTTGCAGGGAGGAGTCCTGGGTAACGGTAAC
AACACCACCGTCTTCGAAGTTCATAACACGTTCCCATTTGAAACCTTCCGGGAAGGACAGTTTCAGGTAGTCCGGGATGTCAGCCGGG
TGTTTAACGTAAGCTTTGGAACCGTACTGGAACTGCGGGGACAGGATGTCCCAAGCGAACGGCAGCGGACCACCTTTGGTAACTTTCA
GTTTAGCGGTCTGGGTACCTTCGTACGGACGACCTTCACCTTCACCTTCGATTTCGAACTCGTGACCGTTAACGGAACCTTCCATACG
AACTTTGAAACGCATGAACTCTTTGATAACGTCTTCGGAGGAAGCCATCTAGTATTTCTCCTCTTTCTCTAGTAGTGCTCAGTATCTC
TATCACTGATAGGGATGTCAATCTCTATCACTGATAGGGAGTTACCGTTCGTATACGATACATTATACGAAGTTAT

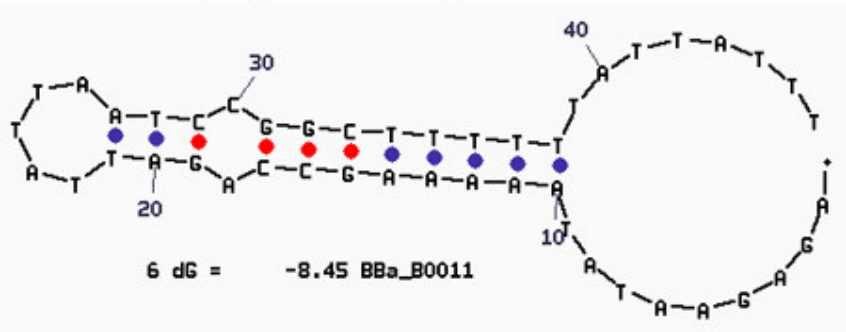
Lox 71

Sequences

-Put in stop codons (light in terms of selection pressure)
-diff terminator sequence (prevent hairpin formation)

Terminator B0021

Aaataataaaaaagccggattaataatctggctttttatattctct = 46bp



Reverse Complement for I13521

Tataaacgcagaaaggcccacccgaaggtgagccagtgtgactctagtagagagcgttcaccgacaaacaacagataaaacga
aaggcccagtctttcgactgagcctttcgttttatttgatgcctggctctagtagcgatctacactagcactatcagcgttat
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cggaggaagccatctagtatttctcctctttctctagtagtgctcagtatctctatcactgatagggatgtcaatctctatca
ctgataggga

Lox Sites

Lox66: ATAACTTGGTATAGCATACATTATACGAACGGTA
Lox71: TACCGTTCGTATACGATACATTATACGAAGTTAT

Need to add 2 random bases to the beginning of the Lox sites to prevent a frameshift mutation.

Lox66: CGATAACTTGGTATAGCATACATTATACGAACGGTA
Lox71: GTTACCGTTCGTATACGATACATTATACGAAGTTAT

Restriction Sites

Gaattcgcggccgcttctagag (to go on the 5' end)
Atgatcatcgccggcgacgtc (to go on the 3' end)

Insert Stop Codon after lox site

TGA is the most commonly used terminator in E.coli *(source NCBI codon usage database)*

Desired Sequence

Restriction sites - Lox66 – stop codon – Terminator Sequence (B0021) – Reverse complement I13521 – Lox71 – Restriction Sites

Desired Sequence

5'Gaattcgcggccgcttctagag
CGATAACTTGGTATAGCATACATTATACGAACGGTA
TGA
Aaataataaaaaagcc
ggattaataatctggctttttatattctct
Tataaacgcagaaaggcccacccgaaggtgagccagtgtgactctagtag
agagcgttcaccgacaaacaacagataaaacgaaaggcccagtctttcgactgagcctttcgttttatttgatgcctggc
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gaccgttaacggaaccttccatacgaactttgaaacgcatgaactctttgataacgtcttcggaggaagccatctagtat
ttctcctctttctctagtagtgctcagtatctctatcactgatagggatgtcaatctctatcactgatagggaGTTACCG
TTCGTATACGATACATTATACGAAGTTATAtgatcatcgccggcgacgtc3'

Primer 1

Primer 1 is the same as the sense streand and consists of
Restriction Sites – Lox Site – Stop Codon – First 20 bases of part B0021

Gaattcgcggccgcttctagag
CGATAACTTGGTATAGCATACATTATACGAACGGTA
TGA
Aaataataaaaaagccg

Primer 2

We need both the sense strand and the anti-sense strand for this primer

Info: 31bp into part B0021 (long due to a run of T and A)
22Bp into part I13521

Same as Sense Strand :

cggattaataatctggctttttatattctct
Tataaacgcagaaaggcccacc

Reversed And Complemantry

GGTGGGCCTTTCTGCGTTTATAAGAGAATATAAAAAGCCAGATTATTAATCCG

Primer 3

Primer 3 contans a section complemantry to the sense strand of the construct and a lox site and some restriction sites. It was made by copying the end of the desired sense strand and making it complementary and reversing it.

GACGTCGCCGGCGATGATCATATAACTTCGTATAATGTATCGTATACGAACGGTAACTCCCTATCAGTGATAGAGATTGAC