Sunday, September 28, 2025

Designing oligonucleotides to disrupt lacZ gene in E coli DH5 alpha

Ali M.

Goal: Knock-out the lacZ gene.

Steps:

Order two oligonucleotides (instructions will be below).

Anneal them → mix forward and reverse oligos, heat and cool so they form a double-stranded insert.

Clone into Cas9 plasmid using Golden Gate (BbsI) or similar cloning strategy.

Transform the plasmid into E. coli DH5α.

Inside the cells, the plasmid will express **Cas9 + our gRNA**, which together target lacZ and cut it at the chosen site.

The cut will usually inactivate lacZ via small indels.

Oligonucleotide design:

Step 1: Obtain the genbank/FASTA link for the E. Coli strand we're targeting. This is the link.

Step 2: Control-F to find the lacZ gene's coordinates. Note the CDS, or coding DNA sequence, maps to β-galactosidase, which is what we want to make non-functional.

```
gene            complement(1239256..1242168)
                /gene="lacZ"
                /locus_tag="NEB5A_06270"
CDS             complement(1239256..1242168)
                /gene="lacZ"
                /locus_tag="NEB5A_06270"
                /inference="EXISTENCE: similar to AA
                sequence:RefSeq:WP_011378569.1"
                /note="forms a homotetramer; hydrolyzes lactose
                disaccharide to galactose and glucose; converts lactose to
                allolactose which is the natural inducer of the lac
                operon; Derived by automated computational analysis using
                gene prediction method: Protein Homology."
                /codon_start=1
                /transl_table=11
                /product="beta-D-galactosidase"
                /protein_id="AOO72618.1"
```

Step 3: Using a python script, I created a new FASTA file that only contained nucleotides in the range of 1239256..1242168.

Step 4: Go to CHOPCHOP, and find the best protospacer. Here's a link to the CHOPCHOP output with the new FASTA file from Step 3.

Step 5: According to CHOPCHOP, our best protospacer is 'CTGACAATGGCAGATCCCAG'. So our forward oligo will be:

CACC CTGACAATGGCAGATCCCAG

And our reverse oligo will be:

AAAC CTGGGATCTGCCATTGTCAG

after we add the overhangs.

Step 6: Order the oligonucleotides on idtdna's website.