Plasmids & Oligos:

Assembled Plasmids:

- pdCas9-DetX (https://benchling.com/s/seq-aerLfXOPGLNikNfdOQuQ) – This plasmid is responsible for the detection of the target DNA within a cell. The negative control mutant is linked. Chloramphenicol selectable.
- pUK21-Tar (https://benchling.com/s/seq-LTWygbfjWahe8b43KFtq) – This plasmid has a high copy number and contains a BsaI cloning site where the target DNA sequence can be inserted. Kanamycin selectable.

Base Plasmids:

- pdCas9 (https://benchling.com/s/seq-BbRRSASjjJzz2a6wFM98) – Expresses "Dead" Cas9 in addition to tracrRNA. Contains a BsaI cloning site within a CRISPR region for the insertion of crRNA sequences.
- pUK21 (https://benchling.com/s/seq-peT4pSLS0FJD7rcEEphW) – A high copy-number plasmid that doesn't naturally contain any BsaI sites.

Synthesized Inserts:

- Adapter Insert (https://benchling.com/s/seq-YFD2nS2QyVp1pTjL5CXx) – A simple insert that can be cloned into pUK21 and adds two BsaI cut sites using a sequence borrowed from pdCas9.
- Signal + Multiplier Insert (https://benchling.com/s/seq-QkLjd0JOHnJzT0XGl1aK) – The longest insert, this sequence adds the RFP signalling gene to pdCas9 and contains the second CRISPR region responsible for the positive feedback loop upon target detection.
- LacI Insert (https://benchling.com/s/seq-GNchUCgjQbV3Q5vzG2q0) – This insert simply adds a LacI coding region to pdCas9 so that the LacO controlling RFP expression functions properly.

Oligonucleotide Scaffolding:

Two, complementary oligonucleotide sequences exist for each crRNA mutant — for a total of 32 sequences. The following templates are written in the 5'-3' direction.

1. AAAC – (crRNA) – G
2. AAAAC – (RC crRNA)

Mutant crRNA Fragments:

1. PC: This sequence has no match anywhere in the cell.
   - `tgagaccagtctcggaagctcaaaggtctc`
2. NC: This sequence perfectly matches the RFP promoter.
   - `tatgcttccggctcgtatgttgtg`
3. B: This sequence has a single point mutation directly next to the PAM.
   - `tatgcttccggctcgtatgttgtc`
4. C: This sequence has a single point deletion directly next to the PAM.
   - `tatgcttccggctcgtatgttgt`
5. D: This sequence has a single point mutation 5 nucleotides from the PAM.

- `tatgcttccggctcgtataattgtg`

6. E: This sequence has a single point deletion 5 nucleotides from the PAM.
   - `tatgcttccggctcgtat ttgtg`
7. F: This sequence has a single point mutation 10 nucleotides from the PAM.
   - `tatgcttccggcttgtatgttgtg`
8. G: This sequence has a single point deletion 10 nucleotides from the PAM.
   - `tatgcttccggct gtatgttgtg`
9. H: This sequence has a single point mutation 15 nucleotides from the PAM.
   - `tatgcttcaggctcgtatgttgtg`
10. I: This sequence has a single point deletion 15 nucleotides from the PAM.
    - `tatgcttc ggctcgtatgttgtg`
11. J: This sequence contains a single mismatching base on the end farthest from the PAM.
    - `aatgcttccggctcgtatgttgtg`
12. K: This sequence contains two mismatching bases on the end farthest from the PAM.
    - `attgcttccggctcgtatgttgtg`
13. L: This sequence contains three mismatching bases on the end farthest from the PAM.
    - `atagcttccggctcgtatgttgtg`
14. M: This sequence contains four mismatching bases on the end farthest from the PAM.
    - `atacttccggctcgtatgttgtg`
15. N: This sequence contains five mismatching bases on the end farthest from the PAM.
    - `atacgttccggctcgtatgttgtg`
16. O: This sequence contains six mismatching bases on the end farthest from the PAM.
    - `atacgatccggctcgtatgttgtg`