Materials & Methods:

In preparing the pdCas9-Det mutants (A-O), the pdCas9-Det was digested with BsaI and then gel purified, and the CRISPR spacer inserts were first phosphorylated and then annealed before finally being ligated with the digested and purified pdCas9-Det. The specifics of this procedure can be found in the Marraffini procedure here: https://media.addgene.org/cms/filer_public/15/9f/159fc921-b752-49fd-bef0-a39f5c33f538/marraffini_pcas9_protocol.pdf

Once all of the mutants were prepared in this way, they were transformed into electrocompetent *E. Coli* cells (strain TBD). The electroporation protocol followed was published by NEB and found here: https://www.protocols.io/view/Electroporation-Protocol-C2986-imsv3v

Once transformed and the recovery period (of one hour @ 37° C w/ shaking) had passed, the cells were streaked on LB-Chloramphenicol (25 µg/mL) plates and left to grow up O/N @ 37° C.

After the plates were incubated overnight, the largest colonies were picked off of the plates and grown up to log-phase density. At this point, aliquots of each mutant were flash-frozen in 10% glycerol.

While still in the midst of log-phase growth (OD600 of 0.2 to 0.6), make the cells chemically competent by following this procedure: https://bitesizebio.com/30145/chemically-competent-cells/ but the freezing and storing steps at the end were neglected. Instead, each of the mutant cultures were split into two at the end of the chemical-competence procedure. Additionally, activate the cells by adding 1mM IPTG to all chilling cell cultures. One culture will be heat shocked with the activator DNA, and the other, with no DNA. The activator DNA is the pUC21-Tar plasmid which has a cloning site for the CRISPR spacer inserts along with a neighboring PAM sequence. The (A-O) mutants of this plasmid are produced in the same way as the (A-O) mutants of the pdCas9-Det plasmid.

For each pair of cultures, say pdCas9-TarX, run both pairs through the heat shock transformation found here: https://www.protocols.io/view/Transformation-Protocol-imsvpm but only add the pUC21-TarX to one of the samples. Transform the other without any DNA as a control. Note that the cells containing the A mutant of the pdCas9-Det plamid must be heat-shocked with the A mutant of the pUC21-Tar plasmid. The mutants (the X's in the original step) must match.

After the heat shock, all cultures are grown up. The control cultures are left as is, and the culture with the added pUC21-Tar plasmid is grown up in Kanamycin media (50 µg/mL) and any changes in culture color are carefully noted and recorded.