This word document is a short explanation of the rotation project completed by GK from January 2023 to March 2023. This project is split into two parts: 1) the A908 mutation cloning and 2) the PER1 GR response dCas12 testing. For more information outside the scope of this document, please reach out to the Reddy lab directly for questions.

**A908 mutation cloning:**

Goals: Produce the A908 mutation with hyper LB/LB, As, and Enhanced As variants.

Methods:

Results:

**PER1 Glucocorticoid Response:**

Goals: Test precision cutting with the cas12 system in the PER1 Glucocorticoid response. It is known that two enhancers upstream of PER1 regulate the reduction of expression of PER1 as a consequence of glucocorticoid application. Essentially, if you cut out the glucocorticoid motif in the enhancers, PER1 cannot respond to glucocorticoid application. This has been shown with cas9 before, see Reddy et al, 2012. We attempted to replicate some of the findings in this paper using cas12.

Reddy TE, Gertz J, Crawford GE, Garabedian MJ, Myers RM. The hypersensitive glucocorticoid response specifically regulates period 1 and expression of circadian genes. Mol Cell Biol. 2012 Sep;32(18):3756-67. doi: 10.1128/MCB.00062-12. Epub 2012 Jul 16. PMID: 22801371; PMCID: PMC3430195.

Methods:

Results: