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:*

Gibson Assembly Cloning – Began by making the A908D mutation in dCas12a, As, Enhanced As, and hyper LB.

PCRs –

1. PSM749+750 with SM120 – size 996bp
2. PSM749+750 with SM132 – size 996
3. PSM241+751 with SM120 – size 1503
4. PSM751+752 with SM132 – size 1384
5. PSM66+753 with SM132 – size 1444
6. PGK1+4 with SM 79 – size 321
7. PGK2+3 with SM79 – size 1299

Reaction –

1. R1 – PCR 1, 3 + Xcm1 and Sbf2 in SM120, size 11669, 2375
2. R2 – PCR 2, 4, 5 + Xba1 and Xcm1 in SM120, size 10401, 3643
3. R3 – PCR 6, 7 + Asc1 and Nhe1 with SM79, size 12221, 1493

*Results:*

As and Enhanced As variants cloning and growth succeeded without issue. Confirmed with PCR on January 25th, 2023. The LB variant did not succeed the same process. Below, you can see the various reactions associated with this test. The very last column with the smudge near the bottom represents the R3, the LB variant. Both As variants (the two multi-cut columns next to the last one) cut successfully. All other cuts shown are individual PCRs in the sample described in methods above.

A picture containing indoor, dark

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**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 because 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. All tests were completed with a dAsCas12 system.

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*:

Designed primers to make plasmids for the cutting out of PER1 enhancers, sub-enhancer regions, PER1ko, and controls. These primers and plasmids are listed in the plasmid and primers folders. The plasmids consisted of the dAsCas12 construct with GFP and the guide RNAs which all had mCherry. These plasmids were transfected with lipofectamine into A549 and 293T cells. After the inclusion of both of these plasmid parts, we tested fluorescence under the fluorescent Gersbach lab microscope to confirm the presence of both parts of our system within cells. With this confirmation, cells were then subjected to puromycin selection. After selection, cells were extracted from media and went through both PCR and qPCR to test outputs.

A picture containing night sky

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Description automatically generated*Results*:

A picture containing night sky

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∆B Transfected – GFP Channel

∆B Transfected – mCherry Channel

∆B Transfected – GFP Channel

Red lights in the dark

Description automatically generated with medium confidenceGreen lights in the dark

Description automatically generated with medium confidenceTransfection efficiency is shown above in 293T cells before selection.

∆A Transfected – GFP Channel

∆A Transfected – mCherry Channel

Selection efficiency was extremely poor, such to the extent that it is not likely to be accurate. Further results from gels are shown below:

Gel – running PCR with A549 cells of the prime targets for the PER1 response, specifically, cutting out both enhancers (∆A+ ∆B), cutting out enhancer A (∆A), enhancer B (∆B), knocking out PER1, and a non-targeting control. Both the ∆A and ∆A+∆B cut well, but the PER1ko and ∆B did not.

1. A picture containing door, dark, night sky

   Description automatically generated1kb ladder
2. ∆A+ ∆B
3. ∆A
4. ∆B
5. PER1ko
6. NT

qPCR test in 293T cells:

PER1ko (left curve) against NT controls (right curve). This shows that NT controls have significantly higher expression in comparison to the PERko.

Chart

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Due to selection efficiency, the final results of the PCRs failed as shown in the gels below:

* Follow-up enhavery other column is genomic primer B (for targeting enhancer B), then NT control, for the following plasmids
  + A picture containing dark, night sky

    Description automatically generated∆A+∆B
  + ∆B
  + B1
  + B1-B2
  + B1-B3
  + B2-B4
  + B3-B4
  + B4
  + NT Transfection
* Follow up enhancer A tests, every other column is genomic primer A (for targeting enhancer A), then NT control, for the following plasmids
  + A picture containing text, dark, night sky

    Description automatically generated∆A+∆B
  + ∆A
  + NT