Cockayne Syndrome (CS) is a rare neurodegenerative disorder where patients exhibit sensitivity to light and premature aging. CS Type II, the congenital form of the disease, is caused by mutations in the ERCC6 gene [1]. ERCC6 regulates the transcription coupled nucleotide excision repair process and because DNA lesions are the result of UV radiation, loss of ERCC6 leads to an increased sensitivity to light. ERCC6 is also thought to play a role in repairing DNA damage caused by oxidative damage [2]. In order to determine how mutated ERCC6 leads to a premature aging phenotype in Cockayne Syndrome patients, the role of ERCC6 in repairing oxidative damage in mice must be determined.

Aim 1: Identify which ERCC6 protein domains are important for managing oxidative stress.

**Approach:** I will use SMART and Pfam to identify known protein domains in the ERCC6 protein. I will screen through ERCC6 mutations and find mutations that are within identified protein domains. Using CRIPSR/Cas9, I will create mice that have induced mutations in one ERCC6 protein domain, and I will do this for all identified protein domains. This will give mice lines that are each defective in one unique protein domain. I will induce oxidative stress in each mouse line, and will determine the cellular response indirectly by measuring levels of 8-hydroxydeoxyguanosine (8-OHdG), an oxidative derivative of guanosine, using the Cell Biolabs 8-OHdG RNA Damage ELISA [3,4].

**Rationale:** CSB has two major protein domains: a SNF2\_N domain and HELICc domain, which have different functions. Deducing which domain helps mitigate oxidative damage levels will help clarify how the protein interacts with oxidative damage.

**Hypothesis:** I hypothesize that because helicase domains play an integral role in DNA repair, a knockout of the ERCC6 helicase domain will result in the most accumulated oxidative damage.

Aim 2: Identify differential expression under oxidative stress conditions with and without ERCC6.

**Approach:** I will create ERCC6 knockout mice. I will induce oxidative stress in these mice and in controls and use Southern blotting to identify mitochondrial DNA degradation (oxidative damage leads to this) in both groups to confirm that the knockouts are deficient in managing oxidative damage [5]. I will use RNA sequencing to analyze levels of gene expression in both groups. I will create two separate gene expression profiles (one for the control and one for the knockout) and compare them to see how expression changes without ERCC6.

**Rationale:** By discovering what other genes are being expressed, I can learn more about what genes ERCC6 interacts with to fix oxidative stress. I can also see what genes compensate for ERCC6 loss and hopefully find candidate genes with therapeutic potential.

**Hypothesis:** I hypothesize that ERCC6 works as a recruiter of other proteins, and we will therefore see expression of excision repair proteins when ERCC6 is present. When it is knocked out, I expect to see some genes being overexpressed to replace the lost ERCC6 function.

References

[1] Laugel, V., Dalloz, C., Durand, M. et al. (2010). Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. *Hum. Mutat.*, 31: 113–126. doi:10.1002/humu.21154

[2] Stevnsner, T., Muftuoglu, M., Aamann, M. D., & Bohr, V. A. (2008). The role of Cockayne Syndrome group B (CSB) protein in base excision repair and aging. *Mechanisms of Ageing and Development*, 129(7-8), 441–448. <http://doi.org/10.1016/j.mad.2008.04.009>

[3] Cell Biolabs 8-OHG RNA Damage ELISA Product Page: <http://www.cellbiolabs.com/8-ohdg-dna-damage-elisa>

[4] Syslová, K., Böhmová, A., Mikoška, M., Kuzma, M., Pelclová, D., & Kačer, P. (2014). Multimarker Screening of Oxidative Stress in Aging. *Oxidative Medicine and Cellular Longevity,* ID 562860. doi:10.1155/2014/562860

[5] Shokolenko, I., Venediktova, N., Bochkareva, A., Wilson, G. L., & Alexeyev, M. F. (2009). Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Research*, *37*(8), 2539–2548. http://doi.org/10.1093/nar/gkp100