**Investigating Genetic Causes for Recurrent Glioblastoma Resistance to Temozolomide Treatment at Single Cell Resolution**

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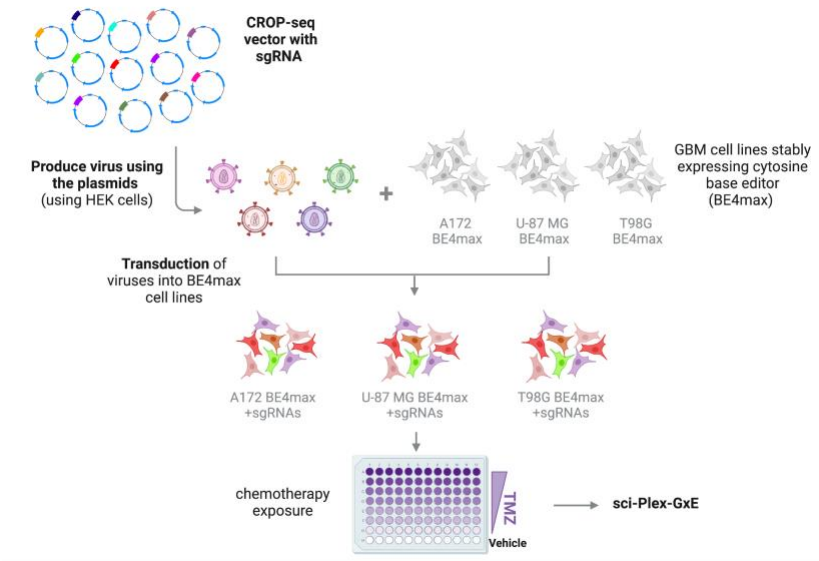
Glioblastoma (GBM) is a universally fatal brain cancer, for which the standard-of-care chemotherapeutic treatment for the primary tumor is temozolomide (TMZ). Unfortunately, after treatment of the primary tumor, virtually all patients have tumor recurrence with a chemoresistant phenotype. In cells that survive the TMZ treatment, it is known that the drug causes G:C to A:T base pair transitions to accumulate in the genome.[1] We hypothesize that these base pair transitions accumulate due to TMZ activity on the primary tumor, leading to TMZ resistance in recurrent GBM. To investigate this hypothesis we will utilize CRISPR base editing to model these G:C to A:T base pair transitions throughout the genome combined with high throughput screening. A recently developed high throughput single-cell resolution chemical screen known as sci-Plex-GxE combines single-cell genetic and chemical screening.[2] Sci-Plex-GxE has been demonstrated to work for CRISPRi, and we aim to extend it to CRISPR base editing. Our project uses the CRISPR base editor BE4Max, which converts a C:G base pair to a T:A base pair in an efficient and localized manner. For BE4max to target the correct, specific area of the genome an sgRNA is also required in this system. The vector used to transduce the sgRNA into the BE4max cell line is the CROP-seq vector. My focus this summer will be molecular cloning of a myriad of sgRNAs into the CROP-seq vector using Gibson ligation. The CROP-seq vector is a lentiviral vector. Therefore, we will transfect HEK293 cells with the CROP-seq+sgRNA construct, which will produce a pooled lentiviral library of our sgRNAs to be used for transduction into the BE4max cell lines.
Schematic demonstrating the project goals, including eventual transduction of the CROP-seq + sgRNA construct into BE4max-expressing GBM cell lines. Current summer work is highlighted in orange.

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(2) McFaline-Figueroa, J. L.; Srivatsan, S.; Hill, A. J.; Gasperini, M.; Jackson, D. L.; Saunders, L.; Domcke, S.; Regalado, S. G.; Lazarchuck, P.; Alvarez, S.; Monnat, R. J.; Shendure, J.; Trapnell, C. Multiplex Single-Cell Chemical Genomics Reveals the Kinase Dependence of the Response to Targeted Therapy. *bioRxiv* March 12, 2023, p 2023.03.10.531983. https://doi.org/10.1101/2023.03.10.531983.