

Perturbing in place: Systems for in situ and cell-type specific single-cell genetic screens

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GBM is a malignant brain tumor that develops from a specific type of brain cell called an astrocyte. GBMs are often very aggressive and spread to surrounding tissues. Only 3.3% and 1.2% of patients survive the illness after two and three years, respectively. ² Some of the most common symptoms are persistent headaches, seizures, memory loss, changes in mood and personality, and speech difficulty.¹ The complexity of the tumor itself is one of the causes of GBM's resistance to therapeutic intervention.

In this study, I applied various cloning methods to construct an in-situ cell-type specific gene editing tool by placing two adjacent sgRNA components into an avian retroviral vector. By modeling how genetic background alters evolution in response to drug exposures, this tool has the potential to identify opportunities for improved GBM therapies. As proof that the final Patchwork plasmid tool effectively delivers the expected genetic perturbations, we inserted either single guides HPRT1 or NTC in place of the sgRNA component. HPRT1 confers resistance to chemotherapy 6-TG and NTC is a non-targeting control. We transfected DF1 chicken cells via Lipofectamine and planned to use the virus produced by these cells to transduce DF1 and T98G dCas9 KRAB +/- tvA mCherry human GBM cells. Assuming the Patchwork plasmid is effective, only cells with tvA receptor (i.e. DF1 and T98G dCas9 KRAB + tvA mCherry) will receive the intended genetic perturbations. Consequently, T98G dCas9 KRAB + tvA mCherry cells transduced with Patchwork (HPRT) will be resistant to 6-TG treatment whereas T98G dCas9 KRAB cells will not.

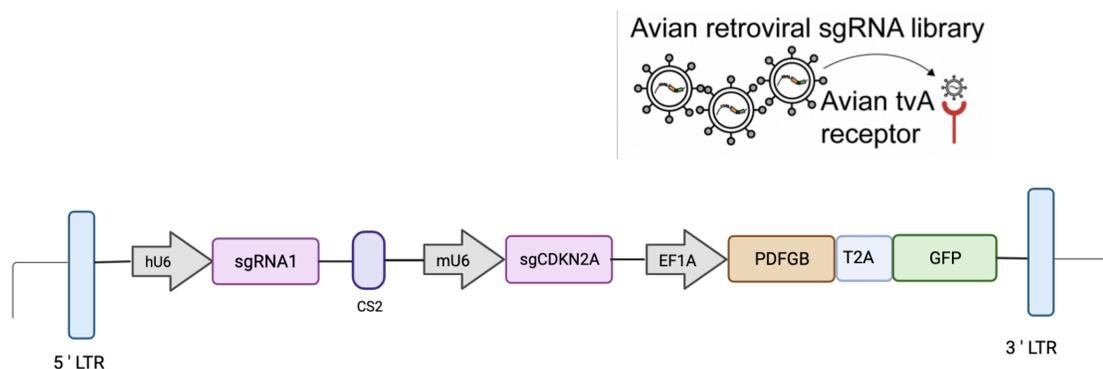


Figure 1. Final result after constructing avian retroviral vector.

References:

- U.S. Department of Health and Human Services. (n.d.). Glioblastoma. Genetic and Rare Diseases Information Center. Retrieved September 19, 2021, from <https://rarediseases.info.nih.gov/diseases/2491/glioblastoma>.

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