GUIDE-Seq/iGuide for CRISPR On/Off Target Analysis

To assess the risks of new human gene editing products, the FDA recommends using multiple orthogonal methods (e.g., in silico, biochemical, cellular-based assays) to identify putative off-target editing sites.1 GUIDE-Seq/iGuide2,3, is one of the techniques that frequently used by our gene and cell therapy clients to analyze on/off-target and effects of CRISPR-Cas9 gene editing. It provides valuable information about the specificity and accuracy of the CRISPR system, serving as a valuable aid in the selection of optimal gRNA, nucleases, and other CRISPR conditions, while also enabling the assessment of the safety of gene-editing-based therapeutics.

How does GUIDE-Seq work?

Cas9 Cleavage and dsDNA Tagging: The gRNA is designed to guide the Cas9 protein to the specific location on the DNA. The cell of interest is treated with the gRNA and other CRISPR reaction components, along with an abundance of a unique double-stranded short DNA. Subsequently, the genome repair mechanism incorporates this dsDNA into the genome on both sides of the potential on/off-target breaks.

Library Preparation and NGS: Genomic DNA is extracted from cells and fragmented with sonication. The resulting DNA fragments undergo a unique Guide-Seq Illumina sequencing library preparation, which utilizes nested PCRs to enrich DNA fragments with the unique dsDNA. The prepared libraries are subsequently sequenced using MiSeq or NextSeq platform.

Data Analysis: A customized Guide-Seq analysis pipeline is to be employed for on/off-target analysis. Analysis of data from negative and positive samples will establish a baseline, allowing the exclusion of false-positive off-target break sites. A comprehensive list of potential off-target sites will be reported.

Critical Success Factors

Indicator Cell Selection: The choice of an indicator cell type is critical in the design of an effective Guide–Seq experiment. It is crucial that the indicator cell line closely mirrors the anticipated gene editing product. Cell lines displaying a strong DNA repair response, such as HEK293 and CD4 T-cells, are advisable for heightened sensitivity in detecting off-target effects.

Control Design: To reduce sequencing background noise during the data analysis phase, it is crucial to incorporate suitable positive and negative controls in GUIDE-seq or iGUIDE experiments. This commonly entails sequencing gene-edited samples alongside both naïve cells and positive controls.

Avance Biosciences’ Experience

Avance Biosciences, a licensed Guide-Seq service provider, boasts extensive experience in assisting gene and cell therapy clients with the comprehensive characterization of their CRISPR-edited in vivo and ex vivo therapeutics. Our successful collaborations with prominent pharmaceutical and biopharmaceutical companies have contributed to the study of on/off-target profiles for their respective gene and cell therapy INDs. Reach out to us today to explore our on/off-target gene editing services and leverage our expertise in navigating the dynamic CRISPR gene editing domain.


* The GUIDE-Seq off-target next-generation sequencing analysis method is licensed from SeQure Dx.