



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

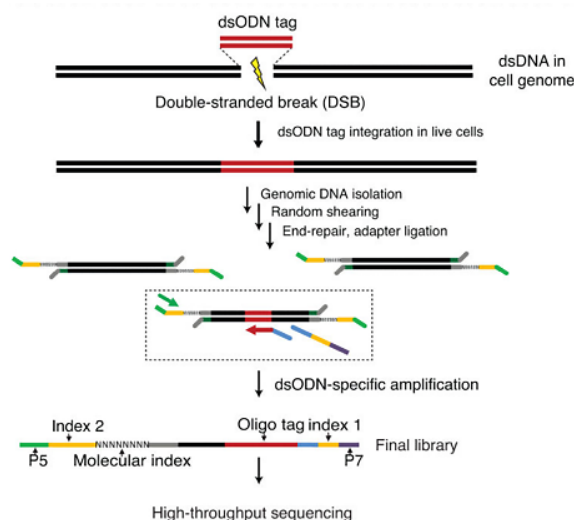
To assess the risks of new human gene editing products, the FDA recommends using multiple orthogonal methods (e.g., in silico, biochemical, cellular-based assays) to identify putative off-target editing sites. GUIDE-Seq/iGuide is one of the techniques that is frequently employed by our gene and cell therapy clients to analyze the on- and off-target 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.

Our Expertise

Avance Biosciences, a licensed Guide-Seq service provider, boasts extensive experience in assisting leading 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.

Workflow

The GUIDE-Seq workflow involves integrating a double-stranded oligodeoxynucleotide (dsODN) at double-stranded breaks, followed by targeted amplification and sequencing to precisely identify and map off-target cleavage sites in CRISPR-edited genomes.



Critical Success Factors

Indicator Cell Selection

- The choice of an indicator cell type is critical in the design of an effective Guide-Seq/iGuide 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 naive cells and positive controls.

Scan to learn more.

