AVANCE BIOSCIENCES

Gene Editing On/Off-Target Analysis

Gene editing is a powerful tool in cell and gene therapy, allowing for precise modifications to treat genetic disorders. Understanding the on-target and off-target effects of genome editing is crucial for ensuring the safety and efficacy of therapeutics. On-target effects are intended changes, while off-target effects are unintended alterations that can cause adverse outcomes. Comprehensive analysis of both is essential to prevent potential toxicity or malignancy. Rigorous on/off-target analysis ensures precise editing, addressing safety concerns and enhancing therapeutic efficacy, paving the way for reliable and successful treatments.

Our Expertise

Our expertise in gene editing on/off-target analysis is demonstrated through our support of numerous biotech and pharmaceutical companies. We utilize advanced techniques such as GUIDE-seq and rhAMPseq™ for comprehensive on/off-target screening, confirm these targets with amplicon sequencing, and assess gene editing translocations using ddPCR and amplicon sequencing. This thorough approach ensures precise and reliable analysis, contributing to the safety and efficacy of gene editing therapies.

Our On/Off Target Analysis Services

To meet evolving regulatory standards and ensure the safety and efficacy of gene editing-based therapies, researchers must take proactive measures to detect and mitigate off-target effects. Avance Biosciences offers a comprehensive range of services to support your development efforts.

Type	Description
In silico Prediction and rhAmpSeq™ Screening	Our team possesses substantial experience in utilizing IDT's rhAmpSeq™ technology to screen potential off-target sites through in silico analysis to predict potential off sites and design rhAmpSeq™ amplicon sequencing panel to experimentally evaluate the likelihood of the predicted sites.
GUIDE-Seq and iGUIDE Screening	We have substantial experience in employing Guide-Seq, licensed from SeQure-Dx, or iGUIDE, for cross-verifying potential off-target sites. In this analysis, a double-stranded DNA tag is integrated into double-strand breaks (DSBs) in the genome, followed by sequencing to identify DSB locations, analyzed together with negative controls and spike-in controls.
Amplicon Sequencing Confirmation	After On/Off target screening, specific PCR primer pairs are developed to further characterize the edited sites. Indels and point mutations will be confirmed, and their relative abundance will also be quantitated. Assay will be meticulously validated in full compliance with CGMP standards.
ddPCR Translocation Study	Leveraging Bio-Rad's QX200 ddPCR platform, we specialize in crafting highly sensitive assays specifically engineered to identify potential genome translocation events following gene editing. Our team boasts extensive experience in validating novel and complex assays in accordance with ICH guidelines, thus ensuring robust support for the characterization of gene editing cell therapy products.

Type	Description
Amplicon Sequencing Translocation Analysis	For translocation analysis of a system that involves many on/off targets, ddPCR method is not an optimum approach due to complexity in the design and validation of multiple ddPCR assays. Amplicon sequencing, on the other hand, can be used to evaluate translocation with high specificity and sensitivity.
Other Analytical Methods	Our expert scientific team is equipped to support you in developing alternative or complementary methodologies for on/off-target analysis tailored to your specific research and regulatory needs. With a skilled NGS team boasting a successful history of custom assay development based on established literature methods, we offer a range of options. Whether you require Digeome-seq, SITE-seq, CIRCLE-seq, or other advanced analysis methods, please reach out to us for further assistance.

Our Experience with Different Gene Editing Technologies

Avance has supported multiple biopharma clients working with various types of gene editing technologies. These technologies are summarized in the table below.

Type	Description
CRISPR-Cas9	CRISPR-Cas9 uses the Cas9 enzyme guided by a single guide RNA (sgRNA) to create targeted DSBs in DNA. However, off-target effects may occur when Cas9 cuts unintended sites, leading to unintended genetic changes.
CRISPR-Cas12a	CRISPR-Cas12a creates DSBs using a different recognition sequence and cutting mechanism compared to Cas9. While Cas12a is generally considered to have fewer off-target effects than Cas9, it is still possible for off-target events to occur.
CRISPR-CasX	CasX proteins are smaller in size, making them potentially easier to deliver into cells using various delivery mechanisms. CasX proteins retain robust DNA-cutting activity and high specificity.
Base Editing	Base editors, such as CRISPR-Cas9 or Cas12a fused with enzymes like cytidine or adenine deaminase, allow precise base conversions (e.g., C to T or A to G) without creating DSBs. Despite generally lower risks of off-target DSBs, base editing can still introduce off-target base changes.
Prime Editing	Prime editing, utilizing a modified Cas9 enzyme (nickase) and a reverse transcriptase, edits DNA without creating double-strand breaks (DSBs). While it generally exhibits fewer off-target effects compared to traditional CRISPR-Cas9 methods, there remains a possibility of unintended edits.
ARCUS	ARCUS gene editing is based on a naturally occurring genome-editing enzyme called I-CreI, derived from a type of homing endonuclease. ARCUS is engineered to be a highly specific and efficient genome-editing tool.
Zinc Finger Nucleases (ZFNs)	ZFNs are engineered DNA-binding proteins that create targeted double-strand breaks (DSBs) using zinc finger domains fused to a nuclease, allowing precise genetic modifications. However, off-target effects can occur if zinc finger domains bind unintended DNA sequences.
Transcription Activator-Like Effector Nucleases (TALENs)	TALENs utilize transcription activator-like effectors (TALEs) to bind specific DNA sequences and induce double-strand breaks (DSBs) using a nuclease domain, enabling precise genome editing. However, TALENs may exhibit off-target effects if TALEs bind to unintended DNA sequences.

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