AVANCE BIOSCIENCES

Integration Site Analysis

NGS-based integration site analysis is crucial for the development of biologics and gene and cell therapies. This advanced technique allows for the precise identification and mapping of viral vector integration sites within the host genome. By utilizing methods such as LAM-PCR, nrLAM-PCR, Target Enrichment Sequencing, and Whole Genome Sequencing (WGS), researchers can comprehensively assess the distribution and frequency of integration events. This information supports the optimization of vector design and delivery methods, ensuring that therapeutic genes are integrated in a controlled and predictable manner.

Our Expertise

Avance Biosciences has a long-standing interest in gene integration studies. Traditionally, we have utilized Southern blot analysis to determine the number of integration sites, supporting cell bank characterization for protein expression in GMP manufacturing. Building on our leadership in NGS applications for biologics development and manufacturing, we have established a series of advanced methods to help our clients understand integration sites. These methods are instrumental in characterizing CHO cell lines, autologous CAR-T cells, plasmid vaccines, among other applications. Through our comprehensive approach, we provide detailed insights that optimize vector design and delivery, ensuring precise and predictable gene integration for therapeutic development.

Methods	Descriptions	Applications
Target Sequencing by Hybridization	Illumina sequencing library is prepared following gDNA fragmentation. Library is amplified by PCR, followed by target pull down with hybridization .	Sensitive and specific method.
EPTS/LM-PCR by NGS	Nontarget DNA removal via magnetic extension primer tag selection (EPTS) precedes solid-phase ligation-mediated PCR (LM-PCR), followed by NGS sequencing	Sensitive and specific method.
LAM-PCR	Restriction digest of gDNA followed by linear amplification and PCR.	Obtaining high sensitivity and specificity is challenging.
nrLAM-PCR	Fragmentation instead of restriction digest of gDNA followed by linear amplification and PCR.	Specificity is improved compared to LAM-PCR, with improved sensitivity.
WGS	Genomic DNA will be extracted from host and sequenced on short-reads or long-reads NGS. Sequencing reads will be analyzed to construct sequences of integration sites.	Low sensitivity; suitable for charactering CHO and other cell lines with single clone origin.

Interesting Facts

Our Target Sequencing by Hybridization method has been validated and demonstrated to have high specificity and sensitivity. It does not rely on the assumption that an LTR is present at the integration sites, although we have predesigned LTR-specific hybridization probes to facilitate off-the-shelf analysis.

For our modified EPTS/LM-PCR method, after capturing fragments with integration sites and completing ligation, NGS amplicon sequencing is performed instead of nested PCR and primer walking with Sanger sequencing, improving assay sensitivity.

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