INTEGRATION SITE ANALYSIS FOR CELL BANKS AND CELL THERAPY LOTS

PathoQuest

PathoQuest is the leading developer and provider of NGS solutions for the biopharmaceutical industry. Our newly validated Integration Site Analysis method characterizes genetic modifications for clone selection, genetic stability and lot release testing.

GMP GOOD MANUFACTURING PRACTICE CERTIFIED

GMP Service available in 2024

MODALITIES TESTED

- Cell banks for mAbs and recombinant biologics, viral vectors, cell therapies, vaccines
- Cell therapy lots
- Gene therapies
- Cultured meat cell banks

BENEFITS OF THIS ASSAY

- ✓ Validated method
- ✓ Single nucleotide precision
- ✓ Resolve concatemers, truncations and inverted repeats more easily than short-read ISA

COMPARISON OF INTEGRATION SITE ANALYSIS METHODS

	FISH	*Sanger Sequencing*	Short-read NGS	Long-read ISA
Single Nucleotide Precision	☆☆☆☆☆	★★★★☆	★★★★★	★★★★★
Transgene location	★★☆☆☆	★★★☆☆	★★★★★	★★★★★
Concatemer & Inverted Repeats	☆☆☆☆☆	★☆☆☆☆	★★★☆☆	★★★★★

As part of the development of a cell line for manufacturing, candidate clones will be selected based on a number of attributes including production yield, number of transgene copies, as well as the structure and location of the transgene in the host cell genome. Indeed, a certain level of genetic characterization of the cell line is expected by the regulatory authorities. However, developers can benefit from a deeper understanding of the integration site of the transgene when making clone selection. For example, transgenes located in areas of instability or regions that are prone to epigenetic silencing may lead to a significant reduction in manufacturing yields over the cell line's manufacturing lifetime.

Classically, integration site analysis has relied on low resolution techniques such as fluorescent in situ hybridization (FISH) or southern blotting. Higher resolution approaches such as Sanger sequencing provide the required precision, but can struggle to identify complex integration events. A targeted analysis based on short-read NGS uses genome crosslinking to generate fragments up to 100kb either side of a known primer site. This method gives good information on insertion site, but can still struggle with concatemers, truncations and inverted repeats.

PathoQuest has validated an enhanced approach that uses targeted DNA cleavage combined with long read nanopore sequencing called long-read ISA. By targeting regions within the transgene expression cassette, both the transgene and the junction site can be comprehensively sequenced. Long read nanopore sequencing has the advantage over short-read NGS methods as it better characterizes concatemers, truncations and inverted repeats.

SAMPLE REQUIREMENTS	SHIPMENT & STORAGE	STANDARD TURNAROUND TIME	FASTTRACK TURNAROUND TIME	OUTPUT
$5x10^6$ cells (≥10µg DNA per sample) Backup sample required BSL1 or 2*	Dry Ice / -80°C	3 weeks**	contact us	GMP CoA Supplemental report detailing integration sites, orientation and configuration

* Biosafety level classifications can vary between regulatory authorities – contact PathoQuest to discuss.
** Turnaround time will depend on sample and testing requirements.

To find out how PathoQuest can better characterize your cells visit **www.pathoquest.com/ISA**

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