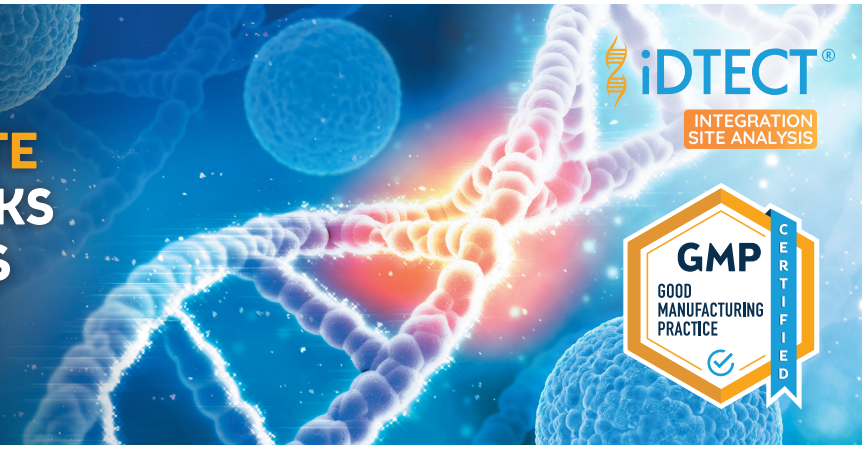
PathoQuest

iDTECT® INTEGRATION SITE ANALYSIS FOR CELL BANKS AND CELL THERAPY LOTS

Clone selection
Genetic stability
Lot release testing



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
- ✓ Resolve concatemers, truncations and inverted repeats more easily than short-read integration site analysis

COMPARISON OF INTEGRATION SITE ANALYSIS METHODS

	FISH	Sanger Sequencing	Short-read NGS	Long-read ISA
Integration Site Resolution	☆☆☆☆☆	★★★★☆	★★★★★	★★★★★
Transgene Location	★★☆☆☆	★★★☆☆	★★★★★	★★★★★
Concatemer & Inverted Repeats	☆☆☆☆☆	★☆☆☆☆	★★★☆☆	★★★★★

As part of the development of a cell line for manufacturing, candidate clones are 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. As such, 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 such as Targeted Locus Amplification (TLA) uses genome crosslinking to generate fragments up to 100kb either side of a known primer site. This method provides some information on insertion site, but still struggles with identification of concatemers, truncations and inverted repeats.

To provide increased resolution, PathoQuest has developed and validated a targeted, nanopore sequencing driven approach for Integration Site Analysis (ISA). This is the first GMP assay based on Oxford Nanopore's long-read sequencing technology, further enhancing the accuracy and reliability of genetic characterization. By targeting regions within the transgene expression cassette, both the transgene and it(s) associated junction site(s) can be comprehensively sequenced. Long read nanopore sequencing has the advantage over short-read ISA methods as it better characterizes concatemers, truncations and inverted repeats, providing a superior and more accurate result.

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	5 weeks**	contact us	GMP CoA Supplemental report detailing the vector insertion site(s) as well as transgene orientation, configuration and general copy number.

* Biosafety level classifications can vary between regulatory authorities – contact PathoQuest to discuss.
** Turnaround time per round.

To find out how PathoQuest can better characterize your cells, visit:
www.pathoquest.com/services/integration-site-analysis

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