PathoQuest

CASE STUDY

CLONALITY INVESTIGATION FOR A CHO PRODUCTION CELL LINE USING iDTECT® INTEGRATION SITE ANALYSIS

iDTECT® INTEGRATION SITE ANALYSIS

Clonality of Master Cell Banks (MCB) for the manufacture of biologics is critical to ensure the consistency of the process and product. The ICH Q5D[1] guideline states that, "for recombinant products, the cell substrate is the transfected cell containing the desired sequences, which has been cloned from a single cell progenitor." There are similar statements in the FDA PTC (1993)[2] and EMA/CHMP (2008)[3] guidelines. Typically, this is achieved at the cell line development stage through limiting dilutions, fluorescence-activated cells sorting (FACS), and single clone selection[4]. An image of the single cell is recorded for regulatory filings.

> *"Combined with other CMC data on the consistency of our product, this elegant assay provided the data we needed to proceed with our clinical trials and avoiding the need to re-derive our cell bank."*
> Late-stage biotech company, USA.

CHALLENGE AND OBJECTIVES

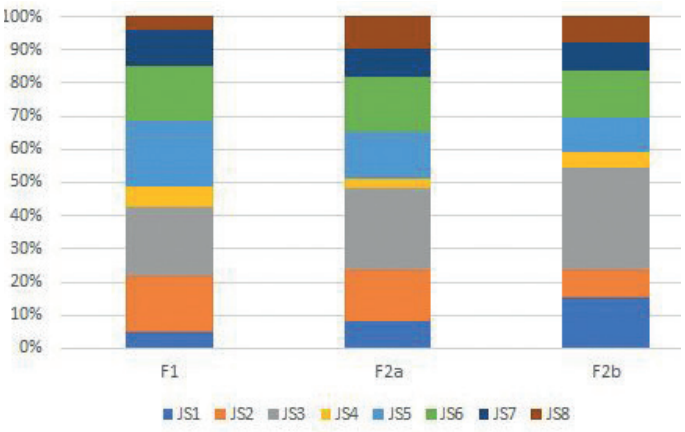
Supporting evidence for cell bank clonality is sometimes unavailable or incomplete. For example, at PathoQuest, we have worked with several customers who have acquired products where the image of the single cell has not been recorded during the original cell line development process. When developing a recombinant cell line, it is extremely unlikely that the transgene(s) will integrate into exactly the same site of the host genome in multiple cells. Thus a unique feature of a subclone is the presence of a consistent pattern of gene integration in all cells of the expanded population. This should also be reproducible in subclones of the presumed subclone, even if other phenotypic properties may vary.

SOLUTION

To meet this challenge, PathoQuest now offers the iDTECT® Integration Site Analysis assay. This method uses targeted DNA cleavage combined with nanopore sequencing to specifically analyze regions within the transgene expression cassette. This enables comprehensive interrogation of both the transgene and the host flanking sequences. In addition, the iDTECT® Integration Site Analysis assay has the advantage over more traditional short-read NGS methods that it can also provide improved characterization of concatemers, truncations, duplications and other structural variations and transgene configurations[5].

OUTCOMES AND BENEFITS

Our iDTECT® Integration Site Analysis assay provides a clear profile of the integration pattern within a cell line, providing confidence in the homogeneity of a subclone by tracking the unique integration sites present.



Example results for clonality investigation by iDTECT® Integration Site Analysis. F1 is the first generation parental subclone analysed, while F2a and F2b are second generation subclones derived from F1. All show consistently the same genome integration (= junction) sites and pattern.

REFERENCES

[1] ICH Q5D Derivation and Characterization of Cell Substrates Used for Production of Biotechnological / Biological Products, (1997)
[2] US FDA Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993).
[3] EMA/CHMP Guideline on Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Products (2008).
[4] Langsdorf et al, "Retrospective assessment of clonal origin of cell lines." Biotechnol Prog (2021);37(4): e3157.
[5] Leitner et al, "Nanopore Cas9-targeted sequencing enables accurate and simultaneous identification of transgene integration sites, their structure and epigenetic status in recombinant Chinese hamster ovary cells." Biotechnol Bioeng (2023 Sep);120(9):2403-241

To find out how we can help you
visit: **www.pathoquest.com/integration-site-analysis**
or contact us: **contact@pathoquest.com**

BioPark – BatB 11 Rue Watt 75013, Paris

466 Devon Park Dr, Wayne, PA 19087, USA

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