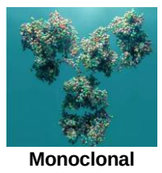
Unveiling Recombinant Cell Line Mysteries: Nanopore Cas9-Sequencing for Integration Site Analysis (ISA) in CHO Cells

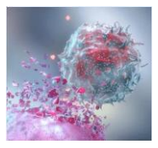
Crispin ZAVALA-ALVARADO[1], Stéphanie LEVON[1], Meissa BARBOUCHE[1], Sandrine MOREIRA[1] and Colette COTE[1]

[1] PathoQuest, Paris, France

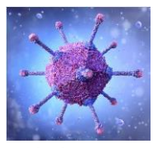
Here we detail a method to characterize the position of transgenes integrated into genetically altered cells & genetic modifications at specific loci within a host genome



Monoclonal Antibodies & Recombinant Proteins



Cell Therapy



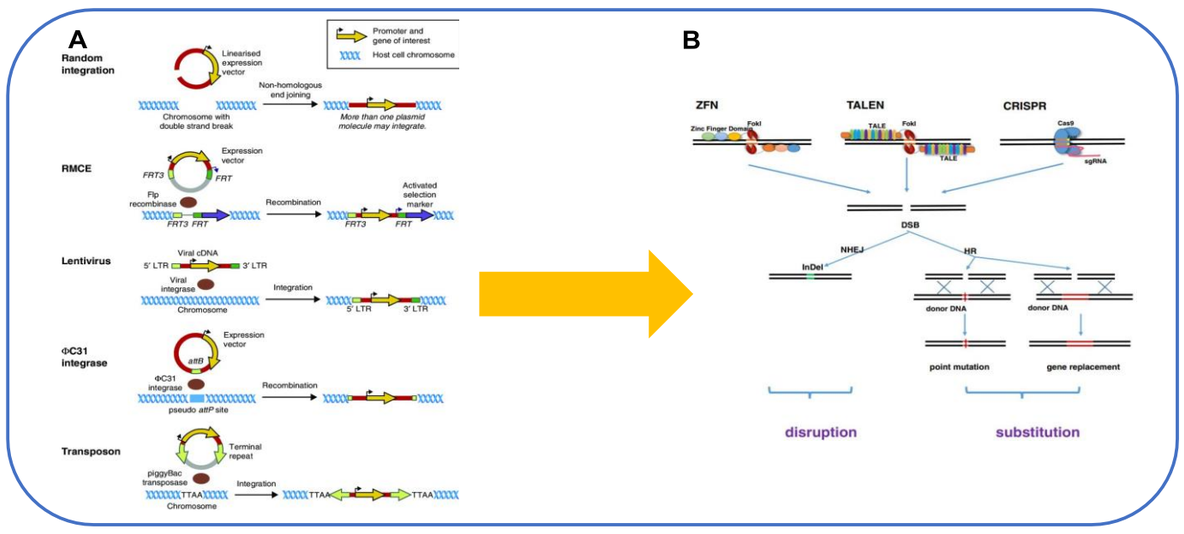
Viral Vectors

Vaccines

Therapeutic mRNAs

Cultivated Meat

Biological processes in biotechnology have evolved, but still rely heavily on genetically modified cell lines for their manufacture. These include the generation of antibodies, recombinant proteins, cell therapy, viral vectors, vaccines, RNA, and cultured meat.



Methods to modify genomes for therapeutic purposes continue to evolve. (A) Classical methods used to modify genomes. (B) Emerging techniques now in use include ZFNs, TALENs, and CRISPR/Cas9-mediated modifications. These methods achieve precise and efficient genome modification by inducing targeted DNA double-strand breaks.

It is critical (& required per ICH Q5B) to characterize both desired & undesired modifications within engineered cell lines.

This is traditionally achieved using a collection of classical, low-resolution assays including:

qPCR/qRT-PCR, Sanger Sequencing, Southern Blotting, FISH Analysis, SKY Karyotyping, *etc.*

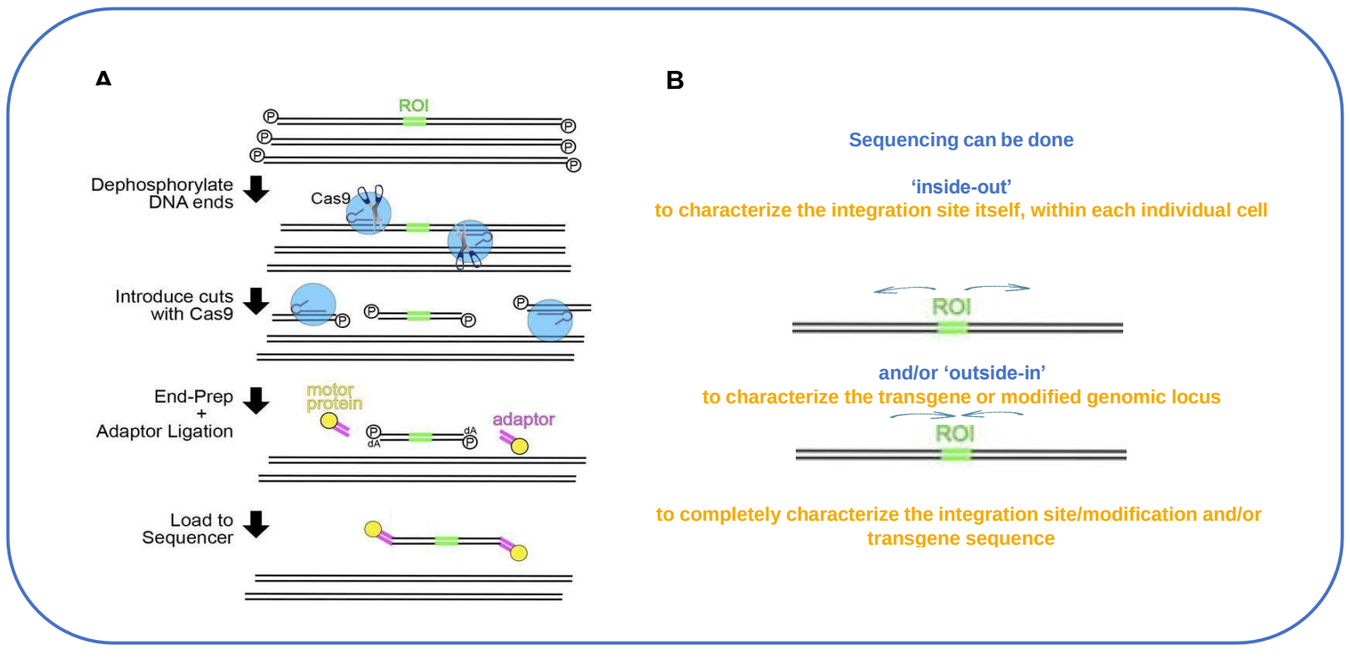
Chinese Hamster Ovary (CHO) cells: the ever-reliable production system(?)

Great for biopharmaceutical manufacturing…

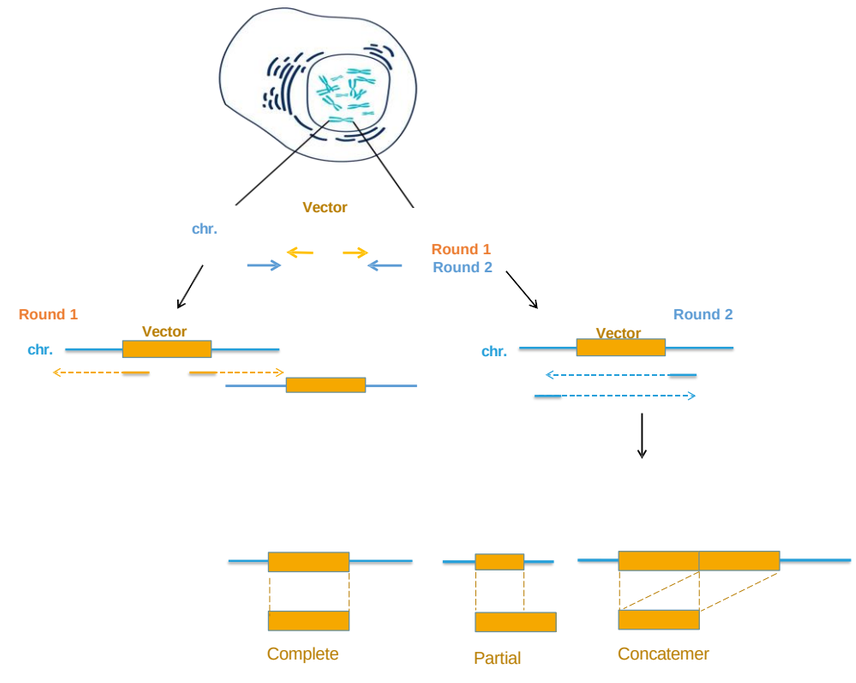
- Grow well in suspension cultures
- Short doubling times
- Highly adaptable at scale
- High producers of therapeutic proteins & monoclonal antibodies
- Very easy to engineer
- High safety profile (decades of supporting data)

…but not so great to characterize

- Cell lines & their derivatives have highly variable genomes, even from parent to child cell line
- Constant rearrangement and duplications
- Poor/incomplete reference sequences available
- Integrations can range from 1 to 100s depending on system
- Ideally want integration in sites with low variation and high transcriptional activity

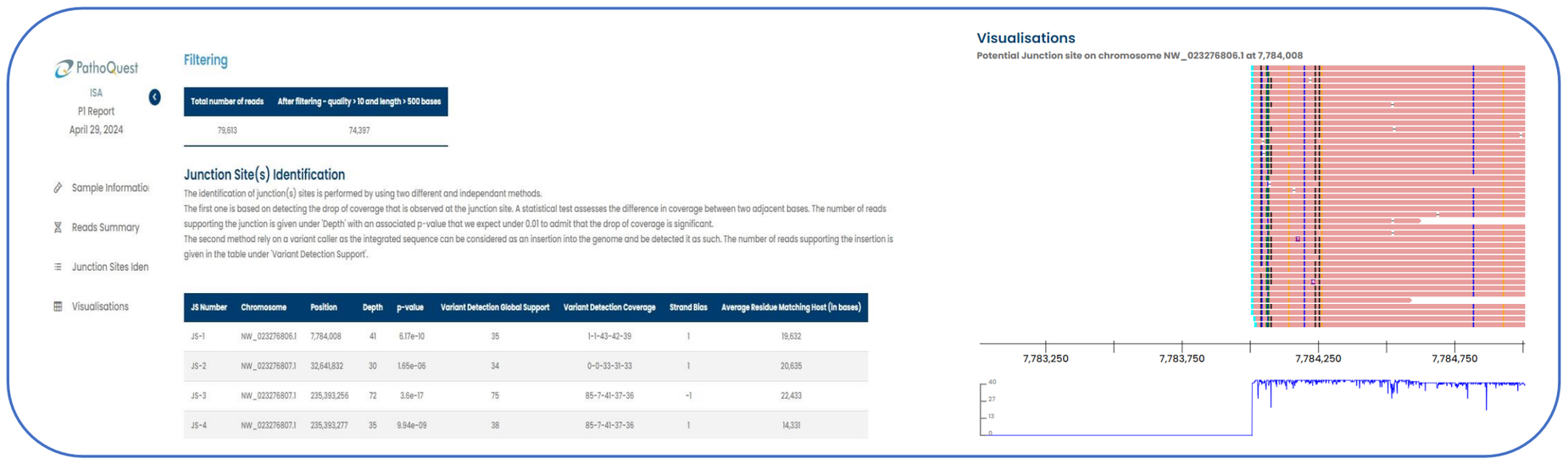


Nanopore Cas9-Sequencing iDTECT® ISA method is ideal for cell line characterization. (A) The Cas9-Sequencing method is illustrated schematically. The process begins with the design and synthesis of guide RNAs (gRNAs) targeting specific genomic loci of interest. These gRNAs, when combined with the Cas9 enzyme, enable targeted cleavage of DNA at desired locations. Following Cas9-mediated cleavage, the adaptors and motor proteins are ligated, and the sample is then loaded onto the GridION instrument. (B) Two rounds of sequencing may be performed to fully characterize the integration site(s): Inside-out (Round 1) and Outside-in (Round 2).



iDTECT® ISA method overview. This method provides information on the identification of insertion sites including the precise position of the junction within the cell (via Round 1) as well as the nature of the integration (via Round 2) such as vector copy number and overall configuration (fully inserted, partially inserted, or if a concatenator is present).

Results You Can Trust



iDTECT® ISA results include the chromosomal coordinates of the insertion sites, accompanied by statistical data supporting their detection, and a visual representation of the insertions, showcasing the position insertion, viewed as a drop in coverage

TAKE HOME MESSAGES:

- Robust, GMP-compliant assay combining the best of Oxford Nanopore Technologies' and PathoQuest's methodologies for integration site characterization.
- Ideal method for biopharmaceuticals to characterize and validate Master Cell Banks (MCB) and End of Production Cells (EOPC) per ICH Q5B requirements.
- Methodology has diverse applications, including characterization of engineered modifications targeted rare diseases. This could not only offer new insights into disease mechanisms but also potential therapeutic strategies for treatment development.