WHEN CORRECT ISN'T COMPLETE

Combining TLA and Nanopore Sequencing to Reveal Complex Targeted Integration Events in a CHO Cell Line

A biotechnology company developing a recombinant protein-producing CHO cell line used a landing pad-based targeting strategy to insert a new genetic construct into a genomic locus. The landing pad had been characterized in an earlier study, identifying the insert location and confirming that one copy of the construct had integrated successfully. However, confirming that the landing pad is present and correct is only part of the story. Developers must also determine whether the new genetic construct is present exclusively at the target locus and whether it integrated as intended, without rearrangements, fragmentation, or other unexpected structural changes. With regulatory agencies placing increasing scrutiny on cell bank characterization and genetic stability, the company partnered with Solvias to perform a comprehensive assessment of the integration event.

THE CHALLENGES

A landing pad is a predefined genomic site designed to accept future genetic insertions. The landing pad itself had already been verified, so the team knew where it was located and that it had integrated correctly. Later, the customer inserted a new construct into this landing pad through a targeting strategy. The challenge was to confirm:

- Did the new construct integrate correctly at the intended site?
- Are there any unintended integrations elsewhere in the genome?

Off-target integration events may be difficult to detect without a construct-targeted enrichment strategy, while structural alterations within the integrated construct may remain hidden when relying solely on conventional confirmation assays.

OUR COLLABORATIVE APPROACH

Step 1: TLA-Based Integration Site Analysis
Targeted Locus Amplification (TLA) works by using primers within the inserted construct, capturing DNA physically linked to that vector, and sequencing the captured fragments. Starting from the vector/transgene, TLA "walks outward" into the surrounding genome, revealing where the construct integrated, junction sequences, and nearby genomic context. Because TLA interrogates DNA connected to the vector sequence, it can identify additional insertion sites.

Step 2: Oxford Nanopore Technology (ONT) Long-Read Structural Reconstruction
Solvias performed Nanopore sequencing to reconstruct the integration locus. The long-read data confirmed the integration site identified by TLA and provided a detailed view of the insertion architecture.

THE RESULTS

The TLA analysis confirmed a single dominant integration site on chromosome 3, corresponding to the intended landing pad location. However, TLA also identified an unexpected vector-vector breakpoint within the integrated sequence. A breakpoint represents a novel junction between two DNA fragments that are not adjacent in the original plasmid. The presence of this novel junction indicated that the targeting event had not resulted in the straightforward insertion of an intact sequence as originally designed.

The Nanopore sequencing was then used to reconstruct the integration locus in greater detail. Rather than a single continuous copy of the vector spanning positions 1 through 13,505, as it would be expected for this complete vector insertion, two overlapping pieces of the plasmid were present and connected in an unexpected configuration. The first fragment corresponded to vector sequence position 13,505-8,671 and the second fragment corresponded to vector sequence position 9,852-1. Both fragments represented only a portion of the original vector rather than a complete copy.

Inserted fragment	Positions integrated with respective orientation
1	vectorX:13,505-8,671
2	vectorX:9,852-1

Together, TLA and Nanopore sequencing provided answers that neither technology could have delivered independently:

- TLA confirmed the insertion site location and ruled out off-target integration.
- Nanopore sequencing revealed the detailed architecture of the insertion.

The combined approach explained the vector-vector breakpoint detected by TLA and fully characterized the underlying structural rearrangement.

CONCLUSION

By leveraging the complementary strengths of TLA and Nanopore sequencing, Solvias provided both genomic localization and structural reconstruction, enabling a level of characterization that neither technology could achieve alone. For developers of engineered cell lines, gene therapies, and other advanced biological products, this integrated approach delivers the confidence needed to verify not only where an insertion occurred, but also what was actually inserted.

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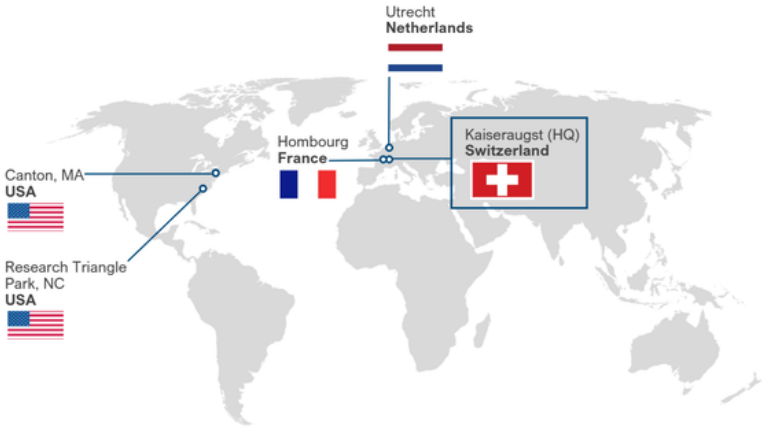
- Founded in 1999
- 800+ team members
- 175+ PhD-level scientists
- GMP, GLP, ISO9001 certified
- 23K m^2 of lab capacity
- 700+ customers worldwide
- 5 centers of excellence



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