Cell lines used for the production of biologic drugs are required to be fully characterized in accordance to the ICH guidelines (Q5D) by the regulatory bodies prior to their approval.

Moreover, throughout development, trial phases and production, master cell banks and working cell banks need to be monitored in order to verify their **clonality**, confirm the **stability** of the recombinant cassette, and rule out the presence of **adventitious agents such as viruses**.

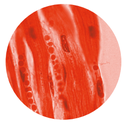
Similarly, certain **gene** and **cell therapy** products involve permanent genome modification of cells prior to grafting or injection.

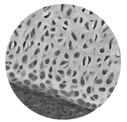
Therefore, transformation events and vectors are required to be characterized to evaluate the probability of side-effects of genome modification and to serve as long-term follow-ups for the stability of such modification.

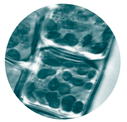
Several studies and working groups have acknowledged **next generation sequencing** (NGS) as a **powerful tool** for analyzing recombinant events and ensuring the absence of dangerous contaminations, such as microorganisms or unwanted cell lines.

IGATech
SERVING HUNGRY SCIENTISTS

IGATech has dedicated more than **10 years** to developing and delivering tools in the field of next generation sequencing and its applications. Our extensive expertise and comprehensive **technology platform offer the flexibility to analyze the structure** of different genomes and their modifications and the power to detect subtle contamination levels in challenging samples.







IGATech
SERVING HUNGRY SCIENTISTS

IGA Technology Services srl
c/o Parco Scientifico e Tecnologico Luigi Danieli
Via J. Linussio 51 - 33100 Udine (Italy)
Tel. +39 0432 629911

www.igatechnology.com
info@igatechnology.com

BIOPHARMA

Cell line characterization

Optimized NGS assays for characterization of biologics, gene therapy, regenerative medicine and biotech products

IGATech

NGS approaches can generate multiple levels of genomic data essential for drug development, from genomic modifications to transcriptome profiling and quantification, epigenetic modifications, and more

Traditional methods like *Sanger sequencing* can only identify a small fraction of the changes present. **NGS allows for a much more complete picture** of biologics during cell line development and for the assessment of safety of the resulting products. Compared to traditional methods, **NGS offers advantages in accuracy, sensitivity, and speed**, which have the potential to make a **significant impact on the fields of oncology, gene therapy and biologics production**.

We provide cell line assay services to contract research organizations and pharmaceuticals companies, clinics, academic institutions, and government agencies.

NEXT GENERATION SEQUENCING

QUALITY CONTROL

Monitor the genetic stability of the cell lines and bacterial strains over time and detect any genetic drift that may occur.

TRANSCRIPTOME ANALYSIS

Profile the transcriptome of master cell banks, which can provide insights into the functional state of the cell line and the pathways that are active.

CHARACTERIZATION

Identify and confirm the identity of the cell line and bacterial strains, including the species and subtype, as well as detecting any contamination or mutations that may have occurred during the development or propagation process.

DETECTING VIRAL CONTAMINATION

Detect the presence of any viral contaminants to ensure the safety of the cells and their products.

SERVICES LIST

Insertional mutagenesis

The precise determination of a transgene integration site is required for several technological applications. Registration and protection of new biotechnology products, GMO application, and risk assessment in gene and cell therapy are some of the areas that require detailed integration information to assess the level of safety of the products and to rule out potential activation of proto-oncogenes. Integration site analysis can also be used as a cell line development screening method to select the most promising clones. Depending on whether a homogeneous (clonal) cell line or a heterogeneous population of transformation events is under analysis, specific methods, and sequencing platforms (Illumina LAM-PCR or Nanopore-Cas9 system) can be applied to reach a complete picture of the insertional landscape of any given genetic background.

Bulk Insertional mutagenesis (LAM-PCR with short-reads): HT assay to determine the integration sites of tens of thousands of independent events from a bulk harvest.

HQ Integration analysis (Cas9-enriched long-reads): LT assay to precisely characterize a monoclonal integration event (any copy number) and determine the integration sites at base-pair resolution along with any local sequence variation. Long reads remove the issue of hard-to-map regions of the genome.

High quality cell line genome assembly

A complete cell line characterization is crucial for both early stages of cell line development and subsequent assessment of its stability. Long-read sequencing can now provide high-quality cell line genome assembly and annotation of gene models and regulatory elements, providing a solid base for cell line optimization, gene editing, metabolic engineering, and analytical development (including vector integrity, insertional profiling and clonality assessment).

Cell line high-quality genome assembly: Long-read based assembly coupled with chromosome reconstruction with Hi-C data. Can be substituted for FISH analysis to characterize large-scale genomic rearrangement and generate a new high-quality reference genome for a proprietary cell line.

Bacterial/yeast genome assembly w/ annotation: Fast-turnaround long-read based assembly to deliver chromosome and plasmid reconstruction and automated annotation.

Integrity analysis

The high depth of coverage provided by NGS can identify thousands of independent copies of the same integration cassette or vector within a given specimen. This allows for the detection of low frequency de novo mutations that might have occurred during cell line propagation or subcloning. We have established efficient protocols for the targeted sequencing of a region of interest (ROI) at very high depth. Specialized bioinformatics tools are then used to detect mutations occurring at very low frequency, avoiding false positives caused by sequencing errors or PCR artifacts.

Vector Cassette characterization (INTEGRITY): Fully sequence tens of kilobases of vector/cassette with high coverage to identify any mutation that can be causative of unwanted missense mutations.

Ultra-low mutation assay (Duplex-Seq): Targeted assay for detection of mutations as low as 0.1% in frequency in specific loci of interest. Sequencing of both DNA strands allows for in silico removal of any sequencing or PCR artifacts in variant calling.

Transcriptome solutions

High-throughput transcriptome profiling using RNA-sequencing (RNA-Seq), has become the preferred method to better understand the functional biology of disease. In cell line development and characterization of cell and gene-therapies, RNA profiling can deliver insight into cell-specific genes, variation in expression levels of genes under different growth conditions, or drug treatment. It can provide information on pathways involved in specific drug responses and in modeling the treatment or in the drug discovery process. Additionally, RNA-Seq is useful for quality control, enabling detection of bacterial, viral, or other eukaryotic cell contaminants in cell harvests.

Single-cell: Transcriptome characterization of cell populations to identify clusters of cells with specific expression profiles and cluster-specific biomarkers (gene expression). Single cell RNA-seq can also play an important role in studying cell line differentiation during cell therapies, in particular in hematopoiesis.

Ribosome profiling: A new frontier in RNA sequencing that allows for specific quantification of transcripts under active ribosome translation and reveals insight into new layers of ribosome regulation which have been shown to play an important role in several pathologies.

Long reads cDNA RNA-seq: the power of long-reads to obtain a complete characterization and quantification of isoforms.

Bulk RNA-seq: our standard RNA sequencing, also available with a dedicated workflow to generate libraries for ultra-low amount of RNA (picograms).