BioReliance

Biologics Safety Testing Services

Genetic Stability Testing for Cell Lines

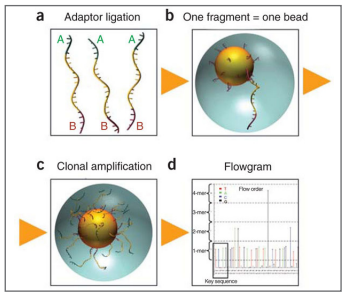
All genetic sequences are susceptible to random mutation. Therefore, regulations stipulate that cell lines used as biopharmaceutical manufacturing substrates must be tested to ensure that product safety and efficacy are not compromised by potential instability of the expression system. Testing methods for genetic stability must be adaptable to the expression system used and specific to the manufacturing process.

BioReliance provides a full range of assays for the characterization of manufacturing substrates in accordance with regulatory guidelines[6–9]. Most agencies recommend that production cell lines be analyzed at both the Master Cell Bank (MCB) and the End of Production Cell Bank (EOP) stage. The studies performed should provide information on the stability of the gene copy number and any nucleotide sequence changes which have occurred in the expression vector or inserted gene that may affect the protein produced. Custom genetic stability studies can be prepared upon request.

BioReliance provides the following GMP and GLP services for genetic stability analysis:

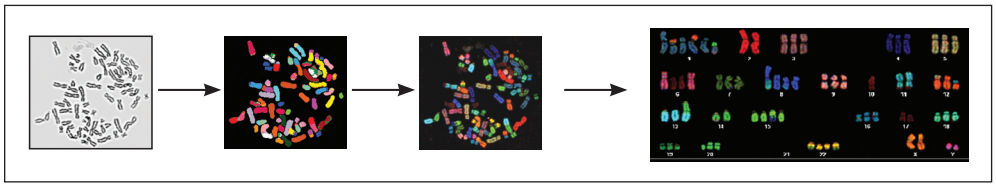
Eukaryotic systems	Prokaryotic systems
DNA & RNA sequencing (Sanger and MP-Seq™)	Genotypic / phenotypic assays
Karyology (Classical & SKY)	DNA sequencing (Sanger and MP-Seq™)
Copy number by Q-PCR	Copy number by Q-PCR
Isoenzyme analysis	Southern blotting
Northern & Southern blotting	Copy number determination by gel electrophoresis
Copy number determination (gel electrophoresis & FISH)	Restriction endonuclease fragment mapping
Northern blotting	Plasmid retention

MP-Seq™ utilizes cutting-edge nucleic acid sequencing technology



*(**a–d**) Genome Sequencer process–specific A and B adaptors are appended to each DNA fragment (**a**) to allow binding to DNA capture beads (**b**). The beads are placed into a water-in-oil emulsion that contains hundreds of thousands of PCR microreactors. Each microreactor contains all reagents necessary for PCR. The entire collection of millions of fragments and beads is amplified in parallel (**c**), with each fragment amplified without the introduction of competing or contaminating sequences. When sequenced on the Genome Sequencer FLX, each clonally amplified fragment generates its own unique sequence read, represented by a flowgram (**d**), with over 400,000 reads generated per instrument run. (Image courtesy of Roche.)*

Spectral Karyotyping



- Nucleic acid sequencing is performed to a GMP standard ensuring highest quality data
- Novel MP-Seq™ (massively parallel sequencing based) methods allow for unprecedented depth of sequence coverage
- Regulatory compliance advice offered by industry experts
- Industry leading turn-around times and reporting

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BioReliance

Biologics Safety Testing Services

Genetic Stability Testing for Cell Lines – Services Offered

Nucleic acid (DNA, RNA) sequencing: Detects point mutations or other changes in nucleotide sequence. Sequencing is performed by traditional capillary-based (Sanger) or by massively parallel sequencing based (MP-Seq™) methods. MP-Seq provides unparalleled depth of coverage by sequencing millions of bases per run (enough to sequence an entire bacterial genome).

Karyology: Metaphases are examined for chromosome number (modal chromosome number, frequency distribution and ploidy); banding pattern; quantification of abnormalities including chromosome and chromatid gaps and breaks; and verification of species/cell line. Analysis is offered by classical and SKY (fluorescent chromosome "paint") methods.

Copy number by Quantitative Polymerase Chain Reaction (Q-PCR): Allows the quantitative determination of the copy number of a nucleic acid target molecule. Calculation of the copy number is achieved by comparing the standard curve generated by known numbers of target molecules with unknown samples.

DNA fingerprinting: Cell line authentication and cross-contamination tests for cell lines of human, rodent and other species of origin can be performed by probing restricted cellular DNA with molecularly cloned, hypervariable sequences. The pattern of hybridization obtained is unique for each cell line.

Copy number determination (by gel electrophoresis): The size of an expression plasmid is determined by restriction endonuclease digestion followed by gel electrophoresis. Estimation of copy number is made in comparison to known concentrations of a reference plasmid and also by comparison to quantitative DNA markers.

Northern & Southern blotting: Northern blotting of mRNA with open reading frame derived probes allows the determination of size distribution and relative abundance of specific mRNA species. Southern blotting provides both structural analysis and copy number of the gene of interest.

Isoenzyme analysis: Isoenzymes from a cell line are electophoretically separated, stained and then compared to controls to determine species of origin and presence or absence of contaminating cells.

Fluorescence in situ hybridization studies (FISH): *In situ* hybridization is a powerful tool for localizing DNA sequences. The technique is sensitive, allowing sequences a few kilobases long to be routinely detected. The relative copy number distribution can be determined for sequences present at more than one site.

Plasmid retention: Determined by propagating a microbial cell line on the appropriate antibiotic selection media and then determining the percentage of total cells plated that survive.

Restriction endonuclease fragment mapping (gel electrophoresis): Restriction mapping detects gross DNA rearrangements or point mutations affecting specific nucleotide sequences recognized by restriction enzymes. The restriction endonuclease electrophoretic pattern is compared with a reference plasmid or against a suitable restriction endonuclease map.

Please contact us for more information or to discuss your genetic stability testing project.

References

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