Reliable and Efficient | Cell Line Genetic Stability Testing to Ensure Product Quality Consistency

Genetic Stability Testing for CHO Cell Line

The genetic stability and clonality of CHO producer cell lines are closely related to the quality consistency of monoclonal antibody products. BRC Biotech offers a comprehensive solutions for cell line development (CLD), clonality, and genetic stability of producer cell line, ensuring stable and consistent product quality.

Regulatory for Genetic Stability Testing

ICH harmonisation for better health

Cell substrate used in biologics production should be evaluated the genetic stability in both master cell bank (MCB) and end of production cell (EOPC) as described in regulatory guidelines, monitoring its genetic locus, coding sequence of the expression cassette or the RNA transcript, the copy number of the transgene. As well, restriction enzyme mapping is suggested to demonstrate integrity and integration pattern of transgene.

Refer to ICHQ5D

World Health Organization

The genetic stability is recommended in WHO by comparing the profiles in both MCB/WCB and ECB/EOPC. The aspects such as the copy number of GOI (gene of interest), the location of GOI, and the sequences using sanger sequencing should be included.

Refer to WHO TRS 978 / Annex3

Traditional Genetic Stability Testing Methods

Detection Items	Standard Solution
Sample for Detection	MCB, EOPC
Transcript Integrity	GOI and Flanking Sequencing RT-PCR
Genome Structure	Restriction enzyme analysis by Southern Blot
Copy number of insertion gene	qPCR
Transcription size	Northern blot
Cell Identification	CO1
Karyotype Analysis	Karyology

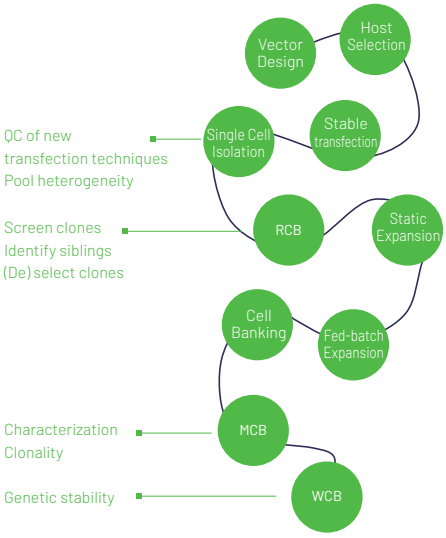
- GMP-compliant testing system
- Full-validated methodology
- Excellent project management
- Meet biological quality requirements
- Comply with the international regulations and guidance
- Professional regulatory /technical support

Genetic Profiling Analysis based on Next Generation Sequencing Technology (NGS)

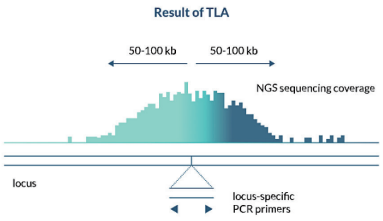
Recombinant-constructed Chinese hamster ovary cells (CHO) are widely used in the recombinant proteins and therapeutic antibodies production. The construction techniques might lead to off-target effects potentially, However, almost all construction techniques can potentially lead to off-target effects, multiple integration sites, and structural variations in the backbone sequence.

According to ICH Q5B and WHO guidelines, it is required to compare the genetic stability between master cell banks (MCBs) and end-of-production cell lines (EOPCs), including the accuracy of coding DNA sequences, insertion site structure stability, and insertion copy number, etc.

Compared with traditional Southern Blotting, FISH and amplicon sequencing, TLA (Targeted Locus Amplification) next generation sequencing technology will bring a new solution for genetic characterization analysis.



Cergentis' proprietary TLA-based assays offer clear advantages over standard approaches, most of which are unable to resolve all essential genetic characteristics in one experiment, e.g. as integration mutagenesis or sequence information. Briefly, genomic DNA is crosslinked, fragmented and circular DNA fragments are generated. The locus of interest is amplified and sequenced with NGS technology, and the sequence data are subsequently analyzed.



Comparing with traditional solutions, TLA-NGS could provide almost all genetic characters of producer cell lines. As the unique authorized domestic partner of Cergentis, BRC Biotech offers:

	Characterization	TLA+NGS	WGS	Amplicon/Capture based	qPCR/ddPCR	Southern Blot	FISH
	Integration site	√	–	–	X	X	√
	Structural variation	√	X	–	X	X	X
	Convergent integration	√	X	X	X	X	X
integrate a vector or GOI	SNVs	√	–	√	X	X	X
	Indels	√	–	–	X	X	X
	Rearrangement	√	X	X	X	√	X
	Tandem	√	√	X	X	X	X
	Copy number	–	√	–	√	X	X

Reference
①. Paula JP de Vree et al. Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping Nature Biotechnology 32: 1019-1025 (2014);
②. Jie Zhu & Diane Hatton New Mammalian Expression Systems. Advances in Biochemical Engineering/ Biotechnology 165: 9-50 (2016)
③. Justin Eyquem et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection Nature 543: 113-117 (2017)
④. 6.Dan Boyd et al. Isolation and characterization of a monoclonal antibody containing an extra heavy-light chain Fab arm mAbs 10(3):346-353 (2018)

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