WHOLE GENOME SEQUENCING

For Cell Authentication and Determination of Product Purity

WHAT IS WHOLE GENOME SEQUENCING (WGS)?

WGS is a powerful tool that combines:

NEXT-GENERATION SEQUENCING

Next-generation sequencing of the genomic DNA of the host cell line that contains the transgene vector combined with…

BIOINFORMATICS TOOLS

…bioinformatics tools that analyze the data to generate…

SURE*scan*® REPORT

…manufacturing and safety intelligence reports for biologic production campaigns/ manufacturing runs.

SELEXIS®

WGS GIVES ASSURANCE AND NEAR CERTAINTY ON A GENETIC LEVEL

- WGS has a 98% statistical confidence limit for cell authenticity
- WGS can determine clonality (product purity) with the same degree of certainty.

SELEXIS®

Using WGS, Selexis Can Generate Reports To:

SURE*scan*® REPORT

IDENTIFY AND PINPOINT INTEGRATION SITES of the transgene across the entire host genome and easily determine copy number of the gene coding sequence

DETECT FOR MUTATIONS (absences of insertions or deletions in the gene) and the integrity of the transgene

DETERMINE WHETHER YOUR CELL BANK IS MADE FROM A SINGLE CLONE OR NOT

And Why Is This Important?

Increasing number of mAb drugs being approved[1]

75

As a result, cell line authentication and purity is becoming a major concern for regulatory agencies

Task force meetings held WCBP (Washington, DC, January 2015)

Not being able to demonstrate the clonality of a cell bank can lead to significant loss of time and money

Contamination and misidentification can jeopardize a program's long years of research and development

1. Current 75 drugs as of Sept 24, 2016

Current EMA/FDA Requirements For Characterization of MCB and WCB

- Verify the correctness of the sequence of the gene coding for the recombinant protein product
- Demonstrate it is integrated in the host cell genome
- Differentiate recombination that is plasmid-to-plasmid vs plasmid-to genome
- Determine the copy number of the gene coding sequence
- Demonstrate absence of insertions or deletions in the gene
- Determine the number of integration sites
- Verify clonality

IDENTITY
phenotypic and genotypic characteristics

PURITY
no contamination by other cell lines; absence of adventitious agents: viruses, mycoplasma, bioburden

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Competitive Analysis

ONLY WGS CAN ENSURE THERE IS NO BIAS IN THE READ OUTS AS COMPARED TO mRNA OR PARTIALLY DIGESTED gDNA SEQUENCING

	WGS	PCR	FISH	Southern
Transgene integrity	✓			✓
Transgene purity	✓	✓	✓	✓
Integration site	✓			
Clonality	✓	✓		
Gene copy Nb	✓	✓	✓	✓
Gene survey	✓		✓	
Adventitous agents	✓			
Future Alterations	✓			

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WGS Overcomes Current Limitations of Other Methods

- ✓ Location of genomic integration sites
- ✓ Assessment of sequence integrity at fusions with genome
- ✓ Identification of possible adverse effects resulting from transgene integration
- ✓ Identification of rearrangements/deletion/structural changes in the genome
- ✓ Detection of any single nucleotide variants (SNV's)
- ✓ Identification of hotspots

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WGS Goes Beyond the Realm of Current Methods

Molecular characterization of inserted DNA and associated native flanking sequences consists of determining:

- the number of integration sites
- the gene copy number at each insertion site
- the DNA sequence of each inserted DNA

✓ WGS also provides a detailed analysis of any genetic rearrangements or genetic change (drift) that may have occurred at or in the integration site or elsewhere that can be deleterious to the life of the cell line

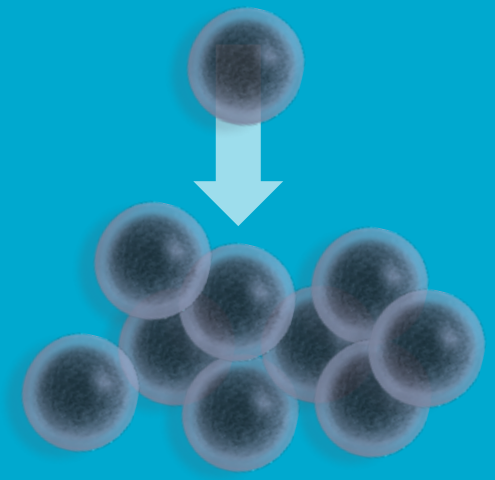
✓ WGS also provides a report on the presence of viral DNA content (adventitious agents) that can be used to monitor potential future viral infections in a process

What is a clone and is it important to ascertain monoclonality?

- ✓ Using WGS, it is possible to determine the lineage of a clade by analyzing the amount of SNV that have been fixed by evolution and thus to prove that a clade is derived from a single cell

- ✓ WGS is the only method that can generate such data as it is possible to sequence and analyze all genomes

DEFINITION OF A CLONE



A clonal population is a collection of individual cells that stemmed from a single cell but that bear different genomes (SNPs, Single Nucleotides Polymorphisms) due to mutations occurring at each cell division (population is a clade)

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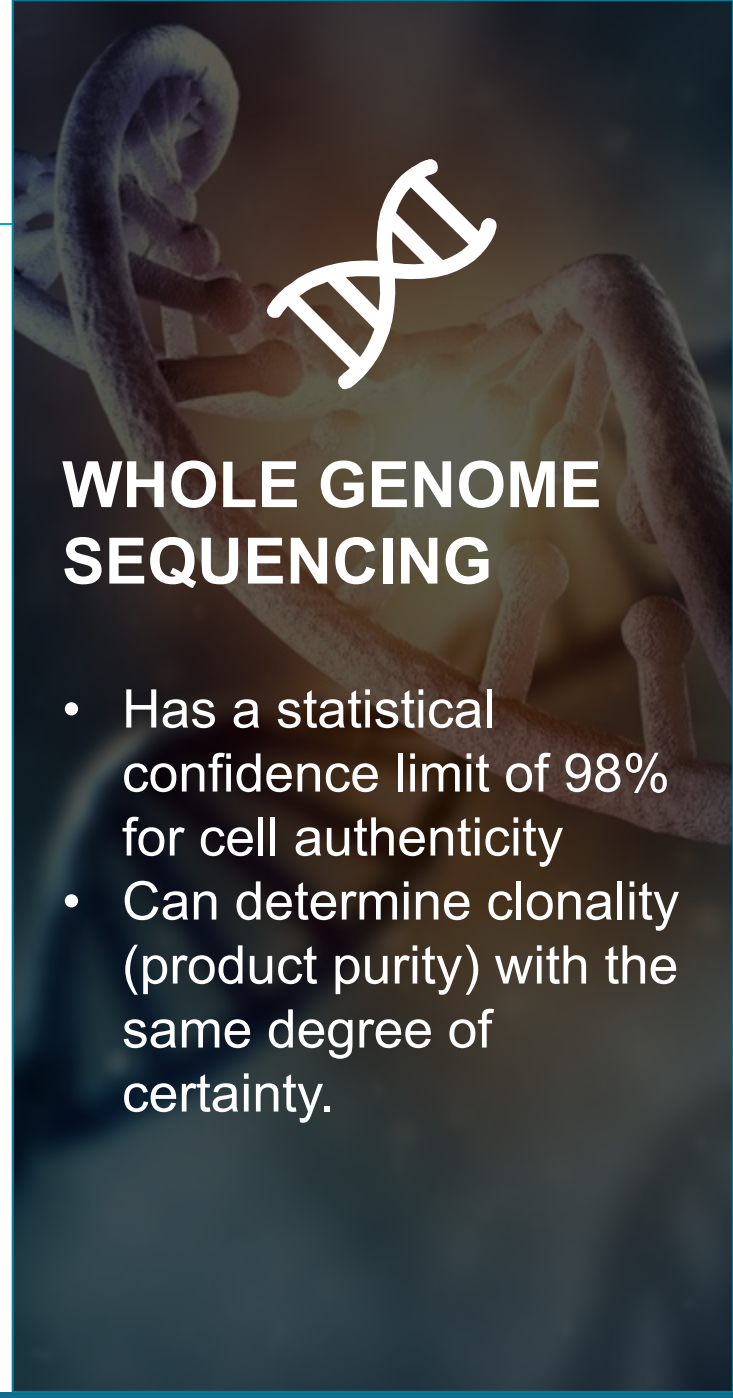
WGS Is More Reliable

- ✓ Analyze whole genomes
- ✓ No impact on integration sites analysis
- ✓ Assessment of the integrity of the transgene copies at all the locations in the genome
- ✓ Higher confidence, no experimental variation
- ✓ Speed, simplicity
- ✓ Greater predicted reproducibility



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The Selexis SURE*technology* Platform™ Offers Assurance

- Efficient therapeutic-producing clones generated with the SURE*technology* Platform
- SURE CHO-M Cell Line™ genome and transcriptome WGS-analyzed
- Early stage clone validation for transgene sequence integrity
- Routine characterization of clonal genome sequence identity
- Perspectives to identify adventitious agents and assess their absence

SELEXIS®

Driven by Excellence.
Guided by Science.

THANK YOU!

For more information: www.selexis.com

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