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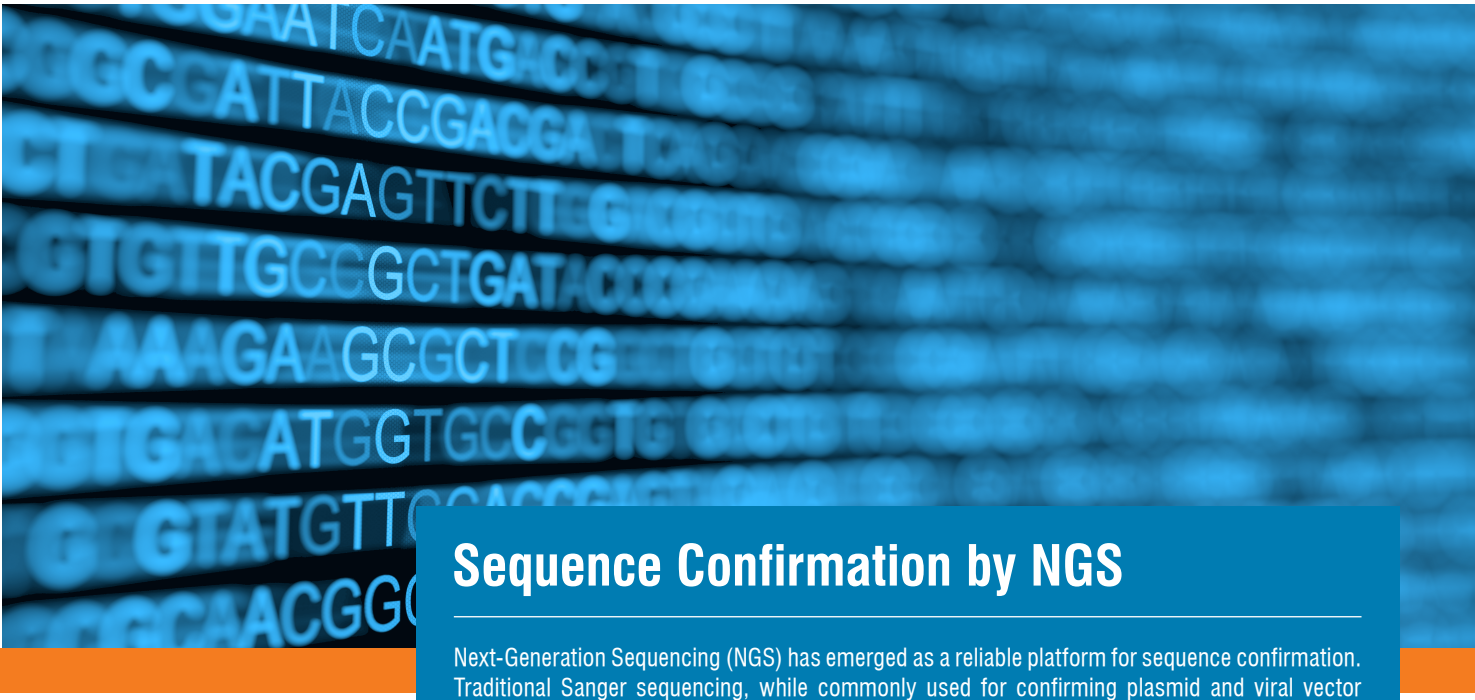

Sequence Confirmation by NGS

Next-Generation Sequencing (NGS) has emerged as a reliable platform for sequence confirmation. Traditional Sanger sequencing, while commonly used for confirming plasmid and viral vector sequences, often struggles with high-quality data production when dealing with hairpin structures or high GC content regions. It also falls short in detecting minor impurities and faces significant challenges with short sequences like sgRNAs used in CRISPR technology. The complexity further increases when sgRNA and mRNA are combined in a CRISPR complex. NGS excels at sequencing single or mixed sequences, offering comprehensive coverage, the ability to overcome secondary or tertiary structures, and the capability to detect minor impurities.

Our Expertise

At Avance Biosciences, we offer advanced and comprehensive NGS ID testing services for plasmids, viral vectors, sgRNA, and mRNA used in gene therapy, vaccine development, and other biopharmaceutical applications. As the first company in the world to introduce Illumina NGS platform in GMP QC environments back in 2012, we are pioneers in applying NGS technology to support drug development and manufacturing. Our NGS ID testing services include the following platform assays:

- Plasmid identification by NGS
- Viral vector identification by NGS
- sgRNA identification by NGS
- mRNA identification by NGS
- Phage Identification by NGS

Features

1. Rapid turnaround time from sample to data in less than 2 weeks
2. High coverage for even the most challenging secondary and tertiary structure or high GC regions
3. High sequence quality with a Q30 quality score
4. PCR-free sequencing library preparation when applicable
5. Validated sequencing analysis pipeline compliant with Part 11 regulations
6. Capable of evaluating DNA or RNA structural integrity
7. Capable of detecting potential contamination
8. Assay validation performed for CGMP sample release testing

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Points to Consider

- Sanger sequencing often fails to produce reliable and accurate results for difficult-to-sequence templates. Any attempt to dissociate challenging secondary and tertiary structures ultimately destabilizes sequencing primer annealing, leading to failed Sanger sequencing results.
- In contrast, random shearing in the NGS library preparation step can break up secondary structures, allowing successful sequencing through these regions, albeit at relatively lower coverage.
- Although NGS ID is a platform method that does not require special sequencing primers or conditions for different samples, it is still necessary to perform a bridge study to define the difficult-to-sequence regions. This helps establish appropriate acceptance criteria for those regions.
- While executing NGS for some of the identification tests is straightforward, ensuring compliance with FDA's data integrity and Part 11 requirements during data analysis is challenging. Avance Biosciences has developed and validated numerous NGS data analysis pipelines, each with enforced audit trails to meet regulatory standards. This approach ensures the reliability and traceability of data generated, supporting rigorous quality assurance in biopharmaceutical development and manufacturing.

Scan to learn more.

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