Center for Breakthrough Medicines

FREQUENTLY ASKED QUESTIONS

NEXT GENERATION SEQUENCING (NGS) FOR HIGH QUALITY PLASMID PRODUCTION

Listen to CBM's on-demand webinar: **High-Quality, Phase-Appropriate Plasmid Manufacturing to Accelerate Advanced Therapies**

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1. Why is NGS important for plasmid manufacturing?

Next-generation sequencing (NGS) is important for plasmid manufacturing because it allows for a more in-depth analysis of the plasmid product, which can provide valuable insights into its quality and stability, and impact to patient safety. NGS can detect a wide range of genetic variations, including single-nucleotide polymorphisms (SNPs), insertions, deletions, and rearrangements, which can be important in ensuring the safety and efficacy of the plasmid product.

2. Where can NGS be used in the plasmids development and manufacturing process?

NGS can be used as an important analytical tool at various stages of plasmid manufacturing, from early research and development to preclinical and clinical development, to routine production and quality control. By using NGS at each stage, manufacturers can monitor the plasmid product for potential genetic variations that could impact its safety or efficacy and make necessary adjustments to ensure that the final product meets the desired specifications.

3. Is NGS better than Sanger sequencing?

NGS and Sanger sequencing are both valuable techniques for DNA sequencing, but they differ in terms of their strengths and limitations. NGS can be performed without preexisting knowledge of the full plasmid sequence, there's no need to optimize multiple sequencing primers, and mutations affecting only a fraction of plasmid population can be precisely quantified. However, NGS data processing usually requires complex equipment and analytical software. Sanger sequencing is performed on 21CFR11-compiant platforms that deliver data more amenable to quality review, which can be important for regulatory compliance. Sanger sequencing is still considered the gold standard for sequencing small DNA molecules or verifying specific mutations in regions on interest, and it can provide a very high level of accuracy.

4. Can NGS Sequencing cover the entire plasmid?

NGS and Sanger sequencing are both valuable techniques for DNA sequencing, but they differ in terms of their strengths and limitations. NGS is generally considered to be faster, more accurate, and more cost-effective than Sanger sequencing for large-scale sequencing projects, as it can sequence millions of DNA fragments simultaneously. However, Sanger sequencing is still considered the gold standard for sequencing small DNA fragments or verifying specific mutations, and it can provide a high level of accuracy for specific regions of a plasmid.

Sanger sequencing is still considered the gold standard for sequencing small DNA fragments or verifying specific mutations, and it can provide a high level of accuracy for specific regions of a plasmid. Sanger sequencing is also more amenable to manual review and quality control, which can be important for regulatory compliance and ensuring the accuracy of the sequencing results.

5. Can NGS detect point mutations?

Illumina NGS sequencing always covers the entire plasmid, but some quality fall-off can be sometimes observed in specific plasmid regions (for example, homopolymer regions). In these rare cases, the use of multiple sequencing techniques, such as custom Sanger sequencing or long-read NGS (PacBio or Oxford Nanopore), may be necessary to reliably cover all regions of the plasmid. With this exception, NGS will provide a high level of accuracy and sensitivity for detecting genetic variations across the plasmid. By using a combination of sequencing approaches, manufacturers can obtain a more comprehensive view of the plasmid product, identify potential issues that may impact its quality or safety, and ensure regulatory compliance and quality oversight.

6. What NGS instruments are available at CBM?

Innovator companies can utilize sequencers from Illumina and PacBio at CBM.

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ABOUT THE CENTER FOR BREAKTHROUGH MEDICINES

CBM is a cell and gene therapy contract development and manufacturing organization (CDMO) based in the heart of Philadelphia's Cellicon Valley. CBM offers preclinical through commercial manufacturing capabilities including process development, plasmid DNA, viral vector manufacturing, cell banking, cell processing, and a full suite of complimentary testing and analytical capabilities. Through a single-source, end-to-end solution, CBM accelerates time to market without compromising quality.

Co-locating manufacturing, process development and analytical services prevents delays and handling errors. CBM's aim was to create one campus, one building, one manufacturing site. Our purpose built 700,000 sq. ft. manufacturing center is future-proofed in terms of infrastructure within and around the site. The current facility sits on over 1 million sq. ft. of space, allowing for future expansion to match the growing demand of the cell and gene therapy industry. Internally, the suites have been designed so that complementary services and labs are adjacent or nearby, to ensure we can accelerate time to market without compromising quality.

Scan or click the QR code to learn more about our plasmid manufacturing