

## NGS for Biologics Development Support

NGS represents a technological leap that has potential to revolutionize drug discovery and development.

Next Generation Sequencing (NGS) has the potential to unlock causative factors from our genetic material. NGS also represents a technological advancement using array-based sequencing that will improve many aspects of scientific research and advance the field of personalized medicine. Because of the sheer magnitude, precision and utilizable nature of the output data, a wealth of information will soon be available from studies completed using NGS.

Example NGS applications:

- CRISPR/cas-9 indel validation and quantitation
- Insertion site study
- Rare mutations in inserted gene
- Adventitious agents testing
- Viral seed sequencing
- E. coli phage identification
- Application Examples
- CRISPR indel amplicon sequencing

Avance Biosciences has developed NGS testing in support of our clients drug development initiatives and GMP manufacturing activities.

### 1. CRISPR indel amplicon sequencing

Working with a leading CRISPR technology-based biopharma company in Cambridge, MA, we developed a quantitative NGS amplicon sequencing assay for the client. In conjunction with a real-time qPCR biodistribution study, we analyzed hundreds of mouse tissue samples to verify that the CRISPR-induced indels and their copies are stable after being injected into the animals. A custom two-step minimum cycles of amplicon sequencing assay was developed and validated per ICH guidelines. More than 500 samples were tested under GLP.

### 2. Insertion site study

Traditionally, Southern blot analysis is the method of choice to satisfy FDA's requirements of characterizing cell line genetic stability. Avance Biosciences is now offering two NGS approaches to analyze the transgene insertion sites.

The first approach performs whole genome sequencing on gDNA extracted from the cell line of interest. Illumina's PCR-free method is used for library preparation with the sequencing performed on a HiSeq. The paired-end sequencing reads generated are analyzed using a proprietary NGS analysis pipeline involving multiple rounds of mapping and assembly to elucidate the insertion junctions. The main advantages of this approach are that a reference genome sequence is not required and this method can be easily validated.

The second approach involves ligation of an adaptor followed by a nested PCR to pull down sequence fragments with insertion junctions. Sequencing can be performed on an Illumina's MiSeq, and the paired-end sequencing reads analyzed to identify insertion junctions. The main advantage is that many samples can be analyzed easily once the assay is established. This method does require knowledge of the host genome sequence.

### 3. Adventitious agents testing

Working with a large pharma company in US, we engaged in an NGS viral testing assay development project to support the client's high-profile viral vaccine application. An assay was designed to test viruses with dsDNA, ssDNA, dsRNA, or ssRNA genomes with a single library preparation process. Multiple exemplary viruses were spiked into the testing sample as part of the qualification process. The qualified assay were used in several batches of sample testing in compliance with GMP requirements.

### 4. Rare mutations in inserted genes

Working with a fast-growing biopharma company in California, we have designed several amplicon sequencing assays to help the client confirm whether mutations exist in their prepared cell lines. Two-step limited cycle PCR was performed to generate NGS libraries, followed by paired-end sequencing performed on Illumina's MiSeq. More than a hundred cell line clonal samples were analyzed. Data analyses corroborated with the client's protein analysis and confirmed minor mutations in several positions.

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Avance Biosciences specializes in assay development, validation, and sample testing services supporting our clients for their drug development initiatives and GMP manufacturing activities. We are a leader in applying state-of-art technologies, such as next generation sequencing (NGS) and digital droplet PCR (ddPCR), to improve the effectiveness and efficiency of biologic tests.

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