

CDMOs & CROs: Leverage CRISPR Functional Assays

for Efficient Process Development and Manufacturing

Key Takeaways:

- CRISPR QC's *in vitro* CRISPR-Cas functional assays provide quantitative data for improved decision making at multiple timepoints in biologics manufacturing.
- The CRISPR Analytics Platform detects RNP formation, DNA binding, and cleavage activity for validating CRISPR designs.
- By identifying optimal enzyme activity and detecting lot-to-lot variability, CDMOs and CROs can pre-screen Cas9 and gRNAs at scale and improve gene editing performance and efficiency.

Successful CRISPR-Cas gene editing is critical for efficient cell and gene therapy discovery, development, and manufacturing. However, there is a lack of functional, effective *in vitro* QC assays to pre-screen or predict CRISPR gene editing efficiency before running costly experiments or production runs. Without QC incorporated into

the development and manufacturing pipeline, manufacturers are left to troubleshoot manufactured lots with suboptimal editing efficiencies and lot-to-lot product variation.

The introduction of robust quality control (QC) measures at critical points in the development pipeline can provide data to help CDMOs and CROs make critical decisions to move processes and products forward, rapidly scale up manufacturing, reduce costly time-consuming optimization cycles, and collect data to support strengthening regulatory requirements.

[The CRISPR Analytics Platform](#) offers a cutting-edge, label-free *in vitro* QC solution for quantifying CRISPR functional activity. [RNP formation](#), [DNA binding](#), and [cleavage activity](#) are measurable, enabling CDMOs and CROs to optimize their CRISPR-Cas systems early in the pre-clinical development or manufacturing process, before investing in costly experimentation and failed production runs.

In this case study, we demonstrate how the CRISPR Analytics Platform enabled the quick identification of Cas9 enzymes with superior cleavage activity, vendors that can deliver Cas9 enzymes with consistent lot-to-lot performance, and pinpoint lot-to-lot variation in gRNA performance - all of which can impact bioprocessing efficiency.

Case #1: Selecting a vendor with the highest Cas9 cleavage activity

Reliable and consistent Cas cleavage activity is crucial for CRISPR efficiency. One of our customers was experiencing inconsistent editing results when using Cas9 proteins from different vendors and needed to identify the vendor with the most active Cas9 enzyme. To explore this variability, we used the CRISPR Analytics Platform to quantify the amplicon cleavage activity of Cas9 proteins from multiple vendors in comparison to the customer's control Cas9 (Figure 1). The data revealed significant variability in cleavage activity across vendors, with one vendor's Cas9 demonstrating approximately three times higher average activity than the customer's control.

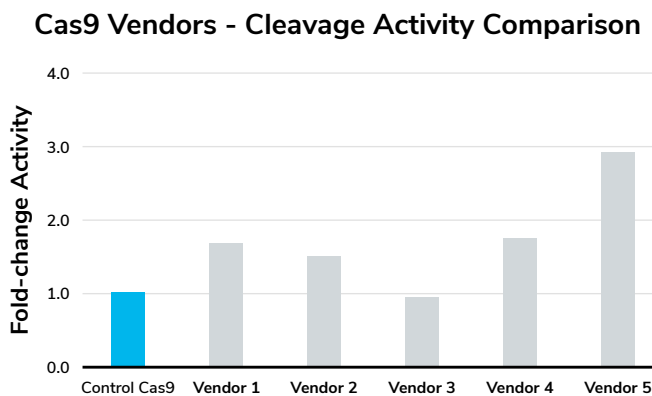


Figure 1. CRISPR QC compared the cutting activity among the customer's current Cas9 protein (control) and several other vendors. The data demonstrated that the Cas9 from Vendor 6 had almost 3x the activity as their current enzyme.

Based on CRISPR Analytics Platform insights, the customer identified an optimal Cas9 vendor for their experiments. Not only did this ensure the highest, most consistent cutting activity for their gene editing work, the customer also streamlined their efforts, saving valuable time and resources.

Case #2: Detecting Cas9 lot-to-lot variation during bioprocessing scale up

CDMOs must use starting materials with reliable performance to consistently manufacture biological materials at scale. One of our customers was generating product lots with variable gene editing performance as they scaled up their bioprocessing operations. To help them understand the root cause of the inconsistency, we partnered with them to measure the lot-to-lot cleavage performance of the Cas9 enzymes used in their processes. We used the CRISPR Analytics Platform to quantify the cutting activity of multiple lots of Cas9 from three different vendors in comparison to the customer's control Cas9. The data revealed lot-to-lot variability in Cas9 performance from two of the three vendors (Figure 2). Vendor A showed consistent cutting performance across 12 different lots, while lots from vendors B and C showed variable performance among the lots evaluated.

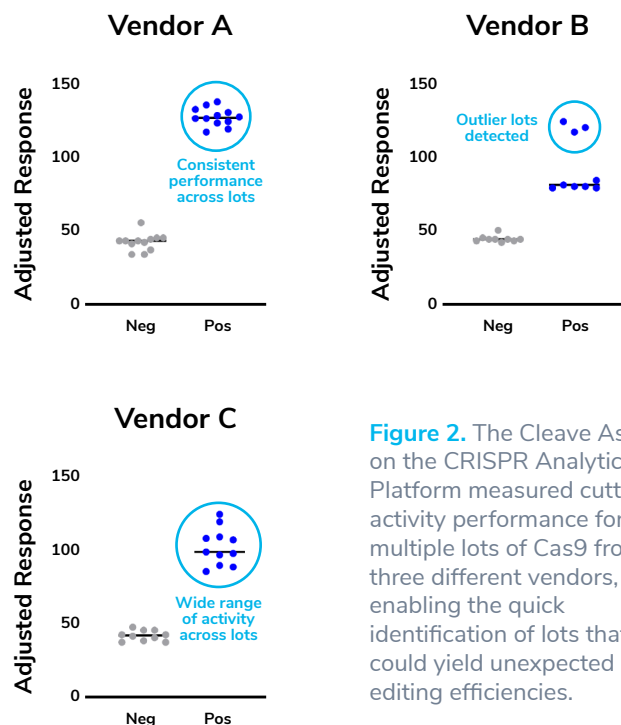


Figure 2. The Cleave Assay on the CRISPR Analytics Platform measured cutting activity performance for multiple lots of Cas9 from three different vendors, enabling the quick identification of lots that could yield unexpected editing efficiencies.

With new insights obtained with the CRISPR Analytics Platform, the customer quickly identified lots of Cas9 that may yield unexpected gene editing performance during bioprocessing. This assay enabled them to functionally qualify Cas9 lots and increase editing efficiency and consistency moving forward.

Case #3: Detecting gRNA lot-to-lot variation prior to manufacturing

The quality of starting materials can have a direct impact on biomanufacturing outcomes. Our customer, a CDMO, routinely received gRNA lots produced by their pharma customer for use in manufacturing. To help them verify that lots were of a consistent quality, we used CRISPR QC's RNP assay to test four different lots of gRNAs prepared at different manufacturing scales in comparison to a negative control. The data showed that one gRNA lot demonstrated much different RNP formation efficiency than the other three lots, indicating variation in the efficiency of the pharma customer's large-scale gRNA production processes (Figure 3).

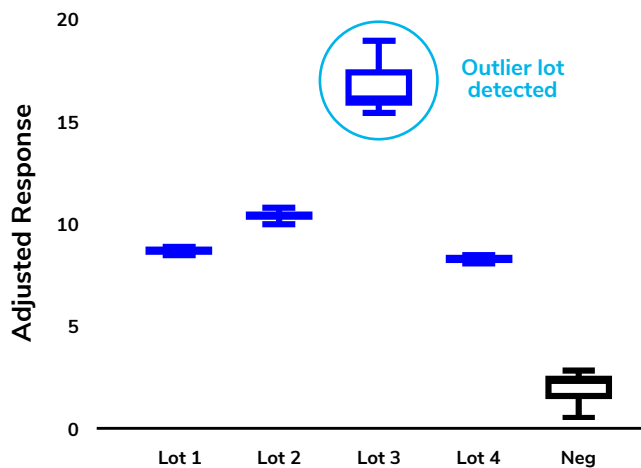


Figure 3. The RNP assay on the CRISPR Analytics Platform indicated an outlier in RNP formation efficiency among gRNA lots received as biomanufacturing starting materials.

This rapid and sensitive QC step enabled our CDMO customer to pre-screen gRNA lot quality prior to manufacturing and protect final product integrity. Simple *in vitro* QC steps can reduce failed lots, wasted materials, and product delivery timelines.

Accelerate Biologics Development and Manufacturing with CRISPR Functional Assays

The cases presented illustrate how the CRISPR Analytics Platform can provide a standardized, repeatable measure of CRISPR-Cas performance. Quantitative data on RNP formation, target DNA binding, and cleavage efficiency enable developers to increase their confidence in CRISPR designs before investing in costly production runs. CDMOs and CROs can partner with CRISPR QC to conduct high-throughput screening of Cas proteins and gRNAs for quality and consistency, identifying the optimal components for gene editing efficiency and biomanufacturing success.



Contact us to learn how the CRISPR Analytics Platform can increase your bioprocessing efficiency.

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