CRISPR QC | Case Study

In vitro CRISPR-Cas Cleavage Assays

Streamline Gene Editing Workflows

Key Takeaways:

- CRISPR QC's *in vitro* CRISPR-Cas cleavage assays can streamline CRISPR workflows by identifying optimal gRNAs and Cas proteins early in the process, saving time and resources typically spent on less effective candidates.
- The CRISPR Analytics Platform revealed significant differences in Cas9 protein activity across different vendors.
- *In vitro* cleavage data from the CRISPR Analytics Platform correlated positively with *in vivo* editing outcomes in transgenic mosquitoes, increasing customer confidence in proceeding with gene editing designs.

CRISPR-Cas gene editing has revolutionized biological research and genetic engineering, enabling the launch of a new class of powerful therapeutics: cell and gene therapies. CRISPR holds enormous potential, but its workflows are complex and quality control (QC) assays are often conducted only after completion of lengthy and expensive cell-based editing experiments. In addition, it is difficult to determine the root cause of failed or inefficient editing experiments. Troubleshooting poor gRNA design, RNP complex assembly, binding to the target sequence, or imprecise cleavage activity wastes time and resources.

Scientists need a rapid, highly sensitive *in vitro* QC assay, early in the developmental pipeline, that provides immediate feedback to optimize CRISPR-Cas performance. CRISPR QC's CRISPR Analytics Platform offers a real-time, label-free method to reliably quantify cleavage activity using amplicons representing target loci. This allows scientists to optimize their CRISPR designs and streamline CRISPR workflows before investing in costly cell-based assays.

In this case study, we explore two examples of how the CRISPR Analytics Platform successfully measured Cas cleavage activity in real time. In one instance, the platform showed variable Cas9 protein activity across vendors, and in the second example, measurements of *in vitro* CRISPR-Cas9 cleavage efficiency paralleled the *in vivo* editing outcomes in the customer's cell lines.

Case #1: Identifying a high-performing Cas9 protein among multiple vendors

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 as compared 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.

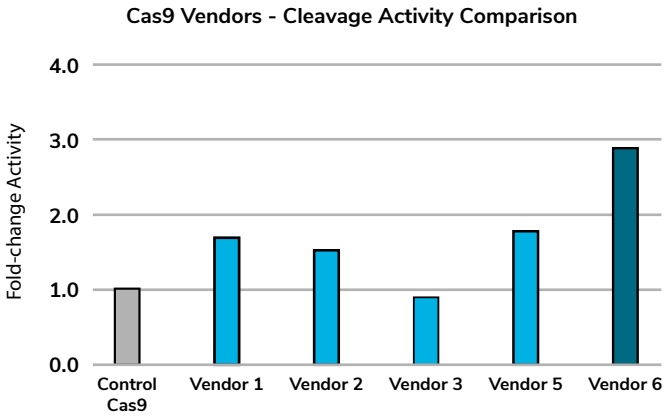


Figure 1. CRISPR QC ran analysis on the cutting activity between the customer's current Cas9 protein (control) and several other vendors. The data demonstrated that the Cas9 from Vendor 6 had almost 3x the activity as their current enzyme.

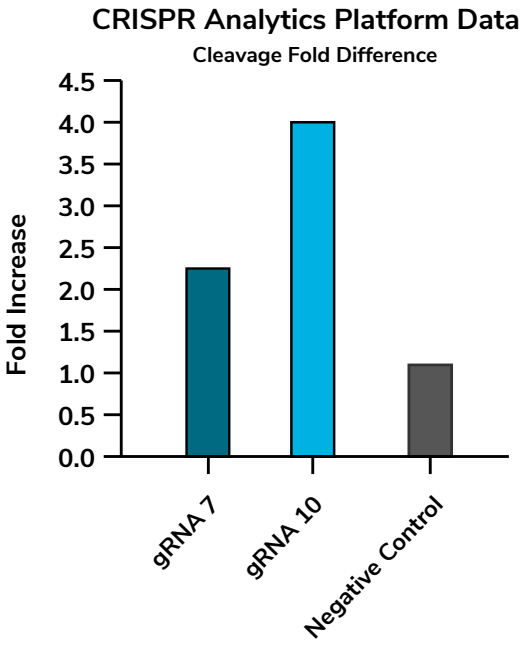
Based on CRISPR Analytics Platform insights, the customer identified an optimal Cas9 vendor for their experiments. Not only did this ensure the highest, most consistent cutting activity for their gene editing work, the customer also streamlined their efforts, saving valuable time and resources.

Case #2: Quantitative *in vitro* cleavage data correlates with *in vivo* editing outcomes

Researchers often rely on *in silico* CRISPR computational design algorithms to generate high-performing gRNAs, yet still experience editing failures *in vivo*. This was the case for another of our customers, a research team developing transgenic sterile male mosquitoes to combat malaria transmission. Their gene editing experiments were frequently failing resulting in substantial delays, with each failed experiment setting the project back by 8 to 12 months. Partnering with them on this issue, we used the CRISPR Analytics Platform to quantify the amplicon cleavage activity of two gRNA candidates. The data revealed that both gRNAs demonstrated cleavage activity at levels well above the negative control, with one gRNA showing approximately twice the activity of the other (Figure 2A).

CRISPR-QC Results Parallel *In-vivo* Editing Outcomes

A)



B)

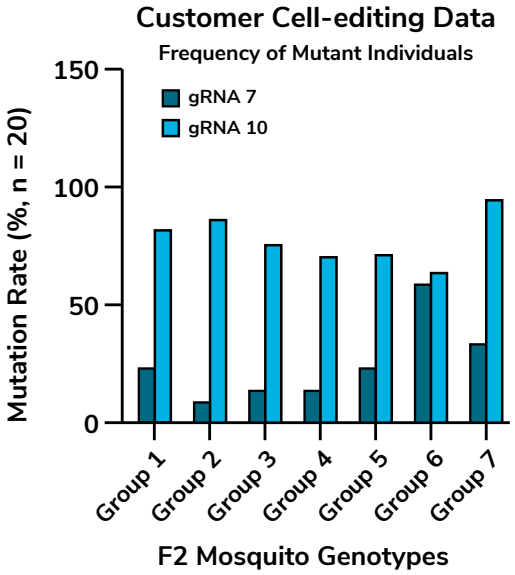


Figure 2. The CRISPR Analytics Platform was used to measure cleavage activity of 2 customer gRNAs. Cleavage rates are reported as fold change in solution conductance normalized to a non-cutting gRNA with the template (negative control) (A). Summary of the frequency of F2 mutant individuals for each candidate gRNA in the transgenic mosquito lines generated by the customer. PCR-based genotyping was utilized to calculate mutation rate (n=20 mosquitos/group).

Based on this data, the team generated transgenic mosquitoes using both gRNA designs. Subsequent PCR-based genotyping of the resulting progeny revealed a positive correlation between the *in vivo* mutational rate and the *in vitro* cleavage activity measured by the CRISPR Analytics Platform for a given gRNA (Figure 2B). This successful validation enabled the research team to proceed with confidence, potentially saving months of work and accelerating their project.

Streamlining CRISPR Workflows with CRISPR QC

The case studies presented here illustrate the transformative impact of the CRISPR Analytics Platform on CRISPR-Cas gene editing success. By partnering with CRISPR QC, scientists gain access to a powerful tool that provides unprecedented biological insights into cleavage efficiency, RNP formation, and target DNA binding. This data enables scientists to optimize gRNA designs, Cas protein selection, and experimental conditions before investing in costly and time-consuming cell-based assays. The CRISPR Analytics Platform provides new knowledge to streamline CRISPR workflows and shape CRISPR-Cas gene editing efforts for improved editing outcomes at the cellular level.

Contact us to learn how the CRISPR Analytics Platform can streamline your gene editing workflows.

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