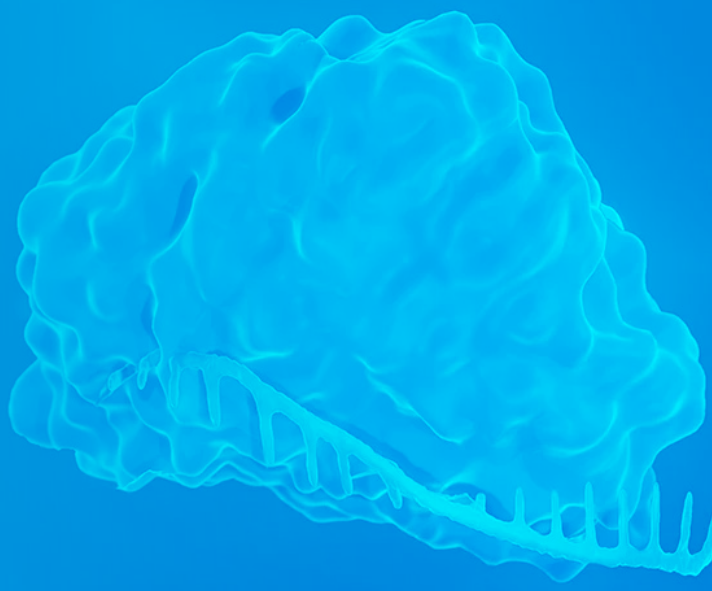
CRISPR QC | Case Study

Optimizing Ribonucleoprotein (RNP) Complexes

and Improving Multiplex Gene Editing Efficiency with Data-Driven gRNA Selection



Key Takeaways:

- A critical gap exists in the CRISPR workflow: the lack of an *in vitro* QC step.
- The CRISPR Analytics Platform is an *in vitro* tool that can measure CRISPR editing efficiency at multiple steps in the CRISPR workflow, including at RNP formation.
- Data-driven selection of stable RNP complexes and optimized combinations of gRNAs can enhance CRISPR efficiency, saving time and resources invested in costly troubleshooting of failed CRISPR experiments.

In recent years, CRISPR-Cas gene editing has revolutionized the field of genetic engineering, creating unprecedented opportunities for novel therapeutic development. Despite the rapidly increasing application of CRISPR-Cas technology, the established CRISPR workflow is missing *in vitro* quality control (QC) metrics, critical for increasing experimental success rates and accelerating therapeutic development.

While many software design tools and computational algorithms are available to help scientists streamline gRNA design, select a Cas protein optimal for an application, and predict on- and off-target editing potential[1], scientists have had few prior methodologies for CRISPR quality control. Frequently, gene editing success can only be confirmed following in vivo experimentation, leaving scientists to troubleshoot failed designs. The optimization process can result in a lengthy and expensive cycle of iterative design, synthesis, transfection condition optimization, and confirmatory testing.

Scientists need accurate, sensitive, and efficient *in vitro* QC steps early in the workflow that can provide tangible, modality-agnostic data that indicates the potential success of CRISPR designs. With this information, scientists are empowered to optimize their designs before investing time and effort into developing ribonucleoprotein (RNP) complexes that are likely to fail or fall short of performance benchmarks.

Here, we demonstrate how CRISPR QC's *in vitro* CRISPR Analytics Platform successfully measured binding efficiencies between candidate gRNAs and Cas9 and showed that failed editing experiments were the result of problems at the gRNA-Cas9 RNP complex formation step.

Case #1: Selecting an optimal gRNA

The optimal binding of a gRNA to Cas, forming a stable RNP complex, is critical for gene editing success. One of our customers noticed that the gRNA selections predicted by *in silico* algorithms were not leading to optimal editing outcomes. To investigate this issue, we used the CRISPR Analytics Platform to characterize how efficiently the customer's gRNA candidates (gRNAs 1-4) bound to Cas9 to form RNPs. The data generated (the measurement of a change in the surface potential of the CRISPR Analytics Platform Chip) revealed that gRNA 2 demonstrated significantly higher performance compared to the other candidates—a difference that was not predicted by design algorithms (Figure 1).

RNP Formation

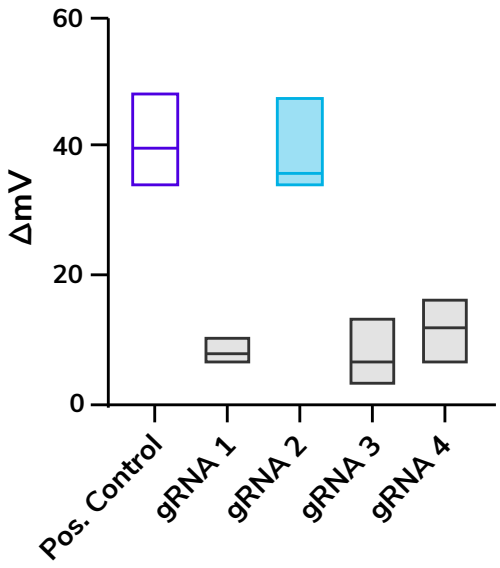


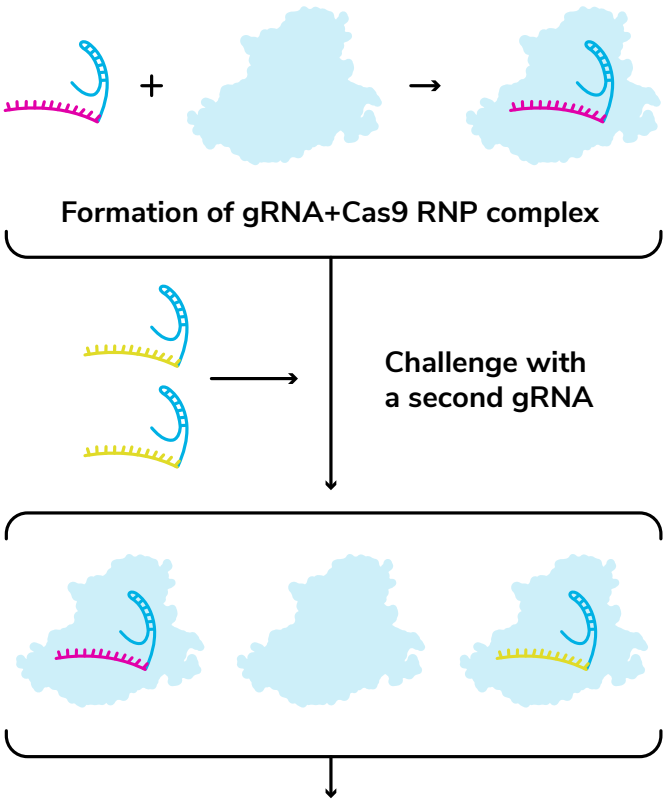
Figure 1. Candidate gRNAs were assayed for RNP formation with Cas9 using CRISPR-Chip analysis, along with a known positive control gRNA. Formation of RNP complexes by candidate gRNAs is reported as the measured change in solution conductance upon adding a particular gRNA to the assay, normalized to Cas9 immobilization for each candidate.

Based on this data, our customer was able to confidently identify an optimal gRNA design, saving them weeks of troubleshooting suboptimal gRNA candidates and streamlining their research.

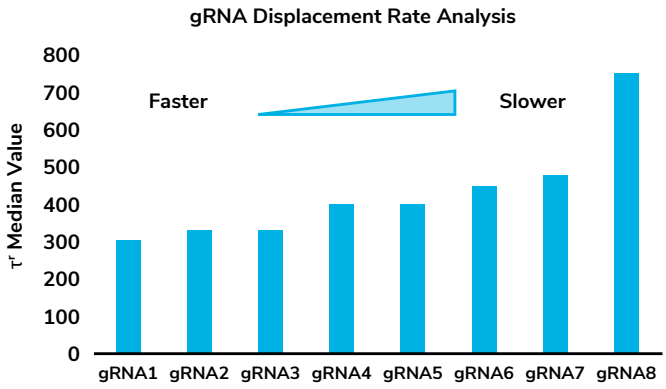
Case #2: Optimizing multiplex gene editing

Resolving inconsistencies in multiplex gene editing experiments can be difficult without quality control metrics. When one of our customers was experiencing inconsistencies in their multiplex gene editing experiments, we used the CRISPR Analytics Platform to characterize the ranking of binding kinetics between the customer's eight unique gRNAs (gRNA 1-8) and Cas9 (Figure 2). The data revealed that specific gRNA candidates had a much faster rate of displacement from Cas9 compared to others. Those with faster displacement rates were more vulnerable to replacement with gRNAs with a higher binding affinity for Cas9, leading to uneven RNP complex formation and skewed editing event frequencies.

A)



B)



Median of the rise time constant for gRNA displacement (τ^r) allows the speed (replacement capacity) at which each gRNA displaces a preloaded gRNA.

High τ^r value indicates longer time to displace preloaded gRNA.

Figure 2. An illustration of the kinetics of RNP formation and the resultant association, dissociation, and displacement events that may result upon challenge with a second gRNA (A). The CRISPR-Chip™ can measure displacement rates of gRNAs from Cas proteins providing an indication of binding affinity for gRNAs and stability of RNP complexes (B).

With this insight, the customer was able to identify gRNAs with similar displacement rates and combine them into an effective multiplex editing design. This data-driven approach allowed them to achieve more consistent and predictable results in their multiplex gene editing experiments.

Incorporating QC into your CRISPR workflow

Integrating quality control steps into your gene editing workflow can significantly enhance the efficiency and reliability of your research. *In vitro* testing provides a standardized, repeatable method to measure CRISPR-Cas activity, offering early insights that validate and increase confidence in your CRISPR designs. This proactive approach can save scientists time and money spent on troubleshooting failed experiments.

Moreover, as regulatory guidelines change, incorporating *in vitro* QC steps is becoming increasingly important. These metrics help ensure the accuracy of CRISPR-Cas edits and address safety concerns by characterizing the type of edit, frequency, and location of edits. By adopting these QC practices, scientists not only optimize their current research but also align with emerging standards in the development of cell and gene therapies.

Enhancing gene editing success with CRISPR QC

Partnering with CRISPR QC offers scientists a powerful advantage in optimizing their gene editing strategies. Our collaborative approach, coupled with our cutting-edge platform, allows for a comprehensive evaluation of each step in the CRISPR workflow. The CRISPR Analytics Platform enables scientists to quantitatively assess the binding and stability of gRNA and Cas nuclease complexes, determine binding efficiency between RNP complexes and target (amplicon) DNA, test RNP CRISPR complex cleavage frequencies in target and non-target DNA samples, and analyze key kinetic rankings underlying successful multiplex editing. By leveraging these tools, we can help scientists quickly obtain actionable insights to optimize their CRISPR strategies.

Whether you're working on basic research or developing therapeutic applications, our platform empowers you to make data-driven decisions, enhancing the efficiency and success rate of your gene editing projects. Contact us to learn how to leverage the CRISPR Analytics Platform for gene editing success.

Get Started

References

Zhang, Y., Zhao, G., Ahmed, FYH., et al. *In Silico* Method in CRISPR/Cas System: An Expedite and Powerful Booster. Front Oncol. 2020; Oct 2:10:584404. Doi:10:3389/fonc.2020.584404.