CRISPR QC | Case Study

Accelerating CRISPR-Cas Optimization

with Quantitative DNA Binding Analysis

Key Takeaways:

- The CRISPR Analytics Platform is an *in vitro* tool for measuring CRISPR editing efficiency, as well as, RNP formation and DNA binding real-time kinetics.
- The CRISPR Analytics Platform identified variable binding affinities among gRNAs designed for the same target DNA sequence.
- Real time data enables scientists to optimize designs before advancing to expensive cell-based experiments and provides a method for troubleshooting failed edits.

The incorporation of CRISPR-Cas gene editing techniques into genomic studies, sustainability research, and therapeutic development is increasing. However, as applications rapidly expand, the need for robust quality control (QC) metrics becomes increasingly important to ensure CRISPR efficiency, specificity, and safety.

Current CRISPR workflows lack effective *in vitro* QC methods for early assessment of gRNA performance, RNP formation and stability, RNP binding affinities for the target sequence, and enzyme cleavage efficiency, leading to wasted time and resources used in developing inefficient gRNAs or conducting costly trial-and-error optimization cycles.

To address this deficiency, CRISPR QC has developed the CRISPR Analytics Platform, a cutting-edge, label-free *in vitro* QC solution for quantifying DNA binding, enabling scientists to optimize their CRISPR-Cas systems early in the development process, before investing in costly cell-based experimentation.

Here, we demonstrate how the CRISPR Analytics Platform successfully detected differences in DNA binding affinities among candidate gRNAs complexed into ribonucleoproteins (RNPs) and enabled scientists to screen out low performance guides and select high-performance gRNA candidates with greater potential for success.

Case Study: Optimizing gRNA selection for improved CRISPR efficiency

CRISPR-Cas gene editing success relies heavily on the efficiency of gRNA design and the binding affinity for a gRNA-Cas complex to a target DNA sequence. One of our customers faced challenges in selecting an optimal gRNA design for their application. Initial gRNA candidates, designed using *in silico* tools, showed inconsistent binding and editing efficiencies despite being designed to target the same genomic region. To address this issue, we used the CRISPR Analytics Platform's DNA Binding assay to assess the binding affinities of several gRNA candidates complexed with Cas9 to target DNA amplicons. The assay included a positive control gRNA and a scrambled negative control for comparison. Results revealed significant variability in target DNA binding affinities among the gRNA candidates, with two gRNAs (5 and 6) demonstrating superior binding compared to others (**Figure 1**).

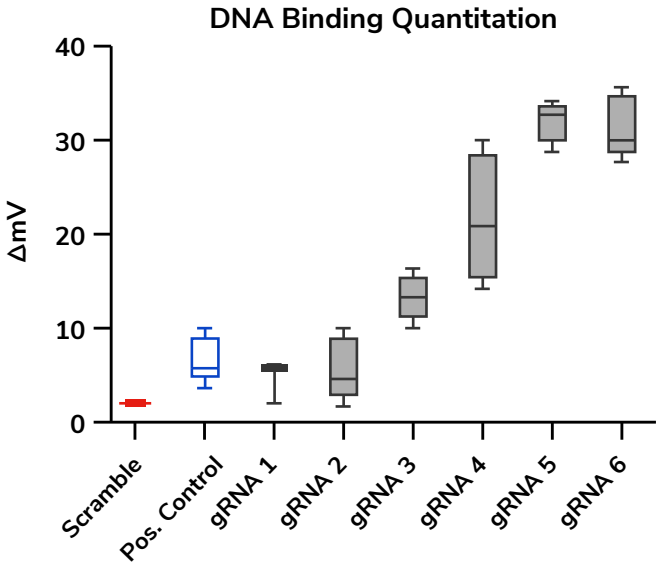

Figure 1. The CRISPR Analytics Platform was used to measure DNA target binding for several gRNA candidates, alongside a positive control gRNA and a scrambled negative control gRNA. Binding to DNA target amplicons by immobilized gRNA-Cas9 RNP complexes (n=4) is reported as the measured change in solution conductance normalized to RNP formation for each candidate.

Informed with quantitative DNA binding data, our customer was able to swiftly select optimal gRNA candidates for further development. This data-driven decision saved valuable time and resources by eliminating the need for extensive trial-and-error experimentation.

Streamlining CRISPR workflows with *in vitro* CRISPR QC assays

Incorporating real time, *in vitro* quality control steps into gene editing workflows offers significant advantages for scientists engaged in CRISPR-Cas applications. With a standardized and reproducible method to robustly measure CRISPR-Cas activity, scientists can gain early insights that increase confidence in their CRISPR designs before moving forward with costly development. This approach can also save time and resources typically spent on troubleshooting failed edits.

Partnering with CRISPR QC and leveraging the CRISPR Analytics Platform can significantly enhance scientists' ability to optimize their CRISPR strategies. This powerful platform enables scientists to conduct rapid, high-throughput CRISPR screening to identify and select the most promising gRNAs for further development, quantitatively assess the binding and stability of gRNA and Cas protein combinations, and determine binding interactions between RNP complexes and target DNA sequences. By measuring key relative kinetics, the platform also supports successful multiplex editing strategies. We partner with you to provide actionable insights for optimizing your CRISPR experiments.

The CRISPR Analytics Platform provides actionable insights that empower scientists to make data-driven decisions, ultimately accelerating research progress and improving the overall efficiency of CRISPR-based experiments.

Contact us to learn how you can enlist our CRISPR analytical services to accelerate gene editing success.

Get Started