Benefit of Quality Control in CRISPR Techniques

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Quality control (QC) analysis is an integral part of nucleic acid and CRISPR applications. Utilizing QC checkpoints at critical junctures in the CRISPR process eliminates unsuitable samples, saves time and money, and maximizes resources. This in turn increases confidence in results and builds consistency in the workflow. Advanced Analytical Technologies, Inc. provides reliable and consistent quality analysis with parallel capillary electrophoresis (CE) instruments. CE instruments provide extreme sensitivity and resolution that make them ideal for QC in CRISPR experiments, from detection of minimal degradation in sgRNA to detection of dsDNA starting product when generating ssDNA end-product for HDR, to determining the length of polyadenylated Cas9 mRNA.

Importance of Quality Control Analysis

The importance of quality control (QC) in laboratory processes is often overlooked, but should be considered an essential part of every procedure. Parallel capillary electrophoresis instruments from Advanced Analytical Technologies, Inc. (AATI) provide the benefit of extremely sensitive, reliable, and automated QC for CRISPR applications, helping to deliver consistent and reproducible gene editing. There are three critical QC checkpoints in the CRISPR workflow, including analysis of the starting material, mutation frequency, and determination of zygosity. Analyzing starting material is essential to the experimental design to confirm it meets or exceeds quality requirements and ensures the experiment is starting off in the right direction. The Fragment Analyzer™ Automated CE System can determine the quality of the sgRNA, ssDNA, or Cas9 polyadenylation starting materials utilized in the CRISPR process. Implementation of QC checkpoints throughout the workflow guarantees that only samples meeting quality requirements continue through the entire downstream process. Detection of low quality, contaminated, or otherwise unsuitable samples allows researchers to rework or remove these samples early in the process. The Fragment Analyzer can aid in determining if the CRISPR experimental design was successful and whether the mutation frequency warrants continuing the experiment. Further on in the CRISPR process, QC analysis can assist in screening for mutations and predicting probable zygosity of diploid single cell lines. Utilizing QC analysis at critical junctures in the CRISPR workflow identifies the quality of the starting material, reduces the number of failed experiments, efficiently utilizes operator time, and saves money.

CRISPR sgRNA Quality Control

One method of CRISPR/Cas9 gene editing employs the delivery of Cas9 complexed *in vitro* with sgRNA directly to a cell line, organism, or tissue. Synthesis of sgRNA usually produces enough material for many transfections. However, multiple freeze thaws, improper handling, or contamination can degrade the sgRNA, preventing efficient gene editing. Thus, quality analysis of sgRNA is performed prior to Cas9 complexing and transfection. The Fragment Analyzer with the DNF-470 Small RNA Analysis Kit or DNF-472 High Sensitivity RNA Analysis Kit automates sgRNA QC steps, providing enhanced resolution. High separation resolution paired with a sensitive detection system allows for easy identification of the smallest amounts of degradation, the sgRNA peak, and secondary structures, without the use of hazardous denaturants (Figure 1).

CRISPR ssDNA Quality Control

The use of strand selective nucleases for the creation of ssDNA templates are particularly useful in CRISPR/Cas homology directed repair (HDR). ssDNA templates, ranging from 500 bases to kilobase length, have been found to greatly increase the number of HDR events when compared to the traditional dsDNA templates. Production of ssDNA templates requires QC steps to ensure complete conversion of dsDNA to ssDNA. Use of AATI's Fragment Analyzer with the DNF-910CP CRISPR Discovery Gel Kit provides critical knowledge about the ssDNA end-product while conserving sample. Starting dsDNA PCR product and ssDNA end-product are easily separated, with small amounts of undigested dsDNA clearly identified (Figure 2).

CRISPR Cas9 Polyadenylation Quality Control

DNA-free CRISPR gene editing is an option allowing researchers to bypass the transfection of plasmids into cells. One method involves transfecting *in vitro* transcribed sgRNA and Cas9 mRNA, relying on the *in vivo* translation of Cas9 mRNA. Polyadenylation (poly(A)) of the Cas9 mRNA prior to transfection is required to protect Cas9 mRNA from degradation prior to *in vivo* translation. The length of the

Figure 1. Electropherogram of sgRNA separated on the Fragment Analyzer™ Automated CE System with the DNF-472 High Sensitivity RNA Analysis Kit.

DG: Degradation; LM: Lower marker; SSP: Secondary structure peak.

Figure 2 Electropherogram overlay of starting dsDNA fragment and ssDNA end-product separated on the Fragment Analyzer™ Automated CE System with the DNF-910CP CRISPR Discovery Gel Kit.

LM: Lower marker; UM: Upper marker.

Figure 3. Electropherogram overlay of Cas9 mRNA with (red trace) and without (black trace) polyadenylation. Separated on the Fragment Analyzer™ Automated CE System with the DNF-472 High Sensitivity RNA Analysis Kit.

LM: Lower marker.

poly(A) tail acts as a timer; longer poly(A) tails result in a longer lasting Cas9 mRNA transcript compared to shorter poly(A) tails. The Fragment Analyzer with the DNF-472 High Sensitivity RNA Analysis Kit can easily size the Cas9 mRNA and determine the approximate length of the poly(A) tail. The length of the poly(A) tail becomes longer with the increased polyadenylation reaction times, as noted by the increase in size of the Cas9 mRNA (Figure 3). The Cas9 mRNA without polyadenylation sized at 4,132 nt, with a 3% difference from the known size of 4,246 nt. Polyadenylation of Cas9 mRNA for 50 minutes displayed a smear representing many different lengths of poly(A) tails with a major peak at 6,196 nt. Secondary structure formations were limited due to the presence of denaturing formamide in the diluent marker of the DNF-472 High Sensitivity RNA Analysis Kit, in addition to heat denaturation.

Summary

QC checkpoints are important in many different nucleic acid applications. Beginning any application with a QC check of the starting materials confirms that the user is starting with good quality material and provides assurance that the downstream process will most likely be successful. QC checkpoints at critical steps throughout the process confirms that the methods are optimized. A final QC check validates the condition of the end-product and increases confidence with the methods in place. QC analysis during CRISPR experiments are crucial to the quality and success of the final product.

CRISPR applications utilize QC checkpoints at several different junctures in the process. Initial quality analysis of sgRNA ensures a high quality starting product essential for efficient gene editing. Quality analysis of the ssDNA end-product ensures a complete conversion of dsDNA to ssDNA, providing a template for CRISPR/Cas HDR. Determination of the approximate length of the poly(A) tail on the Cas9 mRNA, conveys that the reaction occurred, and can be correlated to the duration of transcription. QC analysis at the beginning, throughout, and at the end of an experiment relays important information used to guide the process.

References

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