QUiCKR™: Fast Quantification of Genome-Editing Outcomes using CRISPR-based detection

QUiCKR
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Time Consuming Evaluation of Edits

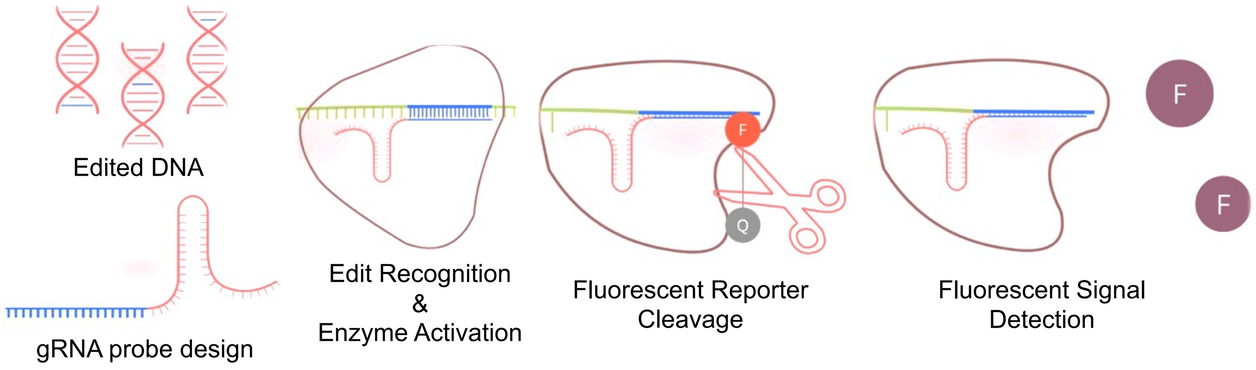
Genome editing is rapidly gaining traction in clinical applications, but the evaluation of editing outcomes remains time consuming and impedes R&D timelines. This is especially important when multiple iterations are needed to optimize editing tools and delivery.

Amplicon sequencing (Amp-Seq) has served as the gold standard for quantification of editing efficiency. However, Amp-Seq is time consuming (~1 week turnaround time), and can be costly. Faster methods have been developed, but these often pose a trade-off of accuracy. The introduction of new workflows into research pipelines also requires additional equipment and/or extensive assay optimization. Hence, there is a critical need for fast, accurate, and cost-effective quantification of gene editing efficiency.

Fast Quantification of Edits

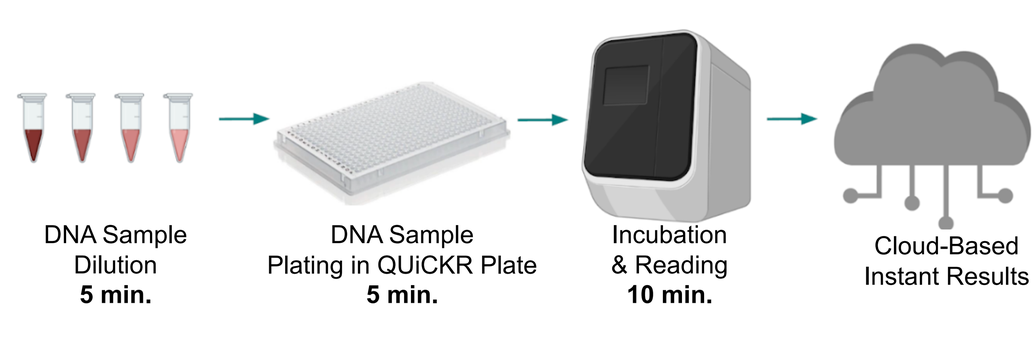
To address this need, we have developed an accurate assay which can be easily run on standard lab equipment for quantification of indels, knock-ins, and off-target activity. The assay is called QUiCKR™ (Quantification Using initial CRISPR Kinetic Rates) and offers results in 20 min with minimal manual steps. The QUiCKR assay measures the kinetic rates of Cas12 trans-cleavage to quantify gene editing efficiency, with amp-seq level accuracy. Pre-plated reagents remain stable during long-term storage. which provides users with ready-to-use reagents, and a fast workflow.

QUiCKR™ Technology



The QUiCKR technology is agnostic to the upstream editing method. We first design gRNA probes to ensure high specificity for the edits of interest. These gRNA probes are complexed with QUiCKR's proprietary Cas12 enzyme and then frozen for storage. After shipping and thawing, the edited sample is added and the CRISPR complex recognizes and hybridizes to specific edits for which it has been designed. Hybridization induces a conformational change of the enzyme, which opens a new cleavage site. The activated site unlocks cleavage of fluorescence reporters, which generates a fluorescence signal, recorded on standard plate readers or thermocyclers.

QUiCKR™ Workflow



The QUiCKR kit includes reagents in pre-plated format to enable a fast and easy-to-use workflow. After DNA amplification, the workflow starts with DNA sample dilution. Once diluted, samples are transferred to the pre-plated QUiCKR reagents. The plate is then read at 37°C for 10 min in standard plate readers or thermocyclers. The raw data output is then uploaded to the QUiCKR analysis platform for instant edits quantification.

On- and Off-target Quantification with QUiCKR, Amp-seq and Sanger-ICE

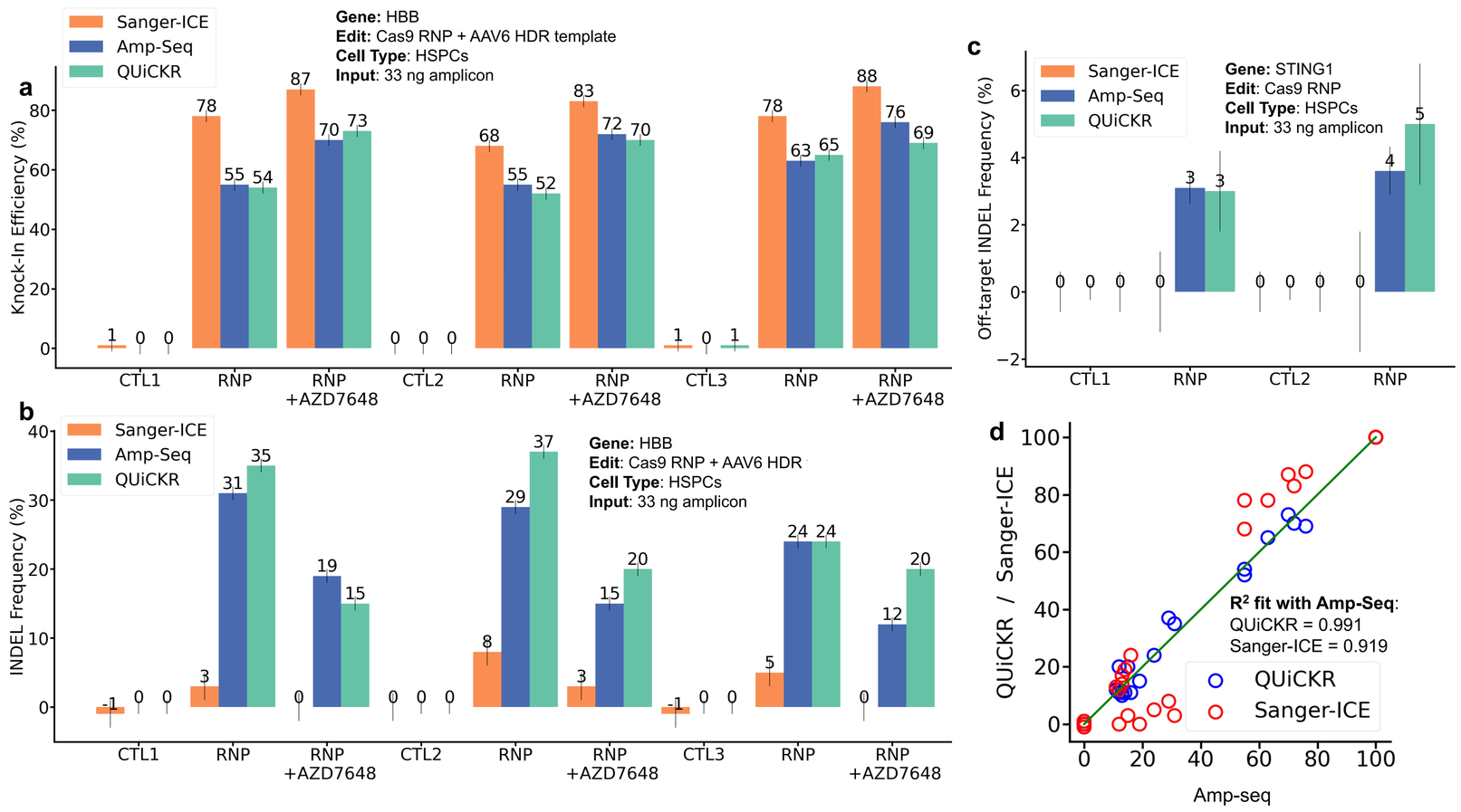


Figure 1. HSPCs were separately edited at HBB, and STING1 loci with Cas9 ribonucleoprotein (RNP) with or without a knock-in AAV6 homology directed repair (HDR) donor template, followed by amplification of the on-target sites and an off-target site. On- and off-target knock-in efficiency (**Fig 1a**), INDEL frequency (**Fig 1b**), and wild-type ratios of off-target INDEL (**Fig 1c**) were quantified using Sanger sequencing with ICE analysis, amp-seq, and the QUiCKR assay.

QUiCKR demonstrated 99.1% R-square fit with amp-seq data over 50 data points and 35 samples (**Fig 1d**). In contrast, ICE showed 91.9% R-square fit with Amp-Seq data and could not capture low levels of off-targets (~3%, **Fig 1c**).

LOD on Single-base Edits

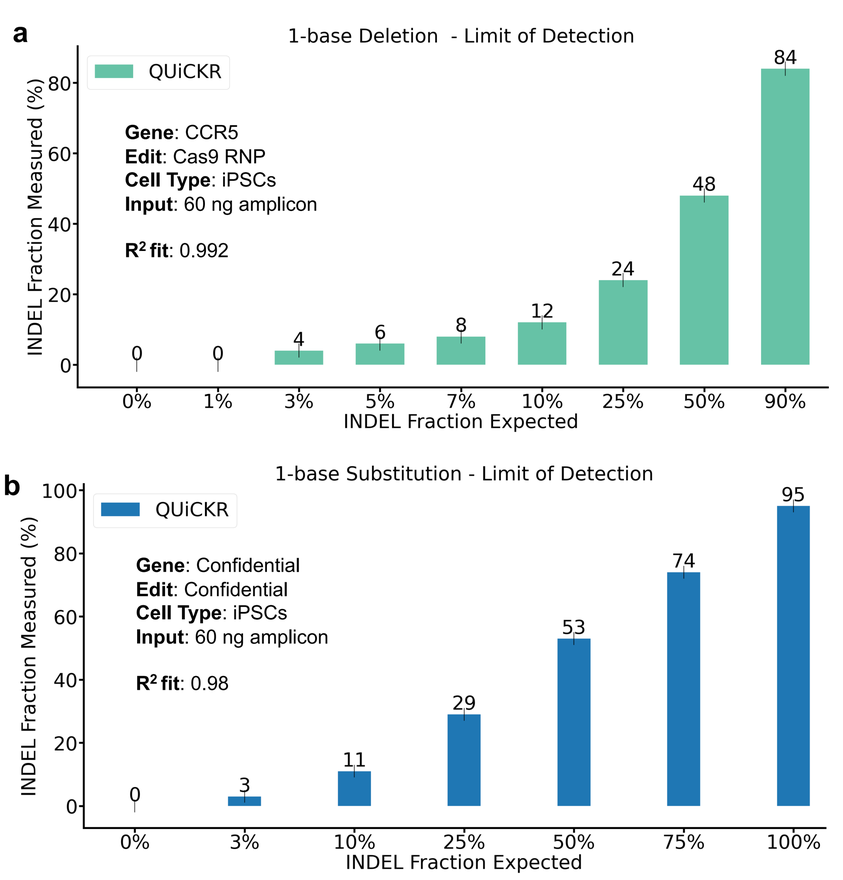


Figure 2. iPSCs were edited at CCR5 locus with Cas9 ribonucleoprotein (RNP) for single base deletion, followed by amplification of the on-target site for both edited and unedited samples. On-target INDEL frequency was quantified via Amp-Seq and mixes of edited and unedited amplicon were prepared for INDEL fractions ranging from 90% to 0%. On-target INDEL frequency was then quantified for all mixes with QUiCKR (**Fig 2a**). The same LOD study was conducted for a single base substitution (**Fig 2b**). LOD was 3% for both edits with 60 ng input. For increased sensitivity, 200 ng input is recommended.

Reagent Stability

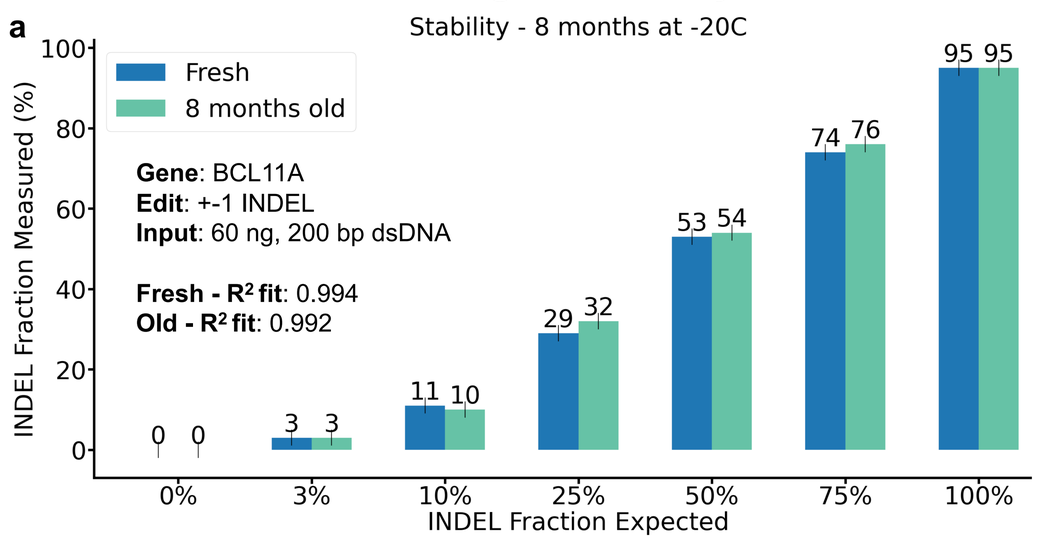
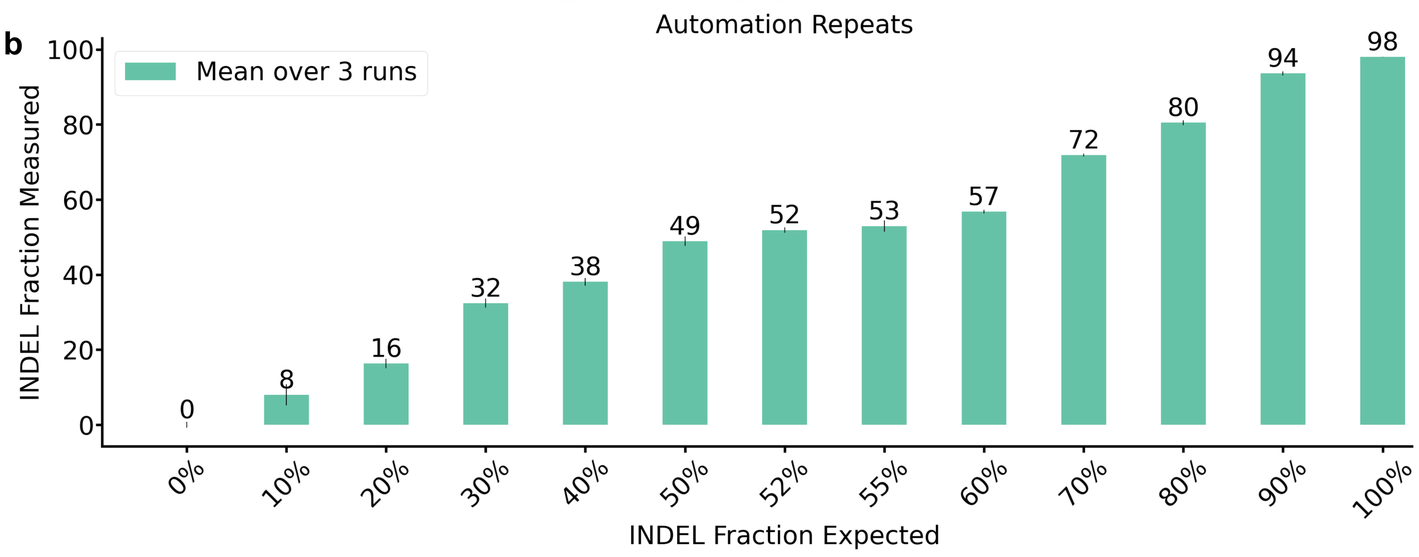


Figure 3. Synthetic dsDNA for wild-type and edited BCL11A were prepared and mixed to obtain INDEL fractions ranging from 100% to 0%. QUiCKR reagents were prepared, and run either fresh or after storage at -20C for 8 months (**Fig 3a**). Similar mixes were prepared to test the repeatability of the assay on liquid-handlers. The average standard error over the 3 runs was 1.0% (**Fig 3b**).

High-Throughput Automation



Conclusion

QUiCKR matches Amp-Seq in accuracy for multiple types of edits yet requires only a 20-min easy-to-use workflow. The assay has the potential to significantly accelerate gene editing research.