# Automating QUiCKR<sup>®</sup> V2 quantification of editing outcomes on Opentrons Flex<sup>®</sup>





# Written by

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# ABSTRACT

QUICKR V2 is a 20-min readout on editing efficiency with Amp-Seq level accuracy. QUICKR kits contain frozen, preplated reagents in 384-well plates, customized to the users' edits of interest. User-edited DNA is added to the QUICKR plates and run on standard fluorescence readers. The raw fluorescence data is then instantly analyzed on QUICKR's cloud based platform. In order to make this editing readout workflow higher throughput, it has been fully automated on the Opentrons Flex.

#### **Key features:**

- · Automating QUICKR V2 on Opentrons Flex reduces hands-on time (from 30 min to 4 min) and human error
- Automation increases throughput from 190 samples an hour when performed manually to 380 samples an hour per fluorescence reader available.
- INDEL fraction percentages yielded 0.93 R-square fit with expected values and 1% standard error across robot runs, demonstrating the reproducibility of the automated workflow.

# **INTRODUCTION**

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.

To address this need, QUICKR Bio has 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 Amp-Seq level accuracy and minimal hands-on steps.

The QUiCKR kit includes reagents in pre-plated format to enable a fast and easy-to-use workflow. 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.

In order to make this editing readout workflow more efficient, it has been fully automated on the Opentrons Flex, a modular, benchtop liquid handler that can automate many laboratory workflows, including the serial dilutions and sample transfers needed to complete the QUICKR workflow. Here, we describe the automated workflow on the Opentrons Flex.

#### **METHODS**

# Workflow Overview



# **Samples Tested**

10 mixes of varying editing efficiencies:

- Gene: CCR5
- Edit: Cas9 RNP
- Cell Type: iPSCs
- Input: 60 ng amplicon

# AUTOMATION ON OPENTRONS FLEX

Initial serial 1:3 dilutions of up to 46 samples were automated on Opentrons Flex (<u>https://library.opentrons.com/p/quickr\_1</u> and Fig. 1, left). Dilutions are performed on 96-well plates, 4 dilution per sample, with 20 µL transfer volumes. Subsequently, the diluted samples were distributed (7 µL transfer volume) to the 384-well QUiCKR plate pre-filled with 14 µL of reagents and mixed, also on Opentrons Flex (<u>https://library.opentrons.com/p/quickr\_2</u> and Fig. 1, right).



**Figure 1.** Deck layout of the Opentrons Flex for QUICKR V2 automation (up to 2 plates). The Protocol Part 1 performs serial 1:3 dilutions of samples filled in Column #1, 5, and 8 with assay buffer (left), and Part 2 conducts liquid transfers to distribute diluted samples to the 384-well assay plate pre-filled with reagents followed by mixing (right).

# **RESULTS AND DISCUSSION**

We automated QUICKR V2 with test samples on Opentrons Flex over 3 separate runs, measuring INDEL fraction percentage relative to expected values (Figure 2). We found measured values yielded a 0.93 R-square fit with expected values, with 1% standard error between runs, demonstrating the reproducibility of the automated workflow.



Figure 2. INDEL fraction of test samples measured over 3 automated runs (mean +/- s.d.)

We found that automating QUICKR V2 on Opentrons Flex reduced hands-on time by 90% (from 30 min to 4 min) while increasing throughput to 380 samples/hour and maintaining accurate results (0.93 R-square fit with expected results) across different runs. The protocols for this automated workflow are available for download from the Opentrons Protocol Library and can be run on the robot with minimal setup time needed.

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