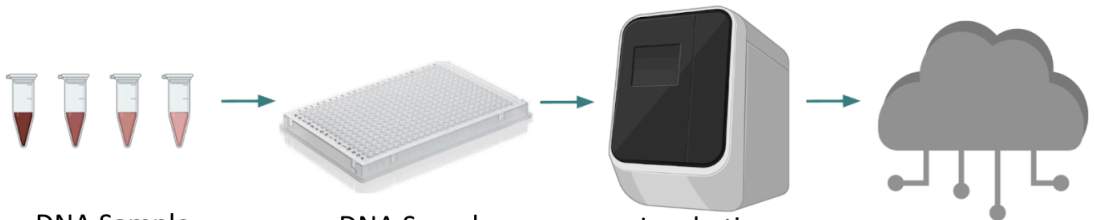
Product Description

QUiCKR V2 Quantifies Editing Efficiency in 20 mins using an enzymatic reaction and fluorescent reporters. The Kit is run on a standard plate reader or thermal cycler capable of capturing kinetic readouts at 37°C. The input of the assay is a customer-specified on-target amplicon. The assay accurately quantifies the INDEL rate on the wild-type on-target site specified for each kit.

Summary

How the assay works

The workflow consists of serial dilutions of DNA samples, followed by transfer of the dilution series to the QUiCKR plate. The plate is run on standard fluorescence readers and the output file is uploaded to the QUiCKR platform for instant analysis.



Specifications

Format: frozen, preplated, 384 (skirted) / 96 (semi-skirted) well plates

Volume: 14 uL / well

Concentration: 1.5X Master Mix

Required Genomic Material:

- 200 ng purified Amplicon (containing both the on-target and reference sites specified at the time of the kit order) in 54 µL water.

Storage Conditions: Between -80°C and -20°C, Avoid Repeated Freeze/Thaw Cycles

Stability: Stable for 6 months at -20°C

Limit of Detection: 2% INDEL rate

Compatibility:

- **Reader Compatibility:** Compatible with most qPCR and plate reader instruments, such as Applied Biosystems, Bio-Rad, etc.
  - Required wavelengths: 493 nm excitation, 517 nm emission
  - Temperature control: 37°C
  - Kinetic Reads: every 30 seconds
- **Automation Compatibility:** Compatible with all liquid handling systems and validated with most liquid handling systems.

Kit Components

Component	Quantity	Description
QUiCKR 96/384 Plate	5	Prelated with QUiCKR Master Mix
10x QUiCKR Buffer	1 x 0.7 mL	Used to dilute initial amplicon
1x QUiCKR Buffer	5 x 3.2 mL	Used for sample serial dilutions
REF DNA control	1 x 220 uL	Positive control
WT DNA control	1 x 220 uL	Positive control

Performance Specifications

Sensitivity: 2% INDEL rate

Specificity: 99.6%

Input quantity: 200 ng amplicon

Assay time: 20 minutes

Hands-on time: 10 minutes

Stability: 6 months at -20°C

Cloud-Based Analysis

The analysis consists of extracting raw fluorescence data from the reader, logging in to the QUiCKR platform and uploading the raw data file for instant quantification of editing efficiency. The detailed steps are as follows:

Figure 1 - Example raw fluorescence data

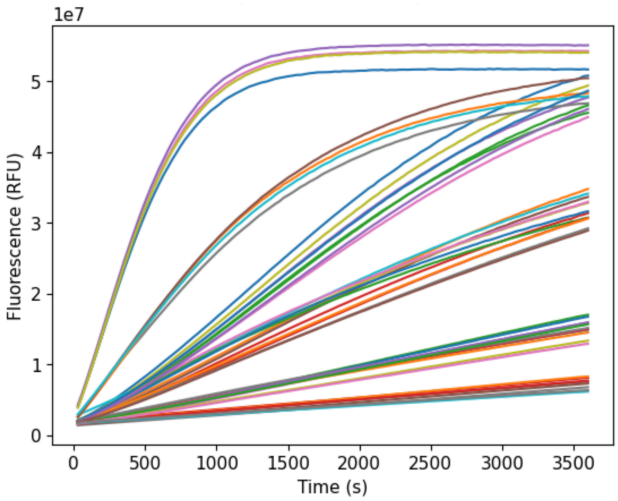


Figure 2 - Example csv file extracted from a QuantStudio 6 Flex

	A	B	C	D	E	F	G	H
35	Instrument	278862619						
36	Instrument	QuantStudio(TM) 6 Flex System						
37	Passive Re							
38	Quantificati	Ct						
39	Signal Smo	true						
40	Stage/ Cycl	Stage 2, Step 1						
41	User Name							
42								
43	Well	Well Positio	Cycle	x1-m1	x2-m2	x3-m3	x4-m4	x5-m5
44	1	A1	1	2,108,331.000	429,761.188	11,030.356	13,290.530	8,788.700
45	2	A2	1	2,023,104.625	377,185.281	13,992.256	16,391.504	-5,274.216
46	3	A3	1	3,990,356.000	484,996.094	28,299.213	37,133.203	7,472.207
47	4	A4	1	4,323,308.000	507,958.625	66,162.820	95,493.203	67,606.859
48	5	A5	1	1,968,534.125	303,103.563	12,161.764	13,671.226	-27,110.455
49	6	A6	1	2,081,800.250	317,989.125	15,999.254	24,555.861	22,137.092
50	7	A7	1	4,058,294.000	441,020.594	23,877.867	36,362.516	28,410.193
51	8	A8	1	4,021,812.250	435,180.094	-12,474.035	-60,244.762	-165,186.000
52	9	A9	1	1,906,175.125	323,694.125	10,846.116	17,609.887	8,414.433
53	10	A10	1	1,898,967.375	339,537.313	10,510.308	15,551.667	13,321.900
54	11	A11	1	4,303,543.500	569,899.000	20,402.961	20,100.963	19,112.682
55	12	A12	1	3,933,360.500	567,830.813	21,869.809	27,857.045	20,069.920
56	13	B1	1	2,014,953.250	370,068.313	25,263.283	35,535.465	19,080.205
57	14	B2	1	2,858,397.750	455,874.719	130,606.859	239,690.922	169,843.406
58	15	B3	1	2,613,223.000	349,092.000	21,349.146	38,484.695	33,221.441
59	16	B4	1	2,622,049.250	334,206.813	16,426.199	26,618.320	-22,819.436
60	17	B5	1	1,787,359.000	273,869.438	10,734.950	16,465.297	15,771.781
61	18	B6	1	1,967,127.125	298,885.844	23,382.568	34,998.168	29,943.189
62	19	B7	1	2,783,933.250	335,698.688	34,207.723	66,116.094	60,699.059
63	20	B8	1	2,649,041.750	329,362.031	3,847.398	-33,883.711	-322,037.656
64	21	B9	1	1,830,018.625	297,604.906	12,255.079	25,469.129	25,895.906

Figure 3 - Platform upload page

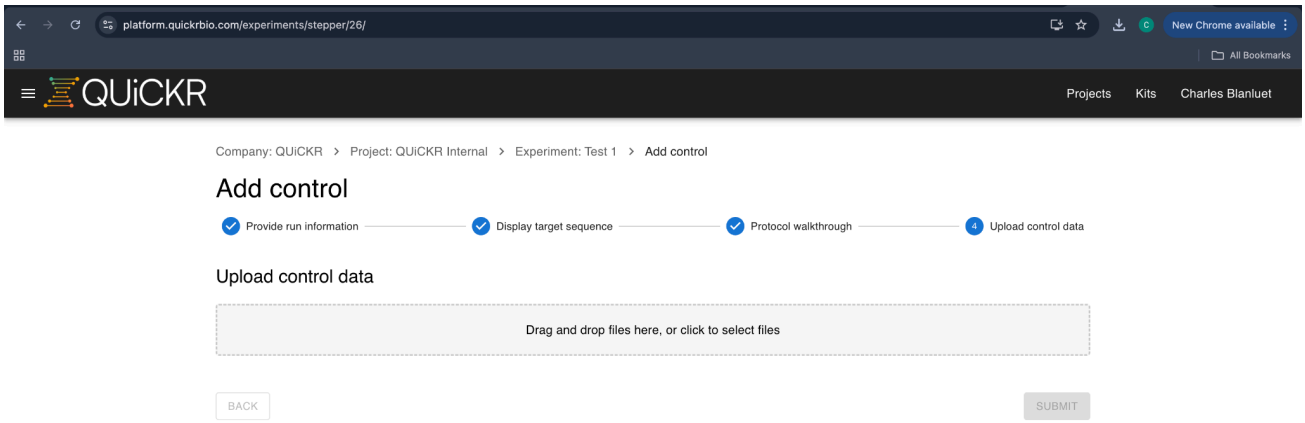


Figure 4 - Example Results

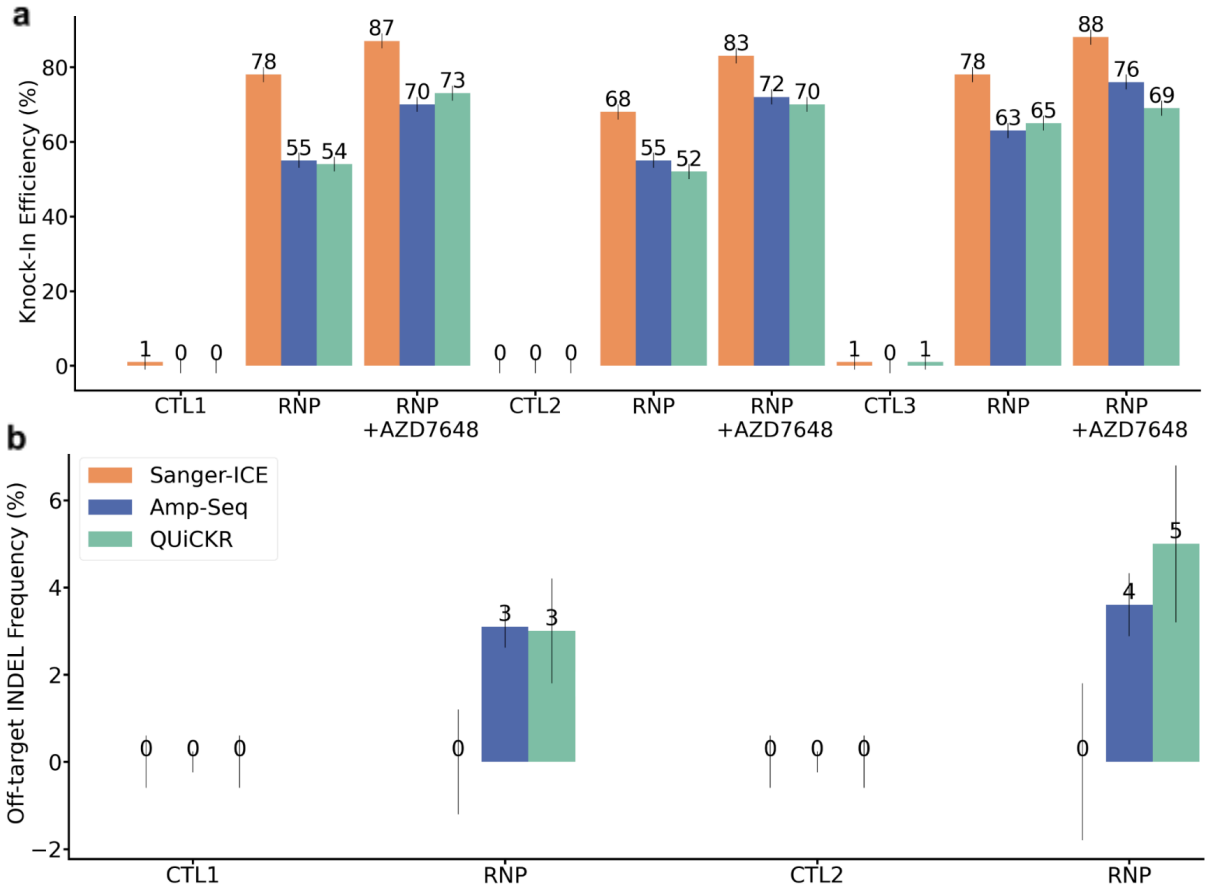


Figure 4.a The HBB gene was edited in HSPCs, followed by genomic DNA extraction and PCR amplification of the edited site. The purified amplicon was analyzed for indel and knock-in

frequencies using amplicon sequencing, Sanger-ICE, and the QUiCKR assay. The QUiCKR assay matched amp-seq in accuracy (97% R-square fit).

Figure 4.b The STING1 gene was edited in HSPCs, followed by genomic DNA extraction and PCR amplification of a known off-target site with low editing. The editing efficiency was quantified using amplicon sequencing, Sanger-ICE, and the QUiCKR assay. The QUiCKR assay and amplicon sequencing yielded comparable results, while Sanger-ICE failed to detect the edit.

Troubleshooting

Symptom	Causes and Comments
Failure to detect fluorescence signal	Improper nucleic acid extraction from samples, resulting in loss of DNA, DNA degradation or both.
	Inhibition of enzyme activity by inhibitors in the sample, e.g., EDTA
	Insufficient amount of DNA in the sample.
	Improper assay set up or execution.
Initial fluorescence is too high	Premature activation of the complex due to too long storage of the plate at too high temperature between DNA addition and readout - ensure the plate is kept cold.
	Fluorescent agent already present in the DNA sample, e.g. from incomplete washing post gel extraction - review purification protocol and ensure the sample does not contain any dyes.
High variability between replicates	Volume or uniformity variations as a result of inaccurate pipetting and/or mixing
	Partial master mix degradation due to non-uniform freeze-thawing of the plate
	Improper calibration of the instrument
	Premature activation in select wells due to reagent mixing at too high temperature