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ICE vs TIDE vs CRISPResso2: Edit Analysis Tool Comparison

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Executive Summary

ICE, **TIDE** and **CRISPResso2** are not three interchangeable calculators for one number. TIDE (Tracking of Indels by DEcomposition) takes two Sanger traces, a control and an edited sample (tide.nki.nl). ICE (Inference of CRISPR Edits) also needs a control and an edited trace file plus a guide sequence (ice.editco.bio). CRISPResso2 takes sequencing reads in FASTQ format plus an amplicon sequence (docs.crispresso.com). The tools therefore start from different raw evidence and report different quantities, and the choice of software cannot be separated from the choice of sequencing modality.

The only public three-way comparison located for this report, a research note published 26 July 2024, used two fibroblast experiments (link.springer.com). In the sheep experiment the percentage of amplicons with indels was 52% (TIDE), 58% (ICE) and 64% (CRISPResso2 on Nanopore reads) (link.springer.com). In the horse experiment it was 81%, 87% and 86% (link.springer.com). Those gaps are 1 to 12 percentage points. In addition, only about 31% and 19% of the Nanopore reads entered the CRISPResso2 indel analysis (link.springer.com), and the authors state that they did not test any tool against ratio dilutions of modified and unmodified DNA (link.springer.com). Agreement between tools is therefore not evidence of accuracy. No benchmark was run for this report.

Licensing differs by tool and should be settled before a for-profit team adopts any of them. The CRISPResso2 repository license says for-profit users need to purchase a license (github.com). The ICE source repository describes itself as free for non-commercial applications and says commercial licensing options are available (github.com). The TIDE web application describes itself as a free web service for research (apps.datacurators.nl), while a separate vendor says companies relying on TIDE in their pipeline choose a Discovery license and offers a GxP tier for regulated environments (tidegenetics.com).

A practical decision rule follows from these facts. A Sanger-based workflow is a reasonable screen when the edit class is non-templated indels, trace quality is good, and the decision is a triage rather than a release threshold. Amplicon sequencing becomes the stronger choice when edit types are templated or mixed,

samples are clonal, or a sponsor threshold sits close to the measured value. The cost model in this report divides all costs by samples that meet predefined evidence criteria, and all prices are supplied by the buyer. This report fabricates no sequencing prices and sets no sensitivity cutoffs.

Introduction and Background

This report is written for research directors, assay-development leads, computational biologists and contract research organization (CRO) data owners who must choose how to quantify on-target edits in research samples. It is a software and data-method selection guide. It does not address wet-lab editing optimization, clinical release testing, functional knockout certification or off-target safety. All statements about tools come from developer documentation, vendor pages and published papers opened during research, as of November 2026.

Four quantities that are often conflated

The first step is to name the requested quantity. Four appear in practice.

- **Trace-decomposed indel fraction.** A model fits a mixture of candidate edits to a mixed Sanger trace. TIDE reports the identity of detected indels and their frequencies (tide.nki.nl). The estimate depends on a model, a window and a trace.
- **Edited aligned-read fraction.** Reads are aligned to a reference amplicon and each read is labeled modified or unmodified. CRISPResso2 describes its core task as determining whether a read is modified or unmodified (github.com). The number depends on which reads survive filtering and alignment.
- **Templated change.** A donor-directed or base-editing outcome is counted. TIDE cannot detect donor-directed "designer" edits, and the developers point to the template-directed TIDER method (tide.nki.nl). ICE accepts a donor sequence for knock-in analysis (www.synthego.com).
- **Functional effect.** ICE reports a knockout (KO) score defined as the proportion of cells with either a frameshift or a 21 or more bp indel (www.synthego.com). That is a sequence-based inference. It is not a direct measurement of protein loss, so a KO score should never be reported as a functionally confirmed knockout.

What this report did and did not do

Research consisted of opening developer documentation, repositories, license files and papers. No tool was run, and no raw dataset was downloaded or reanalyzed. The study accession for the primary comparison is NCBI BioProject PRJNA1109565 (link.springer.com). The static HTML of the hosted ICE application at ice.editco.bio was only a script-rendered shell, and the landing-page text quoted here came from a rendered fetch, so the application's interface, limits and terms were not independently exercised, and which terms govern uploads remains an open question. That observation is not an uptime or service-level finding.

TIDE and TIDER

Capabilities

TIDE is a web tool from the Netherlands Cancer Institute group. The developer page lists two tools, TIDE with two Sanger traces as input and TIDER with three (tide.nki.nl) (apps.datacurators.nl). TIDER needs one additional "reference" sequencing trace to quantify template-directed edits (tide.nki.nl). The application accepts ABIF (.ab1) and SCF (.scf) chromatogram files (apps.datacurators.nl), and it asks for the same number of control and test samples in one submission (apps.datacurators.nl).

Decomposition covers only the sequence downstream of the break site (pmc.ncbi.nlm.nih.gov). In the application the default decomposition window is the largest window possible for the uploaded sequences (apps.datacurators.nl), and the maximum modeled deletion and insertion size defaults to 10 (apps.datacurators.nl). The original paper states that larger indels could not be detected under that setting (pmc.ncbi.nlm.nih.gov). For TIDER, the default window runs from 20 bp before to 80 bp after the break site (apps.datacurators.nl), and non-templated insertions are fixed at a maximum of 5 (apps.datacurators.nl). The application page showed TIDE version 5.3.2 when it was opened (apps.datacurators.nl).

Adoption and availability

No adoption statistics were found in primary sources. The web application states that it does not store data and shows display-only results (apps.datacurators.nl). The TIDER page says uploaded data are used only for the analysis session (apps.datacurators.nl). The page describes the service as free for research (apps.datacurators.nl), so a for-profit team should confirm in writing what that wording covers. This report did not establish how the NKI-hosted application relates to the vendor's TIDE app. A separate vendor site says its public platform is free for academic use, that its research version is free for non-profit research use, and that companies relying on TIDE in their pipeline choose a Discovery license, and it also sells a validated GxP tier for regulated environments (tidegenetics.com).

Strengths and limitations

TIDE's design suits quick, low-cost checks of non-templated indels from ordinary capillary sequencing. The original paper says reliability depends on the purity of the PCR product (pmc.ncbi.nlm.nih.gov), recommends keeping aberrant background signal before the break site low (pmc.ncbi.nlm.nih.gov), and recommends sequencing the opposite strand to confirm results (pmc.ncbi.nlm.nih.gov). Highly repetitive sequence around the target may hamper decomposition (pmc.ncbi.nlm.nih.gov). The paper also notes that only the nucleotide composition of +1 insertions is estimated (pmc.ncbi.nlm.nih.gov).

TIDER extends the approach to templated edits, but its authors state that it can slightly overestimate homology-directed repair (HDR) events in the low range (pmc.ncbi.nlm.nih.gov) and cannot resolve HDR events that carry an additional non-templated indel (pmc.ncbi.nlm.nih.gov). Independent work on clonal samples found that TIDE and a second Sanger-based method can miscall alleles in edited clones, which is a consequence of decomposing diploid or polyclonal traces (pmc.ncbi.nlm.nih.gov).

ICE

Capabilities

ICE was published in The CRISPR Journal in 2022 (pubmed.ncbi.nlm.nih.gov), and its abstract says it determines which proposed edits are supported by the data via regression (pubmed.ncbi.nlm.nih.gov). Its abstract describes a free, open-source tool with batch analysis and support for a variety of editing conditions (pubmed.ncbi.nlm.nih.gov). The preprint reports that ICE strongly correlates with next-generation sequencing of amplicons (www.biorxiv.org), and the hosted form asks for a wild-type or negative control trace (ice.editco.bio). The preprint's regression inference window extends up to 100 bp downstream of the cut site (www.biorxiv.org), and default proposals for a single guide include deletions up to 30 bp and insertions up to 14 bp (www.biorxiv.org). The control trace is aligned using a high-quality window defined by a windowed average Phred score above 30 (www.biorxiv.org).

For templated work, the preprint expects at least 15 bp of matching sequence at both ends of a template (www.biorxiv.org), and base editing is analyzed by supplying a template that mimics the expected outcome (www.biorxiv.org). The Synthego guide describes a knock-in score and a donor of up to 300 bp (www.synthego.com). The repository states that ICE can analyze insertions, deletions, HDR, multiplex edits and base editing (github.com). Outputs named in the preprint are JSON and spreadsheet summaries, plots and alignments (www.biorxiv.org).

Adoption and availability

ICE was originally offered by Synthego, and a Synthego page now states that the tool is hosted by EditCo (www.synthego.com). The EditCo product page says users can upload up to 700 samples at a time and must create a free account to analyze samples (www.editco.bio). The landing page advertises persistent results saved to the user's account (ice.editco.bio). The Synthego page lists the nucleases the tool is configured for, including SpCas9, hfCas12Max, Cas12a and MAD7 (www.synthego.com).

For local use, the repository says ICE can be installed as a Docker container or via pip, and that the version is reported by a command-line option (github.com). The code is described as free for non-commercial applications, with commercial licensing available separately (github.com).

Hosted-data policy is separate from software license. Synthego's terms state that users retain ownership of their data (www.synthego.com), and that the company may de-identify submitted data and aggregate it with data from other users (www.synthego.com). The same terms describe an ICE tool for analyzing Sanger sequence files obtained after CRISPR editing (www.synthego.com). The EditCo privacy policy opened for this report was last revised on 20 March 2024 (www.editco.bio). This report could not confirm which entity's terms govern uploads to the current EditCo-hosted application, so the question remains open for any team handling confidential constructs.

Strengths and limitations

The preprint reports that, in a comparison with amplicon sequencing, the correlation of indel distributions was r2 = 0.93 overall across 92 samples (www.biorxiv.org), and 0.97 for a separate set of 45 HDR samples (www.biorxiv.org). Those are the developers' own results, and they concede amplicon sequencing has better

sensitivity and quantitation (www.biorxiv.org). They also state that ICE and TIDE share an assumption that peak signal is proportional to molarity, which may limit precision (www.biorxiv.org), and that when the true edit is outside the modeled set, both tools give a low goodness-of-fit and may underestimate the actual indel percentage (www.biorxiv.org).

The vendor guide states that a higher model-fit R-squared means more confidence in the ICE score (www.synthego.com). The repository README says ICE uses non-negative least squares regression (github.com), whereas the third preprint version describes Lasso regression (www.biorxiv.org). The two descriptions are not reconciled in the sources opened, which is a reason to record the exact ICE version in every handoff.

CRISPResso2

Capabilities

CRISPResso2 is a command-line toolkit built for sequencing reads. Its paper states that it takes raw FASTQ files as input (pmc.ncbi.nlm.nih.gov) and lists five key innovations (pmc.ncbi.nlm.nih.gov). Its alignment extends the Needleman-Wunsch algorithm with a mechanism that incentivizes indel placement at the cleavage site (pmc.ncbi.nlm.nih.gov). The core tool is designed to be run on a single amplicon (docs.crispresso.com). Related utilities analyze batches, pooled amplicon libraries, and compare or aggregate runs (docs.crispresso.com).

Parameters define what is counted. The default quantification window is 1 bp on each side of the cleavage position, 2 bp in total (docs.crispresso.com). A value of 0 disables the window so that indels across the entire amplicon are considered (docs.crispresso.com). The default window center of -3 suits Cas9 (docs.crispresso.com), and insertions outside the quantification window are not included in the histograms (docs.crispresso.com). Reads must reach a default minimum homology score to align (docs.crispresso.com). HDR analysis needs the full expected amplicon sequence (docs.crispresso.com), and base-editor and prime-editing analysis modes exist (docs.crispresso.com).

Adoption and availability

The latest GitHub release observed was version 2.3.4, dated April 23, 2026 (github.com). Installation routes include a Bioconda environment (docs.crispresso.com), BioContainers images (bioconda.github.io) and a Docker Hub image described as an analysis image for genome-editing outcomes (hub.docker.com).

The hosted web application is a separate offering. Its page restricts use to non-commercial academic research (crispresso2.pinellolab.org). The repository license says the software is free for academic researchers for non-commercial use (github.com), that for-profit users need to purchase a license (github.com), and that it is not offered as a free tool for any commercial or clinical use (github.com). The license names a licensing contact at the vendor Edilytics (github.com) and says the tool shall not be used as a diagnostic decision-making system (github.com).

Strengths and limitations

The documentation says CRISPResso2 assumes reads are already trimmed (docs.crispresso.com). A quality-filter parameter exists, and the troubleshooting page suggests 30 as a reasonable value (docs.crispresso.com). The tool can also mask low-quality bases as N instead of discarding the read (docs.crispresso.com). Its paper reports a negligible false-positive rate in simulations (pmc.ncbi.nlm.nih.gov), but that is a simulation result, not a statement about a sponsor's samples.

The most important limit is the denominator. The output documentation lists reads in input, reads after preprocessing and reads aligned (docs.crispresso.com), and the allele table gives the percentage of all reads having a sequence (docs.crispresso.com). The documentation pages opened do not define in one sentence which of those counts is the denominator for the headline editing percentage, so a team should confirm it from a run. CRISPResso2 also has no documented Sanger-trace input in the pages opened, so .ab1 files would need a different tool.

Feature Comparison

Table 1 below transcribes documented facts only. It does not rank the tools. Cells marked "not stated" mean the page opened did not state the item, not that the tool lacks it.

Tool (version seen)	Input	Reference / control requirement	Intended edit class	Reported quantity and denominator	Quality flags	Exports and logs	Batch / local route	License scope
TIDE (web app 5.3.2)	Two Sanger traces, .ab1 or .scf (tide.nki.nl)	Control trace and test trace, same number of each	Non-templated indels; donor-directed edits are out of scope (tide.nki.nl)	Indel identity and frequency; denominator not stated	Goodness-of-fit and per-indel statistics (pmc.ncbi.nlm.nih.gov)	Display-only results, data not stored	Web application; R code described as open source (apps.datacurators.nl)	"Free web service for research" (apps.datacurators.nl); separate vendor GxP tier (tidegenetics.com)
TIDER	Three Sanger traces	Extra reference trace	Template-directed edits plus non-templated indels (pmc.ncbi.nlm.nih.gov)	Templated fraction and indel spectrum; may overestimate low-range HDR (pmc.ncbi.nlm.nih.gov)	Cannot resolve HDR events with an extra indel (pmc.ncbi.nlm.nih.gov)	Session-only data	Web application; R code on request	Same page wording as TIDE

Tool (version seen)	Input	Reference / control requirement	Intended edit class	Reported quantity and denominator	Quality flags	Exports and logs	Batch / local route	License scope
ICE (EditCo-hosted; landing page announces ICE 4.3 (visible only when the page is rendered in a browser), not independently exercised (ice.editco.bio))	Control and edited trace files plus guide; optional donor	Wild-type or negative control trace	Indels, HDR, multiplex, base editing	Indel percentage, model fit, KO score; denominator not stated	Model-fit R-squared; discordance and quality flags described in the preprint (www.biorxiv.org)	JSON, spreadsheet, plots, alignments (www.biorxiv.org)	Up to 700 samples on the hosted page (www.editco.bio); Docker or pip	Source free for non-commercial use, commercial licensing available; hosted terms (www.synthego.com)
CRISPResso2 (the three-way study ran 2.2.14; latest release seen 2.3.4 (github.com))	FASTQ reads plus amplicon sequence (docs.crispresso.com)	Amplicon reference; guide optional; HDR needs full expected amplicon	Indels, HDR, base editing, prime editing	Modified versus unmodified reads; read counts at each stage; denominator to be confirmed by a run	Guardrails, for example aligned reads below 90% of total (docs.crispresso.com) and fewer than 10000 reads (docs.crispresso.com)	Report, tables, JSON and run log	Batch, pooled, compare and aggregate tools (docs.crispresso.com); Bioconda, Docker (docs.crispresso.com) (hub.docker.com)	Free for academic non-commercial use; for-profit use needs a purchased license

The matrix shows why a single "accuracy" ranking is not meaningful. TIDE and ICE fit a model across a mixed signal, so their numerator and denominator are model contributions. CRISPResso2 counts discrete reads, so its numerator and denominator depend on filtering, masking, windowing and alignment. A team comparing outputs across tools is comparing different quantities that share the label "percent edited".

Performance and Benchmarks

What the direct comparison establishes

The 2024 BMC Research Notes paper compared TIDE, ICE and CRISPResso2 applied to Nanopore reads (called nCRISPResso2) in sheep and horse primary fibroblasts (link.springer.com). Both gRNAs targeted the myostatin gene. The amplicons were large enough that the authors describe them as exceeding the compatibility threshold for Illumina sequencing (link.springer.com). The authors declare no competing interests (link.springer.com). *Table 2* transcribes the two reported experiments and adds percentage-point (pp) differences computed for this report. It is historical data from one study and not a general accuracy estimate.

Experiment (paper figure 1)	TIDE (%)	ICE (%)	nCRISPResso2 (%)	ICE minus TIDE (pp)	nCRISPResso2 minus ICE (pp)	nCRISPResso2 minus TIDE (pp)
Sheep MSTN (link.springer.com)	52	58	64	+6	+6	+12
Horse MSTN (link.springer.com)	81	87	86	+6	-1	+5

Table 2 shows that ICE read higher than TIDE by 6 pp in both experiments, while the CRISPResso2 figure sat 6 pp above ICE in sheep and 1 pp below it in horse. The paper's caption describes the metric for all three tools as the percentage of amplicons with an indel (link.springer.com), but the underlying denominators differ in kind. For the Nanopore analysis, the reads used for indel quantification after default alignment were 139,919 sheep reads (30.7% of total reads) and 47,360 horse reads (19.4% of total reads) (link.springer.com). By subtraction, about 69% and 81% of reads were outside that analyzed set, a figure computed for this report. The two Sanger tools have no read-level denominator at all. The paper reports 456,000 and 244,000 Nanopore reads for the two amplicons (link.springer.com), so the headline numbers in *Table 2* must never be pooled across modalities.

The authors state that ICE and nCRISPResso2 results were more concordant, with 7% variation, than the 12% between the two Sanger tools (link.springer.com). The authors do not define how this variation was calculated, so the figures should not be read as a sum of the experiment-level gaps in *Table 2*. The top five indel outcomes appeared in the same order across the three tools (link.springer.com), using a compared window of 10 bp on either side of the expected cleavage site (link.springer.com).

What the study leaves unknown

- **No ground truth.** The authors state that none of the tools was tested against ratio dilutions of modified and unmodified DNA (link.springer.com). Closeness between tools cannot validate any of them.
- **Two experiments only.** The paper says it presents data from only two gene-editing experiments in sheep and horse fibroblasts (link.springer.com). It does not establish clinical applicability or performance across human samples or edit classes.
- **Single platform and amplicon sizes.** Only R10.4.1 MinION flow cells were evaluated (link.springer.com), and other amplicon sizes were not assessed (link.springer.com).
- **Tool settings entangled with sequencing.** CRISPResso2 was run version 2.2.14 on FASTQ files (link.springer.com), with base calling in super-high-accuracy mode (link.springer.com) and indels below 1% frequency excluded (link.springer.com). The paper's text gives ICE and TIDE only as web interfaces (link.springer.com), with no version numbers or settings. Differences from sequencing, preprocessing and tool settings cannot be separated.
- **Edge masking limits large indels.** Reads need 86% homology (horse) or 88% homology (sheep) over the unmasked interior region after the amplicon edges are masked (link.springer.com), and the authors suggest lowering the minimum alignment score to detect larger indels (link.springer.com).
- **Edit class.** The authors say the commands used are not suitable for identifying substitutions from homology-directed repair (link.springer.com).

- **Platform noise.** Nanopore data are described as noisier than Illumina or PacBio data (link.springer.com), and base-call quality differed between species.

Other published comparisons

Other independent work points the same direction: results depend on the sample type and edit class. A 2024 comparison on artificial templates found DECODR gave the most accurate indel frequencies for most samples, and that TIDE and SeqScreener may suit low frequencies, with a detection limit of a few percent (pmc.ncbi.nlm.nih.gov). Even the best-performing tool output erroneous sequences for some composite events (pmc.ncbi.nlm.nih.gov). In plant samples benchmarked against amplicon sequencing, the authors conclude Sanger sequencing may be unsuitable for precise quantification of low-frequency edits (pmc.ncbi.nlm.nih.gov), TIDE outperformed two other Sanger tools in that setting (pmc.ncbi.nlm.nih.gov), and they caution that amplicon sequencing has its own biases and inaccuracies (pmc.ncbi.nlm.nih.gov). These results come from different sample types and do not combine into a ranking.

The ICE developers' preprint compared TIDE and ICE on 342 samples using default settings (www.biorxiv.org) and reports an r2 of 0.99 on samples selected for high ICE fit (r2 above 0.95) (www.biorxiv.org). Two Sanger tools modelling the same trace are expected to agree, and that does not address accuracy.

DECODR, a Sanger-trace tool without an indel size limit (pmc.ncbi.nlm.nih.gov), is a relevant alternative for teams seeking ICE alternatives. Its authors note that TIDE and ICE give only the amount, not the identity, of inserted bases (pmc.ncbi.nlm.nih.gov), and that DECODR is still limited by input sequence quality (pmc.ncbi.nlm.nih.gov).

A calibration plan that tests truth, not agreement

An author-run exercise should use permissioned public data or synthetic inputs and never proprietary material. It should also never run a commercial-use-restricted tool without the appropriate license. A suitable design has four parts.

- **Known-fraction mixtures.** Mix edited and unedited amplicon DNA at predefined ratios, and send the same material through Sanger and amplicon sequencing.
- **Simulated reads.** Generate FASTQ files with known indel fractions and defined error profiles to test windowing, filtering and denominator behaviour.
- **Public reanalysis.** Where the dataset's terms permit, reanalyze the public accession cited by the primary study and record every parameter.
- **Acceptance limits.** Scientific owners set acceptance limits before the exercise. This report proposes none.

Quality Flags, Escalation and Reproducible Handoff

Reviewing quality flags

Review should be prespecified and applied identically to every sample. The following items need a recorded disposition.

- **Control trace quality.** TIDE's authors advise keeping aberrant background before the break site low (pmc.ncbi.nlm.nih.gov), and ICE's alignment uses a high-quality window from the control (www.biorxiv.org).
- **Capillary artifacts.** Vendor guidance lists heterozygous indels as a cause of mixed signal downstream of the event (documents.thermofisher.com), and saturation can look like mixed sequence (documents.thermofisher.com). Homopolymers raise baseline noise (documents.thermofisher.com), and dye blobs can occur in the first 100 bp (www.thermofisher.com).
- **Windows and limits.** Record the quantification window and indel-size limits for the tool used. CRISPResso2 counts only events inside its window (docs.crispresso.com), and TIDE and ICE have documented indel-size limits (apps.datacurators.nl) (www.biorxiv.org).
- **Read-level flags.** For CRISPResso2, review guardrails such as the aligned-read fraction and total reads (docs.crispresso.com) and the reads-aligned statistics (docs.crispresso.com).
- **Missing and failed samples.** Count failed, excluded and low-fit samples in the batch denominator. Do not drop them silently.

Prespecified escalation checklist

An escalation rule written before data arrive prevents the threshold from moving after results are seen. The triggers below are proposals for a scientific owner to adopt or amend.

- **Fit or quality below owner-set limits.** If a Sanger tool's fit statistic or trace-quality check falls below the limit set in advance, repeat once, then escalate to amplicon sequencing. The tools report fit statistics but do not define a sponsor's pass mark (pmc.ncbi.nlm.nih.gov).
- **Incompatible edit type.** HDR, base editing or prime editing needs a template-aware path: TIDER, ICE with a template, or CRISPResso2 in the relevant mode (pmc.ncbi.nlm.nih.gov) (www.biorxiv.org).
- **Large or structural events suspected.** Short-range PCR assays can fail to detect deletions that remove a primer site (academic.oup.com), and large deletions of many kilobases after Cas9 cutting have been documented and are undetectable by short-range PCR assays (pmc.ncbi.nlm.nih.gov). These events call for an orthogonal assay. Neither Sanger decomposition nor short-amplicon sequencing settles them.
- **Clonal or biallelic samples.** Decomposition of clone traces can miscall alleles (pmc.ncbi.nlm.nih.gov), so clones should go to read-level sequencing.
- **Low-frequency edits.** Sanger tools have detection floors of a few percent in published tests (pmc.ncbi.nlm.nih.gov). If a decision depends on a small fraction, use read-level sequencing.
- **Result near a sponsor threshold.** If the measured value is close to the decision threshold, the uncertainty of the method matters more than the point estimate. Escalate.
- **Long amplicons.** Illumina amplicon workflows are often based on amplicons up to 500 bp (academic.oup.com), with paired-end 250-bp reads recommended in one protocol. Longer targets may need a different platform, as the Nanopore study used.

Reproducible handoff manifest

Evidence handed to a sponsor or CRO should let a third party rerun the analysis. The manifest in *Table 3* draws on documented software outputs and general reproducibility guidance. One widely cited rule is to archive the exact versions of all external programs used (journals.plos.org), and a second is to provide public access to scripts, runs and results where permitted (journals.plos.org). The FAIR principles state that research objects should be findable, accessible, interoperable and reusable (www.nature.com).

Manifest field	What to record	Source or rationale
Sample and reference versions	Sample IDs, amplicon and guide sequences, reference file hash	Both input types depend on the reference (docs.crispresso.com) (ice.editco.bio)
Raw files	.ab1 or FASTQ files, byte-level checksums, accession if deposited	Original submitted files stay retrievable at SRA (www.ncbi.nlm.nih.gov); SRA Lite carries simplified quality scores (www.ncbi.nlm.nih.gov)
Filtering counts	Reads in, after preprocessing, aligned; excluded samples	CRISPResso2 mapping statistics (docs.crispresso.com)
Window and limits	Quantification window, center, indel limits, minimum alignment score	Defaults differ by tool and are listed in each tool's own documentation
Software identity	Tool, version, install route, container digest	ICE reports its version by option; CRISPResso2 writes a JSON of run information (docs.crispresso.com); TIDE page showed 5.3.2 (apps.datacurators.nl)
Exact commands	Full command line or recorded web-form settings with date	CRISPResso2 writes a run log (docs.crispresso.com); web forms need screenshots or exported settings
Reports	HTML or spreadsheet output, plots, alignments	Output types are listed in each tool's own documentation (www.biorxiv.org)
Analyst disposition	Reviewer, date, flags seen, accept, repeat or escalate	Prespecified rules above
License and data terms	License basis for commercial use, hosted-data terms version	(github.com) (www.synthego.com)

Table 3 is deliberately tool-agnostic. For web tools, the weakest element is software identity. The primary comparison paper's text gives no ICE or TIDE version numbers or settings, which illustrates how easily a hosted run becomes unreproducible. A floating Docker tag is a second weak point, since the Docker Hub listing showed a relative update time (hub.docker.com). Pinning an image digest is therefore a recommendation of this report, and it was not tested. If human-derived data are involved, controlled-access routes through dbGaP apply (www.ncbi.nlm.nih.gov), so deposit decisions should be made with the data owner.

Data Analysis and Evidence

Cost and turnaround model

No sequencing prices are quoted in this report because no authoritative current price list was verified. The model below uses symbols, and buyers supply prices, failure rates and review times. Vendor claims about relative cost, for example the Synthego guide's statement of a roughly 100-fold reduction in cost relative to NGS-based amplicon sequencing (www.synthego.com), are unverified by this report and should be replaced by the buyer's own quotes.

- **Per-attempt cost.** For each attempt on a sample, add sequencing, preparation allocation, software or license allocation, compute, analyst review and integration. Call this a for Sanger and b for amplicon sequencing.
- **Accepted-sample cost.** Total accepted-sample cost equals the sum of all costs, including repeats, divided by the number of samples that meet predefined evidence criteria. With a pass rate p per attempt and a fixed batch cost F spread over N samples, the cost per accepted sample is (F/N + per-attempt cost) divided by p.
- **Repeats.** The expected number of attempts per accepted sample is 1 divided by p, assuming independent failures. Repeats raise both cost and turnaround.
- **Turnaround.** Turnaround equals preparation, transit, sequencing, analysis and review time, plus the repeat share of that cycle. The primary study's authors note that external sequencing services can lead to delays.
- **Break-even batch size.** If Sanger has fixed cost A0, per-attempt cost a and pass rate pA, and amplicon sequencing has fixed cost B0, per-attempt cost b and pass rate pB, the break-even batch size is N* = (B0/pB minus A0/pA) divided by (a/pA minus b/pB). It exists only when the Sanger per-accepted-sample cost exceeds the amplicon per-accepted-sample marginal cost.
- **Reflex strategy.** For a Sanger-first workflow that escalates a fraction e of samples, expected cost per accepted sample is the Sanger cost plus e times the amplicon cost. The escalation rate is itself an input to be measured.

Worked arithmetic (Hypothetical Example)

The numbers below are placeholders chosen only to demonstrate the algebra. They are not market prices and not estimates of any real service. Set a = 1 cost unit, A0 = 2 units, pA = 0.90, b = 1 unit, B0 = 20 units, pB = 0.95, so the per-attempt cost is the same for both methods and only the setup cost and pass rate differ. Then B0/pB = 21.05, A0/pA = 2.22, a/pA = 1.11 and b/pB = 1.05. Break-even N* = (B0/pB minus A0/pA) divided by (a/pA minus b/pB), which is exactly 322 with unrounded fractions (3220/171 divided by 10/171); the rounded figures shown would give about 314, so carry full precision. Above 322 samples amplicon sequencing is cheaper on these placeholders. If the buyer's real per-attempt costs make a/pA smaller than b/pB, the denominator is non-positive and amplicon sequencing never wins on cost alone. In that case evidence need, not cost, drives the choice.

Evidence summary

Table 1 and *Table 2* carry the quantitative evidence. The largest gap between tools in the primary study was 12 percentage points across two experiments, and the ICE developers reported r2 values of 0.93 (92 samples) and 0.97 (45 HDR samples) against amplicon sequencing (www.biorxiv.org). No source opened supplies a cost, failure rate or sensitivity cutoff that transfers to a reader's own assay.

Implications and Future Directions

Conditional choices

- **Choose a Sanger tool when** the edit class is non-templated indels, a control trace is available, traces are clean, and the decision is a triage. TIDE or ICE then give an inexpensive first read (tide.nki.nl). Keep TIDER or ICE with a template for templated work.
- **Choose amplicon sequencing with CRISPResso2 when** reads are available or justified, the question needs read-level counts, or the edit class is HDR, base editing or prime editing.
- **Choose a longer-read platform when** amplicons exceed the short-read limit, accepting noisier data and the validation burden the primary study describes.
- **Choose an orthogonal assay when** large deletions, primer-site loss or structural events are plausible.
- **Choose for-profit licensing early.** CRISPResso2 and the ICE source both distinguish non-commercial from commercial use (github.com), so a commercial team should obtain written terms from each vendor before standardizing a pipeline.

Unresolved evidence

Several questions remain open after this research. No head-to-head study was found that tests TIDE, ICE, DECODR and CRISPResso2 against one set of known mixtures. The CRISPResso2 denominator definition, the ICE hosted-data terms for the current host, and ICE and TIDE version history were not established from the pages opened. The hosted CRISPResso application was not exercised, and nor was the ICE application. The impact of preprocessing choices, for example masking base calls below a quality threshold, on headline percentages was not measured here. These gaps are the reason the author-run exercise above is recommended before any tool becomes a release-supporting method.

Where advisory support fits

The decision rule, manifest and escalation criteria should be written before data arrive. Outside advisory help does not replace a scientific owner's acceptance limits or a license review by counsel, and this report has had no outside expert review. The CRISPResso2 license itself states the tool shall not be used as a diagnostic decision-making system.

Frequently Asked Questions (FAQs)

How does CRISPResso2 compare with ICE?

CRISPResso2 counts reads from FASTQ files, while ICE fits a model to Sanger traces (pubmed.ncbi.nlm.nih.gov). In the one three-way study located, ICE and the Nanopore CRISPResso2 workflow differed by 6 pp in sheep and 1 pp in horse (the authors report 7% variation without defining the metric), and the authors describe them as the closer pair. That closeness is not validation, because no ratio-dilution control was run.

How does TIDE compare with ICE for CRISPR analysis?

Both read control and edited Sanger traces. In the study above ICE reported 6 pp higher than TIDE in both experiments (*Table 2*). ICE's developers report r2 of 0.99 between the tools on 342 samples chosen for high ICE fit (r2 above 0.95) (www.biorxiv.org). TIDER adds a reference trace for template-directed work, and ICE accepts a donor sequence.

What is the best CRISPR editing efficiency analysis tool?

No source opened supports a single best tool. Benchmarks on artificial templates, plant samples and fibroblasts favoured different tools. The best choice depends on edit class, sample type, sequencing modality and license.

How can CRISPR edits be quantified from Sanger sequencing?

Sequence the target region in the edited sample and in a control, then upload both traces to a decomposition tool. Record the guide, window, tool version and fit statistic, and follow the review checklist above.

Sanger versus NGS for CRISPR editing quantification: which is better?

Amplicon sequencing offers read-level resolution, and the ICE developers concede it has better sensitivity and quantitation (www.biorxiv.org). Sanger tools are cheaper per sample according to vendor claims and suit screening. Neither method detects every event: short-range PCR misses deletions that remove a primer site (academic.oup.com).

What are alternatives to Synthego ICE?

TIDE and TIDER are Sanger-based alternatives, DECODR removes the indel-size limit, and CRISPResso2 serves read-based work. ICE is now hosted by EditCo (www.synthego.com).

Is CRISPResso2 suitable for amplicon sequencing data?

Yes, FASTQ amplicon reads are its documented input (docs.crispresso.com). It assumes reads are already trimmed (docs.crispresso.com), and for-profit use needs a purchased license.

How should a biotech set up an edit-quantification workflow?

Define the requested quantity, pick tool and modality from *Table 1*, prespecify escalation rules, and keep the *Table 3* manifest for every batch.

Conclusion

ICE, TIDE and CRISPResso2 answer related but different questions. The Sanger tools estimate a mixture from a trace, and CRISPResso2 counts aligned reads. The one public three-way comparison located is small, historical and without ground-truth controls, so it supports the statement that the tools can give similar indel spectra in two fibroblast experiments, and nothing stronger.

A defensible workflow names the requested quantity first, matches tool and modality to the edit class, and writes the escalation triggers before the data exist. It also checks license scope before a for-profit team adopts any tool, and hands over a manifest that records versions, windows, files, counts and dispositions. The cost model rewards teams that measure their own failure and escalation rates. Where a decision sits near the method's uncertainty, the evidence needed is a calibration against known truth, and software agreement alone does not supply it.

Source links

Links cited in this article, in order of first appearance. Full addresses are provided for readers using extracted text.

1. https://tide.nki.nl
2. https://ice.editco.bio
3. https://docs.crispresso.com/latest/core/tool.html
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