



PROTOCOL

General CRISPR Protocol

Designing and running a successful genome editing experiment



Table of Contents

1. How to use this document	3
2. What RNP editing does	3
3. Experimental timeline	3
4. Materials	4
5. Choosing your editing strategy	5
6. crRNA design	6
7. Donor design	8
8. Working with Cas12a	9
9. Choosing a delivery method	9
10. Plasmid-based delivery	10
11. Controls	11
12. What your readout measures	11
13. Checking for unintended on-target effects	12
14. If your experiment underperforms	13
15. Troubleshooting	14
16. References and resources	17



1. How to use this document

This document covers the decisions that determine whether a CRISPR experiment works: how to pick a crRNA, how to design a donor, what your readout is actually measuring, and what quality checks to perform before you trust a clone. It is written for a first CRISPR experiment, but each section stands on its own.

If you have edited before, the sections worth reading are crRNA design, donor design, What your readout measures and Checking for unintended on-target effects. The design rules in the first two differ from what most online tools still recommend, because tools trained on transcribed gRNA do not transfer to the synthetic gRNA used in RNP editing.

This document does not give reaction volumes. Those are in the product protocols for the CCBio Super UniGuide, Super Knock-in Cocktail and Edit Selector Cocktail, each available in an electroporation and a lipofection version.

2. What RNP editing does

You deliver a preassembled complex of recombinant Cas9 protein and a synthetic guide RNA. Inside the cell, the complex finds the target, cuts both strands, and is degraded within hours. Everything that follows is the cell's own repair.

The guide RNA is made of two parts: a crRNA carrying your 20 nt target sequence, and a tracrRNA that is the same for every target. They are annealed before use. A covalently fused single guide RNA (sgRNA) works in the same workflow.

After the cut, the cell takes one of several routes, and they compete:

- **NHEJ** rejoins the ends. Often it leaves a small insertion or deletion (an indel). Sometimes it restores the original sequence exactly, leaving no trace.
- **MMEJ** uses short stretches of microhomology and almost always leaves a deletion.
- **HDR** copies from a template. If you supply a donor DNA carrying your intended change, HDR writes that change into the genome.
- The cell may also **die**, through a p53-dependent response to the DNA break.
- Repair can also change the locus on a larger scale, through **deletions of hundreds of base pairs or more, inversions, translocations or loss of a chromosome arm, or through loss of heterozygosity**, where the break is repaired using the homologous chromosome as a template and the second allele is overwritten.

For a knock-out you want indels, so no donor is needed. For a precise edit you want HDR, which means supplying a donor and accepting that NHEJ and MMEJ are competing for the same break.

RNP delivery is preferred over plasmid delivery because the nuclease is active immediately and disappears quickly, which reduces off-target cutting, and because no foreign DNA can integrate into the genome.

3. Experimental timeline

A typical experiment runs from seeding to genotyping in under two weeks. Knock-in needs longer than knock-out, because precise edits are rarer and the surviving population needs time to expand.



	Day	Step
For all edits	-2 / -1*	Seed cells
	0	Delivery (electroporation or lipofection)
For indels	1 – 3	Recovery and expansion
	2 – 5	Genotyping / Analysis
For knock-ins	3 – 5	Recovery and expansion
	5 – 7	Genotyping / Analysis

The seeding day depends on how fast your cells grow. Aim to be at 60–80% confluence on the day of delivery, and move the seeding day rather than the delivery day.

DNA can be harvested as early as 72 hours after delivery, but cells that were strongly affected by the delivery need longer before the surviving population is representative. If you plan to keep editing cells in culture, set up one well for harvesting and a parallel well for propagation.

4. Materials

Editing components

- Recombinant CRISPR nuclease (Cas9, HiFi-Cas9, Cas9 nickase, or Cas12a)
- crRNA carrying your target sequence, from any oligonucleotide supplier
- tracrRNA. The CCBio Super UniGuide is a universal tracrRNA with an improved backbone and pairs with crRNA from any supplier
- Donor DNA, for precise edits only: ssODN for small edits, dsDNA plasmid or PCR product for larger insertions

Delivery

- Electroporation system and the matching buffer, or
- Lipofection reagent and reduced-serum medium

Cell culture

- Your cell line, at low passage and in active growth
- Culture medium and coating if required
- Cell dissociation reagent. Accutase is gentler than trypsin and is worth using for iPSCs
- ROCK inhibitor (Y-27632), recommended for iPSCs



Analysis

- DNA extraction reagent. A single-tube extract such as QuickExtract is enough for PCR
- PCR primers flanking the target site, around 300 bp of product
- Sanger sequencing or NGS
- For clones taken forward: a ddPCR or qPCR assay for copy number at the target locus

Reagents for difficult targets

- An improved gRNA backbone, an HDR enhancer, or an NHEJ inhibitor, depending on what is limiting. *See section 14. If your experiment underperforms.*

5. Choosing your editing strategy

Three decisions precede any work at the bench: which nuclease, whether you need a donor, and how you will deliver.

Which nuclease

Variant	What it does	When to use it
Cas9	Blunt double-strand break	Default for most applications
HiFi-Cas9	Same cut, higher specificity	Targets with close off-target matches; therapeutic contexts
Cas9 nickase (D10A)	Cuts one strand only	Paired with two gRNAs for a staggered break with fewer off-targets; also useful alongside a wild-type Cas9 gRNA. <i>See section 14. If your experiment underperforms</i>
Cas12a	Staggered break further from the PAM, TTTV PAM	Where no suitable NGG PAM exists, or where large-scale repair is a concern

Whether you need a donor

A knock-out needs no donor. Indels from NHEJ and MMEJ will disrupt the reading frame. A precise change of any size needs a donor, and the donor design matters at least as much as the gRNA. *See section 7. Donor design.*

Cell type

Cell health is the single most common reason an experiment underperforms. Use low-passage cells in active growth.



Cell line	Type	Delivery	Notes
iPSCs and ESCs	Adherent, pluripotent	Either	Sensitive to both the break and the delivery. Dissociate with Accutase, add ROCK inhibitor for the first 24 h, and expect reduced viability
HEK293 and HEK293T	Adherent	Lipofection	Robust and easy to transfect. Hypotriploid, so allele counts are not what you would expect from a diploid line
HeLa	Adherent	Lipofection	Easy to transfect and widely used. Heavily aneuploid
U2OS	Adherent	Lipofection	Easy to transfect. Common in DNA repair work, where the repair pathway balance is itself the readout
HCT116	Adherent	Lipofection	Near-diploid, which makes zygosity and copy number far easier to interpret than in most cancer lines. Mismatch-repair deficient
A549, HepG2, MCF-7	Adherent	Electroporation preferred	Harder to transfect than the lines above. Lipofection works but gives lower and more variable efficiency
K562	Suspension	Electroporation	Standard for screens. Largely triploid with extensive rearrangement, so interpret copy-number results against the line's own baseline
Jurkat	Suspension	Electroporation	Electroporation substantially outperforms lipofection here, as it does for most suspension lines
Primary T cells and HSPCs	Suspension	Electroporation	Particularly sensitive to DNA damage. Low measured editing often reflects cell death rather than poor cutting

Primary cells vary too much for a general rule. Where a cell type is known to be sensitive to DNA damage, as primary T cells are, low measured editing may reflect cell death rather than a poor gRNA.

6. crRNA design

Your crRNA carries 20 nt matching the genomic sequence immediately 5' of an NGG PAM. Within that constraint, pick the guide whose cut site is closest to the position you want to change, then check it against the criteria below.

Check the EVA activity score

Most published gRNA scoring tools were trained on gRNA transcribed from a U6 or T7 promoter. Transcription introduces sequence biases that have nothing to do with how well a guide cuts, and those biases were learned by the models.



For chemically synthesised gRNA, which is what you use in RNP editing, they predict poorly.

The EVA activity score was trained on synthetic gRNA and measures activity as the sum of indels, targeted substitutions and cleavage-induced cell death, rather than indels alone. Scores are precalculated genome-wide and available as UCSC Genome Browser tracks:

- Human (hg38): EVA score track. [Visit website >>](#)
- Mouse (mm39): EVA score track. [Visit website >>](#)

A score below 50 marks an inefficient guide and is worth avoiding. A score above 50 means the guide is unlikely to be what limits your experiment. Around 95% of possible guides in the human genome score above 50 when synthesised, so in most cases you will have a choice.

What the score is built from

Five sequence features reduce synthetic gRNA activity. Knowing them helps when you are choosing between guides with similar scores:

- Strongly negative free energy of the 20 nt spacer, which indicates the guide folds on itself
- Few GA dinucleotides in the spacer
- A low MIT specificity score. Off-target sites compete for the guide, so poor specificity lowers on-target activity as well as raising off-target risk
- G at position 17
- C at position 20, directly before the PAM

What not to filter on

These rules are widely repeated and do not apply to synthetic gRNA:

- **A required G at the 5' end.** This exists because polymerases prefer to initiate on G. Synthetic oligonucleotides have no such constraint.
- **PAM-proximal dinucleotide composition.** A strong effect for transcribed gRNA, largely absent for synthetic.
- **Runs of thymines.** These terminate Pol III transcription and are irrelevant to a synthesised oligo.
- **A low Doench 2016, Rule Set 2 or Rule Set 3 score.** These do not reliably identify poor synthetic guides. In one test, the four worst-performing guides from a 2,314-guide published dataset reached 79–96% activity when supplied as synthetic RNP.

If your only candidate guide has a poor score in one of these legacy tools but a good EVA score, use it.

Off-target assessment

The MIT specificity score is already part of the EVA score, so a guide scoring above 50 has reasonable specificity. For therapeutic or otherwise sensitive work, carry out a dedicated off-target analysis and consider HiFi-Cas9, which reduces off-target cutting without a meaningful loss of on-target activity. CRISPOR is a convenient tool for this: it lists the predicted off-target sites for a given guide with their mismatch counts and CFD scores, and will also design primers for amplifying them. Use it for the off-target output rather than its on-target efficiency scores, which are the transcription-trained predictions described above.



7. Donor design

For precise edits, donor design is as decisive as guide choice. A good guide with a poorly designed donor gives a high indel rate and almost no knock-in.

Donor type and homology arms

Donor	Use for	Homology arms
ssODN	Point mutations, substitutions, edits below about 50 bp	40-100 nt each side
dsDNA plasmid or PCR product	Larger insertions such as tags or cassettes	300-1000 bp each side

Put the edit at the cut site

HDR efficiency falls off steeply with distance. An edit five nucleotides from the cut already has roughly half the efficiency of one at the cut site. If you have a choice of guides, the one that cuts closest to your intended change is usually preferable to one with a marginally higher activity score.

Some base changes are easier than others

The 12 possible substitutions are not equally efficient, and the difference is large enough to be worth designing around:

- C to T is more than 2.5-fold more efficient than G to A.
- T is most readily exchanged for C.
- Changes into purines (A or G) are disfavoured.
- T and G are roughly twice as hard to substitute as A and C.

Where the required change is fixed, this is a limitation to anticipate rather than one you can avoid. Where you have freedom, for example in choosing which silent mutation to introduce alongside your edit, pick the favourable direction.

Avoid donors that fold

HDR efficiency correlates with the predicted free energy of the ssODN. A donor with strongly negative free energy folds on itself and performs poorly. Check your donor sequence with an RNA or DNA folding tool before ordering, and if it folds, shift the homology arms by a few bases and check again.

Block re-cutting

Once HDR has written your edit, the locus is still a target unless the guide can no longer bind. If your intended change does not already disrupt the PAM or the seed region, add a silent mutation to the donor that does. If you want to compare edited with wild-type cells, consider a control donor that introduces the same silent mutation without the intended edit.

For Cas9, a mismatch in the PAM or in the seed region (roughly positions 4-8 upstream of the PAM) most reliably prevents re-cleavage. For Cas12a, mismatches in the first few positions downstream of the PAM are most effective.



This design step also sets you up for enrichment. A donor that changes the PAM or seed region creates a population in which only unedited alleles are still cuttable, which is what the CCBio Edit Selector Cocktail uses to remove unedited cells in a second round.

Predicting HDR efficiency

HDR efficiency can be estimated from three factors: guide activity, donor free energy, and base change type. HDR is always lower than the guide's activity score, and a high-scoring guide can still yield low HDR if the donor is poor. A calculator combining these factors is provided with the source publication. *See section 16. References and resources.*

8. Working with Cas12a

Cas12a is worth considering when no suitable NGG PAM sits near your target, or when unintended large-scale rearrangements would be a problem. Four things change relative to Cas9.

- **No tracrRNA.** Cas12a uses a single crRNA. The hybridisation step is skipped entirely, and the Super UniGuide does not apply.
- **Different PAM and cut position.** Cas12a recognises a T-rich PAM (TTTV) and cuts further from it, leaving staggered ends with 5' overhangs rather than the blunt ends Cas9 produces.
- **Design tools transfer.** This is the one case where a tool trained on transcribed guides predicts synthetic activity well: DeepCpf1 gives reliable predictions for synthetic Cas12a crRNA. The EVA score is Cas9-specific and does not apply.
- **Different repair behaviour.** In a comparison of twenty Cas12a targets, all showed a tendency toward perfect repair rather than large-scale repair, which is the opposite of what Cas9 shows at around half of targets. The likely reason is the staggered cut. For work where chromosome loss or translocation would be unacceptable, this is a substantive argument for Cas12a.

The practical consequence of that last point is a measurement problem rather than an editing problem: a Cas12a guide that cuts well may show few indels because the break is being repaired back to wild type. *See section 12. What your readout measures.*

9. Choosing a delivery method

Electroporation and lipofection deliver preassembled RNP. The choice is mostly about your cells and your equipment.

If your cells grow in suspension, or are known to be hard to transfect, start with electroporation. If you work with a robust adherent line, lipofection is simpler and gentler. For iPSCs both work; dissociating immediately before lipofection and transfecting in suspension (reverse transfection) tends to give the most consistent results.

Whichever you choose, two things matter more than the method:

- **A true single-cell suspension.** Clumps shield cells from the complex and are a common cause of low and variable efficiency. Check under the microscope before you proceed.
- **Minimising time in delivery buffer.** Transfer cells into fresh medium promptly after electroporation.



	Electroporation	Lipofection
Works with	Any cell type, including suspension and primary cells	Adherent lines, iPSCs, and most easily transfected cells
Equipment	Dedicated system and cuvettes	None beyond standard culture
Typical scale	One reaction per cuvette, plated across several wells	One reaction per well
Cell stress	Higher. Expect reduced viability	Lower
Main failure mode	Arcing, or toxicity from the electroporation buffer	Insufficient complex formation, or reagent toxicity

Reaction volumes, reagent amounts and plate formats are in the product protocols rather than here, because they differ between products. Each of the CCBio Super UniGuide, Super Knock-in Cocktail and Edit Selector Cocktail has an electroporation and a lipofection protocol.

10. Plasmid-based delivery

We recommend RNP for almost all applications. If you deliver Cas9 and gRNA encoded on a plasmid instead, the following applies. The CCBio product protocols are written for RNP and their amounts do not transfer.

What changes

- Editing starts only once Cas9 has been transcribed and translated, typically 12-24 h after delivery, and peaks over the following 24-72 h. Add 1-2 days to the recovery and genotyping windows in the timeline above
- The nuclease stays active for days rather than hours, which increases off-target cutting.
- Plasmid DNA can integrate into the genome at the cut site or elsewhere.
- If your plasmid carries a selection marker, selection adds further days before genotyping.

Electroporation

Use 3-5 µg of an all-in-one Cas9/gRNA plasmid per 1×10^6 cells, or 2-3 µg of a Cas9 plasmid plus 1-2 µg of a gRNA plasmid.

Resuspend or elute plasmid DNA at 1 µg/µL or higher, so that the added volume stays within the volume the protocol allots for the RNP mix. Larger volumes of elution buffer change the conductivity of the electroporation mix and can cause arcing and loss of the sample. Use transfection-grade, endotoxin-free DNA; endotoxin carryover reduces viability sharply, particularly in iPSCs.



Lipofection

Lipofectamine RNAiMAX is formulated for RNA and does not transfect plasmid DNA. Use a plasmid-compatible reagent such as Lipofectamine 3000 and follow the manufacturer's instructions for amounts and complex formation. As a starting point for a 96-well reaction, use 100 ng of total plasmid DNA with 0.15–0.3 μ L of lipofection reagent, keeping the volume added per well the same as in the RNP protocol so that the medium is diluted to the same extent. These amounts have not been validated in-house and are intended as a point to titrate from.

Enhancer timing

The CCBio cocktails are optimised for RNP delivery, where cutting begins within an hour or two. With plasmid delivery the break appears considerably later, so the optimal timing for the enhancers has not been established. Start from the amounts in the product protocol and adjust the post-delivery window empirically.

11. Controls

For a first experiment, or a first experiment in a new cell line, include both:

- **Positive control gRNA.** A guide already validated in your cell type. If it fails, the problem is delivery, cell state or reagent handling, not your target.
- **Negative control.** A non-targeting gRNA, or the same reaction without gRNA. This gives you the baseline sequence for comparison and shows how much of any viability loss comes from the delivery itself rather than the cut.

An untreated well is also worth keeping. Sequencing it alongside your edited sample distinguishes real edits from sequencing artefacts and from pre-existing variants in your line, which is particularly important when a result is unexpected.

Controls are most valuable when an experiment fails, so include them in the first experiment at a new site rather than adding them afterwards.

12. What your readout measures

A low indel count does not mean your guide failed to cut. This is the most common misreading of a CRISPR result, and it changes what you should do next.

Why indels undercount activity

The standard readout is a PCR across the cut site followed by sequencing, scored for indels. That misses three outcomes:

- **Perfect repair.** NHEJ frequently rejoins the ends with no change. The sequence is indistinguishable from an uncut allele.
- **Cell death.** Cells in which the target was cut and that subsequently died are no longer present when DNA is extracted.
- **Large-scale repair.** Deletions of hundreds of bases, inversions and translocations remove one or both primer sites, so those alleles do not amplify.



The size of this gap has been measured. In a screen of 78 targets in human iPSCs (Riesenberg et al. 2025, see References), mean indel frequency was 59%, while mean activity, counting targeted substitutions and cleavage-induced cell death alongside indels, was 68%. 93% of guides reached at least about 40% activity. A guide that looks poor on indels alone is often cutting efficiently.

Methods

Method	Measures	Notes
PCR plus Sanger sequencing	Presence of editing	Lowest-cost confirmation that editing has occurred. Amplify roughly 300 bp around the cut site
TIDE or ICE	Indel spectrum and frequency from a Sanger trace	Good for knock-out quantification. Standard mode does not quantify a precise knock-in
NGS of the amplicon	Indels and precise substitutions, separately	The reference method for knock-in and for enrichment
ddPCR	Allele copy number	Absolute quantification against an unlinked reference locus. <i>See section 13. Checking for unintended on-target effects</i>
Mismatch cleavage (T7EI, Surveyor)	Presence of indels	Semi-quantitative, insensitive to small or homozygous changes
Flow cytometry	Fluorescent reporter knock-ins	Only where the edit produces a signal

For quantifying enrichment after a second-round selection, use NGS or ddPCR.

A single-tube extract such as QuickExtract is sufficient as a PCR template. Always sequence an untreated control alongside.

13. Checking for unintended on-target effects

For clones, check target-locus copy number. A clone that sequences as a clean homozygous edit may in fact carry the edit on one allele and have lost the second allele entirely, which PCR across the cut site will not detect.

What can go wrong at the target site

Besides the indel you intended to avoid or create, a break can produce deletions of several hundred bases or more, inversions, translocations, and loss of a chromosome arm. These are collectively common: across a panel of 78 Cas9 targets, roughly half showed a tendency toward large-scale repair. In a validation using single-cell clones, 11-28% of clones from guides with that tendency had lost a copy of the locus, against none of 59 clones from guides without it.



Which guides are at risk

Two signals are predictive, and both can be assessed before the experiment:

- **A high indel rate.** Counterintuitively, guides producing many indels are more likely to also produce large-scale events. Guides producing few indels tend instead toward perfect repair
- **Low microhomology strength at the cut site.** Predictable from the sequence

Cas12a shows the opposite tendency and favours perfect repair, which is one reason to consider it where rearrangements would be unacceptable.

What to check, and when

Check	Method	When
Copy number at the target locus	ddPCR or qPCR, primers flanking the cut, probe avoiding edited positions, against an unlinked reference locus	Every clone taken forward
Loss of heterozygosity	Genotype a nearby heterozygous SNP	Clones from targets in a region where LOH matters
Zygosity of the edit	NGS of the amplicon, or ddPCR	Every clone
Off-target editing	Sequencing of predicted off-target sites	Therapeutic work, or guides with a low specificity score

For a pooled population rather than clones, copy-number checks are less informative, since a minority of rearranged alleles will be diluted out. The check matters most at the point where a single clone becomes your experimental model.

Carry out these checks before a clone becomes the basis of further work. Undetected on-target or off-target effects are not confined to the locus you edited; they propagate into every downstream phenotype, expression measurement and functional assay built on that line, and are far harder to diagnose once an unexpected result has appeared.

14. If your experiment underperforms

Before redesigning anything, determine which of three factors is limiting: the guide is not cutting, the guide is cutting but the repair is not detectable, or the guide is cutting and repair is favouring the wrong pathway. The remedies differ.

The guide may not be cutting

Check the EVA score. Below 50, redesign. Above 50, the guide is probably not the problem, and delivery or cell state is the more likely cause. Confirm with a positive control guide.



The guide is cutting but you cannot see it

Few indels together with a good activity score points to perfect repair. Two approaches recover the signal:

- **Add a neighbouring nicking guide.** A second guide with Cas9-D10A nickase alongside your wild-type Cas9 guide removes the sequence between the two sites, converting invisible perfect repair into detectable editing. Across five targets this raised editing from 0.7-28% to 15-77%, a mean 9.6-fold increase
- **Inhibit NHEJ.** Blocking the end-joining pathway responsible for perfect repair diverts breaks toward MMEJ and HDR

Repair favours the wrong pathway

A high indel frequency with low knock-in indicates that the break is being made but that NHEJ and MMEJ are outcompeting HDR. The options below are ordered by the effort required:

1. Check the donor against the rules in Donor design. Edit distance from the cut and donor folding are the two most common problems.
2. Confirm the edit prevents re-cutting. Without PAM or seed disruption, correctly edited alleles are re-cut.
3. Use reagents that shift the balance. An improved gRNA backbone, like the CCBio Super UniGuide, raises cutting; HDR enhancers such as the CCBio Super Knock-in Cocktail push repair toward the donor.
4. Enrich afterwards rather than optimising further. The CCBio Edit Selector Cocktail removes unedited cells in a second round, which is often faster than further optimisation of the primary HDR rate.

Combining an improved backbone with NHEJ inhibition is effective on difficult targets. Using HiFi-Cas9, four targets went from 2.6-42% to 74-94% editing, a mean 10.6-fold increase. Two of those targets had the worst possible score in a widely used legacy prediction tool.

High cell death

Some loss of viability in the first 24-48 h is expected and is partly the DNA damage response itself. Persistent heavy loss points to delivery conditions, cell state before the experiment, or endotoxin in a plasmid preparation.



15. Troubleshooting

Work through the likely causes in order. Most problems originate in cell state, guide choice or donor design.

Low or no editing detected

Likely cause	What to do
Inefficient gRNA	Check the EVA score. Below 50, redesign closer to the target site. Above 50, look elsewhere first
Cutting masked by perfect repair	Low indels with a good activity score. Add a neighbouring nicking guide, or inhibit NHEJ
Poor delivery	Run a validated positive control gRNA. If it also fails, optimise delivery rather than design
Cells not in active growth	Use low-passage cells at 60–80% confluence. Cells outside log phase edit poorly
Clumped cells	Confirm a true single-cell suspension under the microscope before delivery
Degraded RNA	Use RNase-free consumables, avoid repeated freeze-thaw, keep oligos at -20 °C

Low knock-in with good cutting

Likely cause	What to do
Edit too far from the cut	Efficiency halves by 5 nt. Choose a guide cutting closer, or accept the loss
Donor folds	Check the predicted free energy. Shift the homology arms and recheck
Unfavourable base change	Changes into purines are disfavoured. Where the design allows, pick the favourable direction
Edited allele re-cut	Add a silent mutation disrupting the PAM or seed region
Homology arms too short	40–100 nt each side for ssODN, 300–1000 bp for dsDNA
NHEJ outcompeting HDR	Use an HDR enhancer, or enrich afterwards (CCBio Super Knock-in Cocktail and Edit Selector Cocktail)



High cell death

Likely cause	What to do
Delivery conditions too harsh	Reduce voltage or adjust pulse settings; reduce lipofection reagent
Sensitive cell type	Add ROCK inhibitor for iPSCs, handle gently during dissociation
Prolonged exposure to delivery buffer	Transfer into fresh pre-warmed medium promptly
Residual dissociation reagent	Wash thoroughly before delivery
Expected response to the break	A transient reduction over 24–48 h is expected and partly reflects successful cutting

Inconsistent results between experiments

Likely cause	What to do
Variable cell state	Use a consistent passage number and growth phase
Reagent handling	Thaw properly, spin down before opening, avoid freeze-thaw cycles
No controls	Include positive and negative control gRNAs to separate workflow failure from target difficulty
Reagents not visible in the tube	Small-volume components may be present only as traces. Spin the tube briefly before resuspending

Clone does not behave as expected

Likely cause	What to do
Copy-number loss at the target	Run ddPCR against an unlinked reference locus
Loss of heterozygosity	Genotype a nearby heterozygous SNP
Off-target editing	Sequence predicted off-target sites; consider HiFi-Cas9 for future work
Pre-existing variant in the parental line	Compare against an untreated control from the same stock



16. References and resources

EVA activity score tracks

- Human (hg38): UCSC Genome Browser hub [Visit website >>](#)
- Mouse (mm39): UCSC Genome Browser hub [Visit website >>](#)

The tracks also carry Rule Set 3 scores for the Hsu and Chen tracrRNA backbones, for comparison when working with transcribed gRNA.

Source for the design and quality-control guidance

The gRNA design criteria, the HDR donor rules, and the cryptic repair findings in this document come from:

Riesenberg S, Kanis P, Karlić R, Maricic T. Robust prediction of synthetic gRNA activity and cryptic DNA repair by disentangling cellular CRISPR cleavage outcomes. *Nature Communications* 16, 4717 (2025). [Read paper >>](#)

The EVA and HDR score calculators are provided as Supplementary Data 2 and 3 of that paper.

CCBio product protocols

Each product has a protocol for electroporation and one for lipofection, with reaction volumes, plate formats and timings:

Super Knock-in Cocktail: For precise knock-in via HDR

- Super Knock-in Cocktail Protocol / Electroporation [Go to protocol >>](#)
- Super Knock-in Cocktail Protocol / Lipofection [Go to protocol >>](#)

Super UniGuide: For cleavage efficiency at difficult targets

- Super UniGuide Protocol / Electroporation [Go to protocol >>](#)
- Super UniGuide Protocol / Lipofection [Go to protocol >>](#)

Edit Selector Cocktail: For enrichment of edited cells in a second editing round

- Edit Selector Cocktail Protocol / Electroporation [Go to protocol >>](#)
- Edit Selector Cocktail Protocol / Lipofection [Go to protocol >>](#)

