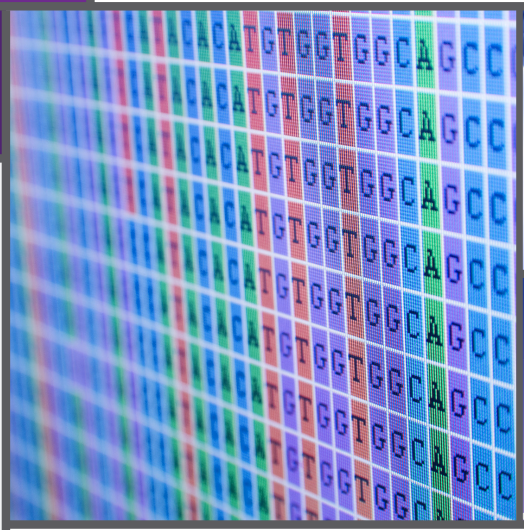
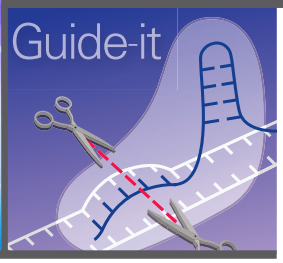
CRISPR/Cas9 Gene Editing Tools

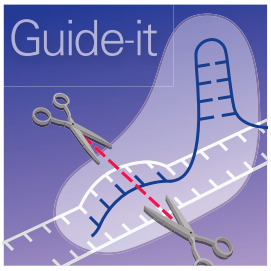




that's GOOD science!®

TaKaRa Clontech

Guide-it™ Products for Successful CRISPR/Cas9 Gene Editing



Gene editing for all researchers

The CRISPR/Cas9 system has democratized genome modification; targeted modification can now be achieved at virtually any genomic locus in virtually any cell type. This powerful method is allowing researchers to ask questions that were previously unaskable, leading to new insights into the basis of fundamental biological processes and to new innovative therapeutic strategies for treating disease—and that's good science!

Why choose Guide-it products?

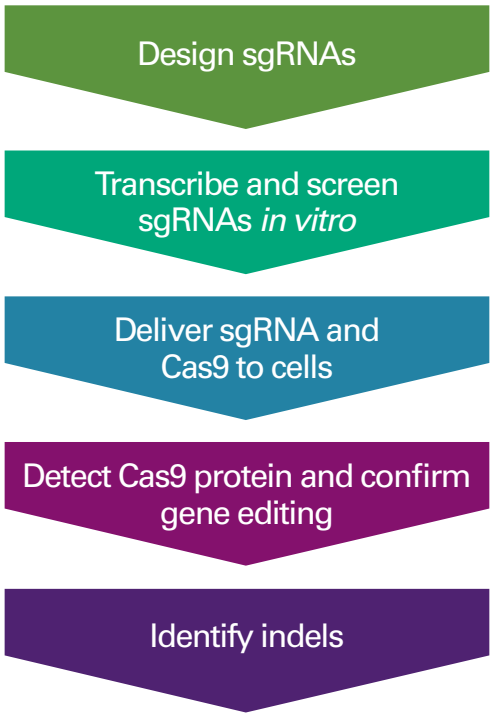
Optimized methods designed for speed and ease of use

- **Complete kits** that don't require additional reagents
- **Backed by our team of technical support scientists,** ready to help at any point in your experiment

Best-in-class tools for your gene editing project—from start to finish

Guide-it products further improve the usability of the CRISPR/Cas9 system by providing straightforward, streamlined methods at every step of your genome editing workflow.

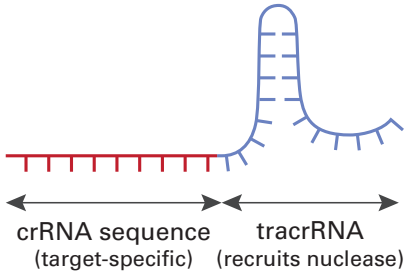
Typical Gene Editing Workflow



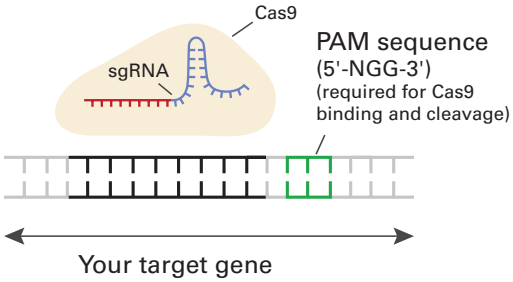
What is CRISPR/Cas9 gene editing?

CRISPR (**c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats)/Cas9 technology is based on an antiviral adaptive immune system in bacteria. The CRISPR/Cas9 system has been harnessed to create a simple, RNA-programmable method to mediate genome editing in mammalian cells. CRISPR/Cas9 editing is mediated by two components: a single-guide RNA (sgRNA) and Cas9 nuclease. The sgRNA directs the Cas9 nuclease to a specific genomic site where it introduces double-stranded DNA breaks. When this DNA damage is repaired by endogenous cellular DNA repair mechanisms, mutant alleles can be introduced.

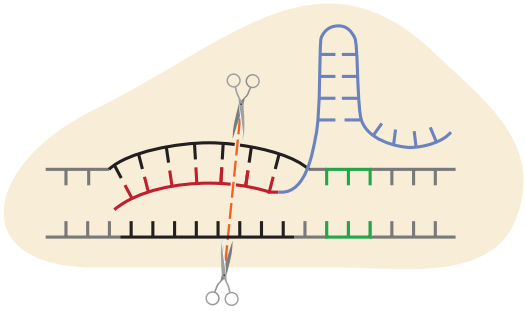
1 sgRNA (single guide RNA)



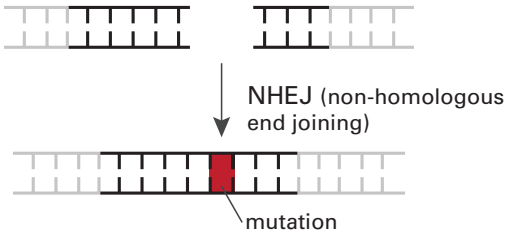
2 sgRNA + Cas9 protein



3 Target-specific cleavage



4 Cellular error-prone repair "knocks out" gene



At the bench: Design the best sgRNA

The first step in any CRISPR/Cas9 experiment is to choose a target sequence in the gene that you want to manipulate, and then design your guide RNA. In theory, selecting a target sequence is simple, but in practice, not all sgRNAs perform with equal efficiency and specificity. It is recommended that for any given target, multiple sgRNAs are designed and tested.

Learn more about best practices for choosing a target site at www.clontech.com/sgRNA-design.

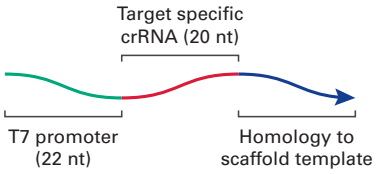
Transcribe and screen sgRNA *in vitro*

Test sgRNA performance before cell delivery

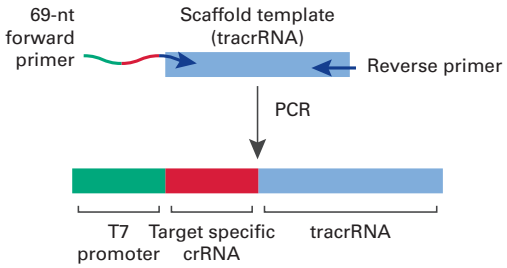
The Guide-it sgRNA *In Vitro* Transcription Kit: Produce high yields of sgRNAs for CRISPR/Cas9 studies

- Produce any sgRNA in <3 hours
- Transcribe >4 µg of sgRNA per *in vitro* reaction

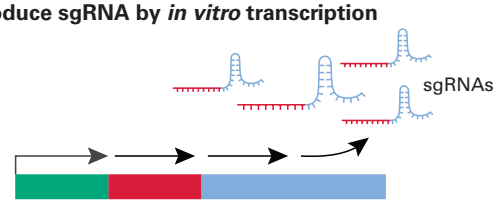
1 Design/purchase a 69-nt forward primer



2 Create template for *in vitro* transcription of sgRNA



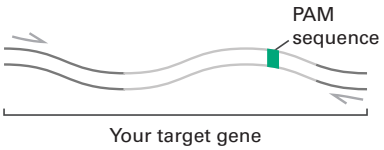
3 Produce sgRNA by *in vitro* transcription



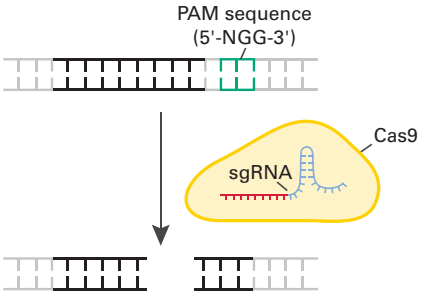
The Guide-it sgRNA *In Vitro* Screening Kit: Test the efficacy of sgRNAs before delivery to cells

- Highly optimized *in vitro* cleavage assay that allows estimation of cleavage efficiency
- Includes high-quality, recombinant Cas9 protein

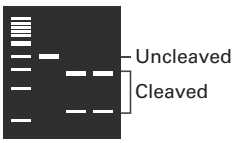
1 Use PCR to generate a target for cleavage



2 Cleave target sequence *in vitro* with recombinant Cas9 and synthesized sgRNA

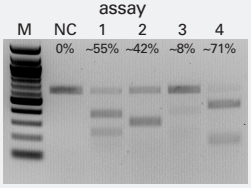


3 Confirm cleavage products by separating on an agarose gel

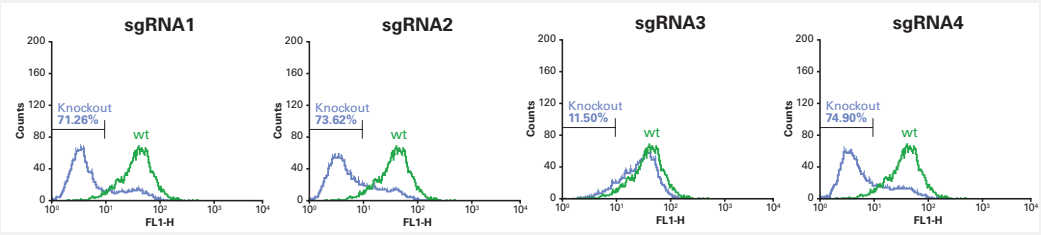


At the bench: Effective sgRNAs are identified *in vitro*

A Guide-it sgRNA Screening Kit *in vitro* Cas9 cleavage assay



B

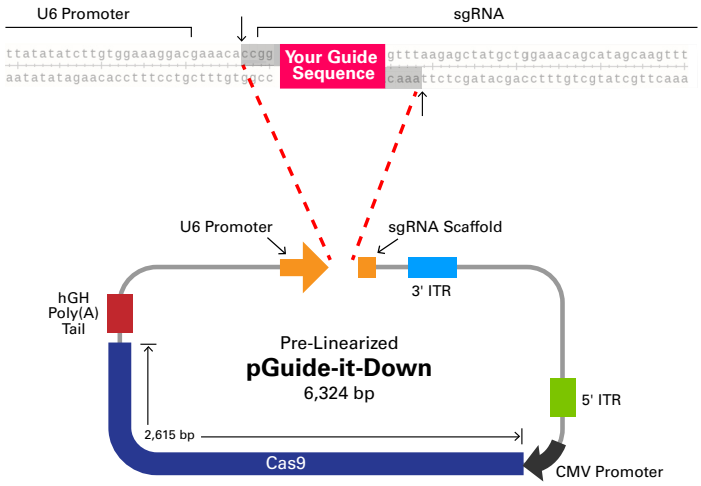


Identifying effective sgRNAs. Panel A. Cleavage efficiency of four different sgRNAs (1–4) targeting *CXCR4*. Negative control (NC) lacked sgRNA. sgRNA3 was predicted to be the least effective. **Panel B.** HeLa cells were cotransfected with plasmids encoding Cas9 and one of the four different sgRNAs. Efficiency of *CXCR4* gene disruption was assessed by a FITC-labeled antibody against CXCR4. In agreement with the Screening Kit results, sgRNA3 resulted in the lowest percentage of knockout cells.

Deliver sgRNA/Cas9 expression cassettes to any target cell

Guide-it CRISPR/Cas9 Systems: Plasmid-based delivery

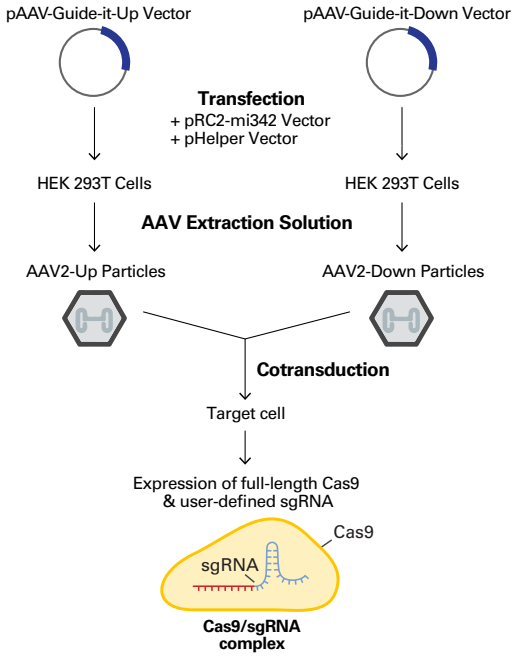
- Complete system for the cloning and expression of sgRNAs and Cas9 in mammalian cells
- Pre-linearized expression plasmids allow for simple, single-step cloning of sgRNAs
- Some vectors also include bright fluorescent reporters, ZsGreen1 or tdTomato (2.5x and 6x brighter than EGFP, respectively) for identification of transfected cells



pGuide-it vector simultaneously expresses Cas9, an sgRNA, and, in some cases, a fluorescent reporter (ZsGreen1 or TdTomato).

AAVpro® CRISPR/Cas9 Helper Free System: AAV-mediated delivery

- Optimized system for producing AAV2 particles to deliver both sgRNA and Cas9 expression cassettes
- Improved results in hard-to-transfect cells
- AAV doesn't integrate, eliminating persistent Cas9 expression and reducing potential off-target effects

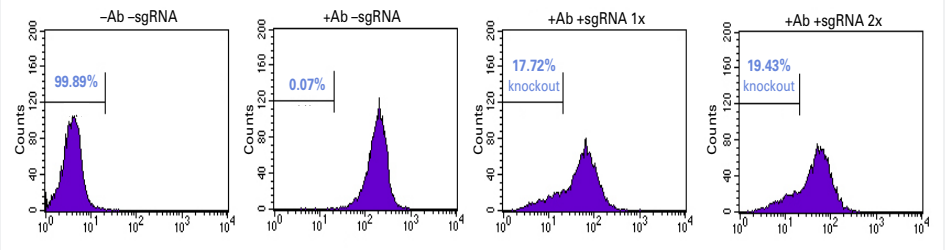


A two-vector system overcomes the size restrictions of the AAV genome. Cas9 is split between two vectors with a 1.6-kb region of homology that mediates recombination in target cells, producing a full-length Cas9 gene expression cassette.

Xfect™ RNA Transfection Reagent for Guide-it gene editing experiments

- Single reagent for transfection of cells lines and primary cells with mRNA and/or sgRNA
- Very low cytotoxicity and high transfection efficiency in primary cells
- Simple, serum-compatible protocol

At the bench: Effective transfection of HT1080 cells with sgRNAs



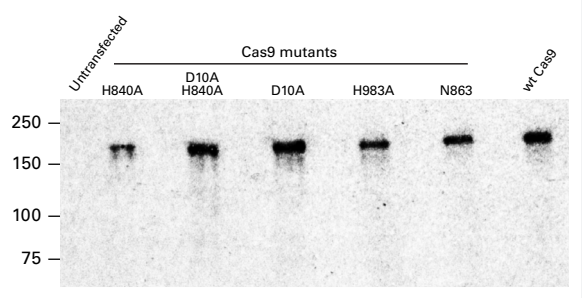
HT1080 cells stably expressing Cas9 (HT1080-Cas9) were transfected with 50 pmol of sgRNA targeting *CD81*, either once (1x) or twice (2x). Seven days later, cells were immunostained with a CD81 antibody (Ab) conjugated to an FITC fluorophore and analyzed by flow cytometry. The percentage of cells that did not bind CD81 was calculated. A control sample, comprised of HT1080-Cas9 cells, was analyzed by flow cytometry, either without (–Ab) or with (+Ab) the CD81 antibody.

Detect Cas9 protein and confirm gene editing

Sensitive detection of Cas9 expression

Cas9 polyclonal antibody: Confirm Cas9 expression in your cells

- Recognizes wild-type (wt) Cas9, as well as nickase and nuclease-deficient mutant versions
- High sensitivity and specificity; recognizes as little as 0.15 ng of recombinant Cas9



At the bench: Wild-type and nickase mutant Cas9 are detected with high sensitivity

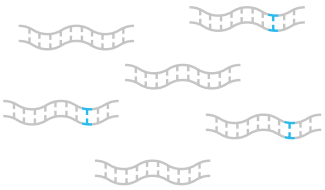
HEK 293 cells were transfected with plasmids encoding either wt or mutant Cas9 transcripts expressed from the CMV promoter; untransfected cells were used as a negative control. After 48 hours, the cells were lysed and Western blotting was performed. Blots were probed with primary anti-Cas9 polyclonal antibody (1:5,000 dilution). For detection, an HRP-conjugated goat anti-rabbit secondary antibody was used.

A streamlined method for detecting mutations

The Guide-it Mutation Detection Kit: PCR-based detection of indels

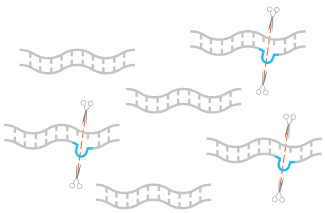
- Complete system for confirming the presence of indels after genome editing
- Ultra-fast PCR-based method—direct amplification from cells without genomic DNA extraction or sequencing
- Includes Guide-it Resolvase enzyme, a mismatch-specific nuclease that provides results that are superior to Cel1 nuclease

1 Amplify genomic DNA from cells using Terra™ PCR Direct Polymerase



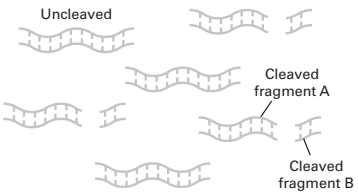
Region of interest is amplified (mutation is marked in blue)

2 Denature and reanneal

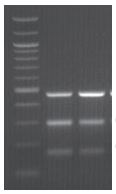


Guide-it Resolvase cleaves imperfectly matched DNA

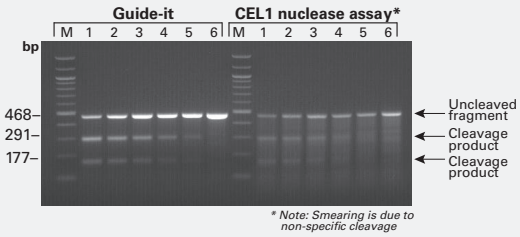
3 Cleaved and uncleaved PCR fragments have different sizes



4 Separate cleaved and uncleaved PCR fragments using agarose gel electrophoresis



At the bench: Improved mutation detection versus the CEL1 assay



HEK 293T cells were transfected with plasmids encoding Cas9 and a sgRNA targeting the *AAVS1* locus. Mutations were easily discernible when using the Guide-it kit. In contrast, the CEL1 assay showed considerable smearing, making it difficult to determine cleavage efficiency and reducing the ability to detect low levels of mutations.

Gene Editing Resources

Visit our website to explore our evolving portfolio of products, learning resources, and technical information for CRISPR/Cas9 gene editing.

www.clontech.com/CRISPR

- New products
- Selection guides
- Technical notes
- Videos
- Protocols
- Learning resources

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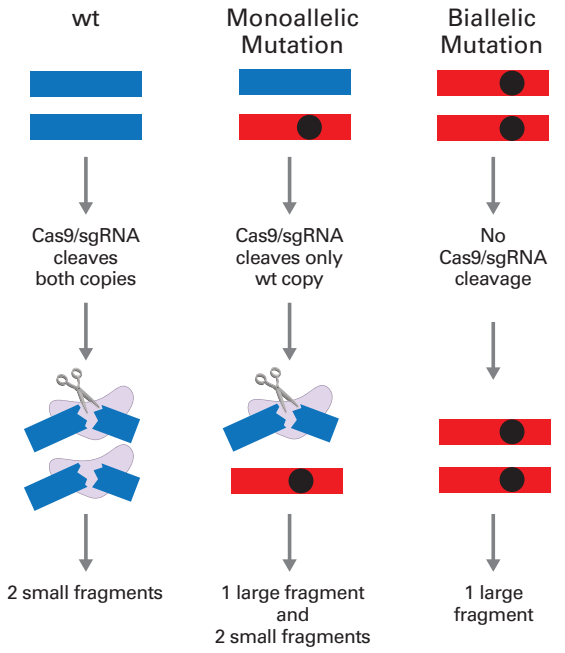
Products • Learning Resources • Technical Information

Determine if one or both copies of your gene have indels

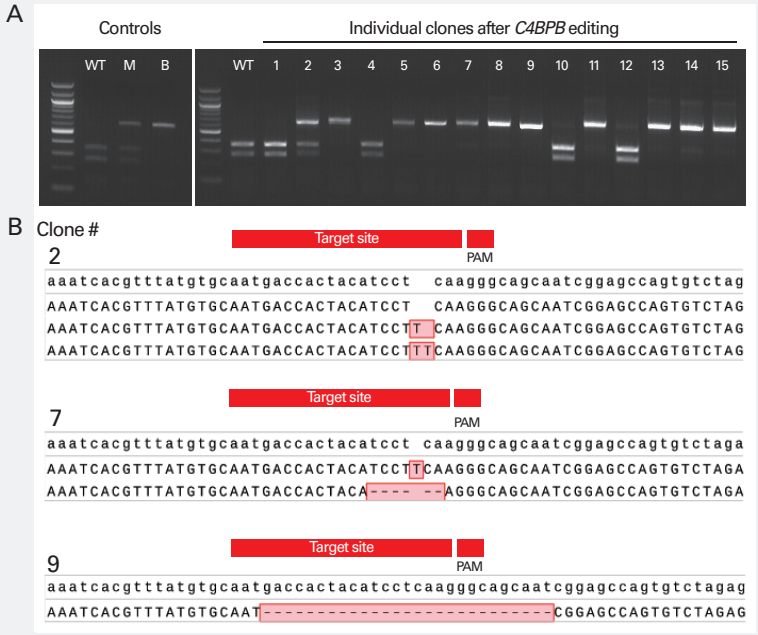
The Guide-it Genotype Confirmation Kit: Simple detection of monoallelic and biallelic mutations

- Streamlined protocol that uses direct amplification of target genomic DNA from cells
- Includes highly purified recombinant Cas9 nuclease for *in vitro* cleavage



The Guide-it Genotype Confirmation Kit includes a Cas9/sgRNA-mediated *in vitro* cleavage reaction for genotype determination after CRISPR/Cas9 gene editing. The same sgRNA that was used for gene editing must be used in the cleavage assay

At the bench: Accurate genotype determination in HEK 293 cells



HEK 293 cells were treated with Cas9 and an sgRNA targeting the *C4BPB* gene to generate 15 single-cell clones. Panel A. The Guide-it Genotype Confirmation Kit was used to determine the genotype, with wt, monoallelic (M), and biallelic (B) control reactions included in the analysis. **Panel B.** Sequencing results are shown for select clones from Panel A. Lowercase letters represent the wt sequence. As predicted, clone 2 is monoallelic and clones 7 and 9 are biallelic.

Characterize indels in four steps

The Guide-it Indel Identification Kit: Characterize the variety of indels created by CRISPR/Cas9 editing

- Complete kit contains all of the components needed to amplify, clone, and prepare modified target sites for DNA sequence analysis
- Ultra-fast protocol includes PCR amplification directly from cells, and the In-Fusion® Cloning system for ligation-free cloning in 15 minutes

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