System Biosciences
Accelerating discoveries through innovations

Genome Engineering

PrecisionX™ Multiplex gRNA Cloning Kit

Create CRISPR/Cas9 constructs with multiple gRNAs simultaneously for better genome editing

The CRISPR/Cas9 gRNA Targeting System

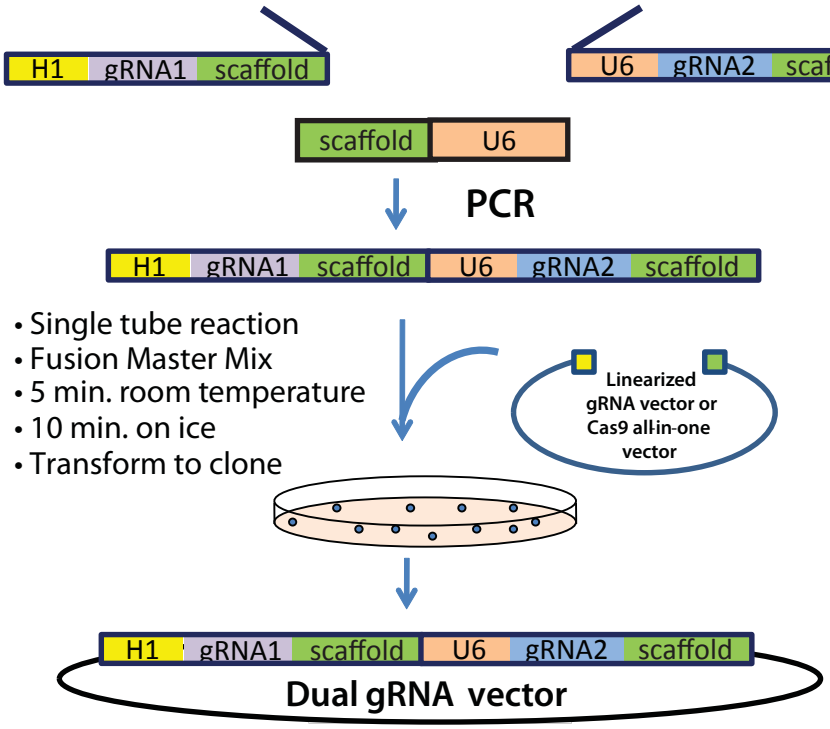
The recent discovery of the CRISPR/Cas9 sysem has provided researchers an invaluable tool to target and modify any genomic sequence with high levels of efficacy and specificity. The system, consisting of a nuclease (Cas9) and a DNA-directed guide RNA (gRNA), allows for sequence-specific cleavage of target sequence containing a protospacer adaptor motif "NGG". By changing the gRNA target sequence, virtually any gene sequence upstream of a PAM motif can be targeted by the CRISPR/Cas9 system, enabling the possibility of systematic targeting of sequences on a genomic scale. The most successful gene targeting using the CRISPR/Cas9 system is through expression of multiple gRNAs to guide the enzyme complex to several locations within the target gene to be cut or nicked.

SBI has developed a revolutionary cloning kit in order to facilitate the cloning of gRNAs into CRISPR/Cas9 vectors, the PrecisionX Multiplex gRNA Cloning Kit (Cat# CAS9-GRNA-KIT). This system allows for the cloning of multiple gRNAs into any Cas9/gRNA "all-in-one" expression vector or gRNA cloning vector of the customer's choice, including SBI's PrecisionX Cas9/gRNA SmartNuclease plasmids and lentivectors. The Multiplex gRNA Cloning Kit is also compatible with the most popular Cas9/gRNA cloning vectors developed in labs across the world, such as pX330, pX335, pX458, and pX459.

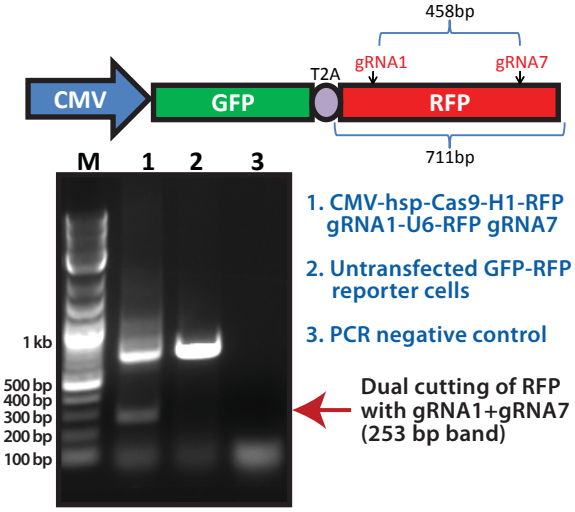
Highlights

- Save time and reagents - no need to clone separate constructs bearing different gRNAs
- Simple two-step PCR and "fusion" reaction to generate multi-cistronic gRNA expression constructs
- Compatible with all types of Cas9/gRNA expression vectors containing H1 or U6 promoters
- Ideal for Cas9 Nickase applications requiring expression of two gRNAs simultaneously for precise targeting
- Enables precise deletion of defined genomic segments and control of multi gene regulation - genome manipulation made simple

How the Multiplex gRNA Cloning Kit Works



Sample Dual gRNA Targeting Data



Functional validation of dual gRNAs cloned into SBI's CAS940A-1 vector (CMV-hspCas9-H1-gRNA) using the Multiplex gRNA Cloning Kit to targeting RFP in a cell line stably expressing a CMV-GFP-T2A-RFP cassette. PCR assay with primers flanking RFP indicate evidence of cutting by gRNAs.

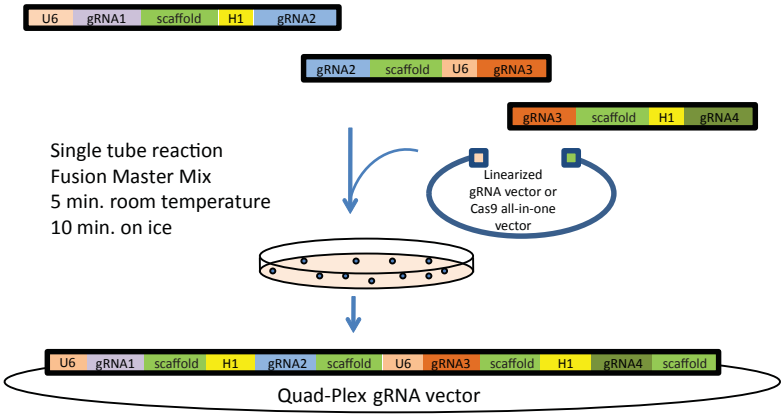
PrecisionX™ Multiplex gRNA Cloning Kit

Generate Tandem CRISPR/Cas9 gRNA Constructs

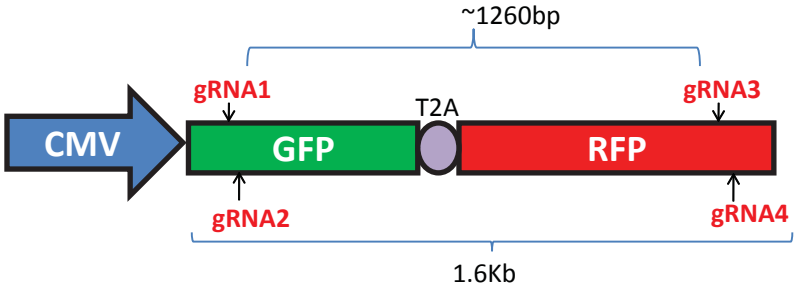
The scalability of the Multiplex gRNA Cloning Kit allows for simultaneous cloning of two or more gRNAs at once into a single vector. This enables researchers to perform more advanced CRISPR/Cas9 techniques such as tandem double-nicking (4 gRNAs total) to remove defined genomic segments using Cas9 Nickase with significantly decreased chances for off-target effects.

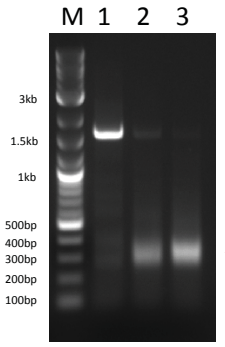
The cloning of four gRNAs will require the researcher to perform three separate PCR reactions with separate primer pairs and blocks. Once the correct size amplicons are generated and gel-purified, they can be mixed at equimolar ratios (1:1:1) based on their concentrations and used as inserts in the subsequent fusion reaction with a suitable linearized destination vector.

How Quad-Plex gRNA Vectors are Built



Sample Tandem gRNA Targeting Data

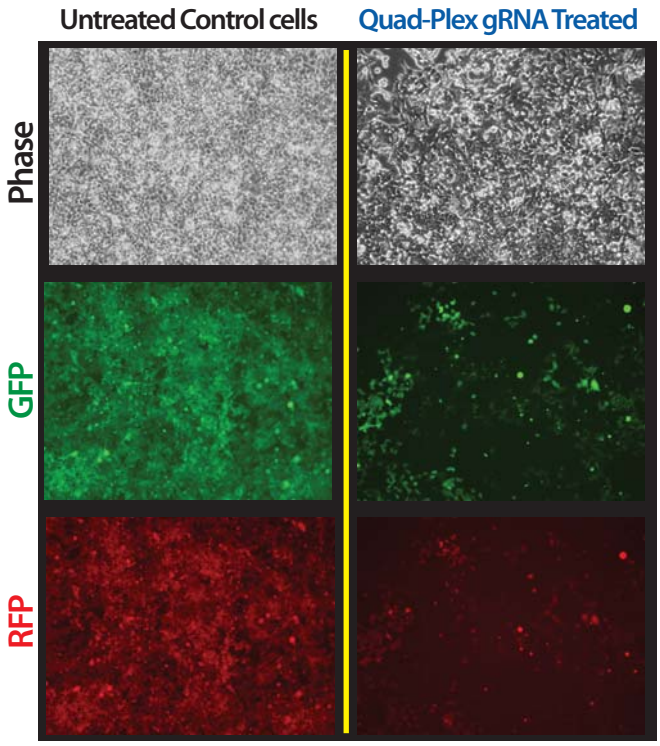




1. Untransfected GFP-RFP reporter cells
2. Lenti-CMV-hspCas9-Nickase-T2A-Puro-H1-GFP gRNA1-U6-GFP-gRNA2-H1-RFP-gRNA3-U6-RFP-gRNA4
3. EF1-hspCas9-Nickase-H1-GFP gRNA1-U6-GFP gRNA2-H1-RFP gRNA3-U6-RFP gRNA4

Paired cutting of GFP/RFP with tandem gRNAs (340 bp band)

Sample Quad-Plex gRNA Targeting Data



Functional validation of tandem Quad-Plex gRNAs cloned into CAS800A-1 Nickase vector (EF1Nickase-H1-gRNA) using the Multiplex gRNA Cloning Kit for targeting GFP and RFP. The gene targets were present in a stable reporter cell line harboring a CMV-GFP-T2A-RFP expression cassette. PCR assays with primers (one at 5' end of GFP and other at 3' end of RFP) indicate evidence of cutting by gRNAs (left panel). Fluorescence images of targeted cells vs control cells taken after 10 days show significant reduction in both RFP and GFP expression (panel above).

We Also Offer Custom Services - *Have SBI Build Your Next Custom Multiplex gRNA Vectors.*

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at www.systembio.com/service or call us at 888-266-5066.

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