GenCrispr sgRNA Synthesis Kit

Cat. No. L00694 Version 07032017

I DESCRIPTION

The CRISPR/Cas9 system is an RNA-guided defense mechanism in bacteria and archaea, which has been widely used for efficient genome editing. In this system, the Cas9 nuclease associates with two RNAs, the CRISPR RNA (crRNA) and the trans-activating crRNA (tracrRNA), to direct sequence-specific cleavage of foreign DNA. The gRNA (guide RNA) is a fusion of the natural crRNA and tracrRNA components. It contains an 18-20 base variable sequence that can be changed to target any DNA sequence that is adjacent to an NGG proto-spacer adjacent motif (PAM) on the 3' end of the target sequence. The gRNAs are synthesized through transcription by T7 RNA polymerase from a DNA template of your choice. The GenCrispr sgRNA synthesis kit is designed to efficiently generate a gRNA DNA template containing a 5' T7 promoter. The kit provides components to perform subsequent *in-vitro* transcription of gRNA DNA template and obtain gRNAs which can be used for *in-vivo* genome editing.

II KIT CONTENTS

Components	Amount provided	
	50-reaction kit	20-reaction kit
10 μM TracrRNA fragment	50 μL	20 μL
10 μM T7 Primer Mix	50 μL	20 μL
10 X PCR Reaction buffer	500 μL	200 μL
High-Fidelity polymerase	25 μL	10 μL
10 μM dNTP mixture	50 μL	20 μL
Control gRNA forward and reverse primers (10 μM)	50 μL	20 μL
50 mM DTT	100 μL	40 μL
10 X Transcription Reaction buffer	100 μL	40 μL
10 mM NTP mix	200 μL	80 μL
GenCrispr T7 RNA polymerase	50 μL	20 μL
RNase inhibitor	25 μL	10 μL
Nuclease-free water	1 mL	1 mL

Materials required but not provided

Target-specific DNA oligonucleotides (see section V A.1)

RNase-free tubes, aerosol tips

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III APPLICATION

The GenCrispr sgRNA synthesis kit is used to generate gRNA DNA template with a T7 promoter and synthesize gRNAs upon *in-vitro* transcription.

IV STORAGE

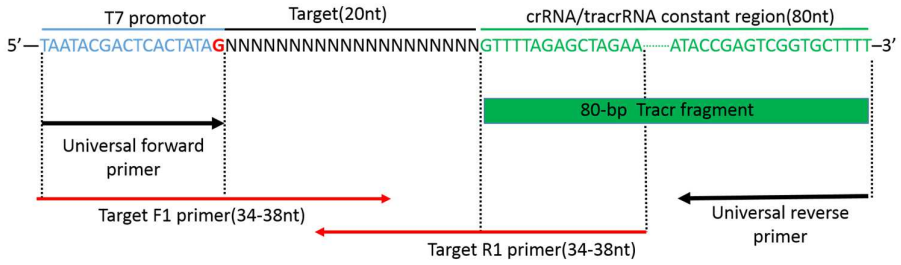
Store all components at -20°C upon receipt.

V PROTOCOL

A. Design the forward and reverse oligonucleotides for PCR assembly

1. The gRNA DNA template sequence is composed of the T7 promoter sequence, the sequence coding the target-specific gRNA and the constant region of the crRNA/tracrRNA.

   The gRNA DNA template sequence:
   TAATACGACTCACTATAGNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAA
   AATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT–



   The T7 promoter sequence is shown in blue.

   Transcription begins at and includes the bold **G** (red) from the T7 promoter sequence.

   The constant region of the crRNA/tracrRNA is shown in green.

   Note: We recommend having at least one G at the start of the transcript to improve gRNA yield from the *in vitro* transcription (IVT) reaction. Although we have observed improved gRNA IVT yields with two to three Gs, usually one G is sufficient. If the target sequence already contains a 5'G, you can choose to keep it, which will result in an extra G being added from the T7 promoter primer. Alternatively, you can remove the first G of the target sequence, which will be added back by the T7 promoter primer.

2. Sequences of the Target F1 forward and Target R1 reverse oligonucleotides required for synthetic gRNA template assembly.
   Target F1: TAATACGACTCACTATAG + first 16-20 nt of the target sequence
   Target R1: TTCTAGCTCTAAAAC + first 19-20 nt of the target sequence reverse complement
   Example:
   HPRT gRNA target sequence: GCATTTCTCAGTCCTAAACA
   Reverse complement: TGTTTAGGACTGAGAAATGC
   HPRT Target F1: TAATACGACTCACTATA**G** + GCATTTCTCAGTCCTA
   HPRT Target R1: TTCTAGCTCTAAAAC + TGTTTAGGACTGAGAAAT
   gRNA sequence after IVT: **G**GCATTTCTCAGTCCTAAACAGTTTTAGAGCTAGA……

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3. Get the Target F1 and the Target R1 oligonucleotides synthesized. We recommend generating three pairs of oligonucleotides for each gene of interest.

B. Assemble the gRNA DNA template by PCR amplification

1. Set up the PCR assembly reaction:

Components	Volume
10 X PCR Reaction buffer	5 μL
10 μM TracrRNA fragment	1 μL
10 μM T7 Primer Mix	1 μL
10 μM Target F1 oligonucleotide	1 μL
10 μM Target R1 oligonucleotide	1 μL
10 μM dNTP mixture	1 μL
High-Fidelity polymerase	0.5 μL
Nuclease-free water	39.5 μL
Total	50 μL

2. Perform assembly PCR using the cycling parameters below:

Cycle step	Temperature	Time	Cycles
Initial denaturation	94 ℃	3 min	1 X
Denaturation	94 ℃	10 s	32 X
Annealing	55 ℃	15 s	
Final extension	72 ℃	1 min	1 X
Hold	4 ℃	Hold*	1 X

C. Perform *in vitro* transcription

3. Set up the following *in vitro* transcription reaction, adding the reaction components in the order given.

Components	Volume
10 x transcription buffer	2 μL
50 mM DTT	2 μL
10 mM NTP mixture	4 μL
gRNA DNA template	200 ng
RNase inhibitor	0.5 μL
T7 RNA polymerase	1 μL
Nuclease-free water	Up to 20 μL
Total	20 μL

4. Incubate at 37 ℃ for 2-3 hours.

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(Optional)

D. Screen the efficiency of sgRNAs by *in-vitro* cleavage assay

The transcribed gRNAs can be directly verified for cleavage efficiency by using the GenCrispr sgRNA screening kit (Cat# L00689).

E. Deliver the sgRNA and Cas9 into cells

The gRNA can be used to form a stable complex with Cas9 Nuclease. This ribonucleoprotein (RNP) complex can be directly delivered into cells using transfection reagents, electroporation, and/or microinjection.

VI NOTES

1. To improve the efficiency of your genome editing experiment, we recommend purification of the gRNA by an RNA clean-up method of your choice before using it for transfection.
2. It is recommended to design at least three different gRNAs for each target gene. Each gRNA has a different gene editing efficiency, using multiple gRNAs simultaneously improves the chances of obtaining successful editing.

VII RELATED PRODUCTS

Cat. No.	Product Name
Z03385	GenCrispr Cas9-C-NLS Nuclease
Z03386	GenCrispr Cas9 Nuclease
Z03388	GenCrispr Cas9-N-NLS Nuclease
Z03389	GenCrispr NLS-Cas9-NLS Nuclease
Z03390	GenCrispr NLS-Cas9-D10A Nickase
Z03393	GenCrispr NLS-Cas9-EGFP Nuclease
Z03396	GenCrispr T7 Endonuclease I
L00688	GenCrispr Mutation Detection Kit
L00689	GenCrispr sgRNA Screening Kit
L00690	High-Efficiency gRNA-Cas9-GFP Plasmid (linear) Assembly Kit
L00691	High-Efficiency gRNA-Cas9-Puro Plasmid (linear) Assembly Kit
L00692	High-Efficiency gRNA-Cas9-GFP Plasmid Assembly Kit
L00693	High-Efficiency gRNA-Cas9-Puro Plasmid Assembly Kit

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