GenCrispr sgRNA Synthesis Kit

Cat. No.: L00694 Version 2018-07-16

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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.

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II Contents

Components	50-reaction	20-reaction
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
RNase-free tubes, aerosol tips

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.

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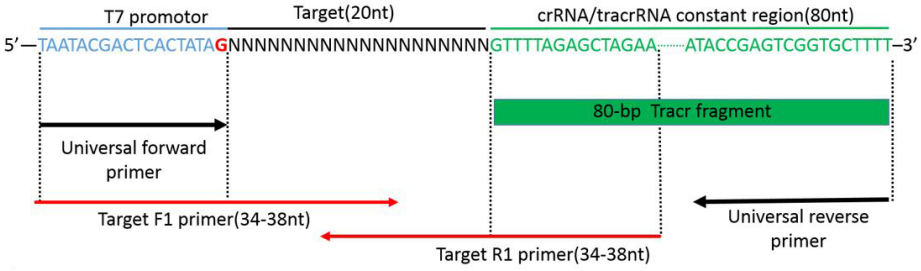
V Protocol

1 Design the forward and reverse oligonucleotides for PCR assembly

1.1 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:
TAATACGACTCACTATA **G** NNNNNNNNNNNNNNNNNNN
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCC
GTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT–



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 ingreen.

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.

1.2 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: GGCATTTCTCAGTCCTAAACAGTTTTAGAGCTAGA……

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

2 Assemble the gRNA DNA template by PCR amplification

2.1 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

2.2 Perform assembly PCR using the cycling parameters below:

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

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3 Perform *in vitro* transcription

3.1 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

3.2 Incubate at 37 °C for 2-3 hours.

(Optional)

4 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. No. L00689).

5 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.

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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 Ordering Information

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

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