HyperScribe™ Two-Step sgRNA Synthesis Kit

Product description

The CRISPR/Cas9 system is a widely used genome editing technology, which uses Cas9 nuclease to cut specific sites of targeted sequences of genomic DNA under the guidance of sgRNA, also known as gRNA (guide RNA). gRNA is a fusion product consisting of 18-20 bp CRISPR RNA (crRNA) sequences that complement the target gene sequences, and Trans-activating crRNA (tracrRNA) sequences that bind to Cas9 nucleases.In the cell, gRNA bound to Cas9 nuclease and guided Cas9 nuclease to cut the target gene at the upstream position of the proto-spacer adjacent motif (PAM) sequence, causing frameshift mutations in the target gene locus and eventually resulting in deletion mutations of the target gene.

This kit is a two-step method for the synthesis of sgRNA based on PCR amplification and in vitro transcription dependent on T7 RNA polymerase. The kit provides a Template Mix, including a Scaffold Template sequence and a downstream primer for amplification of the sequence. Users only need to design and synthesize the Target-specific DNA oligo (i.e. the upstream primer) to obtain the corresponding sgRNA through this kit. After purification, the synthesized sgRNA can be co-transfected with Cas9 mRNA (No.R1015), or gene edited in cells expressing Cas9 protein, or mixed with Cas9 nuclease for enzyme digestion identification experiment.

Composition and storage conditions

Size / Components	20 rxns	100 rxns
2× Template Mix	200 µL	1 mL
Phusion high-fidelity DNA polymerase	10 µL	50 µL
ATP (100 mM)	40 µL	200 µL
GTP (100 mM)	40 µL	200 µL
UTP (100 mM)	40 µL	200 µL
CTP (100 mM)	40 µL	200 µL
T7 RNA polymerase Mix	40 µL	200 µL

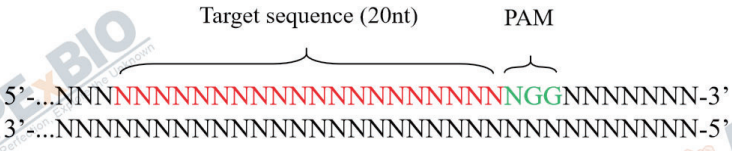
10× Reaction Buffer	40 μL	200 μL
DNase I	20 μL	100 μL
RNase-free H_2O	400 μL	2 mL

Store the components at -20 °C for at least one year.

Experimental operation

1. Target DNA sequence selection and the design of PCR upstream primers (i.e. Target-specific DNA oligo)

(1) The 20 nt sequence adjacent to PAM (NGG) was selected as the target sequence of CRISPR/Cas9 system.



(2) PCR upstream primers (Target-specific DNA oligo) consist of four parts: a) T7 promoter sequence; b) The transcription initiation site needs to add 0-2 G; c) 20 nt sgRNA target sequence; d) 14 nt annealing sequence of Scaffold Template.

***Note:** The T7 promoter requires at least 2 G for effective transcription, and the number of G added depends on the 5' end of the target sequence. If the 5' end of the selected target sequence already contains 2 G, no additional G needs to be added to the transcription initiation site; If it contains 1 or 0 G, 1 or 2 additional G need to be added accordingly.

2. PCR synthesis of DNA templates

(1) Thaw the corresponding components, and then prepare the PCR reaction system on the ice with reference to the table below.

Reagent	Volume	Final Concentration
Template Mix (2×)	10 μL	1×
Target-specific DNA Oligo (10 μM)	1 μL	0.5 μM final
Phusion high-fidelity DNA polymerase	0.4 μL	

RNase-free H_2O	8.6 μL	
Total Reaction Volume	20 μL	

(2) Gently mix the reaction system (gentle suction or slight vortex), and then instantaneously centrifuge to collect residual liquid from the tube wall.

> ***Note:** If there are multiple similar PCR reactions, all components in the table except Target-specific DNA Oligo can be pre-mixed in advance, then packaged into each PCR reaction tube, and Target-specific DNA Oligo can be added at last. The PCR product is then used as a template to produce sgRNA, so it needs to be strictly ensured that RNase contamination is avoided.

> ***Note:** If the PCR instrument used later does not have a hot cap, add mineral oil to cover the prepared reaction system.

(3) PCR reaction. The setting of PCR reaction can be referred to the following table.

PCR Step	Temperature	Time	Cycles
Initial denaturation	98 ℃	1 min	1
Denaturation	98 ℃	15 s	variable
Annealing	55-60 ℃	15 s	
Extension	72 ℃	15-30 s per kB	
Final extension	72 ℃	2 min	1
Hold	4 ℃	+∞	1

(4) After the PCR reaction, 3-5 μL PCR products can be used for 2% or 3% DNA agarose gel electrophoresis to analysis the product quality and yield.

3. In vitro transcription of sgRNA synthesis

(1) Thaw the components and then prepare the in vitro transcription reaction system on the ice with reference to the table below.

Reagent	Volume	Final Concentration
sgRNA Template (PCR Product)	4 μL	
T7 RNA polymerase Mix	2 μL	
ATP (100 mM)	2 μL	10 mM final
GTP (100 mM)	2 μL	10 mM final
CTP (100 mM)	2 μL	10 mM final
UTP (100 mM)	2 μL	10 mM final

10× Reaction Buffer	2 μL	1×
RNase-free H_2O	4 μL	
Total Reaction Volume	20 μL	

(2) Gently mix the reaction system (gentle suction or slight vortex), and then instantaneously centrifuge to collect residual liquid from the tube wall

(3) Incubate at 37 °C for 2-4 h. Generally, the longer the reaction time, the more sgRNA is produced.

(4) Purify the transcripts. The sgRNA can be purified using RNA Clean Beads (No. K1812) or phenol chloroform extraction and ethanol precipitation.

(5) The product quality can be analyzed by RNA gel electrophoresis.

Notes

1. Organic solvents such as phenol and chloroform remaining in DNA will affect transcription efficiency. Try to prepare high-purity DNA templates for transcription.
2. It is recommended to design at least three different gRNAs for each target gene. Using multiple gRNAs at the same time increases the chances of editing success.
3. Strict attention should be paid to avoid RNase contamination during the experimental operation.
4. This product is for scientific use only.

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