HYBRID
LAB NETWORK

In vitro gene editing - sgRNA synthesis

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Synthesis of single guide RNA (sgRNA) - 1.40h

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The T7 RNA polymerase is DNA-dependent RNA polymerase that recognizes and starts transcription at the bacteriophage T7 promoter sequence (5'-TAATACGACTCACTATA-3'). The DNA template, in turn, contains the T7 promoter sequence, followed by the desired sgRNA sequence. One of two different sgRNAs can be produced, sgHL3 or sgHL4, depending on the template used (sgHL3 template or sgHL4 template).

Synthesis of single guide RNA (sgRNA) - Needed Materials:

- 100 ng/µL sgRNA template (sgHL3 template or sgHL4 template);
- Nuclease-free H_2O (i.e. DNase- and RNase- free water);
- 5x Transcription Buffer;
- 50mM DTT (Thermo Scientific) (Dithiothreitol protects T7 polymerase against protein oxidation);
- NTP mix (mix of ribonucleosides, at 20 mM each);
- 40 U/µL NZYRibonuclease Inhibitor (nzytech);
- 20 U/µL T7 RNA polymerase (Thermo Scientific);

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- 1 U/µL DNaseI (Thermo Scientific);
- RNase-free tubes and filter pipette tips;
- Micropipettes;
- 37°C heat block/incubator.

Synthesis of single guide RNA (sgRNA) - Protocol:

a) Assemble the initial reaction mix on ice:

Reagent	**Volume (µL)**
Nuclease-free H_2O	22
5x Transcription Buffer	10
50mM DTT	5
NTP mix (20mM each)	5

b) Mix gently and incubate at 37°C for 5 min.

c) Add the DNA template to the reaction mix:

sgHL3/sgHL4 template (100 ng/µL)	5

d) Mix gently and incubate at 37°C for 1 min.

e) Add Ribonuclease Inhibitor to the mix:

The Ribonuclease Inhibitor inhibits RNase activity during the reaction, to protect the newly transcribed RNA from degradation in the event of RNase contamination.

NZYRibonuclease Inhibitor (40 U/µL)	1

f) Mix gently and incubate at 37°C for 1 min.

g) Add the T7 RNA Polymerase enzyme:

T7 RNA polymerase (20 U/µL)	2
Final Volume (µL)	50

h) Mix gently and incubate at 37°C for 60 min.

i) Add 2µL of DNase I (1 U/mL).

DNaseI degrades all present DNA, thereby removing the sgRNA template that was added to the mixture in the beginning. Following treatment with DNaseI, the only nucleic acids present in the sample should be the synthetized sgRNA molecules.

j. Mix gently and incubate at 37°C for 30 minutes.

k. Place sample tubes on ice.

Proceed with the purification of sgRNA.