In vitro transcription of guide RNAs and 5'-triphosphate removal V.15

Moritz F Schlapansky[1], Eric Aird[1], Mark Dewitt[2], Julia Wong[2], Beeke Wienert[2]

[1]ETHZ - ETH Zurich; [2]UC BERKELEY-IGI

CornLab

Jacob E Corn
ETH Zurich

VERSION 15

MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n2bvjyp5vk5w/v15

External link:
https://doi.org/10.1371/journal.pbio.2005840

Protocol Citation: Moritz F Schlapansky, Eric Aird, Mark Dewitt, Julia Wong, Beeke Wienert 2023. In vitro transcription of guide RNAs and 5'-triphosphate removal. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjyp5vk5w/v15 Version created by Moritz F Schlapansky

MANUSCRIPT CITATION:
Wienert B, Shin J, Zelin E, Pestal K, Corn JE (2018) In vitro–transcribed guide RNAs trigger an innate immune response via the RIG-I pathway. PLoS Biol 16(7): e2005840. doi: 10.1371/journal.pbio.2005840

ABSTRACT

sgRNA template assembly, in vitro T7 transcription, and sgRNA column cleanup to remove 5'-triphosphate groups

Protocol status: Working

Created: Jun 28, 2021

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 51097

Keywords: CRISPR, Cas, guide RNA, gRNA, Cas9, genome editing

GUIDELINES

The primers used are: one long, variable oligo that carries the T7 promoter and desired guide sequence; an 82-nt constant oligo that carries the 3' end of the sgRNA; two short external primers for amplification.

Assembly Oligos:

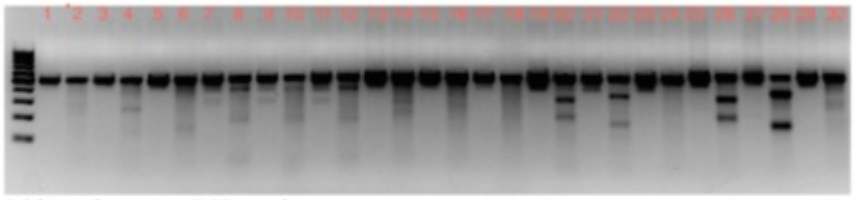
T7FwdVar oligo (5'-TAATACGACTCACTATA**G**--protospacer sequence—GTTTCAGAGCTATGCTGGAAAC-3')

T7RevLong oligo (5'-AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTCAACTTGCTATGCTGTTTCCAGCATAGCTCTGA-3')

Amplification Primers:

T7FwdAmp primer (5'-TAATACGACTCACTATAG-3')

T7RevAmp primer (5'-AAAAAAAGCACCGACTCGGTGC-3')



Odd numbers: just PCR product
Even numbers: with T7E1 treatment
Here's a gel that shows some good guides and some that didn't work; examples of more promising looking guides would be #'s 20, 22, 26, and 28.

Designing the guide sequence: The T7 transcript starts with the G before the dashes in the Fwd assembly oligo. If your target calls for a G at the 5' end of the guide, use the one already included in T7FwdVar design. If your target calls for a different base at the 5' end, put the whole guide sequence in T7FwdVar; there will be an extra G on the end that should have minimal effect, unless your guide is quite short.

Making the template: we have used the Phusion-HF DNA polymerase. Although the amplification primers have quite different Tm's, they work together fine.

MATERIALS

STEP MATERIALS

DNase I (RNase-free) - 1,000 units **New England Biolabs Catalog #M0303S**

HiScribe T7 High Yield RNA Synthesis Kit - 50 rxns **New England Biolabs Catalog #E2040S**

Quick CIP **New England Biolabs Catalog #M0525**

1 sgRNAs need to be purfied before dephosphorylation and transfection. There are different methods one could purify their sgRNAs. We therefore tested different purification kits and found that the **Qiagen miRNeasy Tissue/Cells Advanced Mini Kit** gives the most consistent and highest yields. To purify our sgRNAs with this kit, we follow the manufacturer's instructions with the following modifications:

Note

Yields of from column purification using the Qiagen RNeasy Mini kit can be low as the sgRNAs are small and most kits are designed to bind longer RNAs. Our yields are consistently higher when using the miRNeasy Tissue/Cells Advanced Mini Kit from Qiagen.

1.1 Adjust sgRNA sample to a volume of 100 µl with RNase-free water.
Add 350 µl RLT Buffer to the sample and mix well by pipetting

1.2 Add 450 µl Isopropanol and mix well by pipetting.

1.3 Transfer sample (~900 µl) to an RNeasy mini spin column; spin for 15 sec at 10.000 g.
Discard the flow-through.

1.4 Add 700 µl RWT Buffer; Spin for 15 sec at 10.000 g.
Discard the flow-through.

1.5 Add 500 µl RPE Buffer; Spin for 2 min at 10.000 g.

1.6 Move spin column to a new collection tube and spin for 1 min at 10.000 g to dry the membrane completely.

1.7 Move spin column to an RNAse-free 1.5 ml microfuge tube
Add 33 µl DEPC-treated H2O; spin 1 min
Optional: Repeat the elution to collect any remaining RNA on the column and increase RNA concentration.