

## Single guide RNA synthesis

This is our typical protocol for generating gRNAs singly (one at a time) after designing with CRISPRscan.

Set up the following reaction

Taq buffer (10X)	5 $\mu$ l	
CRISPR tail oligo (100 $\mu$ M)	1 $\mu$ l	(in gRNA synthesis box)
gRNA oligo (100 $\mu$ M)	1 $\mu$ l	
Taq enzyme	0.25 $\mu$ l	
Water	42.75 $\mu$ l	

PCR cycling:

95°C	3 min	
95°C	30 sec	
45°C	30 sec	
72°C	30 sec	x 30 (steps 2-4)
72°C	10 min	
4°C	infinity	

This protocol is in the **General folder** named **GRNA\_2**

## Purify assembled oligos (DNA Clean & Concentrator-5 kit, Zymo Research)

1. Transfer assembled oligo from PCR strip to 1.5 ml tube and add 250  $\mu$ l of DNA binding buffer.
2. Mix briefly by vortexing.
3. Transfer mixture to a Zymo-Spin Column in a collection tube.
4. Centrifuge in desktop centrifuge at max speed for 30 seconds. Discard flow through.
5. Add 200  $\mu$ l of DNA Wash Buffer to the column. Centrifuge at max speed for 30 seconds and discard flow through.
6. Add another 200  $\mu$ l of DNA Wash Buffer, centrifuge for 30 seconds and discard flow through.
7. Add 6  $\mu$ l of RNase-free water directly to the column matrix. Incubate at room temperature for one minute.
8. Transfer the column to a fresh 1.5 ml tube and centrifuge for 30 seconds to elute the DNA.

Attain DNA concentration using the nanodrop (typically 100-200 ng/ $\mu$ l). Can also check integrity of DNA by gel electrophoresis if desired.

## *In vitro* RNA synthesis

Set up RNA synthesis using the T7 MEGAshortscript kit:

rNTPs	0.5 µl of each (2 µl total)
10x transcription buffer	0.5 µl
Purified DNA	2 µl
T7 enzyme	0.5 µl

Incubate at 37°C for 3 hours.

Add 0.5 µl DNase and incubate at 37°C for 30 mins then bring volume to 50 µL with nuclease-free water.

### Purify sgRNA (RNA Clean & Concentrator-5 Kit, Zymo Research)

1. In another tube, add 2 volumes of RNA binding buffer (e.g., 100 µL into a 50 µL rxn)
2. Add an equal volume of 200 proof EtOH (e.g., 150 µL)
3. Transfer to a Zymo-spin column and collection tube, centrifuge for 30 s, discard flow-through
4. Add 400 µL of RNA prep buffer, spin 30 s, discard flow-through
5. Add 700 µL RNA wash buffer, spin 30 s, discard flow-through
6. Add 400 µL RNA wash buffer, spin 30 s, discard flow through
7. Spin dry for 2 min
8. Elute in 15 µL DNase/RNase free H<sub>2</sub>O

Aliquot into 2 µl and store in -80°C. One aliquot can be used for Nanodrop and gel electrophoresis.