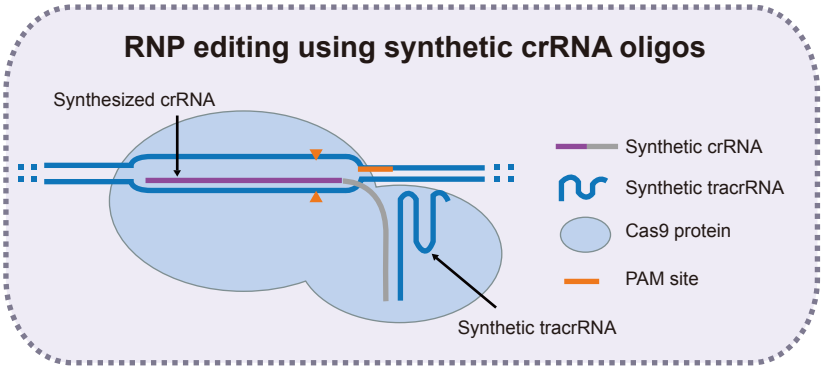
CRISPR Ribonucleoprotein (RNP)
User Manual

Description

This user manual describes how to use GenScript's **CRISPR Ribonucleoprotein (RNP)** products for targeted genome editing. The RNP system is comprised of a Cas9 protein, which creates double strand breaks in the target genome, and two synthetic oligos: CRISPR-targeting RNA (crRNA) and trans-activating crRNA (tracrRNA). There are many advantages to RNP over traditional CRISPR plasmids, including improved transfection efficiency in hard-to-transfect cells, and reduced off-target effects.

GenScript's **CRISPR RNP service** includes two components: (1) a pre-duplexed crRNA:tracrRNA oligo and (2) Cas9 protein. The 20nt crRNAs are designed and synthesized based on the sequence of the target DNA. No in vitro transcription is required - simply complex the crRNA:tracrRNA oligos to the Cas9 protein prior to experimentation.



For additional information about RNP and other CRISPR genome editing service and products, visit ***www.genscript.com/crrna-cas9-protein.html***

Required materials

Material or Reagent	Ordering information
Pre-duplexed crRNA: tracrRNA oligo	GenScript (Cat # SC1838)
Cas9-C-NLS nuclease	GenScript (Cat # SC1841)
Pre-duplexed crRNA:tracrRNA positive control (Human HPRT)	GenScript (Cat # SC1839)
Cas9 Nuclease Reaction Buffer, 10x	Included with SC1838
DEPC Water	General laboratory supplier
Lipofection or Electroporation materials	Preferred laboratory supplier

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Workflow overview

[1]Optional step

Protocol

1 Section A: Test RNP in vitro cutting efficiency

Depending on the target site, it may be necessary to test the CRISPR/Cas9 system prior to experimentation. To verify that the chosen target works, perform in vitro testing prior to introducing RNP into cells or embryos.

1. Prepare the PCR amplicon as the substrate of CRISPR/Cas9 RNP digestion. When designing the amplicon, add at least 200bp on either side of the crRNA target.

▲ **TIP** *A longer amplicon will give a clearer band when the sample is run on a gel to verify successful cutting. We typically use amplicons around 1kb.*

2. Prepare 16 µl of RNP Mix in a RNase-free reaction microcentrifuge tube:

Component	Amount
10X Cas9 Nuclease Reaction Buffer	2 µl
crRNA:tracrRNA	3-10 pmol
Cas9 Nuclease	250 ng
Nuclease-free water	Up to 16 µl

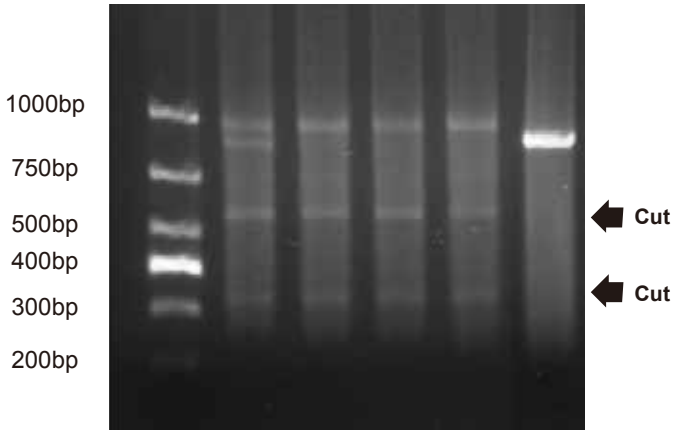
3. Incubate the tube at 37°C for 10 min to allow RNP complexes to assemble.

4. Add 1 µg of PCR amplicon into the 16µl of RNP Mix. Bring the final volume to 20 µl with nuclease-free water and mix gently.

5. Incubate the reaction for at least 30min at 37°C.

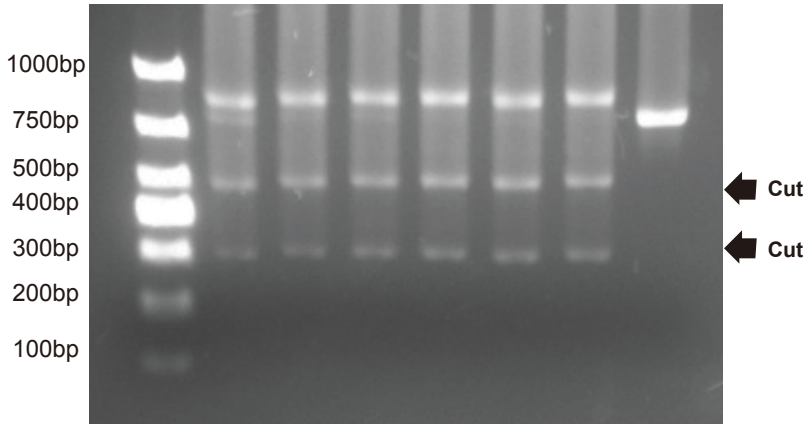
6. Assess the reaction by gel electrophoresis. If the reaction works correctly, two distinct bands will appear on the gel.

▲ ***EXAMPLE*** Incubating RNP with different amounts of crRNA:tracrRNA all cut successfully *in vitro*.



groups	1:1	1:2	1:4	1:8	NC
Protein (pmol)	3	3	3	3	0
crRNA:tracRNA (pmol)	3	6	12	24	0
Cleavage Efficiency (%)	37.82	37.72	38.04	35.85	

▲ ***EXAMPLE*** Incubating RNP with the amplicon for different periods of time all cut successfully *in vitro*.



Time(min)	30	60	90	120	150	180	NC
Cleavage Efficiency(%)	34.12	32.82	32.02	31.99	33.59	33.18	

2 Section B: Test RNP efficacy in cells

RNP testing is best performed in easy-to-handle cell lines, such as HEK293 cells, to confirm gene editing efficiency. For such cells, lipofectamine™ CRISPRMAX™ or electroporation are recommended for transfection, The following protocol is recommended for CRISPRMAX™:

CRISPRMAX™ procedure:

1. Seed well-dissociated cells one day (16–24 h) prior transfection in D10 medium without antibiotics.

▲ ***TIP*** 30–70% confluency at the time of transfection is recommended. If confluency is too high, this can negatively impact transfection efficiency.

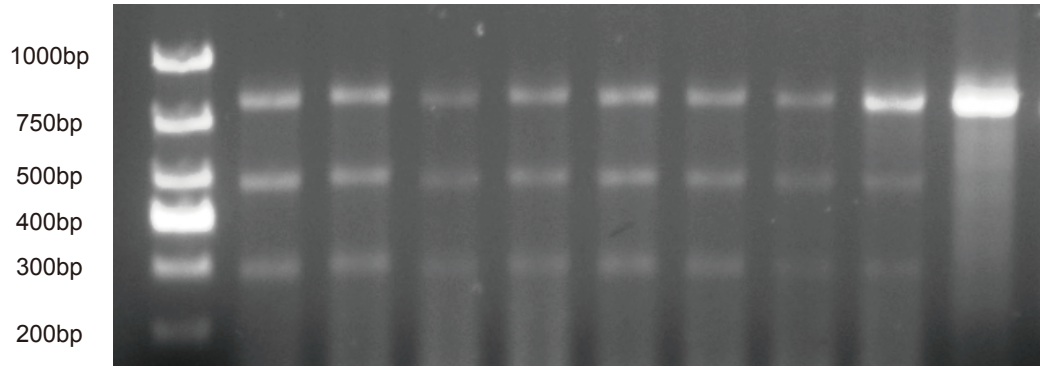
2. Incubate cells with lipofectamine™ as specified in the instructions.

▲ ***TIP*** It is recommended to use the same amount of Cas9 protein and crRNA:tracrRNA. For example, we typically use 3 pmol cas9 protein (240 ng) together with 3 pmol crRNA:tracrRNA for each well of a 24-well plate.

3. Harvest the cells approximately 48 hours after transfection. Extract the genomic DNA for further analysis.

4. PCR amplify the fragment containing the target (for best results, design the primers to target >200 bp away from the target) and test the genome editing efficiency by T7E1 or by Sanger Sequencing.

▲ ***EXAMPLE*** Editing efficiency of different amounts of crRNA:tracrRNA and Cas9 protein at the HRPT target (HPRT positive control) in HEK293T. Editing efficiency in PX458 is also included as a control.



Cas9: gRNA	1:1		1:2		1:4		1:6	PX458	nc
Cas9 (pmol)	6	3	6	3	6	3	3		
crRNA (pmol)	6	3	12	6	24	12	18		
Cleavage (%)	40.46	40.41	33.96	36.92	38.93	37.37	33.13	20.32	

3 Section C: Test RNP efficacy in embryos

RNP testing can also be performed in embryos, such as zebrafish, to confirm gene editing efficiency. The following zebrafish protocol can be used as an example:

1. Prepare 10 µl of RNP Mix in a RNase-free reaction microcentrifuge tube:

Component	Amount
10X Cas9 Nuclease Reaction Buffer	1 µl
crRNA:tracrRNA	~20-40 pmol
Cas9 Nuclease	2-4 µg
Nuclease-free water	Up to 10 µl

Incubate the tube at 37°C for 10 min to allow RNP complexing.

2. Microinject ~1 nl RNP Mix into embryos at the 1-cell stage.

3. When embryos reach 24 hpf, collect at least 5 of the injected embryos and extract their genomic DNA.

4. PCR amplify the fragment containing the target (for best results, design the primers to target >200 bp away from the target) and test the genome editing efficiency by T7E1 or by Sanger Sequencing.

For additional information
or assistance email us at
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or visit
www.genscript.com/CRISPR-Cas9-technology-resource.html

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