Protocol for *in vitro* transcription

Assemble the reaction at room temperature in the following order:

Component	volume
10xTranscription Buffer	2μl
rNTP	2μl
T7 RNA Polymerase Mix	2μl
gRNA PCR	1μg(<142μl)
DEPC H_2O	up to 20μl

Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 2 hours.

Add 2μl DNase to each 20μl reaction, mix and incubate for 30 minutes at 37°C.

Add 115μl DEPC H_2O to each reaction, mix thoroughly and incubate for 30 minutes at 37°C.

Add twice the volume of anhydrous ethanol incubate overnight at -20°C.

Protocol for preparing the dumbbell probes

Probes were prepared by self-templated ligation of 5′ -phosphorylated dumbbell-shaped DNA sequences by T4 DNA ligase.

The ligation reaction was conducted in a volume of 20 μL, produced by adding 1 μL of DNA template (100 μM), 2 μL 10 × T4 DNA ligase reaction buffer (400 mM Tris-HCl, 100 mM MgCl2, 100 mM Dithiothreitol, 5 mM ATP, pH 7.8 at 25 °C), 16 μL DEPC-treated H2O and 1μL of T4 DNA ligase (100 U/μL). Ligation process was done at 16 °C for 2 h and then heated at 65 °C for 10 min to terminate the reaction. Next, Exonuclease I (20 U/μL) and Exonuclease III (100 U/μL) were added to digest the leftover ssDNA and dsDNA to yield closed DNA. The enzymes were denatured by heating at 80 °C for 20 min.

Protocol for purification of dCas9 fusion protein

Transformants of E.coli strain BL21 are grown overnight at 37 ℃ with shaking in LB medium supplemented with suitable concentration of antibiotic. The culture is diluted 50-fold into fresh LB medium with antibiotic and grown at 37 ℃ for 8h to 12h. Then the culture is diluted 100-fold into fresh LB medium with antibiotic and grown in a shaking incubator at 37 ℃. When the OD600 of the culture reaches 0.6 to 0.8, IPTG is added to a final concentration of 200μM. The induced cells are incubated 12h at 16 ℃ with shaking.

Cells are harvested by centrifugation at 6000rpm for 10min at 4 ℃. The cells are resuspended in buffer A (20mM Tris, 500mM NaCl, pH 8.0) and disrupted by ultrasonic cell disrupter. The crude cell lysate is separated into soluble and insoluble fractions by centrifugation at 15000rpm for 50min at 4°C. The soluble fraction pass through 0.22 μm syringe-driven filter units (Millex).

The cleared supernatant is applied to a 5mL bed volume HisTrapTM column equilibrated with 5 bed volumes of buffer A. Unbound protein is removed by washing the column with buffer A. Once the base line is reached, the target protein is eluted by applying a stepped 0 to 0.5M imidazole gradient.

Fractions containing the target protein are concentrated to proper volume, and applied

to 5mL bed volume desalting column equilibrated with 5 bed volumes of buffer D (20mM HEPES, 150mM KCl) to remove imidazole. Then the fractions containing the target protein are concentrated to proper volume.

Protein purity is checked by SDS-PAGE, and the resulting protein is quantified by

spectrophotometry.

Protocol for RCA

Each RCA reaction was conducted in a volume of 25 μL containing 1 μL of prepared probe, 2.5 μL of 10 × phi29 DNA polymerase reaction buffer (500 mM Tris-HCl, pH 7.5 at 25°C, 100 mM MgCl2, 100 mM (NH4)2SO4, 40 mM Dithiothreitol), 0.5 μL BSA (10 mg/mL), 6 μL dNTPs(10 mM for each of dATP, dGTP, dCTP and dTTP) and 2.5 μL of target miRNA solution (various concentrations), 12 μL DEPC-treated H2O and 0.5 μL phi29 DNA polymerase (10 U/μL). The reaction mixture was incubated at 37 °C for 2 h, and then heated at 65 °C for 10 min to stop the reaction. This reaction mixture was used either for gel electrophoresis or for fluorescent spectra measurement.

Protocol for real time fluorescence measurement of RCA

RCA reactions were monitored by real-time fluorescence measurement. Each RCA reaction was conducted at 37 °C in a volume of 25 μL made from 1 μL of prepared probe, 2.5 μL of 10 × phi29 DNA polymerase reaction buffer (500 mM Tris-HCl, pH 7.5 at 25 °C, 100 mM MgCl2, 100 mM (NH4)2SO4, 40 mM Dithiothreitol), 0.5 μL BSA (10 mg/ml), 6 μL dNTPs (10 mM for each of dATP, dGTP, dCTP and dTTP), 2.5 μL of target miRNA solution (10 nM), 12.5 μL DEPC-treated H2O, 0.5 μL phi29 DNA polymerase (10 U/μL), 0.5 μL 25 × Sybr Green I. The fluorescence intensity was recorded on CFX96TM Real-Time System (Bio-Rad, USA) in real time at 1min intervals over a period of 300 min. Excitation wavelength was 494 nm, emission wavelength was 521nm.

Protocol for SDS-PAGE

Pour the Separating Gel

Set up your gel apparatus, prepare separating gel monomer. Add TEMED just prior to pouring gel. Allow to polymerize before adding stacking gel by overlaying gently with water or n-butanol. With higher concentrations of gels, one can immediately pour the stacking gel on the unpolymerized separating gel. Be careful not to mix the two layers. Separating Gels, in 0.375 M Tris, pH 8.8.

	7%	10%	12%	15%
distilled H_2O	5.1 ml	4.1 ml	3.4 ml	2.4 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml	2.5 ml	2.5 ml	2.5 ml
20% (w/v) SDS	0.05 ml	0.05 ml	0.05 ml	0.05 ml
Acrylamide/Bis-acrylamide(30%/0.8% w/v)	2.3 ml	3.3 ml	4.0 ml	5.0 ml
10% (w/v) ammonium persulfate	0.05 ml	0.05 ml	0.05 ml	0.05 ml
TEMED	0.005 ml	0.005 ml	0.005 ml	0.005 ml
Total monomer	10.005 ml	10.005 ml	10.005 ml	10.005 ml

Pour the Stacking Gel

After the separating gel has polymerized, decant the overlay, prepare the stacking monomer, add

the TEMED, and pour. Insert the comb and allow to polymerize completely before running.

Stacking Gels, 4.0% gel, 0.125 M Tris, pH 6.8.

1. 3.075 ml distilled H_2O

2. 1.25 ml 0.5 M Tris-HCl, pH 6.8

3. 0.025 ml 20% (w/v) SDS

4. 0.67 ml Acrylamide/Bis-acrylamide(30%/0.8% w/v)

5. 0.025 ml 10% (w/v) ammonium persulfate

6. 0.005 ml TEMED

7. 5.05 ml Total Stack monomer

For best results:

1. Make ammonium persulfate solution fresh daily.

2. Degas solutions before adding TEMED for 15 min at room temperature.

3. Running the gel

We usually run my gels at constant current, 25-50 mA, depending on gel size. Here"s the recipe for 5X SDS-PAGE running buffer. Dilute to 1X before use.

1. 1 liter 5X Running Buffer, pH 8.3

2. 15 g Tris Base

3. 72 g Glycine

4. 5 g SDS

5. distilled water to 1 liter

Store at room temperature until use.

Sample buffer

Dilute samples at least 1:4 with sample buffer, heat at 95 C for 4 minutes prior to loading. 8 ml

Sample Buffer:

1. 4.0 ml Distilled water

2. 1.0 ml 0.5 M Tris-HCl

3. 0.8 ml Glycerol

4. 1.6 ml Acrylamide/Bis-acrylamide(30%/0.8% w/v)

5. 0.4 ml beta-mercaptoethanol

6. 0.2 ml bromophenol blue

Protocol for testing our system

Each RCA reaction was conducted in a volume of 50 μL containing RCA products, fusion proteins, single-guide RNA, Cas9 buffer and RNase inhibitors, TMB was used as substrate to form blue-colored TMB diamine as a visible signal. This reaction was then halted by addition of 0.16M sulfuric acid and turned the solution into yellow with maximum absorbance at 450nm.

Protocol for western blot analysis

Escherichia coli Rosetta (DE3) cells expressing the sHRP-C-dCas9 fusion and the sHRP-N-dCas9 fusion for 6 h were harvested by centrifugation. Cell pellets were resuspended in PBS, lysed via sonication and centrifuged at 16 000 rpm for 10 min. The supernatant was retained as the soluble cell

lysate. All samples were normalized to the amount of total soluble protein. Immunoblot analysis of soluble lysates was performed with anti-His antibodies (Sigma) to detect sHRP-C-dCas9 or sHRP-N-dCas9 chimeras and anti-HA antibodies (Sigma) for detection of 1,2-PDor mevalonate-related chimeras according to standard procedures.