Materials

$sgRNA_L$ (2813.8 ng/μl, 71.96 μM)
5(6)-Carboxyfluorescein N-hydroxysuccinimide ester (5/6-FAM SE, NHS-Fluorescein, FITC-NHS) was obtained from Mecklin (Shanghai, China).
Dimethylsulfoxide (DMSO)
Sodium bicarbonate ($NaHCO_3$)
sodium acetate (NaAc)
Hydroxymethyl aminomethane hydrochloride (Tris-HCl)
Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na)
Hydrochloric acid (HCl)
Ethyl alcohol

Procedures

1. Preparation of samples and buffers
    a. Dilution of pre-prepared $sgRNA_L$ solution:
    Take the previously prepared purified $sgRNA_L$ solution (2813.8 ng/μl) out of the refrigerator and place it on ice to thaw and prepare for use.
    b. Preparation of FITC labeling reagents (1 mg/ml FITC-NHS):
    The FITC-NHS (Mw:473.39) powder is stored in the -20°C refrigerator and kept away from light. The FITC labeling reagents should be prepared and used on the same day. Usually, 0.1 mg FITC-NHS is weighed and dissolved in 100 μl DMSO solution to be configured as 100 μl of 1 mg/ml FITC labeling reagents, which requires complete avoidance of light throughout the process.
    c. Preparation of labeling buffer (0.2M $NaHCO_3$, pH 9.0):
    0.168 g of $NaHCO_3$ powder is dissolved in 10 ml of water, with pH adjusted to 9.0 by adding HCl. It can be kept for one week at 4°C.
    d. Preparation of 3M sodium acetate (NaAc).
    e. Preparation of dissolving reagents 1 x TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0): The pH is adjusted to 8.0 by adding HCl.

2. Labeling reaction and incubation

RNA labeling reaction system			
	Volume	Mass	Note
2813.8 ng/μl $sgRNA_L$	3.6 μl	10.13 μg	1:1 (v:w) FITC labeling reagent to nucleic acid
Labeling buffer	50 μl		

FITC labeling reagents	10.13µl	10.13 µg	labeling ratio results in a labeling density suitable for most applications.
Milli-Q water	36.27 µl		
Total	100 µl		

a. Prepare the labeling reaction according to the table shown above. Importantly, be sure to add the FITC labeling reagent last. Mix them and centrifuge briefly to concentrate the liquid at the bottom of the tube.
b. Incubate in the dark at 37°C for 1 hour.

Note: After incubation for 30 minutes, centrifuge briefly to reduce the effect of evaporation and maintain the appropriate concentration of reaction components.

3. Purification using Ethanol Precipitation
   a. Add 5 µl of 3M NaAc (0.1 volumes) and 140 µl of ice-cold 100% ethanol (2.5 volumes) to the reaction. Mix well.

   Note: 5M sodium chloride (NaCl) was recommended as well. However, it has been proven that the sedimentation efficiency is lower than 3M NaAc in our previous attempts.

   b. Incubate at -20°C for at least 30 minutes.
   c. Centrifuge at 12,000 rpm for 30 min at 4°C to pellet the labeled nucleic acid.

   Note: If fluorescein is used as the label, at this point, unreacted fluorescein will remain in the supernatant, and the fluorescein-labeled pellet will have a deep yellow color. A white pellet is an indication that the reaction was not successful.

   d. After the sedimentation process, carefully remove the supernatant, and be careful not to touch the sediment. Wash the pellet by adding 500 µl of 70 % ethanol. Centrifuge at 16,000 rpm for 20 min at 4°C and remove the wash solution without dislodging the pellet.
   e. Dry the nucleic acid precipitation in the ultra-clean bench with the lid of centrifuge tubes open for 30 minutes to allow the ethanol to completely evaporate.
   f. Resuspend the pellet in 5 µl of 1 × TE.

   Note: A volume of 100-200 µl 0.1 x TE was recommended for 100-10,000 ng RNA/DNA, but the concentration of the obtained product did not meet the requirements of our subsequent experiments. It is also known that an excessively small volume may result in insufficient nucleic acid dissolution. Therefore, after several attempts, we switched to using a 5 µl volume of 1 x TE for dissolution, resulting in a high concentration of the labeled product.

   g. Use Nanodrop (UV-Vis) to measure absorbances at 260nm, 495nm, and calculate the concentration of the product, recovery rate, and labeling efficiency.

4. Detailed calculation regarding parameters of products
   a. Some profiles need to be searched before the experiments

	Molecular Weight (g/mole)	extinction coefficient ($M^{-1}cm^{-1}$)	nmole/OD_{260nm} (1ml sample)	μg/OD_{260nm} (1ml sample)
$sgRNA_L$	39089.52	1220900.00	0.82	32.02
FITC-NHS	473.39	~70,000 $M^{-1}cm^{-1}$		

   b. Data calculation

	Absorbance	Concentration	Molarity	Purity	Note
$sgRNA_L$	A260-			A260/A280-	Recovery rate-
FITC	A495-		-	-	

Molar concentration = A280 / Extinction coefficient; mg/ml = A280 / (conversion factor of 1g/l).

The labeling efficiency is the molar ratio of fluorescein to nucleic acid.

	Molarity	Concentration	Volume	Mass	Label efficiency	Note
FITC-$sgRNA_L$						

5. Storage and subsequent use

Place the purified and labeled nucleic acids at -20°C in a dark place for long-term storage. It is recommended to aliquot them into small tubes, take one tube at a time for use to avoid repeated freezing and thawing.