Materials

Long ssDNA scaffold (M13mp18) was obtained from Genscript (Jiangsu, China).
Helper strands (S-x, S-G4-x, S-PAM-cap-x, F-cap-x, Apt-cap-x) were obtained from Genscript (Jiangsu, China).
Functional strands (PAM-rich, F-H, aptamer) were obtained from Genscript (Jiangsu, China).
Cas9 Nuclease (10mg/ml) was obtained from KeyGen BioTECH (Jiangsu, China).
sgRNA-linker ($sgRNA_L$)
FITC-$sgRNA_L$
Hemin was obtained from MCE (Shanghai, China).
DMSO was obtained from Biosharp® (Jiangsu, China).
1×TAE
magnesium acetate
potassium chloride
ddH_2O
Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) were obtained from Merck (Germany).

Preparation

1. Helper strands stock solution (20μM, store at -20 ℃)
   Add 25 μL of ddH_2O to each staple tube containing 5 nmol of helper strands. Mix thoroughly to ensure complete dissolution. Next, take 1 μL of this 200 μM helper strands solution and dilute it by adding 9 μL of ddH_2O, resulting in a final concentration of 20 μM.
2. Functional strands stock solution (20μM, store at -20 ℃)
   Add 25 μL of ddH_2O to each staple tube containing 5 nmol of functional strands. Mix thoroughly to ensure complete dissolution. Next, take 1 μL of this 200 μM functional strands solution and dilute it by adding 9 μL of ddH_2O, resulting in a final concentration of 20 μM.
3. Hemin stock solution (255μM, store at -20 ℃ in a dark place)
   Dissolve 0.016g hemin in 100ml DMSO to prepare a 255 μM hemin solution. Use a magnetic stirrer at 45°C to ensure complete dissolution, with the stirring bar rotating at a speed that forms a distinct vortex.
4. $sgRNA_L$ solution (5 μM, store at -20 ℃)
   Use ddH_2O to dilute the $sgRNA_L$ stock solution obtained from in vitro transcription to a concentration of 5 μM.
5. FITC-$sgRNA_L$ solution (5 μM, store at -20 ℃)
   Use ddH_2O to dilute the FITC-$sgRNA_L$ stock solution to a concentration of 5 μM.

6. TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate, pH = 8.3, store at RT)
7. TAE/Mg^{2+}/K^{+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM magnesium acetate, 25 mM potassium chloride, pH = 8.3, store at RT)
8. Pre-treatment of Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa)
    a. Insert the Amicon® Ultra-0.5 device into collection tubes.
    b. Add 500 µL of Milli-Q water to the Amicon® Ultra filter device and cap it. Shake the tubes, and then pour out the Milli-Q water.
    c. Repeat this process 2 times.
    d. Then, add 500 µL of TAE/Mg^{2+} buffer to the filter and place the capped filter device into the centrifuge rotor, aligning the cap strap toward the centre of the rotor; counterbalance with a similar device. Spin the device at 14,000 × g for 10 minutes at 4 °C.