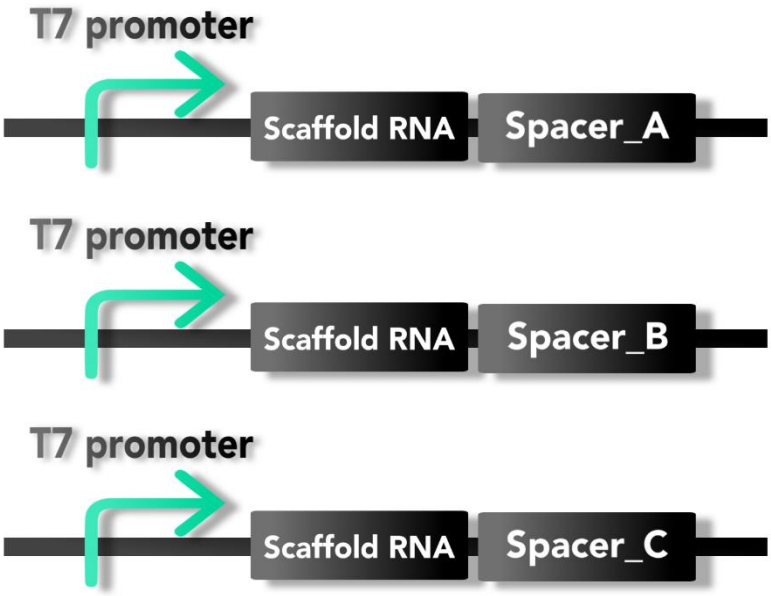
crRNA preparation

We used single strand DNA as template in RNA synthesis.

The templates consist of three main parts: T7 promoter, scaffold RNA template and spacer RNA template:



Scaffold RNA is essential for crRNA assembling with Cas12a. It is conserved among different crRNAs.
Spacer RNA is the region that matches with protospacer (target DNA). Correct spacer RNA sequence determines the binding efficiency and accuracy.

We chose NEB HiScribe™ T7 Quick High Yield RNA Synthesis Kit (E2050S) for high-yield RNA synthesis. The RNA product will then be purified by the following process:

Add 1μL DNase and incubate for 30min.

Add 200μL Trizol per 10μL RNA synthesis product. Then add chloroform to the final concertation 20% (e.g. add 200μL chloroform into 800μL solution). Then centrifuge for 10min in 4℃, speed: 12000g.

The solution will divide into 2 layers. Transfer the upper water layer to a new tube, add isopropanol of the same volume. Place the tube on ice for 10min, and then centrifuge for 10min in 4℃, speed: 12000g.

Add 500μL 75% ethanol solution (in DEPC H_2O). 7000g centrifuge in 4℃ for 5min. Then transfer the liquid out, dry the precipitation in clean environment. Add appropriate volume of ultra clean water to obtain the crRNA solution.