In vitro transcription

Materials:

• T7 High Yield RNA Transcription Kit (Vazyme, catalog no. TR101)
• Nuclease-free, barrier tips and pipets capable of pipetting 0 – 1000 µL
• Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
• Nuclease-free, molecular biology-grade water

Procedure:

1. The components except T7 RNA Polymerase Mix were oscillated and mixed, briefly centrifuged and collected at the bottom of the tube, and stored on ice for later use.
2. The amount of DNA template is between 0.1-1ng. If the product is less than 300bp, it is suggested to increase the amount of DNA template to 2ug.
Prepare reaction according to the following table:

Table

10 x Reaction Buffer	2 µL
ATP Solution	2 µL
GTP Solution	2 µL
CTP Solution	2 µL
UTP Solution	2 µL
DNA template	x µL
T7 RNA Polymerase Mix	2 µL
RNase-free ddH2O	up to 20µl

3. The components were gently mixed with a pipette, collected by centrifugation for a short time, and incubated at 37℃ for 2h.
4. Add 1µL DNase I to the reaction system, and incubate at 37℃ for 15min to digest the template.
5. The synthesized RNA was analyzed by electrophoresis and purified for downstream experiments.