RT-RPA

Aim of the experiment

We used RT-RPA nucleic acid amplification kit to achieve the target sequence concentration detectable by Cas13 protein.RT-RPA kit is based on the Recombinase-aid Amplification technology development of constant temperature detection kits for nucleic acid amplification, at 42 ℃ under the condition of constant temperature specific recognition and RNA amplification target sample, has the advantages of rapid, high sensitivity and specificity is strong, and the reaction components have been mixed and freeze drying into the powder, we easily after freeze-drying experiment, easier to save.

Materials

Single tube reaction system (50 μL) :

Reagent	Dosage
Reaction dry powder	1 tube
Bufer A	41.5 μL
Forward primer(10 μM)	2.0 μL
Reverse primer(10 μM)	2.0 μL
RNA sample	2.0 μL
Buffer B	2.5 μL

Procedure

1. Add 41.5 μL A Buffer 、2.0μL forward primer (10 μM) and 2.0 μL reverse primer (10 uM) to the detection unit tube containing the reaction dry powder.

2. The 2.0 μL RNA sample is added to the detection unit tube.

3. Add 2.5 μL B Buffer to the detection unit tube, cover the tube cover, mix it upside down and thoroughly for 5-6 times, centrifuge it at low speed for 10s.
(Note: The repeatability of the experimental results will be determined by whether this step is well mixed.)

4. Put the detection unit tube in a 42℃ constant temperature metal bath (or a constant temperature water bath pot, incubator, etc.) and incubate for 30 min.

5. After the reaction, 5-10 μL reaction system is used for electrophoresis detection.
(PCR purification Kit or 50 μL phenol: chloroform: isopentyl alcohol (25:24:1) is recommended. The extract was thoroughly mixed and centrifuged at 12000 r/min for 5 min. The supernatant was taken for electrophoresis detection to obtain the best electrophoresis effect.)

6. The gel imager was used for image acquisition and analysis to compare whether the amplified band was the same size as the target segment.(Result analysis and judgment).

7. Concentration measurement.