RPA Reaction System

User's Instruction

Description

Recombinase Polymerase Amplification (RPA) is a kind of amplification method with complex components. It has many components and is sensitive to the proportion of components. When many researchers study RPA amplification methods, the proportion of unstable or even failed amplification is large. In view of the above, we provide the following protocol for reference to facilitate the researchers to optimize and adjust the follow-up research.

RPA Components

Component	Supplier	Cat. No.
T4 UvsX Recombinase (3 μg/μl)	SBS Genetech	UVSX
T4 UvsY Protein (1.5 μg/μl)	SBS Genetech	UVSY
T4 gene 32 protein (5 μg/μl)	SBS Genetech	T4G32
Bsu DNA Polymerase (10U/μl)	SBS Genetech	BSU
Sau DNA Polymerase (10U/μl)	SBS Genetech	SAU
Bst DNA/RNA Polymerase (32U/μl)	SBS Genetech	BSTGF
Creatine Kinase (10 μg /μl)	Sigma	
Exonuclease III (100U/μl)	SBS Genetech	EXN3
2xRPA Buffer Mix	SBS Genetech	UVSX
280mM $Mg(OAC)_2$	SBS Genetech	UVSX

Test Primers and Probes

CPV-F (20μM) CACTTACTAAGAACAGGTGATGAATTTGCTACAG

CPV-R (20μM) AGTTTGTATTTCCCATTTGAGTTACACCACGTCT

Probe (10μM) CCTCAAGCTGAAGGAGGTACTAACTTTGGT[dT-BHQ1]

[THF][dT-FAM]ATAGGAGTTCAACAAG -(C3)

Canine parvovirus type 2, VP2 (Genebank No.: M24003.1)

Protocol

1. Basic RPA Amplification

Component	Volume	Final Concentration
T4 UvsX Recombinase (3 μg/μl)	3 μl	360ng/μl
T4 UvsY Protein (1.5 μg/μl)	0.4 μl	24ng/μl
T4 gene 32 protein (5 μg/μl)	1.5 μl	300ng/μl
Primer F (20 μM)	0.5 μl	400 nM
Primer R (20 μM)	0.5 μl	400 nM
2xRPA Buffer Mix	12.5 μl	1x
Creatine Kinase (10 μg /μl)	0.25 μl	100ng/μl
DNA Polymerase	5-10 U	0.2-0.4 U/μl
ddH2O	Up to 22.75 μl	

Add 1 μl template DNA and 1.25 μl 280 mM $Mg(OAC)_2$ (final concentration 14 mM). Total reaction volume is 25 μl. Mix evenly, centrifuge, and react at 39-42°C for 30min.

2. Fluorescent RPA Amplification

In the fluorescence detection system, add 0.3 μl of 10 μM Probe (final concentration 120 nM) and 50U Exonuclease III (final concentration 2U/μl). The fluorescence detection then can be performed. The application principle of this method is that Exonuclease III cuts the THF site, separates the fluorescence from the quenching group, and reports the fluorescence signal.

Note

- The RPA amplification protocol above belongs to the protein functional test. According to the above experimental operation flow, RPA amplification products can be obtained. Further optimization, including T4 UvsX Recombinase, T4 UvsY Protein, T4 gene 32 protein, polymerase, reaction buffer, and even the sampling sequence, may further improve the amplification performance of RPA.
- As for the selection of DNA polymerase, we tested three common DNA polymerases. When performing fluorescence assay, the recommended selection order is Bst DNA/RNA Polymerase > Bsu DNA Polymerase > Sau DNA Polymerase, and at 42°C, the fastest amplification speed can always be obtained, and the fluorescence signal is stronger. Sau showed better specific amplification ability in Basic RPA Amplification.
- There are still non-specific amplification products in Basic RPA Amplification, which requires further optimization of reaction components and primer sequences. When

the fluorescence probe method is used, the non-specific amplification products usually have no fluorescence signal value due to the presence of specific probes, but the detection sensitivity will be affected at this time. The increase of the concentration of T4 gene 32 protein will significantly reduce the non-specific amplification, but will slow down the amplification speed. At this time, the dosage of T4 UvsX Recombinase and T4 UvsY Protein needs to be increased synchronously to maintain the amplification speed of RPA.

- It is reported in the literature that Nfo Endonuclease (also known as Endonuclease IV, Cat. No. c5027) tends to be used to cut the amplification probe when performing RPA experiments on test strips, but we have not tested it and can not provide corresponding parameters at present.
- RPA reaction buffer is very important for amplification, and slight concentration difference can lead to a significant decrease in amplification efficiency or even failure. RPA buffer mix is a viscous reagent. When using it, be sure to add the sample accurately, and the residual liquid in the tips must be completely injected into the EP tube.

Only for research and not intended for treatment of humans or animals