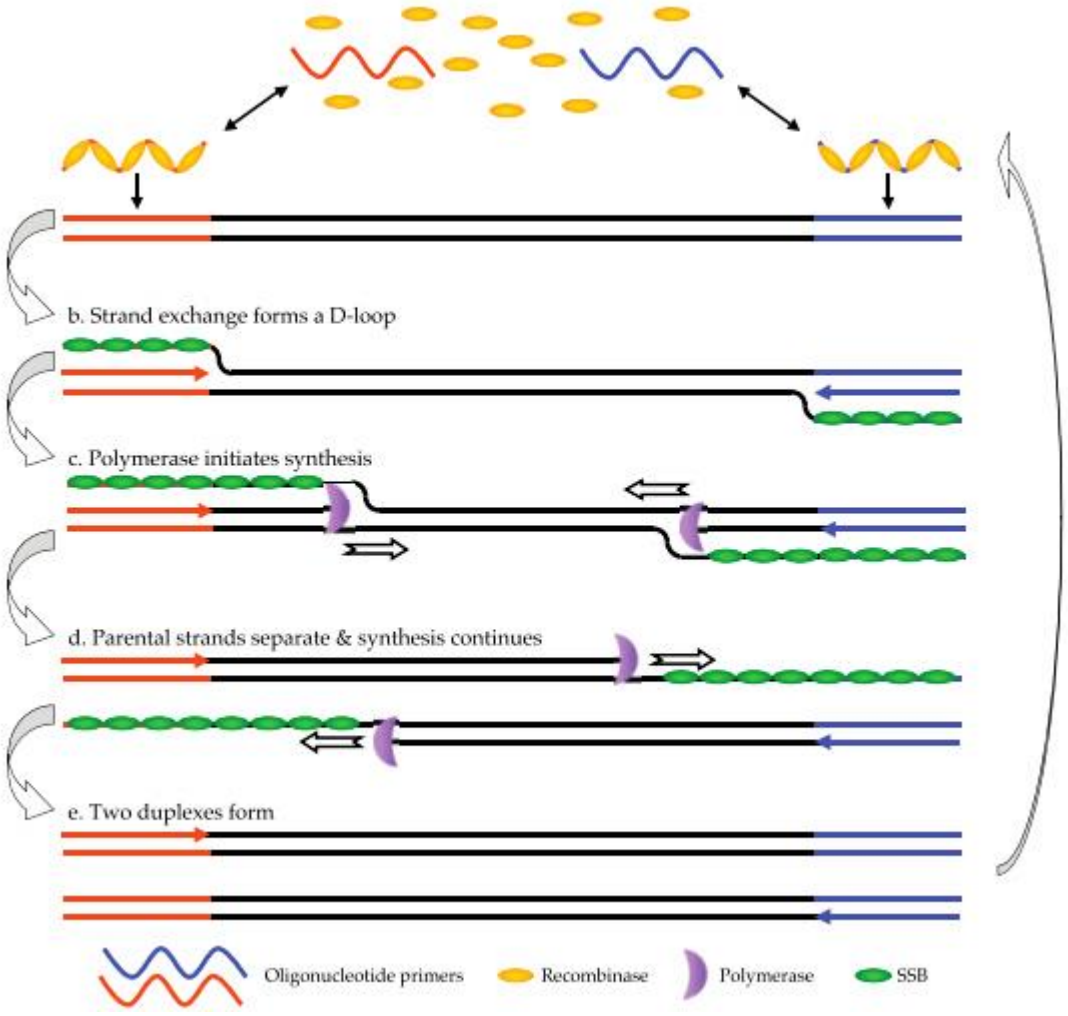
qpcr reaction

Real-time fluorescence quantitative PCR (qPCR) is a new nucleic acid quantitative technique based on conventional PCR. The principle is that fluorescent chemicals are added to the reaction system, and with the continuous accumulation of amplification products in the process of PCR reaction, the fluorescence signal intensity increases in an equal proportion. The whole PCR reaction process is monitored in real time through the change of fluorescence signal, and the results are drawn as fluorescence amplification curve, thus quantitative and qualitative analysis of the initial template.

In our scheme, we use the RPA reaction instead of the pcr reaction in qpcr to amplify the template chain at room temperature. We also design a template chain with fluorescent groups, and the fluorescence concentration increases gradually with the reaction, so as to reflect the concentration of the template.

The RPA Cycle

All steps operate at low constant temperature (optimum37℃）

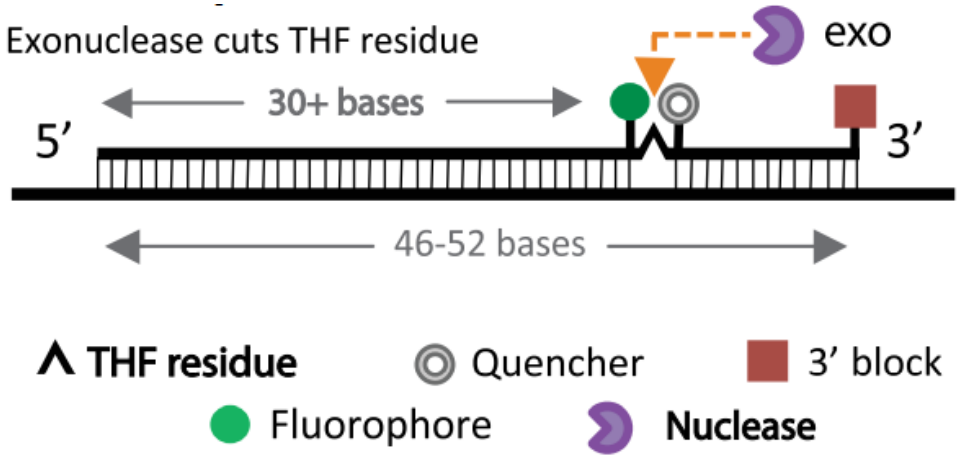


At room temperature and constant temperature, the recombinase and primers form a protein / single stranded nucleotide complex rec / ssDNA, which invades the double stranded DNA template with the help of helper protein and single stranded binding protein SSB.

D-loop region was formed at the invasion site and DNA double strand scanning was started.

After finding the target region complementary to the primer, while the rec / ssDNA complex disintegrates, the polymerase also binds to the 3 'end of the primer to start chain extension.

This process circulates rapidly and efficiently, so as to complete the ultra rapid amplification of the target fragment



When the probe forms a double strand with the complementary sequence, the exo enzyme will cut off the THF site, and the fluorescence signal will be obtained when the fluorescence group is separated from the quenching group.

1. The component of the kit

Buffer A, Buffer B, positive control template, positive control primer probe mix, enzyme reaction tube * 48

Buffer A:

100-800mM tris HCl buffer: solvent for protein and nucleic acid

10-150mM NaCl, 10-150mM KCl, 10-50mM MgCl2: make the solution environment suitable for RPA reaction

Buffer B:

100-800mM tris HCl buffer: solvent for protein and nucleic acid

5-15mM dithiothreitol: reduce DNA dimerization, As the reducing agent and deprotection agent of SULFHYDRYLATED DNA,

5-20% polyvinylpyrrolidone: cosolvent

10-20mM ATP: energy supply for enzymatic reaction,

1-5mM dNTPs : materials of RPA reaction

10-50mM phosphoenolpyruvate: phosphoenolpyruvate can be catalyzed by pyruvate kinase to produce pyruvate and release a large amount of energy

Recombinase polymerase:

500-1500ng/ μ L pyruvate kinase: pyruvate kinase changes phosphoenolpyruvate and ADP into ATP and pyruvate

100-500ng/ μ L BSA: enzyme stabilizer, preventing enzyme decomposition and nonspecific adsorption

50-200ng/ μ LT4 phage DNA helicase gp41 protein: replicative helicase, belonging to SF4 helicase family, preferring to deconvolute in 5 '- 3' direction

100-500ng / μ L Streptomyces coeruleus RecA protein: recombinase

200-1000ng/ μ L single stranded binding protein: SSB binds to the single stranded region

generated by the advance of helicase along the direction of replication fork to prevent the re pairing of newly formed single stranded DNA to form double stranded DNA or protein degraded by nuclease

50-200ng/ μ L E. coli DNA polymerase I : polymerization of 5 '→ 3', exonuclease activity of 3 '→ 5', exonuclease activity of 5 '→ 3',

25-200 pmol primer set

2. **The experimental operation** (preferably with the cooperation of two people)

Preparation :confirm whether the instrument is in use, whether it has authority, and whether the gun is accurate.

（1）According to the required amount,take out the enzyme reaction tube and buffer A,B

（2）Add 29.4 μ of completely melted buffer A (remember it must be completely melted), 11.5 μ of ddH2O, 2 μ of positive and negative primers (10 μ M) to the enzyme reaction tube. At the same time, another student should dilute the template which needs to be added in proportion.

（3） Add 0.6 μ probe (10 μ M) to the enzyme reaction tube and transfer it to the plate of qpcr reaction after centrifugation. Pay attention to the addition of diaphragms to avoid the interference of adjacent pores. At the same time, another student should mix 2.4 μ template and 3 μ Buffer B for use.

（4） After the qpcr instrument was debugged, 4.5 μ template buffer B mixture was added to each hole, and the film was pasted quickly. After centrifugation 2800 rpm for 10s, the fluorescence signal was detected by machine.

（5） If you want to detect protein, change the water to 10.5 μ in the second step, add 1.2 μ protein solution to the template buffer B mixture in the third step, and absorb 5.5 μ in the fourth step.

3. Template assembly and dilution details.

（1）When assembling the template, first calculate the corresponding concentration of each single chain, then use a constant temperature water bath pot to heat 5min at 98℃, then turn off the water bath pot and open the lid, waiting for it to cool naturally.

（2） When diluting, mix well with a gun head, then suck 1 μ to the next gradient of the pcr tube wall, change the gun head to take the droplet down and mix evenly, repeat this operation to reduce the error caused by liquid transfer.

（3）After mixing the gun head, it is necessary to centrifuge again. When mixing with buffer B, it is necessary to blown and suck first if you want to suck the template.