Multienzyme Isothermal Rapid Amplification

Exo kit (DNA)

Guide Manual

（Part number：WLE8202KIT）

Overview of the MIRA technology

Multienzyme isothermal rapid amplification(MIRA) is based on a combination of polymerases and DNA recombination/repair proteins: Recombinases bind to single-stranded nucleic acid and stimulate the resulting protein-DNA complex to search for homologous sequences in duplex DNA with Single Strand Binding protein. Once homology is located, a strand-switching reaction is performed and the oligonucleotide is paired to its complement permitting a polymerase to begin synthesis from the 3’ end. The amplification process is very rapid when optimised and can reach detectable levels of product in a few minutes.

Primer design considerations

The recommended primer length is 30 to 35 nucleotides . It is best to avoid unusual sequence elements within the primer, such as long tracks of one particular nucleotide or a large number of small repeats. oligonucleotides that contain sequence elements that promote secondary structures and primer-primer interactions or hairpins should be discarded.The recommended amplicon length is not exceed 500 bp, and ideally is between 150 -300 bp .

Probe design considerations

The probe sequence does not overlap with the primers recognition site and should be 46-52 nucleotides long. The probe of an oligonucleotide backbone that contains: A dSpacer (tetrahydrofuran, THF) is as a recognition site for the exonuclease , which at least 30 bases are placed 5’ to it, and at least a further 15 bases are located 3’; a flanking dT-fluorophore (typically fluorescein, but any fluorophore available as dT-coupled reagents for oligonucleotide synthesis can be used). The distance between fluorescence group and quenching group is 3-5 bases, located on both sides of THF group.

Materials provided

Materials provided	Content
A buffer	1 tube × 1.6 mL
B buffer	1 tube × 150 μL
Positive control DNA template	1 tube × 30 μL
Positive control primer & probe Mix	1 tube × 70 μL
Reaction unit	48
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Storage conditions

- Transport temperature: ≤ 20 ºC.

- Store at -20 ºC without light，no repeated freezing and thawing.
- Full activity is guaranteed for 14 months.

Set up

1. Add 29.4 μL A buffer (supplied) to a MIRA basic reaction.
2. Add 2 μL forward primer, 2 μL reverse primer and 0.6 μL probe(primer/probe concentration: 10 μM).
3. Add template and water (template and water total volume is 13.5 μL).
4. Add 2.5 μl B buffer (supplied) and mix well, then spin briefly to start reaction.
5. Place reactions in a fluorometer and start run: 39°C, 20 minutes, detect fluorescent signal every 30s.

Reaction mix

A buffer	29.4 μL
Forward primer (10 μM)	2 μL
Reverse primer (10 μM)	2 μL
Probe (10 μM)	0.6 μL
DNA template and water	13.5 μL
B buffer	2.5 μL
Total volume	50 μL

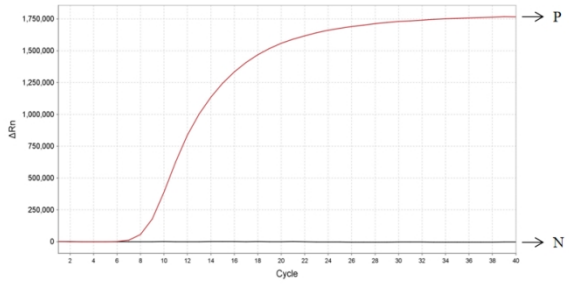

Fig.1 P: positive control; N: negative control

Notes

1. Reactions start as soon as B buffer is added.
2. Set up a negative control for each experiment.
3. For research and development use only.

Ordering information and technical support

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