Application Note 002

Evaluation of isothermal Recombinase Polymerase Amplification incubation temperature tolerance.

Isothermal DNA/RNA amplification technology provides flexibility of use, particularly in the field. TwistDx's proprietary Recombinase Polymerase Amplification (RPA) biochemistry enables such field use. Firstly, it permits transport of reagents (freeze-dried enzymatic pellets) at room temperature to point of testing, even in remote areas. Second it allows amplification and detection of DNA/RNA using low cost, portable equipment. RPA works at an optimal temperature range of 39-42˚C, typically in 3-15 minutes. Low temperature amplification reduces hardware requirements. The tolerance to further reduced temperatures was explored.

Materials and Methods

TwistAmp® Basic kit (50µl) reactions were prepared as follows:

1. Each freeze-dried pellet was rehydrated with 29.5µl rehydration buffer, 7µl primer mix (Basic kit positive control), 8.9µl dH20, 3.6µl magnesium acetate (280mM), 1µl of positive control template (250 copies/ul) or 1µl dH20 for no template control (ntc).
2. Reactions were incubated for an hour at a temperature of 25, 30, 35, 40, or 45˚C in a heat block, reactions were briefly agitated after 4 minutes incubation.
3. Post amplification, reactions were cleaned with a standard PCR clean-up kit and run on a 2% agarose gel.

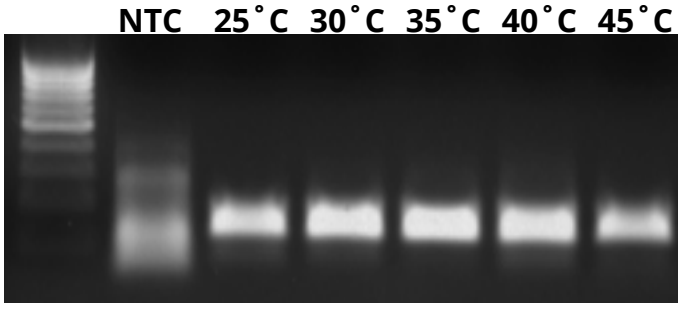

Figure 1

Summary

All reactions amplified a correctly sized product, at all the incubation temperatures tested. This flexible working temperature provides further evidence of RPA suitability for field work. A lower temperature requirement means lower power requirements - key for field applications. Furthermore RPA technology can be applied simply in a lateral flow format (TwistAmp® nfo).

TwistAmp® users have previously reported similar flexibility in RPA temperature requirements with HIV (1, 2) and Schistosoma japonicum (3) targets.

The whole range of TwistAmp® products can be purchased at www.twistdx.co.uk/products

References

1.http://dx.doi.org/10.1371/journal.pone.0108189

2.http://dx.doi.org/10.1371/journal.pone.0112146

3.http://dx.doi.org/10.1186/s13071-016-1745-5

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