

Basic Information

RPA

- 1) Primers must be 30-35 bases
- 2) Works best at constant temperature (37-39°C)
- 3) Amplicons of 80-400bp are preferred
- 4) TwistAmp™ LF Probe or TwistAmp™ exo Probe **1** required - see overleaf

PCR

- 1) Primers typically 18-25 bases
- 2) Thermal cycling required
- 3) Amplicons of 50bp upwards are typical/optimal

Set-up (*single-plex*)²

- 1) Prepare reaction mix in 1.5ml tube:

Primer A (10µM)	2.1 µl
Primer B (10µM)	2.1 µl
TwistAmp™ LF Probe (10µM)	0.6 µl
Rehydration Buffer	29.5 µl
Template and dH ₂ O	13.2 µl
(Total Volume	47.5 µl)

Vortex and spin briefly

- 2) Add reaction mix to freeze-dried reaction. Pipette to mix.
- 3) Add 2.5 µl of 280mM MgAc (supplied) and mix well to start reaction.

WARNING: RPA REACTIONS START AT ROOM TEMPERATURE AS SOON A MAGNESIUM IS ADDED.

- 4) Incubate at 37-39°C for 20-40 minutes.

- 5) Remove strip after 4-6 minutes, invert vigorously 8-10 times to mix & spin briefly, replace in heating device.
- 6) For analysis by lateral flow, dilute reaction products 1/10 with PBST and load 10 µl onto the sample pad of appropriate strip (eg Milenia® HybriDetect strips).
- 7) Place strip in PBST running buffer.

WARNING: IF TUBES ARE OPENED AFTER AMPLIFICATION THERE IS A GREAT RISK OF CONTAMINATION OF WORK SURFACES WITH AMPLICON! ENSURE THAT APPROPRIATE AVOIDANCE MEASURES ARE TAKEN!

- 1 TwistAmp™ exo Probes can also be used with this kit – see manual
- 2 See manual for multiplexing

RPA uses TwistDx's proprietary probe systems

RPA does NOT use PCR probe systems

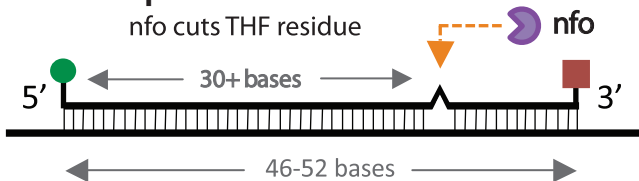
TwistAmp™ exo Probe







Exonuclease cuts THF residue



TwistAmp™ LF Probe

nfo cuts THF residue



- | | | | | | |
|--|-------------|---|----------|---|-------------|
|  | Fluorophore |  | dR group |  | THF residue |
|  | Quencher |  | 3' block |  | Nuclease |

refer to manual for details of probe design