

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™ fpg Probe 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)¹

- 1) Prepare reaction mix in 1.5ml tube:

Primer A (10µM)	2.1 µl
Primer B (10µM)	2.1 µl
TwistAmp™ fpg 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 AS MAGNESIUM IS ADDED.

- 4) Place strip in Twista™ and start run: 37-39°C, 20 minutes.
- 5) Remove strip after 4-6 minutes, invert vigorously 8-10 times to mix, spin briefly & return to Twista™.

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!

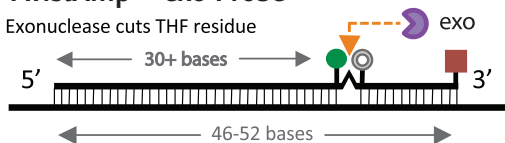
- ¹ See manual for multiplexing

RPA uses TwistDx's proprietary probe systems

RPA does NOT use PCR probe systems

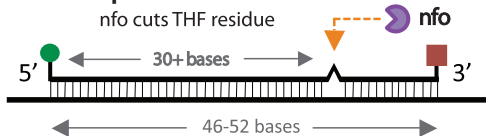
TwistAmp™ exo Probe

Exonuclease cuts THF residue



TwistAmp™ LF Probe

nfo cuts THF residue



TwistAmp™ fpg Probe

4-6 bases

fpg

fpg cuts dR group



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

refer to manual for details of probe design