

TwistAmp® Basic Quick Guide

Part Number: TABASo3Guide | Revision A

Basic Information

RPA

- 1) Primers can be 18-35 bases¹
- 2) Works best at constant temperature (37-39°C)
- 3) Amplicons of 80-400bp are preferred

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)	2.4 µl
Primer B (10µM)	2.4 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) Incubate at 37-39°C for 20-40 minutes.
- 4b) For low template copy number, remove strip after 4 minutes, vortex & spin briefly, replace in heating device.
- 5) After 20-40 minutes, clean amplicons before running on agarose gels.

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!

WARNING: SWITCH OFF HEATED LIDS BEFORE STARTING REACTIONS!

¹ For rapid amplification 30-35 bases are optimal

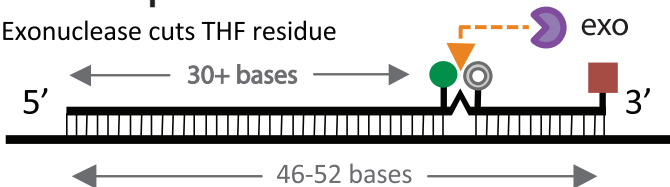
² See manual for multiplexing

RPA uses TwistDx's proprietary probe systems

RPA does NOT use PCR probe systems

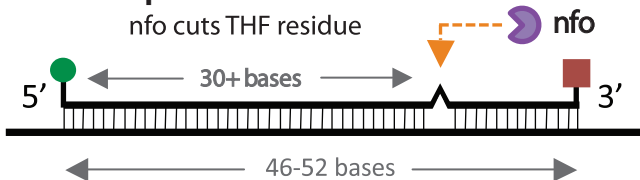
TwistAmp® exo Probe

Exonuclease cuts THF residue



TwistAmp® LF Probe

nfo cuts THF residue



● Fluorophore

● Nuclease

▲ THF residue

◎ Quencher

■ 3' block

refer to manual for details of probe design