TwistDx™

Unwind DNA's possibilities

TwistAmp® Liquid: a versatile amplification method to replace PCR

Here we introduce TwistAmp® Liquid, a new PCR replacement format that makes RPA technology more amenable to a wide range of research applications. In contrast to PCR amplification, RPA takes minutes, rather than hours, and can be run with little to no equipment. TwistAmp® Liquid Basic and Basic RT kits can be used for applications requiring good fidelity, gel electrophoresis or solid phase detection. TwistAmp® Liquid exo and exo RT allow rapid real-time amplification and detection of targets without sacrificing sensitivity or specificity.

Since their launch in 2008, TwistAmp® kits have provided scientists with a simple way to carry out recombinase polymerase amplification (RPA), resulting in over 250 peer-reviewed publications on the technique to date. RPA is an isothermal alternative to polymerase chain reaction (PCR) that uses three proteins (recombinase, single-stranded DNA–binding protein and strand-displacing polymerase) to replace thermal cycling (**Fig. 1**). RPA works best at constant low temperatures (ideally 37–42 °C), is very fast (reaction times of less than 20 minutes) and can be initiated with stable, lyophilized pellets, which makes it ideal for point-of-need testing. Moreover, RPA users have tested TwistAmp® kit reagents in a wide variety of different applications, including those that traditionally utilize PCR (as described in a recent review of RPA applications[1]). Like PCR, at its simplest, RPA uses two opposing primers to exponentially amplify a defined region of DNA. RPA primer pairs follow design rules similar to those of PCR primers, and indeed primers that have been developed for PCR reactions often work well in RPA reactions. To encourage this versatile use of the RPA technology, TwistDx™ has developed and launched TwistAmp® Liquid kits to provide users with a more flexible set of reagents. TwistAmp® Liquid kits are analogous to the premade PCR master mixes found in every molecular biology laboratory freezer, and include a glycerol stock tube of the core RPA protein mixture, a tube of buffer, a tube of the energy mix needed to drive the recombination process and a tube of magnesium acetate cofactor for the proteins.

There are four TwistAmp® Liquid kits available: Basic and Basic RT for amplification without probe, useful for gel electrophoresis, solid-phase amplification and microarray applications with tailed primers and aptamers; and exo and exo RT, which are compatible with probe-based real-time detection methods. All four kits contain the same Core Reaction Mix, which has the same ratio of recombinase, single-stranded binding protein and strand-displacing polymerase as the lyophilized TwistAmp® Basic kit. Because of this, assays developed with lyophilized TwistAmp® Basic kits should of course perform very similarly when the liquid kit is used, but assays developed with

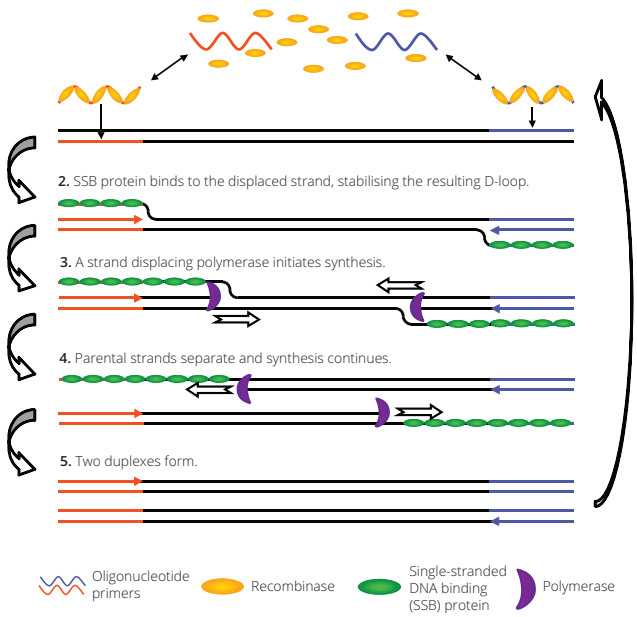

Figure 1 | The RPA cycle. Recombinase proteins form recombination filaments with primers and insert them into complementary sites in the DNA double helix via stand-displacement to create a D-loop. Single-stranded DNA–binding (SSB) proteins stabilize the opening of the double-helix structure and D-loop. The recombinase disassembly allows access for the strand displacing polymerase to extend the 3′-end of the primers along the target sequence. When this sequence is repeated for opposing primers, exponential amplification occurs without the need for thermal denaturing or annealing.

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APPLICATION NOTE

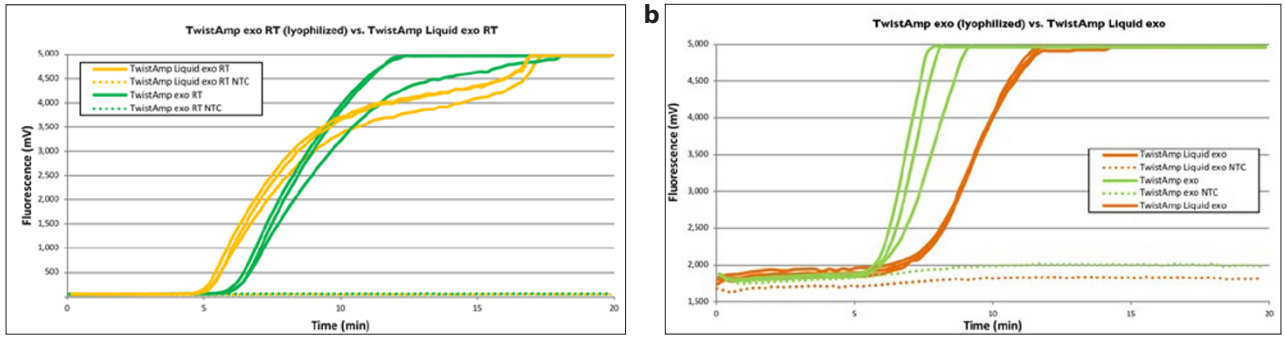

Figure 2 | Amplification of RNA and DNA targets. (**a**) Comparison of TwistAmp® exo RT kits (lyophilized) with TwistAmp® Liquid exo RT kits using respiratory syncytial virus B at 50 copies, alongside results for negative template controls (NTC) containing dH2O in the place of template. (**b**) Comparison between TwistAmp® exo kits (lyophilized) and TwistAmp® Liquid exo kits using 100 copies of apolipoprotein B, alongside NTC data. Reactions were run in a T8-ISO instrument with a 2-mm micro-ball in each reaction for automatic mixing. In this instance the different protein formulations resulted in an earlier onset time of detection for TwistAmp® Liquid exo RT reactions than for lyophilized TwistAmp® exo RT reactions. However, in an alternative assay, the TwistAmp® exo reactions showed an earlier onset time than the TwistAmp® Liquid exo reactions.

the other lyophilized TwistAmp® kits may show a different assay performance when used in liquid kits. **Figure 2** shows that assays run with TwistAmp® exo RT and TwistAmp® Liquid exo RT both detected 50 copies of human respiratory syncytial virus B RNA template. In addition to the Core Reaction Mix, the exo kits contain a tube of exonuclease III to allow the processing of TwistAmp® exo probes for real-time fluorescence detection, and the RT kits contain a tube of reverse transcriptase that allows single-step conversion of RNA into cDNA and subsequent amplification.

TwistAmp® Liquid kits, similarly to their lyophilized counterparts, are formulated to allow amplification of fragments up to 500 base pairs in size, which is sufficient for many common laboratory uses of PCR (different protein formulations are being developed to allow even longer amplicons). This makes RPA an excellent candidate to replace PCR for many standard laboratory cloning and sequencing protocols. When used for Illumina next-generation-sequencing library preparation, RPA leads to fewer errors than proofreading polymerases such as Kapa HiFi and Platinum pfx[2]. Additionally, RPA has been used to generate a longer, 754-base-pair fragment in an experiment that used an isothermal variant of Gibson cloning on an end-to-end automated microfluidic platform developed for synthetic biology[3], thus demonstrating the successful implementation of RPA in an established PCR-focused protocol.

A key advantage of TwistAmp® Liquid over lyophilized TwistAmp® kits is that scientists can use as much or as little of it as they want for a reaction in the consumable of their choosing. Unlike PCR, RPA is isothermal, works at a relatively low optimum temperature and can tolerate off-temperatures and temperature fluctuations. There is no need to preheat the master mix before delivering it into the system, and at a low isothermal temperature the effects of evaporation and condensation are not a major problem. This means that thermal control in small, compartmentalized microfluidic device volumes is simplified: RPA has been shown to work in very small (picoliter) volumes[4]. These unique attributes of RPA also mean that it can be used in reactions with much larger volumes than are ideal for PCR. **Figure 3** shows that a pair of PCR primers[5] successfully amplified a 269-base-pair fragment of *Neisseria gonorrhoeae* in reaction volumes ranging from 2.5 to 5,000 microliters. TwistAmp® Liquid reactions can thus be run in everything from custom-made microfluidic devices to standard laboratory 96-well plates to microcentrifuge tubes, or even larger vessels.

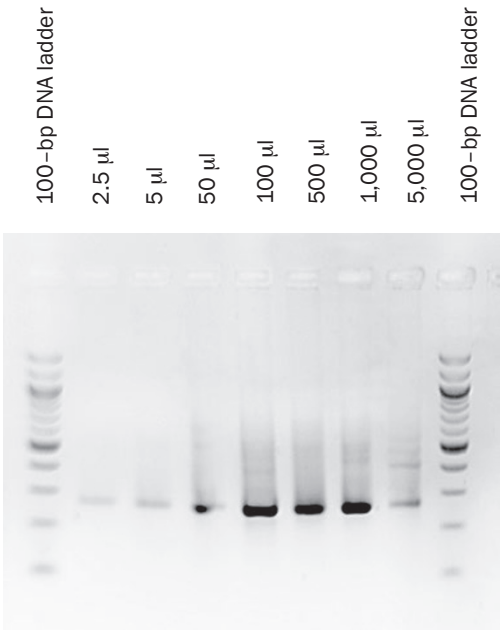

Figure 3 | DNA amplification using TwistAmp® Liquid Basic reactions (20 copies per reaction microliter of *N. gonorrhoeae* DNA) at an incubation temperature of 40 °C on 2% agarose gel. In the experiment shown here, 100% of all samples were loaded except for 100–5,000-µl reactions, for which 50 µl was cleaned up and run on gels.

Additional flexibility is granted to scientists who choose TwistAmp® Liquid kits rather than lyophilized TwistAmp® kits because TwistAmp® Liquid kits do not include deoxyribonucleotide triphosphates (dNTPs). Assays can therefore be run with deoxyuridine triphosphate (dUTP) or labeled nucleotides to allow amplicon capture or colorimetric or electrochemical detection[6–8].

Summary

RPA has been applied in more than 250 externally produced studies, and has been increasingly advantageous for a multitude of applications and fields. Our new TwistAmp® Liquid is the first viable alternative to PCR for laboratory molecular biology research.

The whole range of TwistAmp® products can be purchased at https://www.twistdx.co.uk/products. For more information about RPA technology and assay design, visit https://www.twistdx.co.uk/en/rpa.

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