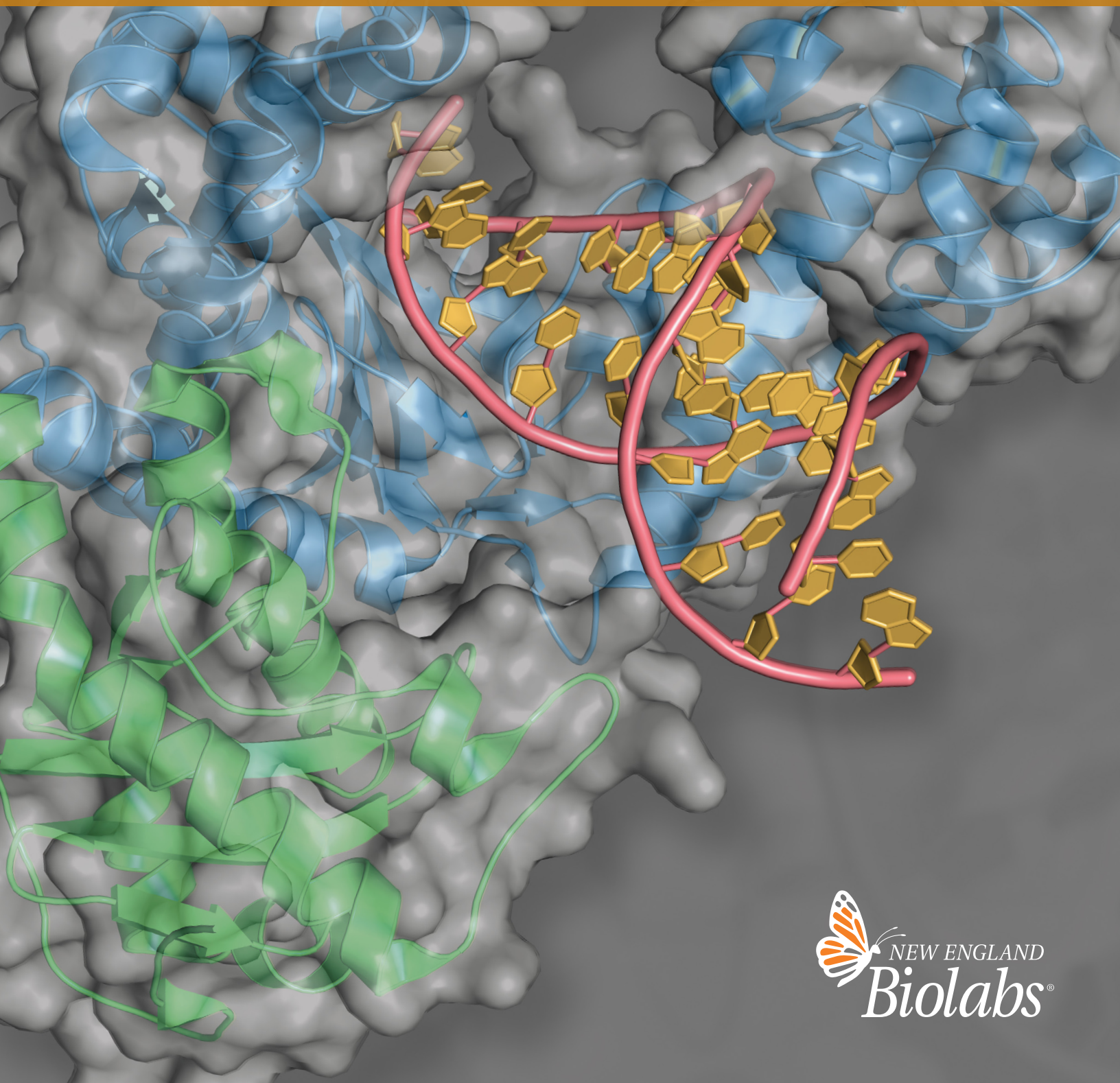
Isothermal Amplification

RAPID NUCLEIC ACID DETECTION



NEW ENGLAND Biolabs®

What is isothermal amplification?

The Polymerase Chain Reaction (PCR) is a well-known approach for amplifying a specific DNA or RNA (RT-PCR) sequence. PCR involves the reiterative cycling of a reaction cocktail between different temperatures to achieve amplification. As routine as PCR is in the molecular biology and molecular diagnostics laboratory, there are other methods of sequence-specific nucleic acid amplification.

These alternative approaches often do not require changing the reaction temperature and are referred to as isothermal amplification methods. Isothermal amplification methods are varied and have different advantages. In general, isothermal techniques are extremely fast and do not require thermocyclers, making them particularly well suited for field applications and point-of-care molecular diagnostics assays.

Advantages

- Fast
- Minimal equipment required
- Robust reactions in the presence of inhibitors
- Options for simplified optical or quantitative detection

Did you know that many of these products can be purchased in large volumes and custom formats? Learn more at **www.neb.com/customizedsolutions**

Not sure which product will work best for your experiment?

NEB offers a selection of *Bst* DNA Polymerase-based products for isothermal amplification. Use this chart to determine which product will work best for your needs.

	5′ → 3′ EXO ACTIVITY	AMPLIFICATION SPEED	ROOM TEMPERATURE SETUP	REVERSE TRANSCRIPTASE ACTVITY	INHIBITOR TOLERANCE	NOTES
***Bst* DNA Polymerase, Full Length**	★★	N/A	N/A	N/A	★	• Nick translation reactions at elevated temperatures
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	• General strand-displacement reactions, original polymerase for LAMP and other diagnostic amplifications
***Bst* 2.0® DNA Polymerase**	N/A	★★	N/A	★★	★★	• Improved LAMP, SDA, and other amplification reactions
***Bst* 2.0 WarmStart™ DNA Polymerase**	N/A	★★	★★★	★★	★★	• Improved performance with the addition of room temperature setup and higher specificity
***Bst* 3.0™ DNA Polymerase**	N/A	★★★	★★	★★★	★★★	• Engineered and fused to a novel nucleic acid binding domain • Fastest, most robust LAMP and RT-LAMP reactions • High reverse transcriptase activity up to 72°C
***Bst*-XT WarmStart DNA Polymerase**	N/A	★★★	★★★	★★★	★★	• Combines the high specificity of *Bst* 2.0 and fast polymerization speed of *Bst* 3.0 products

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

View our **NEB TV webinar** to learn about the benefits of *Bst*-XT WarmStart DNA Polymerase.

Loop-mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) uses 4-6 primers recognizing 6-8 distinct regions of target DNA for a highly specific amplification reaction. A strand-displacing *Bst* DNA polymerase initiates synthesis and 2 specially designed primers form "loop" structures to facilitate subsequent rounds of amplification through extension on the loops and additional annealing of primers. DNA products are very long (>20 kb) and formed from numerous repeats of the short (80–250 bp) target sequence, connected with single-stranded loop regions in long concatamers. These products are not typically appropriate for downstream manipulation, but target amplification is so extensive that numerous modes of detection are possible. Real-time fluorescence detection using intercalating dyes or probes, lateral flow and visual detection are all directly compatible with monitoring a LAMP reaction. Instrumentation for LAMP typically requires consistent heating to the desired reaction temperature and, where needed, real-time fluorescence for quantitative measurements.

RECOMMENDED PRODUCTS

WarmStart® Fluorescent LAMP/RT-LAMP Kit (with UDG)
(NEB #E1708)

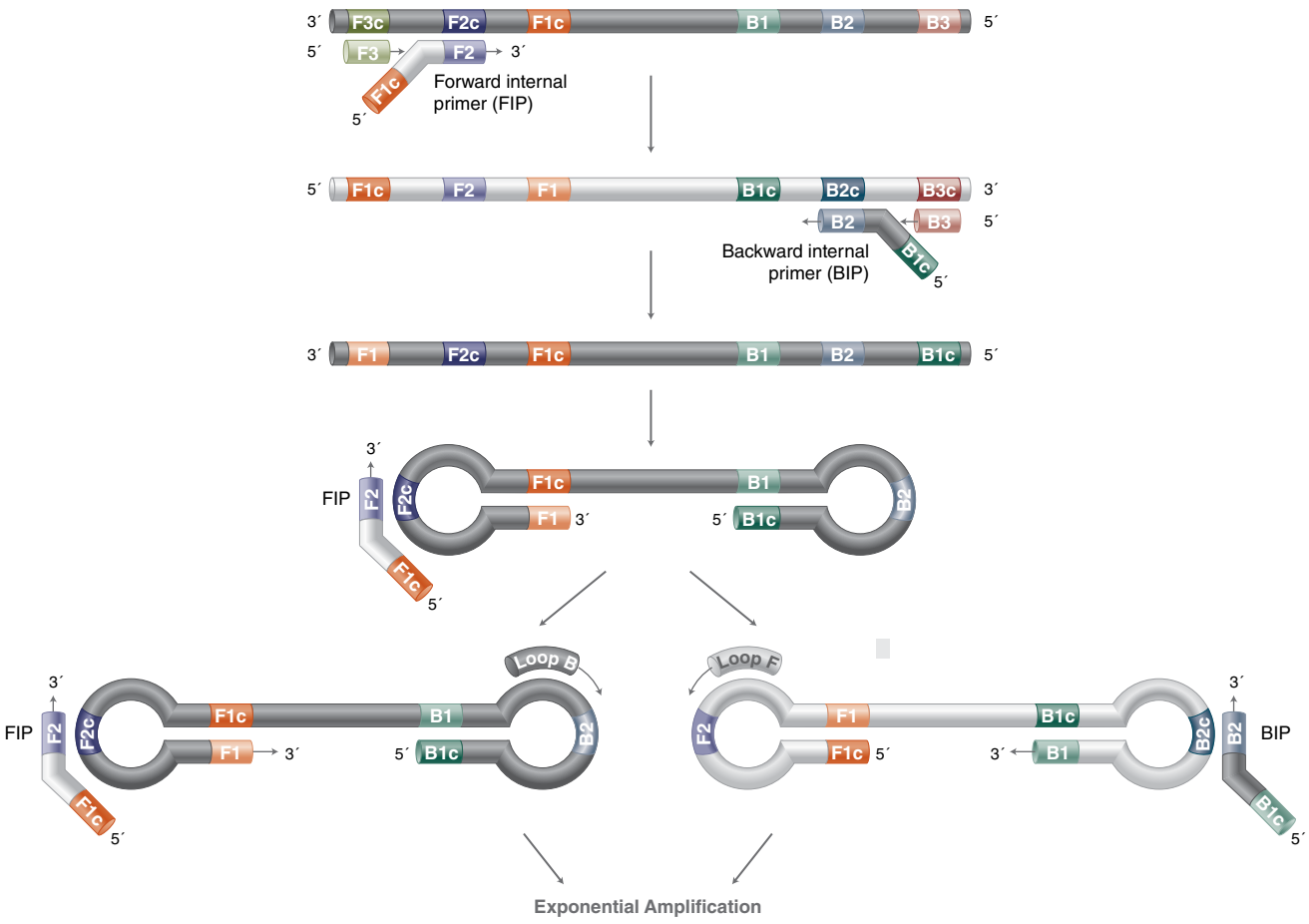
WarmStart Colorimetric LAMP 2X Master Mix with UDG
(NEB #M1804)

LyoPrime WarmStart™ Fluorescent LAMP/RT-LAMP Mix (with UDG)
(NEB #L4401)

WarmStart RTx Reverse Transcriptase
(NEB #M0380)

***Bst* DNA Polymerase**
(NEB #M0275, M0328, M0374, M0537, M0538, M9204)

Overview of Loop-Mediated Isothermal Amplification (LAMP)



Whole Genome Amplification (WGA) and Rolling Circle Amplification (RCA)

WGA and RCA are examples of Multiple Displacement Amplification (MDA). MDA utilizes the strand-displacement activity of DNA polymerases such as phi29 DNA Polymerase, engineered phi29-XT DNA Polymerase, or *Bst* DNA Polymerase for isothermal amplification of an entire genome or circular templates.

As a processive polymerase that can extend for tens of thousands of nucleotides in a single binding event, as well as increased proofreading activity, phi29-XT DNA Polymerase is ideal for accurate amplification of long or circular templates.

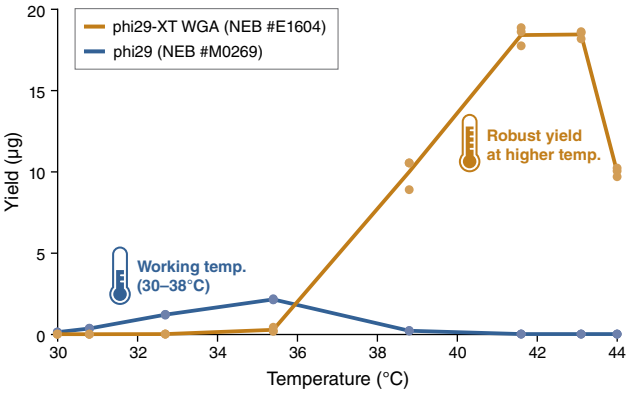
RECOMMENDED PRODUCTS

phi29-XT WGA Kit
(NEB #E1604)

phi29-XT RCA Kit
(NEB #E1603)

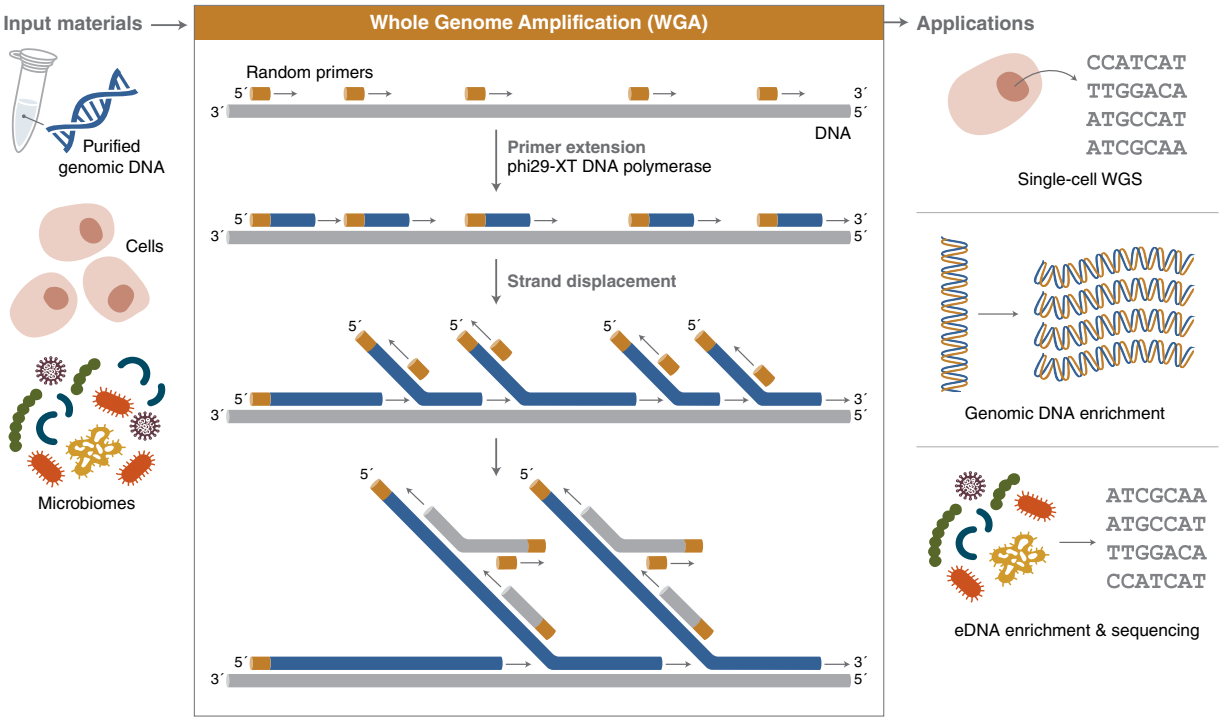
T7 Endonuclease I
(NEB #M0302)

Comparison of phi29-XT and phi29 DNA Polymerase yields



Triplicate WGA reactions were performed with 100 pg human genomic DNA input at the indicated temperatures for 1.5 hours. The reactions were then heat-inactivated at 65°C for 10 minutes. All reactions contained 1 mM dNTPs and 50 µM Exonuclease-Resistant Random Primers. Wild-type phi29 reactions were carried out with 10 units of phi29 DNA Polymerase (NEB #M0269) in 1X phi29 DNA Polymerase Reaction Buffer and 0.1 mg/mL Recombinant Albumin, and phi29-XT reactions were carried out with 1X phi29-XT DNA Polymerase for WGA and 1X phi29-XT Reaction Buffer for WGA. Reaction yields (dots) were quantified using Quant-iT® PicoGreen® dsDNA Reagent and were averaged (lines) to determine the yield at each reaction temperature. While the working reaction temperature of wild-type phi29 DNA Polymerase is typically between 30°C and 38°C, phi29-XT DNA Polymerase generates robust product yields around 42°C. The optimal temperature range for wild-type phi29 increased by approximately three degrees for WGA using human genomic DNA when compared to RCA using pUC19 plasmid as input.

Overview of the phi29-XT WGA Kit



The phi29-XT WGA Kit (NEB #E1604) is a fast, easy-to-use, and highly versatile kit containing all the required components for whole genome amplification (WGA) using a random primer mix. The kit delivers high yields of DNA products from a variety of starting materials including purified genomic DNA, cells, or microbiomes. This kit is ideal for various DNA applications such as single cell whole genome sequencing (WGS), genomic DNA enrichment, and environmental DNA (eDNA) enrichment and sequencing.

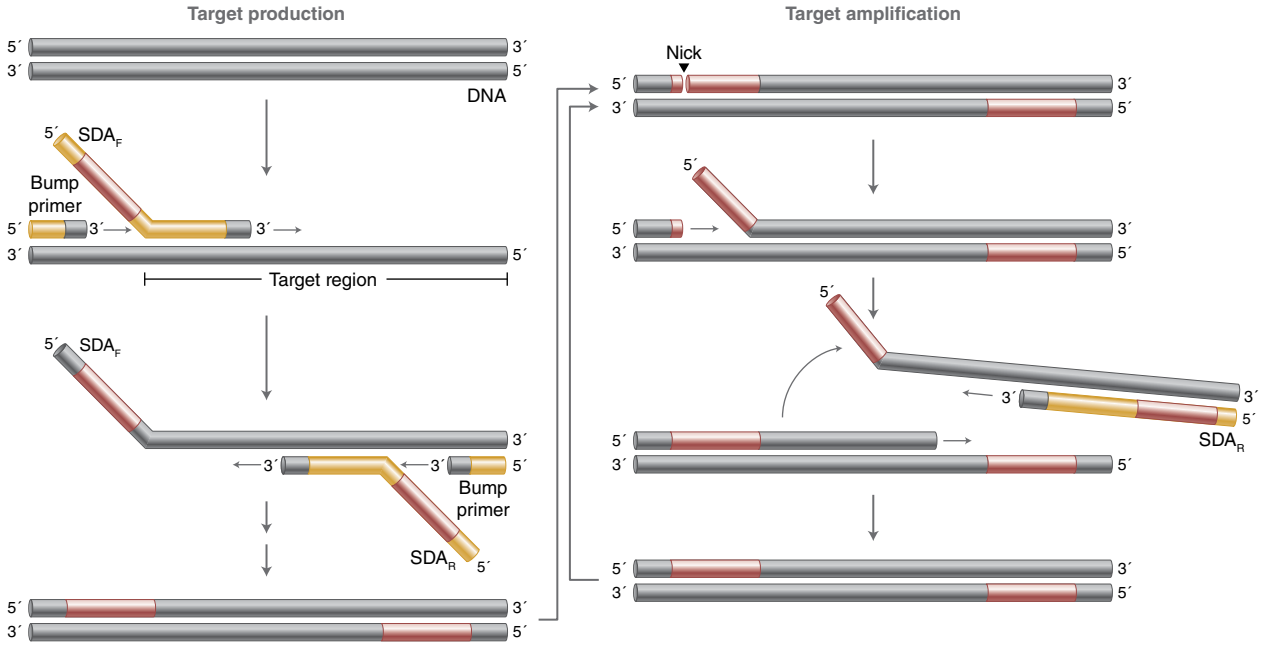
Strand Displacement Amplification (SDA)

SDA relies on a strand-displacing DNA polymerase, typically *Bst* DNA Polymerase, Large Fragment (NEB #M0275) or Klenow Fragment (3'-5' exo–) (NEB #M0212) to initiate amplification at nicks created by a strand-limited restriction endonuclease or nicking enzyme (e.g. WarmStart Nt.BstNBI, NEB #R0725) at a site contained in a primer. The nicking site is regenerated with each polymerase displacement step, resulting in exponential amplification.

RECOMMENDED PRODUCTS

- WarmStart Nt.BstNBI (NEB #R0725)
- *Bst* DNA Polymerase, Full Length (NEB #M0328)
- *Bst* DNA Polymerase, Large Fragment (NEB #M0275)
- *Bst* 2.0 DNA Polymerase (NEB #M0537)
- WarmStart Afu Uracil-DNA Glycosylase (UDG) (NEB #M1282)

Overview of SDA



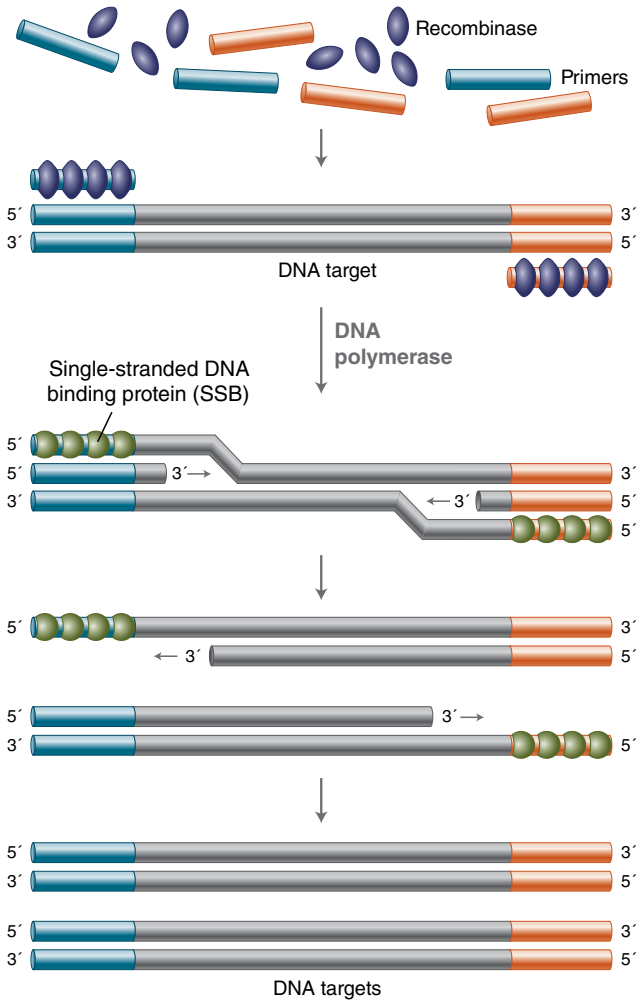
** Target amplification, shown above for SDAF, will also occur simultaneously with SDAR.*

For more information on these and other isothermal amplification methods and products, please visit **www.neb.com/isoamp**

Recombinase Polymerase Amplification (RPA)

Recombinase Polymerase Amplification (RPA) uses a recombinase enzyme to help primers invade double-stranded DNA. T4 UvsX, UvsY, and a single stranded binding protein T4 Gene 32 protein form D-loop recombination structures that initiate amplification by a strand-displacing DNA polymerase. RPA is typically performed at ~37 °C and, unlike other methods, can produce discrete amplicons up to 1 kb.

Overview of RPA



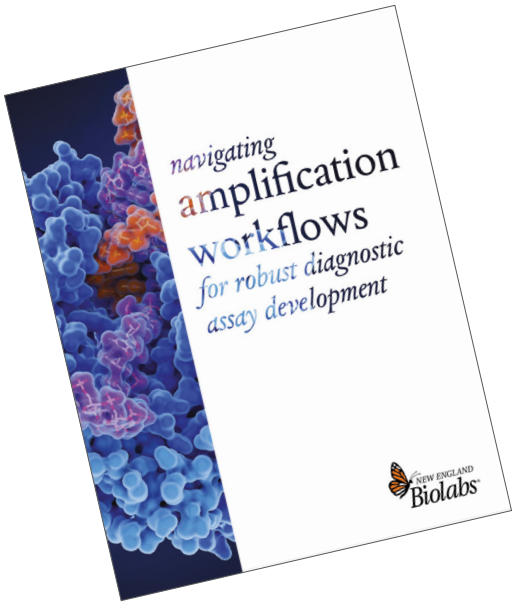
RPA utilizes a recombinase-primer complex, a strand-displacing polymerase, and single-stranded DNA binding proteins to facilitate amplification of discrete DNA products up to 1 kb (primer-to-primer distance).

RECOMMENDED PRODUCTS

- **T4 UvsX Recombinase (Glycerol-free)*** (NEB #M3081)
- **T4 UvsY Protein (Glycerol-free)*** (NEB #M3082)
- ***Bsu* DNA Polymerase, Large Fragment** (NEB #M0330)
- **T4 Gene 32 Protein** (NEB #M0300)

* *Please contact the Customized Solutions Team at www.neb.com/customizedsolutions, to inquire about these products.*

Resources to support your isothermal amplification work



Download our ebook, featuring:

- Comparison of PCR and isothermal amplification technologies
- Examination of assay design optimization parameters
- Considerations for assay lyophilization
- Testimonial from Anne Wyllie, Ph.D., *Research Scientist in Epidemiology of Microbial Disease, Yale School of Public Health*

Register here to receive your copy of the eBook

NEB® LAMP Primer Design

NEB LAMP Primer Design

To begin, use our free online tool to design primers for any of these methods at lamp.neb.com

What products are recommended for molecular diagnostics?

NEB offers a selection of reverse transcriptases (RTs), DNA polymerases, and master mixes for customers to use in the development of rapid and sensitive molecular diagnostics. To support the needs of this market, products are available in fully lyophilized, high concentration, or glycerol-free/lyo-compatible formats. All formats are available for customization.

WarmStart RTx is ideal for LAMP and isothermal amplification workflows and is a core component of a lyophilized LAMP/RT-LAMP mix (NEB #L4401). Luna® RT supports RT-qPCR workflows and is available in a lyophilized format (NEB #L4001). Customers have utilized these enzymes in numerous diagnostic assays, including for COVID-19.

NEB Lyophilization Sciences® is equipped to develop and manufacture lyophilized molecular biology reagents for the life sciences, including research, applied and the molecular diagnostics sectors. The NEB Lyophilization Sciences Team are experts in the design, development and manufacturing of innovative solutions for ambient-stored products.

Products can be lyophilized in multiple formats.

Contact us at www.neb.com/customizedsolutions to find out how we can support your development, or visit www.neb.com/mdx to learn more.

Choose from our selection of products
for your isothermal application.

PRODUCT	NEB #	SIZE
WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	M1708S/L	100/500 reactions
WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)	E1708S/L	100/500 reactions
WarmStart LAMP Kit (DNA & RNA)	E1700S/L	100/500 reactions
LAMP Fluorescent Dye	B1700S	0.25 ml
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800S/L	100/500 reactions
WarmStart Colorimetric LAMP 2X Master Mix with UDG	M1804S/L	100/500 reactions
SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	E2019S	96 reactions
Bst-XT WarmStart DNA Polymerase	M9204S/L	1,600/8,000 units
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/L/M	1,600/8,000/8,000 units
Bst 2.0 DNA Polymerase	M0537S/L/M	1,600/8,000/8,000 units
Bst DNA Polymerase, Large Fragment	M0275S/L/M	1,600/8,000/8,000 units
Bst DNA Polymease, Full Length	M0328S	500 units
phi29-XT RCA Kit	E1603S/L	100/500 reactions
phi29-XT WGA Kit	E1604S/L	25/100 reactions
WarmStart Afu Uracil-DNA Glycosylase (UDG)	M1282S	200 units
WarmStart Nt.BstNBI	R0725S	1,000 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
Nt.BstNBI	R0607S/L	1,000/5,000 units
COMPANION PRODUCTS		
Tte UvrD Helicase	M1202S	0.5 µg
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol of each
Deoxynucleotide (dNTP) Solution Set	N0446S	25 µmol of each

Did you know that many of these products can be purchased in large volumes and custom formats? Learn more at **www.neb.com/customizedsolutions**

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