



DNA Polymerases

DNA Polymerases

from Boca Scientific

Boca Scientific's range of Bioron products

Boca Scientific is dedicated to accelerating research. We are a trusted provider of high-quality biotechnological products and serve research laboratories across the United States and Canada. Boca Scientific works with the long-standing provider of molecular biology products, Bioron, which was established over 20 years ago. Bioron's molecular biology products are adapted for several research applications. Here we review Bioron's range of DNA polymerases and detail how their properties are perfectly adapted to detect targets of clinical value. Examples include pathogens and disease markers.

PCR amplification

Amplification of DNA has been vitally important in studying an organism's genome. Initially invented in the 1980s, PCR allows the amplification of a specific segment of nucleic acid. Standard PCR first involves denaturing DNA using heat (typically 90–95°C) to form two separate strands. Primers — short oligonucleotides complementary to the start and ends of the target DNA — are used to target a specific region in the DNA. The primers anneal to each original strand of DNA, which defines the start point of replication. Each strand can be replicated by exploiting the function of a DNA polymerase enzyme, which can replicate the opposite strand by adding nucleotides.

Extension of the new DNA strands relies on the 5' to 3' polymerase activity, which is common to all DNA polymerase enzymes. This activity enables the addition of deoxy-nucleotides (dNTPs) to extend the new strand in the 5' to 3' direction from the primers. This step occurs at a higher temperature, so DNA polymerases must work in an optimum temperature range of 68–72°C. Each additional cycle allows increasing amounts of DNA to be generated. The quantity of DNA effectively doubles with each cycle. Standard PCR measures the amplified product at the end of the assay — end-point analysis. In contrast, real-time PCR monitors and quantifies each cycle of the amplification reaction.



Boca Scientific's range of DNA polymerases is highly versatile. Each DNA polymerase has unique characteristics, including thermostability, specificity, processivity, strand-displacement activity, and modified nucleotide selection. This whitepaper details the features that make the polymerases ideal for scientific and clinical applications.

SuperHotTaq DNA polymerase for hot start PCR

Nonspecific amplification is problematic for PCR, reducing reproducibility and experimental variation. *Taq* polymerase is the most widely described and used thermostable DNA polymerase. Initially identified in 1976, *Taq* polymerases are thermostable with a half-life of 40 mins at 95°C. One of the limitations of *Taq* polymerase is it has substantial enzymatic activity at 37°C. — known as low-temperature polymerase activity. This results in exactly what one would like to avoid with PCR, where the polymerase amplifies nonspecific products with low stringency (where PCR primers anneal to template sequences that are not perfectly complementary, also known as off-target amplicons).

Another problem associated with PCR applications is primer-dimer formation. Primer dimers occur when primers hybridize with each other via their complementary bases. Off-target amplicons and primers-dimers are particularly troublesome for highly sensitive, multiplexed or genotyping assays, which rely on specific target amplification⁽¹⁾.

The term “hot-start PCR” refers to employing polymerases that exert activity only at higher temperatures and remain inactive at ambient temperatures. Polymerases for hot-start PCR are thermostable and tolerate temperatures above 95°C. Hot-start polymerases use different methods to block the polymerase activity at lower temperatures, avoiding nonspecific amplification. Hot-start methods can suppress the initiation of primer dimers⁽¹⁾.

SuperHotTaq DNA polymerase

SuperHotTaq polymerase is a unique polymerase aimed at reducing nonspecific amplification and the effects of primer dimers. *SuperHotTaq* contains a combination of *Taq* polymerase and anti-*Taq* DNA polymerase monoclonal antibodies. Anti-*Taq* antibodies block the *SuperHotTaq*'s polymerase activity during PCR setup at ambient temperatures (20–22°C). Consequently, nonspecific amplification is reduced as the *SuperHotTaq* remains unreactive at these temperatures. Release of this inhibition on polymerase activity occurs when the temperature is increased above 70°C.

Applications

- ***SuperHotTaq* is suitable for PCR applications with complex genomic or cDNA templates, low copy number targets, and high numbers of thermal cycles**
- **Multiplex, real-time-PCR and diagnostic applications**
- **Effectively amplification of fragments up to 5kb**
- ***SuperHotTaq* can be used and stored at room temperature for several days without cooling**



Published literature using *SuperHotTaq* polymerase

PCR-single-stranded conformational polymorphism (PCR-SSCP) is a method used to detect sequence differences between relatively short stretches of DNA. Together with sequencing, PCR-SSCP is useful in identifying genetic polymorphisms and mutations. *SuperHotTaq* has been employed in a PCR-SSCP method that pre-screened the exons 12 and 18 of the platelet-derived growth factor alpha (PDGFR- α) gene from samples of invasive mammary carcinomas. The PCR products for each exon were purified and consequently analyzed by direct sequencing to screen for PDGFR- α mutations. Researchers showed that the analysis showed genetic alterations at exons 12 and 18, but these were not correlated with increased PDGFR- α protein expression⁽²⁾.

Product	Size (catalog number)
<u><i>SuperHotTaq</i> DNA Polymerase</u>	<u>200 units (Cat# 119002)</u>
	<u>1000 units (Cat# 119010)</u>

Researchers have also employed *SuperHotTaq* in a PCR-SSCP method to study activating mutations in the tyrosine kinase domain (TKD) (exons 18, 19, 20 and 21) of epidermal growth factor receptor (EGFR) from a cohort of 47 metastatic breast carcinomas. Confirmed by sequencing, the PCR-SSCP results showed a lack of activating EGFR mutations, suggesting this is unlikely to be an alternative genetic mechanism for EGFR expression in metastatic breast carcinoma⁽³⁾.

Anti-*Taq* antibodies

Boca Scientific also supplies Anti-*Taq* DNA Polymerase monoclonal antibodies that block polymerase activity at ambient temperatures to prevent nonspecific amplification of DNA.

Product	Size (catalog number)
<u>Anti-<i>Taq</i> DNA Polymerase</u>	<u>100 µg (Cat#121001)</u>
<u>Antibody</u>	<u>500 µg (Cat#121005)</u>

The Anti-*Taq* DNA polymerase antibodies are provided in a glycerol-free formulation, ideal for lyophilization.

Product	Size (catalog number)
<u>Anti-<i>Taq</i> DNA Polymerase</u>	<u>100 µg (Cat#151001)</u>
<u>Antibody, glycerol-free</u>	<u>500 µg (Cat#151005)</u>

Labelling with modified dNTPs

Labelling PCR products are helpful in purifying, monitoring, and analyzing DNA fragments. In-tube labelling can easily be achieved during the PCR process by introducing modified dNTPs. Several chemical moieties, including tags such as fluorophores, biotin and digoxigenin, can chemically modify dNTPs. Specific polymerases can still recognize dNTPs whose bases have been modified, as long as the Watson-Crick base pairing is preserved⁽⁴⁾.

Reverse transcription PCR (RT-PCR)

Both DNA and RNA can be amplified using PCR. The RNA must first be converted to copy DNA (cDNA) for RNA amplification. The cDNA can then serve as a template for the DNA polymerase enzyme. Appropriately named reverse transcription — this process is opposite to the natural transcription process. Correspondingly, the enzymes responsible for this process are called reverse transcriptases (RTs).

RNA-directed DNA polymerases have both DNA polymerase and RT activity. In the presence of reverse transcription primers, dNTPs and a cofactor — either magnesium or manganese ions — RTs can catalyze cDNA synthesis.

Polymerases for RT-PCR

Boca Scientific supply *Tth* polymerase from Bioron, that are ideal for RT-PCR. *Tth* polymerase amplifies DNA up to 3–4 kb at 74°C but also contains reverse transcription activity but no RNase activity.

The *Tth* polymerase is isolated from the eubacteria *Thermus thermophilus* strain HB8 and expressed in the bacterium *E. coli*. The enzyme exhibits an RNA-dependent DNA polymerase activity in the presence of Mn²⁺ ions. Additionally, *Tth* polymerase can incorporate modified dNTPs.

Published literature using *Tth* polymerase

Inhibitors in clinical samples can severely hamper RNA and DNA detection, resulting in low sensitivity and false-negative results. A recent study assessed the capacity of the *Tth* polymerase to detect RNA. The study showed that the *Tth* polymerase is extremely resistant and could detect RNA directly from crude clinical samples or the presence of co-purified inhibitors⁽⁵⁾.

DNA and RNA have been directly detected using *Tth* polymerase from large volumes of whole human blood (WHB) without sample pretreatment, additives or specific sample buffers. The study highlights the effectiveness of *Tth* polymerase not only for RT-PCR but also for PCR-based diagnostic assays. *Tth* polymerase has been shown to be effective in the rapid (within 4 hours) and accurate detection of pathogens from WHB⁽⁶⁾.

Applications

- **Thermostable — replicates DNA at 74°C**
- **Intrinsic reverse transcriptase activity with no RNase activity**
- **Standard PCR, RT-PCR and DNA labelling with modified dNTPs**

Product	Size (catalog number)
<u><i>Tth</i> polymerase</u>	<u>500 units (Cat# 104005)</u>
	<u>2500 units (Cat# 104025)</u>

DFS-Taq DNA Polymerase

Diagnosis of infectious diseases is a crucial application of PCR using polymerases. *Taq* polymerases have a high affinity for DNA and preparations containing contaminating DNA. Possible sources of contaminating DNA are the expression vectors used for recombinant production or from the host genomic DNA. Contaminating DNA is problematic to PCR applications, particularly diagnostic applications, as it can generate false-positive results. In addition, microbial DNA is present at low levels in clinical specimens.

Boca Scientific DFS-Taq polymerase lacks contaminating DNA

DFS-Taq polymerase from Boca Scientific circumvents the generation of false-positive results as it lacks contaminating DNA. This makes it ideal for all types of PCR applications, especially those requiring pathogenic bacteria detection. DFS-Taq has been validated and designated as microbial DNA-free components for PCR amplification⁽⁷⁾.

DFS-Taq polymerase is a thermostable enzyme isolated from eubacterium *Thermus aquaticus* YT-1. The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the presence of magnesium ions and shows 5' to 3' exonuclease activity. DFS-Taq polymerase (DNA Free) is highly purified and free of nonspecific endo- or exonuclease

Applications

- The universal buffer system makes DFS-Taq amenable to different applications
- Suitable for standard PCR, real-time PCR, multiplex PCR and pathogen detection
- Produces a high sensitivity and product yield
- Effectively directs PCR with templates up to 5 kb in length



Product	Size (catalog number)
DFS-Taq polymerase	500 units (Cat# 101005)
	2500 units (Cat# 101025)
	10,000 units (Cat# 101100)

Published literature using DFS-Taq polymerase

A recent study published in *Scientific Reports* employs DFS-Taq polymerases for amplicon sequencing. Amplicon sequencing is a sequencing technique used to describe and compare the phylogeny and taxonomy of bacteria and fungi. Amplicon sequencing target genes of 16S genes ribosomal RNA (rRNA), 18S rRNA and internal transcribed spacer. In the study, DFS-Taq polymerase was used to assess the presence of bacterial DNA using universal primers for 16S rRNA. The study results were significant in generating data on the gut microbial community of European seabass fed with *Gracilaria gracilis*, which improved the general health of the organism⁽⁸⁾.

Another study has also employed DFS-Taq polymerase for amplicon sequencing. This second study compared the structures in bacterial communities from the land plant, *Arabidopsis thaliana* and the algae, *Chlamydomonas reinhardtii*⁽⁹⁾.

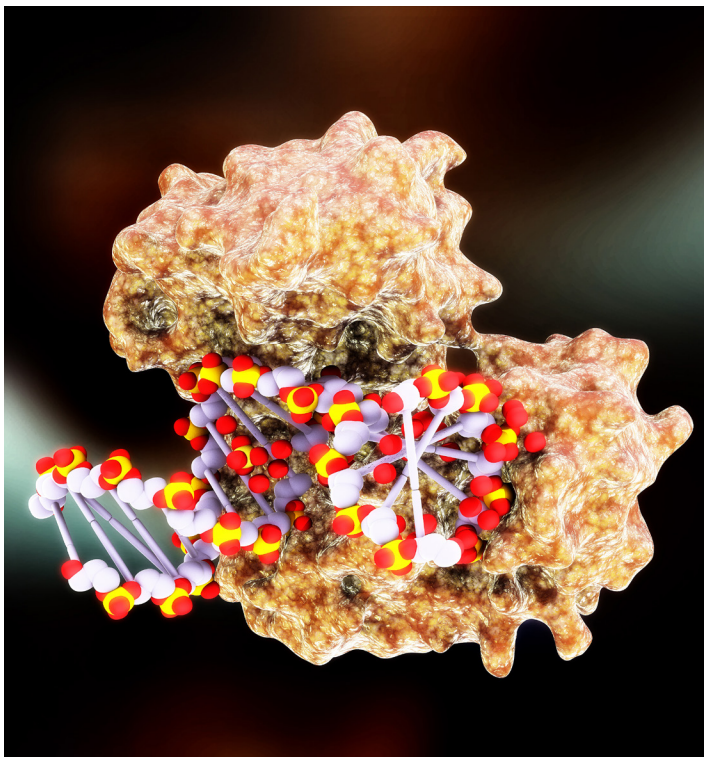
HotStart version of DFS polymerase

A HotStart version DFS-Taq polymerase is also available for reactions needing higher stability and reaction setup at room temperature. DFS-Taq polymerases contain anti-Taq antibodies and are ideal for applications requiring sensitive detection and high product yields; applications include detecting low-abundance target DNA.

Applications

- High sensitivity and free of bacterial DNA
- Suitable for PCR, real-time PCR, multiplex PCR and pathogen detection
- Efficient amplification of single-copy genes; amplifies fragments up to 5 kb in length

Product	Size (catalog number)
DFS HotTaq polymerase	500 units (Cat# 101004)
	2500 units (Cat# 101024)



DNA polymerases with high specificity and precision

Methods such as single nucleotide polymorphisms (SNP) genotyping and DNA sequencing rely on DNA polymerases that are very accurate. These applications rely on the ability of DNA polymerases to incorporate modified nucleotides.

Klen SNPase DNA Polymerase is a modified version of the *Taq* polymerase incorporating a point mutation in the active site of the enzyme. This leads to higher enzymatic specificity and higher precision for incorporating deoxy- and dideoxy-nucleotides. The mutation rate of Klen SNPase is 10–15 fold lower than *Taq* polymerase.

As heat treatment is used for DNA strand denaturation in SNP genotyping, thermostable SNPase HotStart DNA Polymerase is ideal for this application.

Applications

- Specifically designed for SNP genotyping and DNA sequencing
- High specificity and precision with a lower mutation rate than *Taq* polymerase

Product	Size (catalog number)
<u>Klen SNPase DNA polymerase</u>	500 units (Cat# 108205)
	2500 units (Cat# 108225)
<u>SNPase HotStart DNA polymerase</u>	500 units (Cat# 108210)
	2500 units (Cat# 108250)

Stadler *et al.*, used SNPase in a study that needed to pre-amplify DNA containing point mutations to detect circulating cell-free tumor DNA in the plasma of cancer patients⁽¹⁰⁾. The SNPase polymerase was chosen for its extreme base specificity and was coupled with a polymerase for quantitative PCR and dual-labelled hydrolysis probes. Employing SNPase polymerase produced an extremely sensitive and specific technique for detecting and quantifying tumor DNA.



Polymerases for isothermal amplification

There are two main strategies for amplifying DNA, PCR and isothermal amplification. The difference between the two is that PCR requires a PCR instrument, which allows the temperature to be changed for the different phases of the PCR reaction. In contrast, isothermal amplifications do not require expensive thermocycling equipment, as all steps employ a constant temperature.

Different types of isothermal amplification exist, including strand displacement amplification (SDA), rolling circle amplification (RCA), cross-priming amplification (CPA), and loop-mediated amplification (LAMP).

In all types of isothermal amplification, a strand displacement DNA polymerase is employed, which is used to extend the primer and separate the DNA duplex. Isothermal applications are employed in various applications, from clinical testing to cancer research. Isothermal applications are popular in diagnostic testing. It is essential in near-patient and self-testing tests where thermal cyclers are not readily available.

More recently, techniques that combine PCR and the isothermal amplification method have been described. Named polymerase chain displacement reaction (PCDR), PCDR was first described in 2013⁽¹²⁾. The advantage of employing PCDR is that DNA amplification is more than two times per cycle compared with standard PCR, which uses two primers.

Unlike PCR, PCDR uses more than one pair of primers. PCDR uses four primers that flank the sequence of interest (Figure 1). When the outer and inner primers are extended, the extended strand produced from the inner primer is displaced by the extended strand of the outer primer. Only DNA polymerases that possess strand displacement activity and lack a 5' to 3' exonuclease activity can fulfil this function. The 5' to 3' exonuclease means that the inner primer extension product will not be degraded. This reaction is monitored in PCDR by the presence of a dual-labeled probe.

Thermostable enzymes with proofreading capabilities

Ensuring that the amplicon is synthesized accurately for cloning and sequencing is critical. Standard *Taq* polymerase is incapable of high accuracy as it does not possess a proof-reading capacity. Instead, high-fidelity polymerases containing proofreading characteristics provided by the 3' to 5' exonuclease activity are needed. This means that when high-fidelity DNA polymerases are synthesizing DNA, they can check each nucleotide and exercise mismatched nucleotides in the 3' to 5' direction.

DF-*Pfu* DNA Polymerase

The DF-*Pfu* DNA polymerase is isolated from the hyper-thermophilic archaea, *Pyrococcus furiosus*, a thermostable enzyme. DF-*Pfu* DNA polymerase 3' to 5' exonuclease proofreading activity results in the enzyme having an error rate of 1.3×10^{-6} , thus displaying a much lower error rate in PCR than *Taq* polymerase⁽¹¹⁾. Bioron's DF-*Pfu* DNA polymerase is also free of bacterial DNA. DF-*Pfu* DNA polymerase is ideal for high-fidelity synthesis applications, such as DNA sequencing and cloning.

Applications

- High-fidelity synthesis for PCR and primer extension
- Precise amplification of bacterial sequences

Product	Size (catalog number)
DF- <i>Pfu</i> DNA polymerase	500 units (Cat# 108105)
	2500 units (Cat# 108125)

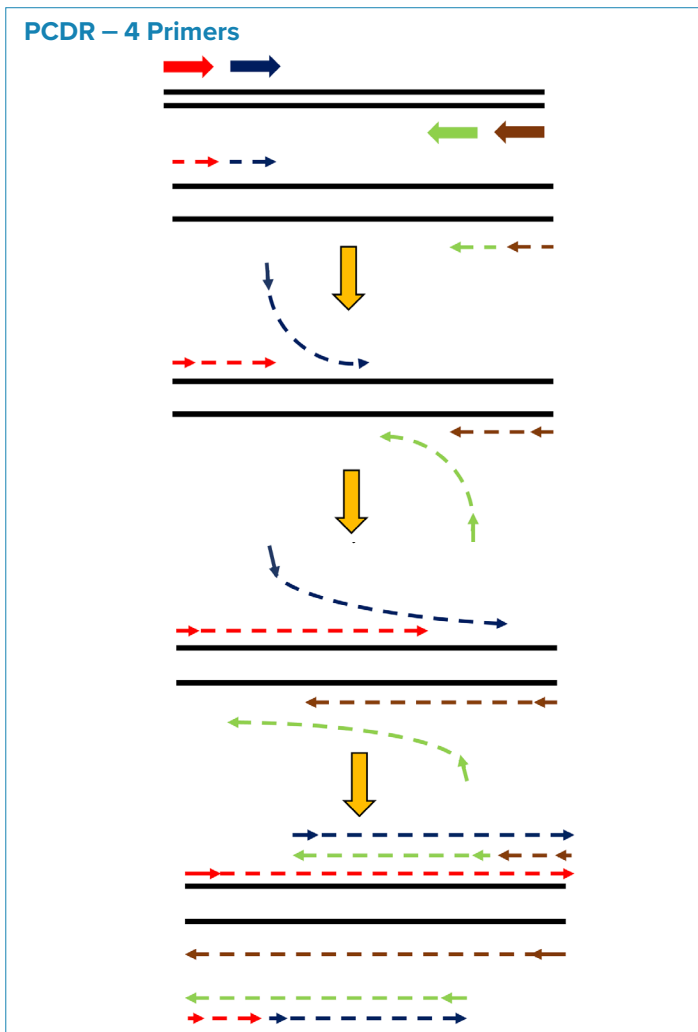


Figure 1. Schematic representation of PCDR

PCDR employs nested primers and strand displacement polymerases for amplification. PCDR produces more amplicons after each amplification cycle, compared with standard PCR⁽¹²⁾.

DNA polymerase strand displacement (SD) polymerase

SD polymerase from Bioron is a novel, artificial polymerase with strong strand displacement characteristics. SD polymerase is also thermostable up to 93°C, which provides better thermostability than polymerases with similar characteristics, such as phi29 or *Bst* DNA polymerases.

Applications

- High sensitivity and product yield
- Suitable for isothermal amplification (LAMP, WGA, RCA), multiple displacement amplification (MDA) and PCDR
- Can be employed for library generation and genome sequencing
- Also suitable for standard, multiplex PCR and real-time PCR (only with intercalating dyes)
- Amplifies up to 30 kb

Product	Size (catalog number)
SD polymerase	Standard, 10 U/μl [<u>200 U (Cat#108702)</u> and <u>1000 U (Cat#108710)</u>]
	High concentration, 50U/μl [<u>1000 U (Cat#108800)</u> and <u>5000 U (Cat#108850)</u>]
	Hot-start, 10 U/μl, [<u>200 U (Cat#108902)</u> and <u>1000 U (Cat#108910)</u>]

Combining PCR and isothermal amplification, both PCDR and PCR-LAMP, have shown that improve the detection of SARS-CoV-2 detection compared with LAMP assays. Overall, the hybrid methods improved virus detection and are ideal for diagnostic kits⁽¹³⁾.

The strong strand displacement activity of the thermostable SD polymerase from Bioron has been instrumental in developing a novel sequencing technique — a single-molecular mutation sequencing approach (SMM-seq). Published in 2022 in *Science*, the SD polymerase is used for library preparation to amplify single-stranded DNA molecules using RCA⁽¹⁴⁾. The researchers showed that the SMM-seq approach could quantitatively identify point mutations in normal cells and tissues.

DNA polymerases from Boca Scientific

Boca Scientific's range of Bioron polymerases is specially selected for their characteristic suited to a range of PCR applications. Boca Scientific have a range of tools for your molecular biology needs. Please [get in touch with us](#) if you need help and further information about any of our products.

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Characteristics	Concentration per μ l	Application			5-3' Exo-nuclease	Strand Displacement	Reverse Transcriptase activity	Hot Start	Amplifications up to	REF	Pack size
		End-point	Real Time	Multiplex							
DFS-Taq	5 U	✓	✓	✗	✓	✗	✗	✗	5 kb	101005 101025 101100	500 U 2,500 U 10,000 U
SuperHotTaq	5 U	✓	✓	✓	✓	✗	✗	AB	5 kb	119002 119010	200 U 1,000 U
SuperHotTaq HC	30 U	✓	✓	✓	✓	✗	✗	AB	5 kb	119030HC	2,000 U
SuperHotTaq HC GF	30 U	✓	✓	✓	✓	✗	✗	AB	5 kb	119030HC-GF	2,000 U
SuperHot Taq UP	5 U	✓	✓	✓	✓	✗	✗	AB	5 kb	129010UP	1,000 U
AptaHotTaq	5 U	✓	✓	✓	✓	✗	✗	Aptamer	5 kb	119602 119610	200 U 1,000 U
AptaHotTaq HC	30 U	✓	✓	✓	✓	✗	✗	Aptamer	5 kb	119630HC	2,000 U
SmartHotTaq	5 U	✓	✓	✓	✓	✗	✗	AB	7 kb	102020 102022	200 U 1,000 U
Tth	5 U	✓	✓	✓	✓	✗	✓	✗	4 kb	104005 104025	500 U 2,500 U
DF-Pfu	5 U	✓	✓	✓	✓	✗	✗	✗	2 kb	108105 108125	500 U 2,500 U
Reverse	200 U	✓	(✓)	✓	✗	✗	✓	✗	7 kb	105150	50,000 U
Reverse GF	200 U	✓	(✓)	✓	✗	✗	✓	✗	7 kb	105250GF	100,000 U
Klen SNPase	25 U	✓	✓	✓	✗	✗	✗	✗	0.5 kb	108205 108225	500 U 2,500 U
SNPase HotStart	20 U	✓	✓	✓	✗	✗	✗	AB	0.5 kb	108210 108250	500 U 2,500 U
SD Polymerase	10 U	✓	(✓)	✓	✗	✓	✗	✗	30 kb	108702 108710	200 U 1,000 U
SD Polymerase	50 U	✓	(✓)	✓	✗	✓	✗	✗	30 kb	108800 108850	1,000 U 5,000 U
SD Polymerase HotStart	10 U	✓	(✓)	✓	✗	✓	✗	AB	30 kb	108902 108910	200 U 1,000 U

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