

# Novel DNA Polymerase Increases Efficiency of Multiple PCR, Isothermal Amplification Methods

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NEW YORK (GenomeWeb) — A team led by scientists from the Russian Academy of Sciences in Moscow has published research demonstrating how a recently commercialized novel DNA polymerase can improve the results of a wide variety of PCR and isothermal amplification methods. According to the researchers, the molecule, called SD Polymerase and sold by German molecular biology firm Bioron, has a high degree of thermostability and strong strand displacement activity, and as such outperformed commonly used polymerases in techniques such as PCR, long-range PCR, loop-mediated isothermal amplification (LAMP), and polymerase chain displacement reaction (PCDR).

The research team, which also included scientists from Russian biotech firms Evrogen and Syntol, as well as from Stockholm University in Sweden, published its findings this month in [Biotechniques](#).

Generally, DNA polymerases are either suitable for PCR amplification or isothermal amplification, but not both. While isothermal methods such as LAMP are promising for point-of-care clinical diagnostic applications because they don't require expensive thermal cycling equipment, it has been shown that LAMP's sensitivity and efficiency are enhanced by heat pre-denaturation of the DNA template.

The polymerases most commonly used in LAMP, such as Bst DNA polymerase and its derivatives, have strong strand displacement activity — a requirement for isothermal techniques. However, these polymerases are generally unstable above 70° C, making them unsuitable for a pre-denaturation step that would normally take place around 90° C.

Meantime, enzymes used in PCR, such as Taq, are highly thermostable but do not exhibit strong strand displacement, making them unsuitable for isothermal methods.

The new SD polymerase is a Taq DNA polymerase mutant that was "created as a result of a successful attempt to combine the thermostability of the enzyme (as for Taq DNA polymerase) and strong strand displacement activity of other enzymes (as for Bst DNA polymerase or Phi29 DNA polymerase)," Anke Fenn, a sales manager for Ludwigshafen, Germany-based Bioron, told *PCR Insider* in an email this week. "SD polymerase seems to be the only ... enzyme [suitable for PCR] which possesses strong strand-displacement activity."

Konstantin Ignatov, corresponding author on the *Biotechniques* study and a researcher at the Vavilov Institute of General Genetics at RAS, said in an email to *PCR Insider* that SD polymerase's particular combination of properties "makes the enzyme unique and would potentially give SD polymerase an advantage over Taq polymerase."

To demonstrate this, Ignatov and colleagues compared the performance of Taq and Bst polymerases to SD polymerase in PCR, including amplification of GC-rich and complex secondary structures,

long-range PCR, LAMP, and PCDR, a recently developed method that was presented in [another \*Biotechniques\* paper](#) published in February 2013.

First, the researchers compared the properties of SD polymerase to Bst polymerase in LAMP, carrying out reactions at 63° C with or without an initial two-minute, 92° C denaturation step on control template DNA. The polymerases demonstrated very similar polymerase activity and strand displacement activity, but only SD polymerase was able to carry out the amplification with the pre-denaturation step.

Next, Ignatov and colleagues synthesized a 135-bp artificial DNA template containing a hairpin structure with 30 complementary base pairs, and compared SD and Taq DNA polymerase efficacy in PCR amplification. The researchers found that only the SD polymerase was able to efficiently amplify the hairpin structure. Further, when used to PCR amplify a 1.3-kb fragment of the *Mycobacterium tuberculosis* genome, SD polymerase provided a higher yield of PCR product than Taq polymerase. The researchers reported that this increased efficiency held true when comparing SD polymerase to Taq polymerases from a number of different commercial suppliers.

The researchers then evaluated the performance of SD polymerase in long-range PCR, a technique that specializes in amplifying long DNA fragments — generally 8 kb or more. However, it is known that the longer the amplified sequence is, the less efficient the PCR reaction is, and conventional polymerases such as Taq and Tth are unsuitable for the method.

Because SD polymerase's strand displacement activity increased the efficiency of PCR, the researchers hypothesized it would do the same for long-range PCR — a hypothesis they vetted by successfully amplifying a 17.5-kb DNA fragment from human genomic DNA, without the addition of any other conventional polymerases.

Finally, the researchers compared SD and Taq polymerases in PCDR, a method of sequence-specific amplification that requires heat denaturation of the DNA, like in conventional PCR, as well as the strand displacement activity of DNA polymerase, as in LAMP. This method also requires the use of at least four primers — outer and inner primers — which enables increased template amplification per cycle compared with standard two-primer PCR.

Using a murine cDNA library as a template and primers for murine G3PDH cDNA, the researchers performed PCDR with both four-primer and six-primer assays, and compared it to standard PCR with two primers. When Taq polymerase was used in the experiments, the levels of PCR and PCDR products were essentially the same. However, when SD polymerase was used, it generated much higher products levels in the PCDR amplification.

In fact, the PCDR method, and not necessarily standard PCR or LAMP, might be the sweet spot for SD polymerase in terms of commercial potential. This is despite the fact that the SD polymerase outperformed conventional polymerases in several amplification methods.

According to Bioron's Fenn, SD polymerase "potentially can be used in the very effective and sensitive technique PCDR, in [long-range] PCR with very low-copy-number templates, [and] in PCR with difficult templates. Probably this enzyme can replace Bst DNA polymerase in some

applications [like] LAMP or [next-generation sequencing]-related techniques." However, Fenn noted that Bioron has not yet studied the various applications of the enzyme in great detail.

Ignatov echoed some of Fenn's comments, noting that he "does not expect that the SD polymerase and other similar enzymes would make strong competitors to Taq in PCR, since Taq has been present on the market for so long, and users would be hesitant to substitute such an established and reliable product as Taq for something they are not familiar with. The same argument works for the case of Bst polymerase in LAMP. Moreover, all current protocols for LAMP are optimized for using Bst or similar enzymes."

Instead, Ignatov said, as new methods of DNA amplification that combine PCR and isothermal techniques evolve — like PCDR — "the interest in SD polymerase will rise, and therein lies its commercial potential. The absence of such enzymes as SD polymerase on the market hampered the development of [these] methods ... [and PCDR] will hugely benefit from the use of SD polymerase, so the development of more methods will hopefully follow."

Patent applications surrounding the PCDR method are owned by [GeneFirst](#), an Oxfordshire, UK-based molecular diagnostics firm that has developed and patented several nucleic acid amplification and sequencing technologies.

Guoliang Fu, corresponding author on the *Biotechniques* paper from February outlining the PCDR method and also CSO for GeneFirst, told *PCR Insider* this week that his company has performed experiments using the SD polymerase with the PCDR method, and that the results were "very good." He also noted that GeneFirst is interested in exploring additional applications combining the two technologies.

Bioron owns patent applications filed around the SD polymerase, and currently holds the commercial rights to the molecule. The polymerase is also available for purchase on [Bioron's website](#).

Fenn said that Bioron recently received a collaborative grant from the Germany Ministry of Science to work with Erlangen University to develop a sensitive test to analyze mutations in circulating DNA. "This grant presumes experiments with SD DNA polymerase as a possible enzyme for the new highly sensitive assay," she added.

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