Polymerase Strand Displacement Reaction with SD DNA Polymerase – New Alternative to PCR

146

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Introduction:

Polymerase Chain Displacement Reaction (PCDR) was described in 2013 by Harris et al. It is a new technique combining thermocycling (like PCR) and isothermal (like SDA or LAMP) approaches to DNA amplification. For PCDR performing, two or more pairs of nested primers are used with a thermostable DNA polymerase that has strong strand displacement activity. When extension occurs from the outer primer, it displaces the extension strand produced from the inner primer by strand displacement activity.

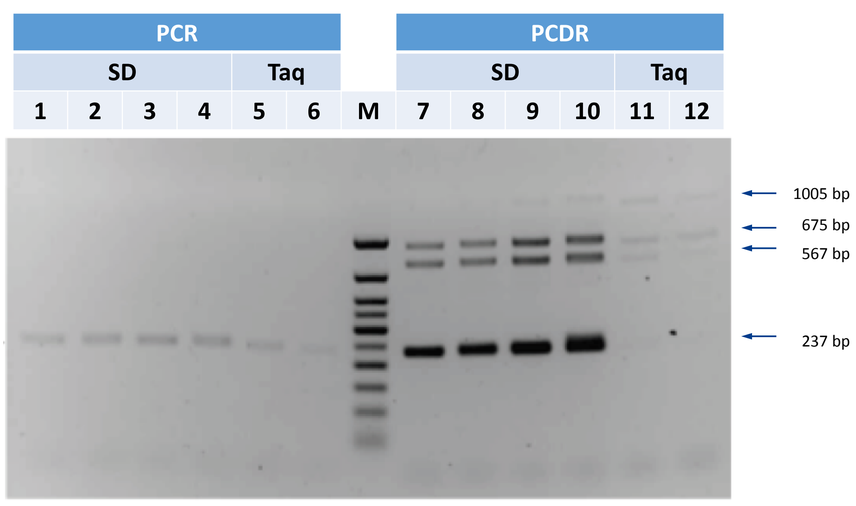
In contrast to PCR, new technique allows to synthesize several new DNA copies from the single template DNA molecule in each amplification cycle. Thus, PCDR is much faster and more effective than PCR. The assays based on this reaction are more sensitive. Besides, due to the use of several nested pairs of primers the specificity of the reaction is increased.

Till recently the main disadvantage of PCDR was the lack of commercially available thermostable DNA polymerase which is suitable for PCR and possesses strong strand-displacement activity. SD DNA polymerase (BIORON GmbH Patent US 9,896,671) allows to fill-in this gap and PCDR started to be a reality.

SD polymerase has been shown to direct effectively PCDR in several diagnostics application (Dengue virus detection, plant pathogen HLB detection). Moreover, recently the PCDR on the basis of SD polymerase has been developed further to so-called tailed tandem repeat PCDR (TTR-PCDR) which looks quite universal and can be applied for the fast and convenient detection of pathogens (J. Wang, et al. (2018) Anal. Methods (35), DOI:10.1039/C8AY01625A).

Thus, PCDR with SD polymerase is a promising method which outperforms PCR in speed and efficiency of amplification and can be used in laboratory practice for research and diagnostics purposes.

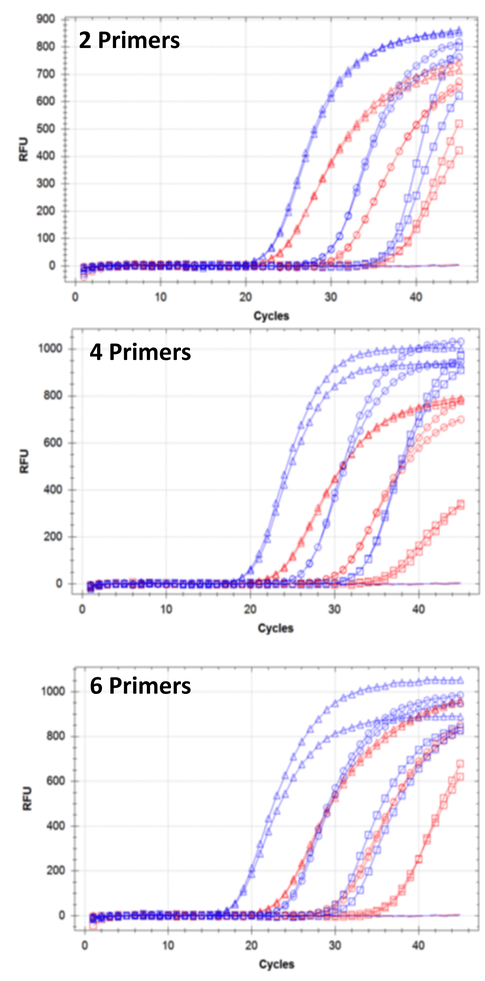
Comparison of PCR and PCDR fragments



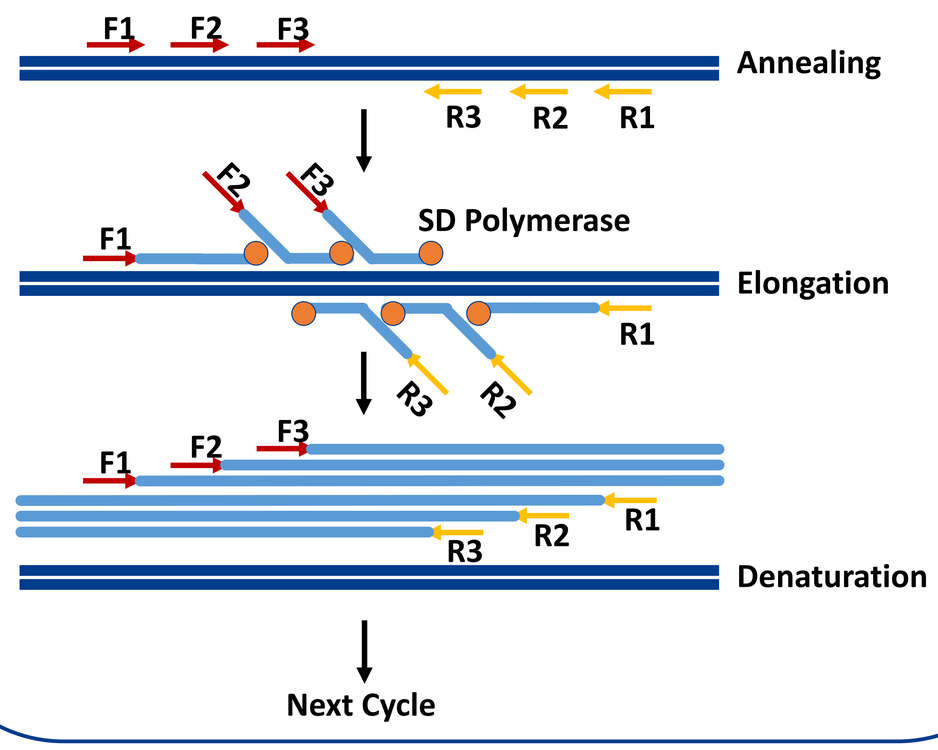
SD DNA polymerase versus *Taq* DNA polymerase in polymerase chain displacement reaction (PCDR). PCR **(lanes 1–6)** and PCDR **(lanes 7–12)** amplifications were carried out with 5, 10, 20, or 40 U of SD polymerase (lanes 1–4 and 7–10) or with 5 and 10 U of *GoTaq* polymerase (lanes 5, 6 and 11, 12). PCR assays contained two primers: F3 and R3. PCDR assays contained four primers: F1, F3, R3, and R1. Amplicon positions are indicated by arrows. **M:** 50 bp ladder.

Comparison of SD and Taq DNA polymerases in qPCR and PCDR

Use of multiple primer pairs in one PCR reaction increase amplification rate and therefore can reduce the Ct value in comparison to a regular PCR. The increase in amplification rate is depending on the number of Primer pairs.
Amplification of murine G3PDH cDNA sequence was carried out with 5 U of SD Polymerase HotStart (blue curves) or GoTaq HotStart (red curves) DNA polymerase. 10 pg (Δ,Δ), 0.1 pg (O,O), 0.001 pg (□, □) of cDNA were used as template.



PCDR schematic first Cycle



Conclusion

The here presented method first published by Harris et al., 2013 was lacking an available enzyme capable of doing strand displacement and possessing heat stability. The here presented SD Polymerase is heat stable until 94°C and is lacking the 5‘-3‘-Exonuclase activity.

The presented method can be used to speed up PCR runs and lower the limit of detection. Recent studies (Lou et al., 2018 and Wang et al., 2018) revealed also the usability of SD Polymerase in combination with Tandem repeat PCR based on the same amplification principle.

When using more unspecific primers the amplification of the whole genome is possible (Blagodatskikh et al.;2017).

References:
Harris et al., 2013; Polymerase Chain Displacement Reaction; Biotechniques, 54(2):93-7
Ignatov et al., 2018; Fragmentation Through Polymerization (FTP): A New Method to Fragment DNA for Next-Generation Sequencing; bioRxiv, 2018
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Blagodatskikh et al., 2017; Improved DOP-PCR (iDOP-PCR): A robust and simple WGA method for efficient amplification of low copy number genomic DNA; PLoS ONE 12(9)
Lou et al., 2018; Development of a Tandem Repeat-Based Polymerase Chain Displacement Reaction Method for Highly Sensitive Detection of ‘Candidatus Liberibacter asiaticus’ Phytopathology, Vol. 108, No. 2, P 292-298