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Meet the Inventors

[Beese, Lorena](#)
[Shi, Yuqian "Yuqian"](#)
[Wang, You "You"](#)

Department

Biochemistry

Publication(s)

External Link(s)

- [From the Lab of Lorena Beese](#)

All-in-one DNA polymerases for hot start PCR

Unmet Need

Polymerase chain reaction (PCR) is among the most common reactions conducted in life sciences, medical, and clinical laboratories. This technique is used for numerous applications such as clinical diagnoses, biological technologies, molecular cloning, and gene synthesis. Current COVID-19 coronavirus testing kits also rely on these techniques. Global PCR, isothermal amplification, DNA sequencing, and reverse transcription markets are estimated to have a net value of \$46.6 billion in 2025. A common problem in all these techniques are artifacts from nonspecific products of DNA polymerases. An issue that leads to low yield of the target product, ambiguous results, and restrictions on the number of samples that can be processed. These artifacts are particularly troublesome when accurate and specific results are essential for diagnosis and decision making, such as when flawed COVID-19 test kits were recalled by Centers for Disease Control (CDC) in February 2020. There is a need for an all-in-one hot start DNA polymerase that eliminates nonspecific activities at room temperature and becomes functional DNA polymerases at working temperature without additional steps or external reagents.

Technology

Duke inventors have developed a novel auto hot start PCR reagent, and method, that increases accuracy while remaining easy to use. This is intended to be used in any kits or reactions that require DNA polymerases to amplify DNA. Specifically, an intervening protein (intein) was fused to DNA polymerase to stably inhibit the polymerase activity at low temperatures. At high temperatures the intein automatically splices and releases the active DNA polymerase to catalyze DNA synthesis. This has been demonstrated in successful DNA amplification using a wide range of PCR conditions and additives.

Other Applications

This technology could also be used in any application that requires DNA polymerases for DNA synthesis and amplification, such as isothermal amplification, DNA sequencing, and reverse transcription.

Advantages

- Hot start function without any external agents
- Inhibits nonspecific reactions for up to 24 hours at room temperature
- Automatic hot start without any additional protocol
- Stable and easy to store

