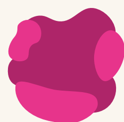
MERCK

What Goes into PCR?

PCR has a lot of moving parts, each with a specific role and purpose. Understanding how these parts contribute to the PCR reaction and modulate each other is instrumental to successful PCR optimization.

A DNA Polymerase

DNA polymerases replicate DNA sequences during the elongation phase, so their function is pivotal to PCR success. Different polymerases have their own incorporation speeds, maximum amplicon lengths, and error rates.



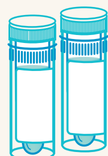
Picking a Polymerase

- ***Taq:*** Originally isolated from *Thermus aquaticus*, *Taq* is widely used for routine PCR processes. It features relatively high thermostability and quick processing speed,[1] but possesses low replication fidelity (1-20 errors per 10,000 base pairs[2]), making it unsuitable for long sequence amplification.
- ***Pfu:*** Originally isolated from *Pyrococcus furiosus*, *Pfu* is highly thermostable. *Pfu* also has proofreading capabilities, giving it extremely high replication fidelity (1-2 errors per million base pairs[1]). However, *Pfu's* drawback lies in its slower processing speed, which is roughly 2-4 times slower than *Taq*.[3]
- **KOD:** A recombinant polymerase derived from *Thermococcus kodakarensis* KOD1 strain, thermostable KOD possesses the fidelity of *Pfu* with much higher processing speeds.[4] However, KOD encounters difficulties with amplicons greater than 5 kb, resulting in lower yields.[5]

Today, laboratory modified and engineered DNA polymerases generally have much higher functionality than their endogenous precursors. A variety of specialized polymerases are available for specific research needs, and specialized applications may necessitate high-performance DNA polymerases.

B Buffers

Buffers ensure an environment that promotes DNA polymerase activity, with specific compositions varying depending on DNA polymerase selection. Tweaking buffer composition by adding chemicals or co-solvents (such as bovine serum albumin) may help when facing difficult samples such as GC-rich templates.

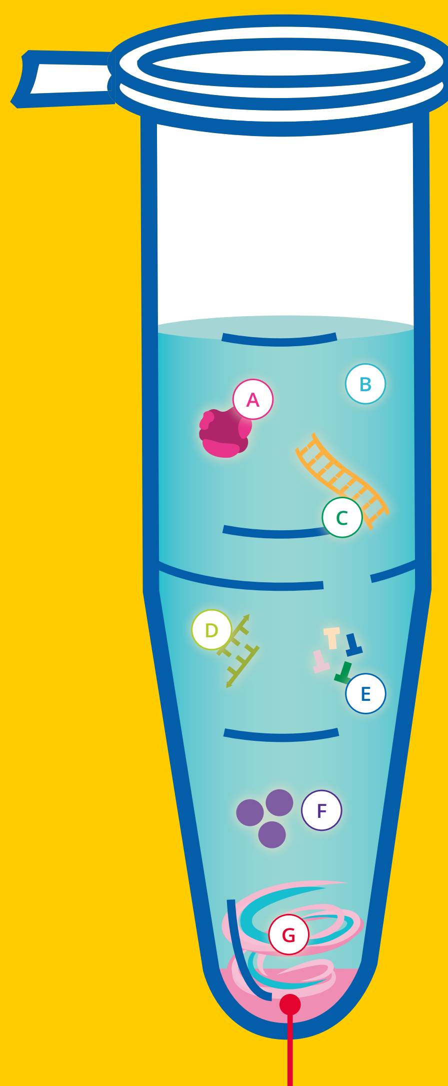


C Template DNA

Template DNA quality and quantity will determine the overall amplification output. DNA fragmentation, excess starting material, and the presence of undesired DNA fractions can all cause non-specific amplification, while low template DNA concentrations result in limited output.

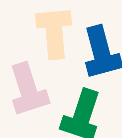
D Primers

Primers guide DNA synthesis, ideally ensuring that only the sequence of interest is amplified. Primer binding sites should be unique; primer thermokinetic properties should roughly match that of the template DNA; and no complementary sequences within a primer or primer pair should exist.



E dNTPs

Free deoxynucleoside triphosphate (dNTP) molecules are the building blocks for DNA amplification. However, excess dNTPs can reduce sequence replication fidelity, especially when using non-proofreading DNA polymerases.

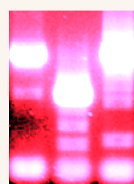


F Magnesium Ions

Magnesium ions are cofactors for DNA polymerase, enabling dNTP incorporation during elongation. Mg2+ ions also facilitate primer-DNA template complexing. Elevated Mg2+ concentrations increase DNA polymerase activity, but this can lead to non-specific PCR products.

G Contaminants

Template DNA quality and quantity will determine the overall amplification output. DNA fragmentation, excess starting material, and the presence of undesired DNA fractions such as genomic DNA can all cause non-specific amplification, while low template DNA concentrations result in limited output.



Identifying Contaminants

- **Extraction reagents:** Common reagents used during lysis and extraction such as phenol and SDS inhibit PCR activity by degrading DNA polymerase enzymes. Phenol can also cross-link to nucleic acids, affecting isolation.[6]
- **Proteinases:** Left over from tissue digestions, proteinases target DNA polymerases. Protease inhibitors or heating the sample prior to DNA polymerase addition can inactivate residual proteinases.
- **Calcium:** Calcium ions inhibit DNA polymerase activity by competing with magnesium ions for binding. Increasing magnesium ion concentrations or adding calcium chelators may remedy this situation.
- **EDTA:** Since it inhibits DNAses, EDTA is often found in storage buffers for nucleic acids. However, it also chelates magnesium ions, thereby reducing DNA polymerase functionality.
- **Chaotropic salts:** Employed to facilitate DNA adsorption to extraction columns, chaotropic salts disrupt protein structures by disrupting hydrogen bonds, and thereby impede enzymatic function.

References

1. F.C. Lawyer et al., "High-level expression, purification, and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5′ to 3′ exonuclease activity," *PCR Methods Appl*, 2(4):275-87, 1993.
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5. M. Nishioka et al., "Long and accurate PCR with a mixture of KOD DNA polymerase and its exonuclease deficient mutant enzyme," *J Biotechnol*, 88(2):141-49, 2001.
6. C. Schrader et al., "PCR inhibitors - occurrence, properties and removal," *J Appl Microbiol*, 113(5):1014-26, 2012.

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