Tips and tricks for PCR success

The polymerase chain reaction, or PCR, allows replication of a single DNA molecule into millions of copies in minutes. It's one of the most well-known techniques in molecular biology with applications ranging from basic research to disease diagnostics, agricultural testing, forensic analysis, and more.

PCR best practices

Set yourself up for success with these expert tips for each stage of the PCR workflow.

Step 1—denaturation

94–98°C

is the typical denaturation temperature, though complex samples may need a higher temperature and/or a longer incubation time (see reaction component considerations).

Having trouble with optimization outside of standard temperature and/or time of incubation ranges? Try using a hot-start PCR protocol with a thermostable enzyme, like Invitrogen™ Platinum™ II Taq Hot-Start DNA Polymerase, or adding extra enzymes or DNA polymerase.

Step 2—annealing

3–5°C

lower than the lowest T_m of your primers is a good rule of thumb for optimal annealing temperature.

The reaction buffer of some DNA polymerases is designed with isostabilizing components that can improve yield and enhance specificity.

A buffer can also enable PCR primer–template annealing at a universal temperature, e.g.,

60°C

Step 3—extension

72°C

—most common extension temperature

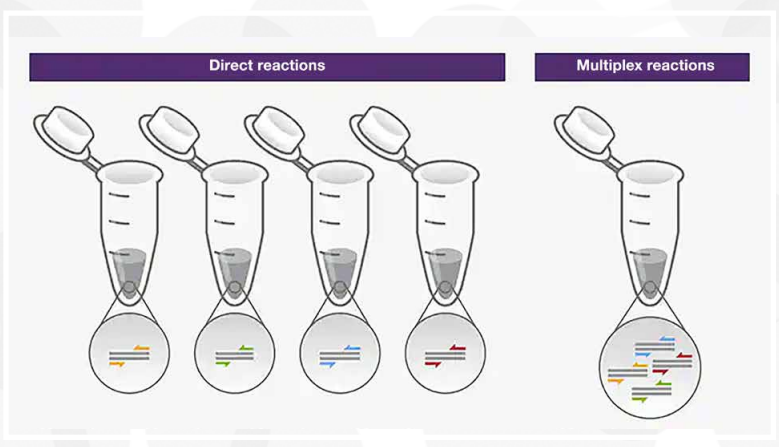
1 min/kb

—recommended extension time

<45 cycles

—to avoid nonspecific bands and accumulation of byproducts

Pro tip — Direct PCR vs. multiplex PCR

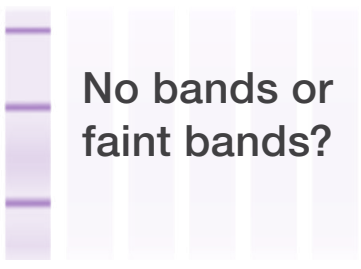


Direct PCR amplifies target DNA directly from samples without nucleic acid isolation, vastly simplifying workflow and preventing DNA loss during purification.

Multiplex PCR allows concurrent amplification of different targets in a single PCR tube to save time, reagents, and samples, and to make simultaneous comparison of multiple amplicons possible.

Learn more about modern PCR methods and the unique benefits of each.

Troubleshooting common problems



No bands or faint bands?

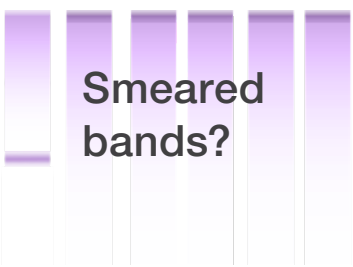
Try this

- Increase denaturing temperature or lower annealing temperature
- Increase cycle count
- Use purification kits to isolate template DNA

Non specific bands?

Try this

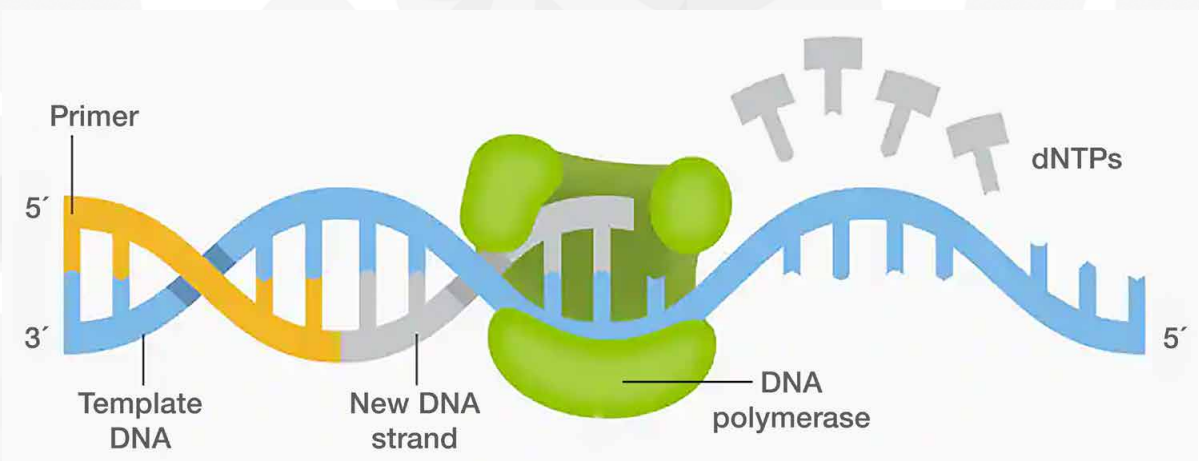
- Reduce cycle count, annealing and/or extension time, amount of template DNA, or [Mg^{2+}]
- Use hot-start DNA polymerases to prevent degradation of primers by the 3′ to 5′ exonuclease activity of proofreading DNA polymerases



Smeared bands?

Try this

- Minimize shearing and nicking of DNA during isolation
- Store DNA in TE buffer (pH 8.0) or molecular-grade water to prevent degradation by nucleases



Did you know?

The oldest DNA ever sequenced came from the permafrost-preserved molar of a long-extinct mammoth that roamed Siberia around 1.2 million years ago. Modern PCR with modifications can help recover even tiny amounts of ancient DNA.

Additional PCR tools and resources

Check out our comprehensive PCR troubleshooting guide for even more solutions.

For additional quick guides with tips and tricks to popular protocols, subscribe to Connect to Science.

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