Qualitative polymerase chain reactions

Reviewed: Jun 8, 2025

Polymerase chain reaction (PCR) can reveal the presence of specific DNA sequences or structural variations such as insertions and deletions when combined with gel-based detection of the resulting amplicon. Even single-nucleotide polymorphisms (SNPs) can be detected in a qualitative manner with allele-specific primer design or restriction fragment analysis. This approach is routinely used to screen bacterial or plasmid clones, or to distinguish known genetic variants in research or diagnostic development contexts.

Two common protocols are described: a Taq polymerase–based method for rapid, low-cost colony screening (*Alternative A*), and a high-fidelity protocol with lower error rates and improved specificity, particularly for GC-rich or long templates. The high-fidelity approach is also compatible with downstream amplicon sequencing (*Alternative B*). Note that relative band intensities cannot be interpreted quantitatively due to variability in template input, enzyme activity, and amplification efficiency.

Buffers and cycling conditions may require optimization depending on the application.

This is a bench card. Full protocol available online.

Procedures

›› ***Primer design***

(1.) Design primers to flank the region of interest, ideally producing an amplicon between 100–1 500 bp.

- Avoid placing a T at the 3 end if possible; it has the weakest stability during extension.
- Avoid long stretches of G or C at the 3 end.
- Aim for a melting temperature of 55–65 °C, with both primers within 2 °C of each other.
- Check for the absence of significant primer-dimer formation or strong hairpin structures.

(2.) *Optional:* For SNP detection, design allele-specific primers with the variable base positioned at the 3 end of the forward or reverse primer.

(3.) *Optional:* For multiplex PCR, design multiple primer pairs with similar annealing temperatures. Avoid cross-reactivity or overlapping product sizes.

Critical: Always validate each primer pair in single-plex reactions before combining them into a multiplex reaction.

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A › *Colony PCR using Taq DNA polymerase*

☐ R0193 10 × Long incubation buffer, 45 mL

(1.) Pick a small amount of a bacterial (or yeast) colony using a sterile pipette tip or toothpick and resuspend it in 40 µL aqueous buffer. Alternatively, dilute a saturated culture about 5- to 10-fold.

Using the same tip, transfer a small amount of the cell suspension into the PCR reaction mix.

Critical: Avoid transferring excess amounts of biomass. Overloading the reaction with DNA may inhibit polymerase activity.

(2.) Prepare 10 µL PCR master mix for each sample:

Ingredient	Stock	Final	For 1 sample	For 8 samples
Long incubation buffer	10 ×	1 ×	1.0 µL	8.0 µL
Betaine	5 M	500 mM	1.0 µL	8.0 µL
$MgCl_2$	50 mM	2.5 mM	0.5 µL	4.0 µL
Forward primer	10 µM	500 nM	0.5 µL	2.0 µL
Reverse primer	10 µM	500 nM	0.5 µL	2.0 µL
dNTP mix	10 mM	400 nM	0.4 µL	3.2 µL
Taq DNA polymerase	5 U/µL	0.1 U/µL	0.2 µL	1.2 µL
Water, reagent-grade			5.0 µL	40.0 µL
Cell suspension, dilute			1.0 µL	8.0 µL

Critical: Taq DNA polymerase lacks hot-start inhibition and may begin extending misprimed or partially annealed products if the reaction is assembled at room temperature. Prepare master mixes on ice and begin thermocycling promptly after setup.

(3.) *Critical:* Include a background or parental strain as a negative control in each PCR run.

(4.) Spin down the reactions briefly and place directly in the thermocycler. Run the following program:

Cycles	Temperature	Duration	Name	Remarks
1 ×	95 °C	3:00 min	Initial denaturation	Set to 7:00 min to lyse yeast or thick bacterial cell walls
20–25 ×	95 °C	0:30 min	Denaturation	
	50–60 °C	0:30 min	Annealing	Set to 5 °C below the primer melting temperature
	72 °C	1:00 min/kbp	Extension	Adjust for target length
1 ×	72 °C	5:00 min	Final extension	
1 ×	12 °C	∞	Hold	

(5.) Proceed with analysis of PCR products as described below.

B › *High-fidelity PCR for sequence validation*

□ R0194 5 × High-fidelity PCR buffer, 40 mL

(1.) Prepare 10 µL PCR master mix for each sample:

Ingredient	Stock	Final	For 1 sample	For 8 samples
High-fidelity PCR buffer	5 ×	1 ×	2.0 µL	16.0 µL
Betaine *(optional)*	5 M	500 mM	1.0 µL	8.0 µL
$MgCl_2$	50 mM	2.5 mM	0.5 µL	4.0 µL
Forward primer	10 µM	500 nM	0.5 µL	2.0 µL
Reverse primer	10 µM	500 nM	0.5 µL	2.0 µL
dNTP mix	10 mM	1.0 mM	1.0 µL	8.0 µL
DNA polymerase, diluted	0.04 U/µL	0.008 U/µL	0.2 µL	1.6 µL
Water, reagent-grade			4.0 µL	32.0 µL
Template	5 ng/µL	1 ng/µL	0.2 µL	1.6 µL

Critical: Avoid exceeding 2 ng of a 5 kbp plasmid per reaction. Excess template can inhibit the polymerase and promote nonspecific amplification. High-fidelity enzymes are effective with as little as 1 pg/µL DNA.

(2.) *Critical:* Include a reaction without the template as negative control in each PCR run.

(3.) Spin down the reactions briefly and place in the thermocycler. Run the following program:

Cycles	Temperature	Duration	Name	Remarks
1 ×	97 °C	1:00 min	Initial denaturation	Set to 2:00 min for GC-rich templates
25–30 ×	97 °C	0:30 min	Denaturation	
	65–68 °C	0:30 min	Annealing	Set to the primer melting temperature
	72 °C	0:15 min/kbp	Extension	Adjust for target length
1 ×	72 °C	5:00 min	Final extension	
1 ×	12 °C	∞	Hold	

Critical: Consult the manufacturer's recommended cycling conditions.

(4.) Proceed with analysis of PCR products as described below.

(5.) If the amplicon will be used for restriction-ligation cloning SOP 0033, purify over a silica column SOP 0021 or by gel extraction SOP 0022 to remove primers, dNTPs, and polymerase before proceeding with restriction digest.

List of references

Recipe (available online) Troubleshooting (available online) Notes (available online)