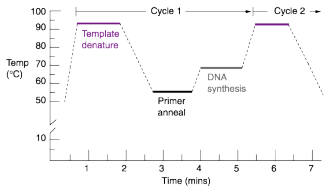
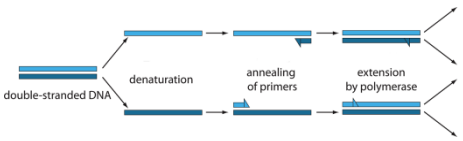
User's Selection Guide

Guidelines for PCR Optimization

PCR (Polymerase Chain Reaction)

PCR is a powerful tool to amplify DNA by DNA polymerase in the presence of template, primers, and building substrates of polynucleotide. The PCR is performed in a thermocycler which repeats incubation steps at different temperatures.





1. Denaturation Step: Denaturation step of double-stranded template DNA by heating at 94-97°C.
2. Annealing Step: Primer pairing step to the complementary template DNA at low temperature (45-68°C).
3. Extension Step: Polymerization step by a thermostable DNA polymerase at an intermediate temperature (68-75°C).

PCR Primer Design

1. PCR primers generally range in length from 18–24 bases.
2. Primers ideally will have a GC-content of 40–60% (T_m = 50~75°C).
3. To minimize the primer-dimer formation, avoid intra- or intermolecular complementary sequences in primers.
4. T_m of the two primers will be within 5°C so that the primers anneal efficiently at the same temperature.
5. Avoid three G or C residues in a row near the 3′-end of the primer to minimize non-specific primer annealing.

You can estimate the T_m of your primers by following formula

$T_m = 81.5 + 16.6 \times (\log_{10}[Na^+]) + 0.41 \times (\%G+C) - 675/n$

$[Na^+]$ = molar concentration salt in the PCR reaction

n = number of bases

The final concentration of the primers in the PCR reaction must be optimized. 5 pmol of each primer in a 20 µl reaction is a good starting point. Higher concentration of primers may increase primer-dimer production.

Cycling Parameters

The most important parameters to be considered are annealing temperature, extension time and cycle number. The annealing temperature is dependent on your primers' T_m. For the most PCR reactions, annealing at 2 to 4°C below primers' calculated T_m is working well. But, we recommend to experimentally determine the optimum annealing temperature by varying temperatures over a range of 45–68°C. The higher temperature minimizes non-specific primer annealing, increasing the amount of specific product produced and reducing the amount of primer-dimer formation.

The optimum PCR cycle number is 25–35 cycles. The exact cycle number should be determined by considering final yield of products and production of non-specific bands. Insufficient cycles may result in low product yield, whereas excess cycles may cause unexpected or diffused products.

Template DNA

An adequate amount of template DNA is between 10 and 100 ng for genomic DNA, 1 and 10 ng for plasmid DNA, 1/20 and 1/200 of cDNA synthesized from 1 µg of total RNA for a total reaction mixture of 20 µl. Larger template DNA amounts usually increase the yield of non-specific PCR products and smeared products.

Taq DNA polymerase

Commercially available Taq polymerases should be selected by considering their unique features, such as error rate, extension length, thermostability, production yield, and so on. For a PCR reaction to prepare insert DNA for a cloning, Pfu rather than Taq polymerase should be used because of its high fidelity. But you should keep in mind that usually Pfu generates lower yield rather than Taq polymerase. Usually 1 unit of Taq polymerase is added in the PCR reaction of 20 µl. Higher concentrations of Taq polymerase may cause synthesis of non-specific products and smeared products.

Pipetting errors are the most frequent cause of excessive enzyme levels. Accurate dispensing of submicroliter volumes of enzyme solutions in 50% glycerol is nearly impossible. Making master mixes is the best solution to overcome this problem. The master mixes will increase the initial pipetting volume of reactants and reduce pipetting errors.

dNTPs

Usually 200 µM of each dNTP (dATP, dCTP, dGTP, dTTP) is added in the PCR reaction mixture. These concentrations must be checked as being equal, because inaccuracies will increase the degree of misincorporation. To decrease error rate in the PCR reaction, lower concentrations of dNTP is required.

Taq ENZYMES & PCR PRODUCTS

Magnesium Concentration

Free magnesium ion is a most important factor affecting the activity of Taq polymerase. Without free magnesium in the PCR reaction, Taq polymerase is inactive. Conversely, excess free magnesium reduces enzyme accuracy and may increase the level of non-specific amplification. Usually 1.0-3.0 mM magnesium ion is working well. But you should keep in mind that several factors can affect the amount of free magnesium in the PCR reaction such as chelating agents present in the sample (e.g., EDTA or citrate), dNTPs, template DNA, and proteins.

PCR Additives

Several PCR additives to improve the reliability and yield of conventional PCR reactions are used. 1-5% formamide in the PCR reaction helps to decrease non-specific amplification. 2-10% DMSO is thought to reduce secondary structure and is particularly useful for GC rich templates. As DMSO decreases the melting point of the primers, the annealing temperature must be lowered when high DMSO concentration is used (10% DMSO decreases the annealing temperature by 5.5-6.0˚C). Non-ionic detergents, 0.1-1% Triton X-100, Tween 20 or NP-40 stabilize Taq polymerase and may also suppress the formation of secondary structure.

TMAC is also used to reduce potential DNA-RNA mismatch. The base analogue 7-deaza-2'-deoxyguanosine may facilitate amplification of templates with stable secondary structures when used in place of dGTP. Proteins such as bovine serum albumin, single strand DNA binding protein, T4 gp32 are known to be effective in increase PCR yield.

Betaine is a common PCR additive provided by many suppliers. 0.5-2 M betaine is effective for GC rich templates and reducing the non-specific amplification.

Standard PCR Condition

For a 20 μl PCR reaction

10x buffer	2 μl
25 mM $MgCl_2$	1 - 2 μl (1.25-2.5 mM final)
2.5 mM dNTPs (each)	1.6 μl (200 μM final)
10 pmol/μl primer F	0.5 μl
10 pmol/μl primer R	0.5 μl
DNA template	1-10 ng
Taq polymerase	1 unit

Adjust final volume to 20 μl with PCR grade distilled water

Most 10x PCR buffers supplied in Taq polymerase contains optimized concentrations of magnesium ion as $MgCl_2$ or $MgSO_4$. In this case, you have no need to add Mg ion separately.

Standard PCR Cycling

Initial Denaturation	95°C	3 min	
Denaturation	95°C	30 sec	25-35 cycles
Primer Annealing	Tm-4°C	30 sec	
Extension	72°C	30-60 sec/1 kbp	
Final Extension	72°C	1 - 10 min	

Trouble Shooting for a Commonly Raised Problem

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
   - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragments
2. Smear bands or smeared background
   - Reduce template concentration : High concentration of template can lead to smearing of PCR products Generally, 0.1-10 ng of plasmid DNA and 1-100 ng of genomic DNA are working well
   - Reduce enzyme concentration in the reaction
   - Increase annealing temperature
   - Set up a reaction mix on ice
3. Non-specific bands
   - Increase annealing temperature
   - Consider using PCR additives, like 1-5% DMSO and 0.5-2 M betaine
   - Confirm specificity of your primers
4. Low yield
   - Increase enzyme concentration in the reaction
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added
5. Mutation is found
   - Increase initial template concentration
   - Reduce PCR cycle number
   - Reduce dNTP concentration added in PCR mix

User's Selection Guide

PCR Enzymes

PCR Purpose

- RT-PCR
- SNP analysis
- DDRT-PCR
- Genotyping
- Primer extension
- Sequencing

Primer Design

- Hair-pin formation
- Internal binding
- Tm & △G
- Compatibility

Optimization

- Amplification efficiency
- Specificity
- Mutation rate

Further Study

- Cloning & Expression
- Differential expression analysis
- Library construction
- Probe synthesis

Economical

HiPi Eco
rTaq Plus

High Specific

rTaq Plus HOT
HiPi Plus
HiPi Super
Pfu Super or Plus

High Fidelity

Pfu HOT
Pfu Plus
Pfu Super

Long PCR

HiPi Super
Pfu Super
HiPi v2
HiPi Plus
Pfu Plus

Time-Saving

HiPi Super
rTaq Plus
Pfu Super
HiPi v2

Colony PCR

rTaq Plus
HiPi Eco
HiPi v2

RT-PCR, gPCR

rTaq Plus
rTaq Plus HOT
HiPi v2
Pfu Plus

PCR for a Cloning

Pfu Plus HOT
Pfu Plus
Pfu Super
HiPi Super
HiPi Plus

Point Mutagenesis

Pfu Plus HOT
Pfu Plus
Pfu Super

Multiplex PCR

rTaq Plus HOT
rTaq Plus

Real-Time PCR

rTaq Plus HOT
Pfu HOT

Economical, Convenient, Reproducible

PCR Premix
One-step RT-PCR premix

Taq ENZYMES & PCR PRODUCTS

Taq ENZYMES & PCR PRODUCTS

User's Selection Guide

Properties of PCR Enzymes

rTaq

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★	★★★	★★	6 kbp	3′-dA

Applications : Confirmation PCR like colony PCR, RT-PCR, genomic PCR without considering fidelity

rTaq Plus

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★	★★★★★	★★★★	10 kbp	3′-dA

Applications : Rapid PCR for colony PCR, RT-PCR, genomic PCR without considering fidelity, multiplex PCR

rTaq Plus HOT

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★★★	★	★★★★★★	★★★★	10 kbp	3′-dA

Applications : RT-PCR, genomic PCR, multiplex PCR, real-time PCR with SYBR dye

HiPi

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★★	★★	★★	★	3 kbp	3′-dA

Applications : General RT-PCR, genomic PCR, colony PCR with increased specificity

HiPi V2

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★★	★★	★★★★	★★★★	10 kbp	3′-dA

Applications : General RT-PCR, genomic PCR, colony PCR with increased specificity

HiPi Plus

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★★★	★★★	★★★★	★★	10 kbp	blunt + 3′-dA

Applications : General RT-PCR, genomic PCR, colony PCR with increased specificity

HiPi Super

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★★★	★★★★	★★★★★★	40 kbp	blunt + 3′-dA

Applications : Long PCR at extremely short running time

Pfu

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★★★★★	★	★	6 kbp	blunt

Applications : PCR for a cloning and point mutagenesis with low error rate

Pfu Plus

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★★★★★	★★★★	★★	12 kbp	blunt

Applications : PCR for a cloning and point mutagenesis with low error rate

Pfu Plus HOT

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★★★	★★★★★	★★★★★	★★	12 kbp	blunt

Applications : PCR for a cloning and point mutagenesis with low error rate

Pfu Super

Specificity	Fidelity	Yield	Speed	Max Size	3′end
★★★	★★★★★★	★★	★★★★	15 kbp	blunt

Applications : Long PCR for a cloning and point mutagenesis with extremely low error rate and short running time