Third Edition

October 2010

Taq PCR Handbook

Taq DNA Polymerase
Taq PCR Core Kit
Taq PCR Master Mix Kit

For standard and specialized PCR applications with minimal optimization

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

***Taq* DNA Polymerase Catalog no.**	**(250 units) 201203**	**(1000 units) 201205**	**(5000 units) 201207**	**(25000 units) 201209**
Taq DNA Polymerase	250 units	4 x 250 units	20 x 250 units	100 x 250 units
QIAGEN® PCR Buffer,* 10x	1.2 ml	4 x 1.2 ml	20 x 1.2 ml	100 x 1.2 ml
CoralLoad PCR Buffer,* 10x	1.2 ml	4 x 1.2 ml	20 x 1.2 ml	100 x 1.2 ml
Q-Solution, 5x	2.0 ml	4 x 2.0 ml	20 x 2.0 ml	100 x 2.0 ml
$MgCl_2$, 25 mM	1.2 ml	4 x 1.2 ml	20 x 1.2 ml	100 x 1.2 ml
Handbook	1	1	1	1

***Taq* PCR Core Kit Catalog no.**	**(250 units) 201223**	**(1000 units) 201225**
Taq DNA Polymerase	250 units	4 x 250 units
QIAGEN PCR Buffer,* 10x	1.2 ml	4 x 1.2 ml
CoralLoad PCR Buffer,* 10x	1.2 ml	4 x 1.2 ml
Q-Solution, 5x	2.0 ml	4 x 2.0 ml
$MgCl_2$, 25 mM	1.2 ml	4 x 1.2 ml
dNTP Mix, 10 mM each	200 µl	4 x 200 µl
Handbook	1	1

***Taq* PCR Master Mix Kit Catalog no.**	**(250 units) 201443**	**(1000 units) 201445**
Taq PCR Master Mix†	3 x 1.7 ml	12 x 1.7 ml
RNase-Free water	3 x 1.7 ml	12 x 1.7 ml
Handbook	1	1

* Contains 15 mM $MgCl_2$.

† Contains *Taq* DNA Polymerase, 2x QIAGEN PCR Buffer, 3 mM $MgCl_2$, and 400 µM of each dNTP.

Shipping and Storage

Taq DNA Polymerase and the *Taq* PCR Core Kit are shipped on dry ice but retain full activity at room temperature (15–25°C) for 2 weeks.

The *Taq* PCR Master Mix Kit is shipped on dry ice but retains full activity at room temperature (15–25°C) for 3 days.

Taq DNA Polymerase, the *Taq* PCR Core Kit, and the *Taq* PCR Master Mix Kit, including buffers and reagents, should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance. The *Taq* PCR Master Mix Kit can also be stored at 2–8°C for up to 2 months.

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding *Taq* DNA Polymerase, the *Taq* PCR Core Kit, the *Taq* PCR Master Mix Kit, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Product Use Limitations

Taq DNA Polymerase, the *Taq* PCR Core Kit, and the *Taq* PCR Master Mix Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Specifications

Enzyme:

QIAGEN *Taq* DNA Polymerase is a recombinant 94 kDa DNA polymerase (deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7), originally isolated from *Thermus aquaticus*, and expressed in *E. coli*.

One unit of *Taq* DNA Polymerase is defined as the amount of enzyme that will incorporate 10 nmol of dNTPs into acid-insoluble material within 30 min at 72°C, under the assay conditions described in the section Quality Control on the following page.

Concentration:	5 units/µl
Substrate analogs:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	2–4 kb/min at 72°C
Half-life:	10 min at 97°C; 60 min at 94°C
5'–3' exonuclease activity:	Yes
Extra A addition:	Yes
3'–5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No
Storage and dilution buffer:	20 mM Tris·Cl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% (v/v) Nonidet® P-40, 0.5% (v/v) Tween® 20, 50% (v/v) glycerol; pH 8.0 (20°C)

Buffers and Reagents:

QIAGEN PCR Buffer:	10x concentrated. Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7 (20°C)
CoralLoad PCR Buffer:	10x concentrated. Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, gel loading reagent, orange dye, red dye; pH 8.7 (20°C).
Q-Solution:	5x concentrated
$MgCl_2$ solution:	25 mM
dNTP Mix:	10 mM each of dATP, dCTP, dGTP, and dTTP; ultrapure quality
RNase-free water:	Ultrapure quality; PCR-grade

***Taq* PCR Master Mix:**

Taq PCR Master Mix:	2x concentrated. Contains *Taq* DNA Polymerase, QIAGEN PCR Buffer (with 3 mM $MgCl_2$), and 400 µM of each dNTP

Quality Control

Enzyme:	(See quality control label inside kit lid for lot specific values)
Unit assay:	Sonicated herring sperm DNA (12.5 µg) is incubated with 0.01–0.1 units of *Taq* DNA Polymerase in assay buffer (25 mM TAPS [tris-(hydroxymethyl)-methyl-amino-propane-sulfonic acid, sodium salt, pH 9.3 at 20°C], 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 200 µM of each dNTP, 100 µCi [α-^{32}P] dCTP) at 72°C for 30 min. The amount of incorporated dNTPs is determined by precipitation with trichloroacetic acid.
Amplification efficiency assay:	The amplification efficiency is tested in parallel amplification reactions and is indicated under "Amp".
PCR reproducibility assay:	PCR reproducibility is tested in parallel amplification reactions. The reactions must yield a single specific product.
Exonuclease activity assay:	Linearized DNA is incubated with *Taq* DNA Polymerase in QIAGEN PCR Buffer. Exonuclease activity per unit enzyme is indicated under "Exo".
Endonuclease activity assay:	Plasmid DNA is incubated with *Taq* DNA Polymerase in QIAGEN PCR Buffer. Endonuclease activity per unit enzyme is indicated under "Endo".
RNase activity assay:	RNA is incubated with *Taq* DNA Polymerase in QIAGEN PCR Buffer. RNase activity per unit enzyme is indicated under "RNase".
Protease activity assay:	*Taq* DNA Polymerase is incubated in storage buffer. Protease activity per unit enzyme is indicated under "Protease".
Self-priming activity assay:	Assays are performed under standard PCR conditions, without primers, using *Taq* DNA Polymerase and human genomic DNA (purified with the QIAamp® DNA Blood Mini Kit). The absence of PCR product is indicated by "No" under "Self-priming".

Buffers and Reagents:

QIAGEN PCR Buffer, 10x:	Conductivity, pH, sterility, and performance in PCR are tested.
CoralLoad PCR Buffer, 10x:	Conductivity, pH, sterility, dye concentrations, and performance in PCR are tested.
Q-Solution, 5x:	Conductivity, sterility, pH, and performance in PCR are tested.
$MgCl_2$, 25 mM:	Conductivity, pH, sterility, and performance in PCR are tested.
dNTP Mix:	Concentration and purity are verified by UV spectroscopy and HPLC. Performance in PCR is tested.
Distilled water:	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.

***Taq* PCR Master Mix Kit:**

PCR reproducibility assay:	The PCR reproducibility assay reactions are performed in parallel using *Taq* PCR Master Mix and individual reagents of the same lot number as the Master Mix components.

Introduction

QIAGEN *Taq* DNA Polymerase used in combination with QIAGEN PCR Buffer or CoralLoad PCR Buffer provides robust performance for reproducible results in a wide range of PCR applications without the need for time-consuming optimization. QIAGEN PCR buffers have been developed to dramatically reduce the need for optimization of individual primer–template systems, saving time and effort. For maximum convenience, the *Taq* PCR Master Mix Kit includes a ready-to-use premixed solution containing *Taq* DNA Polymerase, PCR buffer, and dNTPs.

QIAGEN *Taq* DNA Polymerase

Taq DNA Polymerase is a high-quality recombinant enzyme produced by QIAGEN and sold under a licensing agreement with Hoffmann-La Roche. This enzyme is suitable for standard and specialized PCR applications such as differential display and PCR-based DNA fingerprinting.

QIAGEN PCR Buffer

The innovative QIAGEN PCR Buffer facilitates the amplification of specific PCR products. During the annealing step of every PCR cycle, the buffer allows a high ratio of specific-to-nonspecific primer binding. Owing to a uniquely balanced combination of KCl and $(NH_4)_2SO_4$, the PCR buffer provides stringent primer-annealing conditions over a wider range of annealing temperatures and Mg^{2+} concentrations than conventional PCR buffers.* Optimization of PCR by varying the annealing temperature or the Mg^{2+} concentration is dramatically reduced and often not required.

CoralLoad PCR Buffer

Taq DNA Polymerase and the *Taq* PCR Core Kit are supplied with new CoralLoad PCR Buffer, which has all of the advantages of QIAGEN PCR Buffer (also supplied) but can also be used to directly load the PCR products onto an agarose gel without the need to add a gel loading buffer. CoralLoad PCR Buffer provides the same high PCR specificity and minimal reaction optimization as conventional QIAGEN PCR Buffer. Additionally, it contains two marker dyes — an orange dye and a red dye — that facilitate estimation of DNA migration distance and optimization of agarose gel run time (Figure 1). CoralLoad dyes do not interfere with most downstream enzymatic applications. However, for reproducible results, purification of PCR products prior to enzymatic manipulation is recommended.

* For further information see our comprehensive brochure, *"Critical success factors and new technologies for PCR and RT-PCR"*. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

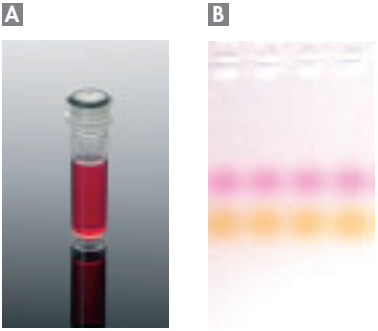


Figure 1. CoralLoad PCR Buffer. CoralLoad PCR Buffer A contains gel-tracking dyes B enabling immediate gel loading of PCR products and easy visualization of DNA migration.

Q-Solution

Taq DNA Polymerase and the *Taq* PCR Core Kit are provided with Q-Solution, an innovative additive that facilitates amplification of difficult templates by modifying the melting behavior of nucleic acids. Q-Solution will often enable or improve suboptimal PCR systems caused by templates that have a high degree of secondary structure or that are GC-rich. Unlike other commonly used additives such as DMSO, Q-Solution is used at just one working concentration. For further information, see the PCR Protocol using *Taq* DNA Polymerase and Q-Solution, page 17.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

All protocols

- dNTPs (e.g., dNTP Mix, PCR Grade, cat. no. 201900)
- Distilled water
- Reaction tubes
- Pipets and pipet tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)
- Primers
  - Primers should be purchased from an established oligonucleotide manufacturer, such as Operon Biotechnologies (www.operon.com). Lyophilized primers should be dissolved in TE to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry. Primer stock solutions should be stored in aliquots at –20°C.

For amplification of long PCR products

- HotStar HiFidelity Polymerase Kit (cat. no. 202602)

Protocol: PCR Using *Taq* DNA Polymerase

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- CoralLoad PCR Buffer is not recommended when downstream applications require fluorescence or absorbance measurements without an intermediate purification of the PCR product (e.g., using QIAquick® PCR Purification Kits or MinElute® PCR Purification Kits).

Things to do before starting

- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C. For convenience, the *Taq* PCR Core Kit includes a ready-to-use ultrapure dNTP Mix, and *Taq* PCR Master Mix provides a premixed solution containing *Taq* DNA Polymerase, QIAGEN PCR Buffer, and dNTPs. High quality, PCR-grade dNTP mix (10 mM) is available separately from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw 10x CoralLoad PCR Buffer or 10x PCR Buffer, dNTP mix, primer solutions, and 25 mM $MgCl_2$ (if required) at room temperature or on ice.**

   Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.

2. **Prepare a master mix according to Table 1, page 14.**

   The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should be included in every experiment. The optimal Mg^{2+} concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the 1x QIAGEN PCR Buffer, will produce satisfactory results. Keep the master mix on ice.

   Note: The Mg^{2+} concentration provided by the supplied PCR or CoralLoad PCR Buffer will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg^{2+} concentration according to Table 2.

Table 1. Reaction Composition Using *Taq* DNA Polymerase

Component	Volume/reaction	Final concentration
Master mix		
10x CoralLoad PCR Buffer* or 10x PCR Buffer*	10 µl	1x
25 mM $MgCl_2$	Variable, see Table 2	See Table 2
dNTP mix (10 mM each)	2 µl	200 µM of each dNTP
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Taq DNA Polymerase	0.5 µl	2.5 units/reaction
Distilled water	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	**100 µl**	**–**

* Contains 15 mM $MgCl_2$.

Note: If smaller reaction volumes are used, please reduce the amount of each component accordingly.

Table 2. Final Mg^{2+} Concentrations

Final Mg^{2+} concentration in reaction (mM):	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Required volume of 25 mM $MgCl_2$ per reaction (µl):	0	2	4	6	8	10	12	14

Note: The optimal Mg^{2+} concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the 1x PCR Buffer and 1x CoralLoad PCR Buffer, will produce satisfactory results.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   Mix gently, for example, by pipetting the master mix up and down a few times. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.

4. **Add template DNA (≤1 µg/reaction) to the individual tubes containing the master mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume (see Appendix E, page 36).

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 100 µl mineral oil.**
6. **Program the thermal cycler according to the manufacturer's instructions.**

   A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Table 3. Thermal Cycler Conditions

			Additional comments
Initial denaturation:	3 min	94°C	
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers (see Appendix B, page 33).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min/kb DNA.
Number of cycles:	25–35		See Appendix C, page 36.
Final extension:	10 min	72°C	

7. **For a simplified hot start, proceed as described in step 7a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.**

7a. **Simplified hot start: Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler.**

In many cases, this simplified hot start improves the specificity of the PCR.

For information on highly specific and convenient hot-start PCR using HotStarTaq® DNA Polymerase, see Appendix F, page 38.

Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

8. **When using CoralLoad PCR Buffer, the PCR product can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel tracking dyes.**

   CoralLoad PCR Buffer contains a gel loading reagent and gel tracking dyes. Refer to Table 4 below to identify the dyes according to migration distance and agarose gel percentage and type.

Table 4. Migration Distance of Gel Tracking Dyes

%TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 (270) bp	~80 bp (<10 bp)
1.0	300 (220) bp	~40 bp (<10 bp)
1.5	250 (120) bp	~20 bp (<10 bp)
2.0	100 (110) bp	<10 bp (<10 bp)
3.0	50 (100) bp	<10 bp (<10 bp)

Protocol: PCR Using *Taq* DNA Polymerase and Q-Solution

This protocol is designed for using Q-Solution in PCR assays. Q-Solution changes the melting behavior of DNA and can be used for PCR systems that do not work well under standard conditions. When using Q-Solution for the first time in a particular primer–template system, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed even if another PCR additive (such as DMSO) was previously used for a particular primer–template system.

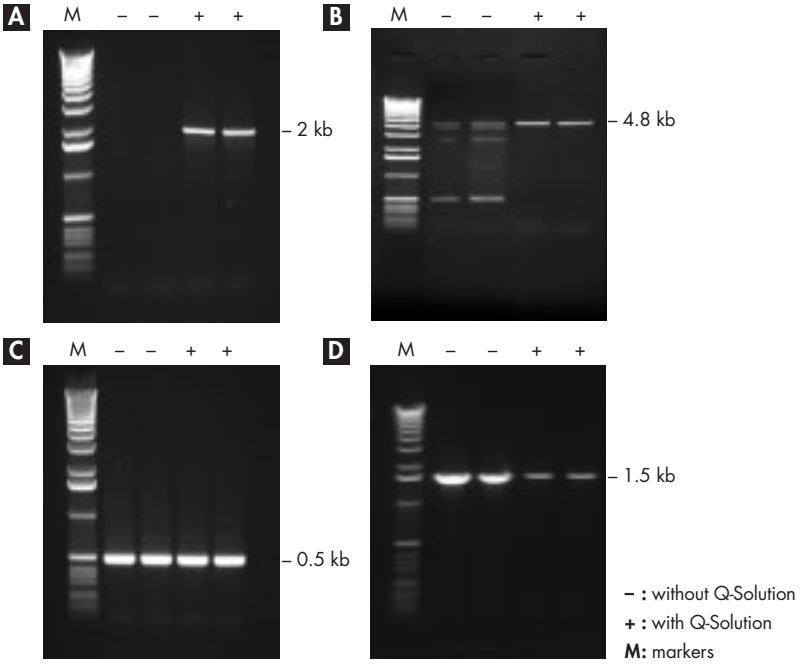
When using Q-Solution, the following effects may be observed depending on the individual PCR assay:

Case A: Q-Solution enables an amplification that previously failed.

Case B: Q-Solution increases PCR specificity in certain primer–template systems.

Case C: Q-Solution has no effect on PCR performance.

Case D: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction. In this case, addition of Q-Solution disturbs the previously optimal primer–template annealing. Therefore, when using Q-Solution for the first time for a particular primer–template system, always perform reactions in parallel with and without Q-Solution.



Important points before starting

- When using Q-Solution for the first time with a particular primer–template system, it is important to perform parallel amplification reactions with and without Q-Solution.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- CoralLoad PCR Buffer is not recommended when downstream applications require fluorescence or absorbance measurements without an intermediate purification of the PCR product (e.g., using QIAquick PCR Purification Kits or MinElute PCR Purification Kits).

Things to do before starting

- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C. For convenience, the *Taq* PCR Core Kit includes a ready-to-use ultrapure dNTP Mix, and *Taq* PCR Master Mix provides a premixed solution of *Taq* DNA Polymerase, QIAGEN PCR Buffer, and dNTPs. High quality, PCR-grade dNTP mix (10 mM) is available separately from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw 10x CoralLoad PCR Buffer or 10x PCR Buffer, dNTP mix, primer solutions, and Q-Solution at room temperature or on ice.**

   Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration. When using Q-Solution, additional $MgCl_2$ is not usually required.

2. **Prepare a master mix according to Table 5, page 19.**

   The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should be included in every experiment.

Table 5. Reaction Composition Using *Taq* DNA Polymerase and Q-Solution

Component	Volume/reaction	Final concentration
Master mix		
10x CoralLoad PCR Buffer* or 10x PCR Buffer*	10 µl	1x
5x Q-Solution	20 µl	1x
dNTP mix (10 mM each)	2 µl	200 µM of each dNTP
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Taq DNA Polymerase	0.5 µl	2.5 units/reaction
Distilled water	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	**100 µl**	**–**

* Contains 15 mM $MgCl_2$.

Note: If smaller reaction volumes are used, please reduce the amount of each component accordingly.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   Mix gently, for example, by pipetting the master mix up and down a few times. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.

4. **Add template DNA (≤1 µg/reaction) to the individual tubes containing the master mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume (see Appendix E, page 36).

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 100 µl mineral oil.**

6. **Program the thermal cycler according to the manufacturer's instructions.**

   A typical PCR cycling program is outlined in Table 6. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Table 6. Thermal Cycler Conditions

			Additional comments
Initial denaturation:	3 min	94°C	
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers (see Appendix B, page 33).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min/kb DNA.
Number of cycles:	25–35		See Appendix C, page 36.
Final extension:	10 min	72°C	

7. For a simplified hot start, proceed as described in step 7a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.

7a. Simplified hot start: Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler.

In many cases, this simplified hot start improves the specificity of the PCR.

For information on highly specific and convenient hot-start PCR using HotStarTaq DNA Polymerase, see Appendix F, page 38.

Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

8. When using CoralLoad PCR Buffer, the PCR product can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel tracking dyes.

CoralLoad PCR Buffer contains a gel loading reagent and gel tracking dyes. Please refer to Table 7 below to identify the dyes according to migration distance and agarose gel percentage and type.

Table 7. Migration Distance of Gel Tracking Dyes

%TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 (270) bp	~80 bp (<10 bp)
1.0	300 (220) bp	~40 bp (<10 bp)
1.5	250 (120) bp	~20 bp (<10 bp)
2.0	100 (110) bp	<10 bp (<10 bp)
3.0	50 (100) bp	<10 bp (<10 bp)

Protocol: PCR Using *Taq* PCR Master Mix

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined.

Important notes before starting

- *Taq* PCR Master Mix provides a final concentration of 1.5 mM $MgCl_2$ which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, prepare a stock solution containing 25 mM $MgCl_2$.
- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw primer solutions.**

   Keep on ice after complete thawing, and mix well before use.

   Optional: Prepare a primer mix of an appropriate concentration (see Table 8, page 22) using the water provided.

   This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA, added at step 4, should be 50 µl per reaction.

2. **Mix the *Taq* PCR Master Mix by vortexing briefly, and dispense 50 µl into each PCR tube according to Table 8, page 22.**

   It is important to mix the *Taq* PCR Master Mix before use to avoid localized differences in salt concentration. *Taq* PCR Master Mix is provided as a 2x concentrate (i.e., a 50-µl volume of the *Taq* PCR Master Mix is required for amplification reactions with a final volume of 100 µl). For volumes smaller than 100 µl, the 1:1 ratio of *Taq* PCR Master Mix to diluted primer mix and template should be maintained as defined in Table 8. A negative control (without template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the Master Mix.**

4. **Add template DNA (≤1 µg/reaction) to the individual PCR tubes.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume (see Appendix E, page 36).

Table 8. Reaction Composition Using *Taq* PCR Master Mix

Component	Volume/reaction	Final concentration
***Taq* PCR Master Mix**	50 µl	2.5 units *Taq* DNA Polymerase 1x QIAGEN PCR Buffer* 200 µM of each dNTP
Diluted primer mix		
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
RNase-free (provided)	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	**100 µl**	**–**

* Contains 1.5 mM $MgCl_2$.

Note: If smaller reaction volumes are used, please reduce the amount of each component accordingly.

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 100 µl mineral oil.**

6. **Program the thermal cycler according to the manufacturer's instructions.**

   A typical PCR cycling program is outlined in Table 9, page 23. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

7. **For a simplified hot start, proceed as described in step 7a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.**

7a. **Simplified hot start: Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler.**

   In many cases, this simplified hot start improves the specificity of the PCR.

   For information on highly specific and convenient hot-start PCR using HotStarTaq DNA Polymerase, see Appendix F, page 38.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

Table 9. Thermal Cycler Conditions

			Additional comments
Initial denaturation:	3 min	94°C	
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers (see Appendix B, page 33).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min/kb DNA.
Number of cycles:	25–35		See Appendix C, page 36.
Final extension:	10 min	72°C	

Protocol: Amplification of Long PCR Products

This protocol has been optimized for amplifying long PCR products (>5 kb)* using a combination of HotStar HiFidelity DNA Polymerase and QIAGEN *Taq* DNA Polymerase. HotStarTaq *Plus* DNA Polymerase can be used in place of QIAGEN *Taq* DNA Polymerase. See *HotStar HiFidelity PCR Handbook* for protocol.

Note: Amplification of long PCR products requires comparably long DNA fragments as template. Amplification of long PCR products is not possible with degraded or highly fragmented DNA.

Important points before starting

- Use the PCR Buffer supplied with QIAGEN *Taq* DNA Polymerase and Q-Solution.
- Use a final primer concentration of 0.5 µM for each primer.
- Use a final concentration of 300 µM of each dNTP.
- An extension temperature of 68°C is strongly recommended.
- The denaturation step should not last longer than 10 s.
- Use 0.2 units of HotStar HiFidelity DNA Polymerase per reaction. If necessary, dilute HotStar HiFidelity DNA Polymerase in 1x HotStar HiFidelity PCR Buffer.

Procedure

1. **Thaw 10x QIAGEN PCR Buffer, dNTP mix, primer solutions, Q-Solution, and 25 mM $MgCl_2$ (if required). Mix the solutions completely before use.**

   Make sure to use the PCR Buffer supplied with QIAGEN *Taq* DNA Polymerase.

2. **Prepare a master mix according to Table 10 on page 25.**

   The optimal Mg^{2+} concentration should be determined empirically, but in most cases a concentration of 1.5 mM, as provided in the 1x PCR Buffer, will produce satisfactory results. Master mix preparation and reaction setup should be performed on ice.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   It is recommended to keep the PCR tubes on ice before placing in the thermal cycler.

* Refers to complex templates (e.g., genomic DNA).

4. **Add template DNA (see Table 11 for recommended amounts) to the individual tubes containing the master mix.**

   For two-step RT-PCR, the volume of cDNA added (from the RT reaction) as template should not exceed 10% of the final PCR volume. See Appendix E, page 36, for more information about RT-PCR.

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.**

Table 10. PCR Components (Master Mix and Template DNA)

Component	Volume/reaction	Final concentration
PCR Buffer,* 10x (supplied with QIAGEN *Taq* DNA Polymerase)	5 µl	1x
Q-Solution, 5x	10 µl	1x
$MgCl_2$, 25 mM	Variable, see Table 12	Variable, see Table 12
dNTP mix (10 mM of each)	1.5 µl	300 µM of each dNTP
Primer A	Variable	0.5 µM
Primer B	Variable	0.5 µM
Taq DNA Polymerase	1 µl	5 units/reaction
HotStar HiFidelity DNA Polymerase (diluted)†	1 µl	0.2 units/reaction
Distilled water	Variable	
Template DNA		
Template DNA, added at step 4	Variable	See Table 11
Total volume	**50 µl**	

* Contains 15 mM $MgCl_2$.

† Dilute enzyme in 1x HotStar HiFidelity PCR Buffer to a concentration of 0.2 units/µl.

Table 11. Optimal Amounts of Starting Templates from Different Origins

Starting template	Optimal range
Human genomic DNA	1 ng – 200 ng
cDNA*	10 ng – 100 ng
Bacterial DNA	10 pg – 10 ng
PCR fragment (1 kb DNA)	10 fg – 1 ng
Plasmid DNA	0.1 ng – 50 ng

* Optimal starting template amount depends on the abundance of the respective molecule in your sample. The lower value refers to highly abundant transcripts and the upper values to low-abundance transcripts. To amplify long cDNA species, it is strongly recommended to use cDNA generated using oligo-dT primers only.

Table 12. Final Mg^{2+} Concentrations

Final Mg^{2+} concentration in reaction (mM):	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Required volume of 25 mM $MgCl_2$ per reaction (µl):	0	1	2	3	4	5	6	7

6. Program the thermal cycler according to the manufacturer's instructions.
Each PCR program must start with an initial heat-activation step at 95°C for 2 min.

Table 13. Thermal Cycler Conditions

			Additional comments
Initial activation step:	**2 min**	**95°C**	HotStar HiFidelity DNA Polymerase is activated by this heating step.
3-step cycling			
Denaturation:	10 s	94°C	
Annealing:	1 min	50–68°C	Approximately 5°C below T_m of primers
Extension:	1 min/kb	68°C	Use an extension time of approximately 1min/kb DNA
Number of cycles:	25–45		
End of PCR cycling:	Indefinite	4°C	

7. **Place the PCR tubes in the thermal cycler, and start the cycling program.**

   After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.

   Note: In contrast to other proofreading DNA polymerases, PCR products generated using HotStar HiFidelity DNA Polymerase can be used directly in TA- or UA-cloning procedures. Cloning efficiency, however, may be slightly lower compared with PCR products generated using *Taq* DNA polymerase.

   For efficient cloning we recommend QIAGEN PCR Cloning Kits. Ligation of the PCR products, transformation, and plating of QIAGEN EZ Competent Cells takes place in just 40 min.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Little or no product		
a)	Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of reagents, including primers and dNTP mix. When using *Taq* PCR Master Mix, ensure that a 1:1 ratio of *Taq* PCR Master Mix to primer–template solution is maintained.
b)	PCR cycling conditions are not optimal	Using the same cycling conditions, repeat the PCR with the Q-Solution. Follow the protocol on page 17.
c)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM increments). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.
d)	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template (see Appendix A, page 32). If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.
e)	Mg^{2+} concentration not optimal	Perform PCR with different final concentrations of Mg^{2+} from 1.5–5.0 mM (in 0.5 mM increments) using a 25 mM $MgCl_2$ solution.
f)	Enzyme concentration too low	Use 2.5 units of *Taq* DNA Polymerase per 100 µl reaction. When using *Taq* PCR Master Mix, always use 50 µl Master Mix per 100 µl reaction.

		Comments and suggestions
g)	Insufficient number of cycles	Increase the number of cycles in increments of 5 cycles.
h)	Incorrect annealing temperature or time	Decrease annealing temperature by 2°C increments. Annealing time should be between 30 and 60 s. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix G, page 38).
i)	Incorrect denaturation temperature or time	Denaturation should be at 94°C for 30–60 s. Ensure that a prolonged initial denaturation is performed as described in step 6 of the protocols (pages 15, 19, and 22).
j)	Extension time too short	Increase the extension time by increments of 1 min.
k)	Hot start may be necessary	Follow the simplified hot-start procedure described in step 7 of the protocols (pages 15, 20, and 22). If higher specificity is required, the use of HotStarTaq DNA Polymerase is recommended (see Appendix F, page 38).
l)	Insufficient starting template	Perform a second round of PCR using a nested-PCR approach (see Appendix D, page 36).
m)	Primer design not optimal	Review primer design (see Appendix B, page 33).
n)	RT reaction error	For RT-PCR, take into consideration the efficiency of reverse transcriptase reaction which averages 10–30%. The added volume of the reverse transcriptase reaction should not exceed 10% of the final PCR volume (see Appendix E, page 36).
o)	PCR overlaid with mineral oil when using a thermal cycler with a heated lid	When performing PCR in a thermal cycler with a heated lid that is switched on, do not overlay the PCR samples with mineral oil, as this may decrease the yield of PCR product.
p)	Problems with thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.

		Comments and suggestions
Product is multi-banded		
a)	PCR cycling conditions not optimal	Using the same cycling conditions, repeat the PCR with Q-Solution. Follow the protocol on page 17.
b)	Annealing temperature too low	Increase annealing temperature in 2°C increments. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix G, page 38).
c)	Hot start may be necessary	Follow the simplified hot-start procedure described in step *7* of the protocols (pages 15, 20, and 22). If higher specificity is required, the use of HotStarTaq DNA Polymerase is recommended (see Appendix F, page 38).
d)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM increments). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.
e)	Primer design not optimal	Review primer design (see Appendix B, page 33).
Product is smeared		
a)	Hot start may be necessary	Follow the simplified hot-start procedure described in step *7* of the protocols (pages 15, 20, and 22). If higher specificity is required, the use of HotStarTaq DNA Polymerase is recommended (see Appendix F, page 38).
b)	Too much starting template	Check the concentration and storage conditions of the starting template (see Appendix A, page 32). Make serial dilutions of template nucleic acid from stock solutions. Perform PCR using serial dilutions.

		Comments and suggestions
		When re-amplifying a PCR product, start the re-amplification round using 1 µl of a $1/10^3$ – $1/10^4$ dilution of the previous PCR. In most cases, a nested PCR approach results in higher specificity and sensitivity for re-amplification (see Appendix D, page 36).
c)	Carry-over contamination	If the negative-control PCR (without template DNA) shows a PCR product or a smear, exchange all reagents. Use disposable tips containing hydrophobic filters to minimize cross-contamination. Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
d)	Enzyme concentration too high	Use 2.5 units of *Taq* DNA Polymerase per 100-µl reaction. When using *Taq* PCR Master Mix, always use 50 µl Master Mix per 100-µl reaction.
e)	Too many cycles	Reduce the number of cycles in increments of 3 cycles.
f)	Mg^{2+} concentration not optimal	Perform PCR with different final concentrations of Mg^{2+} from 1.5–5.0 mM (in 0.5 mM increments) using a 25 mM $MgCl_2$ solution.
g)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM increments). In particular, when performing highly sensitive PCR check for possible degradation of the primers on a denaturing polyacrylamide gel.
h)	Primer design not optimal	Review primer design (see Appendix B, page 33).
i)	PCR of long fragments from genomic DNA	Follow the protocol for amplification of long PCR products (page 24).

Appendix A: Starting Template

Both the quality and quantity of nucleic acid starting template affect PCR, in particular the sensitivity and efficiency of amplification.*

Quality of starting template

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol, chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR, for example the QIAprep® system for rapid plasmid purification, the QIAamp and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids, the RNeasy® system for RNA preparation from a variety of sources, and the Oligotex® System for mRNA isolation. For more information about QIAprep, QIAamp, DNeasy, RNeasy, and Oligotex products, please call QIAGEN Technical Service or your local distributor (see inside front cover).

Quantity of starting template

The annealing efficiency of primer to template is an important factor in PCR. Owing to the thermodynamic nature of the reaction, the primer–template ratio strongly influences the specificity and efficiency of PCR and should be optimized empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events. As an initial guide, spectrophotometric and molar conversion values for different nucleic acid templates are listed in Tables 14 and 15 respectively.

Table 14. Spectrophotometric Conversions for Nucleic Acid Templates

1 A_{260} unit†	Concentration (µg/ml)
Double-stranded DNA	50
Single-stranded DNA	33
Single-stranded RNA	40

† Absorbance at 260 nm = 1.

* For further information see our comprehensive brochure, *"Critical success factors and new technologies for PCR and RT-PCR"*. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Table 15. Molar Conversions for Nucleic Acid Templates

Nucleic Acid	Size	pmol/µg	Molecules/µg
1 kb DNA	1000 bp	1.52	9.1×10^{11}
pUC19 DNA	2686 bp	0.57	3.4×10^{11}
pTZ18R DNA	2870 bp	0.54	3.2×10^{11}
pBluescript® II DNA	2961 bp	0.52	3.1×10^{11}
Lambda DNA	48,502 bp	0.03	1.8×10^{10}
Average mRNA	1930 nt	1.67	1.0×10^{12}
Genomic DNA			
Escherichia coli	4.7×10^{6}*	3.0×10^{-4}	1.8×10^{8}†
Drosophila melanogaster	1.4×10^{8}*	1.1×10^{-5}	6.6×10^{5}†
Mus musculus (mouse)	2.7×10^{9}*	5.7×10^{-7}	3.4×10^{5}†
Homo sapiens (human)	3.3×10^{9}*	4.7×10^{-7}	2.8×10^{5}†

* Base pairs per haploid genome.

† For single-copy genes.

Appendix B: Primer Design, Concentration, and Storage

Standard PCR Primers

Prerequisites for successful PCR include the design of optimal primer pairs, the use of appropriate primer concentrations, and the correct storage of primer solutions. Some general guidelines are given in Table 16, page 34.‡

‡ For further information see our comprehensive brochure, *"Critical success factors and new technologies for PCR and RT-PCR"*. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Table 16. General Guidelines for Standard PCR Primers

Length:	18–30 nucleotides
GC content:	40–60%
T_m:	Simplified formula for estimating melting temperature (T_m): T_m = 2°C x (A+T) + 4°C x (G+C) Whenever possible, design primer pairs with similar T_m values. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.
Sequence:	■ Avoid complementarity of two or three bases at the 3' ends of primer pairs to reduce primer–dimer formation. ■ Avoid runs of 3 or more Gs or Cs at the 3' end. ■ Avoid a 3'-end T. Primers with a T at the 3' end have a greater tolerance of mismatch. ■ Avoid complementary sequences within a primer sequence and between the primer pair. ■ Commercially available computer software (e.g., OLIGO 6, Rychlik, 1999) or web-based tools such as Primer3, Steve Rosen and Helen Skaletsky, 2000 (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi), can be used for primer design.
Concentration:	■ Spectrophotometric conversion for primers: Absorbance of 1 at 260 nm (1 A_{260} unit) ⇒ 20–30 µg/ml ■ Molar conversions: **Primer length** / **pmol/µg** / **20 pmol** 18mer / 168 / 119 ng 20mer / 152 / 132 ng 25mer / 121 / 165 ng 30mer / 101 / 198 ng ■ Use 0.1–0.5 µM of each primer in PCR. For most applications, a 0.2 µM primer concentration will be sufficient.
Storage:	Lyophilized primers should be dissolved in a small volume of distilled water or TE to make a concentrated stock solution. Prepare small aliquots of working solutions containing 10 pmol/µl to avoid repeated thawing and freezing. Store all primer solutions at –20°C. Primer quality can be checked on a denaturing polyacrylamide gel; a single band should be seen.

Degenerate PCR primers

Occasionally, the exact nucleotide sequence of the target-template DNA will not be known, for instance when it has been deduced from an amino acid sequence. To enable such templates to be amplified by PCR, degenerate primers can be used. These are actually mixtures of several primers whose sequences differ at the positions that correspond to the uncertainties in the template sequence.

Hot-start PCR often improves the specificity of PCR amplifications that employ degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. We recommend using HotStarTaq DNA Polymerase for highly specific amplification using degenerate primers (see Appendix F, page 38). Table 17 gives recommendations for further optimizing PCR using degenerate primers. Table 18 shows the codon redundancy of each amino acid.

Table 17. Guidelines for Design and Use of Degenerate Primers

Sequence:	■ Avoid degeneracy in the 3 nucleotides at the 3' end. ■ If possible, use Met- or Trp-encoding triplets at the 3' end. ■ To increase primer–template binding efficiency, reduce degeneracy by allowing mismatches between the primer and template, especially towards the 5' end (but not at the 3' end). ■ Try to design primers with less than 4-fold degeneracy at any given position.
Concentration:	■ Begin PCR with a primer concentration of 0.2 µM. ■ In case of poor PCR efficiency, increase primer concentrations in increments of 0.25 µM until satisfactory results are obtained.

Table 18. Codon Redundancy

Amino acid	Number of codons
Met, Trp	1
Cys, Asp, Glu, Phe, His, Lys, Asn, Gln, Tyr	2
Ile	3
Ala, Gly, Pro, Thr, Val	4
Leu, Arg, Ser	6

Appendix C: Number of PCR Cycles

A cycling program usually consists of 25–35 cycles, depending on the number of copies of the starting template. Increasing the number of cycles does not necessarily lead to a higher yield of PCR product; instead they may increase nonspecific background and decrease the yield of specific PCR product. Table 19 provides general guidelines for choosing the number of cycles.

Table 19. General Guidelines for Choosing the Number of Cycles

Number of copies of starting template*	1 kb DNA	*E. coli* DNA†	Human genomic DNA†	Number of cycles
10–100	0.01–0.11 fg	0.05–0.56 pg	36–360 pg	40–45
100–1000	0.11–1.1 fg	0.56–5.56 pg	0.36–3.6 ng	35–40
$1 \times 10^3 - 5 \times 10^4$	1.1–55 fg	5.56–278 pg	3.6–179 ng	30–35
$>5 \times 10^4$	>55 fg	>278 pg	>179 ng	25–35

* Refer to Table 15 (page 33) to calculate the number of molecules. When starting with cDNA templates, it is important to take into account the efficiency of reverse transcription in cDNA synthesis, which is on average 10–30%.

† Refers to single-copy genes.

Appendix D: Nested PCR

If PCR sensitivity is low, nested PCR often improves product yield. This technique involves two rounds of amplification reactions. The first-round PCR is performed according to the PCR Protocol using *Taq* DNA Polymerase (page 13). Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a $1/10^3 - 1/10^4$ dilution, is subjected to a second round of PCR. The second-round PCR is performed with two new primers that hybridize to sequences internal to the first-round primer–target sequences. In this way, only specific first-round PCR products (and not nonspecific products) will be amplified in the second round. Alternatively, it is possible to use one internal primer and one first-round primer in the second PCR; this is referred to as semi-nested PCR. PCR sensitivity can be further improved using hot-start PCR even without using nested PCR (see page 38).

Appendix E: RT-PCR

To perform PCR using RNA as a starting template, the RNA must first be reverse transcribed into cDNA in a reverse transcriptase (RT) reaction. Failure of the subsequent PCR is often a result of the limitations of the RT reaction. On average, only 10–30% of the original RNA molecules is reverse transcribed into cDNA. The expression level of

the target RNA molecules and the relatively low efficiency of the RT reaction must be considered when calculating the appropriate amount of starting template for subsequent PCR. The volume of the RT reaction transferred should not exceed 10% of the total PCR volume. General guidelines are presented in Table 20 below.

Table 20. General Guidelines for Performing RT-PCR

RNA purification and reverse transcription:	QIAGEN offers the RNeasy system for total RNA isolation, Oligotex Kits for messenger RNA isolation, and Omniscript RT for reverse transcription.* Follow the detailed protocol in the *Omniscript Reverse Transcriptase Handbook*. Or, when using an enzyme from another supplier, follow the manufacturer's instructions. The following guidelines may be helpful. ■ Mix the following reagents in a microcentrifuge tube: 4.0 µl 5x RT buffer 1.0 µl RNase inhibitor (5 units/µl) 2.0 µl DTT (0.1 M) 1.0 µl each dNTP (10 mM) ~1 µg RNA 2.5 µl primer (0.2 µg/µl) reverse transcriptase† Add RNase-free water to a final volume of 20 µl. ■ Incubate following the manufacturer's instructions. ■ Heat the reaction mix at 95°C for 5 min to inactivate the reverse transcriptase.
PCR:	■ Prepare a PCR mixture following steps 1–3 in the protocols. ■ Add 2–5 µl from the RT reaction to each PCR tube containing the master mix. ■ Continue with step 5 in the PCR protocols.

Oligotex is not available in Japan.

* For further information about RNeasy, Oligotex, and Omniscript products, contact your local QIAGEN Technical Services or distributor (see back cover or visit www.qiagen.com).

† Please refer to the manufacturer's instructions for the amount of enzyme required.

Appendix F: Hot-Start PCR

In hot-start PCR, polymerase activity is prevented during reaction setup and starts only after the initial denaturation step. A simplified hot-start method is described in step 7 of the standard protocols (pages 15, 20, and 22).

In cases where higher specificity is required, HotStarTaq DNA Polymerase offers a more reliable and convenient method for hot-start PCR. The enzyme is supplied in an inactivated state which exhibits no polymerase activity. This prevents extension of nonspecifically annealed primers and primer–dimers which form at low temperatures during reaction setup and the initial heating period. HotStarTaq *Plus* DNA Polymerase is activated by a 5 min incubation at 95°C. This high-temperature activation step also denatures nonspecifically annealed primers and ensures highly specific amplification. HotStarTaq *Plus* is also available in a ready-to-use solution, HotStarTaq *Plus* Master Mix Kit.

Appendix G: Touchdown PCR

Touchdown PCR uses a cycling program with varying annealing temperatures. It is a useful method to increase the specificity of PCR. The annealing temperature in the initial PCR cycle should be 5–10°C above the estimated T_m of the primers. In subsequent cycles, the annealing temperature is decreased in increments of 1–2°C per cycle until a temperature is reached equal to, or 2–5°C below, the T_m of the primers. Touchdown PCR enhances the specificity of the initial primer–template duplex formation and hence the specificity of the final PCR product.

To program your thermal cycler for touchdown PCR, you should refer to the manufacturer's instructions.

Appendix H: Purification of PCR Products

After amplification, the PCR sample contains a complex mixture of specific PCR product and residual reaction components such as primers, unincorporated nucleotides, enzyme(s), salts, mineral oil, and probably nonspecific amplification products. Before the specific PCR product can be used in subsequent experiments, it is often necessary to remove these contaminants. The QIAquick system offers a quick and easy method for purifying the final PCR product. Using the MinElute system, PCR products can be purified in higher concentrations due to the low elution volumes needed in this system. For more information about QIAquick and MinElute products, please call QIAGEN Technical Service or your local distributor (see back cover).

Appendix I: Control of Contamination

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

General physical and chemical precautions

- Separate the working areas for setting up the PCR master mix and DNA handling, including the addition of starting template, PCR product analysis, or plasmid preparation. Ideally, use separate rooms.
- Use a separate set of pipets for the PCR master mix. Use of pipet tips with hydrophobic filters is strongly recommended.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution.* Afterwards, the benches and pipets should be rinsed with distilled water.
- Prepare and freeze small aliquots of primer solutions and dNTP mix. Use of fresh distilled water is strongly recommended.

General chemical precautions

- PCR stock solutions can be decontaminated using UV light. This method is laborious, however, and its efficiency is difficult to control and cannot be guaranteed. We recommend storing solutions in small aliquots and using fresh aliquots for each PCR.
- To prevent amplification of contaminating DNA, treat individual reaction mixtures with DNase I or restriction enzymes that cut between the binding sites of the amplification primers used, before adding the template DNA sample.

* Most commercial bleach solutions are approximately 5.25% sodium hypochlorite. Sodium hypochlorite is an irritant and should be handled with caution.

† When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
***Taq* DNA Polymerase — for standard and specialized PCR applications**		
Taq DNA Polymerase (250 U)	250 units *Taq* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	201203
Taq DNA Polymerase (1000 U)	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	201205
Taq DNA Polymerase (5000 U)	20 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	201207
Taq DNA Polymerase Kit (25000 U)	100 x 250 units Taq DNA Polymerase, 10x PCR Buffer,* 10 x CoralLoad PCR Buffer, * 5x Q-Solution, 25 mM $MgCl_2$	201209
***Taq* PCR Core Kit — for complete PCR setup**		
Taq PCR Core Kit (250 U)	250 units *Taq* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix‡	201223
Taq PCR Core Kit (1000 U)	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix†	201225
***Taq* PCR Master Mix Kit — for convenient PCR setup**		
Taq PCR Master Mix Kit (250 U)	3 x 1.7 ml *Taq* PCR Master Mix,‡ 3 x 1.7 ml distilled water	201443
Taq PCR Master Mix Kit (1000 U)	12 x 1.7 ml *Taq* PCR Master Mix,‡ 12 x 1.7 ml distilled water	201445

* Contains 15 mM $MgCl_2$.

† Contains 10 mM of each dNTP.

‡ Provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM of each dNTP.

Ordering Information

Product	Contents	Cat. no.
Related products		
HotStar HiFidelity Polymerase Kit (100 U)	100 units HotStar HiFidelity DNA Polymerase, 5x HotStar HiFidelity PCR Buffer (inc. dNTPs),* 5x Q-Solution, 25 mM $MgSO_4$, RNase-Free Water	202602
HotStar HiFidelity Polymerase Kit (1000 U)	1000 units HotStar HiFidelity DNA Polymerase, 5x HotStar HiFidelity PCR Buffer (inc. dNTPs),* 5x Q-Solution, 25 mM $MgSO_4$, RNase-Free Water	202605
HotStarTaq DNA Polymerase (250)	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203203
HotStarTaq DNA Polymerase (1000)	4 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203205
HotStarTaq DNA Polymerase (5000)	1 x 5000 units HotStarTaq DNA Polymerase, 1 x HotStarTaq Buffer Set (1 x 22ml PCR Buffer, 1 x 40ml Q-Solution, 1 x 22ml $MgCl_2$)	203207
HotStarTaq DNA Polymerase (25000)	100 x 250 units HotStarTaq DNA Polymerase, 100 x 1.2ml HotStarTaq Buffer Set, 100 x 2.0ml Q-Solution, 100 x 1.2ml $MgCl_2$	203209
HotStarTaq *Plus* DNA Polymerase (250 U)	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203603
HotStarTaq *Plus* DNA Polymerase (1000 U)	1000 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203605

* Contains Factor SB, dNTPs, and optimized concentration of $MgSO_4$.

† Contains 15 mM $MgCl_2$.

Ordering Information

Product	Contents	Cat. no.
HotStarTaq *Plus* DNA Polymerase (5000 U)	5000 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203607
HotStarTaq *Plus* DNA Polymerase (25000)	25,000 units HotStarTaq Plus DNA Polymerase, 10x PCR Buffer, 10x CoralLoad PCR Buffer, 5x Q-Solution, 25 mM $MgCl_2$	203609
HotStarTaq Master Mix Kit (250)**	3 x 0.85 ml HotStarTaq Master Mix* containing 250 units HotStarTaq DNA Polymerase total, 2 x 1.7 ml distilled water	203443
HotStarTaq *Plus* Master Mix Kit (250)**	For 250 x 20 µl reactions: 3 x 0.85 ml HotStarTaq Plus Master Mix, containing 250 units of HotStarTaq Plus DNA Polymerase total, 1 x 0.55 ml CoralLoad Concentrate, 2 x 1.9 ml RNase-free Water	203643
QIAGEN OneStep RT-PCR Kit (25)	For 25 reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,† dNTP Mix,‡ 5x Q-Solution, RNase-free water	210210
QIAGEN OneStep RT-PCR Kit (100)	For 100 reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,† dNTP Mix,‡ 5x Q-Solution, RNase-free water	210212
TopTaq™ DNA Polymerase – for highly reliable end-point PCR with unrivalled ease-of-use		
TopTaq DNA Polymerase (250)	250 units TopTaq DNA Polymerase, 10x PCR Buffer*, CoralLoad Concentrate, 5x QSolution, 25 mM $MgCl_2$	200203

* Provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM of each dNTP.

† Contains 12.5 mM $MgCl_2$.

‡ Contains 10 mM of each dNTP.

** Larger kit sizes available; see www.qiagen.com .

Ordering Information

Product	Contents	Cat. no.
TopTaq DNA Polymerase (1000)	1000 units TopTaq DNA Polymerase, 10x PCR Buffer*, CoralLoad Concentrate, 5x QSolution, 25 mM $MgCl_2$	200205
dNTP Mix, PCR Grade (200 µl)	Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (1 x 200 µl)	201900
dNTP Mix, PCR Grade (800 µl)	Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (4 x 200 µl)	201901
QIAquick PCR Purification Kit—For purification of PCR products, 100 bp to 10 kb		
QIAquick PCR Purification Kit (50)**	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
MinElute PCR Purification Kit—For purification of PCR products (70 bp to 4 kb) in low elution volumes		
MinElute PCR Purification Kit (50) **	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
QIAxcel System —for effortless automated DNA fragment and RNA analysis		
QIAxcel System	Capillary electrophoresis device, including computer, and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
QIAxcel kits—for fast high-resolution capillary electrophoresis		
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004

* Provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM of each dNTP.

** Larger kit sizes available; see www.qiagen.com .

Ordering Information

Product	Contents	Cat. no.
QIAxcel DNA Large Fragment Kit (600)	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

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