October 2010

HotStarTaq® PCR Handbook

HotStarTaq DNA Polymerase
HotStarTaq Master Mix Kit

For highly specific hot-start PCR
without optimization

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com .

Contents

Kit Contents

HotStarTaq DNA Polymerase	(250 units)	(1000 units)	(5000 units)	(25000 units)
Catalog no.	**203203**	**203205**	**203207**	**203209**
HotStarTaq DNA Polymerase	250 units	4 x 250 units	1 x 5000 units	100 x 250 units
PCR Buffer, 10x*	1.2 ml	4 x 1.2 ml	1 x 22 ml	100 x 1.2 ml
Q-Solution, 5x	2 ml	4 x 2 ml	1 x 40 ml	100 x 2 ml
$MgCl_2$, 25 Mm	1.2 ml	4 x 1.2 ml	1 x 22 ml	100 x 1.2 ml
Handbook	1	1	1	1

* Contains 15 mM $MgCl_2$

HotStarTaq Master Mix Kit	(250 units)	(1000 units)	(2500 units)
Catalog no.	**203443**	**203445**	**203446**
HotStarTaq Master Mix†	3 x 0.85 ml	12 x 0.85 ml	1 x 25 ml
RNase-Free Water	2 x 1.7 ml	8 x 1.7 ml	2 x 20 ml
Handbook	1	1	1

† Contains HotStarTaq DNA Polymerase, PCR Buffer with 3 mM $MgCl_2$, and 400 µM each dNTP.

Shipping and Storage

HotStarTaq DNA Polymerase is shipped on dry ice but retains full activity at room temperature (15–25°C) for 2 weeks.

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

HotStarTaq DNA Polymerase and HotStarTaq 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, this product can be kept at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance.

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 HotStarTaq DNA Polymerase 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

HotStarTaq DNA Polymerase and HotStarTaq Master Mix 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

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Specifications

Enzyme:

HotStarTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*, cloned in *E. coli*. (Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7).

One unit of HotStarTaq 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% glycerol (v/v), stabilizer; pH 9.0 (20°C)

Buffers and reagents:

PCR Buffer:	10x concentrated. Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7 (20°C).
Q-Solution:	5x concentrated
$MgCl_2$ solution:	25 mM
HotStarTaq Master Mix:	2x concentrated. Contains HotStarTaq DNA Polymerase, PCR Buffer (with 3 mM $MgCl_2$), and 400 µM 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 HotStarTaq 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 minutes. The amount of incorporated dNTPs is determined by precipitation with trichloroacetic acid. HotStarTaq DNA Polymerase is activated by heating for 3 hours at 80°C prior to activity measurement.
Amplification efficiency assay:	The amplification efficiency is tested in parallel amplification reactions and is indicated under "Amp".
PCR reproducibility assay:	PCR reproducibility and specificity are tested in parallel amplification reactions. The reactions must yield a single specific product.
Exonuclease activity assay:	Linearized plasmid DNA is incubated with HotStarTaq DNA Polymerase in PCR Buffer. Exonuclease activity per unit of enzyme is indicated under "Exo".
Endonuclease activity assay:	Plasmid DNA is incubated with HotStarTaq DNA Polymerase in PCR Buffer. Endonuclease activity per unit of enzyme is indicated under "Endo".
RNase activity assay:	RNA is incubated with HotStarTaq DNA Polymerase in PCR Buffer. RNase activity per unit of enzyme is indicated under "RNase".
Protease activity assay:	HotStarTaq DNA Polymerase is incubated in storage buffer. Protease activity per unit of enzyme is indicated under "Protease".
Self-priming activity assay:	Assays are performed under standard PCR conditions, without primers, using HotStarTaq 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:

PCR Buffer, 10x:	Conductivity, pH, sterility, and performance in PCR are tested.
Q-Solution, 5x:	Conductivity, pH, sterility, and performance in PCR are tested.
$MgCl_2$, 25 mM:	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled water:	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.

HotStarTaq Master Mix Kit:

PCR reproducibility assay:	The PCR reproducibility assay described above is performed in parallel using HotStarTaq Master Mix and using the separate reagents with the same lot numbers.

Introduction

HotStarTaq DNA Polymerase has been developed by QIAGEN to provide hot-start PCR for higher PCR specificity. The combination of HotStarTaq DNA Polymerase and the unique QIAGEN PCR Buffer minimizes nonspecific amplification products, primer–dimers, and background. It is ideal for amplification reactions involving complex genomic or cDNA templates, very low-copy targets, or multiple primer pairs. HotStarTaq DNA Polymerase makes hot-start PCR simple and easy, eliminating the extra handling steps and contamination risks associated with conventional hot-start methods.

HotStarTaq DNA Polymerase

HotStarTaq DNA Polymerase is a modified form of the recombinant 94 kDa *Taq* DNA Polymerase from QIAGEN. HotStarTaq DNA Polymerase is provided in an inactive state with no polymerase activity at ambient temperatures. This prevents the formation of misprimed products and primer–dimers at low temperatures. HotStarTaq DNA Polymerase is activated by a 15-minute, 95°C incubation step, which can easily be incorporated into existing thermal cycling programs. HotStarTaq DNA Polymerase provides high PCR specificity and often increases the yield of the specific PCR product. PCR setup is quick and convenient as all reaction components can be combined at room temperature.

If you would like even faster PCR with the same unrivalled performance of HotStarTaq DNA Polymerase, we recommend HotStarTaq *Plus* DNA Polymerase. HotStarTaq *Plus* DNA Polymerase provides a quick, 5-minute activation time with a convenient ready-to-load PCR buffer containing gel tracking dyes (see Ordering Information, page 39).

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.

* 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).

Q-Solution

HotStarTaq DNA Polymerase is provided with Q-Solution, an innovative PCR additive that facilitates amplification of difficult templates by modifying the melting behavior of DNA. This unique reagent will often enable or improve a suboptimal PCR caused by templates that have a high degree of secondary structure or that are GC-rich. Unlike other commonly used PCR additives such as DMSO, Q-Solution is used at just one working concentration, it is nontoxic, and PCR purity is guaranteed. For further information, please read the PCR Protocol Using HotStarTaq DNA Polymerase and Q-Solution, page 17.

Specificity and sensitivity

HotStarTaq DNA Polymerase in combination with QIAGEN PCR Buffer* with its balanced potassium and sodium salts promotes specific primer–template annealing and simultaneously reduces non-specific annealing. Maximum yields of specific products are obtained even when using extremely low template amounts (Appendix D, page 32).

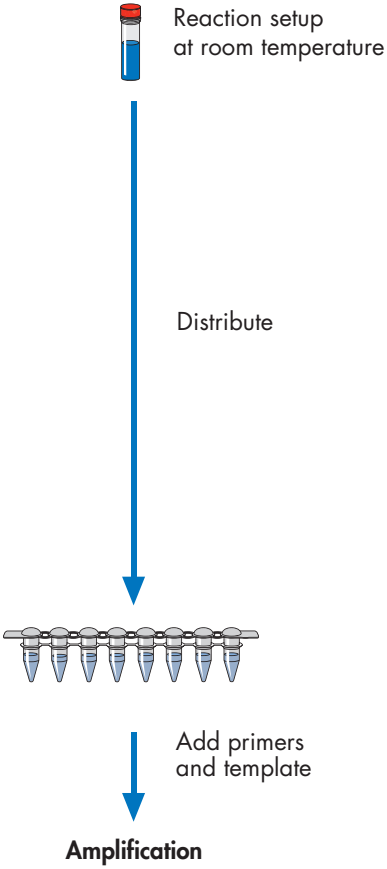
Downstream applications

HotStarTaq DNA Polymerase in combination with QIAGEN PCR Buffer is ideally suited for a wide variety of applications, including challenging applications such as single-cell PCR, nested PCR, or typing studies.

For high-fidelity PCR we recommend the HotStar HiFidelity Polymerase Kit for highly sensitive and reliable high-fidelity PCR without optimization.

* PCR Buffer is provided in HotStarTaq Master Mix.

HotStarTaq Procedure



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

- 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.

HotStarTaq DNA Polymerase Protocols

- Distilled water
- dNTPs (e.g., dNTP Mix, PCR Grade, cat. no. 201900)

Protocol: PCR Using HotStarTaq DNA Polymerase

Important points before starting

- HotStarTaq DNA Polymerase requires an activation step of **15 min at 95°C** (see step 6 of this protocol).
- 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.

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. High-quality, PCR-grade dNTP mix (10 mM) is available from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw 10x PCR Buffer, dNTP mix, primer solutions, and 25 mM $MgCl_2$ (if required).**

   It is important to mix the solutions completely before use to avoid localized concentrations of salts.

2. **Prepare a reaction mix according to Table 1.**

   It is not necessary to keep reaction vessels on ice since HotStarTaq DNA Polymerase is inactive at room temperature.

   The reaction mix typically contains all 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 always be included.

   Note: The Mg^{2+} concentration provided by the supplied 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.

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

   Mix gently (e.g., by pipetting the reaction mix up and down a few times). It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq DNA Polymerase.

4. **Add template DNA (<1 µg/100 µl reaction) to the individual tubes containing the reaction mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume (see Appendix E, page 34).

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.**

Table 1. Reaction Composition Using HotStarTaq DNA Polymerase

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

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

* Contains 15 mM $MgCl_2$

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.

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

Note: Each PCR program must start with an initial heat activation step at 95°C for 15 min.

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 template target or primer pair.

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

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

Table 3. Optimized Cycling Protocol

			Additional comments
Initial activation step:	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
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 28).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 31.
Final extension:	10 min	72°C	

Protocol: PCR Using HotStarTaq 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 the first time for a particular primer–template pair, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed if another PCR additive (such as DMSO) was previously used for a particular primer–template pair.

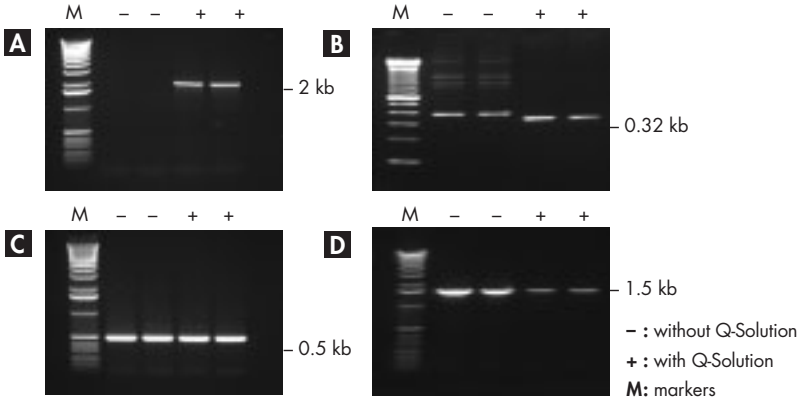
When using Q-Solution, the following effects may be observed depending on the individual PCR assay:

Case A: Q-Solution enables amplification of a reaction which 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

- HotStarTaq DNA Polymerase requires an activation step of **15 min at 95°C** (see step 6 of this protocol).
- 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.

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. High-quality, PCR-grade dNTP mix (10 mM) is available from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw 10x PCR Buffer, dNTP mix, primer solutions, and 25 mM $MgCl_2$ (if required).**

   It is important to mix the solutions completely before use to avoid localized concentrations of salts.

2. **Prepare a reaction mix according to Table 4.**

   It is not necessary to keep reaction vessels on ice since HotStarTaq DNA Polymerase is inactive at room temperature.

   The reaction mix typically contains all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required

Table 4. Reaction Composition Using HotStarTaq DNA Polymerase and Q-Solution

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

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

* Contains 15 mM $MgCl_2$

for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included.

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

   Mix gently (e.g., by pipetting the reaction mix up and down a few times). It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq DNA Polymerase.

4. **Add template DNA (<1 µg/100 µl reaction) to the individual tubes containing the reaction mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume (see Appendix E, page 34).

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.**

   Note: Each PCR program must start with an initial heat activation step at 95°C for 15 min.

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

Table 5. Optimized Cycling Protocol

			Additional comments
Initial activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
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 28).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 31.
Final extension:	10 min	72°C	

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

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

Protocol: PCR Using HotStarTaq Master Mix

Important points before starting

- HotStarTaq DNA Polymerase requires an activation step of **15 min at 95°C** (see step 6 of this protocol).
- HotStarTaq Master Mix provides a final concentration of 1.5 mM $MgCl_2$ in the final reaction mix, 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 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.

Procedure

1. **Thaw primer solutions.**

   Mix well before use.

   Optional: prepare a primer mix of an appropriate concentration (see Table 6) using the water provided. This is recommended if several amplification reactions using the same primer pair are to be performed. The volume of primer mix added to each 50 µl reaction is 25 µl minus the volume of template DNA.

2. **Mix the HotStarTaq Master Mix by vortexing briefly and dispense 25 µl into each PCR tube according to Table 6.**

   It is important to mix the HotStarTaq Master Mix before use in order to avoid localized concentrations of salt. HotStarTaq Master Mix is provided as a 2x concentrate (i.e., a 25 µl volume of the HotStarTaq Master Mix is required for amplification reactions with a final volume of 50 µl). For volumes smaller than 50 µl, the 1:1 ratio of HotStarTaq Master Mix to diluted primer mix and template should be maintained as defined in Table 6. A negative control (without template DNA) should always be included.

   It is not necessary to keep reaction vessels on ice since HotStarTaq DNA Polymerase is inactive at room temperature.

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

4. **Add template DNA (<1 µg/50 µl 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 34).

Table 6. Reaction composition using HotStarTaq Master Mix

Component	Volume/reaction	Final Concentration
HotStarTaq Master Mix	25 µl	2.5 units HotStarTaq DNA Polymerase 1 x 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 Water	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	<1 µg/50 µl reaction
Total volume	**50 µl**	–

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

* Contains 1.5 mM $MgCl_2$.

† The volume of primer mix added to each 50 µl reaction is 25 µl minus the volume of template DNA.

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.**

   Note: Each PCR program must start with an initial heat activation step at 95°C for 15 min.

   A typical PCR cycling program is outlined in Table 7. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Table 7. Optimized Cycling Protocol

			Additional comments
Initial activation step:	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
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 28).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 31.
Final extension:	10 min	72°C	

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

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

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)	HotStarTaq DNA Polymerase not activated	Check whether PCR was started with an initial incubation step at 95°C for 15 min.
b)	Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of reagents, including primers and dNTP mix. In the case of HotstarTaq Master Mix, ensure a 1:1 ratio of HotStarTaq Master Mix to primer-template solution is maintained.
c)	PCR cycling conditions are not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol on page 17.
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 steps). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.*
e)	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template (see Appendix A, page 27). If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.
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 steps) using a 25 mM $MgCl_2$ solution (see Table 2, page 15).

* 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.

		Comments and suggestions
g)	Enzyme concentration too low	When using HotStarTaq DNA Polymerase, use 2.5 units per 100 µl reaction. If necessary, increase the amount of HotStarTaq DNA Polymerase (in 0.5 unit steps). When using HotStarTaq Master Mix, use 25 µl Master Mix per 50 µl reaction.
h)	Insufficient number of cycles	Increase the number of cycles in steps of 5 cycles (see Appendix C, page 31).
i)	Incorrect annealing temperature or time	Decrease annealing temperature in 2°C steps. 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 F, page 35).
j)	Incorrect denaturation temperature or time	Denaturation should be at 94°C for 30 to 60 s. Ensure that the initial 15 min 95°C incubation step was performed as described in step 6 of the PCR protocols (pages 15, 19, and 21).
k)	Extension time too short	Increase the extension time in increments of 1 min. For PCR using genomic DNA, follow suggestion "o) PCR of long fragments from genomic DNA", below.
l)	Insufficient starting template	Perform a second round of PCR using a nested PCR approach (see Appendix D, page 32).
m)	Primer design not optimal	Review primer design (see Appendix B, page 28).
n)	RT reaction error	For RT-PCR, take into consideration the efficiency of the reverse transcriptase reaction, which averages 10–30%. The added volume of reverse transcriptase reaction should not exceed 10% of the final PCR volume (see Appendix E, page 34).
o)	PCR of long fragments from genomic DNA	When amplifying products longer than 4 kb from genomic DNA, increase the concentration of genomic DNA in the reaction (see Appendix A, page 27). Alternatively, use the protocol for amplification of long PCR products using QIAGEN Taq DNA Polymerase and ProofStart® DNA Polymerase or HotStar HiFidelity Polymerase (see the *Taq PCR Handbook* or the *HotStar HiFidelity PCR Handbook*).

p)	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, do not overlay the PCR samples with mineral oil if the heated lid is switched on as this may decrease the yield of PCR product.
q)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.

Product is multi-banded

a)	PCR cycling conditions not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol on page 17.
b)	Annealing temperature too low	Increase annealing temperature in 2°C steps. 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 F, page 35).
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 steps. In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.*
d)	Primer design not optimal	Review primer design (see Appendix B, page 28).

Product is smeared

a)	Too much starting template	Check the concentration and storage conditions of the starting template (see Appendix A, page 27). Make serial dilutions of template nucleic acid from stock solutions. Perform PCR using these serial dilutions. When re-amplifying a PCR product, start the re-amplification round using 1 µl of a 1-in-10^3–10^4 dilution of the previous PCR. In most cases, a nested PCR approach results in higher specificity and sensitivity for reamplification (see Appendix D, page 32).

* 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.

		Comments and suggestions
b)	Carryover contamination	If the negative-control PCR (without template DNA) shows a PCR product or a smear, exchange all reagents. Use disposable pipet 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.
c)	Enzyme concentration too high	When using HotStarTaq DNA Polymerase, use 2.5 units per 100 µl reaction. When using HotStar Master Mix, use 25 µl Master Mix per 50 µl reaction.
d)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.
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 steps) using the 25 mM $MgCl_2$ solution provided (see Table 2, page 15).
f)	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 steps). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.*
g)	Primer design not optimal	Review primer design (see Appendix B, page 28).

* 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.

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, and the RNeasy® system for RNA preparation from a variety of sources. For more information about QIAprep, QIAamp, DNeasy, and RNeasy products, contact one of our Technical Service Departments (see back cover or visit www.qiagen.com).

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 8 and 9 respectively.

Table 8. 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 9. 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 in 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 10.

* 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 10. General Guidelines for Standard PCR Primers

Length:	18–30 nucleotides
G/C 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 mismatches between the 3' end of the primer and the target-template sequence. ■ Avoid runs of 3 or more G or C 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: 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 primer concentration of 0.2 µM will be sufficient.

Table 10. (continued)

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.

* 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.

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 position that correspond to the uncertainties in the template sequence.

Hot-start PCR using HotStarTaq DNA Polymerase often improves the specificity of PCR amplifications that employ degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. Table 11 gives recommendations for further optimizing PCR using degenerate primers. Table 12 shows the codon redundancy of each amino acid.

Table 11. 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 12. 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 between 25 and 35 cycles, depending on the number of copies of the starting template. Too many cycles do 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 13 provides a general guideline for choosing the number of cycles.

Table 13. General Guidelines for Choosing the Number of PCR 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 9 (page 28) 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: Sensitive PCR Assays

PCR can be performed to amplify and detect just a single copy of a nucleic acid sequence. However, amplification of such a low number of target sequences is often limited by the generation of nonspecific PCR products and primer–dimers. The combination of HotStarTaq DNA Polymerase and QIAGEN PCR Buffer increases specificity both at the start of and during PCR. Thus HotStarTaq DNA Polymerase is well suited to such highly sensitive PCR assays.

Nested PCR

If PCR sensitivity is too low, a nested PCR method can increase PCR product yield. Nested PCR involves two rounds of amplification reactions. The first-round PCR is performed according to the PCR Protocol using HotStarTaq DNA Polymerase. Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a 1-in-10^3–10^4 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 and one first-round primer in the second PCR; this method is referred to as semi-nested PCR.

Single-cell PCR

HotStarTaq DNA Polymerase has been shown to successfully amplify a single-copy gene from just a single cell. The recommendations provided in Table 14 are intended to serve as a starting point for performing such a single-cell PCR from genomic template DNA. If the PCR product is undetectable or the product yield is too low, perform a nested PCR.

Table 14. Recommendations for Single-Cell PCR

Isolation and storage of single cells:

- Single cells may be isolated by different methods (e.g., by flow cytometry or by micromanipulation).
- Keep samples cool during the cell-isolation procedure to prevent DNA degradation.
- Transfer cell into a PCR tube that has been filled with 20 µl of 1x PCR Buffer. **Immediately freeze the sample on dry ice.**
- Store cell at –80°C until required for PCR analysis.

PCR setup:

- Prepare a fresh master mix for single-cell PCR (see below).
- Thaw the cells on ice.
- Distribute 30 µl of the master mix into each PCR tube, and place the tubes in the thermal cycler. Immediately start the cycling program with a 10 min incubation step at 95°C to activate HotStarTaq DNA Polymerase for single-cell PCR. 50 cycles of PCR may be required to amplify a single-copy gene in one round of PCR.

Master mix preparation:

Prepare a master mix that has a final volume of 30 µl per PCR, as detailed below.

- Addition of carrier nucleic acid is usually required (e.g., *E.coli* 5S rRNA).
- Use polyacrylamide gel- or HPLC-purified primers only.

Component	Volume/reaction	Final concentration
10x PCR Buffer*	3 µl	1x
25 mM $MgCl_2$	Variable	–
10 mM dNTP	1 µl	200 µM of each dNTP
Primer A	Variable	0.2 µM
Primer B	Variable	0.2 µM
5S ribosomal RNA (*E.coli*)	Variable	50 ng/reaction
HotStarTaq DNA Polymerase	1 µl	5 units/reaction
RNase-free water	Variable	–
Single cell in 1x PCR Buffer	**20 µl**	–
Total volume	**50 µl**	–

* Contains 15 mM $MgCl_2$

Multiplex PCR

Multiplex PCR is a demanding technique that requires extensive optimization of the amounts of DNA polymerase, $MgCl_2$, additional reagents, and primers. Often, cycling parameters need to be changed. In many cases, results are still disappointing and further optimization is required. QIAGEN now offers the QIAGEN® Multiplex PCR Kit, which eliminates the need for optimization, making the development of multiplex PCR assays both simple and fast. The QIAGEN Multiplex PCR Kit is the first kit specifically developed for multiplex PCR and provides an easy-to-use master-mix format. QIAGEN Multiplex PCR Master Mix contains pre-optimized concentrations of HotStarTaq DNA Polymerase and $MgCl_2$, plus dNTPs and an innovative PCR buffer specially developed for multiplex PCR. The new PCR buffer contains a balanced combination of salts and additives, which enables comparable efficiencies for annealing and extension of all primers in the reaction. The kit is highly suited for multiplex PCR applications such as typing of genetically modified animals and plants, microsatellite analysis, determination of bacteria and viruses, or amplification of regions carrying SNPs. For more information about the QIAGEN Multiplex PCR Kit, contact your local QIAGEN Technical Services or distributor (see back cover).

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 reaction (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 reverse transcription 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 15, page 35.

Table 15. 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® Reverse Transcriptase 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 oligo dT primer, 12–18mer (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 to 95°C for 5 min to inactivate the reverse transcriptase.
PCR:	■ Prepare a PCR mixture following steps 1–3 in 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 resin 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: 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 cycle should be 5–10°C above the T_m of the primers. In subsequent cycles, the annealing temperature is decreased in steps of 1–2°C/cycle until a temperature is reached that is 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 G: 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. Gel loading reagent and tracking dyes are effectively removed with the QIAquick and MinElute system. For more information about QIAquick and MinElute products, please call QIAGEN Technical Services or your local distributor (see back cover) or visit www.qiagen.com .

Appendix H: 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 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.
- Prepare and freeze small aliquots of primer solutions and dNTP mix. Use of fresh distilled water 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.

* Most commercial bleach solutions are approximately 5.25% sodium hypochlorate.

† 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.

General chemical precautions

- PCR stock solutions can also 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.
- Another approach to preventing amplification of contaminating DNA is to 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.

Ordering Information

Product	Contents	Cat. no.
HotStarTaq DNA Polymerase (250 U)	250 units HotStarTaq DNA Polymerase, PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203203
HotStarTaq DNA Polymerase (1000 U)	4 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203205
HotStarTaq DNA Polymerase (5000 U)	1 x 5000 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203207
HotStarTaq DNA Polymerase (25000 U)	100 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203209
HotStarTaq Master Mix (250 U)	3 x 0.85 ml HotStarTaq Master Mix, containing 250 units HotStarTaq DNA Polymerase total and providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP; 2 x 1.7 ml distilled water	203443
HotStarTaq Master Mix Kit (1000 U)	12 x 0.85 ml HotStarTaq Master Mix, containing 1000 units HotStarTaq DNA Polymerase total and providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP; 8 x 1.7 ml distilled water	203445
HotStarTaq Master Mix Kit (2500 U)	1 x 25 ml HotStarTaq Master Mix, containing 2500 units HotStarTaq DNA Polymerase total and providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP; 8 x 1.7 ml distilled water	203446

Ordering Information

Product	Contents	Cat. no.
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
TopTaq DNA Polymerase (1000)	1000 units TopTaq DNA Polymerase, 10x PCR Buffer†, CoralLoad Concentrate, 5x QSolution, 25 mM $MgCl_2$	200205
Related Products		
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
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
Taq DNA Polymerase (250 U)*	250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	201203
QIAGEN Multiplex PCR Kit (100)*	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM MgCl2, 3 x 0.85 ml), 5x Q-Solution (1 x 2.0 ml), distilled water (2 x 1.7 ml)	206143
Omniscript RT Kit (10)*	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205110
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205211

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

† Contains 15 mM $MgCl_2$.

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

Ordering Information

Product	Contents	Cat. no.
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
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
QIAxcel DNA Large Fragment Kit (600)	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
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
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
QIAquick Gel Extraction Kit — For gel extraction or cleanup of DNA (70 bp to 10 kb) from enzymatic reactions		
QIAquick Gel Extraction Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704

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

Ordering Information

Product	Contents	Cat. no.
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy Plant Mini Kit (50)*	50 DNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304

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.

Purchase of this product is not accompanied by a license under patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd. No rights to real-time PCR or to any primers, probes or pathogens are conveyed expressly, by implication or by estoppel.

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

Notes

Trademarks: QIAGEN®, QIAamp®, QIAprep®, QIAquick®, CoralLoad®, DNeasy®, HotStarTaq®, MinElute®, Oligotex®, Omniscript®, ProofStart®, Q-Solution™, RNeasy® (QIAGEN Group); TopTaq™ (QIAGEN Group); Nonidet® (Shell Chemicals); pBluescript® (Stratagene Inc.); Tween® (ICI Americas Inc.).

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the HotStarTaq DNA Polymerase and HotStarTaq PCR Master Mix Kit to the following terms:

1. The HotStarTaq DNA Polymerase and HotStarTaq Master Mix Kit DNA Polymerase and HotStarTaq Master Mix Kit may be used solely in accordance with the HotStarTaq PCR Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the HotStarTaq PCR Handbook and additional protocols available at www.qiagen.com .
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see www.qiagen.com .

www.qiagen.com

Australia ▪ Orders 1-800-243-800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Orders 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-926 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 ▪ Offers 01-60-920-928

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789-544 ▪ Fax 02-334304-826 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-436

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 1800-742-4362 ▪ Fax 65-6854-8184 ▪ Technical 1800-742-4368

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)

QIAGEN

1064914 10/2010

Sample & Assay Technologies