January 2019

UCP HiFidelity PCR Kit Handbook

Ultra-clean production master mix for high-fidelity hot-start PCR and microbiome applications

Sample to Insight

QIAGEN

Contents

Kit Contents

UCP HiFidelity PCR Kit	**(100)**	**(500)**
Catalog no.	**202742**	**202744**
Number of reactions	**100**	**500**
UCP HiFidelity PCR Master Mix, 2x	1300 µl	5 x 1300 µl
UCP template tracer, 25x	200 µl	2 x 200 µl
UCP master mix tracer, 125x	50 µl	50 µl
UCP water	1.9 ml	4 x 1.9 ml
Quick-Start Protocol	1	1

Storage

The UCP HiFidelity PCR Kit is shipped on dry ice and should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. When the kit is stored under these conditions and handled correctly, performance is guaranteed until the expiration date printed on the kit label. The UCP HiFidelity PCR Master Mix can also be stored at 2–8°C for up to 6 months or the expiration date printed on the kit label. UCP (ultra-clean production) reagents are depleted of nucleic acids, and appropriate measures should be taken to prevent any contamination during storage or use.

If desired, the UCP master mix tracer can be added to the UCP HiFidelity PCR Master Mix for long-term storage. For details, see section on "Adding UCP master mix tracer to the UCP master mix" and Table 1 (page 9).

Intended Use

The UCP HiFidelity PCR Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of UCP HiFidelity PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Product Specifications

The UCP HiFidelity PCR Kit contains the following:

Component	Description
UCP HiFidelity PCR Master Mix, 2x	Contains UCP HiFidelity PCR Buffer and additives that enable fast cycling and direct loading of the reactions onto agarose gels. Also contains ultra-clean HiFidelity DNA polymerase and dNTP-Mix. UCP master mix is produced under ultra-clean production standards and is further depleted of potentially contaminating DNA.
UCP master mix tracer, 125x	Orange dye allows tracking of master mix addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 50 bp on a 1% agarose gel. UCP master mix tracer is produced under ultra-clean production standards and is further depleted of potentially contaminating DNA.
UCP template tracer, 25x	Blue dye allows tracking of template DNA addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 4000 bp on a 1% agarose gel. UCP template tracer is produced under ultra-clean production standards and is further depleted of potentially contaminating DNA.
UCP water	Ultrapure quality, PCR-grade product depleted of potentially contaminating DNA and filled under ultra-clean production standards.

Introduction

The UCP HiFidelity PCR Kit provides a convenient format for highly sensitive and specific hot-start PCR that need to have low PCR-error rates using any DNA or cDNA template.

Dedicated processes are implemented to enable ultra-clean production of this master mix; these include depletion of potential DNA background to a minimum. Combined with superior inhibitor resistance and minimized GC bias, this makes the kit an ideal choice for microbiome applications such as 16S or 18S amplification. This is complemented with a 70 times higher fidelity compared to Taq polymerase, thus enabling error-free amplification, as needed during cloning, amplicon-sequencing or analysis of genome-editing experiments. Additional features include visual pipetting controls, a fast cycling protocol, room-temperature stability during and after reaction setup and the ability to amplify long amplicons.

UCP HiFidelity PCR Kit components

- UCP HiFidelity PCR Master Mix
  - This ready-to-use master mix reduces hands-on time and contamination risk, because it already contains an ultra-clean HiFidelity DNA polymerase, a sophisticated PCR buffer and dNTPs. The enzyme's proofreading feature provides a significantly improved fidelity that is 70 times higher compared to standard Taq polymerases. Both the polymerase activity and the exonuclease activity are stringently controlled by specific antibodies, thus preventing premature activity or damage and nonspecific annealing of template and primers during reaction setup. At low temperatures, the enzyme's polymerase and exonuclease activities are kept in an inactive state, thus preventing any enzymatic activity at ambient temperatures and until heat activation at 95°C. The high bench and on-board stability makes the kit perfectly suitable for high-throughput applications, and the performance features and high fidelity allow easy integration into various workflows. In addition, the

UCP master mix is depleted of potentially contaminating DNA and has been tested for fungal and bacterial nucleic acids.

- UCP master mix tracer, UCP template tracer and UCP water
  - The blue and orange dyes in the UCP template tracer and in the UCP master mix tracer, respectively, allow visual tracking of pipetted samples during PCR setup to prevent errors. When template colored with the blue dye is added to orange PCR master mix, the color changes to green, confirming that the sample was added. The use of these tracers is optional.
  - The blue UCP template tracer is provided as a 25x concentrate and should be diluted to obtain a 1x final concentration in the sample.*
  - The orange UCP master mix tracer is provided as a 125x concentrate and can be added directly to the master mix vial to obtain a 1x final concentration.† These tracers do not reduce sample stability or PCR performance.
  - Reactions can be directly loaded onto agarose gels after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking of the subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.
  - Additionally, UCP PCR-grade water is provided. All UCP components are depleted of potentially contaminating DNA and filled under ultra-clean production standards.

UCP HiFidelity PCR Kit procedure

The UCP HiFidelity PCR Kit allows fast and easy PCR setup. Whatever the application – gene cloning, site-directed mutagenesis or microbiome studies – you simply need to mix all components together in one tube and start the thermal cycler program (Figure 1, page 8).

* Example: Add 0.2 µl of the blue UCP Template Tracer (25x) to 5 µl of sample before use. If pipetting volumes are too small to handle, the UCP Template Tracer can be prediluted using DNA-free water. In this example, 2 µl of 1:10 prediluted UCP Template Tracer could be added.

† Example: Add 10 µl of the UCP master mix tracer (125x) to 1 tube (1300 µl) of UCP HiFidelity PCR Master Mix (2x). Since the amount of UCP master mix tracer that is added is very small, the concentration of the UCP master mix will not be changed and the UCP HiFidelity PCR Master Mix can be used as indicated in the protocol.

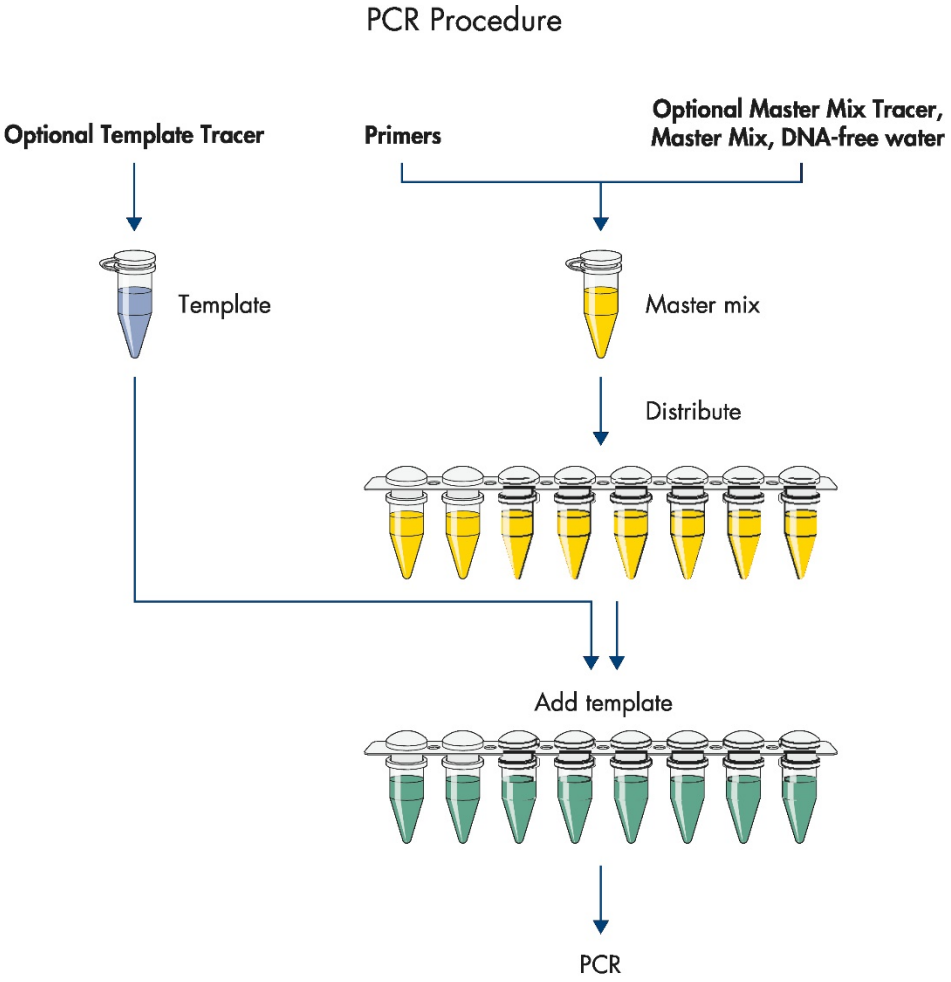

Figure 1. UCP HiFidelity PCR procedure using tracer dyes.

Adding UCP master mix tracer to the UCP master mix

The orange UCP master mix tracer can be added directly to the UCP HiFidelity PCR Master Mix for long-term storage. Since the amount of tracer added is very small (10 µl per 1300 µl of UCP master mix), the concentration of the master mix will not be changed and the master mix can be used as indicated in the protocol (Table 1).

Table 1. Addition of PCR Master Mix Tracer to the UCP Multiplex Master Mix

Volume of UCP HiFidelity PCR Master Mix, 2x	Volume of UCP master mix tracer
1300 µl	10 µl

Special Recommendations for Microbiome Analysis

Preventing unwanted DNA contamination is a key challenge during the analysis of metagenome information using 16S or 18S sequencing. We suggest setting up PCR reactions in a clean PCR cabinet. All surfaces should be UV decontaminated prior to working. All accessories used, e.g., pipettes, racks and PCR disposables, should be dedicated for this particular use and should remain in the PCR cabinet. Please refer to standard publications for further recommendations.

In addition, we recommend including no-template controls (NTCs) and using as much template input and as few PCR cycles as possible. This will help prevent PCR bias. However, in case of small sample amounts or low DNA content, cycle numbers may be increased up to 40 because the background signal from the UCP master mix is exceptionally low.

Equipment and Reagents to Be Supplied by User

The UCP HiFidelity PCR Kit is designed to be used with gene-specific primer sets as well as degenerate primer pools.

- Microcentrifuge tubes or PCR strips
- PCR tubes or plates
- Tubes such as LoBind® (Eppendorf®) or MAXYMum Recovery® (Axygen®) tubes
- Thermal cycler
- Microcentrifuge
- Vortexer
- Pipettes and pipette tips
- UV cabinet (recommended for microbiome applications)

Protocol: HiFidelity PCR Using Standard or Microbial Primer Sets

Important points before starting

- The protocol has been optimized for 0.01 pg – 1 µg of total DNA.
- UCP HiFidelity PCR Kits are designed to be used with a final primer concentration of 0.25 µM for each primer. For ease of use, we recommend preparing a 20x primer mix containing target-specific primers. A 20x primer mix consists of 5 µM forward primer and 5 µM reverse primer in UCP water or TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primers.
- The master mix can also be used for cloning applications, such as site-directed mutagenesis, overlap extension PCR or colony PCR. Dedicated protocols can be found in the appendix section, or visit **www.qiagen.com** for most recent application notes.
- DNA polymerase contained in the UCP master mix requires a heat-activation step of 30 s at 98°C.
- It is not necessary to keep PCR tubes on ice, because nonspecific DNA synthesis cannot occur at room temperature, due to the inactive state of the polymerase.

Procedure

1. Thaw UCP master mix, template DNA or cDNA, primer solutions, UCP water, UCP template tracer (optional) and UCP master mix tracer (optional).

   Mix thoroughly before use by vortexing. For multiplex reactions, we recommend preparing a combined primer mix prior to PCR setup.

2. Prepare a reaction mix according to Table 2. The reaction mix contains all components except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed. It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template) should be included in every experiment.

Table 2. Reaction setup for the UCP HiFidelity PCR Kit

Component	Volume/reaction	Final concentration
UCP HiFidelity Master Mix, 2x	12.5 µl	1x
20x primer mix*	1.25 µl	0.25 µM for each primer
UCP water	Variable	–
Optional: UCP master mix tracer, 125x	0.1 µl	1x
Template DNA (added at step 4)	Variable	0.01 pg – 1 µg/reaction
Total reaction volume	25 µl†	

* A 20x primer mix consists of 5 µM forward primer and 5 µM reverse primer in DNA-free TE buffer or UCP water for each target. Up to 3 primer pairs can be used with this master mix in one reaction.

† For PCR in a 384-well plate, we recommend a final reaction volume of 15 µl. Reduce pipetting volumes accordingly.

3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times or by vortexing for a few seconds. Dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
4. Add template DNA (1 µg – 10 fg per reaction, depending on target abundance) to the individual PCR tubes. The UCP HiFidelity Mix Kit can be used with genomic DNA, cDNA, plasmid DNA, oligonucleotides and other DNA molecules as template. If using cDNA as template, the input volume from undiluted cDNA reaction should not exceed 10% of the total PCR reaction volume.

5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in tables 3 (below) and 4 (page 14).
6. Place the PCR tubes or plates in the thermal cycler and start the PCR program.

   Note: After amplification, samples can be stored at –30 to –15°C for long-term storage.
7. We have evaluated several hints and guides. See appendices A–F.

Table 3. UCP HiFidelity PCR Kit cycling conditions for targets up to 1 kb

Step	Time	Temperature	Comments
Initial PCR activation	30 s	98°C	This heating step activates the DNA polymerase.
3-step cycling			
Denaturation	10 s	98°C	Do not exceed this temperature.
Annealing	10 s	61°C	Approximately 3°C above *T*m of primers.
Extension	15 s	72°C	For PCR products up to 1000 bp, an extension time of 15 s is sufficient. For bigger amplicons, an extension time of 15–30 s/kb shall be used.
Final extension	2 min	72°C	
Number of cycles	≤40		The optimal cycle number depends on the amount of template and the abundance of the target.

Note: The protocol in Table 4 is recommended for amplicons longer than 1 kb or for low-grade multiplex reactions of samples using primer panels with low complexity. In case of high primer *T*m, 2-step cycling can be used instead.

Table 4. UCP HiFidelity PCR Kit cycling conditions for longer amplicons or low-grade multiplex

Step	Time	Temperature	Comments
Initial PCR activation	30 s	98°C	This heating step activates the DNA polymerase.
3-step cycling			
Denaturation	10 s	98°C	Do not exceed this temperature.
Annealing	10 s	61°C	Approximately 3°C above *T*m of primers.
Extension	5 min	72°C	This protocol will work for amplicons of 9 kb. For shorter amplicons, an extension time of 15–30 s/kb shall be used.
Final extension	2 min	72°C	
Number of cycles	≤40		The optimal cycle number depends on the amount of template, the abundance of the target and the application.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Little or no product	
a) Pipetting error or missing reagent	Check the concentrations and storage conditions of reagents, including primers. Repeat the PCR.
b) DNA polymerases not activated	Ensure that the cycling program included the DNA polymerase activation step (30 seconds at 98°C) as described in the cycling protocols (pages 13 and 14).
c) Primer concentration not optimal or primers are degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.2–1.0 µM in 0.1 µM increments.
d) PCR conditions not optimal	Follow the protocol described in Appendix B (page 20).
e) Problems with starting template	Check the concentration, integrity, purity and storage conditions of the starting template (see Appendix A, page 18). If necessary, make new serial dilutions of template DNA 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+} by adding $MgCl_2$ in 0.5 mM increments to the reaction. Do not exceed 3 mM additional $MgCl_2$.
g) Insufficient number of cycles	Increase the number of cycles in increments of 5.

	Comments and suggestions
h) Incorrect annealing temperature or time	Decrease annealing temperature in 2°C increments. Annealing time should be between 10 and 30 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix C, page 27).
i) Incorrect denaturing temperature or time	Denaturation should take place at 98°C for 5–10 seconds. Ensure that the cycling program included the DNA polymerase activation step (30 seconds at 98°C) as described in the cycling protocols (page 11).
j) Insufficient starting template	Increase the template amount used.
k) Primer design not optimal	Review primer design (see Appendix B, page 20). Only use gene-specific primers.
l) Amplifying long fragments	Increase the concentration of template DNA.
m) Reactions overlaid with mineral oil when using a thermal cycler with a heated lid	When using a thermal cycler with a heated lid that is switched on, do not overlay the reactions with mineral oil, because this may decrease the yield of PCR products.
n) Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been programmed correctly.
o) Enzyme concentrations too low	When using UCP master mix, use 12.5 µl master mix per 25 µl reaction.
p) Extension time too short	Increase the extension time in increments of 10 seconds.
Product is multibanded	
a) PCR annealing temperature is 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 C, page 27).
b) Primer concentration not optimal or primers degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.25–1.0 µM in 0.1 µM increments.

	Comments and suggestions
c) Primer design not optimal	Review primer design (see Appendix B, page 20). Use only target-specific primers.
d) Contamination with genomic DNA	Pretreat starting cDNA template with DNase I. Alternatively, use primers located at splice junctions of the target mRNA to avoid amplification from genomic DNA (see Appendix B, page 20).
Product is smeared	
a) Too much starting template	Check the concentration of the starting template (see Appendix A, page 18). If necessary, make new serial dilutions of template DNA from stock solutions. Repeat the PCR using the new dilutions.
b) Carryover contamination	If negative controls (without template) show PCR products or smears, change all reagents. Use disposable pipette 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) Too many cycles	Reduce the number of cycles in increments of 3.
d) Primer concentration not optimal or primers degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.25–1.0 µM 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 20). Use only target-specific primers.
Contamination of 16S PCR	
a) Contamination of NTCs for 16S PCRs	PCR amplification of 16S sequences are especially prone to contamination through operator or environmental causes. To reduce contamination risk, please follow the process recommendations outlined in "Special Recommendations for Microbiome Analysis" (page 9) and in standard publications. In case of positive NTCs, start with replacing single components, e.g., water or primers. In general, we recommend aliquoting reagents and using dedicated pipettes and disposables.

Appendix A: Starting Template

Both the quality and the quantity of nucleic acids used as template affect PCR, particularly 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 compared to single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR. Examples include the QIAprep® system, for rapid plasmid purification, and the QIAamp® and DNeasy® systems, for rapid purification of genomic DNA and viral nucleic acids. Other kits are designed for microbiome research or have undergone ultra-clean production procedures (see "Ordering Information", page 32). For more information about QIAprep, QIAamp and DNeasy products, contact please contact QIAGEN Technical Support at **support.qiagen.com**.

Quantity of starting template

The efficiency with which primers anneal to templates is an important factor in PCR. Due 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 5 and 6 (page 19).

Table 5. 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.

Table 6. 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 bp	1.67	1.0×10^{12}
Genomic DNA	**Size**	**pmol/µg**	**Molecules/µg**
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

Designing primers

When designing primers for multiplex PCR, note the following points:

- Avoid complementarity of 2 or 3 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 and/or C bases at the 3' end
- Avoid complementary sequences within primers and between primer pairs
- Ensure primers are unique to your template sequence. Check similarity to other known sequences with BLAST® (**www.ncbi.nlm.nih.gov/blast/Blast.cgi**).
- Commercially available computer software (e.g., OLIGO 6) or web-based tools such as Primer3 (**frodo.wi.mit.edu/primer3/**) can be used for primer design.

Annealing temperatures

If necessary, perform a gradient PCR to determine the optimal annealing temperature. Otherwise, use the recommendations in Table 7.

Table 7. Recommended annealing temperatures for multiplex PCR

Lowest primer T_m	Annealing temperature
<60°C	Perform gradient PCR over the range of 48–60°C
60–66°C	53–63°C

Distinguishing individual PCR products

Depending on the method of detection, primers should be chosen so that the corresponding PCR products can be easily distinguished from one another (e.g., through size differences), by using primers labeled with different fluorescent dyes or by other appropriate detection procedures.

Successful PCRs

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 8 (below).

Table 8. Guidelines for designing, handling and storing primers

Feature	Description
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 PCR annealing temperatures may be above or below the estimated *T*m. As a starting point, use an annealing temperature 5°C below *T*m. Functionality and specificity of all primer pairs should be checked in individual reactions before combining them in a multiplex PCR assay.

Table continues on next page

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Feature	Description
Location	If detecting mRNA after conversion into cDNA, design primers so that a half of the primer hybridizes to the 3' end of one exon and the other half to the 5' end of the adjacent exon (see Figure 2A, page 24). Primers will anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA. Thus, amplification of contaminating DNA is eliminated. Alternatively, PCR primers should be designed to flank a region that contains at least one intron (see Figure 2B, page 24). Products amplified from cDNA (no introns) will be smaller than those amplified from genomic DNA (containing introns). Size difference in products is used to detect the presence of contaminating DNA. If only the mRNA sequence is known, choose primer annealing sites that are at least 300–400 bp apart. It is likely that fragments of this size from eukaryotic DNA contain splice junctions. As explained in the previous point and Figure 2B (page 24), such primers can be used to detect DNA contamination.
Sequence	Avoid complementarity of two or more 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 three or more G or C nucleotides 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 primers of a primer pair. Commercially available computer software can be used for primer design.

Table continues on next page

Table continued from previous page

<table>
<tr><th>Feature</th><th>Description</th></tr>
<tr><td>Concentration</td><td>Spectrophotometric conversion for primers:
1 A260 unit ≡ 20–30 µg/ml

Molar conversions:

<table><tr><th>Primer length</th><th>pmol/µg</th><th>12.5 pmol (0.25 µM in 25 µl)</th><th>10 pmol (0.25 µM in 20 µl)</th></tr><tr><td>18mer</td><td>168</td><td>37 ng</td><td>30 ng</td></tr><tr><td>20mer</td><td>152</td><td>42 ng</td><td>34 ng</td></tr><tr><td>25mer</td><td>121</td><td>52 ng</td><td>41 ng</td></tr><tr><td>30mer</td><td>101</td><td>62 ng</td><td>50 ng</td></tr></table>
Use 0.25–1.0 µM of each primer in PCR. For most applications, a primer concentration of 0.25 µM will be optimal.</td></tr>
<tr><td>Storage</td><td>Lyophilized primers should be dissolved in a small volume of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to make a concentrated stock solution. Prepare small aliquots of working solutions containing10 pmol/µl to avoid repeated thawing and freezing. For microbiome studies, dissolving in DNA-free water should be considered to prevent DNA intake into the TE buffer used.

Store all primer solutions at –30 to –15°C.</td></tr>
</table>

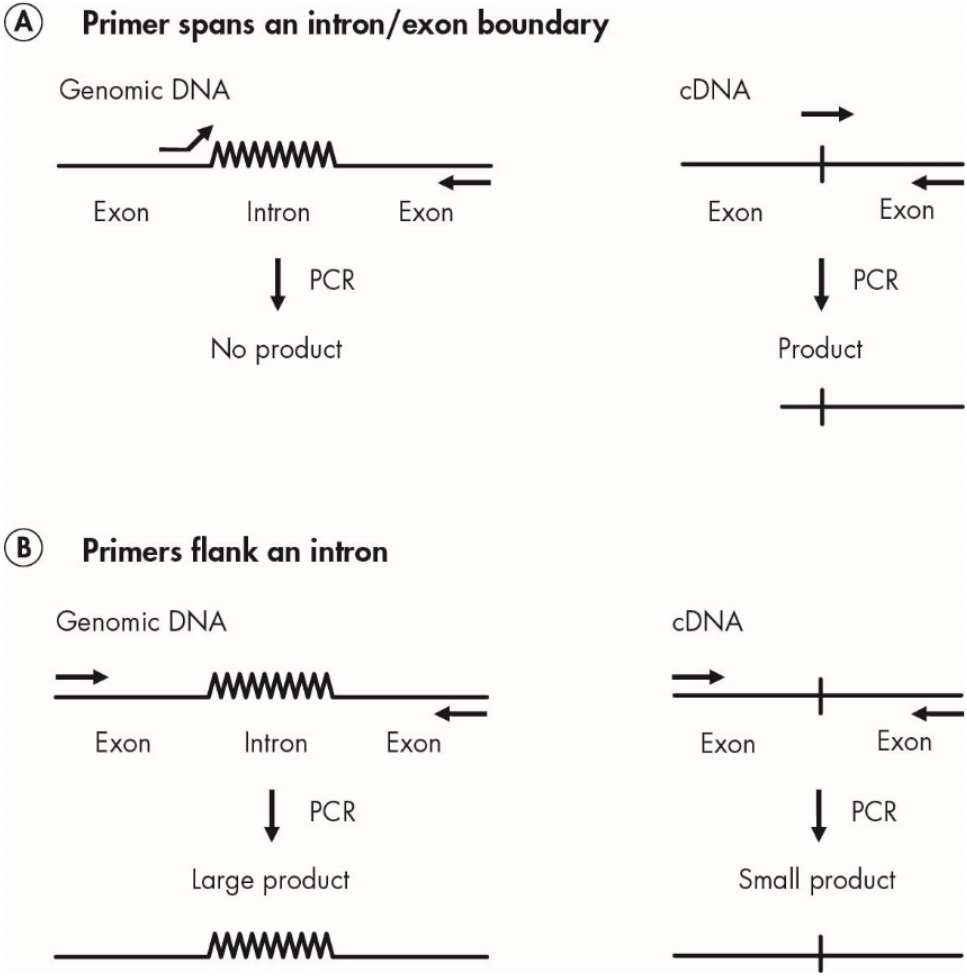

Figure 2. Designing primers for PCR. Primer is designed to **(A)** eliminate or **(B)** detect amplification from contaminating genomic DNA.

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 or when a family of closely related sequences is to be amplified. To amplify such templates using PCR, degenerate primers can be used. These are actually mixtures of several primers, the sequences of which differ at the positions that correspond to the uncertainties in the template sequence.

Hot-start PCR, as enabled by the UCP PCR Kit, often improves amplification specificity in PCR using degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. Table 9 (below) gives recommendations for optimizing PCR using degenerate primers. To help determine the best location for degenerate primers, Table 10 (page 26) lists the codon redundancy of each amino acid.

Table 9. Guidelines for the design and use of degenerate primers

	Description
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.
PCR conditions	When optimizing two-step PCR using degenerate primers, modify PCR conditions in the following order:
Primer concentration	First try a primer concentration of 0.25 µM. If this primer concentration results in poor PCR amplification, increase the primer concentration in increments of 0.2 µM until satisfactory results are obtained.
Template concentration	Increase starting template amount (up to 1 µg).

Table continues on next page

Table continued from previous page

<table>
<tr><th></th><th>Description</th></tr>
<tr><td>Annealing temperature</td><td>Reduce annealing temperature in steps of 2°C.</td></tr>
<tr><td>Concentration</td><td>Spectrophotometric conversion for primers:
1 A260 unit ≡ 20–30 µg/ml.
Molar conversions:
<table>
<tr><th>Primer length</th><th>pmol/µg</th><th>12.5 pmol (0.25 µM in 25 µl)</th><th>10 pmol (0.25 µM in 20 µl)</th></tr>
<tr><td>18mer</td><td>168</td><td>37 ng</td><td>30 ng</td></tr>
<tr><td>20mer</td><td>152</td><td>42 ng</td><td>34 ng</td></tr>
<tr><td>25mer</td><td>121</td><td>52 ng</td><td>41 ng</td></tr>
<tr><td>30mer</td><td>101</td><td>62 ng</td><td>50 ng</td></tr>
</table>
Use 0.25–1.0 µM of each primer in PCR. For most applications, a primer concentration of 0.25 µM will be optimal.</td></tr>
</table>

Table 10. 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: 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 DNA polymerase and PCR buffer in the UCP master mix increases specificity both at the start of and during PCR. Thus, the UCP master mix is well suited to such challenging and highly sensitive PCR assays.

Touchdown PCR

Touchdown PCR uses a cycling program with varying annealing temperatures. It is a useful method for increasing the specificity of PCR. The annealing temperature in the initial PCR cycle should be 5–10°C above the 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 that is equal to the T_m of the primers or 2–5°C below it. 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, refer to the manufacturer's instructions.

Appendix D: Colony PCR

Colony PCR is a useful tool to be used during molecular cloning to verify integration of the insert into the plasmid backbone. The advantage is, that transformed *E. coli* colonies can be analyzed quickly while a liquid culture is prepared from this colony in parallel. A standard protocol for colony PCR is described below. For more information please refer to standard literature (e.g., *Molecular Cloning: A Laboratory Manual*, by Sambrook et al.).

1. Transform your ligation, mutagenesis reaction or sample into competent *E. coli* cells and perform selection using LB-agar plates with appropriate selection antibiotic at 37°C for at least 16 h.
2. On the next day, prepare PCR reaction strips with 10 µl PCR-grade water per tube.
3. Use a sterile pipette tip to remove part of the colony from the plate. Take care not to carry over LB-agar.
4. Swirl the tip briefly in one PCR tube containing water, and then immediately transfer the tip to an appropriate culture tube containing at least 2 ml of growth medium with the respective selection antibiotic.
5. After all colonies have been sampled and appropriate control reactions have been prepared (e.g., NTC, vector of origin as positive control), add 12.5 µl master mix and primers as described below (Table 11).

Table 11. Reaction setup for resuspended colonies

Component	Volume/reaction	Final concentration
UCP HiFidelity Master Mix, 2x	12.5 µl	1x
20x primer mix*	1.25 µl	0.25 µM for each primer
UCP water	Variable	–
Optional: UCP master mix tracer, 125x	0.1 µl	1x
Template suspension (added at step 4)	10 µl	Variable
Total reaction volume	25 µl	

* A 20x primer mix consists of 5 µM forward primer and 5 µM reverse primer in DNA-free TE buffer or UCP water for each target. Up to 3 primer pairs can be used with this master mix in one reaction.

6. Close the PCR tubes and cycle as indicated below. Note that a longer initial denaturation step was added to sufficiently release bacterial DNA from the cells.

Table 12. UCP HiFidelity PCR Kit cycling conditions for colony PCR

Step	Time	Temperature	Comments
Initial PCR activation	3 min	95°C	This heating step activates the DNA polymerase.
3-step cycling			
Denaturation	10 s	98°C	Do not exceed this temperature.
Annealing	10 s	61°C	Approximately 3°C above *T*m of primers.
Extension	15 s	72°C	For PCR products up to 1000 bp, an extension time of 15 s is sufficient. For bigger amplicons an extension time of 15–30 s/kb shall be used.
Final extension	2 min	72°C	
Number of cycles	≤40		The optimal cycle number depends on the amount of template and the abundance of the target.

7. After PCR is finished, analyze the results using the QIAxcel® Advanced System (cat. no. 9002123) or by gel-based analysis

Appendix E: Purification of PCR Products

After amplification, the PCR sample contains a complex mixture of specific PCR products and residual reaction components such as primers, unincorporated nucleotides, enzymes, salts, mineral oil and possibly nonspecific amplification products. Before the specific PCR products can be used in subsequent experiments, it is often necessary to remove these contaminants.

The QIAquick® and MinElute® systems offer a quick and easy method for purifying the final PCR products. For more information about QIAquick or MinElute products, please visit **www.qiagen.com**.

Appendix F: Controlling Contamination

General control of nucleic acid contamination

It is extremely important to include at least one negative control in every round of PCR. This control contains no added template nucleic acid, thus allowing detection of possible contamination of the reaction components.

General physical precautions

Separate the working areas for setting up PCR amplifications and for RNA and DNA handling, including the addition of starting template, PCR product analysis or plasmid preparation. Ideally, use separate rooms.

Use a separate set of pipettes for the PCR master mix. Use of pipette tips with hydrophobic filters is strongly recommended.

Prepare and freeze small aliquots of primer solutions. Use of DNA-free water is strongly recommended.

In case of contamination, laboratory benches, apparatus and pipettes can be decontaminated by cleaning them with 10% (v/v) commercial bleach solution. Afterwards, the benches and pipettes should be rinsed with distilled water.

For 16S or 18S PCR amplification reactions, we recommend setting up in UV cabinets. All surfaces should be UV decontaminated prior to working. All used accessories – for instance, pipettes, racks and PCR disposables – should be dedicated to this particular use and should remain in the PCR cabinet. Please refer to standard publications for further recommendations.

General chemical precautions

PCR stock solutions can also be decontaminated using UV light. However, this method is laborious, 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.

Ordering Information

Product	Contents	Cat. no.
UCP HiFidelity PCR Kit (100)	For 100 x 25 µl PCR reactions: ultra-clean production master mix for high fidelity hot-start PCR and microbiome applications	202742
UCP HiFidelity PCR Kit (500)	For 500 x 25 µl PCR reactions: ultra-clean production master mix for high fidelity hot-start PCR and microbiome applications	202744
Related Products		
UCP Multiplex PCR Kit (100)	For 100 x 20 µl PCR reactions: ultra-clean production master mix for multiplex hot-start PCR and microbiome applications	206742
UCP Multiplex PCR Kit (100)	For 100 x 20 µl PCR reactions: ultra-clean production master mix for multiplex hot-start PCR and microbiome applications	206744
DNeasy PowerSoil® Pro Kit (50)	For 50 preps: isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: isolation of microbial genomic DNA from all soil types	47016
QIAamp UCP DNA Micro Kit (50)	For 50 preps: ultra-clean DNA purification from small sample volumes	56204
QIAamp UCP Pathogen Mini Kit (50)	For 50 preps: microbial DNA purification from whole blood, swabs, cultures and body fluids	50214

Product	Contents	Cat. no.
RNeasy® UCP Micro Kit (50)	For 50 preps: purification of up to 45 µg total RNA from small or low biomass samples	73934
QIAgility® System HEPA/UV (incl. PC)	Robotic workstation for automated PCR setup (with UV light and HEPA filter), notebook computer and QIAgility software: includes installation and training, 1-year warranty on parts and labor	9001532

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.

Visit **www.qiagen.com/geneXpression** to find out more about standardized solutions for gene expression analysis – from RNA preparation to real-time RT-PCR.

Handbook Revision History

Document	Changes	Date
HB-2617-002	Corrected cat. no. for UCP HiFidelity PCR Kit (500) in Kit Contents. Changed UCP master mix tracer volume in tables 2 and 11, from "0.04 µl" to "0.1 µl".	January 2019

Notes

HB-2617-002 01/2019