February 2020

UltraRun LongRange PCR Kit Handbook

For ultrafast hot-start mediated long-range PCR permitting moderate multiplexing

Sample to Insight

QIAGEN

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Kit Contents

UltraRun LongRange PCR Kit	**(100)**	**(500)**
Catalog no.	**206442**	**206444**
Number of reactions	**100**	**500**
UltraRun LongRange PCR Master Mix, 4x	500 µl	5 x 500 µl
Template Tracer, 25x	200 µl	2 x 200 µl
Master Mix Tracer, 125x	50 µl	50 µl
Q-Solution®, 5x	2 ml	2 ml
PCR-grade water	1.9 ml	4 x 1.9 ml
Quick-Start Protocol	1	1

Storage

The UltraRun LongRange PCR Kit is shipped on dry ice and should be stored immediately upon receipt at –15 to –30°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 UltraRun LongRange 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.

If desired, the Master Mix Tracer can be added to the UltraRun LongRange PCR Master Mix for long-term storage. For details, see section "Adding Master Mix Tracer to the Master Mix" (Table 1, page 8).

Intended Use

The UltraRun LongRange 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 UltraRun LongRange PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Product Specifications

The UltraRun LongRange PCR Kit contains the following:

Component	Description
UltraRun LongRange PCR Master Mix, 4x	Contains UltraRun PCR Buffer and additives that enable fast cycling and direct loading of the reactions onto agarose gels. Also contains a blend of Taq DNA Polymerase and a polymerase with proofreading capability as well as a dNTP-Mix. Antibody-mediated hot-start feature of both polymerase and proofreader activity requires a 3-min, 93°C incubation step.
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.
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
Q-solution	5x concentrated
PCR-grade water	Ultrapure quality, PCR-grade

Introduction

The UltraRun LongRange PCR Kit provides a convenient format for highly sensitive and long amplification using genomic DNA or cDNA as starting material. Due to the unique combination of enzymes and an improved buffer concept, low grade multiplexing of up to 6 amplicons in a range from 1-9Kb is possible.

Additional features include visual pipetting controls, a fast cycling protocol, room-temperature stability during and after reaction setup, and a 4x concentrated master mix format, allowing for higher sample input volumes. Furthermore the reactions can be directly loaded on agarose gels, and the optical tracer dyes serve as gel migration indicators.

UltraRun LongRange PCR Kit components

- UltraRun LongRange PCR Master Mix

  This ready-to-use master mix improves hands-on time and process robustness. It contains a blend of Taq DNA polymerase and a polymerase with proofreading capability, as well as sophisticated PCR buffer optimized for long PCR and dNTP-mix. At low temperatures, both polymerase and proofreader activity are kept in an inactive state by antibodies. This provides a stringent hot-start and prevents any enzymatic activity at ambient temperatures and until heat activation at 93°C, which is particularly important to prevent primer or DNA damage caused by the proofreader activity. Due to this functionality, complete reactions including primers and template are stable at room temperature for longer periods improving process handling. Furthermore, the master mix formulation enables direct load of PCR reactions to agarose gels for analysis. The 4x master mix concentration allows greater flexibility for template input volumes over common 2x concentration master mixes.

- Q-Solution

  The UltraRun LongRange PCR Kit includes Q-Solution, an innovative PCR additive that facilitates amplification of difficult templates by modifying the melting behavior of DNA.

This unique reagent often enables or improves 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 1 working concentration, is nontoxic, and PCR purity is guaranteed. Q-Solution changes the melting behavior of nucleic acids and can be used for PCR systems that do not work well under standard conditions. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution, because it can reduce efficiency of PCR reactions that work well under standard buffer conditions.

- Master Mix Tracer and Template Tracer

  The blue and orange dyes in the Template Tracer and in the 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 sample was added. The use of these tracers is optional.

  The blue Template Tracer is provided as a 25x concentrate and should be diluted to obtain a 1x final concentration in the sample*.

  The orange 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 affect 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.

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

† Example: Add 4 µl of the Master Mix Tracer (125x) to 1 tube (500µl) of UltraRun PCR Master Mix (4x). Since the amount of Master Mix Tracer added is very small, the concentration of the Master Mix will not be changed and the UltraRun PCR Master Mix can be used as indicated in the protocol.

UltraRun LongRange PCR Kit procedure

The UltraRun LongRange PCR Kit allows fast and easy PCR setup at room temperature. Long-range amplification up to 30Kb, multiplexing of up to 6 targets in ranges of 1-9 Kb, or amplification of pan bacterial sequences like 16S-ITS-23S can be performed by simply mixing all components in one tube and starting the thermal cycler program (see Figure 1, page 8).

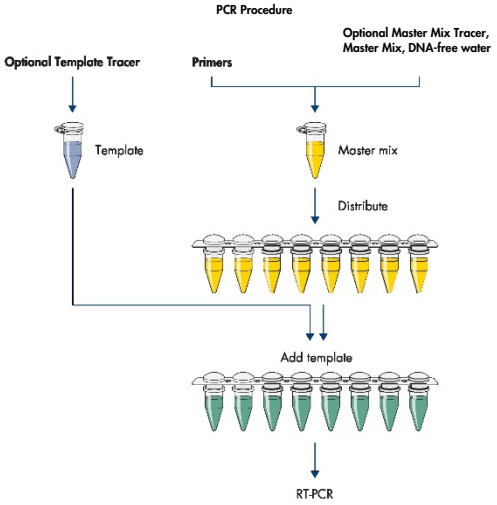

Figure 1. UltraRun LongRange PCR procedure using tracer dyes.

Adding Master Mix Tracer to the Master Mix

The orange Master Mix Tracer can be added directly to the Multiplex PCR Master Mix for long-term storage. Since the amount of tracer added is very small (4 µl per 500 µl of Master Mix), the concentration of the master mix will not be changed and the master mix can be used as indicated in the protocol (see Table 1, page 8).

Table 1. Addition of PCR Master Mix Tracer to the UltraRun LongRange Master Mix

Volume of UltraRun Long Range PCR Master Mix, 4x	Volume of Master Mix Tracer
500 µl	4 µl

Equipment and Reagents to Be Supplied by User

The UltraRun LongRange PCR Kit is designed to be used with gene-specific primers.

- 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: Long Amplicon Generation

Important points before starting

- The protocol has been optimized for 10 pg–1 µg of total DNA.
- The UltraRun LongRange PCR Kit is designed to be used with a final primer concentration of 0.5 µM for each primer. For low-grade multiplexing approaches, the amount may need to be adapted. For ease of use, we recommend preparing a 20x primer mix containing target-specific primers. A 20x primer-mix consists of 10 µM forward primer and 10 µM reverse primer in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primers.
- Depending on the application, up to 6 primer pairs can be used for multiplexed amplification (eg, 4 targets in the range of 2.9 Kb can be multiplexed and amplified in 1 reaction). Higher multiplexing might require adaption.
- The UltraRun LongRange PCR Kit is provided with Q-Solution, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.
- The DNA Polymerase blend in the UltraRun LongRange PCR Master Mix requires a heat-activation step for 3 min at 93°C.
- 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 the polymerases.
- Reactions can be loaded onto agarose gel directly after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking during electrophoresis. The dyes run at about 50 bp (orange dye) or 4000 bp (blue dye) on 1% agarose gel.

Procedure

1. Thaw UltraRun LongRange PCR Master Mix, template DNA or cDNA, primer solutions, water, Q-solution (optional), Template Tracer (optional) and 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 UltraRun LongRange PCR Kit

Component	Volume/reaction	Final concentration
UltraRun LongRange PCR Master Mix, 4x	5 µl	1x
20x Primer Mix*	1 µl	0.5 µM for each primer
PCR-grade water	Variable	–
Optional: Master Mix Tracer, 125x	0.04 µl	1x
Optional: Q-Solution†, 5x	4 µl	1x
Template DNA (added at step 4)	Variable	0.01 ng–1 µg/reaction
Total reaction volume	**20 µl‡**	

* A 20x primer mix consists of 10 µM forward primer and 10 µM reverse primer in TE buffer for each target. Primers can either be pre-mixed and added simultaneously or added separately for each target. If the concentration of the primer mix(es) differ, the respective added volume needs to be adjusted to achieve a final concentration of 0.5 µM for each primer. Up to 6 primer pairs can be multiplexed.

† For templates with GC-rich regions or complex secondary structure.

‡ For PCR in a 384-well plate, we recommend a final reaction volume of 10 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 pg per reaction, depending on target abundance) to the individual PCR tubes. The UltraRun LongRange PCR Kit can be used with genomic DNA, cDNA, plasmid DNA, 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 Table 3 (page 12) and Table 4 (page 13).
6. Place the PCR tubes or plates in the thermal cycler and start the PCR program.
   Note: After amplification, samples can be stored at –15 to –30°C for long-term storage.

   Note: See Appendices A–E (page 17 to 27) for several hints and guides on the use of the kit.

Table 3. UltraRun LongRange PCR Kit cycling conditions: standard 2-step protocol

Step	Time	Temperature	Comments
Initial PCR activation	3 min	93°C	This heating step activates the DNA Polymerase.
2-step cycling:			
Denaturation	30 s	93°C	Do not exceed this temperature.
Annealing/Extension	30 s/kb	65°C*	Use an extension time of 30 s per kilobase DNA for genomic DNA targets.
Final Extension	10 min	72°C	
Number of cycles	≤35		The optimal cycle number depends on the amount of template and the abundance of the target.

* Standard for primers with *Tm* between 58–65°C.

The 3-step cycling may be used in case the annealing temperatures are significantly differing or in case a lower annealing temperature may be beneficial.

Table 4. UltraRun Long Range PCR Kit cycling conditions: 3-step protocol

Step	Time	Temperature	Comments
Initial PCR activation	3 min	93°C	This heating step activates the DNA Polymerase.
3-step cycling:			
Denaturation	30 s	93°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below *Tm* of primers.
Extension	30 s/kb	68°C	Use an extension time of 30 s per kilobase DNA for genomic DNA targets.
Final Extension	10 min	72°C	
Number of cycles	≤35		The optimal cycle number depends on the amount of template and the abundance of the target. For 16S analysis the number should be as low as possible.

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 (3 min at 93° C) as described in the cycling protocols (page 14).
c) Primer concentration is not optimal or primers are degraded	A primer concentration of 0.5 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. For high-grade multiplexing applications, a reduced primer concentration might be needed.
d) Insufficient denaturation of long targets	Increase denaturation time from 30 to 40 or 50 s. Alternatively use Q-solution as described in protocol if secondary structures are too complex to properly melt at longer denaturation steps
e) Problems with starting template	Check the concentration, integrity, purity and storage conditions of the starting template (see Appendix A: Starting Template, page 17). If necessary, make new serial dilutions of template DNA from stock solutions. Repeat the PCR using the new dilutions.
f) Insufficient number of cycles	Increase the number of cycles in increments of 5.
g) Incorrect annealing temperature or time	Decrease annealing temperature in 2°C increments. Annealing time should be between 15 and 30 s. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix C: Sensitive PCR Assays, page 25)

	Comments and suggestions
h) Incorrect denaturing temperature or time	Denaturation should take place at 93°C for 30 s. Ensure that the cycling program included the DNA Polymerase activation step (3 min at 93°C) as described in the cycling protocols (page 12 and 13).
i) Insufficient starting template	Increase the template amount used.
j) Primer design not optimal	Review primer design (see Appendix B: Primer Design, Concentration, and Storage, page 19). Only use gene-specific primers.
k) Amplifying long fragments	Increase the concentration of template DNA.
l) 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, as this may decrease the yield of PCR products.
m) Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been programmed correctly.
n) Enzyme concentrations too low	When using UltraRun Master Mix, use 5 µl Master Mix per 20 µl reaction.
o) Extension time too short	Use 30s/kb of expected amplicon. Increase the extension time in increments of 10 s.
Product is multi-banded	
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 26).
b) Primer concentration not optimal or primers degraded	A primer concentration of 0.5 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.
c) Primer design not optimal	Review primer design (see Appendix B: Primer Design, Concentration, and Storage, page 19). 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: Primer Design, Concentration, and Storage, page 19).
Product is smeared	
a) Too much starting template	Check the concentration of the starting template (see Appendix A: Starting Template, page 17). If necessary, make new serial dilutions of template DNA from stock solutions. Repeat the PCR using the new dilutions.

		Comments and suggestions
b)	Carry-over 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 0.5 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: Primer Design, Concentration, and Storage, page 19). Use only target-specific primers.

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, as 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. For more information about QIAprep, QIAamp and DNeasy products, 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 Table 5 and Table 6 (page 18).

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 (eg, 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.

Table 8. Guidelines for designing, handling and storing primers

Feature	Description
Length	18–30 nucleotides
G/C content	40–60%
Tm:	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.
Location	If detecting mRNA after conversion into cDNA, design primers so that one 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 22). 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 1 intron (see Figure 2B, page 22). 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 22), such primers can be used to detect DNA contamination.

<table>
<tr><th>Feature</th><th>Description</th></tr>
<tr><td>Sequence</td><td>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 3 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.</td></tr>
<tr><td>Concentration</td><td>Spectrophotometric conversion for primers:
1 A_{260} 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 –15 to –30°C.</td></tr>
</table>

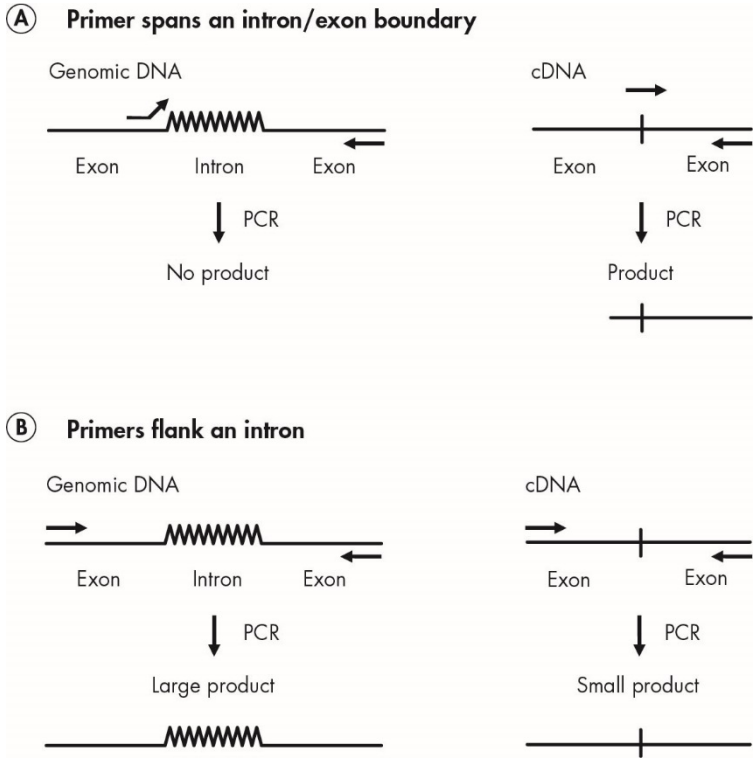

Figure 2. Designing primers for PCR. Primer design to (A) eliminate or (B) detect amplification from contaminating genomic DNA.

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 whose sequences differ at the positions that correspond to the uncertainties in the template sequence.

Hot-start PCR, as enabled by the UltraRun LongRange PCR Kit, often improves amplification specificity in PCR using degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. Table 9 gives recommendations to optimize PCR using degenerate primers. To help determine the best location for degenerate primers, Table 10 (page 24) lists the codon redundancy of each amino acid.

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

<table>
<tr><th></th><th>Description</th></tr>
<tr><td>Sequence</td><td>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.</td></tr>
<tr><td>PCR conditions</td><td>When optimizing two-step PCR using degenerate primers, modify PCR conditions in the following order.</td></tr>
<tr><td>Primer concentration</td><td>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.</td></tr>
<tr><td>Template concentration</td><td>Increase starting template amount (up to 1 µg).</td></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 A_{260} 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 UltraRun LongRange Master Mix increases specificity both at the start of and during PCR. Thus, the UltraRun LongRange 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 to increase 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 steps of 1–2°C per 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, refer to the manufacturer's instructions.

Appendix D: 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 E: Controlling Contamination

General control of nucleic acid contamination

It is extremely important to include at least 1 negative control in every round of PCR. This control contains no added template nucleic acid, which allows detection of possible contamination of the reaction components.

General physical precautions

Separate the working areas for setting up PCR amplifications and 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 example, 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.

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.
UltraRun LongRange PCR Kit(100)	For 100 x 20 µl PCR reactions: master mix kit for ultrafast hot-start mediated long range PCR permitting moderate multiplexing	206442
UltraRun LongRange PCR Kit(500)	For 500 x 20 µl PCR reactions: master mix kit for ultrafast hot-start mediated long range PCR permitting moderate multiplexing	206444
Related products		
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
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(500)	For 500 x 20 µl PCR reactions: ultra-clean production master mix for multiplex hot-start PCR and microbiome applications	206744
AllPrep DNA/mRNA Nano	For 12 parallel isolations of mRNA and gDNA from low biomass input samples	80272
QIAquick PCR Purification Kit – for direct purification of PCR fragments		
QIAquick PCR Purification Kit (50)	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104

Product	Contents	Cat. no.
MinElute PCR Purification Kit – for purification of PCR products (70 bp – 4kb) in low elution volumes		
MinElute PCR Purification Kit (50)	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
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: Ultraclean 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
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.

Notes

Revision History

Date	Changes
02/2020	Initial release

Limited License Agreement for UltraRun LongRange PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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