Quick-Start Protocol March 2016

QIAGEN® LongRange PCR Kit

The QIAGEN LongRange PCR Kit (cat. nos. 206401, 206402 and 206403) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *QIAGEN LongRange PCR Handbook*: **www.qiagen.com/HB-0107**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Choose the most suitable protocol according to amplicon size (see Table 1).
- For cDNA templates, make sure that the RNA used for cDNA synthesis is of sufficiently high quality and is not degraded. For gDNA templates, use high-molecular-weight DNA templates only, with average size >20–50 kb.
- Primers should have annealing temperatures above 60°C.
- Set up all reactions on ice.
- Always use an elongation temperature of 68°C.
- Always perform denaturation at 93°C for 15 s. Do not exceed this temperature.
- Use a final dNTP concentration of 500 µM of each dNTP.
- The QIAGEN 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.

Table 1. Protocol selection according to amplicon size

Size of amplicon	Protocol
0.1–10 kb	Protocol 1: Long-range PCR
>10 kb	Protocol 2: Very long-range PCR

Protocol 1: Long-range PCR (targets up to approximately 10 kb)

1. Thaw 10x LongRange PCR Buffer, dNTP mix, primer solutions, RNase-free water and Q-Solution (optional). Mix thoroughly before use.
2. Prepare a reaction mix according to Table 2.
3. Mix the reaction mix gently but thoroughly, for example by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes.
4. Add template DNA to the individual PCR tubes containing reaction mix, using the recommended amounts shown in Table 3.

Table 2. Reaction setup for long-range PCR and very long-range PCR

Component	Volume/reaction	Final concentration
Reaction mix		
LongRange PCR Buffer, 10x	5 µl	1x*
dNTP mix (10 mM each)	2.5 µl	500 µM of each dNTP
Primer A	Variable	0.4 µM
Primer B	Variable	0.4 µM
RNase-free water	Variable	–
LongRange PCR Enzyme Mix	0.4 µl	2 units/50 µl reaction
Optional: 5x Q-Solution†	10 µl	1x
Template DNA (added at step 4)	Variable	See Table 3
Total reaction volume	50 µl	

* Contains 2.5 mM Mg^{2+}; we strongly recommend starting with this initial Mg^{2+} concentration.

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

Table 3. Amount of template

Type of template	Optimal amount for long-range PCR (0.1–10 kb)	Optimal amount for very long-range PCR (>10 kb)
Human genomic DNA	50–500 ng	100–500 ng
cDNA	50–500 ng	100–500 ng
Bacterial DNA	100 pg–10 ng	100 pg–10 ng
Phage DNA	1–100 ng	1–100 ng
Plasmid DNA	1–20 ng	1–20 ng

Table 4: Cycling conditions for long-range PCR (0.1–10 kb)

Step	Time	Temperature	Comment
Initial activation step	3 min	93°C	
3-step cycling:			
Denaturation	15 s	93°C	Do not exceed this temperature.
Annealing	30 s	62°C	Approximately 5°C below T_m of primers.
Extension	1 min/kb	68°C	1 min per kb DNA for genomic DNA targets.
Number of cycles	35		Will give satisfactory results in most cases.
End of PCR cycling	Indefinite	4°C	

5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 4.
6. Place the tubes immediately into a thermal cycler that is preheated to 93°C and start the cycling program. This simplified hot start ensures PCR specificity.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.
7. Analyze samples using an appropriate detection system such as agarose gel electrophoresis. A TA/UA cloning system can be used for direct cloning of amplified fragments.

Protocol 2: Very long-range PCR (targets longer than 10 kb)

1. Carry out steps 1, 2, 3 and 4 of Protocol 1.
2. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 5.
3. Place the tubes immediately into a thermal cycler that is preheated to 93°C and start the cycling program. This simplified hot start ensures PCR specificity.

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

Table 5. Cycling conditions for very long-range PCR (>10 kb)

Step	Time	Temperature	Comment
Initial activation step	3 min	93°C	
3-step cycling: 38 cycles total*			
First 10 cycles:			
Denaturation	15 s	93°C	Do not exceed this temperature.
Annealing	30 s	62°C	Approximately 5°C below T_m of primers.
Extension	1 min/kb	68°C	1 min per kb DNA for genomic DNA targets.
Next 28 cycles:			
Denaturation	15 s	93°C	Do not exceed this temperature.
Annealing	30 s	62°C	Approximately 5°C below T_m of primers.
Extension	1 min/kb + 20 s in each additional cycle	68°C	1 min per kb DNA for genomic DNA targets.
End of PCR cycling	Indefinite	4°C	

* Amplification for 38 cycles will give satisfactory results in most cases. However, the optimum number of cycles depends on the amount of template DNA.

4. Analyze samples using an appropriate detection system such as agarose gel electrophoresis. For separation of products >25 kb in a standard electrophoresis chamber, a 12-hour run at 40 V is recommended. Generally, efficient separation of very large DNA fragments is achieved only by field inversion or by pulsed field gel electrophoresis. A TA/UA cloning system can be used for direct cloning of amplified fragments.

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