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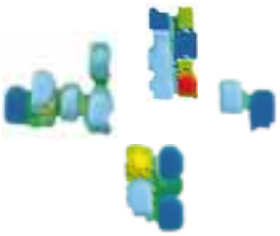


VIII. Optimizing DNA Sequence Quality

Sequencing methods

A detailed description of the equipment, reagents, and conditions used at QIAGEN for automated sequencing is presented in the Appendix. Many of these procedures were derived through comparative studies, some of which are presented below. Others have been taken from the manufacturers' recommendations without further modification. These recommendations do not imply that other equipment, reagents, and conditions should be considered unsuitable.

The automated sequencing results (electropherograms) in this guide have all been obtained with the Applied Biosystems Model 377 or 377XL DNA Sequencers. The general considerations outlined here for optimizing DNA sequencing quality are likely to apply to other types of sequencers, such as the LI-COR and Pharmacia ALF instruments, and their corresponding sequencing chemistries, although this has not been directly confirmed.

A typical sequencing result obtained with a standard primer and dichlororhodamine dye terminator chemistry using optimum template preps is shown in Figure 16. Read lengths in excess of 700 bases were achieved with >99% unedited accuracy.

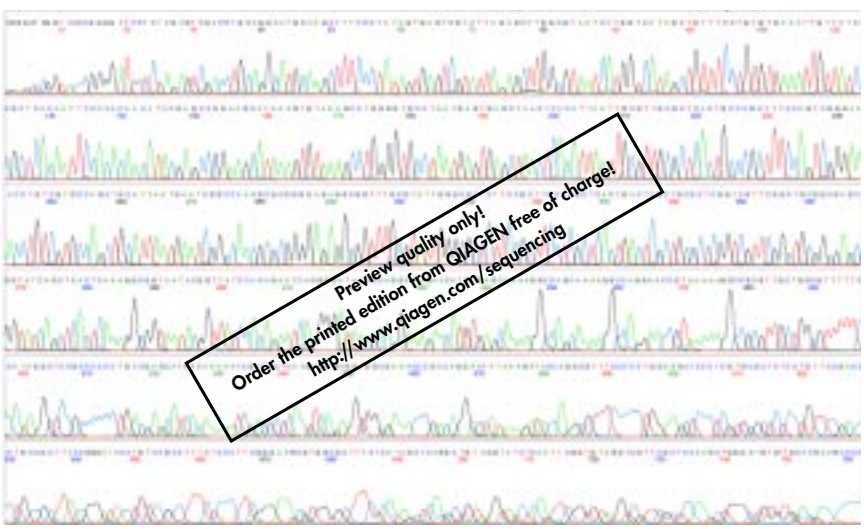
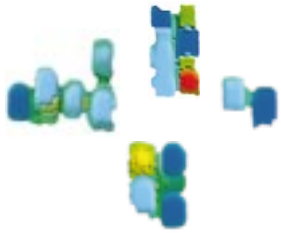


Figure 16. *Typical sequencing result with a 36 cm well-to-read gel. Plasmid pTZ19R was purified using the QIAGEN Plasmid Midi Kit. Sequencing reaction: 0.2 µg plasmid DNA in a Taq FS dRhodamine dye terminator cycle sequencing reaction under standard conditions with the –21M13 primer. Gel: 5% polyacrylamide, 36 cm well-to-read distance, data collection time 9 hours (see Appendix for conditions).*

The quality of the DNA sequence data can be affected by the quality of any of the components of the sequencing reaction, as well as by suboptimal conditions in any one of the three distinct phases of the sequencing process: the enzymatic reaction, electrophoresis, and data collection. The most common factors which limit sequence quality are impure template DNA, incorrect template or primer concentrations, suboptimal primer selection or annealing, and inefficient removal of unincorporated labeled dideoxynucleotides.



The template-primer-polymerase complex

A key factor in the relative success or failure of a sequencing reaction is the number of template-primer-polymerase complexes that are formed in the course of the reaction. The formation of this complex is necessary (but not sufficient) to produce labeled extension products, and many problems with sequencing reactions can be traced to one or more of these elements.

For example, the ability of an oligonucleotide primer to bind to the template and interact with the polymerase is a major determinant of signal strength. Primers should be selected which have one or more G or C residues at the 3'-end, a base composition of approximately 55% GC and no inverted repeats or homopolymeric regions. These factors affect the stability of the primer–template interaction, which in turn dictates the number of primer–template complexes available to the polymerase under a given set of conditions. It is particularly important to use appropriate annealing temperatures when performing linear amplification (cycle) sequencing with thermostable polymerases, since the remaining steps in the reaction are performed at higher temperatures. Primers with >50% AT content may require a lower annealing temperature than the standard 50°C, while primers with >50% GC content may exhibit nonspecific binding at a low annealing temperature.

Template quantity

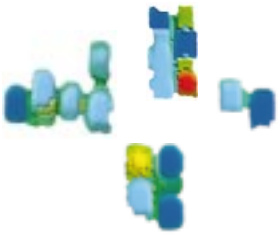
The amount of template needs to be within an appropriate range: if the amount is too low, few complexes form and the signal level is too low to be accurately extracted from the background noise. With manual sequencing, a film can be exposed longer to provide a stronger signal. This is not possible with automated sequencing since the data are collected during the run; however, the signal processing software of the automated sequencers appears to perform a similar function by increasing the size of the peaks to fill the space available in each panel. Unfortunately, any background is also amplified and appears as a chaotic pattern underlying the genuine peaks.

To distinguish high background from low signal, check the signal level numbers shown in the header for each sequence. If the average of these numbers is below a certain threshold (below 50 for ABI-type sequencers), ambiguities, inaccuracies, and limited read length due to low signal level may be observed. Low signal levels can arise from any factor that affects the formation of the template-primer-polymerase complex, but is often due to insufficient or low template.

On the other hand, if the template concentration is too high, the nucleotides in the reaction will be distributed over too many growing chains and an overabundance of short fragments will result. In a dye primer reaction, this will be observed as a higher peak intensity towards the beginning of the sequence. In a dye terminator reaction, most of the fragments will result from premature termination. Consequently, no label will be added and the effect will be observed as a loss of signal strength. In addition, a high concentration of DNA may mean a high concentration of contaminant if the template preparation is impure.

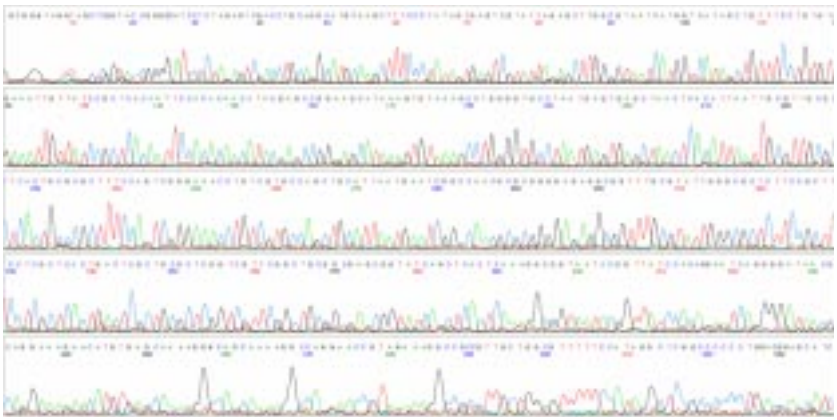
▼ Tip

Check the signal levels in each sequence to distinguish high background from low signal. If the average signal level is below a certain threshold, ambiguities, inaccuracies, and limited read lengths may be observed.



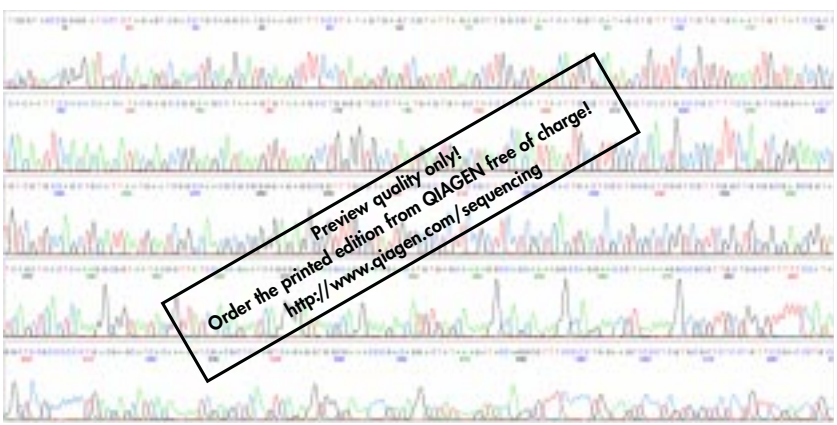
A 50 ng of template DNA

Signal G:34 A:29 C:36 T:29



B 200 ng of template DNA

Signal G:125 A:104 C:140 T:101



C 2.3 µg of template DNA

Signal G:425 A:624 C:817 T:666

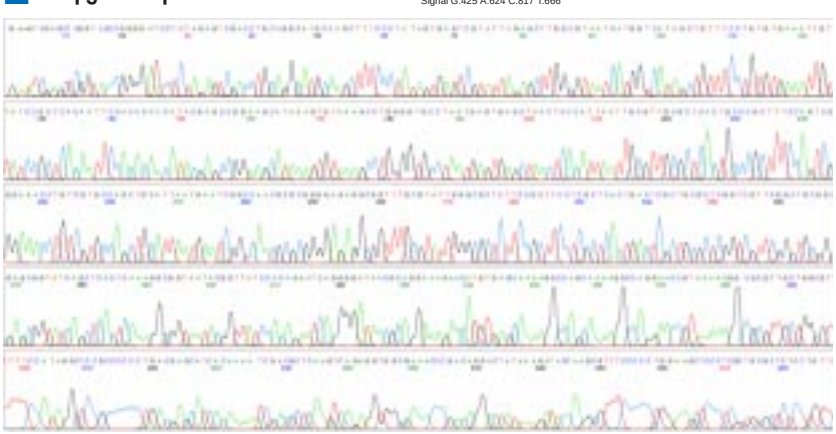


Figure 17. *Taq FS dRhodamine dye terminator sequencing reactions with different amounts of ultrapure plasmid DNA purified using QIAGEN-tip 100.* **A** *50 ng,* **B** *200 ng,* **C** *2.3 µg plasmid pTZ19R; primer: –21M13 primer. Note the different signal intensities in the header of each electropherogram.*

These contaminants may also affect the progress of the polymerase, again leading to a predominance of short, prematurely terminated fragments.

In Figures 17–18, the effects of both template amount and general template quality are illustrated. The ability of data analysis software to remove background noise from a low signal scan has improved considerably in the past few years and even a reaction performed with only 50 ng of standard-quality plasmid template is relatively clean. As expected, reactions performed with the recommended quantity of template produce optimal results, while the effect of using too much DNA depends heavily on the quality of DNA used. Ultrapure templates provide excellent results, even when 3 µg of template DNA is used, while a comparable amount of lower quality DNA produces low signal levels and a relatively short read length. This is because the higher contaminant load in the lower quality DNA becomes inhibitory when the concentration of DNA used is excessive.

Depending on the size of the amplified fragment, the amount of PCR-derived template that is required for a successful reaction may be very small. A good guideline to follow is that 5–10 ng of amplified fragment are required for every 100 bp. Thus, only 10–20 ng of a 200 bp fragment are needed for an optimal reaction. The amount required for a 4 kb fragment would be 400 ng, similar to the amount needed for a plasmid template of the same size. The reason less template is required for short fragments is that most, if not all, of the DNA is read and the concentration of actual template is high relative to the same nanogram amount of a larger fragment.

Ideally, template concentrations should be determined using a fluorometer with a DNA-specific fluorescent dye to avoid errors caused by RNA contamination, and confirmed by comparing an aliquot of the template preparation to standards of known concentration on an agarose gel. Table D in the Appendix provides a list of template amounts found to be optimal for the different sequencing chemistries.

▼ Tip

To avoid errors caused by RNA contamination, template concentration should ideally be determined using a fluorometer with a DNA-specific fluorescent dye. It is also recommended to compare an aliquot of the template prep to standards of known concentration on an agarose gel.

Template quality

Culture

An important initial factor in plasmid template purification is the quality of the culture from which the DNA is extracted. Two elements are of particular importance: i) the conditions under which the culture is grown and ii) the host strain selected. Ideally, bacterial cultures for plasmid preparation should be started from fresh colonies grown on selective media. Subculturing directly from glycerol stocks, agar stabs and liquid cultures may lead to uneven culture growth and possible loss of the plasmid. However, use of a preculture (small culture used to inoculate larger culture from which the plasmid is actually purified) may alleviate some of these problems. The effects of culture medium and growth conditions on plasmid DNA quality are discussed in detail in Section H of the Appendix.

Host strain

Plasmids propagated in host strains such as DH1, DH5α, DH10B, C600 and XL1-Blue are highly suitable for template purification. Strain HB101 and its derivatives, including TG1, TG2 and the JM100 series, contain large amounts of carbohydrate that are released during lysis. These strains also have an intact endA locus and produce relatively large amounts of nuclease (16).

Plasmid purification products based on QIAGEN Anion-Exchange Resin efficiently remove carbohydrates and proteins from crude lysates and are recommended for difficult host strains. In QIAprep Plasmid Kits a special wash buffer, Buffer PB, is used to efficiently remove nucleases and other contaminants from suboptimal host strains, while an optional heating step is used to denature contaminating nucleases in the R.E.A.L. Prep 96 protocol. These additional steps will produce satisfactory results with all host strains, even those that typically do not work well with conventional methods.

The general effect of suboptimal template quality is shown in Figure 18. Template DNA was purified by a boiling prep followed by phenol-chloroform extraction and isopropanol precipitation, then sequenced using dye-terminator chemistry. The signal levels were low relative to those obtained with ultrapure DNA (Figure 17), and short fragments predominated. The presence of contaminants in this preparation made the polymerase more likely to dissociate from the template before a labeled terminator could be inserted, so fewer long chains were likely to be produced. These effects were not observed with all templates purified by lower quality preparation methods, but illustrate the greater variability that is encountered relative to results produced with more highly purified templates.

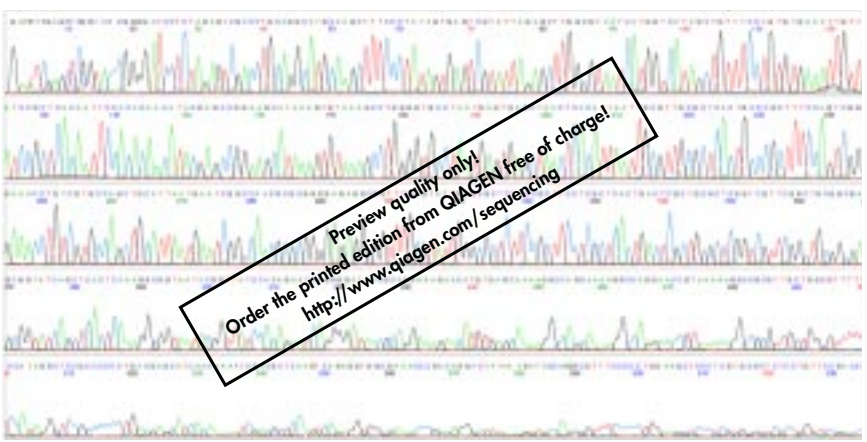


Figure 18. *Taq FS dRhodamine dye terminator reaction with –21M13 primer using approximately 200 ng pTZ19R plasmid DNA prepared by the boiling preparation method. Note how the quality of the plasmid preparation reduces the signal level and results in short fragments, in comparison to plasmid DNA purified using QIAGEN-tip 100 (Figure 17).*

Effect of specific contaminants

Nuclease

To demonstrate how nuclease contamination can affect sequence quality, plasmid DNA was contaminated with DNase I at concentrations between 1 ng/ml and 5000 ng/ml, then incubated for 20 min and precipitated prior to dye terminator cycle sequencing. With increasing DNase I concentrations, the quality of the sequencing scans decreased (Figure 19). At 500 ng/ml DNase I, the signal intensity of longer fragments decreased, while at >1000 ng/ml, read length and data accuracy were greatly reduced. These effects were correlated with the appearance of the template on an agarose gel before sequencing (Figure 20). Low amounts of DNase I caused a shift from supercoiled to linear plasmid, while higher amounts further degraded the DNA. The influence of nuclease contamination was even more severe when radioactive sequencing methods were used (Figure 21). An internal radioactive labeling procedure was used, so pre-maturely terminated extension products arising from truncated templates were detected along with those incorporating a ddNTP terminator. In the laboratory, extensive DNA degradation can also occur with lower DNase I concentrations, depending on the sample storage conditions, the length of time the DNA is stored, and the storage temperature.

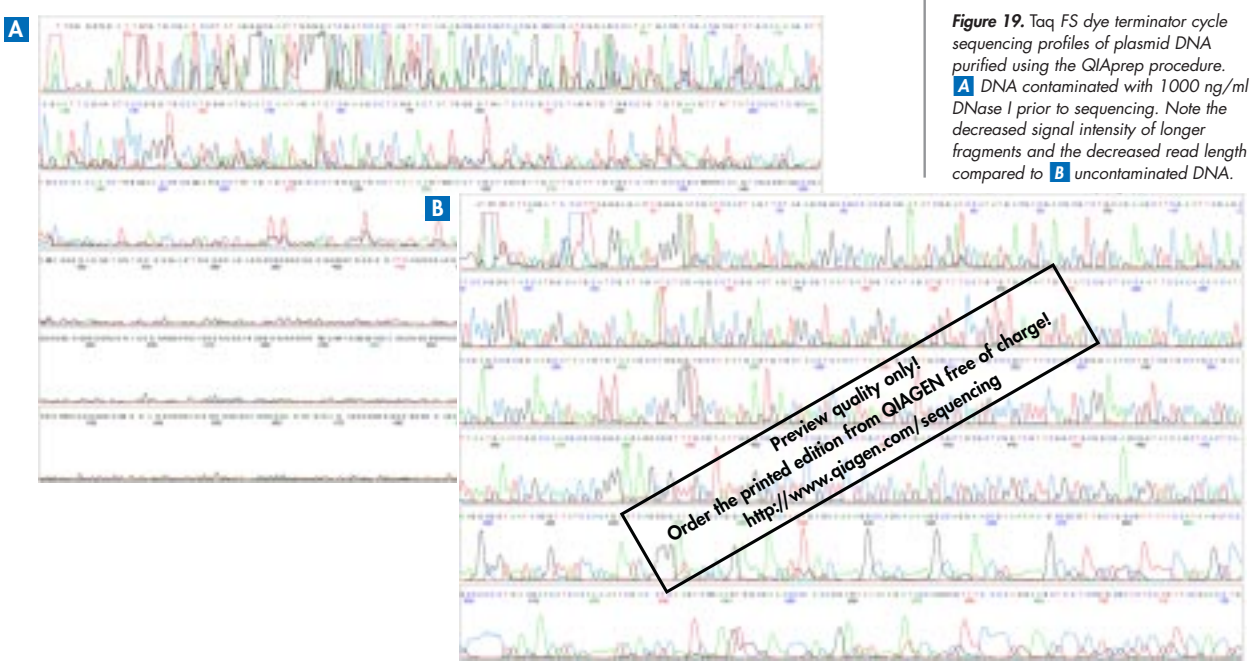


Figure 19. Taq *FS dye terminator cycle sequencing profiles of plasmid DNA purified using the QIAprep procedure.* **A** *DNA contaminated with 1000 ng/ml DNase I prior to sequencing. Note the decreased signal intensity of longer fragments and the decreased read length compared to* **B** *uncontaminated DNA.*

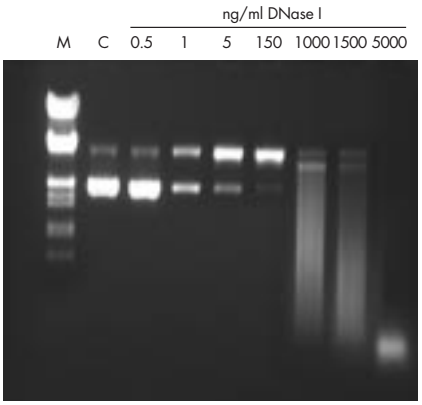


Figure 20. *Agarose gel analysis of DNA samples incubated with the indicated concentrations of DNase I for 20 min.* ***M****: lambda* HindIII-EcoRI *markers.* ***C****: control (untreated purified plasmid DNA). Note that the already low amounts of contaminating DNase I cause a shift from supercoiled to linear plasmid DNA.*

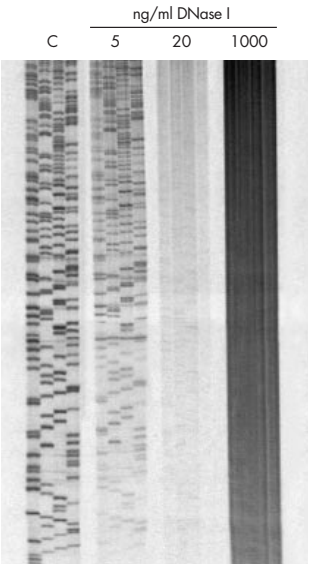


Figure 21. *Effects of nuclease contamination on radioactive sequencing methods. Autoradiogram sequences of plasmid DNA purified with the QIAprep procedure and contaminated with DNase I (at the concentrations indicated) for 20 min prior to sequencing. Double-stranded DNA was sequenced using the Sequenase Version 2.0 DNA Sequencing Kit and [α-^{35}S]dATP.*

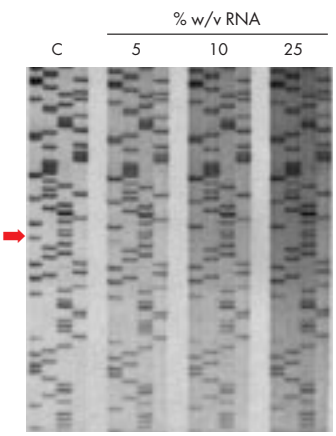


Figure 22. *Effect of RNA contamination on radioactive sequencing. Sequencing profile of plasmid DNA purified using the QIAprep procedure and sequenced in the absence (****C****: control) or the presence of RNA (concentrations indicated) prior to the sequencing reactions. The arrow indicates a low-intensity band which disappears with increasing RNA contamination. Double-stranded DNA was sequenced using the Sequenase Version 2.0 DNA Sequencing Kit and [α-^{35}S] dATP.*

RNA

RNA can be a common contaminant of template preps, particularly in overloading situations where the quantity of cells used exceeds the capacity of the lysis buffers. Figure 22 illustrates the effect of adding 5 to 25% RNA (relative to template) to radioactive sequencing reactions. The RNA-contaminated samples exhibited a high background, which increased with increasing RNA concentration. At high RNA concentrations, some low-intensity bands disappeared, potentially leading to misinterpretation of sequencing data. These effects are due to the contaminating RNA molecules acting as random primers in the sequencing reaction, resulting in a low signal-to-noise ratio. The effect of RNA on automated sequencing results was much less pronounced, most likely because the analysis software effectively removes background noise from the electropherogram (not shown).

Salt

To test the effect of salt contamination on the quality of sequencing data, samples of plasmid DNA were contaminated with 5 to 100 mM sodium acetate and used as templates in fluorescent sequencing dRhodamine dye terminator reactions. The presence of sodium acetate up to a final concentration of 5 mM in the sequencing reactions did not affect the quality of the sequencing data, but higher concentrations of sodium acetate severely inhibited the sequencing reactions (Figure 23). At a final concentration of 20 mM, the signal intensity of the electropherogram as well as the accuracy and the read length were significantly reduced. At concentrations higher than 20 mM, no usable sequencing data could be obtained.

Similar effects were observed if potassium acetate was used in place of sodium acetate. The effects of sodium chloride were less severe: decreases in signal intensity and read length were observed at a concentration greater than 45 mM, with no usable sequence at concentrations above 60 mM (not shown).

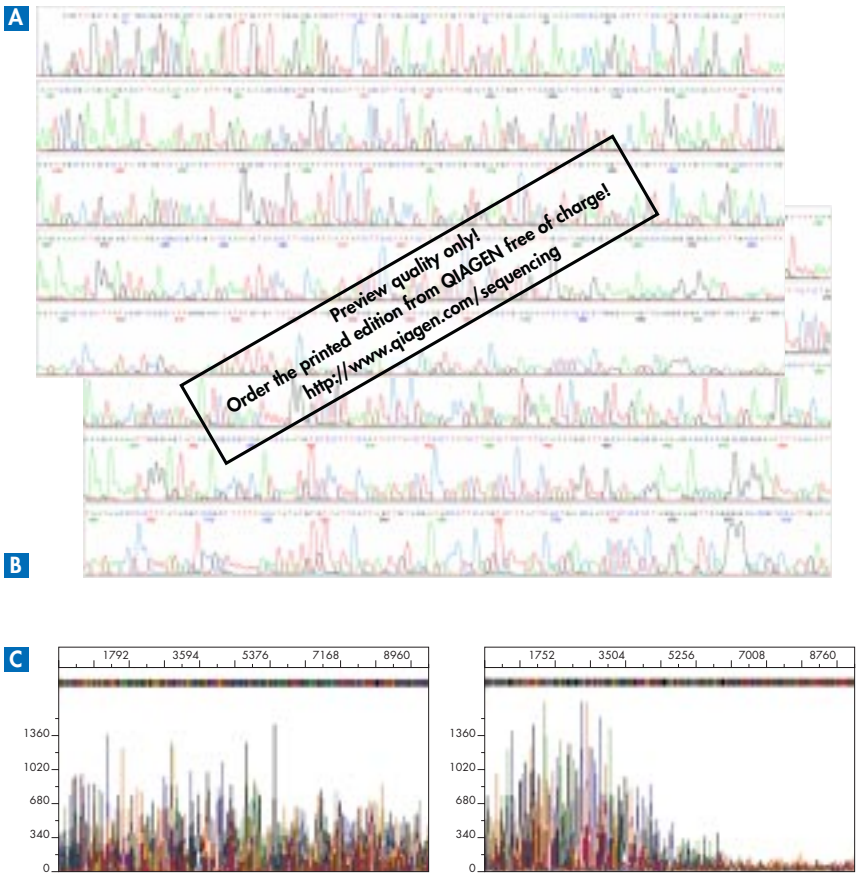


Figure 23. *Effect of salt contamination on Taq FS dye terminator cycle sequencing. The sequencing reactions were performed with 400 ng plasmid pTZ19R using the forward (-20M13) primer and* **A** *20 mM, and* **B** *5 mM sodium actetate in the reaction.* **C** *typical "full view" display of samples that were contaminated with 50 mM NaCl (right panel) compared to a sequence with ultrapure DNA (left panel). Note the decreased signal strength with increasing read length due to salt contamination.*

The observation that sodium acetate has a lower inhibitory concentration than either sodium or potassium chloride indicates that acetate ions inhibit sequencing reactions more than sodium, potassium, or chloride ions.

Salt contamination in template DNA can result from coprecipitation of salt in alcohol precipitations incubated at low temperatures, by insufficient removal of supernatant, or an insufficient 70% wash with ethanol. If traces of salt are suspected, careful precipitation of the template at room temperature, followed by a subsequent room-temperature 70% alcohol wash, can solve the problem.

Ethanol

The effect of ethanol contamination is shown in Figure 24. The presence of 6% ethanol in a dye terminator reaction significantly reduced peak height in the last three panels, resulting in an accurate read length of only 450 bases. The effect of ethanol is less pronounced in a dye-primer reaction, where usable results were obtained even when 10% ethanol was added to the reaction (data not shown). Contamination with >10% ethanol usually resulted in complete failure of the reaction.

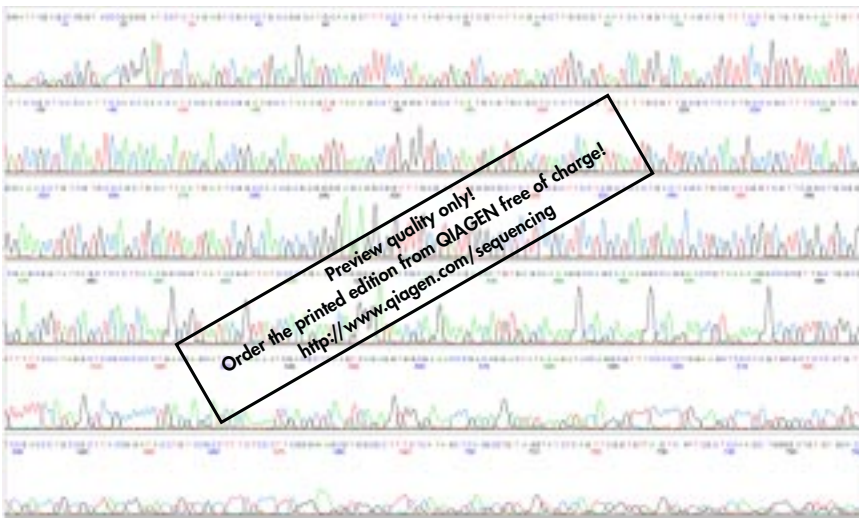


Figure 24. *Effect of ethanol contamination on Taq FS dRhodamine dye terminator cycle sequencing. Sequence of the plasmid pTZ19R using the –21M13 primer in the presence of 6% ethanol. Note the reduced peak height due to ethanol contamination.*

Alcohol contamination in the template can arise from insufficient drying of a DNA pellet after precipitation, or from incomplete removal of ethanol-containing wash buffer from silica membranes or resin during DNA isolation. If no other contaminants are present, ethanol contamination in a template preparation can be removed by evaporation under vacuum with no loss of template quality.

Phenol

Some methods of template purification incorporate phenol extraction as a means to remove proteins and other contaminants from cleared lysates. To assess the effect of small amounts of phenol in a sequencing reaction, we tested sequencing reactions containing between 0.1 and 2.25% (w/v) phenol. This corresponds to 0.01–0.25 µl of saturated phenol solution in a 10 µl sequencing reaction. When the phenol concentration was above 0.7% (v/v) the quality of the automated sequencing data decreased with respect to read length, accuracy, and signal intensity (Figure 25). If the phenol concentration exceeded 1.4% (v/v), no usable sequencing data were obtained. Similar results were observed when radioactive sequencing reactions were tested. A clear decline in the quality of the sequence data was observed with 1% phenol, with numerous sites exhibiting bands across all four lanes. (Figure 26). At phenol concentrations greater than 1.5% no sequence data were obtained.

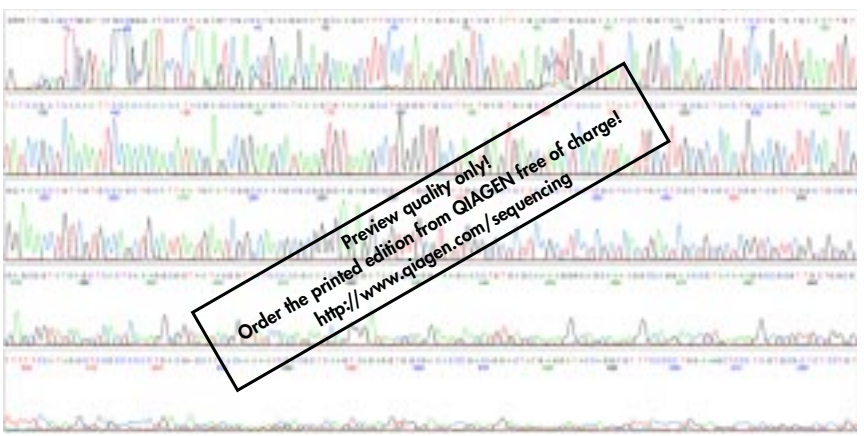


Figure 25. *Effect of phenol contamination on Taq FS dRhodamine dye terminator cycle sequencing. Sequence of the plasmid pTZ19R using the –21M13 primer in the presence of 0.7% (v/v) phenol. Note the reduced signal intensity, read length and accuracy in the presence of phenol.*

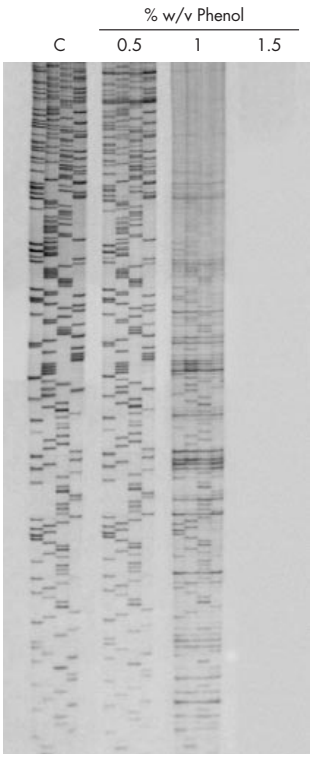


Figure 26. *Effect of phenol contamination on radioactive sequencing. Sequencing profiles of pUC21 plasmid DNA purified using the QIAprep procedure and sequenced in the absence (**C**: control) or presence of phenol (at concentrations indicated) prior to the sequencing reactions. Note that phenol contamination leads to nonspecific chain termination. Double-stranded DNA was sequenced using the Sequenase Version 2.0 DNA Sequencing Kit and [α-^{35}S]dATP.*

Chloroform

Chloroform is often used together with phenol in plasmid preparation methods. However, we observed no effect on the quality of the sequencing profiles when comparable amounts of chloroform were present. Chloroform does not denature proteins as strongly as phenol, and therefore may not affect sequence quality to the same degree.

Contaminants affecting PCR fragment templates

Primers

To examine the effects of common impurities on the sequencing of PCR-amplified templates, different quantities of contaminants such as nucleotides and primers were added to a purified 1000-bp PCR fragment before sequencing. When the purified fragment was contaminated with the primers used for amplification and sequenced from a nested primer (Figure 27), significantly lower sequence quality was observed with 1 pmol of each amplification primer, while contamination with 2 pmol resulted in unreadable sequence. The excessive background signal obtained arises from sequence products primed by the contaminating primers.

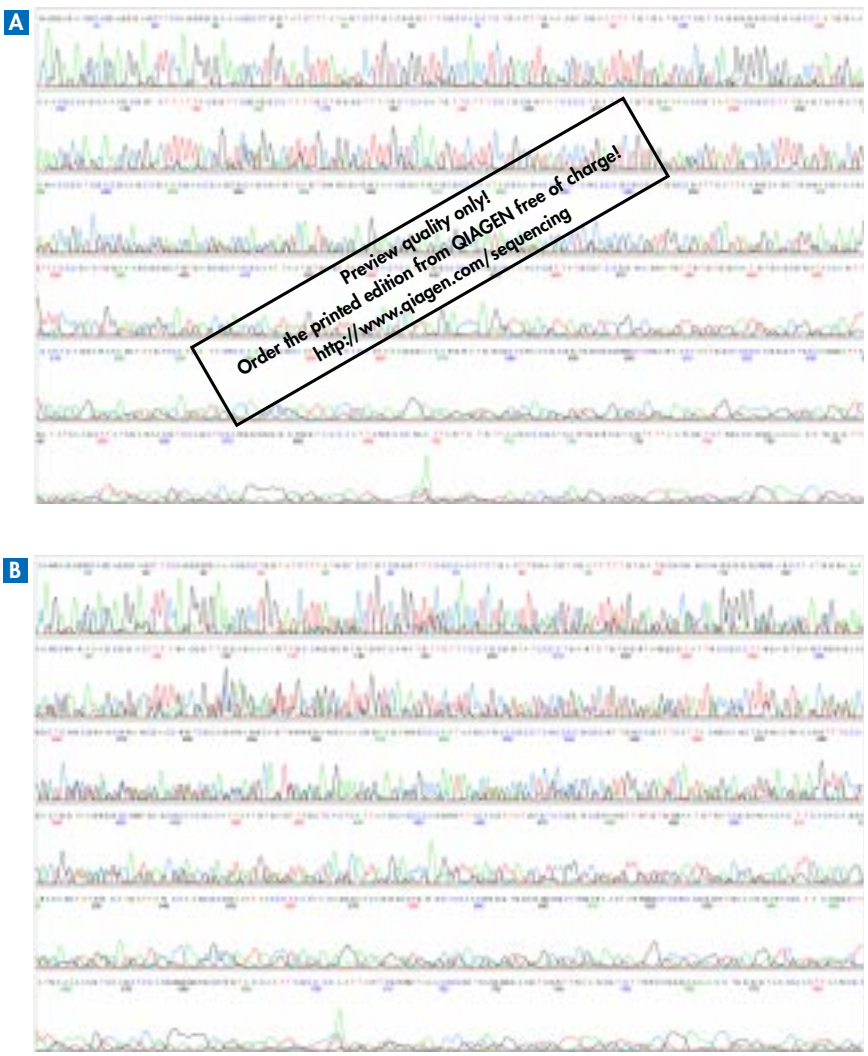


Figure 27. *Effect of primer contamination on fluorescent sequencing. Sequences of 1000-bp PCR fragment contaminated with the amplification primer and sequenced with a nested primer.* ***A*** *contamination with 1 pmol of each amplification primer. Note the reduced sequence quality.* ***B*** *contamination with 2 pmol of each amplification primer. This gives an unreadable sequence.*

dNTPs

In a second set of experiments, nucleotides were added to the purified 1000-bp PCR fragment at concentrations ranging between 2.5 and 100 µM of each dNTP (Figure 28). Contamination with nucleotides at concentrations of 10 µM or more led to a decrease in signal strength and a greater number of ambiguities. An increase in the proportion of long fragments was also observed. At the highest concentration tested, 100 µM, incorporation of dye terminators was inhibited. The presence of contaminating nucleotides alters the ratio of dNTPs to terminators (ddNTPs) in the sequencing reaction, lowering the probability that a ddNTP will be incorporated. This reduces the amount of short extension products that will be produced and may ultimately affect the overall signal level.

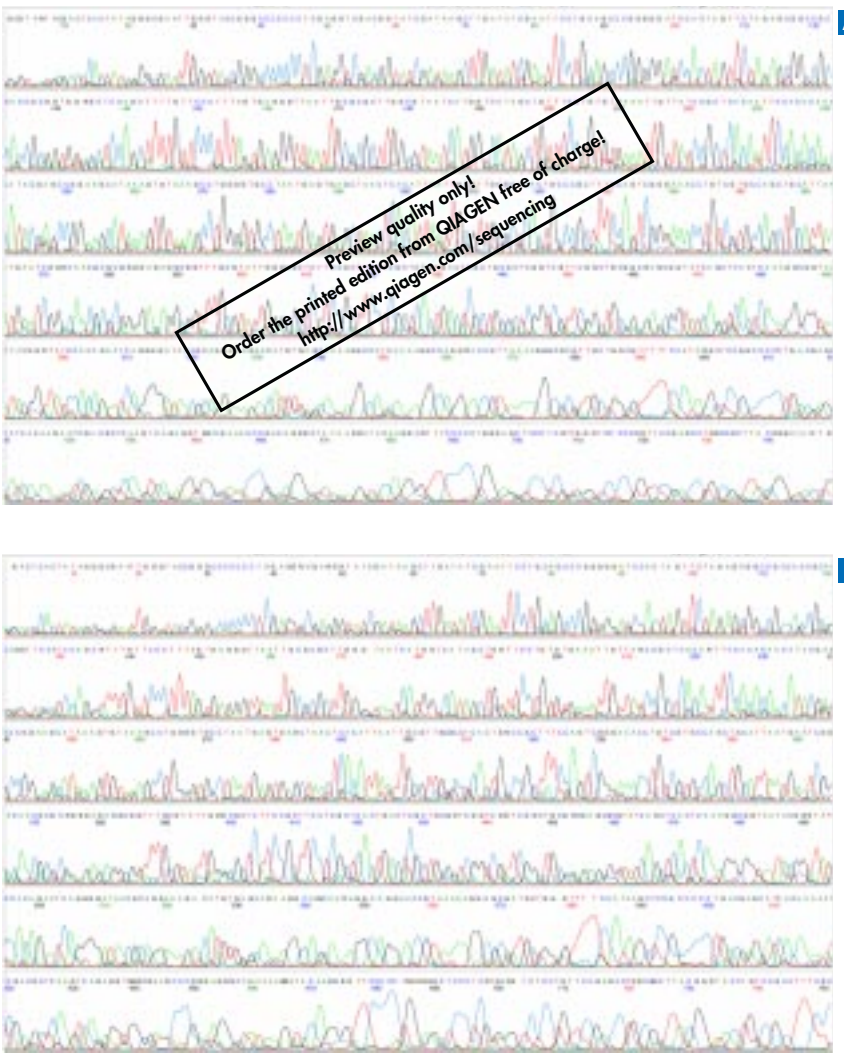
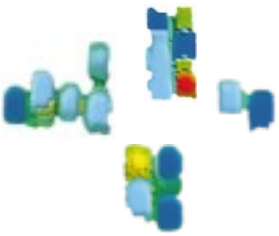


Figure 28. *Effect of nucleotide contamination on fluorescent sequencing. Sequences of 1000-bp PCR fragment were spiked with nucleotide concentrations ranging between 2.5 and 100 µM of each dNTP.* **A** *contamination with 10 µM of each dNTP.* **B** *contamination with 50 µM of each dNTP. Note the decrease in peak height and greater number of ambiguities at ≥10 µM dNTP.*



PCR mix

In a typical amplification reaction, the primers and nucleotides used are not completely consumed. Yields of PCR products are typically 0.5–3 µg, from which it can be calculated that, under standard reaction conditions, 11–14 pmol of each primer and 160–190 µM nucleotides remain following PCR. To reproduce the effect of these residual nucleotides and primers in a sequencing reaction with a PCR-derived template, a simulated post-PCR mixture was prepared containing 11 pmol of each primer, 160 µM nucleotides, and 2.5 U *Taq* DNA polymerase. Different amounts of this mixture, corresponding to 0.5–25% of unpurified amplification reaction, were added to 50 ng of purified 1000-bp DNA fragment, which corresponds to 2–10% of the total yield of a typical PCR product. The contaminated DNA fragments were subsequently sequenced with a nested primer (Figure 29). Samples contaminated with 2.5–5% unpurified reaction gave progressively greater suboptimal quality data, while those contaminated with >5% of the simulated unpurified amplification reaction gave unreadable sequence data. These effects would be even more pronounced in PCR systems using >15 pmol primers, or in low efficiency PCR systems which yield <0.5 µg product. At concentrations normally used for PCR amplification, the *Taq* DNA polymerase contained in the reaction did not affect the quality of the sequencing profiles (not shown).

▼ Tip

Since remaining nucleotides and primers can significantly decrease sequencing data quality, thorough PCR amplification cleanup is recommended.

Priming artifacts

Mispriming

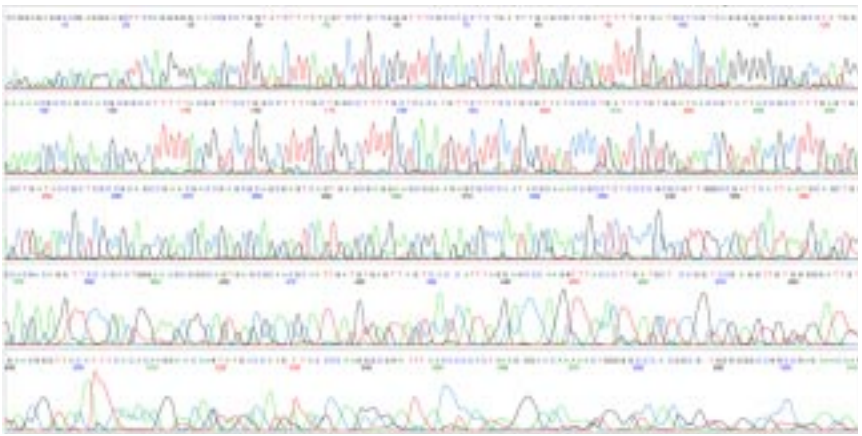
Annealing of a primer to multiple sites on a template results in superimposed sequence ladders. In most instances, the alternate priming site does not perfectly match the primer, and the spurious signal is less intense than the correct signal from the intended priming site. Molecules of the same length, but different base composition, do not run at exactly the same position. This difference is often sufficient to affect the spacing functions of the base-calling software and leads to artifactual peaks. An example of a multiple priming artifact is shown in Figure 30. Multiple priming can be minimized by prescreening vector and insert DNA for sequences closely matching a proposed priming site, and by selecting temperatures that limit the annealing of mismatched primers (50°C or higher). If closely related primer binding sites are inevitable, as when sequencing through a repeated region by primer walking, selection of a primer that has a 3′-base match only at the desired site should produce acceptable results.

▼ Tip

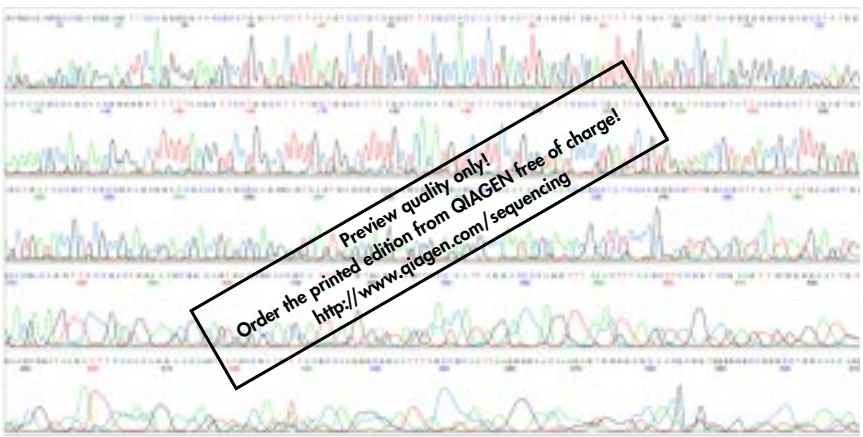
To minimize mispriming, it is advisable to prescreen both vector and insert DNA for sequences closely matching a proposed priming site. An annealing temperature of 50°C or higher should be selected to limit the annealing of mismatched primers.

Priming sites should be selected only from unambiguous sequence regions. Mismatches between the primer and the primer binding site can have serious effects on sequence quality due to a reduction of the stability of primer binding. An example of a sequence obtained using a mismatched primer is shown in Figure 30A. This result was produced with a standard reverse primer (–29Rev dye-labeled reverse M13 primer) on a yeast subclone carried in pTZ18. A comparable reaction using a primer located further

A 2.5% unpurified PCR-amplification reaction



B 5.0% unpurified PCR-amplification reaction



C Control

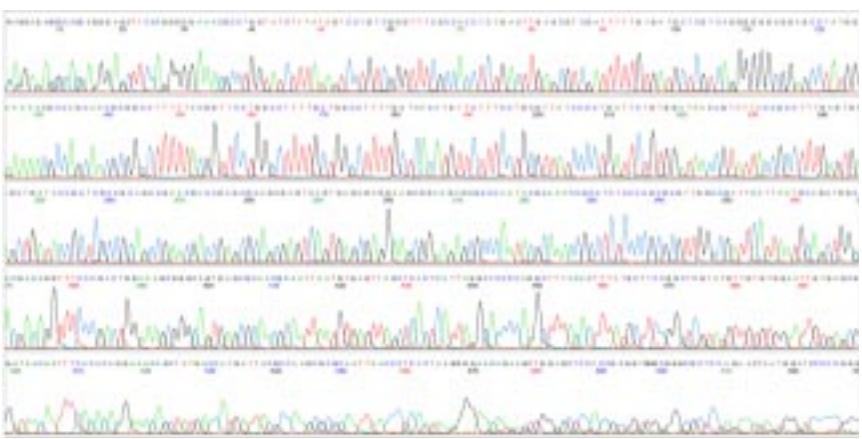


Figure 29. *Simulation of the sequencing of unpurified PCR-amplification reactions. The sequencing reactions of a purified template were spiked with unpurified PCR products.* **A** *contamination with 2.5% unpurified PCR reaction.* **B** *contamination with 5.0% unpurified PCR reaction.* **C** *control.*

upstream (–48Rev) gave normal results (Figure 30B). Subsequent analysis showed that guanosines at two positions (–19 and –12) were deleted from the primer binding site in the vector (5′-CATGGTCATAGCTGTTTCCTG-3′). Similar deletions were also detected in derivatives of pUC18/19 and pGEM3® from some laboratories. The mutation at position –12 in the second codon of the *lac*Z gene may be a common variant of pUC-like vectors and has been reported before (80). In general, mismatches at or near the 3′-end of the primer affect priming efficiency more strongly than mismatches at the 5′-end, since the polymerase requires an annealed 3′-end to initiate elongation.

Figure 30. *Priming artifacts — mispriming in the reverse primer binding site. Yeast subclone from chromosome XII in pTZ18 vector was purified using QIAwell 96 Ultra Plasmid Kit.* ***A*** *sequencing reaction with standard reverse (–29Rev) dye primer. Despite the high-purity template and known vector–primer combination, very low signal with high background noise was observed.* ***B*** *same template sequenced with a more distally located primer (–48Rev).*

Poor quality primer

Similar problems can occur when a primer preparation contains a significant amount of n-1 and shorter derivatives. Primers are synthesized in the 3′ to 5′ direction, and a poor-quality synthesis will result in a population of oligonucleotides with a common 3′-end (the end used for priming) but different 5′-ends. Consequently, chains that terminate at the same position may have different lengths and will run at different positions on the gel. An example of this effect is shown in Figure 31. Note how each peak in the electropherogram contains one signal originating from the full-length primer and a shadow peak representing the n-1 signal.

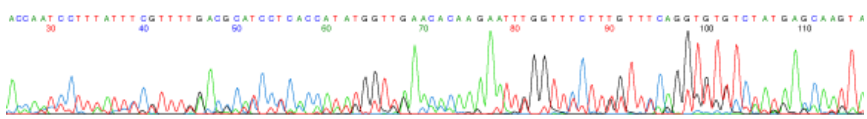


Figure 31. *Primer synthesis artifacts — "n-1" sequences. Primer synthesis artifacts are evident as shadow peaks next to the peak originating from the full-length primer. The formation of shadow peaks from n-1 derivatives is especially noticeable for bases A47, A69, and G91.*

Mixed templates

A variation of the multiple priming site problem occurs when two or more different populations of molecules with a common priming site are present in the template DNA. This happens when more than one recombinant colony or plaque is inoculated into a single culture. When template DNA prepared from such a culture is sequenced into the flanking vector sequences, good vector sequence data will be obtained, but the insert region will show an overlapping band pattern (Figure 32). Such problems can be corrected by retransforming the DNA and inoculating the culture with a single colony or plaque. Similar artifacts will also arise if a plasmid contains more than one vector molecule, or if spontaneous deletions occur during growth.

Figure 32. *Priming artifacts — mixed population bacterial cultures. Shotgun subclones from* Schizosaccharomyces pombe *in pUC18 vector in a Taq FS dRhodamine dye terminator reaction using the –21M13 primer. The double sequence starts directly after the SmaI restriction site used for subcloning (position 42).*

A second source of mixed template arises when PCR-amplified templates contain more than one amplified fragment. This can occur when one or more of the primers recognizes more than one site on the template DNA. Such a problem is most difficult to identify when the fragments are amplified from two or more segments of related DNA since the sizes of the amplified fragments may be the same. Amplification of nonspecific PCR products can be avoided by using optimized PCR buffers with a balanced combination of cations that minimize nonspecific annealing (81). Such a system is available with QIAGEN *Taq* DNA Polymerase and the accompanying optimized PCR buffer. Problems with mixed templates may also be circumvented by using a sequencing primer that binds to a site internal to the primers used for PCR.

Template artifacts

The ease with which a polymerase moves along a template can be affected by the overall base composition of the template, and by specific sequences that influence secondary structure.

Unbalanced base composition

Commercially available reaction mixes are formulated for templates with balanced base compositions. When used with templates that have a high GC- or AT-ratio, premature depletion of one or more nucleotides can occur. This results in a higher proportion of prematurely terminated molecules that are detected as increased background in dye primer reactions, and as reduced signal strength in the latter half of the sequence. These effects can be corrected by increasing the concentration of the nucleotides that are in short supply.

GC-rich templates

Problems with secondary structure are often observed with GC-rich template DNA, which may not melt at the temperature of sequencing reactions. This is particularly true for reactions using modified T7 DNA polymerase.

Figure 33. *Artifacts related to DNA template composition — elongation stop due to secondary structures, and distortion of elongation after a long poly-T stretch.* **A** *sequence with a polymerase elongation stop.* **B** *modified BigDye terminator sequencing protocol that can often resolve elongation stop structures.* **C** *slippage and stuttering effects of polymerase elongation due to a long poly-T stretch. Note that elongation stops can be more easily detected in the "full view" mode of a sequencing file (right panels of* **A** *and* **B** *).*

Effects are seen as one or more strong stops (bands for all four dideoxynucleotides) anywhere along the template. GC-rich regions should always be sequenced by cycle sequencing with a thermostable polymerase, since the elevated temperature will melt hairpins and permit passage of the polymerase. The addition of 5–10% DMSO or formamide to the reaction further reduces the melting temperature of GC-rich regions without otherwise

reducing sequence quality (Ref. 82, Figure 33, for modified protocol see Appendix). GC-rich templates should also be sequenced using dye-terminator chemistry, since prematurely terminated molecules, which constitute most of the spurious signal in a strong stop in a dye primer reaction, will not be detected when dye terminators are used (Figures 34A and 34B). An example of premature termination created by a highly AT-rich region is shown in Figures 34C and 34D.

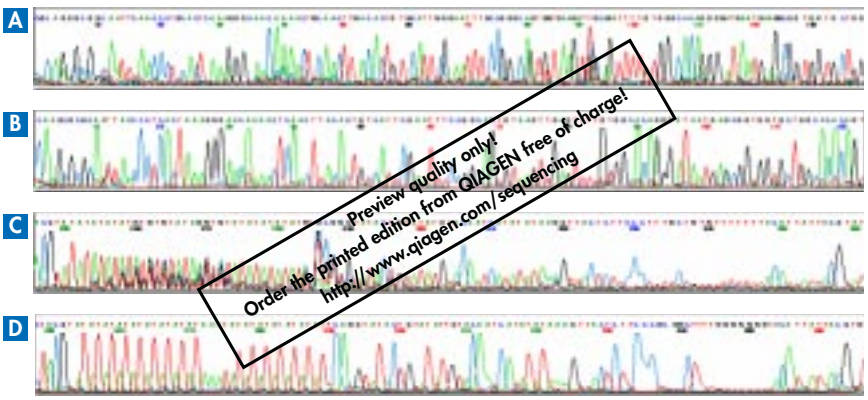


Figure 34. *Artifacts related to DNA template composition — multiple false terminations. Yeast subclone from chromosome XI in pBluescript II vector purified using QIAwell 8 Plus Plasmid Kit.* ***Reaction****: 0.8 µg of plasmid DNA in Taq dye primer and dye terminator cycle sequencing reactions (see Appendix) with Forward (–20M13) primer.* **A** *dye primer reaction with several false stops, compression, and smeared bands.* **B** *same template used in a dye terminator reaction. The sequence-dependent artifacts from reaction A are all resolved or do not interfere with this chemistry.* **C** *dye primer reaction with high background and false stops within and after an AT stretch.* **D** *same template used in dye-terminator reaction. Most sequence-dependent artifacts from reaction* **C** *are resolved except for several ambiguities related to low signal in the T-rich region.*

Secondary structure can also affect the mobility of molecules in a sequencing gel, resulting in sections of the sequence where the bands cannot be adequately resolved ("compressions"). Most commercial kits for sequencing reactions include nucleotide analogs, such as deoxyinosine-5'-triphosphate or 7-deaza-dGTP, that form fewer hydrogen bonds when paired. These analogs reduce the melting temperature of the template, and alleviate many of the problems associated with GC-rich regions.

Homopolymeric regions

The polymerases used for DNA sequencing often exhibit slipping and stuttering when processing long homopolymeric stretches, such as in the poly-A/poly-T tract of a cDNA (Figure 33C). The best results with these sequences are obtained if *Taq* dye terminator chemistry with higher annealing temperatures and longer denaturation times is used (83), and if the homopolymeric tract occurs in the early part of the sequence, when the nucleotide concentration and enzyme activity are likely to be optimal.

Sequencing large templates

BACs, PACs, and P1s

The sequencing of very large plasmids such as BACs, PACs, and P1s presents particular challenges and is a real test of the sequencer's skill. Part of the challenge arises from the fact that the fraction of the large plasmid template DNA that is actually used for sequencing is much lower than that of a small plasmid template. For example, when 200 ng of a 5-kb plasmid is used in a sequencing reaction, the amount of DNA corresponds to 60 fmol. By comparison, 200 ng of a 150-kb BAC clone corresponds to 2 fmol. To achieve

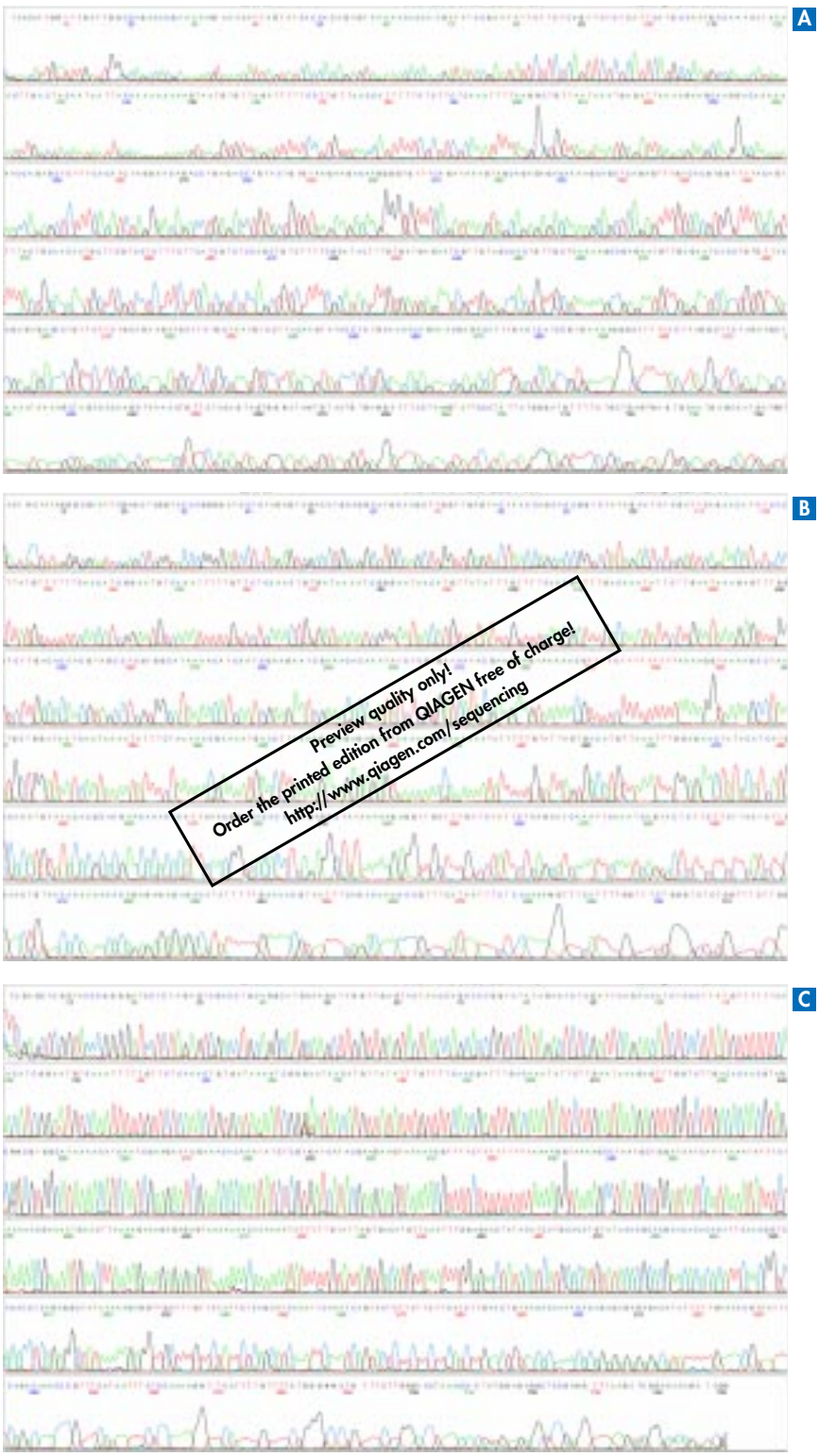


Figure 35. *BAC end sequences of* Arabidopsis thaliana *BAC clones purified using QIAGEN-tip 500 and sequenced using different sequencing chemistries and template concentrations.* **A** *AmpliTaq FS dRhodamine dye terminator sequence using 2 µg template DNA.* **B** *AmpliTaq FS BigDye dye-terminator sequence using 520 ng template DNA.* **C** *AmpliTaq FS BigDye dye-primer sequence using 132 ng template DNA.*

▼ **Tip**

DNA purified using QIAGEN Anion-Exchange Resin works well for large-plasmid sequencing because contaminants that cause inhibition when concentrated in a low-copy plasmid prep are removed most efficiently by anion exchange.

the same molar concentration of BAC template as plasmid template, 6 µg of BAC DNA would have to be used in the sequencing reaction. Since there is a limit on the amount of DNA possible that can be added to a sequencing reaction, the signal level obtained will be much lower if only 200–500 ng of BAC DNA are used. Most large plasmids exhibit very low-copy number in *E. coli*, and it is often difficult to obtain enough BAC DNA from a small culture (e.g. a culture grown in a 96-well block) for more than one round of sequencing.

The method used to prepare large plasmid template DNA can have a significant effect on the quality of the results. DNA purified using QIAGEN Anion-Exchange Resin works well for large-plasmid sequencing because contaminants that cause inhibition when concentrated in a low-copy plasmid prep are removed most efficiently by anion exchange (60–63). Other methods may be used, however, if the concentration of contaminants in the sequencing reaction can be sufficiently diluted.

Recently, a new series of dyes called BigDyes were introduced that exhibit much higher fluorescence yields than conventional dRhodamine dyes (see Section II, *Energy transfer dyes*). The higher fluorescence yield means that for large plasmid sequencing, less template DNA is needed to obtain acceptable signal levels. A comparison of BAC end sequences obtained with conventional dichlororhodamine-labeled terminators and with BigDye-labeled terminators is shown in Figure 35. In this example, 2 µg of template was required with conventional dRhodamine dye terminator sequencing, 520 ng of template was required with the BigDye terminators, while only 132 ng was required with BigDye primer sequencing. This means that smaller culture volumes can be used to obtain template for sequencing and lower quality templates can be diluted to reduce the concentration of contaminants.

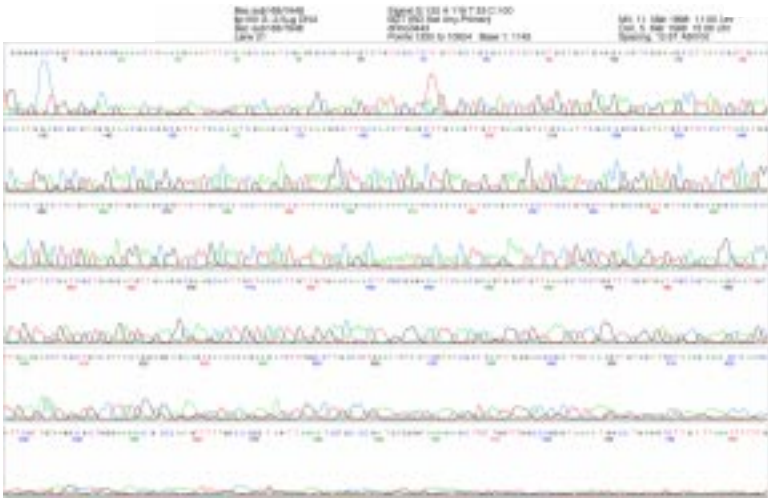
Direct sequencing of genome-size DNA templates

▼ **Tip**

High-quality genomic DNA is essential for successful direct sequencing of genome-size DNA templates, since the amount of template required is high, and significant template contamination would inhibit the sequencing reaction.

The additional sensitivity conferred by energy-transfer dyes like BigDyes raises the possibility of sequencing directly from small genomes such as those of bacteria. This would provide a means of efficiently closing gaps between contigs when using a whole genome shotgun approach (41), and precisely identifying transposon insertion sites and other types of mutations without an intermediate PCR step.

Using protocols from the supplier (84), this was tested with *Bacillus subtilis* (52), which has a genome size of 4.2 Mb, and *E. coli* (85), which has a genome size of 4.7 Mb. The relatively large size of these templates means that the amount of DNA corresponding to the specific region to be sequenced is only one thousandth of a standard 4.2–4.7 kb plasmid template. To overcome the low template concentration, 2.5–3 µg of genomic DNA was used and the number of reaction cycles was increased from 25 to 99. It was observed that the quality of the genomic DNA template is critical for success since, given the amount of template required, a significant contaminant load would inhibit the sequencing reaction. DNA purified on a QIAGEN Genomic-tip 100/G column worked reliably for this purpose (Figures 36 and 37).



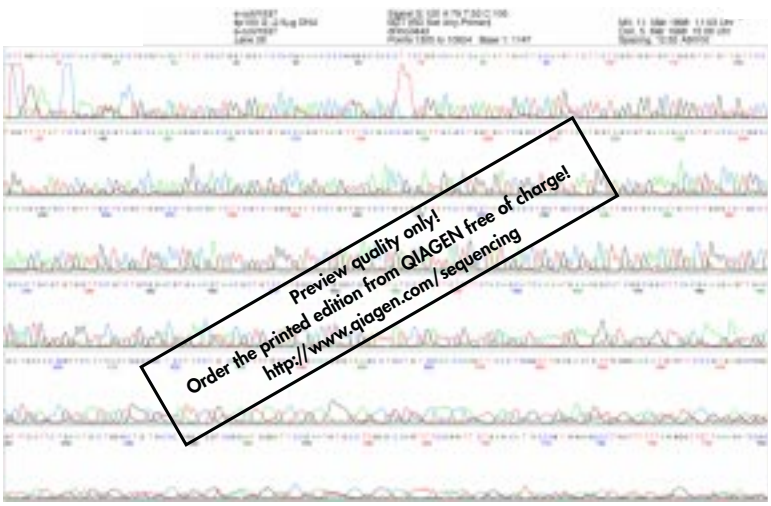


Figure 36. *Direct sequencing of* B. subtilis **A** *and* E. coli **B** *genomic DNA purified with the QIAGEN Genomic-tip 100/G protocol. Sequencing reactions: 2.5 µg genomic DNA; primer* B. subtilis*: 25-mer (CTG TAG GCG TGC GTA AAG GGA TGG A) binding inside the groEL gene; primer* E. coli*: 22-mer (CGC CGC AGG ATT ACA TAG GAC A) binding inside the atpB gene. Sequencing was performed with the ABI protocol, see Appendix.*

```
gb|AE000450|ECAE000450 Escherichia coli K-12 MG1655 section 340 of 400 of the complete genome
Length = 11414

Score = 1027 bits (518), Expect = 0.0
Identities = 602/629 (95%), Positives = 602/629 (95%), Gaps = 5/629 (0%)

Query: 17      ctgnacctgcgtacattctcgctggtggatccacnaaaccccnnngccaccttctggaca   76
                ||| ||||||||||||||||||||||||||||||| |||||||  ||||||||||||||
Sbjct: 10444   ctggacctgcgtacattctcgctggtggatccacaaaaccccccagccaccttctggaca   10385

Query: 77      atcaatattgactccatgttcttctcggtggtgctgggtctgttgttcctggttttattc   136
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 10384   atcaatattgactccatgttcttctcggtggtgctgggtctgttgttcctggttttattc   10325

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Query: 497     caacccgttcaatcactgggccgtcattcctg-caacttantccttgaaagggtaagcct   555
                | | |||||||||||||||  |||||||| ||||||| |||||||| ||||||||||
Sbjct: 9964    c-agccgttcaatcactgggcgttcattcctgtcaacttaatccttgaaggggtaagcct   9906

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                ||   ||  |      |     |
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```
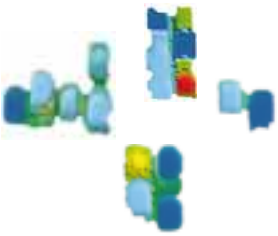

Figure 37. *BLAST sequence comparison of the unedited* E. coli *genomic sequencing reaction (Figure 36B) with the consensus sequence of* E. coli *(85). Note the very good sequence accuracy for the direct genomic sequencing reaction in the main part of the alignment.*



IX. Conclusions

Since the publication of the first edition of *The QIAGEN Guide to Template Purification and DNA Sequencing,* changes in sequencing chemistry and instrumentation have significantly improved both the power, sensitivity, and reliability of automated DNA sequencing. At the same time, the pace of research utilizing DNA sequence data has accelerated, leading to ever higher demands for increased throughput and convenience. QIAGEN will continue to support researchers using DNA sequencing by providing efficient, cost-effective methods for purifying template DNA and automating laboratory procedures. Updates of the methods described in this guide will be provided through an electronic version of the guide located on our website (see below). We welcome your comments on this guide, and look forward to further advances in sequencing and purification methodologies in the years to come.

X. Web Addresses

Sequencing at QIAGEN	http://www.qiagen.com/sequencing
QIAGEN Sequencing Services	http://www.qiagen.com/QSS
QIAGEN homepage	http://www.qiagen.com
QIAGEN Technical Service	http://www.qiagen.com/techservice.html
Useful links to worldwide bioinformatics servers	http://www.qiagen.com/bioinfo/
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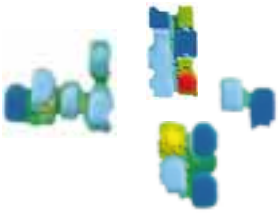


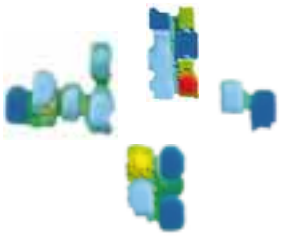
XIII. Ordering Information

Product	Contents	Cat. No.
Plasmid Starter Kits		
QIAGEN Plasmid Starter Kit I	10 QIAGEN-tip 20, 3 QIAGEN-tip100, 1 QIAGEN-tip 500, Reagents, Buffers	12129
Plasmid Mini Kits		
QIAGEN Plasmid Mini Kit (25)	25 QIAGEN-tip 20, Reagents, Buffers	12123
QIAGEN Plasmid Mini Kit (100)	100 QIAGEN-tip 20, Reagents, Buffers	12125
Plasmid Midi Kits*		
QIAGEN Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAfilter Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
Plasmid Maxi Kits*		
QIAGEN Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers	12163
QIAfilter Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12263
Plasmid Mega Kits		
QIAGEN Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers	12181
QIAfilter Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12281
Plasmid Giga Kits		
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAfilter Plasmid Giga Kit (5)†	5 QIAGEN-tip 10000, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12291

Product	Capacity plasmid DNA	Contents	Cat. No.
QIAGEN-tips without buffers			
QIAGEN-tip 20 (25)	20 µg	25 columns	10023
QIAGEN-tip 20 (100)	20 µg	100 columns	10025
QIAGEN-tip 100 (25)	100 µg	100 columns	10043

Product	Contents	Cat. No.
QIAprep Miniprep Kits*	**for high-purity plasmid DNA minipreparation**	
QIAprep Spin Miniprep Kit (50)	For 50 plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
QIAprep Spin Miniprep Kit (250)	For 250 plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106
QIAprep 8 Miniprep Kit (50)‡	For 50 x 8 plasmid minipreps: 50 QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27144
QIAprep 8 Turbo Miniprep Kit (10)‡	For 10 x 8 plasmid minipreps: 10 each: TurboFilter 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27152

* *Other kit sizes available*
† *For low-copy plasmid and cosmid preparations, the QIAfilter/EndoFree Plasmid Mega Kit is a more cost-effective option than the QIAfilter Giga Kit.*
‡ *Requires use of QIAvac 6S*



Ordering Information

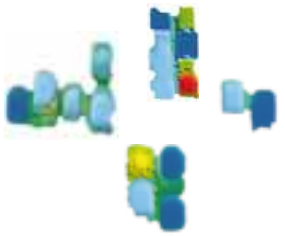
Product	Contents	Cat. No.
QIAprep 8 Turbo Miniprep Kit (50)*	For 50 x 8 plasmid minipreps: 50 each: TurboFilter 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27154
QIAprep 96 Turbo Miniprep Kit (1)†	For 1 x 96 plasmid minipreps: 1 each: TurboFilter 96 and QIAprep 96 Plates; Flat-Bottom Block and Lid, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27190
QIAprep 96 Turbo Miniprep Kit (4)*	For 4 x 96 plasmid minipreps: 4 each: TurboFilter 96 and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27191
R.E.A.L. Prep 96 Kits	**for highest-throughput, lowest-cost plasmid DNA minipreparation**	
R.E.A.L. Prep 96 Plasmid Kit (4)†	For 4 x 96 plasmid minipreps: 4 QIAfilter 96 Plates, Square-Well Blocks, Tape Pad, Reagents, Buffers	26171
R.E.A.L. Prep 96 Plasmid Kit (24)†	For 24 x 96 plasmid minipreps: 24 QIAfilter 96 Plates, Square-Well Blocks, Tape Pads, Reagents, Buffers	26173
R.E.A.L. Prep 96 Accessories		
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96 well-blocks	19571
Square-Well Blocks (8)	96-well blocks with 2.2-ml wells, 8 per case	19572
Square-Well Blocks (24)	96-well blocks with 2.2-ml wells, 24 per case	19573
QIAwell Plasmid Kits‡	**for high-throughput, ultrapure plasmid DNA minipreparation**	
QIAwell 8 Plasmid Kit (50)*	For 50 x 8 plasmid minipreps: 50 QIAwell 8 Strips, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	17124
QIAwell 8 Plus Plasmid Kit (50)*	For 50 x 8 plasmid minipreps, 50 each: QIAwell 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16144
QIAwell 8 Ultra Plasmid Kit (50)*	For 50 x 8 plasmid minipreps, 50 each: QIAfilter 8, QIAwell 8, and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16154
QIAwell 96 Ultra Plasmid Kit (4)†	For 4 x 96 plasmid minipreps, 4 each: QIAfilter 96, QIAwell 96, and QIAprep 96 Plates; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16191
QIAprep M13 Kits‡		
QIAprep Spin M13 Kit (50)	For 50 ssDNA preparations: 50 QIAprep Spin Columns, Buffers, Collection Tubes (2 ml)	27704
QIAprep 8 M13 Kit (50)*	For 50 x 8 ssDNA preparations: 50 QIAprep 8 Strips, Buffers, Extension Tubes, Collection Microtubes (1.2 ml), Caps	27744
QIAprep 96 M13 Kit (4)†	For 4 x 96 ssDNA preparations: 4 QIAprep 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	27781
QIAprep M13 Accessories		
Extension Tubes (100)	For use with QIAGEN 8-well strips, or spin columns on vacuum manifolds: 100/pack	19555

* *Requires use of QIAvac 6S*
† *Requires use of QIAvac 96*
‡ *Other kit sizes available*

Ordering Information

Product	Contents	Cat. No.
QIAGEN Genomic-tips	**for small- to large-scale purification of high-molecular-weight DNA**	
QIAGEN Genomic-tip 20/G	25 columns	10223
QIAGEN Genomic-tip 100/G	25 columns	10243
Accessories		
Genomic DNA Buffer Set	Buffers, including specific lysis buffers for yeast, bacteria, cells, blood, and tissue: Y1, B1, B2, C1, G2, QBT, QC, QF; for 75 mini-, 25 midi-, or 10 maxipreps	19060
QIAquick PCR Purification Kits*	**for direct purification of PCR fragments from amplification reactions**	
QIAquick PCR Purification Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick Nucleotide Removal Kits*	**for cleanup of oligonucleotides and DNA fragments from enzymatic reactions**	
QIAquick Nucleotide Removal Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28304
QIAquick Gel Extraction Kits*	**for purification of DNA fragments (70 bp – 10 kb) from agarose gels**	
QIAquick Gel Extraction Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704
QIAquick Multiwell PCR Purification Kits*	**for high-throughput purification of PCR fragments from amplification reactions**	
QIAquick 8 PCR Purification Kit (50)†	For purification of 50 x 8 PCR reactions: 50 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml), Caps	28144
QIAquick 96 PCR Purification Kit (4)‡	For purification of 4 x 96 PCR reactions: 4 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28181
BioRobot 9600§	System includes: robotic workstation with microprocessor-controlled shaker and vacuum pump; vacuum manifold; QIAsoft Operating System; computer, cables; installation and training; 1 year warranty on parts and labor	900200
BioRobot 9600 Accessories§		
Accessory Pipetting System	Dilutor unit with ceramic-coated, stainless steel pipetting probe for 1–500 µl volumes	900510
High-Speed Pipetting System	Specially designed microprocessor-controlled peristaltic pump with solenoid valves, Reagent Delivery Module for high-throughput applications, bottles and tubing	900520
Tip-Change System	Multifunctional system for error-free use of conducting tips, Tip-Disposal Station for the release and the disposal of used tips; Sensing of tip uptake and disposal using the Tip-Check Station, cooling block for reaction mix; racks for disposable tips	900530
Disposable Tips 300 µl (960)	Conducting disposable tips for use on the BioRobot 9600, pack of 960 tips	990032
Disposable Tips 300 µl (18 x 960)	Conducting disposable tips for use on the BioRobot 9600, pack of 18 x 960 tips	990036

* *Other kit sizes available*
† *Requires use of QIAvac 6S*
‡ *Requires use of QIAvac 96*
§ *The BioRobot 9600 is not available in all countries. Please inquire.*



Ordering Information

Product	Contents	Cat. No.
BioRobot Kits§		
QIAwell 8 Ultra BioRobot Kit (48)	For 48 x 8 plasmid minipreps, 48 each: QIAfilter 8, QIAwell 8, and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids	960134
QIAwell 96 Ultra BioRobot Kit (4)	For 4 x 96 plasmid minipreps, 4 each: QIAfilter 96, QIAwell 96, and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids, Tape Pads	960141
QIAprep 8 Turbo BioRobot Kit (48)	For 48 x 8 plasmid minipreps, 48 each: TurboFilter 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids	962134
QIAprep 96 Turbo BioRobot Kit (4)	For 4 x 96 plasmid minipreps, 4 each: TurboFilter 96, and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids, Tape Pads	962141
R.E.A.L. Prep 96 BioRobot Kit (4)	For 4 x 96 rapid extraction alkaline lysis minipreps: 4 QIAfilter 96 Plates; Flat-Bottom Blocks, Square-Well Blocks, Reagents, Buffers, Tape Pads	961141
QIAquick 8 PCR BioRobot Kit (48)	For purification of 48 x 8 PCR products: 48 QIAquick 8 Strips; Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids	963134
QIAquick 96 PCR BioRobot Kit (4)	For purification of 4 x 96 PCR products: 4 QIAquick 96 Plates; Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids	963141
QIAprep 8 M13 BioRobot Kit (48)	For 48 x 8 ssDNA preparations: 48 QIAprep 8 Strips; Reagents, Buffers; Extension Tubes, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids	962534
QIAprep 96 M13 BioRobot Kit (4)	For 4 x 96 ssDNA preparations: 4 QIAprep 96 Plates; Square-Well Blocks, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids, Tape Pads	962541
QIAGEN 96-Well Centrifugation System		
Plate Rotor 2 x 96*	Rotor for 2 QIAGEN 96 plates for use with Centrifuge 4-15*	81031
Centrifuge 4-15 (230 V)†	Universal laboratory centrifuge with brushless motor (230 V/50 Hz)	81020
Centrifuge 4-15 (120 V)†	Universal laboratory centrifuge with brushless motor (120 V/60 Hz)	81010
QIAvac Vacuum Manifolds		
QIAvac 6S	Vacuum manifold for processing 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder	19503
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder	19504
QIAvac Manifold Components and Replacement Parts		
Vacuum Regulator	For use with QIAvac Manifolds	19530

* *The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuge 4-15 from QIAGEN or the freely programmable models of centrifuges 4-15, 4K15, 6-10, and 6K10 from Sigma Laborzentrifugen GmbH.*

† *Not available in all countries. Please contact your local QIAGEN company or distributor.*

Ordering Information

Product	Contents	Cat. No.
QIAGEN Products for PCR*		
***Taq* DNA Polymerase**		
Taq DNA Polymerase (250)	250 units *Taq* DNA Polymerase, 10x PCR Buffer†, 5x Q-Solution, 25 mM $MgCl_2$	201203
***Taq* PCR Core Kit**		
Taq PCR Core Kit (250)	250 units *Taq* DNA Polymerase, 10x PCR Buffer†, 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix‡	201223
***Taq* PCR Master Mix Kit**		
Taq PCR Master Mix Kit (250)	3 x 1.7 ml *Taq* PCR Master Mix§ containing 250 units *Taq* DNA Polymerase total, 3 x 1.7 ml distilled water	201443

Notice to Purchasers of QIAGEN Taq DNA Polymerase: Limited License

Purchase of *Taq* DNA Polymerase or *Taq* PCR Core Kit is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e. an authorized thermal cycler.

* *Larger kit sizes available.*
† *Contains 15 mM $MgCl_2$.*
‡ *Contains 10 mM each dNTP.*
§ *Provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP*

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* The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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