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QIAGEN News

Innovation Working for You

Biolistic transformation of plants

with anion-exchange–purified plasmid DNA

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Biolistic transformation has become the method of choice for introducing genes into cell organelles. The method involves bombarding target cells with microscopic heavy-metal particles coated with DNA. Chloroplast transformation is significantly less efficient than nuclear transformation using this method, and it is difficult to select even small numbers of chloroplast transformants from bombarded leaf samples. Therefore, in order to optimize parameters for bombardment, a model system involving transient

▶▶ Biolistic transformation, page 11

Effect of DNA Quality on Biolistic Transformation

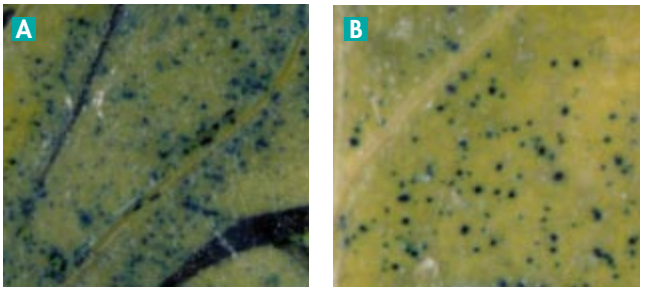

Figure 1 *Transient GUS assay of tobacco leaves bombarded with plasmid pFF19G. This plasmid carries the* uidA *reporter gene driven by the CaMV 35S promoter (3). Plasmid DNA was purified using* **A** *a QIAGEN Plasmid Maxi Kit or* **B** *a silica-slurry–based kit from another supplier.*

PCR optimization: primer design

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Optimal primer sequences and appropriate primer concentrations are essential for maximal specificity and efficiency in PCR. In this study, we demonstrate the effects of primer sequence and concentration on PCR performance and present general guidelines to overcome some of the problems related to primer design.

Primer sequence

The 3′-terminal sequence of the primer molecule is critical for the specificity and sensitivity of PCR. A run of 3 or more G or C bases at this position should be avoided since it may stabilize nonspecific annealing of the primer. Furthermore, a thymidine at the 3′ end is not recommended, since it is more

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What's New?

Mega- and Giga-sized QIAfilter™ and EndoFree™ Plasmid Kits. See page 8.

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PCR primer design

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Effect of Primer–Dimer Formation

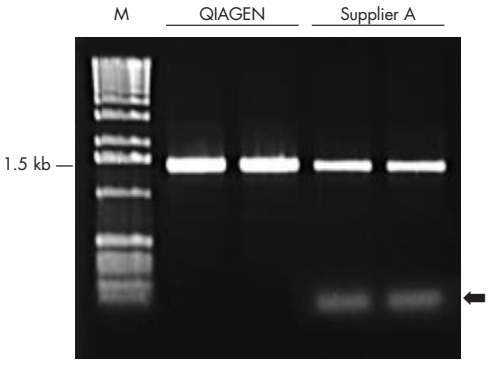

Figure 1 *Primers with a 4-bp overlap at the 3′-terminal position were employed to amplify a 1.5-kb fragment of the human single-copy cystic fibrosis gene using QIAGEN Taq DNA Polymerase and PCR Buffer (**QIAGEN**). The same PCR was performed in parallel using PCR buffer and Taq DNA polymerase from another supplier (**Supplier A**). Equal volumes of PCR product were analyzed on a 1% agarose gel. The arrow indicates primer–dimer product. **M**: markers.*

Effect of Primer Length

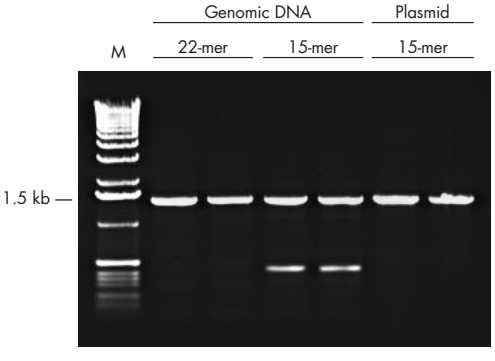

Figure 2 *Amplification of a 1.5-kb fragment from the human single-copy cystic fibrosis gene, using either two 22-mer primers (control) or one 22-mer primer (located 5′ of the fragment) and a 15-mer primer (located 3′ of the fragment). The 15-mer primer comprised the same sequence as the 22-mer control primer minus 7 nucleotides at the 3′ end. PCR was performed either with human genomic DNA or with plasmid DNA containing the gene fragment to be amplified. **M**: markers.*

prone to mispriming than other nucleotides (1). Most importantly, primer pairs should be checked for complementarity. Complementarity between primer sequences at the 3′ end often leads to primer–dimer formation. The creation and subsequent amplification of these primer–dimers reduces the availability of primer to the template molecule resulting in decreased sensitivity or even failure of the PCR (Figure 1). In the illustrated example the PCR is notably less susceptible to primer–dimer formation using QIAGEN® PCR Buffer rather than the PCR buffer from supplier A which contains K^+ as the only monovalent cation.

If mutations are to be introduced via the primer into the PCR product, it is important to leave at least three bases at the 3′ end of the primer which are homologous to the template DNA. Mismatches at these sites will greatly reduce the efficiency of PCR (1, 2). Therefore, when using degenerated primers, it is especially important that the 3′ sequence of the primer should correspond to highly conserved regions of the target sequence. Bases located at the 5′ end of the primer are less critical for primer annealing. Therefore, including recognition sites for restriction enzymes or promoter elements into the primer molecule can be easily achieved by adding these sequence elements to the 5′ end. Usually, such noncomplementary sequences do not change the annealing behavior of the sequence-specific part of the primer.

Primer sequences should also be checked for self-complementarity which could introduce secondary structures like hairpin loops into the primer. Commercially available computer software can be used to search for such complementary sequences.

Primer length

Usually, a primer length of 18–30 bases is optimal for most PCR applications. This is based on the complexity of the target template DNA. Theoretically, a primer of 18 bases represents a unique DNA sequence amongst $4^{18} = 7 \times 10^{10}$ nucleotides and should hybridize at only one position in most eukaryotic genomes which consist of approximately 10^9–10^{10} base pairs. A shorter primer such as a 15-mer would have a higher chance of annealing at more than one complementary site within the genome. This may lead to amplification of nonspecific PCR products. In contrast, when using the same shorter primer with a less complex DNA template such as plasmid DNA, PCR only generates the specific PCR product (Figure 2).

T_m and annealing temperature

The optimal annealing temperature often varies from the estimated T_m (3; Table 2) even when using pairs of primers with a similar T_m value. As a starting point, an annealing temperature 5°C below the T_m can be used. This is usually then adjusted to improve specificity and yield in a series of tedious optimization experiments. Using QIAGEN PCR Buffer such optimization of annealing temperature is often unnecessary. This is because QIAGEN PCR Buffer provides a wider temperature window for specific annealing than other commercially available PCR buffers (4; Figure 3).

Primer concentration

The concentration of primer in the amplification reaction should be between 0.1 and 0.5 µM. For most PCR applications, including sensitive PCR assays and the amplification of longer PCR products, 0.2 µM of each primer produces satisfactory results (Figure 4A). Primer concentrations which are too high increase the chance of mispriming. Subsequent extension of misprimed molecules results in nonspecific PCR products (Figure 4B). Table 1 gives a range of molar conversions for easy calculation of primer concentration.

Effect of PCR Buffers on Annealing Temperature

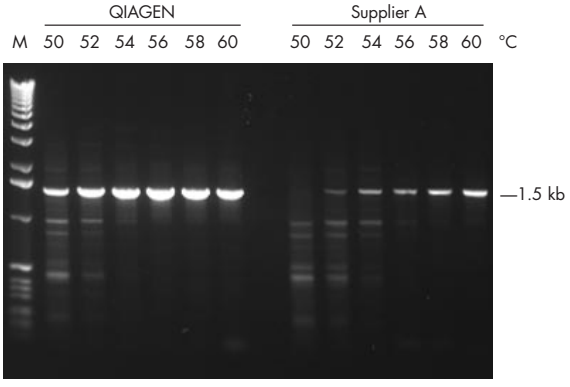

*Figure 3 Amplification of the human single-copy cystic fibrosis gene at the indicated annealing temperatures using QIAGEN PCR Buffer and QIAGEN Taq DNA Polymerase (**QIAGEN**). The same PCR was performed in parallel using PCR buffer and Taq DNA polymerase from another supplier (**Supplier A**). **M**: markers.*

Effect of Primer Concentration

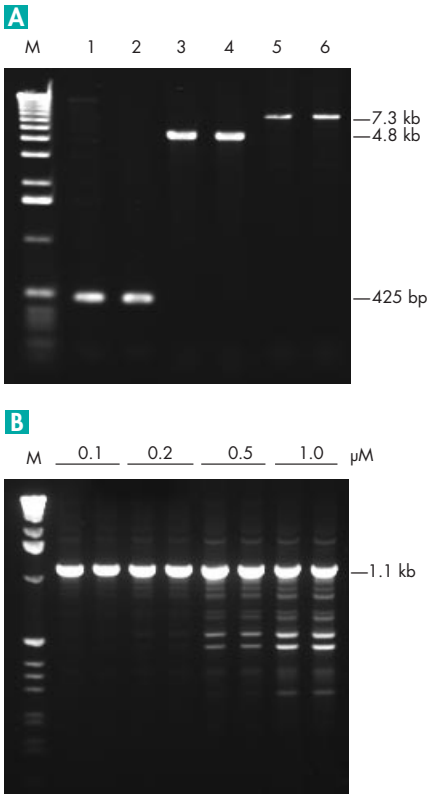

*Figure 4 **A** Amplification of a 425-bp fragment of the HIV pol gene from 10 copies of HIV DNA in 1 µg of human genomic DNA (**1, 2**), a 4.8-kb fragment of the human tissue plasminogen activator gene (**3, 4**), and a 7.3-kb fragment of the human interleukin-9-receptor gene (**5, 6**) using 0.2 µM each primer. **B** Amplification of a 1.1-kb fragment of the human hugl-2 gene employing the primer concentrations indicated. **M**: markers.*

Table 1. Molar conversions for primer concentration

Primer length	pmol/µg	20 pmol*
18-mer	168	119 ng
20-mer	152	132 ng
25-mer	121	165 ng
30-mer	101	198 ng

** 20 pmol primer in a 100-µl PCR gives a primer concentration of 0.2 µM.*

PCR primer design

References

1. *Kwok, S.* et al. *(1990) Effects of primer–template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies. Nucleic Acids Res.* ***18****, 999.*
2. *Sommer, R. and Tautz, D. (1989) Minimal homology requirements for PCR primers. Nucleic Acids Res.* ***17****, 6749.*
3. *Suggs, S.V.* et al. *(1981) ICN-UCLA Symp. Mol. Cell. Biol.* ***23****, 683.*
4. *PCR optimization. (1997) QIAGEN News* ***2****, 1.*

Table 2. Guidelines for the design and use of primers

Sequence:	◆ Avoid runs of 3 or more G or C at the 3′ end ◆ Avoid a T at the 3′ end ◆ Avoid mismatches at the 3′ end ◆ Avoid complementary sequences within a primer and between primers
Length:	18–30 nucleotides
GC content:	40–60%
T_m:	T_m = 2°C x (A+T) + 4°C x (C+G)
Conc.:	0.1–0.5 µM (0.2 µM)

Conclusions

PCR specificity and efficiency can be greatly affected by the way primers are designed and used. A summary of the guidelines for design of PCR primers is provided in Table 2. Even when primers are designed to have similar annealing properties, the PCR may yield nonspecific PCR products, low amounts of specific product, or fail completely. The subsequent time-consuming optimization procedures required can often be avoided when using QIAGEN *Taq* DNA Polymerase and PCR Buffer which allow for specific annealing within a wider temperature window than other PCR buffers. ■

Ordering Information

Product	Contents	Cat. No.
***Taq* DNA Polymerase**		
Taq DNA Polymerase (250)	250 units *Taq* DNA Polymerase, 10x PCR Buffer*, 5x Q-Solution, 25 mM $MgCl_2$	201203
Taq DNA Polymerase (4 x 250)	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer*, 5x Q-Solution, 25 mM $MgCl_2$	201205
Taq DNA Polymerase (20 x 250)	20 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer*, 5x Q-Solution, 25 mM $MgCl_2$	201207
***Taq* PCR Core Kits**		
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 Core Kit (4 x 250)	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer*, 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix†	201225
***Taq* PCR Master Mix Kits**		
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 H_2O	201443
Taq PCR Master Mix Kit (4 x 250)	12 x 1.7 ml *Taq* PCR Master Mix‡ containing 1000 units *Taq* DNA Polymerase total, 12 x 1.7 ml distilled H_2O	201445

* *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*

Purchase of *Taq* DNA Polymerase, *Taq* PCR Core Kit, or *Taq* PCR Master Mix 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.