January 2015

QIAGEN® PCR Cloning Handbook

For

QIAGEN PCR Cloning Kit

QIAGEN PCR Cloning*plus* Kit

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

QIAGEN PCR Cloning Kit	**(10)**	**(40)**
Catalog no.	**231122**	**231124**
Ligation Master Mix, 2x	50 µl	200 µl
pDrive Cloning Vector	0.5 µg	2.0 µg
Distilled water	1.7 ml	1.7 ml

QIAGEN PCR Cloning*plus* Kit	**(10)**	**(40)**
Catalog no.	**231222**	**231224**
BOX1		
Ligation Master Mix, 2x	50 µl	200 µl
pDrive Cloning Vector	0.5 µg	2.0 µg
Distilled water	1.7 ml	1.7 ml
BOX2		
QIAGEN EZ Competent Cells	10 tubes, 50 µl each	40 tubes, 50 µl each
SOC medium	2 x 1.9 ml	6 x 1.9 ml

Shipping Conditions

QIAGEN® PCR Cloning Kits are shipped on dry ice. Please verify upon shipment arrival that dry ice is still present in the shipping container. The QIAGEN PCR Cloning*plus* Kit is packaged in two boxes: Box I contains reagents for ligation of PCR products into the pDrive Cloning Vector, and Box II contains QIAGEN EZ Competent Cells and SOC medium.

Storage

The QIAGEN PCR Cloning Kit and Box I of the QIAGEN PCR Cloning^{plus} Kit (containing Ligation Master Mix and pDrive Cloning Vector) should be stored at –15 to –30°C in a constant temperature freezer or at –70°C **immediately** upon receipt. Box II of the QIAGEN PCR Cloning^{plus} Kit (containing QIAGEN EZ Competent Cells) should be stored at –70°C or below **immediately** upon receipt. Care should be taken to avoid thawing of competent cells. When stored under these conditions and handled correctly, the kit components can be kept at least until the expiration date (see inside of kit lid) without showing any reduction in performance.

Product Use

QIAGEN PCR Cloning Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. A copy of the QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call one of the QIAGEN Technical Service Departments or your local distributor (see back cover for contact information).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any

difficulties regarding QIAGEN PCR Cloning Kits, or QIAGEN products in general, please do not hesitate to contact us. QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information, please call one of the QIAGEN Technical Service Departments or your local distributor (see back cover for contact information).

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 QIAGEN PCR Cloning Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAGEN PCR Cloning Kits combine ligation technology with a unique combination of time-saving features for fast, easy, and highly efficient cloning of PCR products generated using *Taq* and other non-proofreading DNA polymerases. The pDrive Cloning Vector provides superior performance through UA-based ligation and allows easy analysis of cloned PCR products. The Ligation Master Mix contains all other reagents and cofactors required for optimal ligation in a convenient, pre-mixed format. QIAGEN EZ Competent Cells, provided in ready-to-use aliquots in the QIAGEN PCR Cloning^{plus} Kit, are capable of high-efficiency transformation and can be plated immediately onto agar/ampicillin plates following transformation.

The QIAGEN PCR Cloning Principle

QIAGEN PCR Cloning Kits take advantage of the single A overhang at each end of PCR products generated using *Taq* and other non-proofreading DNA polymerases. The pDrive Cloning Vector, supplied in a linear form with a U overhang at each end, hybridizes with high specificity to such PCR products. Optimal conditions for rapid and efficient hybridization and ligation are provided by the advanced buffer design of the Ligation Master Mix.

The QIAGEN PCR Cloning procedure is fast and easy — PCR products are simply mixed with pDrive Cloning Vector and Ligation Master Mix and then incubated at 4–16°C for 30 minutes (e.g., in a refrigerator). Special preparation of PCR products is not required. Transformation and plating using QIAGEN EZ Competent Cells takes only 10 minutes, making the total procedure, from PCR product to plating of transformed cells, just 40 minutes.

Product Description

pDrive Cloning Vector

The pDrive Cloning Vector is supplied in a linear form, ready-to-use for direct ligation of PCR products. This vector allows ampicillin and kanamycin selection, as well as blue/white colony screening. The vector contains several unique restriction endonuclease recognition sites around the cloning site, allowing easy restriction analysis of recombinant plasmids. The vector also contains a T7 and SP6 promoter on either side of the cloning site, allowing in vitro transcription of cloned PCR products as well as sequence analysis using standard sequencing primers. In addition, the pDrive Cloning Vector has a phage f1 origin to allow preparation of single-stranded DNA. A map of the pDrive Cloning Vector and

the sequence of the region surrounding the cloning site are provided in Figure 1 (page 10–11). See "pDrive Cloning Vector restriction sites" (Appendix, page 26) for additional information.

Reagents

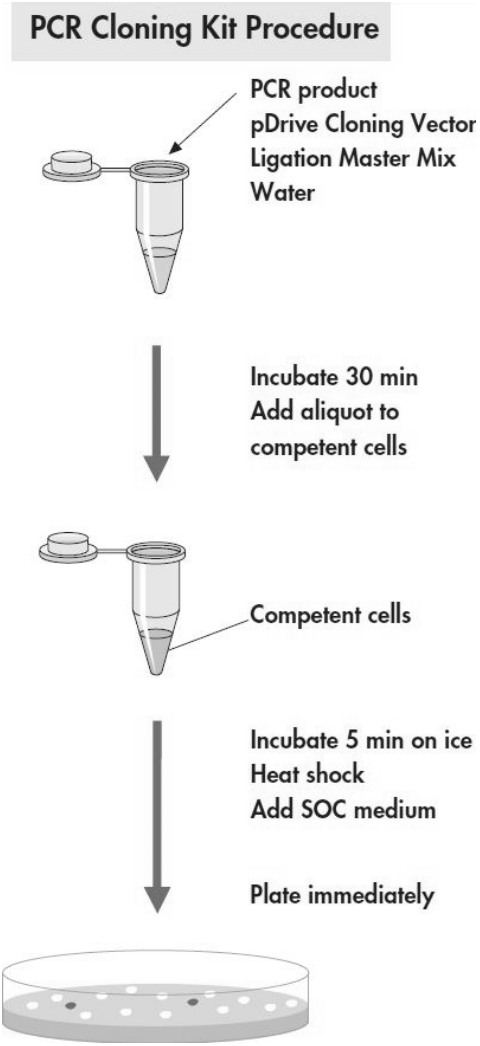
Ligation Master Mix, 2x: The Ligation Master Mix contains all reagents and cofactors required for ligation in a convenient pre-mixed format.

SOC medium: Tryptone, yeast extract, NaCl, KCl, glucose, $MgCl_2$, $MgSO_4$, sterile water.

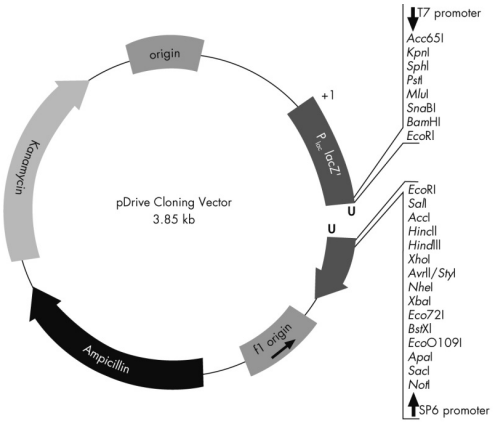
QIAGEN EZ Competent Cells

The genotype of QIAGEN EZ Competent Cells is: [F'::Tn*10*(Tc^r) *proA*$^+$*B*$^+$ *lacI*^{q}ZΔ*M15*] *recA1* end *A1 hsdR17* (r_{K12}^- m_{K12}^+) *lac glnV44 thi-1 gyrA96 relA1* (see Table 1, page 12, for further details).

QIAGEN EZ Competent Cells are capable of high-efficiency transformation (>1.0 x10^8 colony forming units per microgram DNA), and are supplied in ready-to-use aliquots for single transformation reactions. The cells are compatible with ampicillin and kanamycin selection and blue/white screening following transformation with pDrive Cloning Vector. When using ampicillin selection, QIAGEN EZ Competent Cells do not require an incubation step following transformation, but instead can be directly plated.


PCR Cloning Kit Procedure
PCR product
pDrive Cloning Vector
Ligation Master Mix
Water
Incubate 30 min
Add aliquot to
competent cells
Competent cells
Incubate 5 min on ice
Heat shock
Add SOC medium
Plate immediately

A



B

Element	Position (bp)
Multiple cloning site	266–393
LacZ α-peptide	216–593
T7 RNA polymerase promoter	239–258
T7 transcription start	256
SP6 RNA polymerase promoter	398–417
SP6 transcription start	400
Ampicillin resistance gene	1175–2032
Kanamycin resistance gene	2181–2993
pUC origin	3668
Phage f1 origin	588–1043
Primer binding sites:*	
M13 forward (–20)	431–447
M13 forward (–40)	451–467
M13 reverse	209–224
T7 promoter primer	239–258
SP6 promoter primer	400–418

* Primer sequences are provided in the Appendix

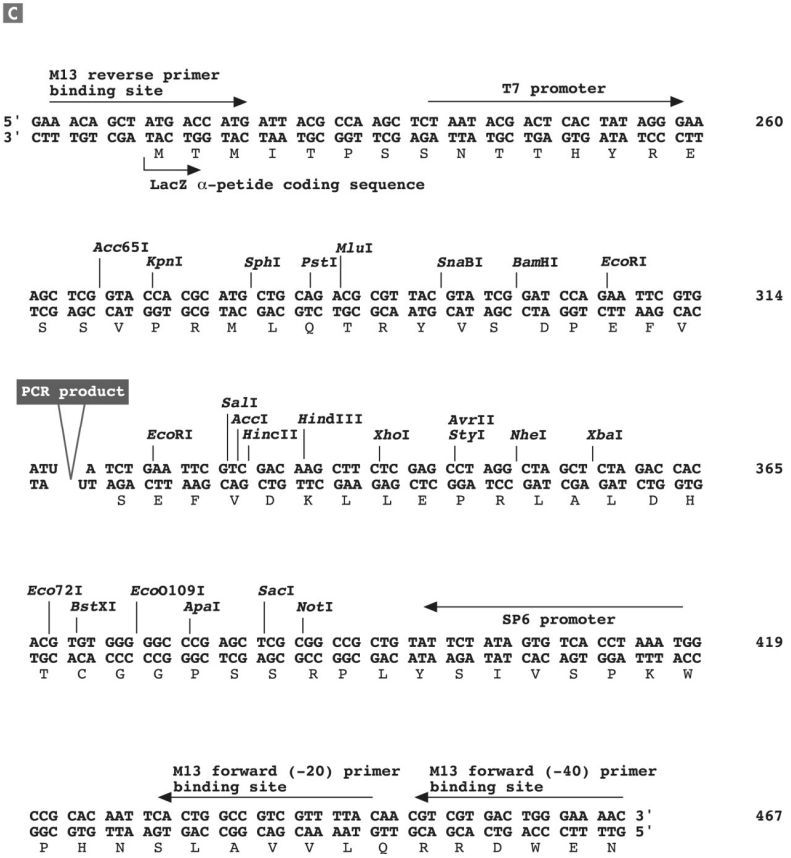


Figure 1. pDrive Cloning Vector Map. [**A**] Representation of the linearized pDrive Cloning Vector with U overhangs. The unique restriction endonuclease recognition sites on either side of the cloning site are listed. [**B**] Positions of various elements in the pDrive Cloning Vector. [**C**] DNA sequence of the region surrounding the cloning site. The amino acid sequence of the LacZ α-peptide is also given. The positions of the T7 and SP6 promoter sites and the M13 forward and reverse sequencing primer binding sites are provided, as are the cutting positions of the unique restriction endonucleases.

Table 1. Genotype of QIAGEN EZ Competent Cells

Genotype	Description/phenotype
F'	Presence of the low-copy-number F plasmid [F'::Tn *10*(Tc^r) *proA*$^+$*B*$^+$*lacI*^{q}ZΔ*M15*]
Tn *10*(Tc^r)	Transposon conferring tetracyclin resistance
lacI^q	Overproduction of the lac operon repressor, which controls expression from P_{lac}
lacZΔ*M15*	Expression of N-terminally deleted β-galactosidase. This protein and LacZ α-peptide (encoded by the pDrive Cloning Vector) provide β-galactosidase activity. Cells transformed by pDrive Cloning Vector which does not contain a PCR product will express LacZ α-peptide, and will form blue colonies when grown in the presence of X-gal/IPTG. In contrast, cells transformed by pDrive Cloning Vector which does contain a PCR product will not express LacZ α-peptide, and will form white colonies.
recA1	Abolished homologous recombination
end A1	Abolished nonspecific endonuclease I activity, and hence improved DNA quality in plasmid preparations
hsdR17	This mutation prevents restriction, but not protective methylation, of unmethylated DNA or DNA containing foreign methylation patterns in *E. coli* cells.
lac	Inability to utilize lactose
glnV44	Suppressor of amber (UAG) mutation; required for growth of some phage vectors. Formerly called *supE*.
thi-1	Requires thiamin (vitamin B1) for growth in minimal medium
gyrA96	Mutation in DNA gyrase; resistance to naladixic acid
relA1	Relaxed mutation, permits RNA synthesis in the absence of protein synthesis

Protocol: QIAGEN PCR Cloning Kit Ligation

Important notes before starting

- Use of PCR products generated with proofreading DNA polymerases (i.e., DNA polymerases with 3'-5' exonuclease activity) will dramatically lower ligation efficiency as these PCR products do not have an A overhang.
- We recommend using a molar ratio of 5–10 times more PCR product DNA than pDrive Cloning Vector DNA for ligation (Table 2). However, less PCR product may also be sufficient. PCR products can be concentrated using MinElute™ Kits.
- Purification of PCR products prior to ligation (e.g., using QIAquick®, MinElute PCR Purification, or Gel Extraction Kits) is optional but recommended, as this will generally result in higher transformation efficiency. See "Purification of PCR products" (Appendix, page 25).
- The 5'-terminal base of the PCR primers can affect addition of an A overhang to PCR products by non-proofreading DNA polymerases. See "Effect of the 5'-terminal base of PCR products on cloning efficiency" (Appendix, page 23).
- Background colonies may appear following transformation if the PCR template was plasmid DNA containing a resistance gene for the antibiotic used for colony selection (i.e., an ampicillin- or kanamycin-resistance gene). In these cases the PCR product should be gel-purified prior to ligation to remove template plasmid DNA. Gel-purification can be avoided by using kanamycin for selection if the template plasmid contains the ampicillin-resistance gene, and vice versa.
- If **electrocompetent** cells will be used instead of QIAGEN EZ Competent Cells for transformation, we strongly recommend inactivating the ligase prior to electroporation by incubating the ligation-reaction mixture for 10 min at 70°C (see protocol step 4).

Table 2. Guide for the amount of PCR product to use in the ligation reaction

PCR product size	5-times molar excess*	10-times molar excess*
100 bp	6.5 ng	13 ng
200 bp	13 ng	26 ng
500 bp	32.5 ng	65 ng
1000 bp	65 ng	130 ng
1500 bp	97.5 ng	195 ng
2000 bp	130 ng	260 ng
3000 bp	195 ng	390 ng

* Calculated for 50 ng pDrive Cloning Vector using the following equation:

$$\text{ng PCR product required} = \frac{50\ \text{ng} \times \text{PCR product size (bp)} \times \text{molar ratio}}{3851\ \text{bp}}$$

Procedure

1. **Thaw 2x Ligation Master Mix, pDrive Cloning Vector DNA, and distilled water (provided). Place on ice after thawing.**
   It is important to mix the solutions completely before use to avoid localized concentrations of salts. Keep 2x Ligation Master Mix on ice and immediately store at –15 to –30°C or –70°C after use.
2. **Prepare a ligation-reaction mixture according to the following table:**

Component	Volume/reaction
pDrive Cloning Vector (50 ng/µl)	1 µl
PCR product	1–4 µl*
Distilled water	variable
Ligation Master Mix, 2x†	5 µl
Total volume	**10 µl**

* Purified PCR product. If using non-purified PCR product, do not add more than 2 µl PCR product.

† We recommend adding the Ligation Master Mix last.

3. **Briefly mix the ligation-reaction mixture then incubate for 30 min at 4–16°C (e.g., in a refrigerator, water bath, or thermal cycling block).**

   Mix gently, for example by pipetting the ligation-reaction mixture up and down a few times.

   Note: Increasing the ligation time to 2 h can result in a 2–3 fold increase of recombinants. This might be especially useful for PCR fragments longer than 2 kb. If the total number of recombinants is not essential, however, the ligation time can be as short as 15 min.

4. **Proceed with the "Transformation Protocol" (page16) or store ligation-reaction mixture at –15 to –30°C until use.**

   IMPORTANT: The "Transformation Protocol", page 16 is for use with QIAGEN EZ Competent Cells. If **electrocompetent** cells will be used, we **strongly recommend** inactivating the ligase in the ligation-reaction mixture prior to electroporation. Incubate the ligation-reaction mixture for 10 min at 70°C, then proceed with electroporation. Alternatively, the MinElute Reaction Cleanup Kit can be used to remove ligase from the ligation-reaction mixture. The ligase does not need to be inactivated when using QIAGEN EZ Competent Cells.

Protocol: QIAGEN PCR Cloning*plus* Kit Transformation Protocol

Important notes before starting

- This protocol is for use with QIAGEN EZ Competent Cells. It is not for use with electrocompetent cells. If electrocompetent cells will be used, we **strongly recommend** inactivating the ligase in the ligation-reaction mixture prior to electroporation. See step 4 of the "Ligation Protocol" (page13) for details.
- Competent cells are extremely sensitive to temperature and mechanical stress. **Do not allow QIAGEN EZ Competent Cells to thaw at any point prior to transformation**. Keep thawed cells on ice. Avoid excessive and/or rough handling, especially pipetting. Mix cells by **gentle** flicking.
- Thaw SOC medium and warm to room temperature. Store at –15 to –30°C or –70°C after use.
- Prepare fresh LB agar plates containing either ampicillin (100 µg/ml LB agar) or kanamycin (30 µg/ml LB agar) as a selection marker. Include IPTG (50 µM) and X-gal (80 µg/ml) for blue/white screening of recombinant colonies. See "Appendix" (page 31) for recipes.

Procedure

1. **Thaw the appropriate number of tubes of QIAGEN EZ Competent Cells on ice. Thaw SOC medium and warm to room temperature.**
   IMPORTANT: Competent cells should only be thawed on ice. Do not allow unused QIAGEN EZ Competent Cells to thaw. Test whether cells are thawed by gently flicking the tube. Proceed immediately to the transformation step once the cells have thawed.
2. **Add 1–2 µl ligation-reaction mixture per tube of QIAGEN EZ Competent Cells, mix gently, and incubate on ice for 5 min.**
   Mix gently, for example by flicking the transformation mixture a few times.
3. **Heat the tube(s) in a 42°C water bath or heating block for 30 s without shaking.**
4. **Incubate the tube(s) on ice for 2 min.**

5. **Add 250 µl room temperature SOC medium per tube and directly plate 100 µl each transformation mixture onto LB agar plates containing ampicillin.**

   Note: For **kanamycin** selection, incubate the cells at 37°C for 30 min with shaking prior to plating to allow recombinant outgrowth.

   The transformation mixture can be plated using a sterile bent glass rod or a specialized spreader. It is generally recommended to plate different amounts of each transformation mixture onto separate plates (e.g., 100 µl and 20 µl) to ensure good separation of colonies for subsequent single-colony isolation. For more efficient plating of small volumes of transformation mixture (<50 µl) we recommend pipetting 100 µl LB medium onto the plate, and then pipetting the transformation mixture into the liquid LB.

6. **Incubate the plate at room temperature until the transformation mixture has absorbed into the agar. Invert the plate and incubate at 37°C overnight (e.g., 15–18 h).**

   Note: For blue/white screening, we recommend a second incubation at 4°C (e.g., in a refrigerator) for a few hours. This "cold" incubation step enhances blue color development and thereby facilitates differentiation between blue colonies and white colonies.

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

	Comments and suggestions
No colonies on agar plate	
a) Missing reagent	Check that all reagents were included in the ligation reaction and that a portion of the ligation mixture was added to the competent cells prior to transformation.
b) Low transformation efficiency of competent cells	Use highly competent cells (>1.0 x 10^8 cfu/µg circular plasmid DNA). If not using QIAGEN EZ Competent cells, test transformation efficiency by transforming the cells using a defined amount of circular plasmid DNA.
c) Incorrect transformation procedure	Make sure that the appropriate transformation procedure was used, i.e., heat shock at 42°C for chemically competent cells (such as QIAGEN EZ Competent Cells) or electroporation for electrocompetent cells.
d) Ligase was not inactivated prior to electroporation	If electrocompetent cells are used form transformation, **we strongly recommend** inactivating the ligase in the ligation-reaction mixture prior to electroporation. Following ligation, incubate the ligation-reaction mixture for 10 min at 70°C, then proceed with electroporation. Alternatively, MinElute Reaction Cleanup Kits can be used to remove ligase from the ligation-reaction mixture.
e) Incorrect antibiotics	Check that the LB agar plate used contained the appropriate antibiotic.

	Comments and suggestions
f) Incorrect incubation temperature(s)	Ensure that all incubation steps were performed at the correct temperature.
g) Incorrect DNA polymerase used for PCR	QIAGEN PCR Cloning Kits are designed for cloning of PCR products generated by non-proofreading DNA polymerases (e.g., *Taq*DNA polymerase). Proofreading DNA polymerases generate predominantly blunt-ended PCR products, which will not efficiently ligate to the pDrive Cloning Vector. An A overhang can be added to such PCR products by treatment with an appropriate polymerase.
Low number of white colonies	
a) Incorrect DNA polymerase used for PCR	QIAGEN PCR Cloning Kits are designed for cloning of PCR products generated by non-proofreading DNA polymerases (e.g., *Taq*DNA polymerase). Proofreading DNA polymerases generate predominantly blunt-ended PCR products, which will not efficiently ligate to the pDrive Cloning Vector. An A overhang can be added to such PCR products by treatment with an appropriate polymerase.
b) Inappropriate insert: vector ratio	Quantitate the PCR product by agarose gel analysis (see "Quantitation of PCR products", Appendix, page 25). We recommend a molar ratio of insert to vector of 5:1 to 10:1. In rare cases further optimization of the insert:vector ratio may be necessary.
c) Ligation time too short	Increasing the ligation time can yield higher numbers of recombinant colonies
d) Inefficient addition of single A overhang by the DNA polymerase	For efficient addition of an A overhang during the PCR procedure, we recommend a final extension step for at least 10 min. The efficiency of addition of a single A to PCR products by DNA polymerase is also sequence dependent. See "Effect of the 5'-terminal base of PCR primers on cloning efficiency", Appendix, page 23.

	Comments and suggestions
e) Presence of inhibitor in PCR mixture	Purify the PCR product prior to ligation, e.g., using the QIAquick ot MiniElute system. See "Purification of PCR products", Appendix, page 25.
f) Overexposure of PCR products to UV light	For gel-purification, use long-wave UV light to visualize PCR products and ensure that the DNA is exposed for as short a time as possible. Overexposure of DNA to UV induces pyrimidine dimers which reduce the efficiency of the procedure.
g) Incorrect ligation temperature	Ensure that the temperature used for ligation does not exceed 16°C. Higher temperatures, e.g., greater than 25°C, give rise to increased background and fewer recombinants.
h) Nuclease contamination	Nucleases may degrade the U overhang. Use only the provided distilled water and Ligation Master Mix, which are tested for absence of nucleases, in the ligation reaction.
Only white colonies on the plate	
a) Inappropriate bacterial strain used for blue/white screening	Ensure that the bacterial strain used for transformation has a *lacI*^{q}ZΔM15 genotype. This mutation, which is present in QIAGEN EZ Competent Cells, is usually located on the F' plasmid and is a prerequisite for blue/white screening.
b) Insufficient/no X-gal and/or IPTG	Check that the agar plates contain sufficient X-gal/IPTG. Use freshly prepared plates.

	Comments and suggestions
c) Cells transformed with residual plasmid DNA from PCR reaction	Background colonies may appear following transformation if the PCR template was plasmid DNA containing a resistance gene for the antibiotic used for colony selection (i.e., an ampicillin- or kanamycin-resistance gene). In these cases the PCR product should be gel-purified prior to ligation to remove template plasmid DNA, e.g., using the QIAquick or MinElute systems. Gel purification can be avoided by using kanamycin for selection if the template plasmid contains the ampicillin-resistance gene, and vice versa. Alternatively, screen ampicillin-resistant white colonies for kanamycin resistance, and vice versa.
d) Degraded antibiotics	The antibiotic present in the agar plate may have lost its full activity, allowing untransformed colonies to grow. Use freshly prepared plates. Add antibiotic to cooled agar (<55°C) before pouring plates.
Only blue colonies on the plate	
a) Inappropriate bacterial strain used for blue/white screening	Ensure that the bacterial strain used for transformation carries a *lacZ* mutation (e.g., QIAGEN EZ Competent Cells). $LacZ^+$ strains are not appropriate for blue/white screening.
b) Functional LacZ α-peptide fusion protein	In rare cases the cloned PCR product will be expressed as a fusion protein with functional LacZ α-peptide. The length of such cloned fragments is usually a multiple of 3 bases (including the 3'-A overhang).
c) Nuclease contamination	Nucleases may degrade the U overhang. Use only the provided distilled water and Ligation Master Mix, which are tested for absence of nucleases, in the ligation reaction.
d) PCR products not added to the ligation-reaction mixture	Ensure that the PCR product was added to the ligation-reaction mixture.

	Comments and suggestions
Clones contain the PCR product in only one orientation	Expression of the PCR product in the appropriate reading frame (e.g., as a fusion protein with LacZ α-peptide) might be toxic for the cells. Omitting IPTG from the growth medium will decrease the expression level from P_{lac}, and therefore potentially allow the cells to survive. However, blue/white selection is usually not possible in the absence of IPTG.
White colonies do not contain the PCR product of interest	
a) Cells transformed with residual plasmid DNA from PCR reaction	Background colonies may appear following transformation if the PCR template was plasmid DNA containing a resistance gene for the antibiotic used for colony selection (i.e., an ampicillin- or kanamycin-resistance gene). In these cases the PCR product should be gel-purified prior to ligation to remove template plasmid DNA, e.g., using the QIAquick or MinElute systems. Gel purification can be avoided by using kanamycin for selection if the template plasmid contains the ampicillin-resistance gene, and vice versa. Alternatively, screen ampicillin-resistant white colonies for kanamycin resistance, and vice versa.
b) Non-specific PCR products or primer-dimer cloned into pDrive Cloning Vector	Increase the stringency of the PCR conditions, for example by increasing the annealing temperature or using a hot-start procedure (e.g., HotStarTaq™ DNA Polymerase). Alternatively, gel-purify the PCR product prior to ligation.
c) Nuclease contamination	Nucleases may degrade the U overhang. Use only the provided distilled water and Ligation Master Mix, which are tested for absence of nucleases, in the ligation reaction.

Appendix

Generation of PCR products

PCR products that will be cloned using QIAGEN PCR Cloning Kits should be generated using a thermostable DNA polymerase that does not possess proofreading activity, such as *Taq* DNA polymerase. Such polymerases attach a single A overhang to their reaction products, which can hybridize to the U overhang of the pDrive Cloning Vector. For efficient addition of an A overhang during the PCR procedure, we recommend a final extension step for 10 min at 72°C (as described in the PCR protocols for QIAGEN *Taq* DNA Polymerase and HotStarTaq DNA Polymerase).

High-specificity PCR is also a prerequisite for fast and easy cloning of PCR products. QIAGEN *Taq* DNA Polymerase and HotStarTaq DNA Polymerase are provided with innovative QIAGEN PCR Buffer, which supports specific primer annealing in each PCR cycle without the need for tedious optimization procedures. The use of a hot-start procedure can further increase PCR specificity. HotStarTaq DNA Polymerase, a modified form of QIAGEN *Taq* DNA Polymerase, provides high specificity in hot-start PCR. HotStarTaq DNA Polymerase is supplied in an inactive state that has no polymerase activity at ambient temperatures. This prevents extension of nonspecifically annealed primers and primer-dimers formed at low temperatures during PCR setup and the initial PCR cycle. HotStarTaq DNA Polymerase is activated by a15-minute incubation at 95°C that can be easily incorporated into any existing thermal-cycler program. For more information about QIAGEN *Taq* DNA Polymerase or HotStarTaq DNA Polymerase, please call your local QIAGEN Technical Service Department or distributor (see back cover for contact information).

Effect of the 5'-terminal base of PCR primers on cloning efficiency

Thermostable DNA polymerases possessing no proofreading activity, such as *Taq* DNA polymerase, commonly add a single deoxynucleotide to the 3'-terminus of PCR products. Although deoxyadenosine (A) is generally added, this activity may also be dependent on the 5'-terminal base(s) of the primers used for PCR. The highest cloning efficiency will be obtained using PCR primers with a 5'-terminal deoxyadenosine.

General guidelines for PCR primer design and usage

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 provided in Table 3 (see also QIAGEN News 1997 No. 5, 1).

Table 3. General guidelines for standard PCR primers

Length	18–30 nucleotides
G/C content	40–60%
T_m:	Simplified formula for estimating melting temperature (T_m): T_m = 2°C x (A+T) + 4°C x (G+C) Whenever possible, design primer pairs with similar T_m values. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.
Sequence	■ Avoid complementarity of two or three 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 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 primer pair. ■ Commercially available computer software (e.g., Primer Designer 1.0, Scientific Software, 1990; Oligo, Rychlik and Rhoads,1989) can be used for primer design.
Concentration	■ Spectrophotometric conversion for primers: 1 A_{260} unit = 20–30 µg/ml

	■ Molar conversions: **Primer length** **pmol/µg** **20 pmol** 18mer 168 119 ng 20mer 152 132 ng 25mer 121 165 ng 30mer 101 198 ng ■ Use 0.1–0.5 µM of each primer in PCR. For most applications, a primer concentration of 0.2 µM will be sufficient.
Storage	Lyophilized primers should be dissolved in a small volume of distilled water or TE to make a concentrated stock solution. Prepare small aliquots of working solutions containing 10 pmol/µl to avoid repeated thawing and freezing. Store all primer solutions at –15 to –30°C. Primer quality can be checked on a denaturing polyacrylamide gel; a single band should be seen.

Purification of PCR products

After amplification, the PCR sample contains a complex mixture of specific PCR product and residual reaction components such as primers, unincorporated nucleotides, enzyme(s), salts, mineral oil, and possibly non-specific amplification products. Removal of these contaminants may increase cloning efficiency. The QIAquick system offers a quick and easy method for purifying PCR products. The MinElute system should be used if the PCR product needs to be concentrated, e.g., due to inefficient PCR. See page 33 for "Ordering Information". For more information about QIAquick or MinElute Kits, please call your local QIAGEN Technical Service Department or distributor (see back cover for contact information).

Quantitation of PCR products

DNA concentration is generally determined either by spectrophotometric measurement of the adsorption at 260 nm or by agarose gel analysis. Absorbance readings (A_{260}) should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. An absorbance of 1.0 at

260 nm corresponds to 50 µg DNA/ml. Since spectrophotometric measurement cannot differentiate between PCR products, primers, and oligonucleotides, the PCR product should be purified prior to measurement. The QIAquick and MinElute systems offer quick and easy methods for purifying PCR products (see page 33 for "Ordering Information").

PCR products can be quickly and easily quantitated by agarose gel analysis. The sample is run in an agarose gel side-by-side with known amounts of DNA of the same size (e.g., an appropriate DNA ladder). The DNA can be visualized under UV light following staining with ethidium bromide*, and the DNA concentration of the sample estimated by visual comparison of the intensity of the sample band with that of the standards. More precise quantitation can be obtained by densitometric measurement of band intensity and comparison with a standard curve.

Preparation of recombinant plasmid DNA

Many downstream applications require high-purity or ultrapure DNA. QIAGEN offers a complete range of plasmid preparation systems ensuring the highest quality plasmid DNA, for example, the QIAprep® miniprep system for fast and simple preparation of up to 20 µg high-purity DNA, and the HiSpeed™ Plasmid Kit for fast purification of ultrapure DNA. See page 33 for "Ordering Information". For more information about plasmid purification products, please call your local QIAGEN Technical Service Department or distributor (see back cover for contact information).

pDrive Cloning Vector restriction sites

The pDrive Cloning Vector is supplied in a linear form with a U overhang at each end. Insertion of a PCR product destroys the EcoRV site of the vector, and therefore use of EcoRV for restriction mapping of recombinant plasmid is not recommended. The first base of the lacZ 5' upstream region is designated as nucleotide number one. Table 4 lists restriction enzymes that do not cut the pDrive Cloning Vector, and Table 5 lists restriction enzymes that cut the vector 1–5 times. The complete pDrive Cloning Vector sequence is available on the QIAGEN website (www.qiagen.com/pDriveVectorSequence).

* Toxic and/or mutagenic. Take appropriate safety measures.

Table 4. Restriction enzymes that do not cut the pDrive Cloning Vector

*Aat*II	*Bgl*II	*Bss*HII	*Dsa*I	*Nco*I	*Sfi*I
*Acc*III	*BPu*1102I	*Bst*1107I	*Eco*47III	*Nde*I	*Sgr*AI
*Age*I	*Bse*RI	*Bsr*BI	*Ehe*I	*Pac*I	*Spe*I
*Asc*I	*Bsg*I	*Bst*API	*Fse*I	*Pin*AI	*Srf*I
*Ava*I	*BsiW*I	*Bst*EII	*Hpa*I	*Pme*I	*Sse*8387I
*Bbe*I	*BsmF*I	*Bst*HPI	*Kas*I	*Ppu*MI	*Stu*I
*Bbs*I	*Bsp*119I	*Bsu*36I	*Mfe*I	*Psh*AI	*Swa*I
*Bdv*CI	*Bsp*MI	*Btr*I	*Msc*I	*Sac*II	*Tth*111I
*Bal*I	*Bsr*BRI	*Csp*I	*Mun*I	*Sac*DI	*Xcm*I
*Bcu*I	*Bsr*GI	*Csp*45I	*Nar*I	*Sex*AI	

Table 5. Restriction enzymes that cut the pDrive Cloning Vector 1–5 times

Enzymes that cut once					
*Acc*I	328*	*Eco*31I	1896	*Psp*AI	2484
*Acc*65I	266	*Eco*52I	387	*Psp*OMI	374
*Ade*I	812	*Eco*72I	367	*Pst*I	283
*Alw*NI	3316	*Eco*ICRI	382	*Sac*I	384
*Apa*I	378	*Eco*NI	2524	*Sal*I	327
*Avr*II	345	*Eco*O109I	374	*Sap*I	3842
*Bam*HI	299	*Eco*RV†	316	*Sca*I	1481
*Bcg*I	1424	*Esp*3I	2628	*Sgf*I	2612
*Bpm*I	1893	*Fbl*I	328	*Sma*I	2486
*Bpu*10I	2629	*Hin*cII	329	*Sna*BI	293
*Bsa*I	1896	*Hin*dIII	333	*Sph*I	278
*Bsm*BI	2628	*Hsp*92I	1422	*Sst*I	384
*Bsp*DI	2303	*Kpn*I	270	*Sty*I	345
*Bsp*LU11I	3725	*Mlu*I	285	*Van*91I	2875
*Bst*XI	369	*Nae*I	915	*Xba*I	356
*Cci*NI	387	*Ngo*MIV	913	*Xho*I	339
*Cfr*9I	2484	*Nhe*I	350	*Xma*I	2484
*Cla*I	2303	*Not*I	387	*Xma*III	387
*Dra*III	812	*Nru*I	2269		
*Ech*HKI	1962	*Psi*I	684		

* Numbers refer to the position of the 3' base of the restricted DNA in each recognition sequence. The first base of the lacZ 5' upstream region of the pDrive Cloning Vector is designated as nucleotide number one.

† Insertion of a PCR product destroys the EcoRV site of the vector. Use of EcoRV for restriction mapping of recombinant plasmid is therefore not recommended

Enzymes that cut twice					
*Alw*44I	1290, 3411	*Bsr*DI	1728, 1902	*Nsp*I	278, 3729
*Apa*LI	1290, 3411	*Bss*SI	1293, 3552	*Ppu*10I	2458, 2724
*Ava*II	1598, 1820	*Dra*I	767, 3623	*Psp*1406I	1360, 1733
*Bcn*I	1125, 3527	*Eco*47I	1598, 1820	*Pvu*II	54, 517
*Bgl*I	577, 1844	*Eco*57I	1296, 3183	*Sin*I	1598, 1820
*Bmr*I	456, 1911	*Eco*RI	306, 321	*Taq*I	1478, 1484
*Bsa*MI	2496, 2573	*Fsp*I	567, 1739	*Xmn*I	1362, 3001
*Bse*MI	1728, 1902	*Nsi*I	2462, 2728		
Enzymes that cut three times					
*Alw*26I	1120, 1896, 2628	*Bsr*FI	913, 1877, 2566	*Pvu*I	548, 1593, 2612
*Ava*I	339, 377, 2484	*Cfr*10I	913, 1877, 2566	*Rsa*I	268, 1481, 2447
*Bsa*AI	293, 367, 812	*Csp*6I	267, 1480, 2446	SspI	604, 1157, 2537
*Bsm*AI	1120, 1896, 2628	*Dra*I	1384, 2076, 2095		
*Bsp*HI	1122, 2130, 3005	Eco88I	339, 377, 2484		

Enzymes that cut four times					
*Afl*II	285, 364, 366, 3725	*Bsr*HKAI	384, 1294, 1379, 3415	*Eco*64I	130, 266, 849, 2009
*Ban*I	130, 266, 849, 2009	*Bsr*BI	186, 956, 1120, 3796	*Hae*II	4, 963, 971, 3485
*Ban*II	378, 384, 887, 2267	*Ear*I	526, 1163, 2425, 3842	*Mbi*I	186, 956, 1120, 3796
*Bsa*WI	1666, 2747, 3372, 3519	*Eco*24I	378, 384, 887, 2267		
Enzymes that cut five times					
*Ase*I	46, 105, 1787, 2811, 3000	*Fau*I	34, 76, 546, 949, 1018	*Nci*I	1426, 1777, 2485, 2486, 3348
*Bcn*I	1426, 1777, 2485, 2486, 3348	*Hga*I	293, 1029, 1430, 3035, 3613	*Sml*I	339, 1337, 3080, 3357, 3619
*Eae*I	35, 387, 417, 433, 1569	*Msl*I	367, 413, 1191, 1550, 1709	*Vsp*I	46, 105, 1787, 2811, 3000

Recipes

X-gal stock solution (40 mg/ml)

Dissolve 400 mg 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside in 10 ml dimethylformamide.* Protect the solution from light by wrapping in aluminium foil or by using a brown bottle. Store at –15 to –30°C.

IPTG stock solution (100 mM)

Dissolve 238.3 mg isopropyl β-D-thiogalactopyranoside in 10 ml deionized water. Filter-sterilize and store in aliquots at –15 to –30°C.

Ampicillin stock solution (100 mg/ml)

Dissolve 5 g ampicillin sodium salt in 50 ml deionized water. Filter-sterilize and store in aliquots at 4°C.

Kanamycin stock solution (30 mg/ml)

Dissolve 1.5 g kanamycin monosulfate salt in 50 ml deionized water. Filter-sterilize and store in aliquots at 4°C.

LB (Luria Bertani) agar plates (1000 ml = approx. 40 plates)

Dissolve 10 g tryptone, 5 g yeast extract, 10 g NaCl, and 15 g agar in 950 ml deionized water. Adjust the pH of the solution to 7.0 and add deionized water to 1000 ml. Autoclave the solution for 20 min.

To pour plates, allow autoclaved LB agar to cool to 55°C. Add 1 ml ampicillin stock solution and/or kanamycin stock solution, as appropriate. If blue/white screening will be performed, add 2 ml X-gal stock solution and 0.5 ml IPTG stock solution. Mix, pour agar into plates, allow to set, invert, and store at 4°C under sterile conditions.

We recommend using freshly poured plates (<1 week old) for plating cells. Long-term storage of plates can result in decreased antibiotic activity.

Primer sequences

M13 forward (–20):	5' GTAAAACGACGGCCAGT 3'
M13 forward (–40):	5' GTTTTCCCAGTCACGAC 3'
M13 reverse:	5' AACAGCTATGACCATG 3'
SP6 promoter:	5' CATTTAGGTGACACTATAG 3'
T7 promoter:	5' GTAATACGACTCACTATAG 3'

* Toxic and/or mutagenic. Take appropriate safety measures.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
QIAGEN PCR Cloning Kits — for direct cloning of PCR products		
QIAGEN PCR Cloning Kit (10)	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), Distilled water (1.7 ml)	231122
QIAGEN PCR Cloning Kit (40)	For 40 reactions: 2x Ligation Master Mix (200 µl), pDrive Cloning Vector (2.0 µg), Distilled water (1.7 ml)	231124
QIAGEN PCR Cloning*plus* Kit (10)	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), Distilled water (1.7 ml), QIAGEN EZ Competent Cells (10 tubes, 50 µl each), SOC medium (2 x 1.9 ml)	231222
QIAGEN PCR Cloning*plus* Kit (40	For 40 reactions: 2x Ligation Master Mix (200 µl), pDrive Cloning Vector (2.0 µg), Distilled water (1.7 ml), QIAGEN EZ Competent Cells (40 tubes, 50 µl each), SOC medium (6 x 1.9 ml)	231224
Related products for PCR		
HotStarTaq DNA Polymerase — for highly specific hot-start PCR		
HotStarTaq DNA Polymerase, (250)*	250 units HotStarTaq DNA Polymerase 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203203

* Larger kit sizes available

† Contains 15 mM $MgCl_2$

Product	Contents	Cat. no.
QIAGEN *Taq* DNA Polymerase — for standard and specialized PCR applications		
Taq DNA Polymerase (250)*	250 units *Taq* DNA Polymerase,10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	201203
QIAGEN OneStep RT-PCR Kit — for fast and efficient one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 one-step RT-PCR PCRs: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,‡ dNTP Mix,§ 5x Q-Solution, RNase-free water	210210
Omniscript RT Kit — for standard reverse transcription		
Omniscript RT Kit (10)*	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,** RNase-free water	205110
Sensiscript RT Kit — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,** RNase-free water	205211
Related products for PCR product cleanup		
QIAquick PCR Purification Kit — for direct purification of PCR fragments		
QIAquick PCR Purification Kit (50)††	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104

* Larger kit sizes available
† Contains 15 mM $MgCl_2$
‡ Contains 12.5 mM $MgCl_2$
§ Contains 10 mM each dNTP
** Contains 5 mM each dNTP
†† Larger kit sizes and/or different kit formats available

Product	Contents	Cat. no.
QIAquick Gel Extraction Kit — for gel extraction of DNA fragments		
QIAquick Gel Extraction Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704
MinElute PCR Purification Kit — for purification of PCR products in low elution volumes		
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
MinElute Gel Extraction Kit — for gel extraction of DNA fragments in low elution volumes		
MinElute Gel Extraction Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28604
MinElute Reaction Cleanup Kit — for cleanup of DNA from enzymatic reactions		
MinElute Reaction Cleanup Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28204
Related products for plasmid DNA preparation		
QIAprep Miniprep Kits — for high-purity plasmid minipreps		
QIAprep Spin Miniprep Kit (50)*	50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
HiSpeed Plasmid Kit — for ultrafast purification of plasmid or cosmid DNA		
HiSpeed Plasmid Midi Kit (25)*	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Modules plus Syringes, Reagents, Buffers	12643

* Larger kit sizes and/or different kit formats available

Notes

Notes

Notes

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