

# QIAGEN News

Innovation Working for You

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## New QIAGEN® PCR Cloning Kits — better results fast!

New QIAGEN® PCR Cloning Kits combine the latest 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 ligation and is optimized for rapid and efficient cloning.

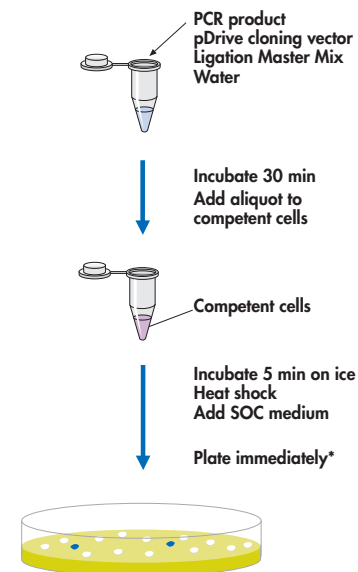
QIAGEN PCR Cloning Kits are available in two formats. The QIAGEN PCR Cloning Kit and the QIAGEN PCR Cloning<sup>plus</sup> Kit include all reagents required for efficient cloning of

PCR products. The QIAGEN PCR Cloning<sup>plus</sup> Kit additionally includes QIAGEN EZ Competent Cells in convenient, ready-to-use aliquots. These cells are capable of high-efficiency transformation (>10<sup>8</sup> colony forming units per microgram DNA) and can be plated immediately onto agar/ampicillin plates following transformation.

### QIAGEN PCR Cloning Kit advantages:

- ◆ **Fast and easy procedure** — just 40 minutes from PCR product to plated cells
- ◆ **Low background** — high specificity through UA basepairing
- ◆ **Choice of formats** — with or without competent cells
- ▶▶ **Fast and easy cloning of PCR products, page 18**

### PCR Cloning Kit Procedure



\*Using QIAGEN EZ Competent Cells

## New ProofStart™ DNA Polymerase — a new concept for high-fidelity PCR

QIAGEN introduces the new ProofStart™ DNA Polymerase, a robust proofreading enzyme for high-fidelity PCR (Figure 1, page 20).

### The new ProofStart DNA Polymerase offers:

- ◆ **Robust PCR performance** — modified enzyme to prevent primer degradation
- ◆ **High-fidelity PCR** — very low error rates
- ◆ **Minimal optimization** — through built-in hot start and optimized PCR buffer
- ◆ **Easy handling** — set up your reactions at room temperature

Proofreading polymerases are the enzymes of choice for applications that require error-free PCR products, such as cloning, site-directed mutagenesis, and mutation-detection analysis. However, these DNA polymerases are typically more difficult to use than *Taq* DNA polymerase due to their innate exonuclease activity. Although this activity is essential for reducing error rates, it also degrades primers, thereby causing low specificity and background smearing in PCR.

- ▶▶ **ProofStart DNA Polymerase, page 20**



**References:**

1. Ho, P.S. et al. (1985) G-T wobble base pairing in Z-DNA at 1.0 Å atomic resolution: the crystal structure of d(CGCGTG). *EMBO J.* **16**, 3617.
2. Kneale, G. et al. (1985) G-T base-pairs in a DNA helix: the crystal structure of d(G-G-G-T-C-C). *J. Mol. Biol.* **20**, 805.
3. Kwok, S. et al. (1990) Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *Nucleic Acid Res.* **18**, 999
4. Sugimoto, N., Nakano, M., and Nakano, S. (2000) Thermodynamics-structure relationship of single mismatches in RNA/DNA duplexes. *Biochemistry* **19**, 11270

continued from page 1

**Fast and easy cloning procedure**

QIAGEN PCR Cloning Kits are designed for fast and easy cloning of PCR products generated using *Taq* and other non-proofreading DNA polymerases that leave a single

A overhang on their reaction products. The pDrive Cloning Vector is supplied in a linear form with a U overhang at each end. This U overhang hybridizes with high specificity to the A overhang of the PCR products. Optimal conditions for efficient hybridization and ligation are provided by the advanced buffer design of the Ligation Master Mix, which contains all other reagents and cofactors required for ligation in a convenient premixed format.

The QIAGEN PCR Cloning Kit procedure is simple — just mix the PCR product directly with pDrive Cloning Vector and the Ligation Master Mix, incubate for 30 minutes at 4–16°C (e.g., in a refrigerator) and then transform bacteria with the ligation mix. 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. This is significantly faster than conventional sticky- and blunt-end ligation methods as well as T-based cloning methods.

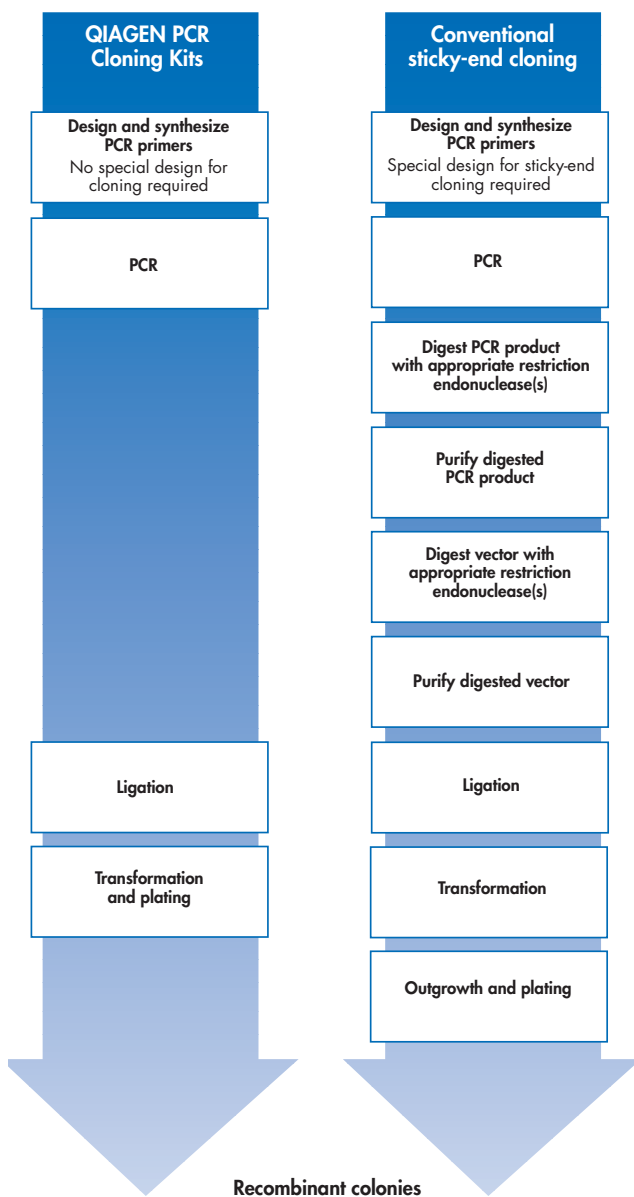
**Eliminate laborious restriction cloning**

QIAGEN PCR Cloning Kits eliminate problems associated with sticky-end cloning techniques. Generation of PCR products and vector with the appropriate ends for sticky-end ligation through use of restriction endonucleases is a time-consuming and laborious process. Furthermore, due to problems with poor digestion of PCR-product ends by restriction endonucleases, cloning efficiency is often low. Conventional blunt-end cloning techniques are generally not a viable choice as they require removal of the A overhang from the PCR product and suffer from a lower cloning efficiency. In contrast, the pDrive Cloning Vector takes advantage of the existing sticky end of PCR products generated using *Taq* and other non-proofreading DNA polymerases, so special preparation of PCR products is not required. This makes the QIAGEN PCR Cloning Kit procedure faster and far less labor-intensive than conventional ligation methods (Figure 1).

**Efficient ligation through UA-cloning**

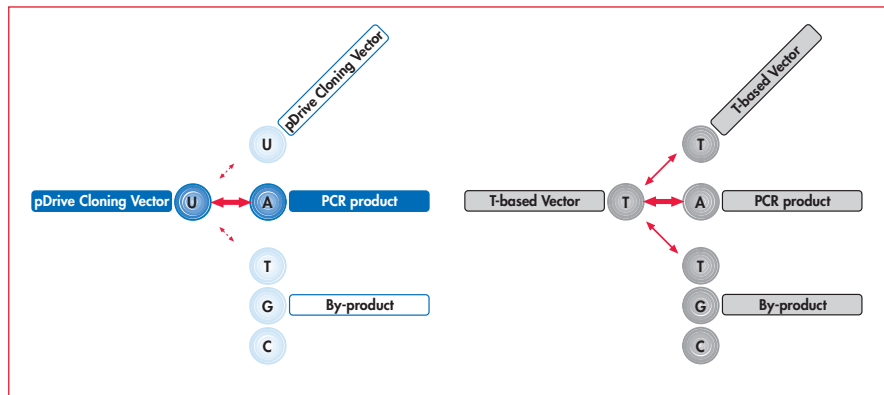
The pDrive Cloning Vector provides high-specificity cloning of PCR products. Of the four DNA bases, T is the most likely to

**Save Time Using QIAGEN PCR Cloning Kits**



**Figure 1** Comparison of the number of steps involved in conventional sticky-end cloning techniques and in the QIAGEN PCR Cloning Kit procedure.

Highly Specific Basepairing with the pDrive Cloning Vector



**Figure 2** Model of basepairing specificities for T and U. The T and U overhangs of T-based vectors and the pDrive Cloning Vector, respectively, have different specificities (indicated by the size of the arrows) for different 3' single nucleotide overhangs of other DNA species.

basepair with non-complementary bases, i.e., G, C, and T (1–4). This means that vectors with a T overhang are more likely to self-anneal and to anneal to undesirable by-products such as primers, annealed primer pairs, and incomplete PCR products (see Figure 2). In contrast, the higher cloning efficiency of the pDrive Cloning Vector indicates that U has a lower tolerance for nonspecific basepairing, reducing the number of false-positive colonies.

In addition, the pDrive Cloning Vector has a host of features, such as a large number of

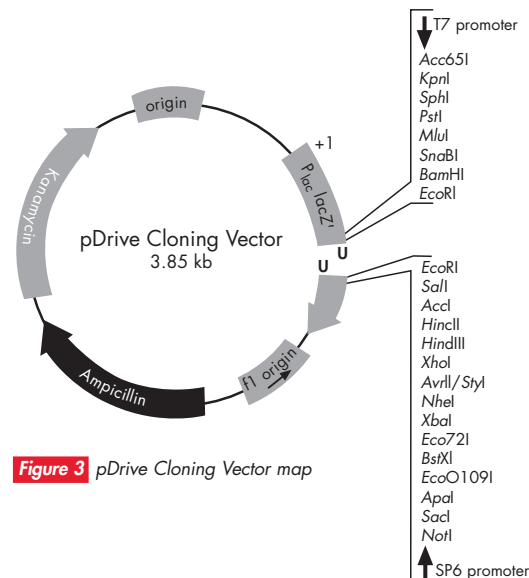
unique restriction enzyme recognition sites, universal sequencing primer sites, and promoters for in vitro transcription, designed to facilitate analysis of cloned PCR products (Figure 3).

**QIAGEN PCR Cloning Kits** combine highly efficient cloning with maximum convenience and ease of use, and are the ideal choice for cloning of PCR products. Order your kit today! ■

Reader Inquiry No. 01201

See also "Three new QIAexpress® vectors for more flexibility in protein applications", page 7, for details about our new UA-cloning-based expression vector.

Easy Analysis of Cloned PCR Products



**Figure 3** pDrive Cloning Vector map

Ordering Information

Product	Contents	Cat. No.
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 <sup>plus</sup> 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 <sup>plus</sup> 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