Quick-Start Protocol April 2016

QIAGEN® PCR Cloning Kit

The QIAGEN PCR Cloning Kit (cat. nos. 231122 and 231124) should be stored at –30 to –15°C in a constant-temperature freezer or at –70°C immediately upon receipt.

Further information

- *QIAGEN PCR Cloning Handbook*: **www.qiagen.com/HB-1912**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

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. The HotStar HiFidelity PCR Polymerase Kit (cat. no. 202602) provides the only proofreading DNA polymerase that generates PCR products with an A overhang for cloning.
- The 5'-terminal base of the PCR primers can affect addition of an A overhang to PCR products by *Taq* DNA polymerases.
- We recommend using a molar ratio of 5–10 times more PCR product DNA than pDrive Cloning Vector DNA for ligation (Table 1). However, less PCR product may also be sufficient.
- PCR products can be concentrated using QIAGEN's MinElute® Kits.
- Purification of PCR products prior to ligation is optional but recommended, as this will generally result in higher transformation efficiency.

- 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.

Table 1. Guide for the amount of PCR product to use in the ligation reaction

	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:

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

1. Thaw 2x Ligation Master Mix, pDrive Cloning Vector DNA and distilled water (provided) and place on ice. It is important to mix the solutions thoroughly before use. Keep 2x Ligation Master Mix on ice and immediately store at at –30 to –15°C or –70°C after use.
2. Prepare a ligation-reaction mixture according to Table 2.

Table 2. Ligation reaction setup

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).

   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, the ligation time can be as short as 15 min.

4. Proceed with the transformation protocol or store ligation-reaction mixture at –20°C until use.

   IMPORTANT: 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 and 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 (included in the QIAGEN PCR Cloning*plus* Kit).

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.