Quick-Start Protocol April 2016

# QIAGEN® PCR Cloning*plus* Kit

Box I (containing Ligation Master Mix and pDrive Cloning Vector) of the QIAGEN PCR Cloning*plus* Kit (cat. nos. 231222 and 231224) should be stored at –30 to –15°C in a constant-temperature freezer or at –70°C immediately upon receipt. Box II (containing QIAGEN EZ Competent Cells) should be stored at –70°C or below 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 –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, however, 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.

## Transformation protocol

### Notes before starting

- The QIAGEN PCR Cloning*plus* Kit 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 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.
- 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 –30 to –15°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.

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.
3. Mix gently by flicking the transformation mixture a few times.
4. Heat the tube(s) in a 42°C water bath or heating block for 30 s without shaking.
5. Incubate the tube(s) on ice for 2 min.
6. 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.

   **Note**: 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.
7. 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.

Scan QR code for handbook.

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