In-Fusion™ Dry-Down PCR Cloning Kit
Protocol-at-a-Glance

(PT3754-2)

Please read the *User Manual* (PT3754-1) before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

A. Cloning Procedure

1. Mix your PCR fragment and vector together at a 2:1 molar ratio in 10 µl of deionized H_2O.
2. Set up In-Fusion cloning reactions:
   a. Cut one tube off the strip, and peel back the aluminum seal.
   b. Add the 10 µl of vector + insert DNA (from Step 1). Mix well by pipetting up and down.
3. Incubate reactions at 42°C or at room temperature for 30 min, then transfer tubes to ice.
4. Proceed with Transformation (Section B). If you cannot transform cells immediately, store cloning reactions at –20°C until you are ready.

B. Transformation

1. Dilute the In-Fusion reaction mixture with 40 µl TE buffer, and mix well.
2. Transform competent cells with 2.5 µl of diluted reaction mixture as follows:
   a. **Using Fusion-Blue™ Competent Cells:**
      - Thaw one vial of frozen Fusion-Blue Competent Cells on ice. Tap tube gently to ensure that the cells are suspended.
      - Add 2.5 µl of the diluted reaction mixture to the cells. Mix gently to ensure even distribution of the DNA solution. Leave the tube on ice for 30 min.
      - **Do not add more than 5 µl of diluted reaction to 50 µl of competent cells.**
      - Heat shock the cells in a water bath at 42°C for 45 sec, and then place them directly on ice for 1 min.
      - **Competent cells should yield >1 x 10^8 cfu/µg. If not, replace with a fresh batch of cells.**
   b. **If using other competent cells with In-Fusion™ Kits**, follow the transformation protocol provided by the manufacturer **(do not add more than 5 µl of diluted reaction to 50 µl of competent cells)** and proceed to Step 3.
3. After heat shocking, add 450 µl of SOC medium to the cells. Incubate at 37°C for 60 min while shaking at 250 rpm.
4. Take 1/20–1/10 of the cells (25–50 µl) from each transformation. Bring the volume up to 100 µl with SOC medium, and plate by spreading on separate LB/X-Gal/Amp plates. Spread the remaining cells from each transformation on separate, LB/X-Gal/Amp plates. Incubate all plates at 37°C overnight.
5. The next day, pick white colonies from each experimental plate and isolate plasmid DNA using a standard method of your choice.

(PR591044; published 30 September 2005)