

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 H₂O.
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 10⁸ 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.

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. Clontech Laboratories, Inc. products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

Clontech Laboratories, Inc. has the exclusive right to make, use and sell the In-Fusion™ PCR Cloning System.

Fusion-Blue™ Cells are manufactured and tested by Novagen for Clontech Laboratories, Inc.

Clontech, Clontech Logo and all other trademarks are property of Clontech Laboratories, Inc.

Clontech is a Takara Bio Company. ©2005

(PR591044; published 30 September 2005)