

In-Fusion™ Ready Vector Cloning Protocol-at-a-Glance

(PT3865-2)

These protocols are provided for cloning PCR fragments into In-Fusion™ Ready vectors using our In-Fusion PCR Cloning Kits (Cat. Nos. 631774 & 631775) in Section I and our In-Fusion Dry-Down PCR Cloning Kits (Cat. Nos. 639602, 639604 & 639605) in Section II.

I. Cloning Protocol for In-Fusion™ PCR Cloning Kits

A. PCR Amplification for In-Fusion™ Ready Cloning

The sense and antisense primers that will be used to amplify the gene of interest via PCR must contain a specific 15 nucleotide sequence 5' to the sequence of the gene of interest, as shown below.

Sense primer: 5'-AAGGCCTCTGTCGAC followed by sequence of amplification target-3'

Antisense primer: 5'-AGAATTCGCAAGCTT followed by sequence of amplification target-3'

Note: The PCR product obtained from amplification using these primers is also ready for In-Fusion cloning into all other Clontech prelinearized In-Fusion Ready vectors if the gene of interest contains an initiating ATG. For additional information regarding In-Fusion cloning, see Section V and subsequent sections of the In-Fusion PCR Cloning Kit User Manual (PT3650-1).

B. Cloning Procedure

1. Dilute the In-Fusion enzyme 1:10 with In-Fusion Enzyme Dilution Buffer.
2. Combine the following in an eppendorf tube:

2	µl	10X In-Fusion Reaction Buffer
2	µl	10X BSA (500 µg/ml)
1	µl	Linearized In-Fusion Ready vector (100 ng/µl)
50–100	ng	Purified PCR product
1	µl	Diluted In-Fusion Enzyme
x	µl	Sterile H ₂ O
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20	µl	Total Volume
3. Incubate reactions at room temperature for 30 min, then transfer tubes to ice.
4. Incubate the reactions on ice for 30 min.
5. Proceed with transformation (Section C). If you cannot transform cells immediately, then store cloning reactions at –20°C until you are ready.

C. Transformation

1. Dilute the In-Fusion reaction mixture with 40 µl TE Buffer. Mix well.
2. Transform competent cells with 2.5 µl of diluted reaction mixture.
 - a. Thaw one vial of frozen competent cells on ice. Tap tube gently to ensure that the cells are suspended.
 - b. 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.
 - c. Heat shock the cells in a water bath at 42°C for 45 sec, and then place them directly on ice for 1 min.
3. Add 450 µl of SOC medium to the cells and then 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 different volumes on LB plates containing the appropriate antibiotic. Spread the remaining cells from each transformation on additional plates. Incubate all plates at 37°C overnight.
5. The next day, pick colonies from each experimental plate and isolate plasmid DNA using a standard method of your choice.

II. Cloning Protocol for In-Fusion™ Dry-Down PCR Cloning Kits

A. PCR Amplification for In-Fusion™ Ready Cloning

The sense and antisense primers that will be used to amplify the gene of interest via PCR must contain a specific 15 nucleotide sequence 5' to the sequence of the gene of interest, as shown below.

Sense primer: 5'-AAGGCCTCTGTCGAC followed by sequence of amplification target-3'

Antisense primer: 5'-AGAATTCGCAAGCTT followed by sequence of amplification target-3'

Note: The PCR product obtained from amplification using these primers is also ready for In-Fusion cloning into all other Clontech prelinearized In-Fusion Ready vectors if the gene of interest contains an initiating ATG. For additional information regarding In-Fusion cloning, see Section V and subsequent sections of the In-Fusion Dry-Down PCR Cloning Kit User Manual (PT3754-1).

B. Cloning Procedure

1. Combine your PCR fragment (50 ng) and vector (100 ng) 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 C). If you cannot transform cells immediately, store cloning reactions at -20°C until you are ready.

C. 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 different volumes on LB plates containing the appropriate antibiotic. Spread the remaining cells from each transformation on additional plates. Incubate all plates at 37°C overnight.
5. The next day, pick colonies from each experimental plate and isolate plasmid DNA using a standard method of your choice.

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

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