In-Fusion® Ready Vector Cloning Protocol-At-A-Glance (PT3865-2)

This protocol is provided for cloning PCR fragments into In-Fusion Ready vectors using In-Fusion HD Cloning Plus (Cat. Nos. 638909, 638910, 638911 and 638920).

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 the In-Fusion HD Cloning Kit User Manual.

B. Cloning Procedure

1. Combine the following in an eppendorf tube:

1 µl	Linearized In-Fusion Ready Vector (100 ng/µl)
__ µl	(50 ng) Purified PCR product
2 µl	5x In-Fusion HD Enzyme Premix
__ µl	Sterile H_2O
10 µl	Total Volume

2. Incubate the reactions for 15 min at 50°C, then place on ice.
3. Proceed with transformation (Section C). If you cannot transform cells immediately, store cloning reactions at –20°C until you are ready.

C. Transformation

1. Transform Stellar™ competent cells with 2.5 µl of 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 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 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.
2. Add 450 µl of SOC medium to the cells and then incubate at 37°C for 60 min while shaking at 250 rpm.
3. Take 1/100 of the cells (5 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.
4. The next day, pick colonies from each experimental plate and isolate plasmid DNA using a standard method of your choice.

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