Protocol-at-a-Glance

In-Fusion™ Advantage PCR Cloning Kit
Protocol-at-a-Glance (PT4065-2)

Please read the In-Fusion Advantage User Manual (PT4065-1) before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

Following PCR, verify on an agarose gel that your target fragment has been amplified. If a single band of the desired size is obtained, you can **EITHER** treat your PCR product with Cloning Enhancer (**follow Protocol I**), **OR** treat your PCR product with DpnI and spin-column purify (**follow Protocol II**). However, if non-specific background or multiple bands are visible on your gel, we recommend that you isolate your target fragment by gel extraction, then spin-column purify (**follow Protocol II**).

Protocol I. In-Fusion PCR Cloning Procedure w/Cloning Enhancer Treatment

If a single prominent band of the desired size is obtained, you can treat your PCR product with Cloning Enhancer and follow the procedure outlined below. DO NOT follow this procedure if your PCR product requires purification.

A. Procedure for Treating Unpurified PCR Inserts with Cloning Enhancer

1. Add 2 µl of Cloning Enhancer to 5 µl of PCR product.
2. Incubate at 37°C for 15 minutes, then at 80°C for 15 minutes in a PCR thermal cycler.
   If you used more than 100 ng of DNA as a template in the PCR reaction, extend the 37°C incubation step to 20 minutes. If you are using a water bath or heat block instead of a thermal cycler, extend each of the incubation steps to 20–25 minutes.

B. In-Fusion Cloning Procedure for Cloning Enhancer-Treated PCR Inserts

3. Determine the amount of linearized vector and Cloning Enhancer-treated PCR insert to use in your In-Fusion cloning reaction using the recommendations in the table below.

Recommended Amount of VECTOR		Recommended Amount of CLONING ENHANCER TREATED INSERT*	
Vector Size	**Recommended Nanograms (ng)**	**Insert Size**	**Recommended Microliters (µl)**
<4 Kb	100 ng	<1 Kb	1 µl
4–6 Kb	100 to 150 ng	1–4 Kb	1–2 µl
6–10 Kb	200 ng	4–8 Kb	4 µl
>10 Kb	Up to 400 ng	8–12 Kb	7 µl

** If you obtain a low product yield from your PCR reaction, we recommend adding more of the Cloning Enhancer-treated insert (up to 7 µl).*

4. Set up the In-Fusion cloning reaction as outlined below:

Reaction Ingredients	Volumes
5X In-Fusion Reaction Buffer	2 µl
In-Fusion Enzyme	1 µl
Vector	_____ µl
Insert	_____ µl
dH_2O (as needed)*	_____ µl
Total Volume	10 µl

** Adjust the total reaction volume to 10 µl using deionized dH_2O as needed.*

For reactions with larger volumes of vector and insert (> 7 µl of vector + insert), double the amount of reaction buffer and enzyme, and add dH_20 for a total volume of 20 µl.

5. Incubate the reaction for **15 min at 37°C, followed by 15 min at 50°C**, and place on ice.

C. Transformation

6. Bring the reaction volume up to 50 µl* with TE buffer (pH 8), then mix well. Proceed with the transformation reaction (Step 7). If you cannot transform cells immediately, store the cloning reactions at –20°C until you are ready.

   **For some cell strains, it may be better to dilute the reaction to 100 µl with TE buffer.*

7. Transform competent cells with 2.5 µl of the diluted reaction mixture from step 6. **DO NOT add more than 5 µl of the diluted reaction to 50 µl of competent cells**—more is not better. *If you obtain low cloning efficiency, we recommend further dilution of the reaction (see Step 6*) for better results.*

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Protocol II. In-Fusion PCR Cloning Procedure w/Spin-Column Purification

If a non-specific background smear or multiple bands are visible on your gel, isolate your target fragment by gel extraction, then spin-column purify and follow the procedure outlined below. If you obtain a single band of the desired size, you can choose to treat your PCR product with DpnI and spin-column purify (following the procedure outlined below) instead of using the Cloning Enhancer.

A. Procedure for Spin-Column Purification of PCR Inserts

1. If non-specific background is visible on your gel, isolate your target fragment by gel extraction, then spin-column purify (see Step 2). If a single band of the desired size is obtained, add 1 µl of DpnI to 50 µl of the PCR reaction and incubate at 37°C for 60 min, then spin-column purify.
2. Spin-column purify your PCR product (e.g. insert) by using a silica-based purification system, such as NucleoSpin® Extract II. During purification, avoid nuclease contamination and exposure of the DNA to UV light for long periods of time.

B. In-Fusion Cloning Procedure for Spin-Column Purified PCR Inserts

3. Mix your purified PCR insert and linearized vector together in a 2:1 molar ratio. We recommend using between 50-200 ng of insert, and 100-400 ng of vector. Clontech provides an online tool to assist in determining the correct amounts of insert and vector to achieve a 2:1 ratio (view the online tool at **http://bioinfo.clontech.com/infusion**).

Recommended Amount of VECTOR	
Vector Size	**Recommended Nanograms (ng)**
<4 Kb	100 ng
4–6 Kb	100 to 150 ng
6–10 Kb	200 ng
>10 Kb	Up to 400 ng
Control Vector (2.7 Kb)	50 ng (1 µl)

4. Set up your In-Fusion cloning reaction as outlined below:

Reaction Ingredients	Volumes
5X In-Fusion Reaction Buffer	2 µl
In-Fusion Enzyme	1 µl
Vector	_____ µl
Insert	_____ µl
dH_2O (as needed)*	_____ µl
Total Volume	10 µl

** Adjust the total reaction volume to 10 µl using deionized dH_2O as needed.*

For reactions with larger volumes of vector and insert (> 7 µl of vector + insert), double the amount of reaction buffer and enzyme, and add dH_20 for a total volume of 20 µl.

5. Incubate the reaction for **15 min at 37°C, followed by 15 min at 50°C**, then place on ice.

C. Transformation

6. Bring the reaction volume up to 50 µl* with TE buffer (pH 8), then mix well. Proceed with the transformation reaction (Step 7). If you cannot transform cells immediately, store the cloning reactions at –20°C until you are ready.

   **For some cell strains, it may be better to dilute the reaction to 100 µl with TE buffer.*

7. Transform competent cells with 2.5 µl of the diluted reaction mixture from step 6. **DO NOT add more than 5 µl of diluted reaction to 50 µl of competent cells**—more is not better. *If you obtain low cloning efficiency, we recommend further dilution of the reaction (see Step 6*) for better results.*