I. Introduction

Takara Bio's **Cloning Enhancer** (Cat. Nos. 639615 & 638926) eliminates the need for PCR insert purification prior to the In-Fusion® cloning workflow. Since treatment with Cloning Enhancer occurs in the same tube as the PCR reaction, it minimizes the risk of losing PCR product during purification and avoids UV damage or nicking on PCR product. PCR samples treated with Cloning Enhancer yield significantly more recombinant clones than those left untreated.

II. General Considerations

- Please read the In-Fusion Snap Assembly User Manual before using this Protocol-At-A-Glance.

  NOTE: This abbreviated protocol is provided for your convenience and is not intended for first-time users.

- Following PCR, verify by agarose gel electrophoresis that your target fragment(s) has been amplified.
  - If a single band of the desired size is obtained, you can treat your PCR product with Cloning Enhancer.
  - If nonspecific background products or multiple bands are visible on your gel, we recommend that you instead isolate your target fragment by gel extraction using a NucleoSpin Gel and PCR Clean-Up Kit (e.g., Takara Bio, Cat. No. 740609.50).

III. Protocol

There are two sets of protocols listed in the sections below: a two-step (Section III.A) and a one-step (Section III.B) process. The one-step process requires specific criteria to make a sample eligible for use.

- To use the one-step process (Section III.B), the answer must be "yes" to **both** of the following conditions:
  1. PCR was used to amplify BOTH the vector and the insert(s).
  2. You obtained both a PCR-amplified vector AND PCR-amplified insert(s) without nonspecific background products.
- If you did not PCR amplify your vector, use the two-step procedure in Section III.A.

A. Two-Step Cloning with Cloning Enhancer

1. Treating Unpurified PCR-amplified Insert(s)

Before setting up the In-Fusion cloning reaction, treat unpurified PCR-amplified insert(s) as follows:

a. Add 2 µl of Cloning Enhancer to 5 µl of the PCR product in PCR tube(s) or a plate.

b. Incubate in a PCR thermal cycler using the following program:

37°C	15 min
80°C	15 min
4°C	Hold

NOTES:
- If you used more than 100 ng of DNA as a template in the PCR reaction, extend the first (37°C incubation) step to 20 min.
- If you are using a water bath or heat block rather than a thermal cycler, extend each of the incubation steps to 20–25 min.

SAFE STOPPING POINT: Store treated PCR reactions at –20°C until you are ready to proceed to cloning.

2. Cloning Treated PCR-Amplified Insert(s)

IMPORTANT: Only do this protocol after having performed the protocol in Section A.

a. Set up the In-Fusion cloning reaction:

2 µl	5X In-Fusion Snap Assembly Master Mix
N µl*	Linearized vector
1–2 µl†	Cloning Enhancer-treated insert(s)
Up to *N* µl	dH_2O (as needed‡)
10 µl	**Total volume**

*Use 50–200 ng of linearized vector (*N*), as calculated from the volume of your solution.
†1–2 µl of Cloning Enhancer-treated insert(s) is optimal, regardless of their length. The total volume of Cloning Enhancer-treated inserts should not exceed 4 µl per 10 µl reaction.
‡Use enough dH_2O to bring the total reaction volume to 10 µl.

b. Mix the reaction gently.

c. Incubate the reaction in a PCR thermal cycler for 15 min at 50°C, then place on ice.

NOTE: The In-Fusion reaction is completed within the required 15 min incubation. Longer incubation times do NOT increase cloning efficiency, even with multiple-insert cloning reactions.

d. Continue to the Transformation Procedure in the In-Fusion Snap Assembly User Manual (Section VI). If you cannot transform cells immediately, store the cloning reactions at –20°C until you are ready.

For more details, please read the In-Fusion Snap Assembly User Manual.

B. One-Step Cloning with Cloning Enhancer

If you obtain both a PCR-amplified linearized vector and PCR-amplified insert(s) without nonspecific background products, you may use the following protocol to perform the Cloning Enhancer treatment and In-Fusion reaction in the same tube.

NOTE: Only perform this protocol if you have not performed the procedures in Sections A on the given sample solution.

1. In PCR tube(s) or a plate, set up the In-Fusion cloning reaction:

2 µl	5X In-Fusion Snap Assembly Master Mix
1–2 µl*	PCR-amplified linearized vector
1–2 µl*	PCR-amplified insert(s)
1 µl	Cloning Enhancer
Up to *N* µl	dH_2O (as needed†)
10 µl	**Total volume**

*The total volume of PCR-amplified vector and insert(s) should not exceed 4 µl per 10 µl reaction.
†Use enough dH_2O to bring the total reaction volume to 10 µl.

2. Mix the reaction gently.

3. Incubate the reaction in a PCR thermal cycler using the following program:

37°C	15 min
50°C	15 min
4°C	Hold

4. After removing from the thermal cycler, place the tubes or plate on ice.
5. Continue to the Transformation Procedure in the In-Fusion Snap Assembly User Manual (Section VI). If you cannot transform cells immediately, store the cloning reactions at –20°C until you are ready.

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