TonkBio Fast-Fusion Cloning Kit

Catalog No.: TB10012
Storage: Store at –20ºC
Size: 10 rxns/50 rxns/100 rxns

Reagents Supped

- Fast-Fusion Enzyme
- 10× Fast-Fusion Buffer

Description

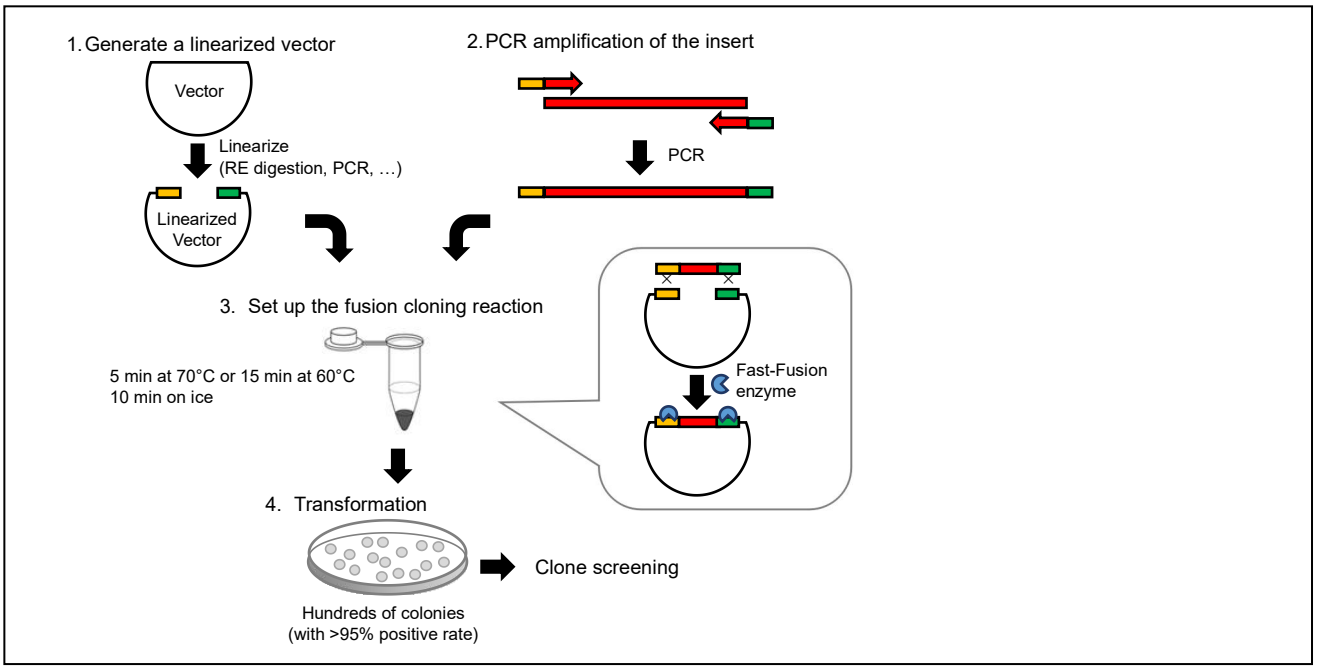
The TonkBio Fast-Fusion Cloning Kit is designed for fast, directional cloning of DNA fragment into any vector at any site. It can simplify and accelerate your experiments without requiring PCR product cutting with restriction enzymes allowing flexible options for restriction enzyme sites when linearizing the vector. Additionally, the linearized vector and PCR product can be cyclized in only 5 minutes at 70°C. Compared to the conventional cloning method, the 15 base tract is added at the ends of PCR primers corresponding to the ends of the linearized vector.

Applications

- PCR cloning into any vector
- Gene transfer from one vector to another
- Add adaptor, linker and tag before or after the inset
- High throughput cloning

Protocol

A. Overview



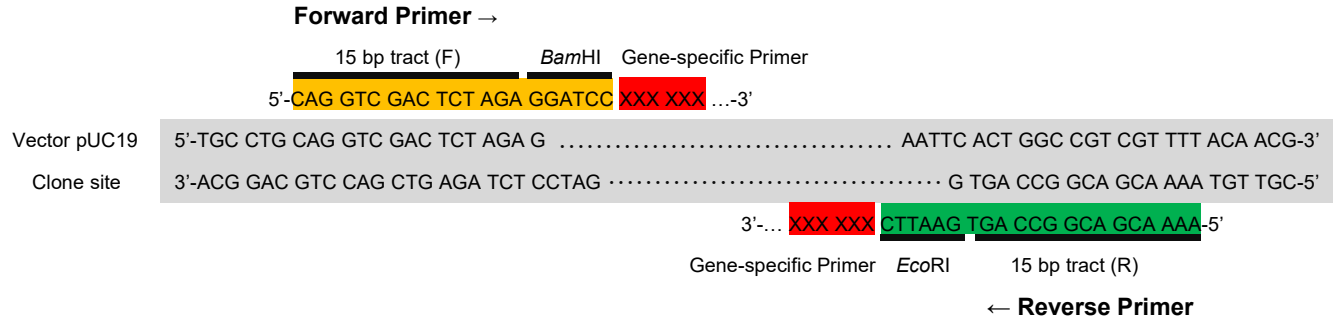
B. Preparation of Linearized Vector

Complete linearization of the vector is critical to achieve a successful fusion cloning reaction. Incomplete linearization of the vector will result in high background. The linearized vector can be generated by PCR or restriction enzyme digest (single or double digest) and should be purified using a gel or PCR purification kit.

C. Primer Design

Example of primers designed for Fusion cloning

PCR primers must contain 15−21 bp sequences that are homologous to the end of the vector. Consider the following parameters:



D. PCR Requirements

This fusion method is not affected by the presence or absence of A-overhangs, so you can use *Taq* or other high fidelity DNA polymerase for amplification. When PCR is finished, use an agarose gel to confirm that you obtained a single DNA fragment. Purify the appropriate band to remove any extra primers or primer dimmers. Quantify the amount of DNA by measuring against a known standard or DNA mass ladder.

E. Fast-Fusion Reaction

Set up the following reaction in a sterile, nuclease-free microcentrifuge tube on ice. Spin down briefly after each add addition to collect the reagents at the bottom of the tube.

Component	Volume
10× Fast-Fusion Buffer	1 μl
Fast-Fusion Enzyme	1 μl *
Linearized vector	20−100 ng
Insert	10−100 ng
Nuclease-free water	To 10 μl

2:1 molar ratio of inset:vector presents the highest fusion efficiency. The optimal quantity of vector and insert is as follows:

- **Linearized Vector = [0.01×the length of vector (bp)] ng**
- **Insert = [0.02×the length of insert (bp)] ng**

Fusion Reaction Conditions: Incubate the fusion mixture as follows:

Thermocycle Instrument		Water Bath
70°C, 5 min	60°C, 15 min	37°C, 15 min
4°C, ∞	4°C, ∞	On ice

Incubation in thermocycle instrument is recommended. *If the fusion reaction is incubated at 37°C in water bath，2 μl Fast-Fusion Enzyme should be added for a 10 μl reaction. Immediately proceed the transformation step or store the reaction tube at −20°C.

F. Transformation

We strongly recommend the use of competent cells with a transformation efficiency ≥ 1×10^8 cfu/μg.

1. Transform 100 μl competent cell with 10 μl of the Fast-Fusion reaction mixture.
2. Incubate on ice for 30 min.
3. Heat shock at 42°C for 60–90 s (please follow the transformation protocol provided with your cells).
4. Transfer on ice for 2–3 min.
5. Add 900 μl SOC or LB antibiotic free medium.
6. Shake at 37°C for 1 hour at 200–250 rpm in shaking incubator.
7. Spread 100–200 μl culture to LB plate containing the appropriate antibiotic. (If the efficiency of cells is lower than the recommended amount, centrifuge the culture at 6000 rpm for 5 min, resuspend the pellet in 100 μl medium, and then, spread the entire suspension.)
8. Incubate the plate at 37°C overnight
9. The next day, pick individual isolated colonies from the plate. To determine the presence of insert, analyze the DNA by restriction digestion or PCR screening.

G. Troubleshooting Guide

Problem	Possible explanation	Solution
No or few colonies obtained from the transformation	Primer sequences are incorrect	● Check primer sequences to ensure that they provide 15 bp of homology with the region flanking the insertion site.
	Suboptimal PCR product	● Optimize your PCR amplification reactions so that you generate pure PCR product. ● Use a different method to purify your PCR product.
	Low DNA concentration in reaction	● It is imperative to obtain as high a DNA concentration as possible in your fusion reaction
	Inhibitory contaminants from PCR product or linearized vector	● Both the PCR product and the linearized vector should be purified.
	Transform with too much reaction mixture	● Make sure the volume of reaction mixture is no more than 10 % of competent cells
	Low transformation efficiency of competent cells	● Repeat transformation experiment with efficient competent cells
	Wrong antibiotic used or too much antibiotic in the medium	● Choose plates with the appropriate concentration of the right antibiotic.
Large numbers of colonies contained no insert	Incomplete linearization of your vector	● Repeat digestion of vector with more time and gel purify
	Contamination of plasmid with same antibiotic resistance in PCR fragment	● Linearizing the template DNA before performing PCR. ● Treat the PCR product with *Dpn*I before purification.
	Plates too old or contained incorrect antibiotic	● Be sure that your antibiotic plates are fresh. ● Check the antibiotic resistance of your fragment.
Clones contain incorrect insert	PCR products contain nonspecifically amplified fragments	● Optimize the PCR reaction to improve the specificity or screen more colonies for the correct clones