



For general laboratory use. Not for use in diagnostic procedures. FOR IN VITRO USE ONLY.

Fast Seamless Cloning Kit

User Manual

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Overview

The Fast Seamless Cloning Kit provides a fast, high-throughput cloning of PCR products and generation of precise, directional constructs with any vectors that are ready for any purpose. The Fast Seamless cloning technology allows cloning your gene or sequence of interest into any vector in one day without the need for restriction enzymes, ligase, or blunt-end polishing.

Kit Contents and Store

Component \ Package	<input type="checkbox"/> S020	<input type="checkbox"/> S050	<input type="checkbox"/> S100
Fast Seamless cloning Enzyme (Freeze-drying)	2000U /vial	5000U/vial	10000U/vial
Dissolve Buffer	According to package		
10X Reaction Buffer	50µl	250µl	500µl
pUC19 (Amp+), Linearized 50ng/µl	6 µl	6 µl	6 µl
480-bp Control Insert 50ng/µl	6 µl	6 µl	6 µl
Store at -20°C			

PCR primer design

It is important to design primers properly to ensure the success of the Fast Seamless Cloning reaction. The design of primers of PCR amplification for cloning of your sequence of interest is based on the same principles as the design of PCR primers for any sequence. The only difference is that simply add the 14-18 bases of vector sequence to the 5' end of your sequence-specific PCR primers when designing primers. After PCR clean up, the resulting PCR- amplified insert is ready for Fast Seamless Cloning.

Guidelines for universal primer design (Figure 1 and 2)

- Count the homology from the 5' end.
- For 5' overhangs and blunt ends, count all bases including bases complementary to the 5' overhang in the sequence homology.
- For 3' overhangs, do not count the 3' overhang in the sequence homology.
- For the site of linearization includes restriction enzyme overhangs, bases complementary to the 5' overhang count towards homology but the 3' overhang does not. (Figure 2)

Fast Seamless Cloning Procedure

I . Dissolution of the freeze-drying Fast Seamless Cloning Enzyme

To prepare a 200U/μl Fast Seamless Cloning Enzyme "working stock" solution, for example, in the kit S020, the Fast Seamless Cloning Enzyme must be dissolved by 10μl Enzyme Dissolve Buffer which has been kept at 0°C before use. Mix the components well by gently pipetting up and down 5 times with a 100μl pipette (which has been kept at 0°C) and keep the tube on ice at least 10 min to ensure the enzyme is well dissolved. Avoid creating bubbles. The Fast Seamless Cloning Enzyme working stock (200 U/μl) should be stored at -20°C after use.

Notes:

- The Enzyme dissolve buffer should be kept at 0°C by place on ice before use.
- The Fast Seamless Cloning Enzyme working stock (200 U/μl) can be stored at -20°C for up to six months. It may be thawed and refrozen twice without a loss in activity.

****We have offered 200U/μl Fast Seamless Cloning Enzyme "working-stock" solution in this package! Yes / No**

II · Fast Seamless Cloning Procedure

In general, maximum cloning efficiency is achieved when 50–300 ng of PCR product is used in the cloning reaction. The cloning efficiency will be reduced, if you use amounts outside of this range in the reaction.

1. Set up Fast Seamless gene cloning reactions:

Component	Cloning Reaction	Control (-)	Control (+)
10X Reaction Buffer	2 μl	2 μl	2 μl
Linearized Vector	50–300ng	50–100ng	2 μl PUC19, Linearized (50ng)
PCR-amplified DNA	50–300ng	50–100ng	2 μl Control Insert (50ng)
Fast Seamless Cloning Enzyme	0.5 μl	---	0.5 μl
ddH ₂ O	X μl	X μl	X μl
Total volume	20 μl	20 μl	20 μl

Note: The optimal amount of vector were showed as follow table when various length of inserts were used in the cloning reaction.

Size of Linearized Vector	Mass of Vector
<4K	100ng
4K–6K	150ng
6K–8K	200ng
8K–12K	250ng
>12K	300ng

Note: Insert :vector molar ratios between 3 and 9 are optimal for single insertions. Ratios below 3:1 result in lower cloning efficiency.

2. Incubate the reactions for 30 min at 25°C followed by 15 min at 42°C and then transfer the tubes to ice.

3. Proceed with transformation immediately. If you cannot transform cells immediately, store cloning reactions at –20°C until you are ready.

III . Transformation

1. Thaw one vial of 100 µl frozen competent cells on ice. Tap tube gently to ensure that the cells are suspended.

2. Add 3 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.

3. Heat shock the cells in a water bath at 42°C for 90 sec, and then place them directly on ice for 2 min.

4. Add 500 µl of LB medium to the cells and then incubate at 37°C for 60 min while shaking at 150 rpm.

5. Centrifuge at 4500 x g (2000 rpm) for 2 min. Discard the supernatant fluid. Tap tube gently to ensure that the cells are suspended with the remaining liquid.

6. Take 25–50 µl suspended cells from each transformation and spread the cells on separate LB/X-Gal/IPTG (control) plates containing of ampicillin or other appropriate medium for your cloning vector. Incubate all plates at 37°C overnight.

7. The next day, pick white colonies (10 or more) from each experimental plate and Isolate plasmid DNA using a standard method to determine the presence of insert. Analyze DNA by restriction digest or PCR screening.

Note:The positive control plates should have many white colonies, with a few blue colonies (less than 20%).

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Trouble Shooting

A. No or Few Colonies Obtained from Transformation		
Description of Problem	Explanation	Solution
Low transformation efficiency	Suboptimal PCR product	Repeat PCR amplification and purify product using a different method of purification.
	Bacteria were not competent	Check transformation efficiency. You should obtain $>1 \times 10^6$ cfu/ug; otherwise use fresh competent cells.
Low quality DNA fragments	Low DNA concentration in reaction	we recommend using more than 100 ng of vector, depending on its size (see Table).
	Wrong molar ratio	Insert : vector molar ratios between 3 and 9 are optimal for single insertions. Ratios below 3:1 result in lower cloning efficiency.
	Primer sequences are incorrect	Check primer sequences to ensure that they provide 15 bases of homology with the region flanking the insertion site.
Incorrect antibiotic	Plates too old or contained incorrect antibiotic	Be sure that your antibiotic plates are fresh (<1 month old) and in proper antibiotic concentration.
B. Large Numbers of Colonies Contained No Insert		
Description of Problem	Explanation	Solution
Large numbers of colonies obtained with no insert	Incomplete linearization of your vector	It is important to remove any uncut vector prior to use in the In-Fusion reaction. If necessary, recut your vector and gel purify.
	Contamination of In-Fusion reaction by plasmid with same antibiotic resistance	If your insert was amplified from a plasmid, closed circular DNA (vector) may have carried through purification and contaminated the cloning reaction. To ensure the removal of any plasmid contamination, the PCR product can be treated with DpnI to remove the parental vector template after PCR amplification.

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