Hyper Assembly Cloning Kit User Manual

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1. Introduction

Hyper Assembly Cloning Kit is designed for fast, directional cloning of one or more target fragment of DNA into any vector. This method employs the 3'-5' exonuclease activity of Hyper Assembly Enzyme to get 15-20nt complementary arms at the ends of PCR-generated inserts and linearized vectors, then fuse the DNA fragments to get the recombinant vectors. These 15-20-bp overlaps can be engineered by designing primers for amplification of the desired sequences.

- Clone any insert into any location within any vector you choose
- Efficiently clone a broad range of fragment sizes
- Clone multiple DNA fragments simultaneously into any vector in a single reaction
- No restriction digestion, phosphatase treatment, or ligation required

A general outline of the protocol used for Hyper Assembly Cloning Kit is illustrated in Figure 1.

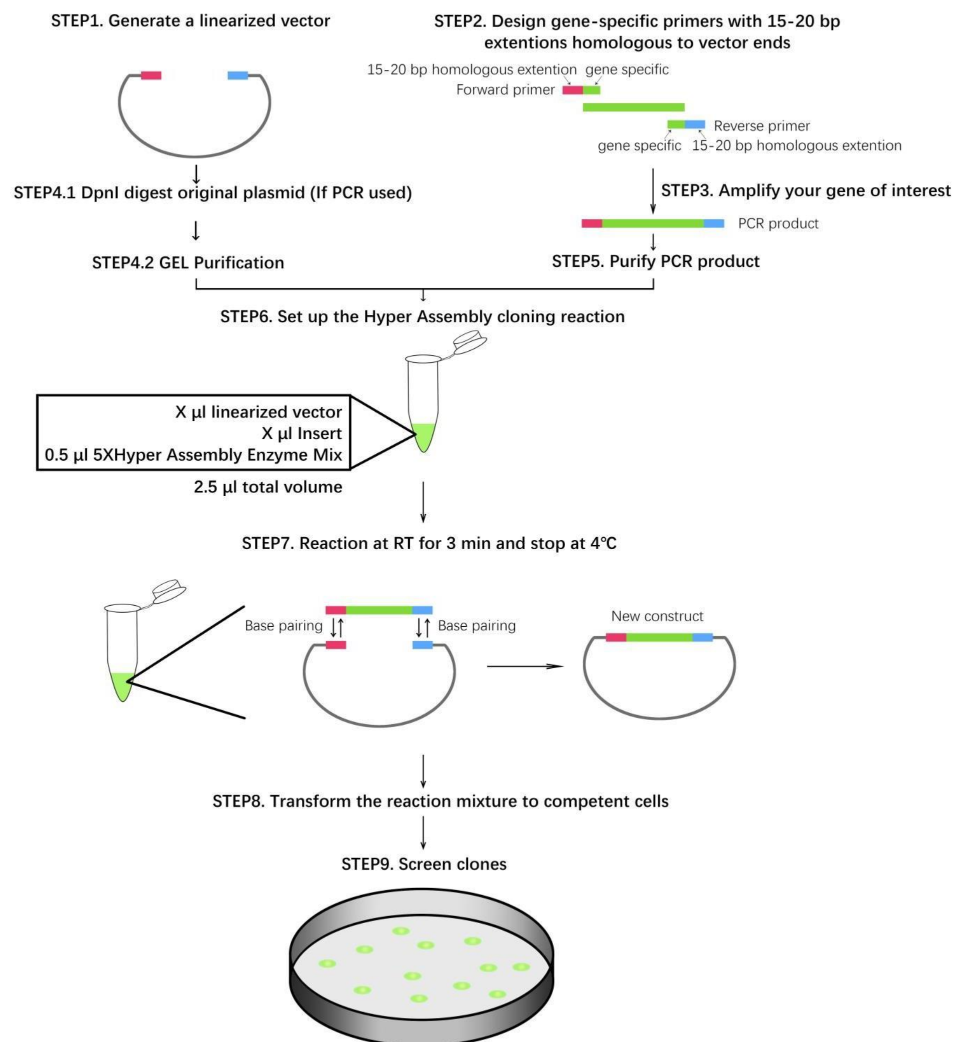

Figure 1. Hyper Assembly Cloning Protocol Overview

2. Components

Component	Size	Volume	Storage
5XHyper Assembly Enzyme Mix	25 rxns	12.5μl	Store at -20℃
	50 rxns	25μl	
	100 rxns	50μl	
Dpn 1	20,000 units/ml	5μl	

3. Preparation of a Linearized vector

To achieve a successful Hyper Assembly cloning reaction, we must first generate a linearized vector. The linearized vector can be generated using restriction enzymes (single or double digestion) or by PCR. You can use our Phusion high-fidelity DNA polymerase (**Cat: K1031**) to amplify the linearized vector or your target gene.

1) Preparation of a linearized vector by Restriction Digestion

Generally speaking, two enzymes cut well than any single enzyme. Efficiency of digestion will always be better if the restriction sites are as far apart as possible. In addition, increasing the enzyme digestion time and the digestion reaction volume will reduce the background.

a) For many enzymes, incubation from 3h to overnight can increase linearization and reduce background.

b) After digestion, purify the linearized vector using any available PCR purification Kit (e.g., PCR Clean-Up Kit). Figure 2, example of linearized vector prepared by double restriction enzymes (BamHI and XhoI).

c) [Control] Check the background of your vector by transforming 5–10 ng of the linearized and purified vector into competent cells. If the background is high, continue digesting the vector for a longer time after the addition of more restriction enzyme(s). Incubate 2 hours to overnight. Gel purify the remainder of the vector and transform again.

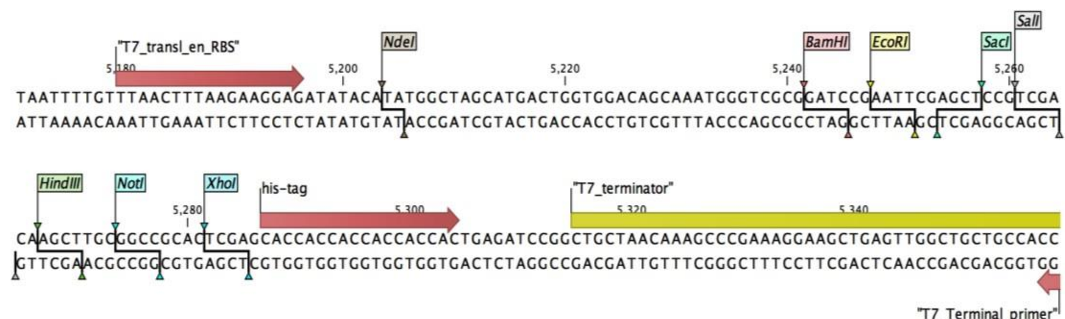

Figure 2: Example of linearized vector prepared by restriction enzymes (BamHI and XhoI). The generated linearized vector has different sticky ends

2) Preparation of a linearized vector by PCR

A linearized vector obtained by PCR is with Blunt ends and the new construct has no extra or unwanted base pairs. And we can choose where to insert the target fragment. The linearized vector should be treated by DpnI before purification of the PCR product. An example of linearized vector prepared by PCR is illustrated in figure 3.

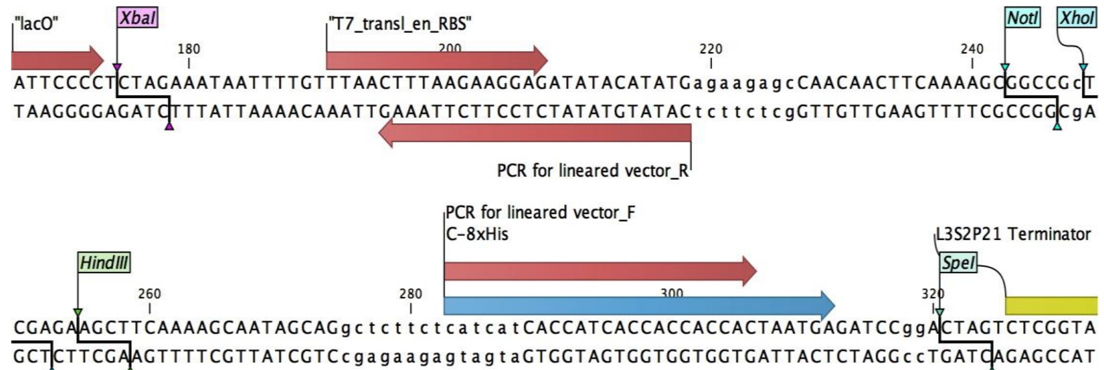

Figure 3: Example of primers designed for linearized vector. The linearized vector is with blunt ends. Note: the concentration of the linearized vector is recommended 30ng/μL~50ng/μL.

4. Preparation of target inserts

Primer design and quality are critical for the success of the Hyper Assembly cloning reaction. The Hyper Assembly cloning reaction allows you to join two or more fragments, e.g. vector and insert (or multiple fragments), as long as they share 15-20 bases of homology at each end. Therefore, primers must be designed in such a way that they generate PCR products containing ends that are homologous to those of the vector (or each other). Figure 4 outlines the guidelines for primer design.

Guidelines for universal primer design:

a) To determine the 15-20b homology sequence to be incorporated into each primer, start at the 5' end of each DNA strand in the linearized vector (*). The region of homology for a particular primer consists of bases that are complementary to the first 15-20 bases at the 5' end of a particular DNA strand. This means that the bases complementary to 5' overhangs are included in the primer sequence, but the bases in 3' overhangs are not.

b) Run agarose gel to test the PCR product, if there are no unspecific strips, purify the target inserts using any available PCR purification Kit (e.g., PCR Clean-Up Kit). If there are many unspecific strips, we will change the conditions of PCR reaction or redesign the primers. We don't use the DNA gel extraction kit to purify the target fragments if the unspecific strips are weak, it will have no effect on the Hyper Assembly cloning reaction. In short, after purifying the PCR product, you should run agarose gel to check the quality of the product. And the concentration of the target fragments are recommended 80~100ng/μL or more.

Figure 4. Universal primer design for Hyper Assembly cloning reaction. The PCR-generated target fragment requires that the PCR insert shares 15-20 bases of homology with each of the linearized vector. This sequence homology is added to the insert through the PCR primers. For vector with sticky ends, bases complementary to 5' overhangs are included in the primer sequence; bases in the 3' overhangs are not. Because we use the 3'-5' exonuclease activity of Hyper Assembly enzyme, the bases at 3' overhangs will be deleted (Brackets in this figure indicate bases to be included in the 15-20bp region of homology)

5. Materials & Reagents Not Supplied

1) Linearized vector: 30~50ng/μL, prepared by restriction digestion or by PCR, store at -20℃.
2) PCR-generated target DNA fragments: 80~100ng/μL, store at -20℃.
3) DH5a competent cells, store at -80℃.
4) LB medium, store at RT.
5) LB/antibiotic plates, store at 4℃.

6. Procedure

1) Reaction setup for Hyper Assembly cloning:

Linearized vector(30~50ng/μL)	1μL
Target DNA fragment(80~100ng/μL)	1μL
5XHyper Assembly Enzyme Mix	0.5μL
Total	2.5μL

Note: We recommend the molar ratio of linearized vector and target DNA fragment with **1:2-5**, which produces the most cloning efficiency.

2) Reaction setup for negative control

Linearized vector(30~50ng/μL)	1μL
ddH2O	1μL
5XHyper Assembly Enzyme Mix	0.5μL
Total	2.5μL

Note: Use ddH20 to replace target DNA fragment.

3) The both reaction setups for Hyper Assembly cloning and negative control, mix well by pipette and react at RT for 3min, and stop the reaction at 4℃.
4) Transform the both 2.5μL reaction mixtures to the DH5a competent cells (100μL) using a standard transformation protocol. Incubate all of the plates overnight at 37°C.

5) The next day, pick individual isolated colonies from each experimental plate. Isolate plasmid DNA using a standard method of your choice (e.g. miniprep). To determine the presence of an insert, analyze the DNA by restriction digestion or PCR screening. (the results should be as shown in figure 5).
6) The strips of the recombinant vectors should larger than the empty vector, and pick 3 shifted recombinant vectors (3 colonies) to sequence.

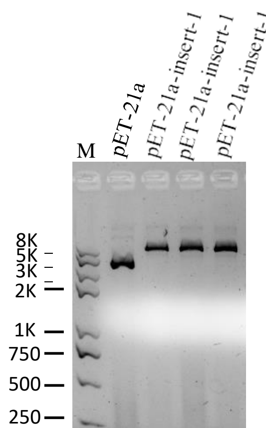

Figure 5. pET-21a is the empty circle vector as the negative control, and the pET-21a-inserts are the successful recombinant vectors (3 colonies)

7. Trouble shooting

1) The presence of no colonies on plates:
    a) To check the concentration and quality of insert fragments and the linearized vector.
    b) Check the reaction time, you should ensure the reaction time at RT is 3min and no more than 4min, if you do many Hyper Assembly cloning reactions once.
2) The presence of many (hundreds) of colonies on the negative control is indicative of incomplete vector linearization (eg., You need digest the PCR-generated linearized Vector by DpnI).