2025 NEFU_China

Connection

ABSTRACT

This protocol is used to connect two or more pieces of DNA together

BEFORE STARTING

Setup a small box with ice, put DNA and enzymes on it.
Prepare the water bath to 37℃ to have Gibson assembly.

1. Select the appropriate connection method according to the experimental situation。

1.1 C116 Gibson connection

This protocol is based on C116 ClonExpress® II One Step Cloning Kit by Vazyme.

Preparation of linearized vectors

2. Select an appropriate cloning site on the vector that will be linearized.

3. Vector linearization: the linearized vector can be obtained by digesting the circular vector with restriction enzymes or by reverse PCR.

PCR of the inserts DNA fragments

4. Amplify the insert DNA fragments with homologous sequences (for homologous recombination) of vector-upstream or -downstream by PCR using high fidelity DNA polymerase.

Calculate amount and ratio of linearized vectors and Inserts

5. Detect DNA concentration of linearized vectors and inserts by Nanodrop. 6.Calculation of the amount of vectors:
The optimal amount of vector for the recombination with ClonExpress II is 0.03 pmol, and the optimal amount of insert is 0.03 pmol (molar ratio of vector to insertion is 1:1), as roughly calculated as follows:

The optimal mass of vector = [0.02 ×number of base pairs] ng (0.03 pmol) The optimal mass of insert = [0.02 ×number of base pairs] ng (0.03 pmol)

Recombination

7. Dilute linearized vectors and inserts before recombination to make sure the loading accuracy. The volume of each component loaded should be no less than 1 μl.
8. Setup the following reaction on ice:

	A	B
1	Linearized Vectors	X μl
2	Inserts	Y1+Y2 ..... Yn μl
3	2 ×clonExpress Mix	4 μl
4	ddH2O	Add to 20 μl

9. Use the palm centrifuge to mix the solution in PCR tube.
10. Incubate at 50℃ for 15 min and immediately place the tube at 4℃ or on ice.

This procedure is used to connect two pieces of DNA.Incubate at 50℃ for 30 min and immediately place the tube at 4℃ or on ice.This procedure is used to connect three or more pieces of DNA.

1.2 Goldengate connection

Preparation of linearized vectors

2. Select an appropriate cloning site on the vector that will be linearized.

3. Vector linearization: the linearized vector can be obtained by digesting the circular vector with restriction enzymes or by reverse PCR.

PCR of the inserts DNA fragments

4. Amplify the insert DNA fragments with homologous sequences of vector-upstream or -downstream by PCR using high fidelity DNA polymerase.

Recombination

5. Dilute linearized vectors and inserts before recombination to make sure the loading accuracy. The volume of each component loaded should be no less than 1 μl.

6. Setup the following reaction on ice:

	A	B
1	Linearized Vectors	10ng
2	Inserts	Y1+Y2 ..... Yn μl
3	BSA Enzyme	0.5μl
4	BSA Buffer(1g/L)	1 μl
5	T4 DNA ligase	0.2μl
6	10 ×Ligase Buffer	1 μl
7	ddH2O	Add to 10 μl

9. Use the palm centrifuge to mix the solution in PCR tube.

10. .Program the thermocycler as follows:

	A	B
1	Temperature	Time
2	37℃	90 min
3	55℃	15 min
4	80℃	15 min

immediately place the tube at 4℃ or on ice.