

## 2024 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°C 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 =  $[0.02 \times \text{number of base pairs}] \text{ ng}$  (0.03 pmol)

The optimal mass of insert =  $[0.02 \times \text{number of base pairs}] \text{ ng}$  (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  $\mu\text{l}$ .

8. Setup the following reaction on ice:

	A	B
1	Linearized Vectors	X $\mu\text{l}$
2	Inserts	Y1+Y2.....Yn $\mu\text{l}$
3	2 $\times$ clonExpress Mix	4 $\mu\text{l}$
4	ddH2O	Add to 20 $\mu\text{l}$

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

10. Incubate at 50°C for 15 min and immediately place the tube at 4°C or on ice.

This procedure is used to connect two pieces of DNA. Incubate at 50°C for 30 min and immediately place the tube at 4°C 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°C	90 min
3	55°C	15 min
4	80°C	15 min

immediately place the tube at 4°C or on ice.