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Overlap & Gibson ligation

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ABSTRACT

This protocol is used to lagate two pieces of DNA together without digesting the fragment with restriction endonucleases.

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MATERIALS TEXT

DNA fragments
Primers
ClonExpress II One Step Cloning Kit (Vazyme)
2×High Fidelity Master Mix (MCLAB)
Nanodrop
Thermocycler
Water bath
DdH2O

SAFETY WARNINGS

Please wear gloves for the experiment, don't try to touch the lid after PCR program initiation.

BEFORE STARTING

Set up a small box with ice, put DNA and enzymes on it.
Prepare the water bath to 37°C to have Gibson assembly.

1 Ligation of two DNA fragments by using cases below.
Step 1 includes a Step case.
Overlap PCR
Gibson Assembly

Preparation of linearized vectors

step case

Overlap PCR

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:
Molar ratio of vector to insertion is 1:1

Recombination & PCR

7 Set up the following reaction on ice (50µl):

A	B
Forward Primer (10 µM)	1µl
Reverse Primer (10 µM)	1µl
Fragment1(vector)	X
Fragment2(insertion)	Y
2×High Fidelity Master Mix (MCLAB)	25µl
ddH2O	Add to 50µl

The primer is used to amplify recombinant DNA fragment/circular DNA.

8 Program the thermocycler as follows:

A	B
Temperature	Time
95/98℃	5 min
95/98℃	30 s
Tm-3~5℃	30 s
72℃	1kb/min
72℃	5~10 min
16℃	∞

Repeat 30 times in 3-5 steps

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

10 Put the PCR tube into the thermocycler and Run the program.

11 Using agarose gel electrophoresis to confirm if correct construct was present.