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Gibson Assembly

宏亮 董¹

¹Northeast Forest University

2019 iGEM NEFU_China

Tech. support email: shengyiyanwork@gmail.com

宏亮 董

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

This protocol includes two different kits. Choose one to use.

MATERIALS

The old kit: Trelief™ SoSoo Cloning Kit Ver.2

The new kit: ClonExpress® II One Step Cloning Kit

SAFETY WARNINGS

Please wear gloves for the experiment.

The old kit

1 Set up a small box with ice, put DNA and Gibson Mix into it before going into the metal bath or the Bio-rad S1000TM thermocycler cyclers .

2 Add the following reagent to a PCR tube (4 µl).

Gibson Mix	2 µl
insert	X µl
vector	Y µl

$X+Y=2\text{ ul}$, $X : Y=3 : 1\sim5 : 1$.

Temperature : Time= 50°C : 15-30 min

The new kit

3 Set up a small box with ice, put DNA and Gibson enzyme and Gibson buffer into it before going into the metal bath or the Bio-rad S1000TM thermocycler cyclers .

4 Add the following reagent to a PCR tube (10 µl).

Gibson enzyme	1 µl
Gibson buffer	2µl
insert	X µl
vector	Y ul

$X=0.02\times \text{vector length /vector concentration}$

$Y=0.02\times \text{insert length /insert concentration}$

Temperature : Time= 37°C : 30-60 min