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Gibson Assembly

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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.



- 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 μ l).

Gibson Mix	2 μ l
insert	X μ l
vector	Y μ l

$X+Y=2$ μ l , $X : Y=3 : 1\sim 5 : 1$.

Temperature : Time= 50°C : 15-30 min

- 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 μ l).

Gibson enzyme	1 μ l
Gibson buffer	2 μ l
insert	X μ l
vector	Y μ l

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

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

Temperature : Time= 37°C : 30-60 min