In-Fusion Advantage PCR Cloning Kit

Modified from introduction of manufacturer

Reagents:

In-Fusion Advantage PCR Cloning Kit: Clontech Cat. No. 1008471A

Cloning Enhancer: Clontech Cat. No. 1007103A

One Shot TOP10 Chemically Competent Cell: Invitorgen Cat. No. C4040-03

Day 1

1. Linearize vector by one or two restriction enzyme O/N.
2. PCR amplify insert. Hot start polymerase, 10pg~1ng plasmid DNA is recommended.

Day 2

Check and purify linearized vector.

Day 3

Cloning Enhancer Treatment: 1h

1. Add 2 ul of Cloning Enhancer to 5 ul of the PCR reaction.
2. Incubate at 37°C for 15 minutes, then at 80°C for 15 minutes in a PCR thermal cycler. (<100ng plasmid DNA as template)

In-Fusion Cloning Procedures: 1h

1. Set up the In-Fusion cloning reaction:

5X In-Fusion Reaction Buffer	2 µl
In-Fusion Enzyme	1 µl
Vector	x µl (<4kb, 100ng; <10kb, 200ng)
Cloning Enhancer-Treated PCR Insert	x µl (molar ratio)
ddH2O (as needed)	x µl
Total Volume	10 µl

(For reactions with > 7 µl of vector + insert), double the amount of reaction buffer and enzyme, and add ddH20 for a total volume of 20 µl)

2. Mix the reaction and incubate the reaction for 15 min at 37°C, followed by 15 min at 50°C, then place on ice.

3. Bring the reaction volume up to 50µl* with TE buffer (pH 8), and mix well. Take 25 ul to a new tube and add TE buffer to 50 ul.
4. Continue Transformation Procedures with 2 diluted reaction or store the cloning reactions at –20°C until you are ready.

*Negative control: add 1ul of the linearized pUC19 control vector included in the kit into cloning reaction without insert.

*Positive control: add 1ul of the linearized pUC19 control vector and 2ul of the 2kb control insert included in the kit into cloning reaction.

Transformation: 2h

1. Put 2 µl of the diluted In-Fusion reaction mixture into 20 ul of GC10 competent cells (invitrogen). Don't add more than 5 ul of reaction to 50 ul of cells.
2. Transformation.

*Control: Transform a circular vector as positive control and a linearized vector as negative control

Day 4

Pick up individual colonies and culture at 37°C O/N.

Day 5

1. Miniprep plasmid DNA.
2. Digest the plasmid DNA and check the insert.
3. Plasmid DNA sequencing.