LiClone™ One Step DNA Assembly Kit

Cat. #: M0010 Size: 25 rxns

Email: support@lifesct.com Tel: 240-715-2985

LifeSct.com
Life Scientists' Service Center

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Quick Workflow

The Amount of Linearized Vectors and Inserts

◇ Simple homologous recombination and single-fragment homologous recombination:

The optimal mass of vector required = [0.02 × number of base pairs] ng (0.03 pmol)

The optimal mass of insert required = [0.04 × number of base pairs] ng (0.06 pmol)

◇ Multi-fragment (2 - 5) homologous recombination:

The optimal mass of vector required = [0.02 × number of base pairs] ng (0.03 pmol)

The optimal mass of each insert required= [0.02 × number of base pairs] ng (0.03 pmol)

Recombination

1. The amount of DNA can be roughly calculated according to the above formula.
   Dilute linearized vectors and inserts before recombination to assure the loading accuracy. The volume of each component loaded should be no less than 1 μl.

2. Prepare the following reaction on ice:

Components	Recombination	Negative control-1	Negative control-2	Positive control
Linearized Vector	X μl	X μl	0 μl	1 μl
Insert (n≤5)	$Y_1+Y_2+...+Y_n$ μl	0 μl	$Y_1+Y_2+...+Y_n$ μl	1 μl
2× LiClone Mix	5 μl	0 μl	0 μl	5 μl
ddH_2O	To 10 μl	To 10 μl	To 10 μl	To 10 μl

1. Gently pipette up and down for several times to mix thoroughly (**DO NOT VOTEX!**). Spin briefly to bring the sample to the bottom of the tube before reaction.

2. **Single-fragment homologous recombination:** Incubate at 50°C for 5 min and chill the tube immediately at 4°C or on ice.
   Multi-fragment homologous recombination: Incubate at 50°C for 15 min and chill the tube immediately at 4°C or on ice.
   ▲Increase the volume of reaction system to 20 μl if the total volume of vector and insert is more than 5μl. For single-fragment homologous recombination, increasing the recombination time to 15min may be helpful to improve the recombination efficiency when the amount of DNA is between 300 ng and 400 ng. For multi-fragments homologous recombination, prolonging the recombination time to 15min, but no more than 1h, can improve the recombination efficiency.

Transformation

1. Place the competent cells on ice.
2. Pipet 5 - 10 μl of the recombination products to 100 μl competent cells, flip the tube for several times to mix thoroughly (DO NOT VOTEX!), and then place the tube still on ice for 30 min.
   ▲The volume of transformation products should not be more than 1/10 of the volume of competent cells.
3. Heat-shock the tube at 42°C for 45 sec and then immediately chill on ice for 2-3 min.
4. Add 900 μl of SOC or LB medium (without antibiotics) to the tube. Then, shake at 37°C for 1h at 200 - 250 rpm.
5. Preheat the LB plate which contains appropriate selection antibiotic at 37°C.
6. Centrifuge the culture at 5,000 rpm for 5 min, drop 900 μl of supernatant. Then, re-suspend the pellet with 100 μl of remaining medium and plate it on an agar plate which contains appropriate selection antibiotic.
7. Incubate at 37°C for 12 - 16 hours.

Please download the whole protocol: https://www.lifesct.com/download/Manuals/M0010-1.pdf

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