DNA Cloning (Gibson Assembly, Transformation, Plating and Incubation) V.2

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

ABSTRACT

2023 NUS-Singapore iGEM Team followed this protocol to construct the plasmid of interest or to clone the plasmid of interest.

MATERIALS

1. NEBuilder HiFi DNA Assembly Master Mix
2. Competent cells made by 10β E. coli.
3. LB media.

SAFETY WARNINGS

- Proper laboratory PPE must be worn at all times.
- Thermal gloves shall be worn when handling competent cells from the -80°C fridge.
- Since cells like 10β E. coli are used in this protocol, a Biosafety Cabinet (BSC) is required to ensure safety.

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Gibson Assembly

1 Calculate the volumes of respective DNA fragments to assemble based on their length and concentration. (The maximum final volume of the mixed fragments is 5 µL for each reaction.)

2 Add the DNA fragments into a PCR tube according to the volumes obtained from the calculation.

3 Add 5 µL of NEBuilder HiFi DNA Assembly Master Mix into the same PCR tube.

4 Vortex to mix the solution and centrifuge the PCR tube to spin down the remaining solution on the wall.

5 Incubate the PCR tube at 50°C for 01:00:00. 1h

Transformation

6 Switch on the water bath and set the temperature to 42°C.

7 Prepare a box of ice.

8 Take an Eppendorf tube that contains pre-made competent cells from the -80°C fridge.

9 Immediately place the Eppendorf tube with competent cells into the ice box for 00:05:00. 5m

10 Add 20 µL of the Gibson Assembly product or 1 µL of pure DNA plasmid into the Eppendorf tube containing the competent cells.

11 Tap the bottom of the Eppendorf tube to mix the solution.

12 Leave the Eppendorf tube in ice for 00:10:00. 10m

13 Place the Eppendoft tube into a foam floating.

14 Place them into the water bath for 00:00:45 at 42°C for heat shock. 45s

15 Place the Eppendorf tube into the ice immediately.

16 Add 1 mL of the LB media into the Eppendorf tube.

17 Place the Eppendoft tube into the incubator at 37°C for 01:00:00 for recovery. 1h

18 Centrifuge the Eppendorf tube to form a cell pellet (no specific speed and time).

Plating and Incubation 1h

19 Prepare an LB agar plate with the correct antibiotics.

20 Remove 950µL of the LB solution from the Eppendorf tube that contains the cell pellet, leaving about 100µL in the Eppendorf tube.

21 Resuspend the cells by pipetting the solution.

22 Spread the cells onto the agar with the L-spreader.

23 Place the petri dish in the incubator at 37°C for Overnight to allow the colonies to grow. 1h