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Golden Gate Assembly V.2

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NUS iGEM[1]

[1]National University of Singapore

NUS iGEM
National University of Singapore

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

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Abstract

2023 NUS-Singapore iGEM team followed this protocol to assemble DNA oligos containing Golden Gate restriction sites with a plasmid backbone that contains the same restriction sites. The restriction enzymes utilised in this protocol are the Type IIS restriction enzymes, specifically BsaI. The use of Type IIS restriction enzymes ensures that there is no scar or extra sequence at the junctions between the assembled fragments.

Guidelines

This protocol outlines the Golden Gate procedures with a sample volume of 20 µL per reaction.

Protocol materials

BsaI-HF®v2 **New England Biolabs Catalog #**R3733S

T4 DNA Ligase **New England Biolabs Catalog #**M0202S

10X NEB T4 DNA ligase buffer **New England Biolabs**

Troubleshooting

Safety warnings

- Proper lab PPE must be worn at all times.
- Thermal gloves shall be worn when handling items from -20 °C fridge.

Golden Gate Assembly

1 Prepare an ice box.

2 Place the 10X NEB T4 DNA ligase buffer **New England Biolabs**, BsaI-HF®v2 **New England Biolabs Catalog #**R3733S, and T4 DNA Ligase **New England Biolabs Catalog #**M0202S in ice.

3 Add the following reagents into a PCR tube:

	Item	Volume
	DI Water	10µL
	Plasmid	1µL
	PCR Extract Oligo	5µL
	BsaI-HFv2 Enzyme	1µL
	T4 DNA Ligase	1µL
	T4 Ligase Buffer	2µL

Note

Reagents with enzymes such as BsaI-HFv2 Enzyme and T4 DNA Ligase must be kept at a low temperature (in ice) when they are in-use to prevent the enzymes from denaturation.

4 Put the sample into the Thermal Cycler and run it with the following conditions:

*Set "Lid Temperature" to 105 °C and set "Volume" to 20 µL

	Temperature	Duration
	37°C	5 minutes
	16°C	5 minutes
	Go to step 1, repeat the cycle 40 times	

	Temperature	Duration
	37°C	1 hour
	60°C	15 minutes
	12°C	Infinite Loop

Transformation

1h 15m 45s

5 Prepare a box of ice.

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

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

8 Add the whole Golden Gate Assembly product (20 µL) o into the Eppendorf tube containing the competent cells.

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

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

11 Place the Eppendoft tube into a foam floating.

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

13 Place the Eppendorf tube into the ice immediately

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

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

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

Plating and Incubation 1h

17 Prepare an LB agar plate with the correct antibiotics.

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

19 Resuspend the cells by pipetting the solution.

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

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