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Version 1

Golden Gate Assembly V.1

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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 Bsal. 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 μ L per reaction.

Protocol materials

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

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

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

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** ,  Bsal-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	14µL
Plasmid	1µL
PCR Extract Oligo	1µL
Bsal-HFv2 Enzyme	1µL
T4 DNA Ligase	1µL
T4 Ligase Buffer	2µL

Note

Reagents with enzymes such as Bsal-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



	Temperature	Duration
	Go to step 1, repeat the cycle 40 times	
	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 Eppendof^t 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