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## Golden Gate Assembly

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### ABSTRACT

Protocol for golden gate assembly of modular plasmids from Yeast Toolkit, Bee Toolkit and Marine Modification Kit platforms.

### OPEN ACCESS

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**protocols.io**

<https://protocols.io/view/golden-gate-assembly-b5i2q4ge>

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

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**PROTOCOL integer ID:**  
58682

**Keywords:** Cloning, Golden Gate Assembly, Plasmid

### Day 1

- 1 Streak out the plasmid parts that will be used in the assembly onto LB agar plates with the appropriate antibiotics (Chloramphenicol 100µg/mL for Type 1-7 parts; Kanamycin or Gentamicin 100µg/mL for Type 8 backbone parts). Incubate overnight at 37°C.

## Day 2

- 2 Inoculate a single colony into 25mL of LB plus antibiotics in the late afternoon/early evening. Incubate overnight at 37°C while shaking at 200rpm.

## Day 3

- 3 Spin the culture at 5000g for 20 minutes. Remove the supernatant, resuspend the pellet in 1mL water. Perform a plasmid miniprep (Zyppy miniprep or Omega E.Z.N.A. Plasmid Mini Kit II) following the standard kit protocols.
- 4 Measure the Plasmid DNA concentration on a spectrophotometer.
- 5 Perform the Golden Gate Assembly:  
Dilute backbone plasmid parts and add 10fmol to the reaction  
Dilute the insert plasmid parts and add to 20fmol of each insert to the reaction  
Add 2µL T4 Ligase Buffer (Promega)  
Add 1µL T4 Ligase  
Add 1µL Bsal-HF\_V2 or BsmBI-HF endonuclease  
Add X water up to a 20µL reaction

### Note

Follow the reaction protocol listed on the Barrick Lab website:

[https://barricklab.org/twiki/bin/view/Lab/Old\\_Golden\\_Gate\\_Assembly](https://barricklab.org/twiki/bin/view/Lab/Old_Golden_Gate_Assembly)

- 6 Run the thermocycler program for Bsal/BsmBI as follows:

A	B	C
Step	Temperature	Time
1	37/42°C	5 minutes
2	16°C	5 minutes

A	B	C
Cycles 1-2	Repeat 30x	
3	37/55°C	10 minutes
4	80°C	10 minutes

### Note

The thermalcycler program used for this protocol can be found on the Barrick Lab website [here](#), along with other great troubleshooting tips.

## Day 4

- 7 Dilute 2x or electroporate 2µL of the GGA directly into electrocompetent cells (i.e. SM10<sup>pir</sup>, S17<sup>pir</sup>). Recover 1+ hours and plate on LB Agar media containing the correct antibiotic concentrations. Incubate at 37°C overnight.

## Day 5

- 8 Screen colonies for correct insert and perform colony PCR with primers spanning assembly junctions.
- 9 Streak out clones that yield a band onto LB Agar media containing the correct antibiotic concentrations and incubate at 37°C overnight.

## Day 6

- 10 Inoculate a single colony into 25mL LB broth containing the correct antibiotic concentrations and incubate at 37°C overnight.

## Day 7

- 11 Store overnight culture in cryovials for long term storage. Add 500µL of culture to 500µL of 50%

glycerol and store in -80°C freezer.

- 12 With remaining culture, Centrifuge at 5000g for 20 minutes. Remove the supernatant, resuspend the pellet in 1mL water. Perform a plasmid miniprep (Zyppy miniprep or Omega E.Z.N.A. Plasmid Mini Kit II) following the standard kit protocol. Elute with water.
- 13 Measure the Plasmid DNA concentration on a spectrophotometer.
- 14 Send the miniprepped plasmid for Sanger or Oxford Nanopore long-read plasmid sequencing to confirm the construct. Store the remaining miniprep for downstream applications (i.e. shuttle into different electrocompetent cell for conjugation).