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Golden Gate Assembly (Protocol for NEB® Golden Gate Assembly Mix)

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

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Abstract

Golden Gate Assembly and its derivative methods exploit the ability of Type IIS restriction endonucleases (REases) to cleave DNA outside of the recognition sequence. The inserts and cloning vectors are designed to place the Type IIS recognition site distal to the cleavage site, such that the Type IIS REase can remove the recognition sequence from the assembly.

Guidelines

For complex (>10 fragment) assemblies, high efficiencies are achievable with increased ligase and BsaI-HFv2 levels (1000 units T4 DNA Ligase, 30 units BsaI-HFv2), as listed in this protocol. For assemblies involving 10 fragments and less, the standard amounts (500 units T4 DNA Ligase, 15 units BsaI-HFv2) are sufficient. Note the reaction volume of 25 µl is used to allow sufficient volume for precloned insert additions, if needed.

Materials

MATERIALS

- NEB 10-beta Competent E.coli (High Efficiency) - 6×0.2 ml **New England Biolabs Catalog #**C3019I
- NEB Golden Gate Assembly Mix - 15 rxns **New England Biolabs Catalog #**E1600S
- T4 DNA Ligase **New England Biolabs Catalog #**M0202
- NEB 10-beta/Stable Outgrowth Medium - 4×25 ml **New England Biolabs Catalog #**B9035S
- BsaI-HFv2 **New England Biolabs Catalog #** R3733L

pGGA Destination Plasmid*
LB Agar plates with chloramphenicol

* Included in the NEB Golden Gate Assembly Mix (**NEB #E1600**)

Troubleshooting

Safety warnings

Wear laboratory coat, gloves and googles. Always check the safety warnings indicated by the Mix supplier.

Assembly Reactions

1 Set up 25 µl assembly reactions as follows:

		Assembly Reaction	Negative Control (If desired)
	pGGA Destination Plasmid*, 75 ng/µl	1 µl (75 ng)	1 µl (75 ng)
	24 precloned inserts cloned into pMiniT 2.0, 100 ng/ul each plasmid	0.75 µl (75 ng) each, (18 µl total)	-
	T4 DNA Ligase Buffer (10X)	2.5 µl	2.5 µl
	T4 DNA Ligase, 2000 U/µl	0.5 µl (1000 units)	0.5 µl (1000 units)

	BsaI-HFv2, 20 U/µl	1.5 µl (30 units)	1.5 µl (30 units)
	Nuclease-free H2O	1.5 µl	19.5 µl

*or user provided

2 Mix gently by pipetting up and down 4 times.

3 Briefly microcentrifuge (1 second) to bring material to the bottom of tube.

4 Transfer to thermocycler and program as follows: (5 min 37ºC → 5 min 16ºC) x 30 cycles followed by 5 minutes 60ºC. If reactions are done overnight, add a 4ºC terminal hold to the protocol, but repeat the final 5 minutes 60ºC step the next day before the transformations.

Transformation

5 For each assembly, thaw a 50 µL tube of NEB 10-beta competent *E. coli* cells on ice for 5–10 minutes.

6 Add 2 µL of the assembly reaction; gently mix by flicking the tube 4-5 times.

7 Incubate on ice for 30 minutes.

8 Heat shock at 42ºC for 30 seconds.

9 Place back on ice for 5 minutes.

10 Add 950 µL of room temperature NEB 10-beta/Stable Outgrowth Medium. Incubate at 37ºC for 60 minutes, shaking vigorously (250 rpm) or using a rotation device.

Plating

11 Warm LB agar plates containing chloramphenicol (for pGGA) at 37ºC for 15 minutes.

12 Mix the cells thoroughly by flicking the tube and inverting, then spread 100 µL outgrowth onto each plate.

13 Incubate the plates overnight at 37ºC, or 24 hours at 30ºC, or 48 hours at 25ºC.