Golden Gate Assembly protocol

Protocols

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Fragment amplification via PCR

Introduction

This is the protocol for fragment amplification with NEB® Hot Start Taq 2X Master Mix to get your insert ready for golden gate assembly using NEB® Golden Gate Assembly Kit.

Material

- Reverse and Forward primers
- Template DNA (100-200 ng/µL)
- NEB Hot Start Taq 2X master mix
- Nuclease-free water
- Bucket of ice
- Promega Wizard® SV Gel and PCR Clean-Up System kit

Procedure

1- Add to a sterile thin-walled PCR tube on ice:

component	50 µL reaction
20 µM Forward Primer	1.5 µL
20 µM Reverse Primer	1.5 µL
Template DNA	2 µL
NEB Hot Start Taq 2X master mix	25 µL
Nuclease-free H20	20 µL

Gently mix the reaction and perform a quick spin to collect all liquid to the bottom of the tube.

2- Transfer PCR tubes to a PCR machine and run the following thermocycling protocol:

Step	Temperature (°C)	time
Initial denaturation	95°C	30 secondes
35 cycles	95 °C 55°C 68 °C	30 secondes 30 secondes 50 secondes
Final extension	68 °C	5 minutes
Hold	10°C	infinite

3- Perform a PCR clean-up using the Promega Wizard® SV Gel and PCR Clean-Up System.
Add 50µL of Membrane Binding Solution to your PCR amplification product and follow the 5.A. DNA Purification by Centrifugation part of the protocol given in the kit.

4- Nanodrop your sample to make sure of the purity of your sample and to know the concentration.

Golden Gate cloning

Introduction

This is the protocol for Golden Gate assembly using NEB® Golden Gate Assembly Kit (BsaI-HF®v2).

Material

- Golden Gate plasmid (150 ng/µL)
- Insert (150 ng/µL)
- NEB® T4 ligase buffer
- NEB® Golden Gate assembly mix

Procedure

1- Add to a sterile thin-walled PCR tube on ice:

Component	Negative control	Assembly Reaction
Golden gate plasmid	0.5 µL	0.5 µL
Insert	-	1 µL
T4 ligase buffer	2 µL	2 µL
Golden Gate Assembly mix	1 µL	1 µL
Nuclease-free H20 (up to 20µL)	16.5 µL	15.5 µL

Gently mix the reaction and perform a quick spin to collect all liquid to the bottom of the tube.

2- Transfer PCR tubes to a PCR machine and run the following thermocycling protocol:

Temperature (°C)	time
37°C	5 minutes
60 °C	5 minutes
10 °C	infinite

Bl21(DE3) *E. coli* heat shock transformation

Introduction

This is the protocol for the heat shock transformation of Bl21(DE3) *E. coli* with your plasmid assembled using the golden gate method previously detailed.

Material

- Bucket of ice
- Bl21(DE3) *E. coli* (120 µL)
- Plasmid (~100 ng/µL)
- Terrific Broth (TB) media
- Round-bottom tube
- Petri dishes (LB + antibiotic of your plasmid resistance cassette)

Procedure

1- Store all your material on ice under the hood. Bacteria should thaw slowly (~10 minutes).

At the same time, prepare aliquots of TB and incubate them at 37°C. Turn on your water bath at 42°C.

2- Add 2µL of the plasmid at the bottom of the P20 pipette

3- Add 120µL of the bacteria, gently tap the tube to mix.

4- Leave to incubate on ice for 30 minutes. Gently tap the tube every 10 minutes.

5- Heat shock 30 seconds in a water bath at 42°C

6- Leave 2 minutes on ice

7- Add 150µL of the TB media

8- Incubate on the shaker 220 RPM, 37°C for 90 minutes

9- Plate 120 µL on your petri dish

10- Incubate at 37°C overnight