Golden Gate reaction protocols

Pipetting scheme for Lvl 0:

0.5 µL of DNA insert (~60 ng/ µL)
0.5 µL of entry Vector (15 ng/ µL)
1 µL T4 DNA Ligase buffer (NEB),
0.5 µL T4 DNA Ligase (NEB),
0.5 µL Esp3I (NEB)
water to 10 µL.

Pipetting scheme for Lvl 1/2:

0.5 µL of each DNA insert
1 µL T4 DNA Ligase buffer (NEB),
0.5 µL T4 DNA Ligase (NEB),
0.5 µL BsaI V2 (NEB) / Esp3I (NEB)
water to 10 µL. (20 µL also possible)

Esp3I (NEB) is used for Level 0 and Level 2
BsaI V2 (NEB) is used for Level 1

For an improved assembly efficiency, the amount of DNA inserts can optionally be normalized to equimolar concentrations (~20 fmol each) or use 75 ng of each insert (antibiotic resistance part should be diluted 1:10, 7.5-10 ng).

pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)

50 ng of 5000 bp dsDNA is about 0.015 pmols.
50 ng of 500 bp dsDNA is about 0.15 pmols.

Troubleshoot/Overnight Protocol

Step 1	37°C	2 min	x 50
Step 2	16°C	5 min	
Cycle steps 1 & 2 x 50			
Step 3	50°C	10 min	
Step 4	80°C	10 min	
Total Time ~6h (370min)			

Improved Protocol

Step 1	37°C	1.5 min	x 15
Step 2	16°C	3 min	
Cycle steps 1 & 2 x 15			
Step 3	50°C	5 min	
Step 4	80°C	10 min	
Total Time 82.5 min			

Rapid Protocol (for Lvl 0 for example)

Step 1	37°C	20 min	x 5-10
Step 2	37°C	1.5 min	
Step 3	16°C	3 min	
Cycle steps 2 & 3 x 5-10			
Step 4	50°C	5 min	
Step 5	80°C	10 min	
Total Time 37.5-60 min			

Transformation:

Transformations were performed using 2-5 µL of each assembly reaction added to 50 µL competent cells. Cells should be recovered for 1h (amp) to 2h (Kan, Chloramphenicol).

For additional information, help or suggestions join our slack workspace through this QR-Code!