Golden Gate Assembly

Introduction

Golden Gate is an assembly method using Type IIS restriction enzymes and predefined overlap sequences to assemble multiple pieces of DNA in one reaction. Golden Gate design is beyond the scope of this protocol; this protocol is the reaction setup only.

Materials

- 200 µl thin-wall PCR strip tubes, one per reaction
- Backbone plasmid
  - Usually pDONR-GG L1_lacZ_L2 or pDONR-GG L4_lacZ_R1
- Parts being assembled, either in plasmids or gBlocks
- Nuclease-free water
- T4 ligase buffer, 1.5 µl per reaction
  - Found in single-use aliquots of 10 (??) µl in the -20° freezer. T4 ligase buffer does NOT like to be thawed and frozen again; once you use an aliquot, discard what's left!
- 10X BSA, 1.5 µl per reaction
  - Found in the -20° freezer. ...and that's Bovine Serum Albumin, NOT the BsaI enzyme!
- BsaI (or AarI) restriction enzyme, 1 µl per reaction
  - Leave the BsaI and T4 ligase in the freezer until the very end, and add after the rest of the reaction has been set up.
- T4 ligase, 1 µl per reaction
  - Many protocols (both online and in the lab) call for high-concentration T4 ligase. This is only necessary if you're doing VERY LARGE Golden Gate reactions (5-8 parts). You do NOT need the HC ligase for most of the GGs you'll be doing.
  - Leave the BsaI and the T4 ligase in the freezer until the very end, and add after the rest of the reaction has been set up.
- An **empty** 10µl or 200µl tip box
- Ice in an ice bucket

Procedure

Record your reaction setup.

1. A Golden Gate works best when there are equimolar amounts of each part in the reaction. By convention, we use **50 fmol** of each part. For each part (plasmid and gBlock), compute the concentration (fmol/ul) and the volume required for 50 fmol in the table below. If you resuspended your gBlocks at 50 fmol/ul, you can omit them in this table.

Table1

	A	B	C	D	E
1	**Part**	**Size (kb)**	**Concentration, (ng/ul)**	**Concentration, (fmol/ul)**	**Volume for 50 fmol**
2	pDONR-GG	3.4	100	44.6	1.1
3					
4					
5					

2. Record the reaction setup for each reaction below. For each reaction, include:

    -- 50 fmol of each part
    -- 1.5 ul T4 ligase buffer
    -- 1.5 ul 10X BSA
    -- 1 ul BsaI
    -- 1 ul T4 ligase
    -- Water to a total volume of 15 ul.
    -- **NOTE:** If you're using **AarI**, you need to add the **AarI oligo** as well!

Table2

	A	B	C	D
1	**A19 L4_PhlF_R1**			
2	pDONR-GG L1_L2	1.1 ul		
3	BT-01 gBlock	1 ul		
4	BT-02 gBlock	1 ul		
5	Water	6.9 ul	x	
6	T4 ligase buffer	1.5 ul	x	
7	10X BSA	1.5 ul	x	
8	BsaI or AarI	1 ul		
9	T4 ligase	1 ul		
10				

Set up your thermocycler

3. Program a thermocycler with the following program:

    NOTE: If you are using **AarI,** use **50 cycles** instead of 15.

Table3

	A	B
1	Heat lid	70°
2	Start cycle	15X
3	--	37° for 1′ 30″
4	--	16° for 3′ 0″
5	Close Cycle	
6	50° for 5′	
7	80° for 10′	
8	Store at 8°	

Set up your reactions

4. Fill an empty tip box with ice, put the grey platform back on, then fill with water until just under the rack.
5. Label one thin-walled PCR tube per reaction.
6. Add the reaction components. **Add the water first; add the restriction enzyme and buffer last.**
7. Cap the tubes. Flick a few times to mix, then pulse spin.
8. Load the tubes in the thermocycler and start the program.

Transform

9. Transform as usual. **Plate only 10 ul of the outgrowth in a 200 ul puddle of water, or you will get a lawn of colonies.**