Protocol for Golden Gate assembly with Echo liquid handler

Description: This protocol explains how to set up a golden gate assembly using an Echo liquid handler

Material:

- Labcyte Echo liquid handler
- Thermal cycler
- Echo source plate 384PP
- 96 or 384-well plate compatible with the Echo machine
- Parts to be assembled
- T4 DNA ligase
- T4 DNA ligase buffer
- BsaI-HFv2 restriction enzyme

Source and Destination plate layout

Before the experiment, the layout of the source and destination plate contents should be prepared. This will make writing the Cherry-picking file easier. Prepare a table with the arrangement of the reagents and parts in the wells of the source plate. Prepare a second table with assembly constructs. Here is a simple example:

384-well Source Plate

	1	2	3	...	24
A	Promoter	T4 ligase buffer			
B	RBS 1	Ligase			
C	RBS 2	BsaI			
D	CDS	Water			
E	Terminator				
F	Vector				
...					
P					

96-well Destination Plate

	1	2	...	12
A	**Construct 1** (P1, RBS 1, CDS, Term, Vector)	**Construct 2** (P1, RBS 2, CDS, Term, Vector)		
...				
H				

Preparation of the parts

- Dilute each part to 40 fmol.
- Add 50 µL of each part to the Echo source plate wells.
- Add 50 µL of T4 DNA ligase, T4 DNA Ligase Buffer, BsaI-HFv2 restriction enzyme to the different source plate wells.

Writing the Cherry Pick file

- Set up a spreadsheet file with the following headings:

Source Plate Name	Sample Group	Source Well	Destination Plate	Destination Well	Transfer Volume	Part name

- Enter Source Plate name. *Ex: 1.*
- Sample group indicates the fluid calibration. Refer to the fluid calibration protocol to verify that the fluid calibration is correct. *Ex: 384PP_Plus_AQ_GP2 for the enzymes (T4 ligase and BsaI-HFv2), and 384PP_Plus_AQ_SP2 for the DNA parts and buffer.*
- Enter destination plate name. *Ex: 1.*
- Enter the destination well in which the part is to be assembled.
- Enter the transfer volume to be transferred from the Source Plate to the Destination plate (in nL).

  Ex:
  100 for each part
  50 for the vector
  100 for the T4 DNA ligase
  100 for the T4 DNA ligase buffer
  50 for the restriction enzyme
- Optional: Enter the part name.
- Save file as csv

Here is an example of a complete Cherry-pick file:

Source Plate Name	Sample group	Source Well	Destination Plate Name	Destination Well	Transfer Volume	Part name
1	384PP_Plus_AQ_SP2	A1	1	A1	100	Promoter 1
1	384PP_Plus_AQ_SP2	A1	1	A2	100	Promoter 1
1	384PP_Plus_AQ_SP2	B1	1	A1	100	RBS 1
1	384PP_Plus_AQ_SP2	C1	1	A2	100	RBS 2
1	384PP_Plus_AQ_SP2	D1	1	A1	100	CDS
1	384PP_Plus_AQ_SP2	D1	1	A2	100	CDS

1	384PP_Plus_AQ_SP2	E1	1	A1	100	Terminator
1	384PP_Plus_AQ_SP2	E1	1	A2	100	Terminator
1	384PP_Plus_AQ_SP2	F1	1	A1	50	Vector
1	384PP_Plus_AQ_SP2	F1	1	A2	50	Vector
1	384PP_Plus_AQ_SP2	A2	1	A1	100	T4 Ligase Buffer
1	384PP_Plus_AQ_SP2	A2	1	A2	100	T4 Ligase Buffer
1	384PP_Plus_AQ_GP2	B2	1	A1	100	T4 Ligase
1	384PP_Plus_AQ_GP2	B2	1	A2	100	T4 Ligase
1	384PP_Plus_AQ_GP2	C2	1	A1	50	BsaI
1	384PP_Plus_AQ_GP2	C2	1	A2	50	BsaI

Running cherry-pick protocol:

- Open the cherry-picking software of the Echo and create a new protocol.
- Choose 384PP as a source plate and 384_AQ_BP2 as a source plate type.
- Choose the destination plate type. Refer to the Destination Plate calibration protocol for calibration of the plate.
- Import the previously created cherry-picking csv file in the pick list tab.
- Save your protocol.
- Run simulation to verify the protocol.
- Run protocol.
- Seal the plate.

Golden-gate reaction:

- Incubate the assembly in the thermal cycler with the following cycle:

Step	Temperature	Duration
1 Initial digestion	37°C	30 min
2 Digestion	37°C	2 min
3 Ligation	16°C	5 min
Go to step 1 50-100x		

4 Final Digestion	37°C	30 min
5 Enzyme inactivation	0°C (BsaI/Esp3I) 65°C (BbsI)	20 min
6 Hold	120°C (BsaI/Esp3I) 65°C (BbsI)	Infinite hold