Golden Gate assembly with the Echo liquid handler

In this document you can find how to set up the Labcyte Echo liquid handler for a Golden Gate assembly.

Material

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

Preparation of the parts

Each of the parts should be purified and diluted to 40fmol.

- Add 50uL of each part to an Echo source plate 384PP following the Cherry Pick spreadsheet*.

**See Cherry Pick application section*

- Add 50uL of T4 DNA ligase, T4 DNA ligase buffer and BsaI-HFv2 restriction enzyme to 3 different wells of the source plate.

They can be pipetted back after the protocol is performed to save resources.

The dead volume of a well is 15uL and the maximum volume of a well is 65uL. Make sure to add enough components for all the reactions. If a specific component is required excessively, split it in different wells and adjust the protocol accordingly.

Protocol creation

See the Echo Cherry Pick User Guide for more detailed information on setting up the protocol.
Set up a spreadsheet as follows:

Source plate name	Sample group	Source well	Destination plate name	Destination well	Transfer volume	Part name (optional)

Avoid adding columns such as Plate type or Plate barcode as the application often has trouble uploading the protocol.

- For a Golden Gate assembly, a single source plate is used.

Example of a source plate name: Source 1

- Sample group: type of fluid to be transferred. Use 384PP_Plus_AQ_GP2 for the enzymes (T4 ligase and BsaI-HFv2), use 384PP_Plus_AQ_SP2 for the DNA parts and buffer.

The calibration of the machine varies depending on the fluid viscosity. Samples in water require less pulse than samples stored in glycerol which are more viscous. Enzymes are typically stored in 50% glycerol.

- Source well: the position of each part on the source plate.

Example of a source well: A1

- One or multiple destination plates may be used.

Example of a destination plate name: Dest 1

- Destination well: the well of the destination plate in which a plasmid is to be assembled

Example of a destination well: C2

- Transfer volume: volume to be transferred from the source well to the destination well. The volumes are in nL.

For a regular Golden Gate 8 part assembly with parts at a concentration of 40 fmol/µl
(If fewer parts are used, fill the reaction to 1µl using ddH2O):
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

- The part name is optional but can be helpful to set up the spreadsheet

Save the file as .csv

Cherry Pick application

Open the Cherry Pick application and follow the instructions in this guide.
In the protocol tab:

- Choose 384PP as a source plate and 384_AQ_BP2 as a source plate type.

Plate calibration works with a wide range of fluids with this plate type.

- There are no control plates for this protocol
- There are no replicates for this protocol
- Choose biorad_96PCR as a destination plate

In the Pick list/Samples tab:

- Import the previously created .csv file
- Click Automap (this option does not import the Part name column)
- Click import

In the Pick list/Plate Preview tab:

- Verify the position of your samples

- Verify the position of the destination wells

Save your protocol.
Before starting the protocol, run a final simulation to verify it.
Click the run button then simulate.

Running the protocol
Beforehand, ensure that all the buttons of the Echo Liquid Handler application are green.
Run the protocol.
Immediately close the wells.
Incubate in the thermal cycler with the following cycle:

Step	Temperature	Duration
1 Initial digestion	37°C	30min
2 Digestion	37°C	2min
3 Ligation	16°C	5min
GOTO 1 50-100x		
4 Final digestion	37°C	30min
5 Enzyme inactivation	80°C (BsaI/Esp3I) 65°C (BbsI)	20min
6 Hold	12°C	Infinite hold