Gibson Assembly

Introduction

Gibson assembly protocol from the ThermoFischer GeneArt™ Gibson Assembly HiFiCloning® Kit. This kit can be used for up to 5 inserts. Suitable for fragments from 0.5-32kb.

Materials

- GeneArt™ Gibson Assembly HiFi Master Mix
  - Contains positive control (10ng of 1.5kb insert (kanamycin insert) and 30ng of 2.7 kb vector containing an ampicillin resistance gene.
  - Select for 4.2kb assembled construct on LB agar plates with 100ug/mL ampicillin or 50ug/mL kanamycin
- DNA fragments to assemble
- Linearized E.coli cloning vector
- Positive control (included in kit)
- Sterile deionized water

Procedure

1. Determine Concentration of DNA (preparation for step 3)
2. Determine concentration of DNA insert solution by OD260 or fluorescence and use concentrations to calculate volume required to achieve and use concentrations to calculate the volume required to achieve the required molar ratio of insert to vector.
3. Use following formula to calculate molarities:

   pmols = ((weight in ng) * 1000)/ ((fragment length in bp)* 660)

4. Cloning Reaction
5. Thaw master mix on ice
6. Vortex master mix immediately before use
7. In a microcentrifuge tube on ice set cloning reaction as following:

	1-3 inserts assembly	4-5 inserts assembly	Positive Control
Recommended DNA molar ratio	vector:inster = 1:1	vector:inster = 1:1	-
Amount of each fragment	0.08pmol vector and 0.08pmol each insert = X uL		10 µL
GeneArt™ Gibson Assembly HiFi Master Mix	10 µL	10 µL	10 µL
Deionized water volume	(10-X) µL	(10-X) µL	-
Total Volume	20 µL	20 µL	20 µL
Incubation time at 50C	15min	60min	15min

8. Mix the reactions by vortexing, spinning down and then incubate at 50C for 15 min (1-3 inserts) or 60 min (4-5 inserts). Incubate positive control for 15 min.
9. *Note that incubation of 60min for only one insert will increase the possibility of efficiency!
10. After incubation, place the reaction mix on ice and immediately proceed to the transformation step or store at -20C.