Gibson Assembly® HiFi 1-Step Kit Quick Reference Manual

SYNTHETIC GENOMICS®

Complete product information and additional resources are available at **www.sgidna.com/hifi_kit**.

Catalog Numbers GA1100-10, GA1100-50, GA1100-S, GA1100-10MM, GA1100-50MM

Products and Storage Conditions

Gibson Assembly® HiFi 1 Step Kit

Quantity	Component	Cat. GA1100-S (5 Reactions)	Cat. GA1100-10 (10 Reactions)	Cat. GA1100-50 (50 Reactions)	Storage Temperature
		Volume			
1 tube (each)	GA HiFi 1 Step Master Mix (2X)	25 µL	50 µL	250 µL	−20°C
	GA Positive Control (2X)	10 µL (2 Control Rxns)	10 µL (2 Control Rxns)	25 µL (5 Control Rxns)	

Gibson Assembly® HiFi 1 Step Master Mix (2X)

		Cat. GA1100-10MM (10 Reactions)	Cat. GA1100-50MM (50 Reactions)	
1 tube	GA HiFi 1 Step Master Mix (2X)	50 µL	250 µL	−20°C

Guidelines for Assembly

- Use approximately 10–100 ng of each DNA fragment (including the cloning vector) in equimolar amounts:

Fragment size	Amount	pmols
≤1 kb	20–40 ng	0.04
1–5 kb	10–25 ng	0.008–0.04
5–8 kb	25 ng	0.005–0.008
8–20 kb	25–100 ng	
20–32 kb	100 ng	0.005

- Keep GA 1-Step Master Mix (2X) on ice at all times.
- For the assembly of multiple fragments, create a master mix of fragments in the proper ratios to minimize pipetting error.

Gibson Assembly® HiFi 1-Step Method

1. Thaw GA 1-Step Master Mix (2X) on ice.
2. Dilute your DNA fragments with nuclease-free water in PCR tubes to a total volume of 5 µL according to the “Guidelines for Assembly”.
3. **Vortex the master mix immediately before use** after it is thawed.
4. In a tube on ice, combine 5 µL of DNA fragments and 5 µL of GA 1-Step Master Mix (2X). Mix the reaction by pipetting.
5. (Optional) For the positive control, combine 5 µL of the Positive Control (2X) and 5 µL of GA 1-Step Master Mix (2X) in a tube on ice. Mix the reaction by pipetting.
6. Vortex and spin down all reactions.
7. Incubate the reactions at 50°C for 1 hour.
8. After the incubation period, store reactions at −20°C or proceed to transformation.
9. (Optional) Analyze the assembly reaction with electrophoresis of 5–10 µL of the reaction on a 0.8–2% agarose gel. A high molecular weight ladder is indicative of a successful assembly reaction.

Transformation Recommendations

- Use Epicentre TransforMax™ EPI300™ electrocompetent cells with the Bio Rad Gene Pulser Xcell electroporator.
- Dilute the assembled product 1:5 with nuclease-free water before transformation and follow the “Transformation Procedure for Electrocompetent EPI300™ Cells (Recommended Procedure)”.

Guidelines for Transformation

- For transformation with electrocompetent cells other than EPI300™ cells, we recommend using 2% (v/v) of the assembly reaction per transformation (e.g. use 1 µL of the assembly reaction per 50 µL of high efficiency competent cells). Follow the protocol and electroporation parameters supplied with your electrocompetent cells.
- For transformation using chemically competent cells, follow the procedure “Transformation: Chemically Competent Cells” on page 2.
- Before starting, prepare ice buckets, tubes, and pipettors so that the transformation steps can be completed quickly and efficiently.

Transformation Procedure for Electrocompetent EPI300™ Cells (Recommended Procedure)

1. Prepare 15 mL snap cap tubes with 1 mL SOC per tube.
2. Chill electroporation cuvettes on ice.
3. While keeping the tubes on ice, pipet 2.5 µL of the diluted assembly reaction into a clean 1.5 mL microfuge tube (See "Transformation Recommendations").
4. Thaw TransforMax™ EPI300™ Electrocompetent *E. coli* (Epicentre® EC300110) on ice. Mix gently.
5. Add 30 µL of thawed, electrocompetent cells to each cold tube containing the diluted reaction (from step 3, above). Mix gently with the end of a pipette tip and return the tube to ice.
6. Incubate the cells and DNA on ice for one minute.
7. After the incubation, pipet the cell/DNA mixture into a chilled cuvette. Tap the cuvette gently onto the benchtop 2–3 times, insert the cuvette into the electroporator, close the lid, and press the pulse button.

   Note: The pulse settings for electrocompetent TransforMax™ EPI300™ cells are 1200 V, 25 µF, 200 Ω, 0.1 cm cuvette.
8. During the pulse (≈ 2 seconds), remove about 800 µL SOC from the snap cap tube (step 1). Add the SOC to the cuvette immediately after the end of the pulse.
9. Thoroughly pipet the mixture up and down. Add the mixture back to the snap cap tube containing about 200 µL SOC, and repeat steps 5–8 for the remaining tubes. Work as quickly as possible until the cells are transferred into the snap cap tube.
10. Incubate the tubes with shaking at about 200 RPM for 1 hour at 37°C to allow the cells to recover.
11. Proceed to "Plating Procedure".

Transformation: Chemically Competent Cells

1. While keeping tubes on ice, pipet 1 µL of the assembly reaction (from step 8 of the "Gibson Assembly® HiFi 1-Step Method") into a clean 1.5 mL microfuge tube.
2. Thaw chemically competent cells on ice. Mix gently.
3. Add 50 µL of thawed, chemically competent cells to each cold tube containing the assembly reaction (from step 1). Mix gently with the end of a pipette tip.
4. Incubate the cells and DNA on ice for 30 minutes without mixing.
5. Heat shock the cell/DNA mixture according to the instructions provided with your competent cells.

   Note: For most competent cells, the heat shock parameters are 42°C for 30 seconds.
6. Return tubes to ice for 2 minutes
7. Add 950 µL of room temperature SOC media to the tubes.
8. Incubate the tubes with shaking at about 200 RPM for 1 hour at 37°C to allow the cells to recover.
9. Proceed to "Plating Procedure".

Plating Procedure

1. Pre-warm LB plates in an incubator upside down for 10–15 minutes.
2. After the incubation, plate 1/10–1/100 of the transformation reaction (10–100 µL out of 1 mL) onto LB agar plates with appropriate antibiotics. See "Recommended Plating Volume" below.
3. (Optional) For the positive control, plate 1/100 volume of the transformed reaction onto LB plates containing 100 µg/mL ampicillin or carbenicillin with 40 µg/mL X-Gal and 0.1 mM IPTG.
4. Incubate plates at 37°C upside down, overnight.
5. Pick colonies for screening.

Recommended Plating Volume

Number of fragments	Plating volume	For example, we normally plate... (Based on a 1000 µL transformation mixture)	Notes
1–2	1/50	2 µL and 20 µL	• Plate two plates: one low and one high volume • Spin down the reaction before plating
3–5	1/10	10 µL and 100 µL	
> 5	1/2	100 µL and 500 µL	

Technical Services: For technical assistance, please contact technical services at techservices@sgidna.com.

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