Gibson Assembly® HiFi kit — Quick reference manual

For additional product information, an online primer design tool, and a detailed protocol, visit telesisbio.com

Catalog numbers GA1100-10, GA1100-50, GA1100-S, GA1100-10MM, GA1100-50MM, GA1100-B05

Products and storage conditions

Gibson Assembly® HiFi kit

Quantity	Component	Cat. GA1100-S (5 reactions)	Cat. GA1100-10 (10 reactions)	Cat. GA1100-50 (50 reactions)	Storage temperature
		Volume			
1 tube (each)	Gibson Assembly HiFi master mix A (2X)	25 µL	50 µL	250 µL	Aliquot and store at -20 °C
	Gibson Assembly positive control	10 µL (2 control rxns)	10 µL (2 control rxns)	25 µL (5 control rxns)	

Gibson Assembly® master mix (2X)

Important: Upon receipt, place Gibson Assembly master mix (2X) on ice to thaw. Briefly vortex and centrifuge the thawed master mix. Then, aliquot the master mix to reduce the number of freeze-thaw cycles. Properly aliquoted Gibson Assembly master mix is stable up to six months when stored at −20 °C.

Guidelines for assembly

- For a typical Gibson Assembly HiFi reaction, combine 25–50 ng of vector with approximately 10–300 ng of insert. For best results, we recommend balancing the molar ratio of the DNA fragments. For fragments > 1 kb, use an equimolar ratio. For DNA fragments ≤ 1 kb, we recommend using a 5-fold molar excess of insert. To precisely determine the pmol or ng of DNA for a fragment of a given size, use the following formulas:
- pmol DNA = [ng DNA/(660 x # of bases)] x 1000
- ng of DNA = [pmol DNA x (660 x # of bases)]/1000
- Refer to Amount of DNA to use in Gibson Assembly reaction table for approximate pmol of DNA for a given fragment size and amount.
- Keep Gibson Assembly HiFi 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.

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Gibson Assembly® HiFi method

Thaw master mix (2X) on ice.

1. Dilute your DNA fragments with nuclease-free water in PCR tubes to a total volume of 5 µL according to the guidelines for assembly
2. Vigorously vortex the master mix for 15 seconds immediately before use, after it is thawed.
3. In a tube on ice, combine 5 µL of DNA fragments and 5 µL of master mix (2X). Mix the reaction by pipetting up and down.
4. (Optional) For the positive control, combine 5 µL of the positive control (2X) and 5 µL of master mix (2X) in a tube on ice. Mix the reaction by pipetting up and down.
5. Vortex and spin down all reactions.
6. Incubate the reactions at 50 °C for one hour.
7. After the incubation is complete, store the reactions at –20 °C or dilute reactions for downstream applications such as PCR or *E. coli* transformation (see the protocols on the following pages).
8. (Optional) Analyze the assembly reaction by performing gel electrophoresis with 5–10 µL of the reaction on an 0.8–2% agarose gel.

Transformation guidelines

We recommend transformation with *E. cloni* 10G chemically competent cells (Lucigen cat. no. 60107) or TransforMax™ EPI300™ electrocompetent *E. coli.* (Lucigen cat. no. EC300110). If you use competent cells other than the recommended cells, follow the transformation protocol provided with the competent cells. Use cells with a transformation efficiency ≥ 1 × 109 CFU/µg pUC19.

Because some ingredients in the buffer mix can negatively impact the survival of some competent cells, we recommend diluting the assembly reaction before performing the transformation. Dilute Gibson Assembly HiFi assemblies up to 5-fold. You may need to empirically determine the optimal level of dilution, depending on the type of cells used.

Transformation with *E. cloni* 10G chemically competent cells (recommended)

1. Pre-chill 15 mL disposable polypropylene culture tubes (one tube for each transformation reaction).
2. Thaw cells on ice for 5 to 15 minutes.
3. Add 40 µL of thawed, competent cells to each cold tube.
4. Add 2 µL of the diluted assembly reaction to each cold tube of competent cells. Mix by briefly stirring (do not pipette up and down).
5. Incubate the cells and DNA on ice for 30 minutes. Do not mix.
6. Heat shock the mixture in a 42 °C water bath for 45 seconds.
7. Return tubes to ice for two minutes.
8. Add 950 µL room temperature recovery media to the cells in the culture tube.
9. Incubate the tubes with shaking at about 250 rpm for 90 minutes at 37 °C to allow cells to recover.
10. Proceed to plating procedure.

Transformation with TransforMax™ EPI300™ electrocompetent *E. coli*

1. Add 1 mL SOC media to 1.5 mL microcentrifuge tubes (one tube per reaction). Place tubes on ice for 10 minutes.
2. Chill clean electroporation cuvettes on ice.
3. Pipette 30 µL of EPI300™ cells directly between the slit of the cuvettes on ice (one cuvette per reaction).
4. Add 2 µL of the diluted assembly reaction to the cells in the cuvette. Mix by pipetting up and down gently two times.
5. Incubate cuvette on ice for one minute.
6. Gently tap cuvette on a benchtop two times to make sure all contents are at the bottom of the cuvette in between the slit.
7. Insert the cuvette into a BioRad electroporator or equivalent, and press *Pulse*. Pulse settings for EPI300™ cells are 1200 V, 25 uF, 200 Ω, 0.1 cm cuvette.
8. During the pulse (~2 seconds), remove 800 µL SOC from a pre-chilled 1.5 mL tube (step 1). Immediately add the SOC to the cuvette after the pulse.
9. Mix the cells and SOC by pipetting up and down. Add the mixture back into the tube containing the remaining SOC.
10. Incubate the cells for one hour at 37 °C with shaking at 200 rpm.
11. Proceed to plating procedure.

Plating procedure

1. Pre-warm LB plates in an incubator upside down for 10 to 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.
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

Always plate two plates (one low- and one high-volume).

Number of fragments	**Plating volume** (fraction of the total transformation mixture)	**E.g. we typically plate...** (based on a 1,000 µL transformation mixture)
1–2	1/50	2 µL and 20 µL
3–5	1/10	10 µL and 100 µL

Reference material

Amount of DNA to use in Gibson Assembly® reaction

Refer to the following table for approximate pmol of DNA for a given fragment size and amount.

Fragment size	ng of DNA	pmol of DNA
0.5 kb	20	0.061
	40	0.121
1 kb	10	0.015
	25	0.038
5 kb	10	0.003
	25	0.008
8 kb	25	0.005
	50	0.009
10 kb	25	0.004
	50	0.008
15 kb	50	0.005
	100	0.010
20 kb	50	0.004
	100	0.008
30 kb	50	0.003
	100	0.005

For technical assistance, contact help@telesisbio.com.