Gibson Assembly® Ultra kit — Quick reference manual

Complete product information and additional resources are available at telesisbio.com

Catalog numbers GA1200-S, GA1200-10, GA1200-50, GA1200-10MM, GA1200-50MM, GA1200-B05

Products and storage conditions

Quantity	Component	Cat. GA1200-S (5 reactions)	Cat. GA1200-50 (10 reactions)	Cat. GA1200-50 (50 reactions)	Storage temperature
		Volume			
1 tube (each)	Gibson Assembly Ultra master mix A (2X)	25 µL	50 µL	250 µL	Aliquot and store at –20 °C
	Gibson Assembly Ultra master mix B (2X)	50 µL	100 µL	500 µL	
	Gibson Assembly positive control (2X)	10 µL (2 control rxns)	10 µL (2 control rxns)	25 µL (5 control rxns)	

Gibson Assembly® Ultra master mixes (2X)

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

Catalog numbers	GA Ultra master mix A (2X)	GA Ultra master mix B (2X)	Number of reactions
	Volume		
GA1200-10MM	50 µL	100 µL	10
GA1200-50MM	250 µL	500 µL	50
GA1200-B05	5 mL	10 mL	1,000

Guidelines for assembly

- For a typical Gibson Assembly Ultra 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

- The total volume for the combined DNA fragments in the assembly reaction is ≤ 5 µL.
- To assemble multiple fragments and minimize pipetting error, create a master mix of fragments in the proper ratios.

Part number 43011 | Revision 1.1 | Effective 06DEC2022

Gibson Assembly® Ultra kit method

1. Thaw the Gibson Assembly Ultra master mix A (2X) on ice.
2. Dilute your DNA fragments with nuclease-free water in PCR tubes to a total volume of 5 µL.
3. Vortex the thawed master mix immediately before use.
4. In a 0.2 mL PCR tube on ice, combine 5 µL of DNA fragments and 5 µL of Gibson Assembly Ultra master mix A (2X). Mix the reaction by pipetting up and down.
5. (Optional) Set up a positive control reaction by aliquoting 5 µL of Gibson Assembly positive control (2X) into a 0.2 mL PCR tube on ice. Add 5 µL of Gibson Assembly Ultra master mix A (2X) and mix the reaction by pipetting up and down.
6. Vortex and spin down all reactions.
7. Program the following conditions into a thermocycler and transfer assembly reaction tubes to start:

		Overlap size	
3' end chew back	**1 cycle**	< 80 bp	≥ 80 bp
		37 °C for 5 min	37 °C for 15 min

Inactivation	1 cycle	75 °C for 20 min (for all overlap sizes)
Slowly cool		0.1 °C/sec to 60 °C
Anneal		60 °C for 30 min
Slowly cool		0.1 °C/sec to 4 °C

8. Thaw the Gibson Assembly Ultra master mix B (2X) on ice. Vortex and briefly centrifuge the thawed master mix immediately before use.
9. While keeping the tubes on ice, add 10 µL of Gibson Assembly Ultra master mix B (2X) to the reaction from step 7. Mix the reaction by pipetting up and down.
10. Incubate the reaction using the following conditions:

Repair	1 cycle	45 °C for 15 min

11. After the incubation is complete, store reactions at –20 °C or proceed to transformation.
12. (Optional) Analyze assembly reactions with agarose gel electrophoresis. A high molecular weight smear is indicative of a successful assembly reaction.

Guidelines for transformation

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 Ultra assemblies up to 2-fold. You may need to empirically determine the optimal level of dilution, depending on the type of cells used.

Transformation with Lucigen ***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 2 minutes.
8. Add 950 µL room temperature recovery media to the cells.
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 ten 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 1/2–1/50 of the transformation reaction (20–500 µL out of 1 mL) onto LB agar plates with appropriate antibiotics.
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
> 5	1/2 (Spin down reaction before plating)	100 µL and 500 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

Part number 43011 | Revision 1.1 | Effective 06DEC2022

TelesisBio