Gibson Assembly® Ultra Kit

Quick Reference Manual

SGI-DNA

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

Catalog Numbers GA1200-S, GA1200-10, GA1200-50, GA1200-10MM, GA1200-50MM, GA1200-B05, GA1200-B10, GA1200-B20, GA1200-B30, GA1200-B40, GA1200-B50

Products and Storage Conditions

Gibson Assembly® Ultra Kit

Quantity	Component	Cat. GA1200-S (5 Reactions)	Cat. GA1200-10 (10 Reactions)	Cat. GA1200-50 (50 Reactions)	Storage Temperature
		Volume			
1 tube (each)	GA Ultra Master Mix A (2X)	25 µL	50 µL	250 µL	Aliquot and store at –20°C
	GA Ultra Master Mix B (2X)	50 µL	100 µL	500 µL	
	GA Positive Control (2X)	10 µL (2 Control Rxns)	10 µL (2 Control Rxns)	25 µL (5 Control Rxns)	

GA Ultra Master Mixes (2X)

IMPORTANT: Upon receipt, place the GA Ultra Master Mixes (2X) on ice to thaw. After briefly vortexing and centrifuging the thawed master mixes, aliquot the master mixes to reduce the number of freeze-thaw cycles. Properly aliquoted GA Ultra Master Mix (MM) A and B are stable for up to 6 months when stored at –20°C.

Catalog Number	GA Ultra MM A (2X)	GA Ultra MM 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	1000
GA1200-B10	10 mL	20 mL	2000
GA1200-B20	20 mL	40 mL	4000
GA1200-B30	30 mL	60 mL	6000
GA1200-B40	40 mL	80 mL	8000
GA1200-B50	50 mL	100 mL	10,000

Guidelines

- 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. Refer to the table on page 2 for approximate pmol of DNA based on the size and amount of a given fragment. To precisely determine the pmol or ng of DNA based on fragment 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.

Gibson Assembly® Ultra Procedure

1. Thaw GA Ultra Master Mix A (2X) on ice.
2. Dilute 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 GA Ultra Master Mix A (2X). Mix the reaction by pipetting.
5. (Optional) Set up a positive control reaction by aliquoting 5 µL of GA Positive Control (2X) into a 0.2 mL PCR tube on ice. Add 5 µL of GA Ultra Master Mix A (2X) and mix the reaction by pipetting.
6. Vortex and spin down all reactions.
7. Transfer assembly reaction tubes to a thermocycler and program the following conditions:

		Overlap size:	
		< 80 bp	≥ 80 bp
3' end Chew Back	1 cycle	37°C for 5 min	37°C for 15 min
Inactivation	1 cycle	75°C for 20 min (for all overlap sizes)	
Slowly Cool	1 cycle	0.1°C/sec to 60°C	
Anneal	1 cycle	60°C for 30 min	
Slowly Cool	1 cycle	0.1°C/sec to 4°C	

8. Thaw GA Ultra Master Mix B (2X) on ice and **vortex the thawed master mix immediately before use.**
9. While keeping tubes on ice, add 10 µL of GA Ultra Master Mix B (2X) to the reactions from step 7. Mix the reaction by pipetting.
10. Incubate the reactions 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 × 10^9 CFU/µg pUC19.

Because some of the 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 5–15 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 pipet 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 10 minutes.
2. Chill clean electroporation cuvettes on ice.
3. Pipet 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 1 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 10–15 minutes.
2. After the incubation, plate 1/2–1/50 of the transformation reaction (20–500 µL 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)	For example, we normally plate*...
1–2	1/50	2 µL and 20 µL
3–5	1/10	10 µL and 100 µL
> 5	1/2 †	100 µL and 500 µL

*Based on a 1000 µL transformation mixture

† Spin down the reaction before plating

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 ng	0.061
	40 ng	0.121
1 kb	10 ng	0.015
	25 ng	0.038
5 kb	10 ng	0.003
	25 ng	0.008
8 kb	25 ng	0.005
	50 ng	0.009
10 kb	25 ng	0.004
	50 ng	0.008
15 kb	50 ng	0.005
	100 ng	0.010
20 kb	50 ng	0.004
	100 ng	0.008
30 kb	50 ng	0.003
	100 ng	0.005

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

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Gibson Assembly®: US Patent Nos. 7,776,532, 8,435,736, and 8,968,999.

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

SGI-DNA, Inc.

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