INSTRUCTIONS

ClonEasy™

One Step Cloning Kit

Package Contents: 1 vial

ClonEasy™ One Step Cloning Kit					
Catalog No.	Package	Price	Quantity/Unit	Form	Sipping and Storage Guidelines
R-GC01	20 T	320	1 kit.	Liquid.	Shipped at 4°C. Recommended storage at -20°C, effective for 2 years.
R-GC02	50 T	580			
R-GC03	100 T	896			

Storage

ClonEasy is shipped stored at -20 °C. Avoid repeated freezing and thawing. The expiry date is specified on the product label.

DESCRIPTION

Gibson Assembly is a molecular cloning method, which allows for the joining of multiple DNA fragments in a single, isothermal reaction, regardless of fragment length or end compatibility. The method can simultaneously combine up to 15 DNA fragments based on sequence identity, requiring that the DNA fragments contain 20~40 base pairs and overlap with adjacent DNA fragments. The entire reaction completes at 50 °C, for most parts of enzyme mix can work at this temperature. After one hour or less, the reaction product is ready to transform into competent cells immediately. For these reasons, this kit has been rapidly adopted by the synthetic biology community. The Gibson Assembly Master Mix consists of three different enzymatic activities that perform in a single buffer:

• An exonuclease, chewing back the 5’ ends of the fragment, thus generating long overhangs which allows the single stranded regions with homology to anneal

• A polymerase, filling in the gaps

• A DNA ligase, sealing the nicks of the annealed and filled-in gaps

The end-result is a double-stranded fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation.

ADVANTAGES

1. Fewer steps. One tube reaction.

2. No scar between joined fragments.

3. No PCR clean-up step required.

4. Combine many DNA fragments at once.

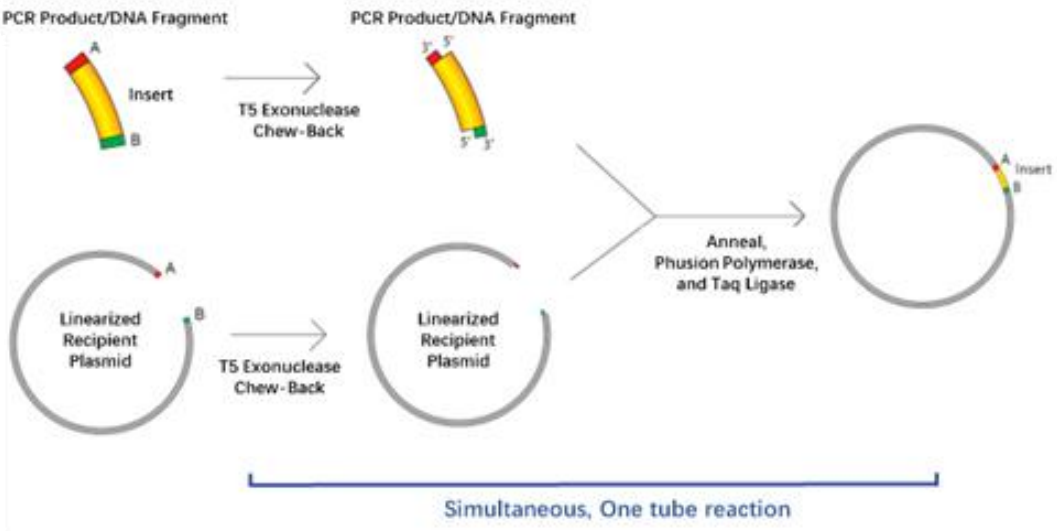
5. Generate wild type and mutant constructs at the same time.

6. High transformation efficiencies for inserts up to 20 kb.

7. Easily adapted for multiple DNA manipulations, including site-directed mutagenesis.

PROCEDURE

Overview of Gibson Assembly Cloning Method:



Step 1. Linearize vector

Linearize vector by restriction digestion

1. Choose the proper site for insertion of your target fragment.

2. Set up the mixture for enzyme digestions.

3. Gel-purify the linearized vector.

Note:

Double enzyme digestion of vector DNA with two restriction endonucleases is the best approach to reduce the uncut vector background. If significant amounts of undesired product exist, gel purify DNA segments. If you want to save time, PCR purification or even the raw PCR mix can work fine in an assembly.

Generate linearized vector by PCR

1. Choose the proper site for insertion of your target fragment and design primers.
2. Perform PCR to amply the vector.

Note:

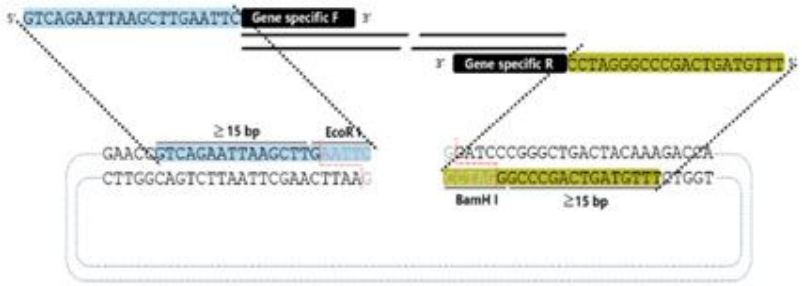
It's better to gel-purify the PCR products to remove the circle vector templates.

Step 2. PCR amplification of insert fragments

1. In accordance with the sequence of the selected sites, design primers.
2. Amply the insert fragments by PCR.
3. Run PCR product on a gel to check for size and yield.

Note:

To achieve efficient assembly of PCR fragments into a vector, it's better to use a 15–25 nt overlap with a Tm equal to or greater than 48°C (assuming A-T pair = 2°C and G-C pair = 4°C) (Figure 2).



Design primers to split an antibiotic resistance gene to effectively create an extra part. Any colonies should have at least the correctly assembled antibiotic gene. This is a nice trick that can cut down on background and enrich correctly assembled plasmids.

Step 3. Combine segments through Gibson Assembly Reaction

1. Set up the following reaction on ice:

Recommended Amount of Fragments Used for Assembly			
	2–3 Fragment Assembly	4–6 Fragment Assembly	Positive Control
Total Amount of Fragments	0.02–0.5 pmols[a], X µl	0.2–1 pmols, X µl	10 µl
Gibson Assembly Master Mix (2×)	10 µl	10 µl	10 µl
Deionized H_2O	10-X µl	10-X µl	0

Total Volume	20 µl[b]	20 µl[b]	20 µl

a: Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.

b: If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled. Following incubation, store samples on ice or at –20°C for subsequent transformation.

Note:

Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases.

Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend the following formula: pmols = (weight in ng) × 1,000 / (base pairs × 650 daltons)

50 ng of 5000 bp dsDNA is about 0.015 pmols.

50 ng of 500 bp dsDNA is about 0.15 pmols.

The mass of fragments can be measured using the NanoDrop instrument, absorbance at 260 nm or estimated from agarose gel electrophoresis followed by ethidium bromide staining.

Step 4. Transform the assembly DNA into bacteria and screen for the correct plasmid product by Restriction Digest.

Gibson Assembly Transformation Protocol

1. Thaw chemically competent cells on ice.
2. Add the chilled assembly product to the competent cells. Mix by gently pipetting up and down or by flicking the tube 4-5 seconds not vortex.
3. Place the mixture on ice for 30 minutes. No mix.
4. Heat shock at 42℃ for 30 seconds. No mix.
5. Transfer tubes to ice for 2 minutes.
6. Add room-temperature SOC media to the tube.
7. Incubate the tube at 37℃ for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Spread 100 µl of the cells onto the selection plates.
9. Incubate overnight at 37℃.

Electrocompetent Cells Transformation Protocol

1. Thaw electrocompetent cells on ice.
2. Transfer 50 µl of electrocompetent cells to a pre-chilled electroporation.
3. Cuvette with 1 mM gap.
4. Dilute assembled products 3-fold with H_2O prior electroporation.
5. Mix gently by pipetting up.
6. Once DNA is added to the cells, carry out electroporation immediately. It is not necessary to incubate DNA with cells.
7. Add 950 µl of room temperature SOC media to the cuvette immediately after electroporation.
8. Place the tube at 37℃ for 60 minutes. Shake vigorously (250 rpm) or rotate.
9. Spread 100 µl of the cells onto the selection plates.
10. Incubate overnight at 37℃.

Step 5. Sequence the important regions of the final plasmid, especially the seams between the assembled parts.

1. Pick several single clones and incubate at 37℃.
2. Sequence the important regions of the final plasmid, especially the seams between the assembled parts.

Contact Information

Genemedi Biotech. Inc.

For more information about reagents, please visit: https://www.genemedi.net/i/reagent
For more information about Genemedi products and to download manuals in PDF format, please visit our web site: www.genemedi.net
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