Golden Gate Assembly Kit (BpiI)

Cat. No.: G1522813 | Pack size: 10 reactions; 50 reactions | Storage: -20°C

Product Information

Attribute	Value
Pack Size(s)	10 reactions; 50 reactions
Specifications & Purity	BioReagent
Grade	BioReagent
Application	Molecular Cloning
Stability And Storage	Store at -20°C long term (24 months).
Storage Conditions	Store at -20°C
Shipped In	Ice chest + Ice pads

Product Description

The Golden Gate Assembly Kit is a series of products containing multiple Type IIS restriction endonucleases, among which BpiI is adopted in this kit. All products in this series work based on the Golden Gate Assembly principle: specific sticky ends are generated via unique cleavage characteristics of Type IIS restriction enzymes, and then ligated by T4 DNA Ligase. It is especially suitable for assembling difficult-to-clone sequences, including repetitive sequences, high-GC sequences, TAL (transcription activator-like) effector genes, ultra-short sequences (<100 bp), etc.

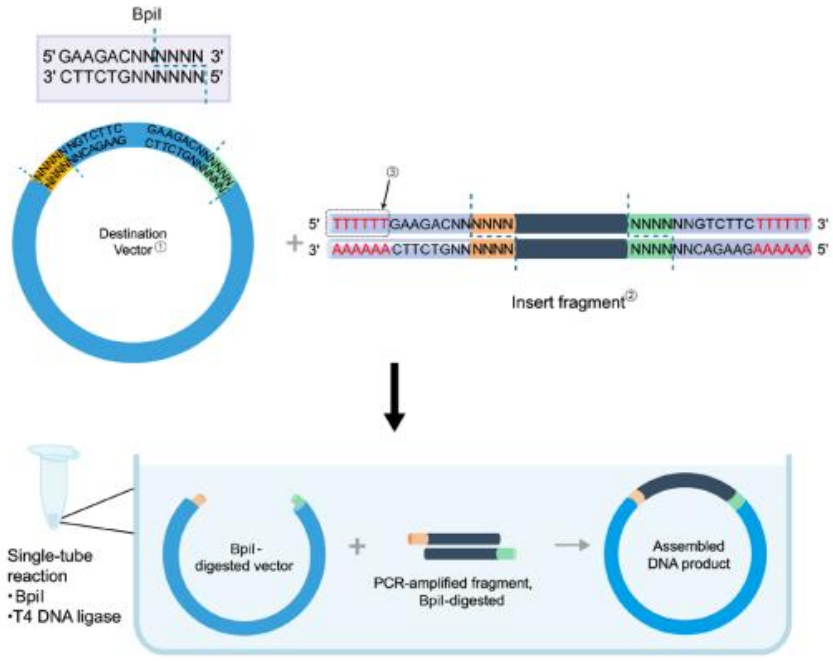
Different from traditional restriction enzymes, Type IIS restriction endonucleases recognize non-palindromic sequences and cleave DNA at a certain distance downstream of recognition sites. They produce arbitrary sticky ends outside recognition sequences, enabling customized cleavage sequence design.

The cloning procedure is as follows: Type IIS restriction enzyme recognition sites are designed outside target gene cleavage sites. These recognition sites are removed after digestion and will not remain in inserted fragments, so inserted fragments cannot be recleaved after ligation with vectors. Vectors carry sticky ends complementary to target gene cleavage sites, allowing ligation without introducing extra sequences to achieve seamless cloning.

Based on the above principle, the Golden Gate Assembly Kit (BpiI) integrates all enzymes required for digestion and ligation in a premixed format for convenient operation. It supports ligation of up to 16 fragments in a single reaction, fully meeting diverse experimental requirements.

Experimental Principle

Taking single DNA fragment insertion as an example:



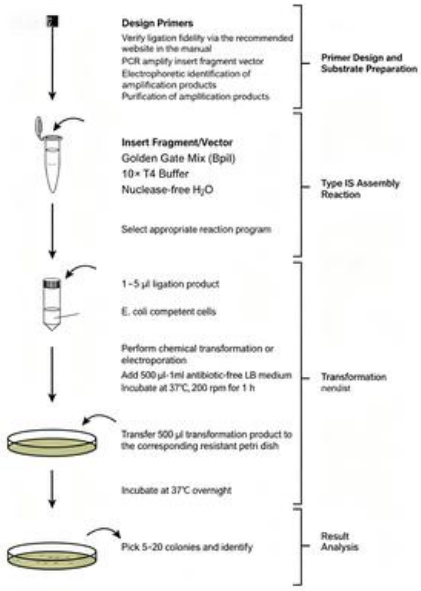
Note 1. The vector is obtained by enzymatic digestion, and vectors with BpiI sites are required. Alternatively, vectors can be prepared by PCR; primer design is described below.

Note 2. Insert fragments are amplified by PCR, and restriction sites are added to fragment ends via primers. High-fidelity DNA polymerase is recommended to ensure accurate amplicons.

Note 3. Red "TTTTTT" indicates protective bases, adjustable for different enzymes; 6 bases are recommended.

Note 4. The figure only shows single-fragment ligation. Multi-fragment assembly follows the same principle, and more fragments can be connected by altering sticky end sequences.

Experimental Procedure



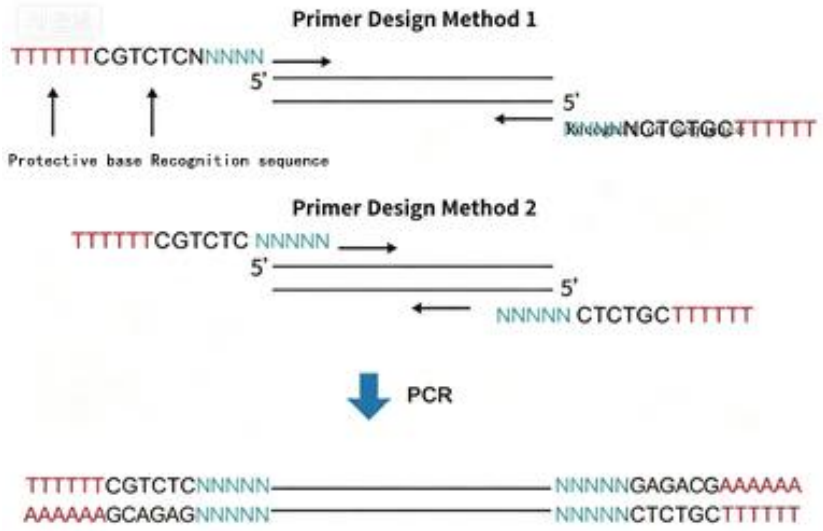
Precautions

1. Cloning efficiency is greatly affected by type, quantity and length of inserted DNA. Although conditions can be optimized, cytotoxicity and intracellular recombination of partial exogenous DNA cannot be completely avoided.
2. Ligation fragments are usually amplified by PCR, and can also be obtained by pre-cloning or gene synthesis. Regardless of sources, correct orientation of restriction sites between adjacent fragments must be guaranteed for accurate sticky-end ligation.
3. The kit easily assembles repetitive or ultra-short difficult-to-clone sequences, but cloning efficiency declines gradually with increasing fragment quantity. Efficiency drops significantly when more than 10 fragments are cloned. It is recommended to pick and screen around 20 colonies to obtain target ligation products.
4. Pre-cloned DNA fragments show higher cloning efficiency than PCR-amplified fragments, especially for repetitive sequences with >80% homology.
5. A thermal cycler is recommended for thermal cycling reactions with proper programs to ensure smooth reactions.

Primer Design Guide

BpiI recognition sequences are introduced via PCR and added to the 5' end of primers. Protective bases are required at the end of recognition sequences to ensure stable binding and cleavage of restriction enzymes. The number and type of protective bases are flexible, and 6 bp protective bases

are recommended for most routine digestions. Since cleavage sites locate downstream of recognition sequences with arbitrary sequences, two common primer design strategies are illustrated below:



Primer Design Method 1: Protective bases and restriction sites (TTTTTTGAAGACNNNNN) are introduced by primers, while the remaining sequences (>15 bp) originate from inserted fragments. The two parts form complete primers.

Primer Design Method 2: Protective bases and recognition sequences (TTTTTTGAAGACN) are introduced by primers, and "NNNN" comes from inserted fragment sequences.

Note 1: The core difference between the two methods is whether "NNNN" belongs to inserted fragments. For seamless cloning, if both fragments and vectors are PCR-amplified, "NNNN" must derive from either fragments or vectors, meaning fragments and vectors adopt different primer designs.

Note 2: Sticky-end sequences "NNNN" greatly affect ligation specificity. Although 256 combinations exist theoretically, palindromic sequences should be excluded. Sticky ends can be designed via online tools: https://goldengate.neb.com/

Additional Primer Design Requirements

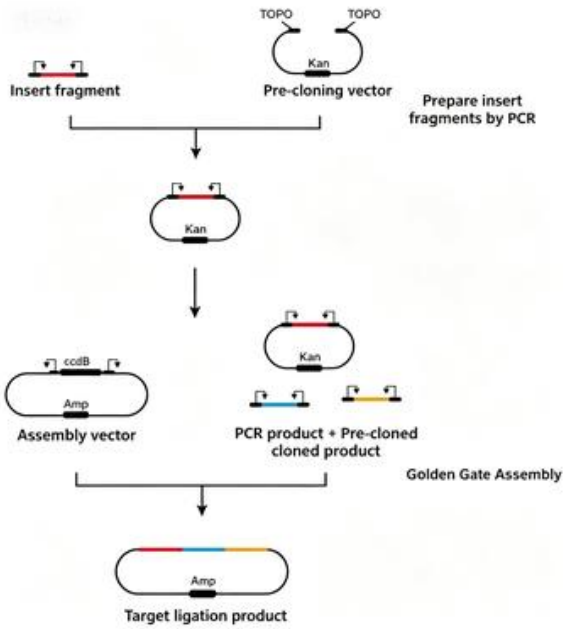
1. Amplicons are generally controlled below 5 kb to guarantee accurate amplification.
2. The Tm value of primer regions matching templates should be 58–60 ℃ for optimal PCR performance.
3. No complementary sequences are allowed inside or between primers to avoid hairpin structures.
4. Primer quality critically affects subsequent ligation. A single base mutation in sticky ends may cause ligation failure. Reliable gene synthesis suppliers are highly recommended.

PCR Precautions

1. Optimize PCR conditions for each fragment to obtain single specific amplicons.
2. Gel purification of target DNA is mandatory if multiple PCR bands appear, otherwise assembly fails or cloning efficiency decreases sharply. Minimize UV exposure during gel cutting, or use blue-light gel cutting to reduce DNA damage.
3. Long fragments (>5 kb) are easily damaged during gel extraction. Multi-fragment ligation is preferred over single large-fragment cloning.
4. Elute DNA with ddH_2O or 10 mM Tris buffer (pH 8.0). TE buffer is prohibited to avoid inhibiting downstream ligation.
5. Unpurified PCR products can be used directly for ligation, but only for single-fragment cloning. Purification is required for multi-fragment assembly.

Pre-Cloning Guide

Pre-cloning refers to inserting PCR-amplified DNA fragments into intermediate donor vectors (~3 kb), then assembling them with other fragments in the Golden Gate reaction system. Pre-cloning is commonly performed via TOPO cloning: PCR products are ligated into TOPO vectors by topoisomerase. Vectors contain selection markers for positive clone screening. Other cloning methods are also acceptable according to experimental habits. The following shows the TOPO pre-cloning workflow:



Note 1. Black arrows in fragments and vectors indicate restriction cleavage directions from recognition sites to cutting sites.

Note 2. ccdB is an E. coli lethal gene. It kills bacteria without inserted fragments, while inserts disrupt ccdB expression and allow positive colonies to grow.

Pre-cloning is recommended for repetitive/homologous sequences with >80% identity. Stepwise pre-cloning into target vectors is advised when more than 5 fragments are inserted.

Protocol

1. Prepare Reaction Systems on Ice

Components	Ligation Reaction	Negative Control[a]
Vector	0.05 pmol[b]	0.05 pmol
Insert fragment	0.1 pmol[c]	/
10× T4 DNA Ligase Buffer	2 µL	2 µL
Golden Gate Mix (BpiI)	1 µL	1 µL
Nuclease-free H_2O	To 20 µL	To 20 µL

a. Negative controls are optional for Golden Gate Assembly. A reaction without inserts serves as negative control if required.

b. 60 ng vector for 2000 bp; dosage can be calculated proportionally for other lengths.

c. Molar ratio: Insert : Vector = 2:1; ratio between inserts=1:1. Swap dosages if fragments are longer than vectors.

Note: More fragments lead to lower ligation efficiency and positive rate. Overlong fragments or vectors also reduce efficiency.

Mix thoroughly by vortexing after system preparation, then run reactions in a thermal cycler with programs matched to fragment quantity.

2. Recommended Thermal Programs

Number of inserted fragments	Reaction protocol
1	42°C, 5 min → 65°C, 5 min
2~4	42°C, 1 h → 65°C, 5 min
5~10	(42°C, 1 min → 22°C, 1 min) × 30~60 → 65°C, 5 min

Note: Assembly of >10 fragments can be completed in one reaction, but colony count and positive rate decline significantly. Stepwise assembly is suggested for high success rate.

Optional Isothermal Programs

Number of inserted fragments	Reaction protocol
1	30℃, 5 min → 65℃, 5 min
2~10	30℃, 1~2 h → 65℃, 5 min

Note: Isothermal reactions perform equally well for single-fragment cloning. Efficiency decreases slightly with more fragments, but remains above 70% for 2–5 fragments. Thermal cycling programs are preferred when available.

3. Completed reaction mixtures can be used for transformation directly, or stored at -20 ℃.

4. Recombinant Product Transformation

Add 5–10 µL reaction mixture into 100 µL competent cells, mix gently, and incubate on ice for 30 min. Heat shock at 42 ℃ for 45–60 sec, then ice-bath for 5 min. Add 500 µL SOC or LB medium, incubate at 37 ℃ with shaking (200 rpm) for 40–60 min. Spread bacterial suspension evenly onto antibiotic plates, and incubate inverted at 37 ℃ overnight.

Note 1. Positive rates vary among competent cells. Cells with transformation efficiency >10^8 CFU/µg are recommended.

Note 2. Colony quantity depends on concentration and purity of PCR products and linearized vectors.

Note 3. A large number of white single colonies grow on positive control plates, while few colonies appear on negative control plates.

5. Positive Clone Identification

Enzymatic digestion and PCR are two routine screening methods.

Enzymatic Digestion: Pick 5–20 colonies and culture overnight in 1 mL resistant LB medium. Extract plasmids, digest with suitable restriction enzymes, and analyze results via agarose electrophoresis to screen target plasmids. 5–10 colonies are sufficient for conventional ligation. 20 colonies are required for repetitive/homologous sequences or >10-fragment assembly.

PCR Detection:

1. Design specific detection primers. Amplicons are recommended between 500 bp–2 kb. Forward and reverse primers are located on vectors and inserts for single-fragment cloning, and both on vectors for multi-fragment cloning.

2. Pick 5–20 colonies and culture overnight. Transfer 1 µL bacterial culture into 30 µL PCR system.
3. Extend initial denaturation to 95℃ for 10 min (standard: 95℃ 3 min), keep other steps unchanged. Detect amplicons by agarose electrophoresis.
4. Extract plasmids from positive bacterial cultures. Sequencing verification is highly recommended to avoid PCR errors.

Troubleshooting

Problem Description	Cause	Solution
Low transformation efficiency	Low competence of competent cells	Use freshly prepared or properly stored competent cells.
Low transformation efficiency	Improper ratio of DNA fragments	Prepare the reaction system according to the optimal dosage and ratio recommended in the instruction manual. Concentration determination of vectors and inserts: for purified linearized vectors and inserts with single and sharp electrophoretic bands, spectrophotometric instruments such as ultra-micro nucleic acid protein analyzers can be used. Concentration data is reliable only when A260/A280=1.8~2.0. Agarose electrophoresis can be adopted to measure concentrations of unpurified samples.
Low transformation efficiency	Insufficient purity of DNA fragments	Purify vectors and inserts. Metal ion chelators such as EDTA inhibit seamless cloning reactions. Dissolve purified products in ddH_2O, and avoid Tris-EDTA buffer.
Low transformation efficiency	Excessive reaction products	The volume of seamless cloning reaction mixture shall not exceed 10% of competent cell volume in transformation system.
Low transformation efficiency	Overlong or excessive fragments	Golden Gate Assembly works well for ligation of less than 10 fragments. Ligation efficiency drops sharply with overlong fragments, and declines greatly when fragment number exceeds 10. This kit is recommended for ligation within 10 kb.

Problem Description	Cause	Solution
Most clones contain no inserts	Incomplete vector linearization	Increase dosage of rapid restriction enzymes, prolong incubation time and purify digested products by gel extraction when linearizing vectors via enzymatic digestion.
Most clones contain no inserts	Contamination of antibiotic-resistant plasmids	Use pre-linearized plasmids as templates for insert PCR amplification. Treat PCR products with methylation-sensitive enzymes such as DpnI, or purify products via gel extraction.
Most clones contain no inserts	Insufficient antibiotic resistance on plates	Adopt appropriate antibiotics and freshly prepared antibiotic agar plates.
Most clones contain no inserts	Faulty adapter design	Ligation efficiency varies among sticky ends. Improper adapter design causes non-specific ligation. Use professional software to predict ligation fidelity in advance and reduce non-specific amplification.
Most clones carry incorrect inserts	Non-specific PCR amplicons	Optimize PCR system to improve amplification specificity, or purify overlapping-sequence PCR primers by gel extraction.
Most clones carry incorrect inserts	Faulty adapter design	Adapter types greatly affect ligation accuracy for multi-fragment assembly and easily induce non-specific ligation. Predict ligation fidelity in advance with software to avoid non-specific amplification.
Most clones carry incorrect inserts	Amplification errors of fragments or vectors	This ligation relies on 4-base sticky ends and requires high amplification fidelity. Base mutations cause faulty or failed ligation. High-fidelity DNA polymerase is recommended for amplifying ligation substrates.

FAQs

1. What is the maximum fragment number per single assembly reaction? The maximum tested quantity is 15 fragments, with extremely low colony count and positive rate. No more than 5

fragments are recommended for reliable results. Split assembly steps if multi-fragment ligation fails.

2. What is the length range of inserted fragments? Valid inserts range from 20 bp to 10 kb. Longer fragments have lower efficiency. Total vector-plus-insert length is recommended below 13 kb.
3. Can reaction duration be adjusted? Yes. 5 min at 42℃ suffices for single-fragment ligation. 30–60 cycles are used for multi-fragment assembly. Do not exceed 60 cycles to avoid non-specific ligation.
4. Why incubate at 65℃ for 5 min? T4 DNA Ligase is inactivated at 65℃, while restriction enzymes remain active to digest empty plasmids and reduce background clones.
5. Can unpurified PCR products be used directly for assembly? Yes, but only for single-fragment cloning with ≤1 µL input. Residual DNA polymerase may fill sticky ends and cause non-specific ligation. Primer dimers and non-specific amplicons also interfere with target assembly.
6. Can Golden Gate products act as PCR templates? Yes. Circular assembled plasmids are suitable for PCR, rolling circle amplification and other DNA amplification reactions.
7. How to handle internal BpiI sites in inserted fragments? Perform synonymous mutation on endogenous BpiI sites, or switch to other Type IIS enzymes including BbsI, BsaI, BspQI. This kit only matches BpiI and cannot be used with other enzymes.
8. How to select suitable Golden Gate Assembly Kits? Choose kits according to endogenous Type IIS sites in target sequences. Select enzymes with zero or minimal recognition sites to minimize mutation work.
9. Performance comparison with conventional enzyme-ligase cloning? This kit is specially optimized for Golden Gate Assembly, with higher efficiency and simpler operation for ≤10-fragment ligation.
10. What factors affect Golden Gate efficiency? Fragment quantity, length and substrate purity are key influencing factors. Fragments ≤10 kb and purified substrates are highly recommended.
11. Advantages and applications of pre-cloning? Pre-cloned fragments stored in circular plasmids are more stable than PCR products and suitable for long-term storage. Stepwise pre-cloning improves success rate for long or numerous fragments, despite longer experimental cycles.

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