TA-Enhancer Cloning Kit

Manual (5th edition)

Code No. 316-08271 (25 reactions)

Ⅰ Product Description

Many of the PCR products synthesized using PCR enzymes with terminal transferase (TdT) activity, such as Taq DNA polymerase, have a single base addition of deoxyadenosine (dA) at the 3' end, allowing complementary pairing with thymidine (dT) projecting at the 3' end of the T vector for convenient cloning of the PCR product. This method is known as TA cloning.

[TA-Enhancer Cloning Kit] is a TA cloning kit that combines T vectors and ligation reagents. The ligation reagents are highly efficient for TA cloning by the Nippon Gene's proprietary buffer composition and the PprA protein contained in the 10x Enhancer Solution.

■ Features

- TA cloning, which has been considered to be inefficient, can be performed efficiently.
- Blue and white judgment using LacZ is possible.
- When the PCR-product is inserted into the pANT Vector, the restriction sites of Hind lll and Bgl ll are newly formed.

Ⅱ Product description

Contents	(25 reactions)	Storage
pANT Vector (25 ng/μl)	45 μl x 1	-20℃
5 x Ligation Mix	100 μl x 1	-20℃
10 x Enhancer Solution	50 μl x 1	-20℃
Control Insert DNA (10 ng/μl)	10 μl x 1	-20℃

- Packaging units are the number of times when 20 μl of the reaction system is used.
- 5 x Ligation Mix and 10 x Enhancer Solution are available for purchase as TA-Blunt Ligation Kit (Code No. 311-06543)

Ⅲ Preservation

Store at -20℃

- 5 x Ligation Mix and 10 x Enhancer Solution will not be frozen at -20°C, so there is no concern that freezing and thawing will reduce the stability. There is no need for cumbersome thawing.
- Operate on ice when using.

Ⅳ Protocol

<Operation Procedure>

1. Preparation of insert DNA

① Amplify the desired sequence using a TdT-active PCR-enzyme such as Hot-Start Gene Taq NT. Check the type of PCR enzyme because the 3' protruding end is smoothed when the PCR enzyme with proofreading activity is used.

② A portion of the reaction solution is subjected to agarose gel electrophoresis to identify the amplified product.

③ Purify the PCR-products using ISOSPIN PCR Product, etc. If a non-specific band is found, cut the band of the desired size out of the gel and purify it using ISOSPIN Agarose Gel, etc.

④ Dilute part of the PCR product with ddH_2O or TE (pH 8.0) to a suitable concentration※1)

2. Ligation

① Prepare the ligation solution in a new tube with the following contents.※2)

Reaction solution	PCR products	Positive Control
pANT Vector(25 ng/μl)	1.8 μl	1.8 μl
PCR products	X μl	-
Control Insert DNA(10 ng/μl)	-	2 μl
5 x Ligation Mix	4 μl	4 μl
10 x Enhancer Solution	2 μl	2 μl
ddH_2O	Up to 20 μl	Up to 20 μl

◆ The following is the amount of insert that was best obtained under certain conditions as a result of ligation and transformation of insert DNA of various lengths in the Nippon Gene. Refer to this manual when preparing the ligation solution. ※3)

Insert size	200 bp	500 bp	1 kbp	3 kbp
Insert Amount (ng)	20.0~32.0	24.0~50.0	8.0~100.0	24.0~50.0

For 20μl Reaction System (pANT Vector 45 ng)

② React the ligation reaction at 16°C for 30 minutes.

3. Transformation (using ECOS™ Competent E.coli JM 109)

The ligation reaction solution can be used for transformation. The volume of reaction solution used for transformation should not exceed 10% of the competent cell. In addition, the Nippon Gene's ECOS™ Competent E.coli allows the use of a 6-minute fast protocol (the 6-minute fast protocol is only valid for ampicillin). When using ECOS™ Competent E.coli, the amount of DNA solution to be added should be 5% or less of the capacity of the competent cell.

<ECOS™ 6 Minute Protocol>

① Melt the competent cell on ice.
② Add the ligation reaction solution to the ECOS™ Competent E.coli JM 109. ※4), ※5)
③ Vortex for 1 second.
④ Incubate on ice for 5 minutes.
⑤ Incubate at 42°C for 45 seconds.
⑥ Vortex for 1 second.
⑦ Apply evenly reacted ECOS™ Competent E.coli to the LB-plate and incubate for 16 hours at 37°C.

<Preparation of plate for blue-white determination>

Prepare the LB plate (approx. 20 ml) with the following contents.

- 20 µl of ampicillin 50 mg/ml (final concentration 50 µg/ml)
- 40 µl of X-gal 20 mg/ml (40 µg/ml final)
- 1 M IPTG 4 µl (final concentration 0.2 mM)

4. Insert check by Colony PCR

The insert size in the E. coli plasmid can be estimated by colony PCR in order to identify recombinants that contain the desired DNA fragment. M13 Primer is available for pANT Vector.

The combination of 2x M13 Primer Mix and Gene RED PCR Mix Plus allows for rapid and easy colony-PCR.

① Adjust the reagent to the tube with the following composition and dispense 50 µl into the PCR tube.

Gene RED PCR Mix Plus (2x)	25 µl
2xM13 Primer Mix	25 µl
Total	50 µl

② A toothpick or tip gently pushes the colony and suspend into the reaction solution.
Be careful not to bring in large amounts of colony.

③ Perform PCR under the following conditions:

94°C	3 min	
94°C	20 sec	25 cycles
55°C	20 sec	
72°C	10 sec/kbp (10 sec for 1 kbp or less)	

④ After completion of PCR, apply each reaction solution to the agarose gel and perform electrophoresis.

◆ Control Insert DNA is 600 bp.
When Nippon Gene's 2x M13 Primer Mix is used, the amplified product is confirmed at 760 bp.

Ⅴ Notes

※1) Lysis of DNA in high salt buffers and EDTA-enriched buffers significantly reduces ligation efficiency.
Prepare the DNA solution with ddH_2O or TE (pH 8.0).

※2) Measure accurately the amount of 10x Enhancer Solution to be added.
If more or less than the specified amount is added, the ligation efficiency may be significantly reduced.

※3) The ligation efficiency may differ depending on the purity of DNA used in the ligation reaction.

※4) The volume of reaction solution used for transformation should be no more than 1/10 of the volume of competent cell.
When using *ECOS™ Competent E.coli,* the volume of the reaction solution used for transformation should be 5% or less of the volume of the competent cell. The use of large amounts of reactants may reduce transformation efficiency.

※5) If the volume of the reaction solution reaches or exceeds 1/10 of the volume of the Competent cell, perform the ligation reaction followed by treatment with phenol/chloroform/isoamyl alcohol (25:24:1) or chloroform/isoamyl alcohol (24:1). Collect the DNA by ethanol precipitation, and dissolve the DNA in ddH_2O or TE (pH 8.0) to make the content of the competent cell 1/10 or less.

Ⅵ pANT Vector

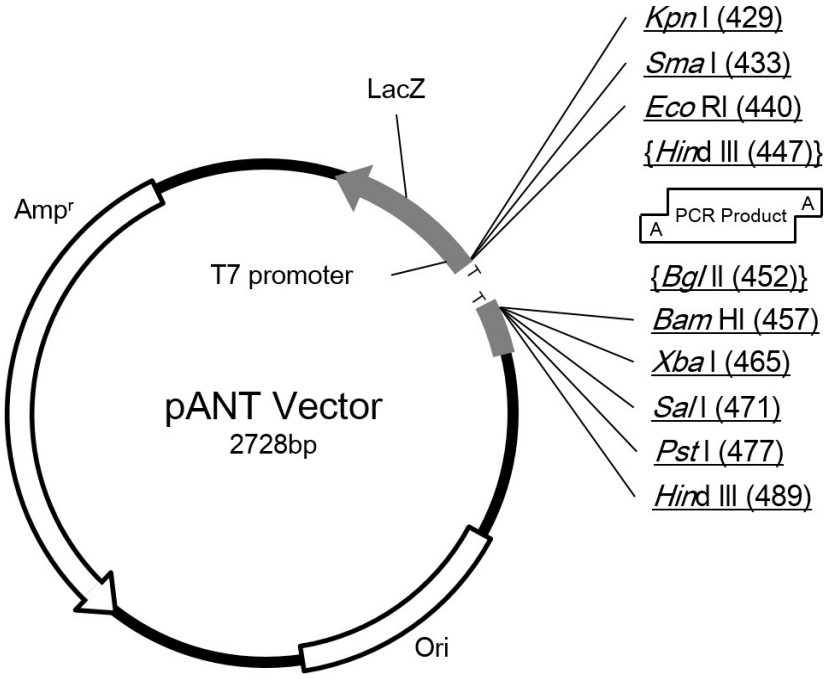

Fig. 1 pANT Vector map

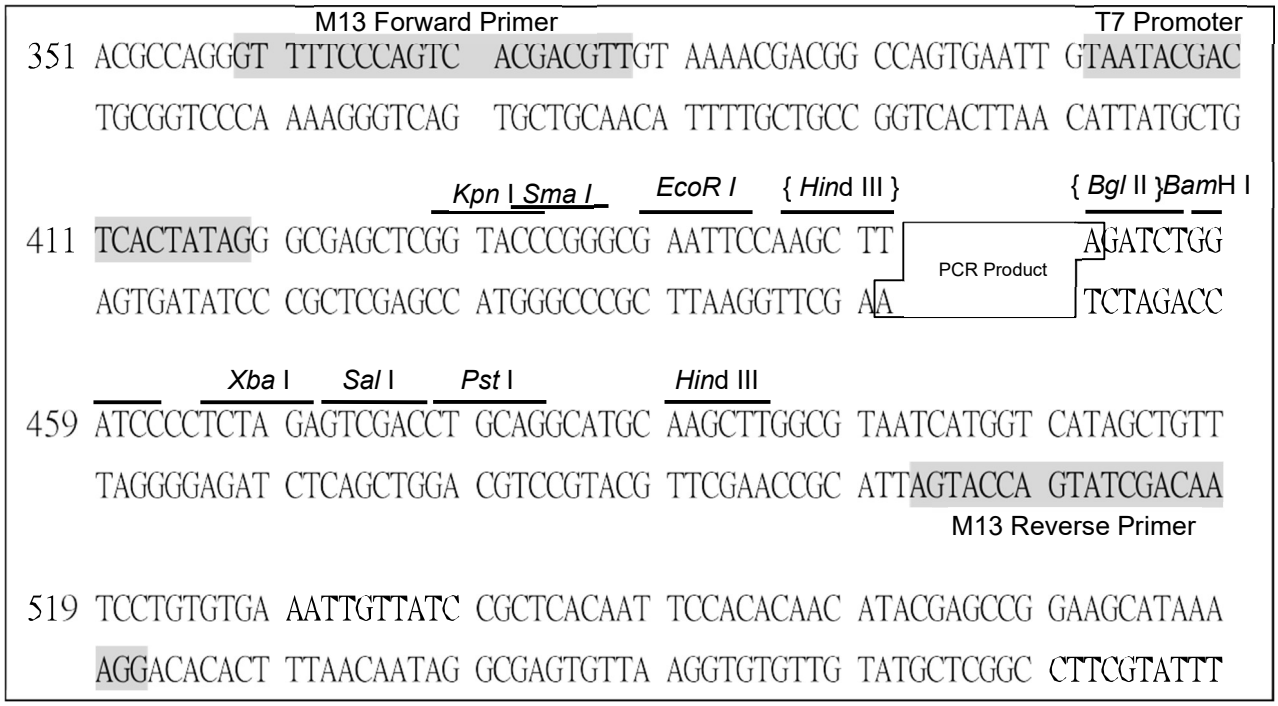

Fig. 2 pANT Vector multicloning site

Multiple cloning region	429-494
M13 forward primer	359-378
M13 reverse primer	502-521
T7 promoter	402-419

Ⅶ Q & A

Q: What should I do when I save the ligation product?
A: Store frozen at -20°C. After thawing, it can be used in transformation experiments.

Q: I have a lot of samples. Can I use it after making a premix with 10 x Enhancer Solution and 5 x Ligation Mix in advance?
A: You can create and use a premix. However, prepare the premix immediately before use, and use it immediately after preparation.

Q: Can ligation reactions be performed at 4°C overnight?
A: It can also be ligated at 4°C overnight (16 hours).

Q: Can the ligation product be precipitated with ethanol as it is?
A: Ethanol precipitation of ligation products can markedly reduce colony counts.
Purify phenol/chloroform and then precipitate ethanol.

Ⅷ Troubleshooting

Problem	Possible cause	Countermeasures
Have no colonies	Terminal mismatch	Be sure to add dA to the 3' end of insert using TdT-active PCR enzyme. (Refer to Manual IV-1.)
	Low transformation efficiency of competent cells	Use competent cells with a transformation efficiency of 1 x 10^7 cfu/μg or more.
	The ligation product was directly precipitated with ethanol.	After treatment of the ligation product with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), collect the DNA by ethanol precipitation and dissolve the DNA in ddH2O or TE (pH 8.0), and then transform.
Few white colonies, no white colonies	Terminal mismatch	Be sure to add dA to the 3' end of insert using TdT-active PCR enzyme. (Refer to Manual IV-1.)
	The dT at the 3' end of the vector is deleted.	· Avoid leaving the pANT Vector at room temperature or repeatedly freezing and thawing it. · Avoid using nucleases that degrade dT.
	Ligation time is short.	· Increase the ligation time. · Perform overnight reaction at 4°C.
	Improper insert volume	Refer to the amount of insert in the manual for ligation. (Refer to Manual IV-1.)
	Inappropriate amount of Enhancer Solution used	The amount of Enhancer Solution added should be 1/10 of the amount of the reaction system.
	Inappropriate amount of vector used	Please use 1.8 μl a pANT Vector in a 20 μl system.
	High concentration of chelating agent such as EDTA	Dissolve the DNA in water or TE (pH 8.0) without chelating agent and perform the ligation reaction.
	DNA solution with high salt concentration	Dissolve the DNA in salt-free water or TE (pH 8.0) and perform the ligation reaction.
	PCR products contain contaminants	Purify the PCR-products with ISOSPIN PCR Product, etc.
	lacZ gene expression by frameshift	Check out the blue colony or the pale blue colony. Inserts may be included.
	Pyrimidine dimers	When cutting from an agarose gel, shorten the UV irradiation time.
Almost white colonies, all white colonies	Inappropriate concentration of ampicillin, X-gal,IPTG	Check the concentration of ampicillin/ X-gal/IPTG and prepare again. (Refer to Manual IV-3.)
Low transformation efficiency	With a large amount of reaction solution	The reaction volume used for transformation should be 1/10 of the competent cell. *When using ECOS™ Competent E. coli, add* Reduce the volume of reaction to 5% or less of the competent cell.
	Low transformation efficiency of competent cells	Use competent cells with a transformation efficiency of 1 x 10^7 cfu/μg or more.

The information, product specifications, and prices are subject to change without notice.

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