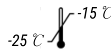
pLUG-Prime® TA-Cloning Vector Kit II

For fast ligation using TA-cloning vector

RUO Research Use Only REF 11063 -25 ℃ -15 ℃



DESCRIPTION

- pLUG-Prime TA-Cloning Vector Kit II offers a quick, reliable and efficient method for cloning a variety of DNA sequence
- Possible to separate insert DNA from vector using *Eco* RI restriction enzyme
- More accurate results / Accept a wide range of inserts with different sizes
- Two types of ligation buffers provided for your convenience

INTRODUCTION

TA-cloning technology exploits the terminal transferase activity of some DNA polymerases such as *Taq* DNA polymerase and other non-proofreading DNA polymerase. These enzymes preferentially add a 3'-end A-overhang to PCR products. This allows the direct insertion of such PCR products into the prelinearized cloning vector, which has a T-overhang on each 3'-end. This eliminates the need for restriction enzyme digestion of the vector or insert, primers with built-in restriction sites, or specially designed adapters, resulting in a much more efficient and robust cloning procedure. This technique is especially useful when compatible restriction sites are not available for the subcloning of DNA fragments. pLUG-Prime® TA-Cloning Vector Kit II contain several engineered restriction-enzyme recognition sites around the TA-cloning site allowing easy restriction analysis of recombinant plasmids or re-cloning to another vector. Especially, the restriction-enzyme recognition sites of pLUG-Prime® TA-Cloning Vector Kit II around the TA-cloning site are more general and simple, which is useful in downstream application such as re-cloning to another vector.

❖ **Features of the pLUG-Prime® TA-Cloning Vector Kit II**

- **LacZ alpha sequence**

The fragment of lacZ alpha sequence in the pLUG-Prime® TA-Cloning Vector Kit II is able to complement beta-galactosidase activity. The lacZ alpha sequence reduces the time to screen for positive clones.

- **Multiple cloning region**

The multiple cloning region is located around the TA-cloning site in the pLUG-Prime® TA-Cloning Vector Kit II. The restriction-enzyme recognition sites of pLUG-Prime® TA-Cloning Vector Kit II around the TA-cloning site are located in mirror-repeat pattern.

KIT CONTENTS and STORAGE CONDITION

Components	Concentration	Volumes
TA-Cloning Vector II (20 reactions)	25 ng/μl	40 μl
Control insert DNA	10 ng/μl	10 μl
T4 DNA ligase	2 U/μl	20 μl
10X Ligation Buffer A	-	50 μl
10X Ligation Buffer B	-	50 μl
Forward Primer (M13-F)	10 μM	50 μl
Reverse Primer (M13-R)	10 μM	50 μl
Storage Conditions : -20℃		

It is recommended for the products to be stored at -20℃. Always avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter stability of product.

CHARACTERISTICS

- High cloning efficiency
- High percentage of true white colony
- Credible blue/white colony selection
- Rapid procedure (rapid ligation)
- Allowing easy re-cloning to another vector
- Allowing convenient sequencing (M13 F/R priming sites)

Cloning into the pLUG-Prime® TA-Cloning Vector Kit II

•Optimizing insert to vector ratio

For the ligation reaction, the optimal molar ratio of insert (i.e., PCR product) to vector has to be optimized. We recommend using the 5-10 fold molar excess of PCR product over TA-cloning vector. In some cases, a lower ratio of PCR product to vector may be sufficient for efficient ligation. The pLUG-Prime® TA-Cloning Vector Kit II is about 2.73 Kb and the kit suggests adding 50ng (2 μl) of the vector for the ligation reaction.

• Calculated for 50ng vector using the following equation :

$$\text{ng PCR product required} = \frac{\text{50ng} \times \text{PCR product size (bp)}}{\text{Vector size (bp)}} \times \text{molar ratio}$$

Table 1. Guide for the amount of PCR product to use in the ligation reaction

PCR product size (bp)	5-times molar excess (ng)	10-times molar excess (ng)
500	45.8	91.6
1,000	91.6	183.3
1,500	137.5	274.9
4,500	412.4	824.8

Protocol for ligation using the pLUG-Prime® TA-Cloning Vector Kit II

1. **Centrifuge pLUG-Prime TA-Cloning Vector Kit II and /or PCR DNA tubes to collect contents at the bottom of the tubes.**
2. **Vortex the ligation buffer vigorously before use.**
3. **Set up the following items as described below :**

Components	Standard control	Positive control
10X Ligation buffer A	1 μl	1 μl
10X Ligation buffer B	1 μl	1 μl
TA-Cloning Vector II	2 μl	2 μl
PCR product	X μl	-
T4 DNA ligase	1 μl	1 μl
Control DNA	-	3 μl
Deionized Water	Up to 10 μl	

* To obtain higher white colonies having insert, we recommend to use the gel-purified PCR product.

4. Mix the reactions by pipetting.

5. Incubate the reactions for 5 to 15min at 22℃. Alternatively, if the maximum of transformants is required, incubate the reactions overnight at 4℃.

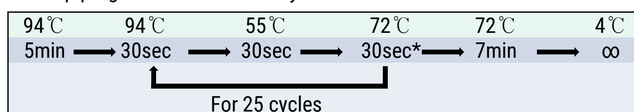
6. Implement transformation with appropriate competent cells.

Note :

1. To facilitate the A-addition of PCR product, add 10 minutes of extension at 72℃ to the end of the PCR cycling.
2. The use of fresh PCR product is strongly recommended. Purification of PCR products prior to ligation will generally result in higher transformation efficiencies. There are several choices to clean up the PCR product. During gel-purification of PCR-products, avoid long exposure to UV-light. To reduce nicking of the DNA, crystal violet staining is an alternative for band visualization.
3. PCR products generated using proofreading DNA polymerases such as *Pfu* DNA polymerase can be used in TA-cloning procedures after the addition of a 3'-end A-overhang.
4. We recommend using the 5-10-fold molar excess of PCR product over TA-cloning vector. In some cases, a lower ratio of PCR product to vector may be sufficient for efficient ligation.
5. If you see precipitate in the ligation buffer, warm it briefly at 37℃ to dissolve the salts. It is important to mix the solutions completely before use to avoid localized concentrations of salts.
6. The pLUG-Prime® TA-Cloning Vector Kit II is compatible with a wide range of chemically competent cells which are available from iNtRON. In particular, DH5α competent cells are appropriate to blue/white colony selection and exhibit the high transformation efficiency. Use the 3-10μl aliquot of ligation mixture to transform competent cells.

PROTOCOL for Colony PCR

1. Pick an isolated colony with a sterile toothpick, and then, dilute the colony in 20μl of DW. Use the appropriate diluted colony as PCR template.
2. Set up program of the thermal cycle



* The setting time of 72℃(for example 30 sec) is different according to length of insert DNA.
(In general, DNA polymerase can synthesize 1Kb DNA in 1minute.)

3. Check the PCR products by using 1% agarose gel :

※ When you check the band in the gel, consider the length of insert DNA between M13 forward primer and M13 reverse primer in the vector.

MAP & MULTIPLE CLONING SITE

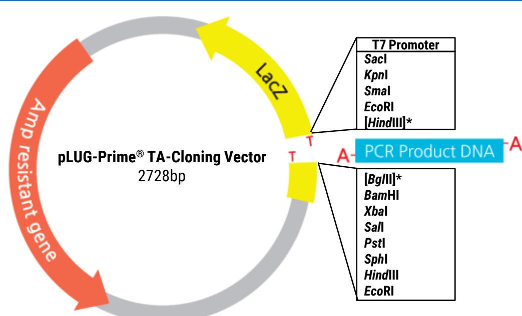


Figure 1 : Map and sequence reference points of the pLUG-Prime® TA-Cloning Vector II

* Before the insert is incorporated into the pLUG-Prime® TA-Cloning Vector II, there is only one *Hind*Ⅲ site and no *Bgl*Ⅱ site. After the incorporation, the T and A nucleotide on the insert will complement with the sequence on the vector and generate these two new sites. This merit of pLUG-Prime® TA-Cloning Vector II makes cloning more economical and convenient.

Multiple Cloning Region	434 to 490
LacZ Operator	531 to 548
LacZ gene	511 to 149
Amp^r gene	2528 to 1671
T7 promoter	402 to 439
M13 forward primer	359 to 375
M13 reverse primer	507 to 528
β-lactamase coding region	1524 to 2528

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301  TACGCCAGCT  GGCGAAAGGG  GGATGTGCTG  CAAGGCGATT  AAGTTGGGTA
     ATGCGGTCGA  CCGCTTTCCC  CCTACACGAC  GTTCCGCTAA  TTCAACCCAT
                 M13 Forward Primer
351  ACGCCAGGGT  TTTCCCAGTC  ACGACGTTGT  AAAACGACGG  CCAGTGAATT
     TGCGGTCCCA  AAAGGGTCAG  TGCTGCAACA  TTTTGCTGCC  GGTCACTTAA
          T7 Promoter         Sac I      Kpn I Sma I  EcoR I   Hind III
401  GTAATACGAC  TCACTATAGG  GCGAGCTCGG  TACCCGGGCG  AATTCCAAGC
     CATTATGCTG  AGTGATATCC  CGCTCGAGCC  ATGGGCCCGC  TTAAGGTTCG
                      Bgl II    BamH I   Xba I    Sal I   Pst I   Sph I
451  T T  Insert  -A GATCTGGAT  CCCCTCTAGA  GTCGACCTGC  AGGCATGCAA
     A A- DNA      T CTAGACCTA  GGGGAGATCT  CAGCTGGACG  TCCGTACGTT
     Hind III     EcoR I
491  GCTTGGCGGA  ATTCTGGTCA  TAGCTGTTTC  CTGTGTGAAA  TTGTTATCCG
     CGAACCGCCT  TAAGACCAGT  ATCGACAAAG  GACACACTTT  AACAATAGGC
                             M13 Reverse Primer
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Figure 2 : Multiple cloning site sequence of the pLUG-Prime® TA-Cloning Vector II

SUGGESTIONS

1. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes by preparing single-use aliquots of Ligation Buffers.
2. *Pfu* DNA polymerase possesses proofreading activity; it does not have the terminal transferase-like activity like Taq DNA polymerase. Ligation reactions using *Pfu* amplified DNA containing no A-tails will result in no positive colonies.
3. Methods for increasing the ligation efficiency :

   A. A-tailing :

Purified PCR product	X μl
10X PCR buffer	10 μl
10mM dATP	2 μl
Taq	1μl

   I. Add deionized water to a final volume of 100ul.
   II. Incubate at 72℃ for 1 hrs.
   III. Purify the A-tailed DNA and use it in the ligation reaction.

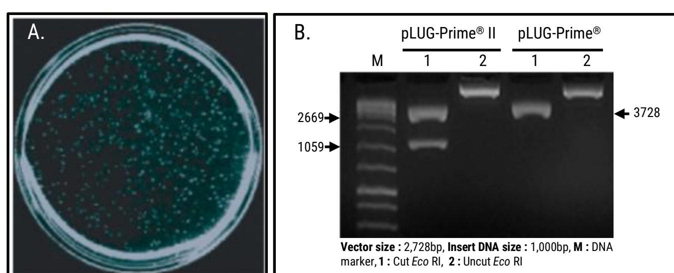
   B. If the maximum of tranformants is required, incubate the reactions overnight at 4℃.

   C. The optimal efficiency can be achieved by using a 1:3 molar ratio of vector DNA to the insert DNA.

   D. Use competent cells with higher efficiency such as MacCell™ (>10^8 cfu/μg DNA) series for transformation.

4. Using the colony PCR technique, clones can be screened easily and precisely.

TECHNICAL DATA



A. Colonies transformed by using pLUG-Prime® TA-Cloning Vector Kit II.
(The colony, which is the result of TA-cloning by using pLUG-Prime® TA-Cloning Vector Kit II, was used as the template DNA for PCR.)

B. The gel analysis of the PCR products ligated by pLUG-Prime® TA-Cloning Vector Kit II.

Restriction Enzyme sites of pLUG-Prime® TA-Cloning Vector Kit II

Enzyme	Position	Enzyme	Position	Enzyme	Position	Enzyme	Position	Enzyme	Position
Aat II	2664	*Ava* I	434	*Cfr* 10I	1822	*Mam* I	457	*Sph* I	488
Acc 65I	430	*Ban* II	428	*Dra* II	2718	*Nar* I	237	*Ssp* I	2546
Acc I	473	*Bam* HI	458	*Eam* 1105I	1742	*Nde* I	185	*Xba*I	466
Afl III	849	*Bcg* I	2281	*Ecl* 136II	426	*Pst* I	482	*Xma* I	434
Ahd I	1742	*Bpm* I	1812	*Eco* 0109I	2718	*Sac* I	428	*Xmn* I	2341
Alw NI	1265	*Bsa* BI	457	*Hin* cII	474	*Sal* I	472		
Asp 700	2341	*Bsa* I	1803	*Hin* dII	474	*Sap* I	733		
Asp 718	430	*Bsp* MI	485	*Ksa* I	236	*Sca* I	2222		
Asp EI	1742	*Bsr* FI	1822	*Kpn* I	434	*Sma* I	436		

Restriction Enzyme sites of pLUG-Prime® TA-Cloning Vector Kit II

AatI	*BbsI*	*BsmFI*	*BstXI*	*EcoNI*	*MunI*	*PaeR7I*	*SexAI*	*Tth111I*
AccIII	*BclI*	*BsmI*	*Bsu36I*	*EcoRV*	*NaeI*	*PflMI*	*SfiI*	*Van91I*
AflII	*BfrI*	*Bsp120I*	*CelII*	*EspI*	*NcoI*	*PinAI*	*SfuI*	*XcmI*
AgeI	*BlnI*	*BspDI*	*ClaI*	*HpaI*	*NgoMI*	*PmaCI*	*SgrAI*	*XhoI*
ApaI	*Bpu1102I*	*BspEI*	*Csp45I*	*KspI*	*NheI*	*PmeI*	*SnaBI*	*XmaIII*
AscI	*BpuAI*	*BsrGI*	*DraIII*	*MfeI*	*NotI*	*PmlI*	*SpeI*	
AspI	*BsaAI*	*BssHII*	*DsaI*	*MluI*	*NruI*	*Ppu10I*	*SspBI*	
AsuII	*BseAI*	*Bst1107I*	*EagI*	*MluNI*	*NsiI*	*PpuMI*	*StuI*	
AvrII	*BsgI*	*BstBI*	*EclXI*	*MroI*	*NspV*	*RsrII*	*StyI*	
BbrPI	*BsiWI*	*BstEII*	*Eco47III*	*MscI*	*PacI*	*SacII*	*SwaI*	

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
No colony was obtained from transformation	
1)Bacteria were not competent	• Check the transformation efficiency of competent cells used. Vortex the ligation buffer vigorously before use. We recommend using highly competent cells (≥1.0×10^8 cfu/μg circular plasmid DNA).
2)Incorrect transformation procedure	• Make sure that the appropriate transformation procedure was used.
3)Use of incorrect antibiotic on plates	• Agar plates should include ampicillin; Check the ampicillin concentration added to the plates; Use fresh antibiotic plates.
Low number of white colonies	
1)A-tailing of the PCR product was not efficient	• Verify that PCR amplification was performed using *Taq* DNA polymerase; Use fresh PCR products. Efficiency may be reduced within a day because of loss of A-overhang of PCR products.
2)Insert to vector ratio was too low	• Use the 5-10-fold molar excess of PCR product over TA-cloning vector.
3)Ligation mixture used in transformation was insufficient	• Increase the amount of the ligation mixture added to the transformation reaction or increase the amount of the transformants plated.
4)Presence of inhibitor in PCR mixture	• The high salt content of PCR reactions can inhibit ligation and transformation. Though your PCR product is shown as single band, purify the PCR product prior to ligation.
5)Overexposure of PCR product to UV-light	• If the PCR product is gel-purified, the PCR product should be exposed to long-wave UV-light for as short a time as possible. Overexposure of PCR product to UV-light will lead to the formation of pyrimidine dimers that can not be ligated efficiently.

Problem / Possible cause	Recommendation
White colonies did not have insert	
1)Cells transformed with residual plasmid DNA from PCR reaction	• If the PCR template was plasmid DNA containing the resistance gene for the antibiotic used for colony selection, colonies may be shown on the following transformation. In that case, PCR product should be gel-purified prior to ligation reaction.
2)Non-specific PCR products or primer-dimers cloned into TA-cloning vector	• Gel-purify the PCR product prior to performing the TA-cloning reaction; Verify that the PCR primer design and quality.
Only white colonies were obtained	
1)No IPTG or X-gal in plates	• X-gal has to be included in the plate for blue/white colony selection. If you use competent cell whose genotype shows *lacI*^{q}, it requires IPTG to induce expression from the *lac* promoter.
2)Inappropriate bacterial strain was used for blue/white colony selection	• Ensure that the bacterial strain used for transformation has *lac*IqZΔM15 genotype.
Most colonies were blue or light blue	
1)The insert does not interrupt the reading-frame of the *lacZ* gene	• If the insert is small (<300bp) and the number of its bases including the 3'-end A-overhang is multiple of 3, the recombinant colony may be light blue.
2)Inappropriate bacterial strain was used for blue/white colony selection	• Ensure that the bacterial strain used for transformation carries *lacZ* mutation. Phenotype of colony is always blue which is in *lacZ*$^+$ strains.
3)Self-ligation of TA-cloning vector	• Frequent freezing/thawing may induce loss of 3'-end T-overhang in the TA-cloning vector. Loss of the T-overhangs is the cause of the ligation of vector itself in blunt-end and the colony is shown up as blue; Nuclease may degrade the T-overhang in TA-cloning vector. Use only the provided distilled water and ligation buffer in the ligation reaction.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
MEGAquick-spin™ PLUS Total Fragment DNA Purification Kit	50/200 col.	17289/17290
pLUG-Prime® TA-Cloning Vector Kit	20 rxn.	11061
DNA-spin™ Plasmid DNA Purification Kit	50/200 col.	17096/17098
DNA-midi™ GT Plasmid DNA Purification Kit	25 col.	17254
MacCell™ DH5α (10^7)	1 ml	15052
MacCell™ DH5α (10^8)	1 ml	15053
MacCell™ DH5α (10^9)	1 ml	15054
Maxime™ PCR PreMix (i-Taq)	96 tubes	25025
Maxime™ PCR PreMix (i-StarTaq)	96/480 tubes	25165/25167
Maxime™ PCR PreMix (i-MAX Ⅱ)	96 tubes	25265
LINKeed® Rapid DNA Ligation Kit (Version 2.0)	30 rxn.	15023

Technical support : +82-505-550-5600

Review date : 2014. 4. 1