コスモ・バイオ株式会社

Handbook for TA-cloning

pLUG® TA-cloning Vector Kit

pLUG®-Multi TA-cloning Vector Kit

Mar 2008

iNtRON Biotechnology

Table

Kit Contents and Storage

pLUG® and pLUG®-Multi TA-cloning vector kits

pLUG® TA-cloning Vector Kit	
Cat. No.	11041
pLUG® TA-cloning vector	20 µl
5X Ligation buffer	100 µl
T4 DNA ligase	20 µl
Distilled water	1 ml

pLUG®-Multi TA-cloning Vector Kit	
Cat. No.	11051
pLUG®-Multi TA-cloning vector	20 µl
5X Ligation buffer	100 µl
T4 DNA ligase	20 µl
Distilled water	1 ml

Shipping conditions

pLUG® and pLUG®-Multi TA-cloning vector kits are shipped in a refrigerated condition.

Storage recommendations

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

Notice to Purchaser

These products are for laboratory research use only.

Related Products

Additional materials listed are available from iNtRON Biotechnology. More information is available on the Internet at www.intronbio.com.

PCR-related products

	Product	Size	Cat. No.
Dried PreMix	*Maxime*™ PCR PreMix (*i-Taq*™)	96 tubes	25025
		480 tubes	25026
	Maxime™ PCR PreMix (*i-StarTaq*™)	96 tubes	25165
		480 tubes	25167
DNA polymerase	*i-Taq*™ DNA polymerase	250U	25021
		500U	25022
	i-StarTaq™ DNA polymerase	250U	25161
		500U	25162
	i-Max™ II DNA polymerase	250U	25261
	i-Max™ DNA polymerase	250U	25041
	i-StarMax™ DNA polymerase	250U	25171
Master mix solution	2X PCR Master mix Solution (*i-Taq*™)	1 ml	25027
		5 ml	25028
	2X PCR Master mix Solution (*i-StarTaq*™)	1 ml	25166
	2X PCR Master mix Solution (*i-Max*™ II)	1 ml	25266
dNTPs	dNTP set	1 ml/each dNTP	32111
	dNTP mixture	2 ml	32292

Transformation-related products

	Product	Size	Cat. No.
Competent cells	DH5α (10^7)	1 ml	15045
	DH5α (10^9)	1 ml	15046
	JM109 (10^7)	1 ml	15047
	JM109 (10^9)	1 ml	15048
	TOP10 (10^7)	1 ml	15049
	TOP10 (10^9)	1 ml	15050
Blue/white colony selection	α–Complementation Solution	100 rxn.	15032

Introduction

Overview of TA-cloning

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.

Characteristics

pLUG® and pLUG®-Multi TA-cloning vectors were designed for the convenient and direct PCR cloning and serving a credible blue/white colony selection. The credible blue/white colony selection could be realized by iNtRON's proprietary DCS™ technology. DCS™ technology gives high percentage of true white colonies with an anticipated recombinant plasmid. DCS™ technology excludes the possibility of selection of colonies having a parental plasmid used for the preparation of TA-cloning vector or a re-circulated plasmid after loss of T-overhang of TA-cloning vector.

Note: *False white colony without cloned DNA may be occurred by the undesirable mutation in the lacZ alpha gene or its regulatory elements during the plasmid propagation and the insertion of non-specific PCR products or primer-dimers into cloning vector, which may shift reading-frame.*

pLUG® and pLUG®-Multi TA-cloning vectors 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®-Multi TA-cloning vector around the TA-cloning site are located in mirror-repeat pattern, which is useful in re-cloning to another vector.

pLUG® and pLUG®-Multi TA-cloning vector kits offer:

- **High cloning efficiency**
- **High percentage of true white colony**
- **Credible blue / white colony selection**

Introduction

How to works

The PCR product and TA-cloning vector with a T-overhang on each 3′-end can be ligated under the action of T4 DNA ligase. The mechanism of T4 DNA ligase is to form covalent phosphodiester bonds between 3'-hydroxyl ends of A-overhang in PCR product with 5'-phosphate ends of T-overhang in TA-cloning vector. ATP is required for the ligase reaction.

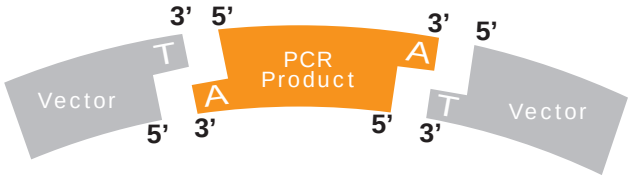
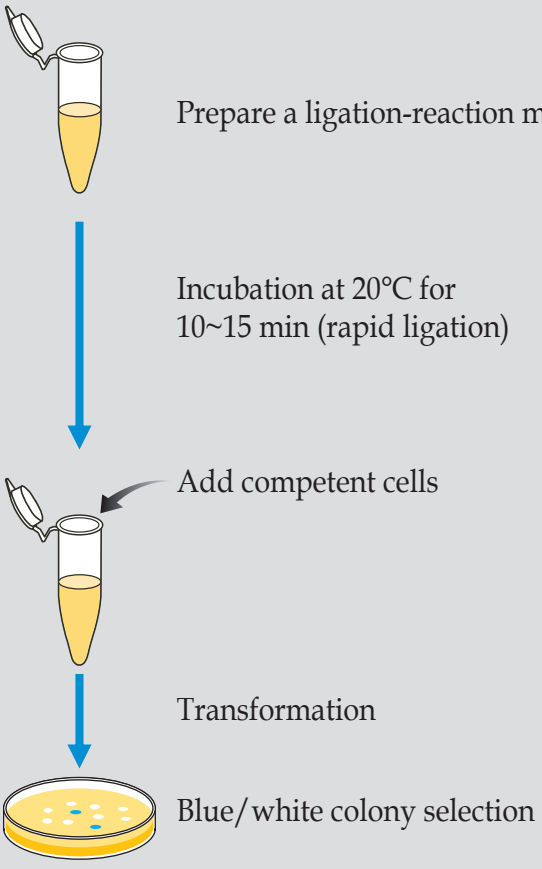


Figure 1. Schematic diagram for TA-cloning

Flow-chart of TA-cloning



Component	Volume(μl)
TA-cloning vector	1
PCR product	1 ~ 4
Distilled water	Adjust to 7
5X Ligation buffer	2
T4 DNA ligase	1
Total volume	**10**

Figure 2. Overview of TA-cloning using the pLUG® or pLUG®-Multi TA-cloning vector.

Features of the pLUG® and pLUG®-Multi TA-cloning Vectors

M13 forward / reverse priming sites

The M13 forward/reverse priming sites in the pLUG® and pLUG®-Multi TA-cloning vectors allow convenient sequencing.

LacZ alpha sequence

The fragment of lacZ alpha sequence in the pLUG® and pLUG®-Multi TA-cloning vectors 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® and pLUG®-Multi TA-cloning vectors. The restriction-enzyme recognition sites of pLUG®-Multi TA-cloning vector around the TA-cloning site are located in mirror-repeat pattern.

Origin of replication

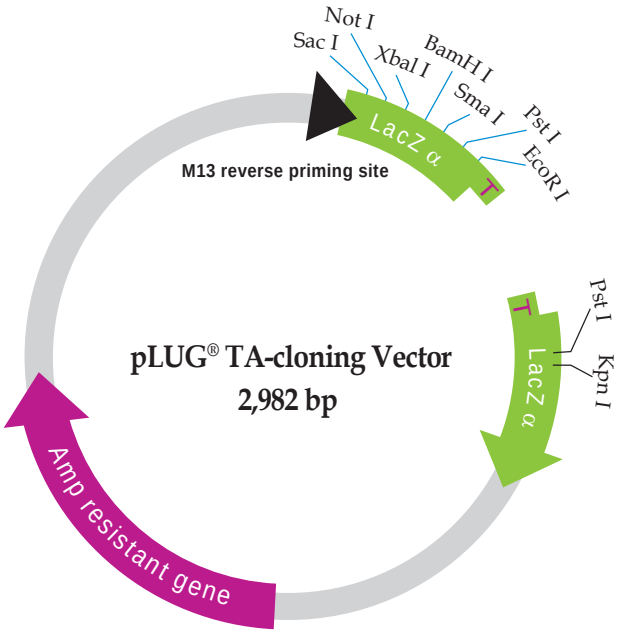
The pLUG® TA-cloning vector has *f1* and *ColE1* origins of replication. The pLUG®-Multi TA-cloning vector has *ColE1* origin of replication which is responsible for the replication of plasmid.

Ampicillin resistance gene

The pLUG® and pLUG®-Multi TA-cloning vectors have *bla* gene encoding for beta-lactamase that confer resistance to ampicillin.

Circle Map and Multiple Cloning Sites of the pLUG® and pLUG®-Multi TA-cloning Vectors

Map and features of pLUG® TA-cloning vector



Element	Position (bp)
LacZα fragment	216 ~ 643
LacZα start cordon	216
M13 reverse priming site	204 ~ 220
M13 forward priming site	439 ~ 454
Multiple cloning site	272 ~ 404
Ampicillin resistance gene	1,188 ~ 2,308
f1 origin of replication	594 ~ 1,050
ColE1 origin of replication	2,042 ~ 2,082

Figure 3. Map of pLUG® TA-cloning vector

Multiple cloning sequence of pLUG® TA-cloning vector

LacZα Start

```
CAGGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGC
GTCCTTTGTCGATACTGGTACTAATGCGGTTCGAGCTTTAATTGGGAGTGATTTCCCTTGTTTTCG
```

M13 reverse priming site

```
Sac I          Not I   Xba I       BamH I  Sma I   Pst I   EcoR I
TGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATA
ACCTCGAGGTGGCGCCACCGCCGGCGAGATCTTGATCACCTAGGGGGCCCGACGTCCTTAAGCTAT

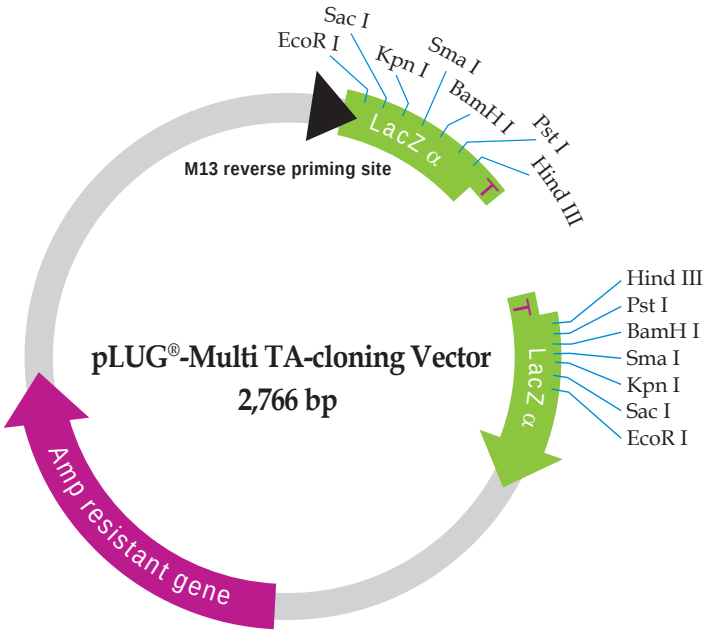
Hind III                    Hind III        Sal I
TCAAGCTTCCAGAGCT PCR AGACGTGGCAAGCTTATCGATACCGTCGACCTTCAGGGGGGG
AGTTCGAAGGTCTCGA Product TCTGCACCGTTCGAATAGCTATGGCAGCTGGAAGTCCCCCCC

Kpn I
CCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGT
GGGCCATGGGTTAAGCGGGATATCACTCAGCATAATGTTAAGTGACCGGCAGCAAAATGTTGCAGCA
```

M13 forward priming site

Circle Map and Multiple Cloning Sites of the pLUG® and pLUG®-Multi TA-cloning Vectors

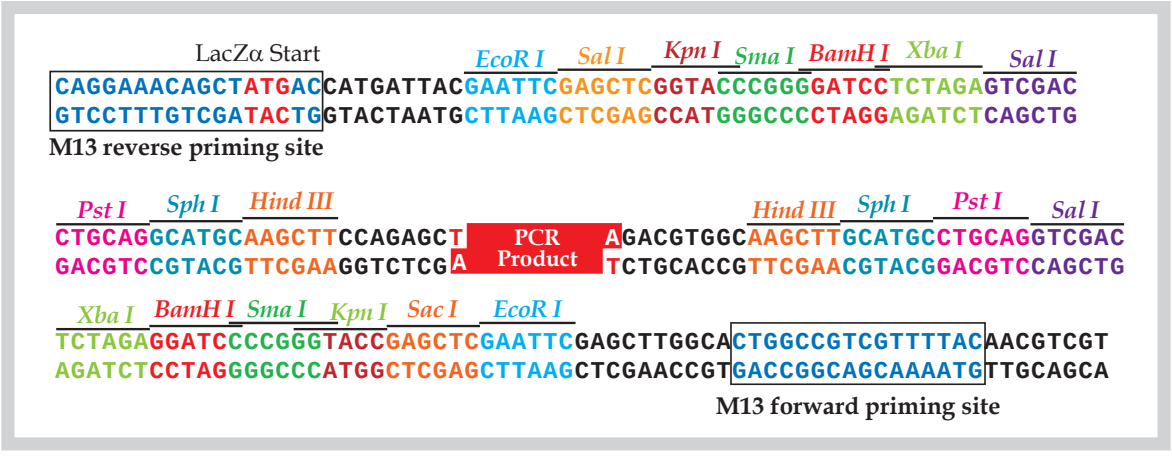
Map and features of pLUG®-Multi TA-cloning vector



Element	Position (bp)
LacZα fragment	216 ~ 619
LacZα start cordon	216
M13 reverse priming site	204 ~ 220
M13 forward priming site	371 ~ 386
Multiple cloning site	230 ~ 360
Ampicillin resistance gene	965 ~ 1,825
ColE1 origin of replication	1,826 ~ 1,866

Figure 4. Map of pLUG®-Multi TA-cloning vector

Multiple cloning sequence of pLUG®-Multi TA-cloning vector



TA-cloning Protocol

<Rapid ligation method>

1. Thaw 5X ligation buffer, TA-cloning vector, and distilled water.
2. Prepare a ligation-reaction mixture according to the following scheme:

Component	Volume (μl)
TA-cloning vector	1
PCR product	1 ~ 4
Distilled water	Adjust to 7
5X Ligation buffer	2
T4 DNA ligase	1
Total volume	**10**

3. Briefly mix the ligation-reaction mixture by pipetting and incubate the tube at 20°C for 10~15 minutes.
4. Proceed transformation according to the standard protocol or store ligation-reaction mixture at -20°C until use.
5. For blue/white colony selection, the standard protocol can be used.

<Standard overnight-ligation method: Optional>

1. Thaw 10X ligation buffer, TA-cloning vector, and distilled water.
2. Prepare a ligation-reaction mixture according to the following scheme:

Component	Volume (μl)
TA-cloning vector	1
PCR product	1 ~ 4
Distilled water	Adjust to 8
10X Ligation buffer	1
T4 DNA ligase	1
Total volume	**10**

3. Briefly mix the ligation-reaction mixture by pipetting and incubate the tube overnight at 4°C or 16°C (e.g., in a water bath or heating block).
4. Proceed transformation according to the standard protocol or store ligation-reaction mixture at -20℃ until use.
5. For blue/white colony selection, the standard protocol can be used.

TA-cloning Protocol

Producing PCR products

• *PCR primer*

Though a conventional PCR primer can be routinely used in TA-cloning, the use of primer with high purity is recommended. According to recent reports*,** the different bases in the 5′-end of the forward and reverse primers could effect on the TA-cloning efficiency of PCR products. The addition of base A on the 5′-end of reverse and/or forward primers improved cloning efficiency. Moreover, when two bases (AA, AT, AC, AG) were added to the 5′-end of pair primers, TA-cloning efficiency was improved markedly. To facilitate the A-addition of PCR product, add a 10 minute extension at 72°C to the end of the PCR cycling.

* *Adenosine added on the primer 5′ end improved TA cloning efficiency of polymerase chain reaction products. Analytical biochemistry 363: 163-165, 2007*

** *Change of 5′ terminal nucleotides of PCR primers causing variable TA-cloning efficiency. Journal of integrative plant biology 49: 382-385, 2007*

• *PCR product*

The use of fresh PCR products in TA-cloning is strongly recommended. The potential presence of exonucleases may cause degradation of the nucleotide overhangs of PCR products, reducing the cloning efficiency. While it is not recommended, some PCR products can be successfully cloned after 1 week of storage at +4°C.

For efficient cloning, PCR products need to be purified to eliminate residual dNTP, enzyme, primers, and salt as well as undesired non-specific bands. Purification of PCR products prior to ligation will generally result in higher transformation efficiencies. There are several choices to clean up the PCR product. When you get a single band on a gel, the PCR product can be puirified with *PCRquick-spin*™ PCR product purification kit (Cat. No.17202). If you do not obtain a single discrete band from your PCR, you may purify the PCR product with *MEGA-spin*™ agarose gel extraction kit (Cat. No.17183). 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.

• *Blunt-ended PCR products*

PCR products generated using proofreading DNA polymerases such as *Pfu* and *Vent* can be used in TA-cloning procedures after the addition of a 3′-end A-overhang.

The addition of a 3′-end A-overhang can be performed as follows:

1. Add 1 unit of *Taq* DNA polymerase to the blunt-ended PCR product sample (~100 µl) and mix well. It is not necessary to change the buffer.
2. Incubate at 72°C for 10 minutes.
3. Purify the PCR product with a silica-based spin column or phenol extraction following ethanol precipitation.

TA-cloning Protocol

Cloning into the pLUG® or pLUG®-Multi TA-cloning vector

• *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 a 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® and pLUG®-Multi TA-cloning vectors are about 3 kbp and the kit suggests adding 50 ng (1 µl) of the vector.

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

PCR product size (bp)	pLUG® TA-cloning vector		pLUG®-Multi TA-cloning vector	
	5-times molar excess (ng)*	10-times molar excess (ng)*	5-times molar excess (ng)*	10-times molar excess (ng)*
500	41.9	83.8	45	90
1,000	83.8	167.7	90	180
1,500	125.8	251.5	135	270

** Calculated for 50 ng vector using the following equation:*

ng PCR product required

= [50 ng X PCR product size (bp) X molar ratio] / [vector size (bp)]

• *Ligation-reaction*

Set up a 10 µl ligation-reaction. The 5X ligation buffer was optimized for rapid ligation which requires short ligation times at room temperature.

If you see precipitate in the ligation buffer, warm it briefly at 37°C to dissolve the salts. It is important to mix the solution completely before use to avoid localized concentrations of salts. If using non-purified PCR product, do not add more than 2 µl PCR product.

Transforming the competent cells

The pLUG® and pLUG®-Multi TA-cloning vectors are compatible with a wide range of chemically competent cells which are available from iNtRON Biotechnology. In particular, DH5α, JM109 and TOP10 competent cells are appropriate to blue/white colony selection (i.e., blue/white screening capability) and exhibit the high transformation efficiency. A 3~10 µl aliquot of ligation mixture may be used to transform competent cells. Transforming the competent cells can be performed according to a standard transformation method. Transformants should be plated onto LB plates with ampicillin, X-gal and IPTG.

TA-cloning Protocol

Background colonies may appear following transformation if the PCR template was plasmid DNA containing a resistance gene for the antibiotic used for colony selection (i.e., an ampicillin-resistance gene). In this case the PCR product should be gel-purified prior to ligation to remove template plasmid DNA.

Screening of a recombinant plasmid

Colonies with a recombinant plasmid will be white. Screening a recombinant colony with cloned DNA can be performed with white colony by colony PCR or enzymatic analysis of the plasmid isolated from its culture.

- ***Colony PCR can be performed as follows:***
  1. Pick a white colony with an autoclaved toothpick or pipette tip and swirl it into 20~30 µl of dH_2O in a microcentrifuge tube.
  2. Heat in a boiling water bath or hot (90~100°C) heating block for 2 minutes (can be used directly for PCR).
  3. Spin for 2 minutes at top speed in microcentrifuge and transfer the supernatant into a new microcentrifuge tube.
  4. Use 1~2 µl of supernatant as a template in 20 µl PCR reaction and perform the PCR.
  5. Subject the PCR product to agarose gel electrophoresis.

- ***Enzymatic analysis can be performed as follows:***
  1. Pick a white colony with an autoclaved toothpick or pipette tip and swirl it into 1~3 ml LB media containing ampicillin.
  2. After cultivation at 37°C, perform the isolation of plasmid DNA using our *DNA-spin*™ plasmid DNA extraction kit.
  3. Treat the isolated plasmid with appropriate restriction enzyme located around the TA-cloning site.
  4. Subject enzyme digest to agarose gel electrophoresis.

Troubleshooting Guide

If you do not obtain the results you expect, refer to following table. Please contact iNtRON Technical Support Department if you have questions on use of the products.

☛ *E-mail: consult@intronbio.com. Tel: +82-31-778-7830.*

Problem	Possible cause	Suggestion
No colony was obtained from transformation	Bacteria were not competent	Check the transformation efficiency of competent cells used. We recommend use highly competent cells (≥1.0 X 10^8 cfu/μg circular plasmid DNA)
	Incorrect transformation procedure	Make sure that the appropriate transformation procedure was used
	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	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 after as little as 1 day of storage because of loss of A-overhang of PCR products
		Addition of extension time at 72°C to the end of the PCR cycling will be helpful for increasing of A-tailing
	Insert to vector ratio was too low	Use of a 5~10 fold molar excess of PCR product over TA-cloning vector is recommended for ligation
	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
	Ligation reaction time was too short	Extend the ligation reaction time. Increasing the ligation time can yield higher numbers of recombinant colonies
	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 a single band, purify the PCR product prior to ligation, e.g., using the *PCRquick-spin*™ PCR product purification kit
	Overexposure of PCR product to UV-light	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 cannot be ligated efficiently

Troubleshooting Guide

Problem	Possible cause	Suggestion
White colonies did not have insert	Cells transformed with residual plasmid DNA from PCR reaction	If the PCR template was plasmid DNA containing a resistance gene for the antibiotic used for colony selection, colonies may appear following transformation. In that case, PCR product should be gel-purified prior to ligation reaction
	Non-specific PCR products or primer-dimers cloned into TA-cloning vector	Gel-purify the PCR product prior to performing the cloning reaction. Use only specified band to the ligation reaction
		Verify that the PCR primer design and quality
Only white colonies were obtained	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 which genotype shows $lacI^q$, it requires IPTG to induce expression from the *lac* promoter
	Inappropriate bacterial strain was used for blue/white colony selection	Ensure that the bacterial strain used for transformation has lacIqZΔM15 genotype
Most colonies were blue or light blue	The insert did 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 colony.
	Inappropriate bacterial strain was used for blue/white colony selection	Ensure that the bacterial strain used for transformation carries a lacZ mutation. Phenotype of colony is always blue which is $lacZ^+$ strains
	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 results in blunt-end ligation of vector itself and the colony with this plasmid is blue
		Nuclease may degrade the T-overhang in TA-cloning vector. Use only the provided distilled water and ligation buffer in the ligation reaction
White colonies do not grow in liquid culture	Ampicillin-sensitive satellite colonies	Pick large white colonies. Be sure that ampicillin is fresh

Appendix

Sequences of the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG® TA-cloning vector (2,982 bp)*

```
                                                                                                    10
GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC
                                                                                                   110
GCAATTAATG TGAGTTAGCT CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT TGTGAGCGGA TAACAATTTC
                                                                                                   210
ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCGA AATTAACCCT CACTAAAGGG AACAAAAGCT GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG
                                                                                                   310
AACTAGTGGA TCCCCCGGGC TGCAGGAATT CGATATCAAG CTTCCAGAGC *TA*GACGTGGC AAGCTTATCG ATACCGTCGA CCTTCAGGGG GGGCCCGGTA
                                                                                                   410
CCCAATTCGC CCTATAGTGA GTCGTATTAC AATTCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT TACCCAACTT AATCGCCTTG
                                                                                                   510
CAGCACATCC CCCTTTCGCC AGCTGGCGTA ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG AATGGCGAAT GGCAAATTGT
                                                                                                   610
AAGCGTTAAT ATTTTGTTAA AATTCGCGTT AAATTTTTGT TAAATCAGCT CATTTTTTAA CCAATAGGCC GAAATCGGCA AAATCCCTTA TAAATCAAAA
                                                                                                   710
GAATAGACCG AGATAGGGTT GAGTGTTGTT CCAGTTTGGA ACAAGAGTCC ACTATTAAAG AACGTGGACT CCAACGTCAA AGGGCGAAAA ACCGTCTATC
                                                                                                   810
AGGGCGATGG CCCACTACGT GAACCATCAC CCTAATCAAG TTTTTTGGGG TCGAGGTGCC GTAAAGCACT AAATCGGAAC CCTAAAGGGA GCCCCCGATT
                                                                                                   910
TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCGAGAAAG GAAGGGAAGA AAGCGAAAGG AGCGGGCGCT AGGGCGCTGG CAAGTGTAGC GGTCACGCTG
                                                                                                  1010
CGCGTAACCA CCACACCCGC CGCGCTTAAT GCGCCGCTAC AGGGCGCGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT
                                                                                                  1110
TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG
                                                                                                  1210
TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA
                                                                                                  1310
CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC
                                                                                                  1410
TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
                                                                                                  1510
AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC
                                                                                                  1610
GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG
                                                                                                  1710
ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA
                                                                                                  1810
CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
                                                                                                  1910
CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC
                                                                                                  2010
AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT
                                                                                                  2110
TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC
                                                                                                  2210
AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC
                                                                                                  2310
TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC
                                                                                                  2410
TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG
                                                                                                  2510
TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG
                                                                                                  2610
AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
                                                                                                  2710
GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA
                                                                                                  2810
AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA
                                                                                                  2910
TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GA
```

Appendix

Sequences of the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG®-Multi TA-cloning vector (2,766 bp)*

```
                                                                                                    10
GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC
       110        120        130        140        150        160        170        180        190        200
GCAATTAATG TGAGTTAGCT CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT TGTGAGCGGA TAACAATTTC
       210        220        230        240        250        260        270        280        290        300
ACACAGGAAA CAGCTATGAC CATGATTACG AATTCGAGCT CGGTACCCGG GGATCCTCTA GAGTCGACCT GCAGGCATGC AAGCTTCCAG AGC*TA*GACGT
       310        320        330        340        350        360        370        380        390        400
GGCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC GAGCTTGGCA CTGGCCGTCG TTTTACAACG TCGTGACTGG
       410        420        430        440        450        460        470        480        490        500
GAAAACCCTG GCGTTACCCA ACTTAATCGC CTTGCAGCAC ATCCCCCTTT CGCCAGCTGG CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC
       510        520        530        540        550        560        570        580        590        600
AGTTGCGCAG CCTGAATGGC GAATGGCGCC TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTC ACACCGCATA TGGTGCACTC TCAGTACAAT
       610        620        630        640        650        660        670        680        690        600
CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA
       710        720        730        740        750        760        770        780        790        800
GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTTATAGG
       810        820        830        840        850        860        870        880        890        900
TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA TACATTCAAA
       910        920        930        940        950        960        970        980        990       1000
TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC
      1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
TTTTTTGCGG CATTTTGCCT TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTTACATCG
      1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT
      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG
      1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC
      1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC
      1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
GATGCCTGTA GCAATGGCAA CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT
      1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG
      1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG
      1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG
      1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG
      2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC
      2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
      2210       2220       2230       2040       2250       2260       2270       2280       2290       2300
TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC
      2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC
      2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT
      2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG
      2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG
      2710       2720       2730       2740       2750       2760
AGCTGATACC GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGA
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Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG® TA-cloning vector*

Table 2. Restriction enzymes that cut the pLUG® TA-cloning vector.

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
Acc113I	1	1487	agt/act
Acc16I	2	572, 1745	tgc/gca
Acc65I	1	397	g/gtacc
AccB1I	4	130, 397, 855, 2015	g/gyrcc
AccBSI	5	189, 297, 965, 1129, 2930	gagcgg
AccI	1	377	gt/mkac
AclNI	1	302	a/ctagt
AcsI	3	326, 620, 631	r/aatty
AcyI	1	1428	gr/cgyc
AflIII	1	2856	a/crygt
AhdI	1	1968	gacnnn/nngtc
Alw21I	4	276, 1300, 1385, 2546	gwgcw/c
Alw44I	2	1296, 2542	g/tgcac
AlwNI	1	2447	cagnnn/ctg
Ama87I	1	314	c/ycgrg
ApaI	1	395	gggcc/c
ApaLI	2	1296, 2542	g/tgcac
ApoI	3	326, 620, 631	r/aatty
AseI	3	46, 105, 1793	at/taat
AsnI	3	46, 105, 1793	at/taat
Asp700I	1	1368	gaann/nnttc
Asp718I	1	397	g/gtacc
AspEI	1	1968	gacnnn/nngtc
AspHI	4	276, 1300, 1385, 2546	gwgcw/c
AvaI	1	314	c/ycgrg
AviII	2	572, 1745	tgc/gca
BamHI	1	308	g/gatcc
BanI	4	130, 397, 855, 2015	g/gyrcc
BanII	3	276, 395, 893	grgcy/c
BanIII	1	368	at/cgat
BbiII	1	1428	gr/cgyc
Bbv12I	4	276, 1300, 1385, 2546	gwgcw/c
BcgI	2	370, 1452	cgannnnnntgc
BcoI	1	314	c/ycgrg
BglI	2	582, 1850	gccnnnn/nggc
BpmI	2	274, 1883	ctggag

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
Bsa29I	1	368	at/cgat
BsaAI	1	818	yac/gtr
BsaHI	1	1428	gr/cgyc
BsaI	1	1901	ggtctc
BsaOI	6	292, 553, 1450, 1599, 2522, 2946	cgry/cg
BsaWI	3	1672, 2503, 2650	w/ccggw
BscI	1	368	at/cgat
Bse118I	2	919, 1883	r/ccggy
BseCI	1	368	at/cgat
Bsh1285I	6	292, 553, 1450, 1599, 2522, 2946	cgry/cg
BshNI	4	130, 397, 855, 2015	g/gyrcc
BsiEI	6	292, 553, 1450, 1599, 2522, 2946	cgry/cg
BsiHKAI	4	276, 1300, 1385, 2546	gwgcw/c
BsiI	2	1304, 2688	ctcgtg
BsoBI	1	314	c/ycgrg
Bsp106I	1	368	at/cgat
Bsp120I	1	391	g/ggccc
Bsp143II	3	969, 977, 2616	rgcgc/y
BspCI	2	553, 1599	cgat/cg
BspDI	1	368	at/cgat
BspHI	2	1128, 2136	t/catga
BspLU11I	1	2856	a/catgt
BspXI	1	368	at/cgat
BsrBI	5	189, 297, 965, 1129, 2930	gagcgg
BsrDI	2	1732, 1914	gcaatg
BsrFI	2	919, 1883	r/ccggy
BssAI	2	919, 1883	r/ccggy
BssSI	2	1304, 2688	ctcgtg
BstD102I	5	189, 297, 965, 1129, 2930	gagcgg
BstDSI	1	280	c/crygg
BstH2I	3	969, 977, 2616	rgcgc/y
BstI	1	308	g/gatcc

Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG® TA-cloning vector (Continued)*

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
BstMCI	6	292, 553, 1450, 1599, 2522, 2946	cgry/cg
BstSFI	6	320, 412, 1037, 1722, 2400, 2591	c/tryag
BstX2I	7	308, 1321, 1338, 2106, 2118, 2204, 2215	r/gatcy
BstXI	1	284	ccannnnn/ntgg
BstYI	7	308, 1321, 1338, 2106, 2118, 2204, 2215	r/gatcy
BstZI	1	289	c/ggccg
Bsu15I	1	368	at/cgat
CciNI	1	289	gc/ggccgc
Cfr10I	2	919, 1883	r/ccggy
Cfr42I	1	283	ccgc/gg
Cfr9I	1	314	c/ccggg
CfrI	4	35, 289, 438, 1575	y/ggccr
ClaI	1	368	at/cgat
DraI	3	1390, 2082, 2101	ttt/aaa
DraII	1	391	rg/gnccy
DraIII	1	818	cacnnn/gtg
DrdI	2	773, 2754	gacnnnn/nngtc
DsaI	1	280	c/crygg
EaeI	4	35, 289, 438, 1575	y/ggccr
EagI	1	289	c/ggccg
Eam1104I	2	541, 1179	ctcttc
Eam1105I	1	1968	gacnnn/nngtc
EarI	2	541, 1179	ctcttc
Ecl136II	1	274	gag/ctc
EclHKI	1	1968	gacnnn/nngtc
EclXI	1	289	c/ggccg
Eco24I	3	276, 395, 893	grgcy/c
Eco255I	1	1487	agt/act
Eco31I	1	1901	ggtctc
Eco32I	1	334	gat/atc
Eco52I	1	289	c/ggccg
Eco57I	3	387, 1286, 2334	ctgaag
Eco64I	4	130, 397, 855, 2015	g/gyrcc
Eco88I	1	314	c/ycgrg

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
EcoICRI	1	274	gag/ctc
EcoO109I	1	391	rg/gnccy
EcoRI	1	326	g/aattc
EcoRV	1	334	gat/atc
FriOI	3	276, 395, 893	grgcy/c
FspI	2	572, 1745	tgc/gca
GsuI	2	274, 1883	ctggag
HaeII	3	969, 977, 2616	rgcgc/y
Hin1I	1	1428	gr/cgyc
HincII	1	378	gty/rac
HindII	1	378	gty/rac
HindIII	2	338, 361	a/agctt
Hsp92I	1	1428	gr/cgyc
KpnI	1	401	ggtac/c
Ksp632I	2	541, 1179	ctcttc
KspI	1	283	ccgc/gg
MflI	7	308, 1321, 1338, 2106, 2118, 2204, 2215	r/gatcy
MroNI	1	919	g/ccggc
MslI	4	282, 1197, 1556, 1715	caynn/nnrtg
Msp17I	1	1428	gr/cgyc
MspA1I	6	54, 282, 522, 1332, 2273, 2518	cmg/ckg
NaeI	1	921	gcc/ggc
NgoAIV	1	919	g/ccggc
NgoMI	1	919	g/ccggc
NotI	1	289	gc/ggccgc
NspBII	6	54, 282, 522, 1332, 2273, 2518	cmg/ckg
NspI	1	2860	rcatg/y
Ple19I	2	553, 1599	cgat/cg
PshBI	3	46, 105, 1793	at/taat
Psp124BI	1	276	gagct/c
Psp1406I	2	1366, 1739	aa/cgtt
PspAI	1	314	c/ccggg
PspALI	1	316	ccc/ggg
PspOMI	1	391	g/ggccc
PstI	1	324	ctgca/g

Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG® TA-cloning vector (Continued)*

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
PvuI	2	553, 1599	cgat/cg
PvuII	2	54, 522	cag/ctg
RcaI	2	1128, 2136	t/catga
SacI	1	276	gagct/c
SacII	1	283	ccgc/gg
SalI	1	376	g/tcgac
ScaI	1	1487	agt/act
SfcI	6	320, 412, 1037, 1722, 2400, 2591	c/tryag
Sfr303I	1	283	ccgc/gg
SmaI	1	316	ccc/ggg
SpeI	1	302	a/ctagt
SspI	2	610, 1163	aat/att
SstI	1	276	gagct/c
SstII	1	283	ccgc/gg
VneI	2	1296, 2542	g/tgcac
VspI	3	46, 105, 1793	at/taat
XbaI	1	296	t/ctaga
XhoII	7	308, 1321, 1338, 2106, 2118, 2204, 2215	r/gatcy
XmaI	1	314	c/ccggg
XmaIII	1	289	c/ggccg
XmnI	1	1368	gaann/nnttc

Table 3. Restriction enzymes that do not cut the pLUG® TA-cloning vector.

AatI, AatII, AccB7I, AccIII, AfeI, AflII, AgeI, AocI, Aor51HI, AscI, AspI, AtsI, AvrII, BalI, BbeI, BbrPI, BbsI, BbuI, Bbv16II, BclI, BfrI, BglII, BlnI, BlpI, BpiI, Bpu1102I, Bpu14I, BpuAI, BsaBI, BsaMI, Bse21I, Bse8I, BseAI, BsePI, BseRI, BsgI, Bsh1365I, BsiMI, BsiWI, BsmBI, BsmI, Bsp119I, Bsp13I, Bsp1407I, Bsp1720I, Bsp19I, Bsp68I, BspEI, BspMI, BspTI, BsrBRI, BsrGI, BssHII, BssT1I, Bst1107I, Bst98I, BstBI, BstEII, BstPI, BstSNI, Bsu36I, CelII, CpoI, Csp45I, CspI, CvnI, Eco105I, Eco130I, Eco147I, Eco47III, Eco72I, Eco81I, Eco91I, EcoNI, EcoO65I, EcoT14I, EcoT22I, EheI, ErhI, Esp1396I, Esp3I, FauNDI, FbaI, FseI, HpaI, KasI, Kpn2I, Ksp22I, LspI, MamI, MfeI, MluI, MluNI, Mph1103I, MroI, MscI, MspCI, MunI, Mva1269I, NarI, NcoI, NdeI, NheI, NruI, NsiI, NspV, PacI, PaeI, PaeR7I, Pfl23II, PflMI, PinAI, PmaCI, Pme55I, PmeI, PmlI, Ppu10I, PpuMI, PshAI, Psp5II, PspEI, PspLI, PstNHI, RsrII, SapI, SbfI, SexAI, SfiI, Sfr274I, SfuI, SgfI, SgrAI, SmiI, SnaBI, SphI, SplI, SrfI, Sse8387I, SseBI, SspBI, StuI, StyI, SunI, SwaI, Tth111I, Van91I, Vha464I, XcmI, XhoI, Zsp2I

Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG®-Multi TA-cloning vector*

Table 4. Restriction enzymes that cut the pLUG®-Multi TA-cloning vector.

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
AatII	1	833	gacgt/c
Acc113I	1	1271	agt/act
Acc16I	2	506, 1529	tgc/gca
Acc65I	2	242, 343	g/gtacc
AccB1I	5	130 242 343 525 1799	g/gyrcc
AccBSI	3	189, 913, 2714	gagcgg
AccI	2	264, 323	gt/mkac
AcsI	2	230, 355	r/aatty
AcyI	3	526, 830, 1212	gr/cgyc
AflIII	1	2640	a/crygt
AhdI	1	1752	gacnnn/nngtc
Alw21I	6	240, 353, 587, 1084, 1169, 2330	gwgcw/c
Alw44I	3	583 1080 2326	g/tgcac
AlwNI	1	2231	cagnnn/ctg
Ama87I	2	246, 339	c/ycgrg
ApaLI	3	583, 1080, 2326	g/tgcac
ApoI	2	230, 355	r/aatty
AseI	3	46, 105, 1577	at/taat
AsnI	3	46, 105, 1577	at/taat
Asp700I	1	1152	gaann/nnttc
Asp718I	2	242, 343	g/gtacc
AspEI	1	1752	gacnnn/nngtc
AspHI	6	240, 353, 587, 1084, 1169, 2330	gwgcw/c
AvaI	2	246, 339	c/ycgrg
AviII	2	506, 1529	tgc/gca
BamHI	2	251, 334	g/gatcc
BanI	5	130, 242, 343, 525, 1799	g/gyrcc
BanII	2	240, 353	grgcy/c
BbeI	1	529	ggcgc/c
BbiII	3	526, 830, 1212	gr/cgyc
BbuI	2	279, 314	gcatg/c
Bbv12I	6	240, 353, 587, 1084, 1169, 2330	gwgcw/c
BcgI	1	1236	cgannnnnntgc
BcoI	2	246, 339	c/ycgrg
BglI	2	516, 1634	gccnnnn/nggc
BpmI	1	1667	ctggag
BsaHI	3	526, 830, 1212	gr/cgyc
BsaI	1	1685	ggtctc
BsaOI	5	487, 1234, 1383, 2306, 2730	cgry/cg
BsaWI	3	1456, 2287, 2434	w/ccggw
Bse118I	1	1667	r/ccggy
Bsh1285I	5	487, 1234, 1383, 2306, 2730	cgry/cg
BshNI	5	130, 242, 343, 525, 1799	g/gyrcc
BsiEI	5	487, 1234, 1383, 2306, 2730	cgry/cg
BsiHKAI	6	240, 353, 587, 1084, 1169, 2330	gwgcw/c
BsiI	3	781, 1088, 2472	ctcgtg
BsmBI	2	714, 768	cgtctc
BsoBI	2	246, 339	c/ycgrg
Bsp143II	2	529, 2400	rgcgc/y
BspCI	2	487, 1383	cgat/cg
BspHI	3	807, 912, 1920	t/catga
BspLU11I	1	2640	a/catgt
BspMI	2	272, 323	acctgc
BsrBI	3	189, 913, 2714	gagcgg
BsrDI	2	1516, 1698	gcaatg
BsrFI	1	1667	r/ccggy
BssAI	1	1667	r/ccggy
BssSI	3	781, 1088, 2472	ctcgtg
BstD102I	3	189, 913, 2714	gagcgg
BstH2I	2	529, 2400	rgcgc/y
BstI	2	251, 334	g/gatcc
BstMCI	5	487, 1234, 1383, 2306, 2730	cgry/cg
BstSFI	5	269, 316, 1506, 2184, 2375	c/tryag
BstX2I	8	251, 334, 1105, 1122, 1890, 1902, 1988, 1999	r/gatcy
BstYI	8	251, 334, 1105, 1122, 1890, 1902, 1988, 1999	r/gatcy
Cfr10I	1	1667	r/ccggy

Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG®-Multi TA-cloning vector (Continued)*

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
Cfr9I	2	246, 339	c/ccggg
CfrI	3	35, 372, 1359	y/ggccr
DraI	3	1174, 1866, 1885	ttt/aaa
DraII	1	772	rg/gnccy
DrdI	2	669, 2538	gacnnnn/nngtc
EaeI	3	35, 372, 1359	y/ggccr
Eam1104I	2	475, 963	ctcttc
Eam1105I	1	1752	gacnnn/nngtc
EarI	2	475, 963	ctcttc
Ecl136II	2	238, 351	gag/ctc
EclHKI	1	1752	gacnnn/nngtc
Eco24I	2	240, 353	grgcy/c
Eco255I	1	1271	agt/act
Eco31I	1	1685	ggtctc
Eco57I	2	1070, 2118	ctgaag
Eco64I	5	130, 242, 343, 525, 1799	g/gyrcc
Eco88I	2	246, 339	c/ycgrg
EcoICRI	2	238, 351	gag/ctc
EcoO109I	1	772	rg/gnccy
EcoRI	2	230, 355	g/aattc
EheI	1	527	ggc/gcc
Esp3I	2	714, 768	cgtctc
FauNDI	1	578	ca/tatg
FriOI	2	240, 353	grgcy/c
FspI	2	506, 1529	tgc/gca
GsuI	1	1667	ctggag
HaeII	2	529, 2400	rgcgc/y
Hin1I	3	526, 830, 1212	gr/cgyc
HincII	2	265, 324	gty/rac
HindII	2	265, 324	gty/rac
HindIII	2	281, 304	a/agctt
Hsp92I	3	526, 830, 1212	gr/cgyc
KasI	1	525	g/gcgcc
KpnI	2	246, 347	ggtac/c
Ksp632I	2	475, 963	ctcttc
MflI	8	251, 334, 1105, 1122, 1890, 1902, 1988, 1999	r/gatcy
MslI	3	981, 1340, 1499	caynn/nnrtg

Enzyme Name	# of Site	Positions of Sites (bp)	Recognition Sequence
Msp17I	3	526, 830, 1212	gr/cgyc
MspA1I	6	54, 456, 650, 1116, 2057, 2302	cmg/ckg
NarI	1	526	gg/cgcc
NdeI	1	578	ca/tatg
NspBII	6	54, 456, 650, 1116, 2057, 2302	cmg/ckg
NspI	4	279, 314, 727, 2644	rcatg/y
PaeI	2	279, 314	gcatg/c
Ple19I	2	487, 1383	cgat/cg
PshBI	3	46, 105, 1577	at/taat
Psp124BI	2	240, 353	gagct/c
Psp1406I	2	1150, 1523	aa/cgtt
PspAI	2	246, 339	c/ccggg
PspALI	2	248, 341	ccc/ggg
PstI	2	273, 320	ctgca/g
PvuI	2	487, 1383	cgat/cg
PvuII	2	54, 456	cag/ctg
RcaI	3	807, 912, 1920	t/catga
SacI	2	240, 353	gagct/c
SalI	2	263, 322	g/tcgac
SbfI	2	273, 320	cctgca/gg
ScaI	1	1271	agt/act
SfcI	5	269, 316, 1506, 2184, 2375	c/tryag
SmaI	2	248, 341	ccc/ggg
SphI	2	279, 314	gcatg/c
Sse8387I	2	273, 320	cctgca/gg
SspI	1	947	aat/att
SstI	2	240, 353	gagct/c
VneI	3	583, 1080, 2326	g/tgcac
VspI	3	46, 105, 1577	at/taat
XbaI	2	257, 328	t/ctaga
XhoII	8	251, 334, 1105, 1122, 1890, 1902, 1988, 1999	r/gatcy
XmaI	2	246, 339	c/ccggg
XmnI	1	1152	gaann/nnttc

Appendix

Restriction sites in the pLUG® and pLUG®-Multi TA-cloning vectors

• *pLUG®-Multi TA-cloning vector (Continued)*

Table 5. Restriction enzymes that do not cut the pLUG®-Multi TA-cloning vector.

AatI, AccB7I, AccIII, AclNI, AfeI, AflII, AgeI, AocI, Aor51HI, ApaI, AscI, AspI, AtsI, AvrII, BalI, BanIII, BbrPI, BbsI, Bbv16II, BclI, BfrI, BglII, BlnI, BlpI, BpiI, Bpu1102I, Bpu14I, BpuAI, Bsa29I, BsaAI, BsaBI, BsaMI, BscI, Bse21I, Bse8I, BseAI, BseCI, BsePI, BseRI, BsgI, Bsh1365I, BsiMI, BsiWI, BsmI, Bsp106I, Bsp119I, Bsp120I, Bsp13I, Bsp1407I, Bsp1720I, Bsp19I, Bsp68I, BspDI, BspEI, BspTI, BspXI, BsrBRI, BsrGI, BssHII, BssT1I, Bst1107I, Bst98I, BstBI, BstDSI, BstEII, BstPI, BstSNI, BstXI, BstZI, Bsu15I, Bsu36I, CciNI, CelII, Cfr42I, ClaI, CpoI, Csp45I, CspI, CvnI, DraIII, DsaI, EagI, EclXI, Eco105I, Eco130I, Eco147I, Eco32I, Eco47III, Eco52I, Eco72I, Eco81I, Eco91I, EcoNI, EcoO65I, EcoRV, EcoT14I, EcoT22I, ErhI, Esp1396I, FbaI, FseI, HpaI, Kpn2I, Ksp22I, KspI, LspI, MamI, MfeI, MluI, MluNI, Mph1103I, MroI, MroNI, MscI, MspCI, MunI, Mva1269I, NaeI, NcoI, NgoAIV, NgoMI, NheI, NotI, NruI, NsiI, NspV, PacI, PaeR7I, Pfl23II, PflMI, PinAI, PmaCI, Pme55I, PmeI, PmlI, Ppu10I, PpuMI, PshAI, Psp5II, PspEI, PspLI, PspOMI, PstNHI, RsrII, SacII, SapI, SexAI, SfiI, Sfr274I, Sfr303I, SfuI, SgfI, SgrAI, SmiI, SnaBI, SpeI, SplI, SrfI, SseBI, SspBI, SstII, StuI, StyI, SunI, SwaI, Tth111I, Van91I, Vha464I, XcmI, XhoI, XmaIII, Zsp2I

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Room703, Jungang Induspia V, Sangdaewon-Dong,
Joongwon-Gu, Seongnam, Gyeonggi-Do, 462-120 KOREA
TEL : 82-505-550-5600 | FAX : 82-505-550-5660
www.intronbio.com