Cat. # 6028

For Research Use

TaKaRa

Mighty TA-cloning Kit

Product Manual

v201812Da

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I. Discription

Most PCR-amplified products obtained using a *Taq*-based DNA polymerase have a single base, deoxyriboadenosine (dA), added at the 3′ end. Among the methods for cloning PCR products with 3'-A overhangs, the TA cloning method utilizes a T-vector with a single deoxyribothymidine (dT) addition at the 3′ end. This cloning mechanism takes advantage of the complementarity between the single dT overhang of the vector and the single dA overhang at the end of the PCR product. The Mighty TA-cloning Kit allows for fast, easy cloning of PCR products with this TA cloning method. The DNA Ligation Kit <Mighty Mix> is used for the ligation reaction, enabling highly efficient ligations to be performed simply and quickly.

II. Components (20 reactions)

1. pMD20-T vector (50 ng/μl)	1 μg (20 μl)
2. Ligation Mighty Mix＊1	50 μl x 2
3. Positive Control Insert＊2	10 μl

＊1 The Ligation Mighty Mix has the same composition as the DNA Ligation Kit <Mighty Mix> (Cat. # 6023).

＊2 The Positive Control Insert is comprised of DNA fragments approximately 200-bp in length with dA overhangs at the 3′ end (amplified with *TaKaRa Ex Taq*® using *E. coli* genomic DNA as a template). (10 ng/μl)

III. Materials Required but not Provided

- Chemical competent cells or electrocompetent cells (*E. coli*)
- SOC bacterial culture medium
- LB plates with ampicillin, X-Gal, and IPTG

IV. Storage

-20℃

V. Vector Map

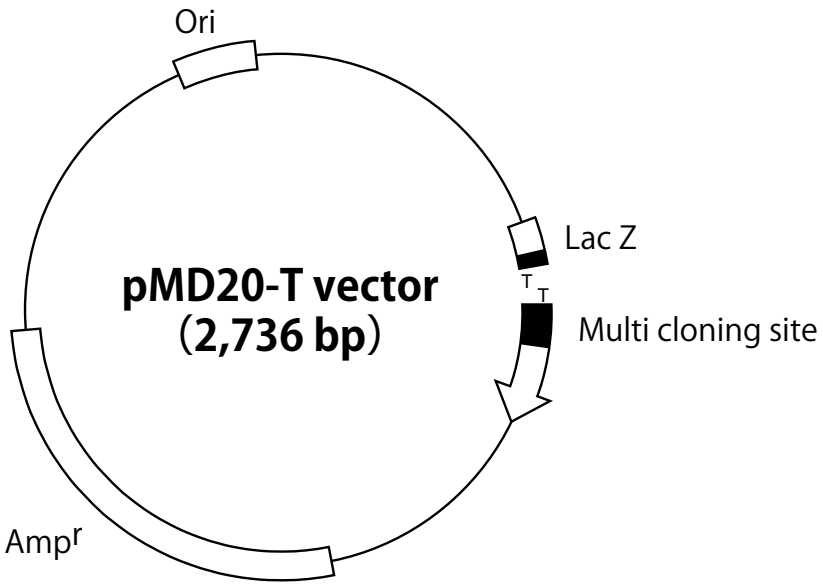

Figure 1. Vector map of pMD20-T

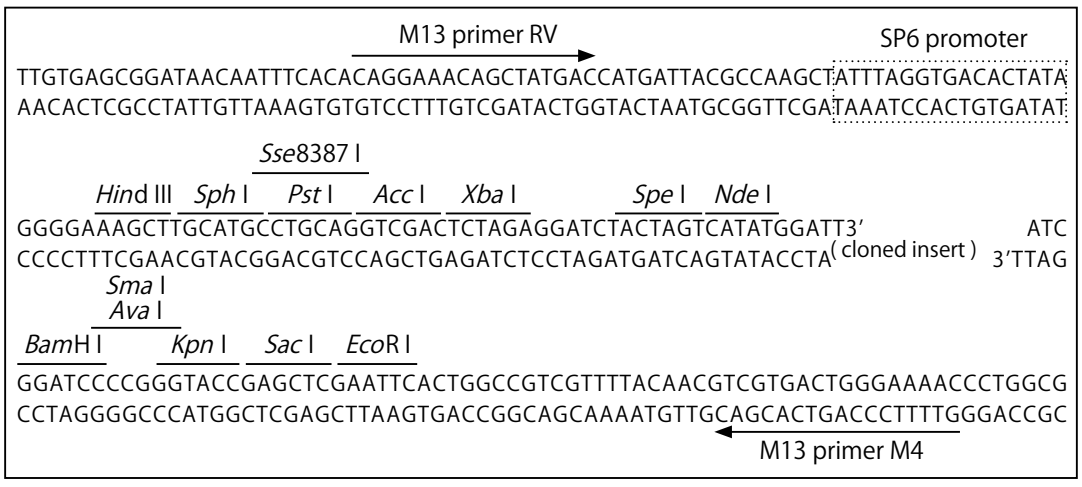

Figure 2. Multiple cloning site in pMD20-T

VI. Precautions

1. Before use, thaw the Ligation Mighty Mix on ice and gently mix.
2. If PCR is performed using a plasmid template with the same selection marker (AmpR in the pMD20-T vector) as the cloning vector, we recommend isolating and purifying the amplified product by gel electrophoresis. This step will avoid the generation of *E. coli* transformants containing the template plasmid.
3. Minimize exposure of the amplified DNA fragment to UV light when isolating and purifying the PCR product via agarose gel electrophoresis. UV irradiation can damage DNA and reduce cloning efficiency.
4. After transformation, there is a possibility that light blue colonies will be present on the LB + Amp/X-Gal/IPTG plates, depending on the length or orientation of the cloned fragment. In this case, perform colony PCR to confirm the presence of the insert DNA.

VII. Protocol

(1) PCR amplification and purification

Perform PCR using Terra™ PCR Direct Polymerase Mix, or *TaKaRa Taq™, TaKaRa Ex Taq*, *TaKaRa Ex Taq* Hot Start Version, *TaKaRa LA Taq®*, or SpeedSTAR™ HS DNA Polymerase, etc. to amplify your target DNA. Confirm the amplified product by gel electrophoresis using a portion of the reaction mixture.

- If the amplified product yields a single band, proceed to the ligation reaction (Section 2).
- If primer dimers are present, perform a simple purification using NucleoSpin Gel and PCR Clean-up, and then proceed to the ligation reaction (Section 2).
- If a non-specific amplified band is present, cut the target band from the agarose gel and purify the insert from the gel using NucleoSpin Gel and PCR Clean-up. Following this purification, proceed to the ligation reaction (Section 2).

(2) Ligation reaction

1. Add 1 μl*1 of the PCR product above to a new microtube.
2. Add 1 μl of pMD20-T vector and 3 μl*1 of sterile purified water to the tube, and mix.
   *1 Adjust accordingly so that the PCR product and sterile purified water are 4 μl of the total volume.
3. Add 5 μl of Ligation Mighty Mix and gently mix.
4. Incubate at 16℃ for 30 minutes.
5. Transform 100 μl of *E. coli* competent cells*2 using the total volume from Step 4. If you use electroporation to transform, do so only after purifying the DNA via phenol/chloroform extraction followed by ethanol precipitation.
   *2 Use *E. coli* HST08 Premium Competent Cells or *E. coli* JM109 Competent Cells. (If using *E. coli* HST08 Premium Competent Cells, IPTG is not required.)
6. Plate the transformation mixture on LB plates containing ampicillin, X-Gal, and IPTG.*3
   *3 Add an adequate amount of SOC Medium and plate on multiple LB plates.
7. Incubate the plate at 37℃ overnight and select white colonies as candidates.

(3) Confirmation of insert DNA

Colony PCR allows you to easily check the insert size in the plasmid in the *E. coli* transformants.
The M13 primers M4 and RV can be used to perform colony PCR in this system using EmeraldAmp® PCR Master Mix or SapphireAmp® Fast PCR Master Mix.

VIII. Control Reactions

1. Add 1 μl of Positive Control Insert DNA to a new microtube.
2. Add 1 μl of pMD20-T vector and 3 μl of sterile purified water to the tube, and mix.
3. Add 5 μl of Ligation Mighty Mix and gently mix.
4. Incubate at 16℃ for 30 minutes.
5. Transform 100 μl of *E. coli* competent cells＊1 using the total volume from Step 4. If you use electroporation to transform, do so only after purifying the DNA via phenol/chloroform extraction followed by ethanol precipitation.

   ＊1 Use *E. coli* HST08 Premium Competent Cells or *E. coli* JM109 Competent Cells. (If using *E. coli* HST08 Premium Competent Cells, IPTG is not required.)
6. Plate the transformation mixture on LB plates containing ampicillin, X-Gal, and IPTG.
7. Incubate the plate at 37℃ overnight and select white colonies as candidates.＊2

   ＊2 If using *E. coli* JM109 Competent Cells with a transformation efficiency of 1 x 10^8 colonies/ μg pUC119 DNA, you will obtain about 1 - 5 x 10^4 white colonies per 50 ng of vector.

IX. Related Products

Mighty TA-cloning Reagent Set for PrimeSTAR® (Cat. # 6019)*
Mighty Cloning Reagent Set (Blunt End) (Cat. # 6027)
T-Vector pMD20 (Cat. # 3270)
E. coli HST08 Premium Competent Cells (Cat. # 9128)
E. coli HST08 Premium Electro-Cells (Cat. # 9028)
E. coli JM109 Competent Cells (Cat. # 9052)
E. coli JM109 Electro-Cells (Cat. # 9022)
M13 Primer M4 (Cat. # 3832A/B)
M13 Primer RV (Cat. # 3830A/B)

Terra™ PCR Direct Polymerase Mix (Cat. #639270, 639271)
MightyAmp™ DNA Polymerase Ver.3 (Cat. # R076A/B)*
TaKaRa Ex Taq® (Cat. # RR001A/B)
TaKaRa Ex Taq® Hot Start Version (Cat. # RR006A)
TaKaRa LA Taq® (Cat. # RR002A)
TaKaRa LA Taq® Hot Start Version (Cat. # RR042A)
TaKaRa Taq™ (Cat. # R001A/B)
TaKaRa Taq™ Hot Start Version (Cat. # R007A/B)

SpeedSTAR™ HS DNA Polymerase (Cat. # RR070A/B)
EmeraldAmp® PCR Master Mix (Cat. # RR300A)*
EmeraldAmp® MAX PCR Master Mix (Cat. # RR320A)
SapphireAmp® Fast PCR Master Mix (Cat. # RR350A)
NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)

＊ Not available in all geographic locations. Check for availability in your area.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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