日本語データシート

Product Name: **FEW^{Blue} TA Cloning Kit (pTAC-1)**

(Previous name：TA PCR Cloning Kit (pTAC-1))

Kit Component

Cat. #	Product	Size
DS125	FEW^{Blue} TA Cloning Kit (pTAC-1)	20 reactions
	pTAC-1 Vector, linearized	20 µl (50 ng/µl)
	2 × Ligation Buffer	100 µl
	Ligase Mixture	20 µl
	M13 BDFw Primer	100 µl (3.2 pmol /µl)
	M13 BDRev Primer	100 µl (3.2 pmol /µl)
DS125L	FEW^{Blue} TA Cloning Kit (pTAC-1), Large	80 reactions
	pTAC-1 Vector, linearized	20 µl (50 ng/µl) × 4
	2 × Ligation Buffer	100 µl × 4
	Ligase Mixture	20 µl × 4
	M13 BDFw Primer	100 µl (3.2 pmol /µl) × 4
	M13 BDRev Primer	100 µl (3.2 pmol /µl) × 4

Storage Conditions: -20ºC

Introduction

The cloning of PCR products into plasmids is a routine step in many molecular biology processes. The FEW^{Blue} TA Cloning Kit is a PCR cloning kit based on T-A pairing of PCR products and T vector.

The procedure is fast and easy. PCR products are simply mixed with pTAC-1 Vector, 2 × Ligation Buffer and Ligase mixture, then incubated at 16 ºC for 30 min. The ligation products can be used directly to transform Chemical Competent Cells.

Procedure of PCR Cloning

1. DNA Amplification

Quality of PCR product is critical for the success of the TA cloning. Several important notes are shown as follows.

- The amount of 3'-A overhang of PCR products directly affects the ligation efficiency between PCR products and the T vector. The high extension activity of 3'-A overhang of PCR products by non-proofreading DNA polymerase will be obtained using PCR primer with a 5'-terminal A. We strongly recommend attaching an A at the 5'-end of your PCR primers.
- We recommend performing a final extension step for 10 min at 72 ºC during the PCR reaction for efficient addition of 3'-A overhang of PCR products.
- Direct cloning of PCR products generated with proofreading DNA polymerases will result in lower cloning efficiency because these PCR products don't have the 3'-A overhang necessary for TA Cloning. See Cloning with blunt-end PCR product in Additional Information.
- The PCR product should be checked by the agarose gel electrophoresis before TA cloning.
- The PCR sample contains a complex mixture which interferes with the cloning. We recommend removing the contaminants by a silica-based spine column to increase the cloning efficiency

and decrease false-positive colonies derived from the primer-dimmers or other short reaction products.

- If your PCR template is a plasmid DNA containing ampicillin resistance gene, the PCR product should be cut by incubating 50-100 µl PCR reaction mixture with 10 - 20 units of *Dpn*I for 30 min at 37 ºC without changing the buffer, followed by a silica-based spin column purification.

2. Ligation Protocol

1. Set up the 10 µl ligation reaction mixture on ice as follows:

pTAC-1 vector (50 ng/µl)	1 µl
PCR product	X µl*1
2 × Ligation Buffer	5 µl
Ligase Mixture	1 µl*2
Distilled water	variable
Total volume	10 µl

2. Incubate at 16 ºC for 30 min.
3. Transform Chemical Competent Cells (50 µl) with the ligation product (5 µl).*3

*1 We recommend using a molar ratio of 2–6 times more PCR product DNA than pTAC-1 Vector DNA for ligation (50 ng, 0.028 pmol). For example, more than 36 ng (0.056 pmol) of 1,000 bp PCR product can be used. However, less PCR product may also be sufficient because of the high cloning efficiency of this kit as shown in the Example on page 3. We recommend checking the amount and quality of PCR product DNA from the gel analysis. When performing electrophoresis, we recommend using a molecular weight marker (e.g., DynaMarker ® DNA Low D (#DM112) or DynaMarker ® DNA High D (#DM122)) that allows you to estimate the amount of DNA based on the degree of band staining.

*2 We recommend adding Ligase Mixture last.

*3 The ligation products can be used directly for the transformation of Chemical Competent Cells. The ligation reaction mixture can be stored at –20 ºC, until transformation.

3. Transformation Protocol

Follow the instructions provided with your competent cells for the transformation procedure.

We recommend using DynaCompetent Cells JetGiga DH5α (#DS230), which is the competent cells with high transformation efficiency (> 1 × 10^9 CFU / 100 µl / tube)*. (See the Example on P.3)

Transformation of DynaCompetent Cells JetGiga DH5α takes only 6 min. The total procedure, from PCR product to plating, takes ~ 36 min.

*Since the amount of competent cells used for one transformation is 50 µl, the transformation efficiency is > 5 × 10^8 CFU / 50 µl / tube.

4. Clone Screening

Pick up the colonies and grow each of them overnight in 3-5 ml LB medium containing 100 µg/ml of ampicillin. Isolate the plasmid and analyze by restriction enzyme digestion or sequencing.

Alternatively, colony PCR can be performed to screen the transformants by the sequence primers supplied

with the kit or your PCR primers of the target gene.

5. Sequencing

For sequencing your insert in pTAC-1 vector, two sequence primers are provided with the Kit.

M13 BDFw Primer; 5'- CAGGGTTTTCCCAGTCACGAC-3'
M13 BDRev Primer; 5'- CGGATAACAATTTCACACAGG -3'

M13 BDFw Primer anneals to pTAC-1 vector 45 bases upstream of *Eco*RI site. Whereas M13 BDRev Primer anneals to pTAC-1 vector 26 bases down stream of *Hin*dIII site.

Additional Information

1. Cloning with Blunt-end PCR Product

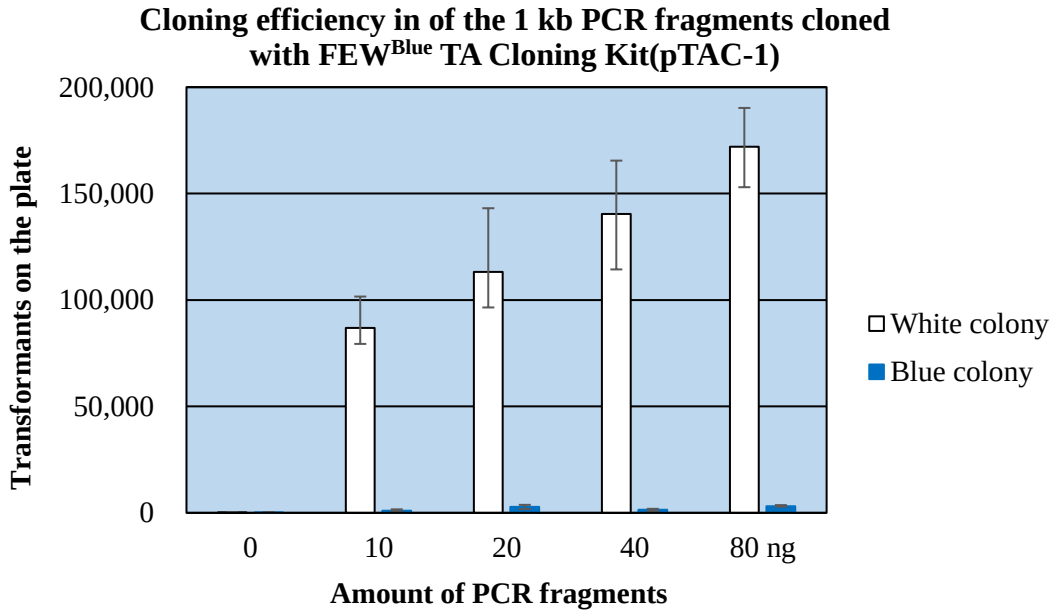
Amplification by Proofreading polymerases gives blunt-end PCR products. For cloning of such blunt-end PCR products into the T-vector, the 3'-A overhangs should be added as follows.

1. Add 1 unit of *Taq* polymerase to the blunt-end PCR product sample (~100 µl). *1
2. Incubate at 72 ºC for 10 minutes.
3. Purify with a silica-based spin column. *2

*1 It isn't necessary to change the buffer.
*2 When using a spin column for purification, the PCR product may be diluted depending on the elution volume. If necessary, the eluate should be concentrated by ethanol precipitation.

2. Example

Differing amounts of about 1 kb PCR fragments were cloned according to the standard protocol using the FEW^{Blue} TA Cloning Kit (pTAC-1). 50 µl of $^{DynaCompetent\ Cells}$ JetGiga DH5α was transformed and the whole amount was spread onto LB agar plates. The white bars and the blue bars show the numbers of white colonies and blue colonies, respectively. It shows low background and high cloning efficiency.



Troubleshooting

Problem	Possible Cause	Solution
Few or no colonies	Incorrect antibiotic.	Check selective growth medium.
Low number of white colonies	Proofreading DNA polymerase was used for PCR.	The PCR product generated with proofreading DNA polymerases do not have the 3'-A overhang necessary for TA Cloning. The 3'-A overhang should be attached by Taq polymerase. See "Cloning with Blunt-end PCR Product".
	Not enough PCR fragment	Check the amount of PCR fragment by electrophoresis or spectrophotometory and use a sufficient amount. Often spectrophotometer measurement of DNA following spin-column purification is inaccurate due to some contamination. We strongly recommend checking by the electrophoresis.
	Presence of inhibitor in PCR products.	Purify the PCR product just by silica-based spin column.
	Cloned insert is not tolerated by *E.coli.*	Incubate the plate and liquid culture not at 37 ºC but at room temperature.
	Excess volume of the ligation reaction mixture was added to the competent cell.	The volume of the ligation product should be no more than 5 % of that of the chemical competent cell.
Only white colonies	All colonies contain insert.	(Cloning is successful.)
	No X-Gal in plate	Check that the plate contains sufficient X-Gal.
	Contamination of plasmid DNA containing Amp^r used as PCR template	We recommend cutting template plasmid with *Dpn*I in PCR reaction mixture or gel-purifying the PCR products. See "DNA Amplification" page 3.
	Inactivation of antibiotic	Use the fresh selective growth medium.
White colonies with blue center or light blue colonies	Leaky expression of the *lacZ* fragment	Pick out these types of colonies and check inserts as some of them may contain insert. In this case, conversely clear white colonies may contain no insert.
Half of the colonies are clear white and The other half of the colonies are white colonies with blue center or light blue colonies.	Leaky expression of the *lacZ* fragment according to insert direction in the vector.	Pick out both types of colonies and check inserts as some of them may contain insert with just directional difference in the vector. Such insert may have SD sequence and an inflame start codon.
Do not grow in liquid culture.	These are satellite colonies.	Be sure to pick out large white colonies and check the ampicillin plate.
	Keep the plate long before picking colonies.	Plasmids containing an insert are shed from the cell or kill the cell on the plate as the cloned insert is not tolerated by *E.coli* to some degree. Pick out colonies from fresh plate.
White colonies do not contain insert.	Primer-dimmers or Non-specific PCR products were cloned.	Improve the PCR reaction condition to obtain a single and discrete band on the gel.
White colonies do not contain plasmid.	Cloned insert is not tolerated by *E.coli* to some degree.	Plasmids containing the insert are rapidly shed from the cell immediately after running out of ampicillin in the liquid culture. Add more ampicillin to the culture medium. In rare cases, even if plasmids containing the insert are rapidly shed from the cell, the cells grow in liquid culture containing enough ampicillin. It may happen that ampicillin resistance gene of the plasmid is integrated into the chromosome. In the case of the latter, incubate the plate and liquid culture not at 37 ºC but at room temperature.

Related Products:

DS230	DynaCompetent® Cells JetGiga DH5α ・Size：100 µl × 10 ・Rapid transformation (6 minutes) and extremely high efficiency (>1×10^9 CFU/100 µl/tube) ・High stability against a freeze–thaw cycle		
DS210	DynaCompetent® Cells JM109	DM122	DynaMarker® DNA High D
DM112	DynaMarker® DNA Low D		

About pTAC-1 vector

pTAC-1 is identical to pUC19 except for multiple cloning site, T7 primer binding site and *lacZ*α gene stop codon.

Sequence around cloning Site

M13-BDFw Primer binding site

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TTG GGT AAC GCC AGG GTT TTC CCA GTC ACG ACG TTG TAA AAC GAC GGC
AAC CCA TTG CGG TCC CAA AAG GGT CAG TGC TGC AAC ATT TTG CTG CCG
 Q   T   V   G   P   N   E   W   D   R   R   Q   L   V   V   A
```

T7 Primer binding site

EcoRI SacI KpnI SmaI

```
CAG CGC GTA ATA CGA CTC ACT ATA GGG CGA ATT CGA GCT CGG TAC CCG
GTC GCG CAT TAT GCT GAG TGA TAT CCC GCT TAA GCT CGA GCC ATG GGC
 L   A   Y   Y   S   E   S   Y   P   S   N   S   S   P   V   R
```

XhoI BamHI

```
GGA TCT CGA GGC CAG ATC T   A ATT GTG GAT CCG CTC
CCT AGA GCT CCG GTC TAG A   T TAA CAC CTA GGC GAG
 S   R   S   A   L   D       N   H   I   R   E
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XbaI SalI PstI PaeI HindIII

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TAG AGT CGA CCT GCA GGC ATG CAA GCT TGG CGT AAT CAT GGT CAT AGC
ATC TCA GCT GGA CGT CCG TAC GTT CGA ACC GCA TTA GTA CCA GTA TCG
 L   T   S   R   C   A   H   L   S   P   T   I   M   T   M
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LacZα-Peptide

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TGT TTC CTG TGT GAA  ATT GTT ATC CGC TCA CAA
ACA AAG GAC ACA CTT  TAA CAA TAG GCG AGT GTT
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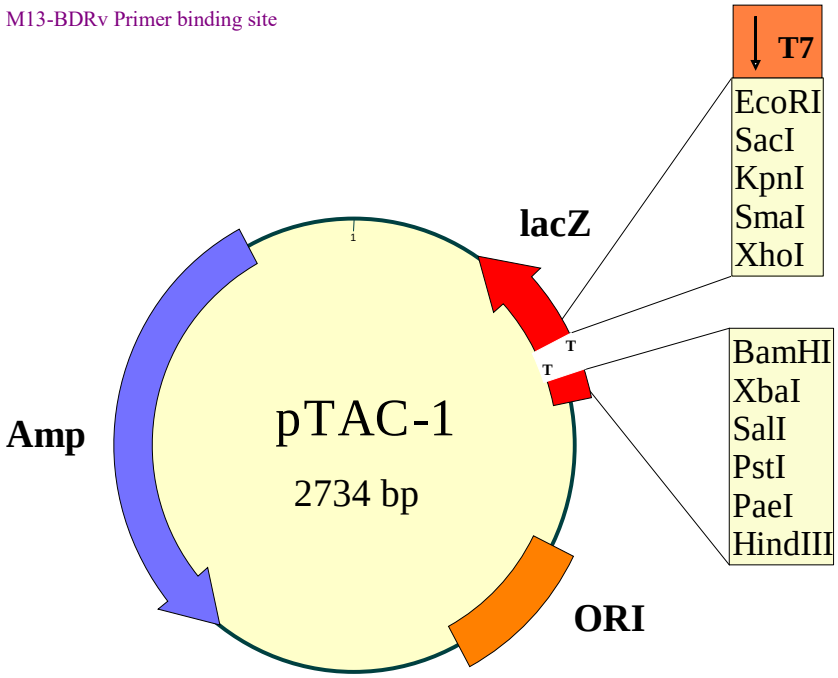
M13-BDRv Primer binding site



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www.biodynamics.co.jp