

Product Name: **FEW^{Blue} TA Cloning Kit (pTAC-2)**
(Previous name: TA PCR Cloning Kit (pTAC-2))

日本語データシート



Kit Component

Cat. #	Product	Size
DS126	FEW^{Blue} TA Cloning Kit (pTAC-2)	20 reactions
	pTAC-2 Vector, linearized	20 μ l (50 ng/ μ l)
	2 \times Ligation Buffer	100 μ l
	Ligase Mixture	20 μ l
	M13 Forward Primer	100 μ l (3.2 pmol/ μ l)
	M13 Reverse Primer	100 μ l (3.2 pmol/ μ l)
DS126L	FEW^{Blue} TA Cloning Kit (pTAC-2), Large	80 reactions
	pTAC-2 Vector, linearized	20 μ l (50 ng/ μ l) \times 4
	2 \times Ligation Buffer	100 μ l \times 4
	Ligase Mixture	20 μ l \times 4
	M13 Forward Primer	100 μ l (3.2 pmol/ μ l) \times 4
	M13 Reverse Primer	100 μ l (3.2 pmol/ μ l) \times 4

Storage Conditions: -20°C

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-2 Vector, 2 \times Ligation Buffer and Ligase mixture, then incubated at 16 °C 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 °C 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-dimers 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 μ l PCR reaction mixture with 10 - 20 units of *DpnI* for 30 min at 37 °C without changing the buffer, followed by a silica-based spin column purification.

2. Ligation Protocol

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

pTAC-1 vector (50 ng/ μ l)	1 μ l
PCR product	X μ l ^{*1}
2 \times Ligation Buffer	5 μ l
Ligase Mixture	1 μ l ^{*2}
<u>Distilled water</u>	<u>variable</u>
Total volume	10 μ l

2. Incubate at 16 °C for 30 min.

3. Transform Chemical Competent Cells (50 μ l) with ligation product (5 μ l).^{*3}

^{*1} We recommend using a molar ratio of 2–6 times more PCR product DNA than pTAC-2 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 °C, until transformation.

3. Transformation Protocol

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

We recommend using *DynaCompetent Cells* JetGiga DH5 α (#DS230), which is the competent cells with high transformation efficiency ($> 1 \times 10^9$ cfu / 100 μ l / tube). (See the Example on P.3)

Transformation of *DynaCompetent Cells* JetGiga DH5 α 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 μ l, the transformation efficiency is $> 5 \times 10^8$ cfu / 50 μ l / tube.}

4. Clone Screening

Pick up the colonies and grow each of them overnight in 3-5 ml LB medium containing 100 μ g/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-2 vector, two sequence primers are provided with the Kit.

M13 Forward Primer; 5'- TGTAACGACGGCCAGT-3'

M13 Reverse Primer; 5'- CAGGAAACAGCTATGAC -3'

M13 Forward Primer anneals to pTAC-2 vector 77 bases upstream of the cloning site. Whereas M13 Reverse Primer anneals to pTAC-2 vector 92 bases down stream of the cloning 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.

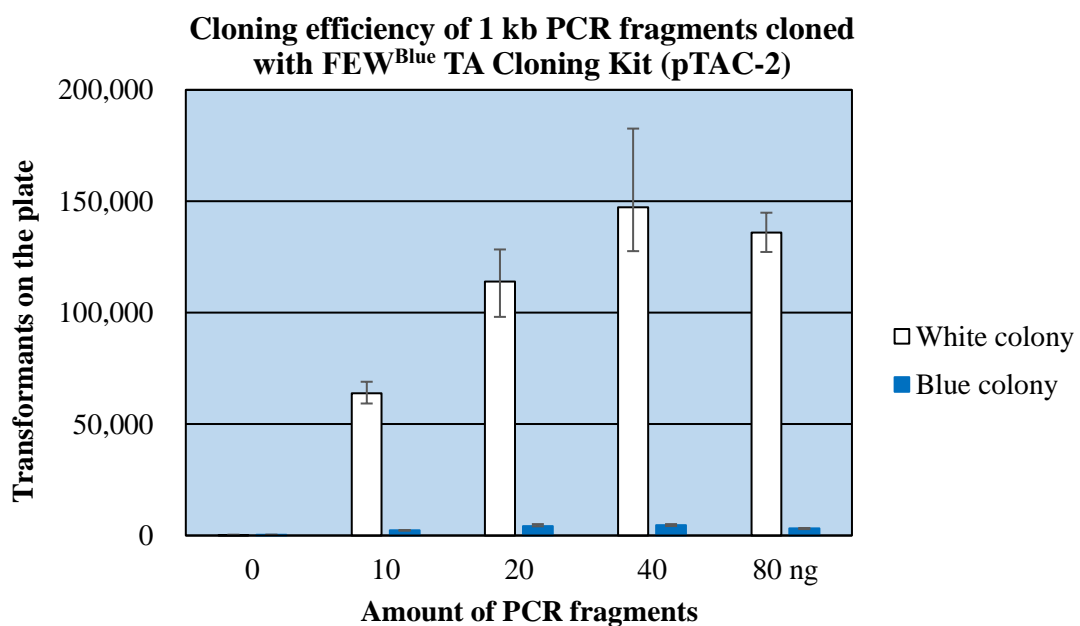
1. Add 1 unit of *Taq* polymerase to the blunt-end PCR product sample (~100 μ l). *¹
2. Incubate at 72 °C for 10 minutes.
3. Purify with a silica-based spin column. *^{2,3}

*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-2). 50 μ l of *DynaCompetent Cells* JetGiga DH5 α 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 spectrophotometry 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 <i>E.coli</i> .	Incubate the plate and liquid culture not at 37 °C 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 <i>DpnI</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 <i>lacZ</i> 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 <i>lacZ</i> 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 inframe 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 <i>E.coli</i> 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 <i>E.coli</i> 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 °C 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-2 vector

pTAC-2 is identical to pUC19 except for multiple cloning site, T7 primer binding site, SP6 primer binding site and *lacZa* gene stop codon.

Sequence around cloning Site

