Product Name: DynaExpress TA PCR Cloning Kit (pTAC-1)

Kit Component

Cat. #	Product	Size
DS125	DynaExpress TA PCR Cloning Kit (pTAC-1)	20 reactions
Box 1 (-20℃)	pTAC-1 Vector, linearized	20 µl (50 ng/µl) ×1
	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	DynaExpress TA PCR Cloning Kit (pTAC-1), Large	80 reactions
Box 1 (-20℃)	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
DS120	DynaExpress TA PCR Cloning Kit (pTAC-1), with Jet Competent Cell	20 reactions
Box 1 (-20℃)	pTAC-1 Vector, linearized	20 µl (50 ng/µl) × 1
	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)
Box 2 (-80℃)	Jet Competent Cell (DH5α)	100 µl × 20
Bottle (RT/-80℃)	Recovery Medium	10 ml

Storage Conditions:
Box 1 should be stored at -20℃. Box 2 should be stored at -80℃ directly from a dry ice shipping box, and stable at -80℃ with little or no loss in transformation efficiency for 12 months from the date of receipt.
Bottle should be stored at room temperature (or -80℃). To avoid precipitation in medium, slow freezing or freeze-thaw cycle (for example, storage around -20℃) should not be done, although transformation efficiency is not very affected by precipitation.

Competency of Competent Cell:
Jet Competent Cell (DH5α) 100 µl > 2.0×10^8 cfu/ µg・100 µl cell (pUC19)

Genotype of *E coli* strain :
DH5α : *supE*44, Δ*lac*U169(φ80*lacZ*ΔM15), *hsdR*17, *recA*1, *endA*1, *gyrA*96, *thi*-1, *relA*1

Introduction

The cloning of PCR products into plasmids is a routine step in many molecular biology processes. The DynaExpress TA PCR Cloning Kit is a PCR cloning kit based on T-A pairing of PCR products and T vector. This kit is manufactured by new technologies of ligation and transformation. The 2 × Ligation Buffer which contains all other reagents and cofactors, provides highly efficient ligation of PCR products into plasmid. Jet Competent Cell has a benefit to save a time for cloning.

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℃ for 30 min. The ligation products can be used directly to transform Jet Competent Cells. Transformation of Jet Competent Cells takes only 10 min without heat shock. The total procedure, from PCR product to plating, takes ~ 40 min.

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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 10min at 72℃ 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℃ 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℃ for 30 min.
3. Transform Jet competent cells with 4 µl of ligation product.*[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 4. We recommend checking the amount and quality of PCR product DNA from the gel analysis.

*[2] We recommend adding Ligase Mixture last.

*[3] The ligation products can be used directly for the transformation of competent cells. The ligation reaction mixture can be stored at –20℃, until transformation.

3. Transformation Protocol

1. Spread 25 µl of 20 mg/ml X-Gal on the LB agar plates and allow the reagent to be absorbed. *1
2. Thaw the Jet competent cell on ice (100 µl in a tube of each transformation).
3. Add 4 µl of ligation reaction mixture directly to the competent cells and mix by flicking gently.
4. Incubate the tube on ice for 5 minutes.
5. Transfer 50 µl of the cell to a new 1.5 ml sterilized tube containing 0.45 ml of Recovery medium and mix the tube contents by vortex for one second. *2
6. Incubate the tube at room temperature for 5 minutes.
7. Spread 10 µl - 200 µl of the cell to a LB agar plate containing ampicillin and X-Gal.
8. Incubate the plate at 37°C overnight.

*1 For blue-white color screening, spread X-Gal and IPTG on the LB agar plates and allow the reagent to be absorbed 30 minutes prior to inoculating cells. As DH5α does not have *lacl*$_q$, IPTG is not required basically.

*2 Recovery medium is pre-warmed at room temperature to 37°C.

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 (~100µl). *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 A silica-based spin column purification kit such as PCR cleaning Kit (Q-Biogene), Rapid PCR Purification Kit (Marligen) or MiniElute PCR Purification Kit (Quagen) is useful.

*3 It can be used for TA cloning. If necessary, the eluate should be concentrated by ethanol precipitation.

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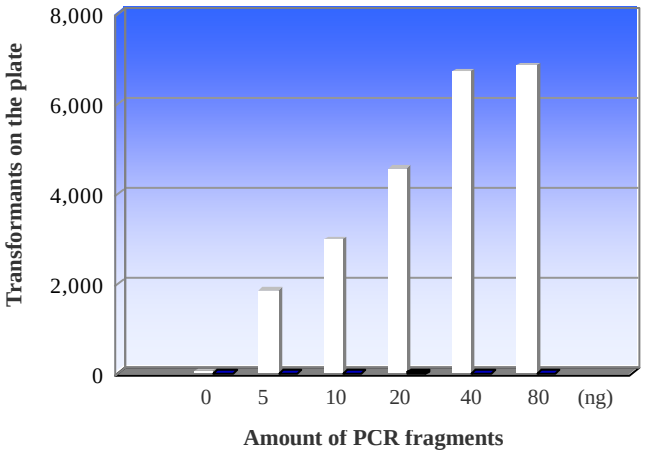
2. Example

Differing amounts of about 1 kb PCR fragments were cloned according to the standard protocol using the DynaExpress TA PCR Cloning Kit. About half of the amounts of transformed competent cells (200 µl) were spread onto LB agar plates. The white bars and the blue bars show the numbers of white colonies and blue colonies, respectively.



Troubleshooting

Problem	Possible Cause	Solution
Few or no colonies	The cells have lost competence due to improper storage or shipping conditions.	Check the frozen cell suspension in the tube. If there is the cell precipitate at the tube bottom, the competent cell has already been thawed somewhere and lost competence. Alternatively, check transformation efficiency using a plasmid such as pUC19.
	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 ℃ 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	Leaky expression of the *lacZ* fragment according to	Pick out both types of colonies and check inserts as some of them may contain insert with just directional

half of the colonies are white colonies with blue center or light blue colonies	insert direction in the vector.	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. Alternatively, gel-purify the PCR fragment with the Gel Indicator DNA Extraction kit (code No. DM550)
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℃ but at room temperature.

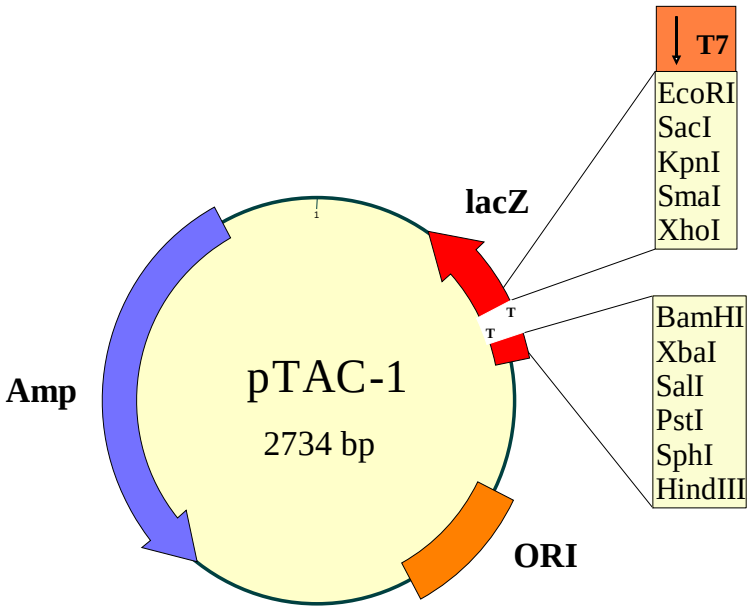
Related Products:

DM112	DynaMaker® DNA Low D	DM132	DynaMaker® for Plasmid D
DM122	DynaMaker® DNA High D	DS210	Competent Cell JM109
DS220	Competent Cell DH5α		

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



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