Product Name: DynaExpress TA PCR Cloning Kit (pTAKN-2)

Kit Component

Cat. #	Product	Size
DS130	DynaExpress TA PCR Cloning Kit (pTAKN-2)	20 reactions
Box 1 (-20℃)	pTAKN-2 Vector, linearized	20 µl (50 ng/µl) ×1
	2 × Ligation Buffer	100 µl
	Ligase Mixture	20 µl
	M13 Forward Primer	100 µl (3.2 pmol /µl)
	M13 Reverse Primer	100 µl (3.2 pmol /µl)
DS130L	DynaExpress TA PCR Cloning Kit (pTAKN-2), Large	80 reactions
Box 1 (-20℃)	pTAKN-2 Vector, linearized	20 µl (50 ng/µl) ×4
	2 × Ligation Buffer	100 µl × 4
	Ligase Mixture	20 µl × 4
	M13 Forward Primer	100 µl (3.2 pmol /µl) × 4
	M13 Reverse Primer	100 µl (3.2 pmol /µl) × 4

Storage Conditions: -20℃.

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. The 2 × Ligation Buffer which contains all other reagents and cofactors, provides highly efficient ligation of PCR products into plasmid.

The procedure is fast and easy. PCR products are simply mixed with pTAKN-2 Vector, 2 × Ligation Buffer and Ligase mixture, then incubated at 16℃ 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 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.

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- If your PCR template is a plasmid DNA containing kanamycin 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:

pTAKN-2 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 chemical competent cells directly with the ligation product.*[3], *[4]

*[1] We recommend using a molar ratio of 2–6 times more PCR product DNA than pTAKN-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.

*[2] We recommend adding Ligase Mixture last.

*[3] The ligation products can be used directly for the transformation of chemical competent cells. The volume of the ligation products should be not more than 5% of that of the chemical competent cell. The ligation reaction mixture can be stored at –20 ℃, until transformation.

We recommend the use of Competent Cell DH5α (code No. 220) or Competent Cell JM109 (code No. 210) for efficient cloning (see the Example on page 3).

*[4] The effective concentration range of kanamycin in the medium is known to be relatively narrow as compared with ampicilline. The concentration of kanamycin in agar plate should be about 25 µg/ml.

4. Clone Screening

Pick up colonies and grow each of them overnight in 3-5 ml LB medium containing 25 µg/ml of kanamycin. 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 pTAKN-2 vector, two sequence primers are provided with the Kit.

M13 Forward Primer; 5'- TGTAAAACGACGGCCAGT-3'
M13 Reverse Primer; 5'- CAGGAAACAGCTATGAC -3'

M13 Forward Primer anneals to pTAKN-2 vector 77 bases upstream of the cloning site. Whereas M13 Reverse Primer anneals to pTAKN-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.

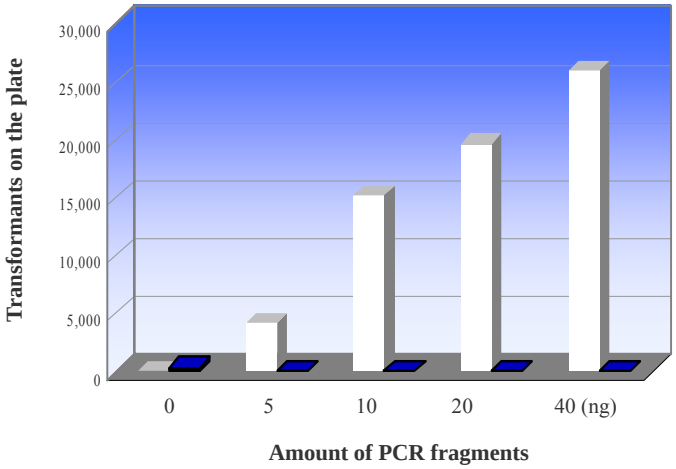
1. Add 1 unit of *Taq* polymerase to the blunt-end PCR product sample (~100µl). *[1]
2. Incubate at 72 °C for 10 minutes.
3. Purify with a silica-based spin column. *[2, 3]

Ver. 1.0

*1 It isn't necessary to change the buffer.
*2 A silica-based spin column purification kit such as PCR Purification Kit (Biodynamics Lab Inc code No. DS350), 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.

2. Example

Differing amounts of about 1 kb PCR fragments were ligated to pTAKN-2 vector according to the standard protocol using the DynaExpress TA PCR Cloning Kit. Four µl of the ligation products were used to transform 100 µl of Competent Cell DH5α (code No.220). About quarter of the amounts of transformed competent cells 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. Alternatively, gel-purify the PCR product using Gel Indicator DNA Extraction kit (code No. DM550).
	PCR fragment has been damaged by UV during the gel-purification.	We recommend the Gel Indicator DNA Extraction kit (code No. DM550) to obtain gel-purified PCR products without UV exposure.

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	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 Kan^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. 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	DM550	Gel Indicator™ DNA Extraction Kit
DS220	Competent Cell DH5α	DS210	Competent Cell JM109
DS350	PCR Product Purification Kit		

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About pTAKN-2 vector

pTAKN-2 is identical to pTAC-2 except for Kanamycin resistance gene.

Sequence around cloning Site

M13 (-21) Forward Primer Binding Site

```
TTC CCA GTC ACG ACG TTG TAA AAC GAC GGC CAG TGA GCT AGT GTA ATA
AAG GGT CAG TGC TGC AAC ATT TTG CTG CCG GTC ACT CGA TCA CAT TAT
 E   W   D   R   R   Q   L   V   V   A   L   S   S   T   Y   Y
```

T7 Primer Binding Site | NotI | EcoRI | SacI | KpnI | SmaI

```
CGA CTC ACT ATA GGG CGC GGC CGC AGA ATT CGA GCT CGG TAC CCG GGA
GCT GAG TGA TAT CCC GCG CCG GCG TCT TAA GCT CGA GCC ATG GGC CCT
 S   E   S   Y   P   A   A   A   S   N   S   S   P   V   R   S
```

XhoI | BamHI

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

XbaI | SalI | PstI | SphI | HindIII | NotI

```
TAG AGT CGA CCT GCA GGC ATG CAA GCT TGC GGC CGC GTA TTC TAT AGT
ATC TCA GCT GGA CGT CCG TAC GTT CGA ACG CCG GCG CAT AAG ATA TCA
 L   T   S   R   C   A   H   L   S   A   A   A   Y   E   I   T
```

SP6 Primer Binding Site | M13 Reverse Primer Binding Site

```
GTC ACC TAA ATA GCA TGG CGT AAT CAT GGT CAT AGC TGT TTC CTG TGT
CAG TGG ATT TAT CGT ACC GCA TTA GTA CCA GTA TCG ACA AAG GAC ACA
 D   G   L   Y   C   P   T   I   M   T   M
```

LacZ α -Peptide