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

Kit Component

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS125</td>
<td>DynaExpress TA PCR Cloning Kit (pTAC-1)</td>
<td>20 reactions</td>
</tr>
<tr>
<td><strong>Box 1 (-20°C)</strong></td>
<td>pTAC-1 Vector, linearized</td>
<td>20 µl (50 ng/µl) × 1</td>
</tr>
<tr>
<td></td>
<td>2 × Ligation Buffer</td>
<td>100 µl</td>
</tr>
<tr>
<td></td>
<td>Ligase Mixture</td>
<td>20 µl</td>
</tr>
<tr>
<td></td>
<td>M13 BDFw Primer</td>
<td>100 µl (3.2 pmol/µl)</td>
</tr>
<tr>
<td></td>
<td>M13 BDRev Primer</td>
<td>100 µl (3.2 pmol/µl)</td>
</tr>
<tr>
<td>DS125L</td>
<td>DynaExpress TA PCR Cloning Kit (pTAC-1), Large</td>
<td>80 reactions</td>
</tr>
<tr>
<td><strong>Box 1 (-20°C)</strong></td>
<td>pTAC-1 Vector, linearized</td>
<td>20 µl (50 ng/µl) × 4</td>
</tr>
<tr>
<td></td>
<td>2 × Ligation Buffer</td>
<td>100 µl × 4</td>
</tr>
<tr>
<td></td>
<td>Ligase Mixture</td>
<td>20 µl × 4</td>
</tr>
<tr>
<td></td>
<td>M13 BDFw Primer</td>
<td>100 µl (3.2 pmol/µl) × 4</td>
</tr>
<tr>
<td></td>
<td>M13 BDRev Primer</td>
<td>100 µl (3.2 pmol/µl) × 4</td>
</tr>
<tr>
<td><strong>Box 2 (-80°C)</strong></td>
<td>Jet Competent Cell (DH5α)</td>
<td>100 µl × 20</td>
</tr>
<tr>
<td><strong>Bottle (RT/-80°C)</strong></td>
<td>Recovery Medium</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

Storage Conditions:
Box 1 should be stored at -20°C. Box 2 should be stored at -80°C directly from a dry ice shipping box, and stable at -80°C 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°C). To avoid precipitation in medium, slow freezing or freeze-thaw cycle (for example, storage around -20°C) should not be done, although transformation efficiency is not very affected by precipitation.

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

Genotype of E. coli strain:
DH5α: supE44, Δ lacU169(ϕ 80lacZ Δ M15), hsdR17, recA1, endA1, gyrA96, thi-1, relA1

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°C 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.
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 µl 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 µl ligation reaction mixture on ice as follows:
   - pTAC-1 vector (50 ng/µl) 1 µl
   - PCR product X µl
   - 2 × Ligation Buffer 5 µl
   - Ligase Mixture 1 µl
   - Distilled water variable
   - Total volume 10 µl

2. Incubate at 16°C for 30 min.
3. Transform Jet competent cells with 4 µl of ligation product.*

*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°C, 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 lacIq, 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 EcoRI site. Whereas M13 BDRev
Primer anneals to pTAC-1 vector 26 bases downstream of HindIII 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 ºC 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.
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.

<table>
<thead>
<tr>
<th>Troubleshooting</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Few or no colonies</td>
<td>The cells have lost competence due to improper storage or shipping conditions.</td>
<td>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.</td>
</tr>
<tr>
<td>Low number of white colonies</td>
<td>Proofreading DNA polymerase was used for PCR</td>
<td>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”.</td>
</tr>
<tr>
<td>Not enough PCR fragment</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Presence of inhibitor in PCR products.</td>
<td></td>
<td>Purify the PCR product just by silica-based spin column.</td>
</tr>
<tr>
<td>Cloned insert is not tolerated by E.coli.</td>
<td></td>
<td>Incubate the plate and liquid culture not at 37 ( ^\circ )C but at room temperature.</td>
</tr>
<tr>
<td>Excess volume of the ligation reaction mixture was added to the competent cell.</td>
<td></td>
<td>The volume of the ligation product should be no more than 5 % of that of the chemical competent cell.</td>
</tr>
<tr>
<td>Only white colonies</td>
<td>All colonies contain insert. (Cloning is successful.)</td>
<td>No X-Gal in plate Check that the plate contains sufficient X-Gal.</td>
</tr>
<tr>
<td>Contamination of plasmid DNA containing Amp(^r) used as PCR template</td>
<td>We recommend cutting template plasmid with DpnI in PCR reaction mixture or gel-purifying the PCR products. See “DNA Amplification” page 3.</td>
<td></td>
</tr>
<tr>
<td>Inactivation of antibiotic</td>
<td>Use the fresh selective growth medium.</td>
<td></td>
</tr>
<tr>
<td>White colonies with blue center or light blue colonies</td>
<td>Leaky expression of the ( lacZ ) fragment</td>
<td>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.</td>
</tr>
</tbody>
</table>
| 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

<table>
<thead>
<tr>
<th>Insert direction in the vector.</th>
<th>difference in the vector. Such insert may have SD sequence and an inflame start codon.</th>
</tr>
</thead>
</table>

Do not grow in liquid culture

<table>
<thead>
<tr>
<th>These are satellite colonies.</th>
<th>Be sure to pick out large white colonies and check the ampicillin plate.</th>
</tr>
</thead>
</table>

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.

<table>
<thead>
<tr>
<th>Primer-dimmers or Non-specific PCR products were cloned.</th>
<th>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)</th>
</tr>
</thead>
</table>

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 plasmid.

<table>
<thead>
<tr>
<th>Cloned insert is not tolerated by <em>E. coli</em> to some degree.</th>
<th>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.</th>
</tr>
</thead>
</table>

**Related Products:**

<table>
<thead>
<tr>
<th>DM112</th>
<th>DynaMaker® DNA Low D</th>
<th>DM132</th>
<th>DynaMaker® for Plasmid D</th>
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<tbody>
<tr>
<td>DM122</td>
<td>DynaMaker® DNA High D</td>
<td>DS210</td>
<td>Competent Cell JM109</td>
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<tr>
<td>DS220</td>
<td>Competent Cell DH5α</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**About pTAC-1 vector**

pTAC-1 is identical to pUC19 except for multiple cloning site, T7 primer binding site and *lacZα* gene stop codon.
Sequence around cloning Site

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 CGG
Q T V G P N E W D R R Q L V V A

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

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

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

TGT TTC CTG TGT GAA ATT GTT ATC CGC TCA CAA
ACA AAG GAC ACA CTT TAA CAA TAG GCG AGT GTT

Amp
lacZ
pTAC-1
2734 bp
ORI

New Feature