BioDynami AnyPCR Cloning Kit (with 3.0 kb cloning vector)

Catalog No. 20006S: 12 reactions (without competent cells)
Catalog No. 20006L: 48 reactions (without competent cells)
Catalog No. 20007S: 12 reactions (with DH5α competent cells)
Catalog No. 20007L: 48 reactions (with DH5α competent cells)

Storage: Two different storage temperatures required
At -20°C and -80°C (competent cells) for periods up to 6 months.
Avoid many freeze-and-thaw cycles.

Description

The BioDynami AnyPCR Cloning Kit provides a highly efficient cloning strategy for any type of PCR product: blunt ends, sticky ends, 5’-phosphorylated ends, and 5’-non-phosphorylated ends. The versatile cloning ability of the kit makes PCR cloning very easy, as it skips the requirements for regular PCR cloning such as identifying type of DNA polymerase, finding the right type of PCR cloning kit, using phosphorylated PCR primers, and treating PCR products with polynucleotide kinase.

Our kit has an extremely low background, eliminates the use of X-GAL/IPTG screening (blue-white colony screening), and makes it free of false negatives and false positives.

Our unique components in the kit make it possible to simplify the regular transformation procedure. Our kit with the simplified transformation protocol is compatible with any competent cells.

Features of the kit:
- Compatible with any types of DNA polymerase
  - Non-proofreading enzymes such as Taq DNA polymerases
  - Proofreading enzymes
- Work with any types of PCR products
  - Blunt ends
  - Sticky ends
- Phosphorylation of PCR primers is not necessary
  - Both phosphorylated primers and non-phosphorylated primers can be used
  - Treatment of PCR product with polynucleotide kinase is not necessary
- Extremely low background
- Fast and simple transformation protocol

Related products
Component

- 2X PCR Cloning Solution
- PCR Cloning Enzyme
- Positive Control
- Sequencing Primers
  - KS1 primer: CACTAAAGGAACAAAAGCTGGGT
  - KS2 primer: CTCACTATAGGCAGATGGAGCT
- DH5α Competent Cells (Cat. # 20007 only)

Storage Condition

- Two different storage temperatures required. Store kit at -20°C, competent cells at -80°C. Stable up to 6 months. Avoid many freeze-and-thaw cycles.

Reagent & Equipment Needed (not provided in this kit)

- LB plates containing 50 μg/mL ampicillin or carbenicillin
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g., plates, spreaders)
- Competent cells (for Cat.#20006)
Protocol

PCR cloning reaction
1. Add the following (including reactions for positive control and negative control) into PCR tubes and mix by gently pipetting several times.

<table>
<thead>
<tr>
<th></th>
<th>PCR Sample</th>
<th>Positive Control</th>
<th>Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insert fragment</td>
<td>PCR fragment 3.5 ul*</td>
<td>Positive control 3.5 ul**</td>
<td>water 3.5 ul</td>
</tr>
<tr>
<td>2X PCR Cloning Solution</td>
<td>5 ul</td>
<td>5 ul</td>
<td>5 ul</td>
</tr>
<tr>
<td>PCR Cloning Enzyme</td>
<td>1.5 ul</td>
<td>1.5 ul</td>
<td>1.5 ul</td>
</tr>
<tr>
<td>Total volume</td>
<td>10 ul</td>
<td>10 ul</td>
<td>10 ul</td>
</tr>
</tbody>
</table>

2. Incubate at 20°C for 1 hour.

* PCR fragment amount: Insert size <1 kb: 45 ng
  Insert size 1-2 kb: 90 ng
  Insert size 2-3 kb: 135 ng
  Insert size 3-4 kb: 180 ng
  Insert size 4-5 kb: 225 ng
  Insert size 5-7 kb: 300 ng
  Insert size >7 kb: 400 ng

** Positive Control: 1.1 kb PCR fragment

Transformation:
1. Thaw competent cells on wet ice.
2. Add 2 ul of the ligation reaction to 50 ul of competent cells on ice. Gently tap tubes several times to mix, and quickly put tubes back on ice.
3. Incubate on ice for 30 minutes.
4. Put tubes in a 42°C water bath for 42 seconds.
5. Transfer tubes to ice for 2 minutes.
6. Plate transformed cells on an agar plates containing 50 μg/mL of ampicillin or carbenicillin.
7. Incubate the plate overnight at 37°C.

Analysis of positive clones
1. Pick 2~6 colonies and culture overnight at 37°C in LB medium containing 50 μg/mL of ampicillin or carbenicillin.
2. Isolate plasmid DNA.
3. Analyze the PCR inserts by restriction digestion, PCR screening, or proceed directly to sequencing.

Restriction digestion
Multiple cloning sites of the vector can be found on page 4.

PCR screening
Commercial colony PCR kit can be used to identify inserts directly from colonies.

Sequencing
The insert can be sequenced in order to confirm the correct orientation or the insert is not mutated. Sequencing primers are included in the kit. Refer to the vector map on page 4 for the sequence surrounding the multiple cloning sites. If the primers included in the kit do not completely sequence the insert, synthesize additional primers may needed.
Multiple Cloning sites of pAnyPCR3.0 and sequencing primers

ATAACAAATTCACACAGGAAACAGGCTATGACCATGATTACGCCAACGGCGCAATTAAACCCCT
TATGTTAAAGGTGATCTCTTTCGATACTGTAATGACGGTTTCGCGGTATAATTGGGA

KS1 primer
CACCTAAAGGGAAACAAAGCTGGGTACCGGGCCCTCCCTGAGGTCAGGTATCAGATACCA
GTGATTTCCTTGTCTTGCACCACATGGGCGGGGGGAGCTCCAGCTGCCCATAGCTATGGT

PCR Product
ATATCAATGCGCTGCAGCCCGGGGATCCACTAGTTCTAGAG
GGCCGT

SacI
CACCCCGGTTGGAGCTCCAATTGC GCCCTA TAGTGAGTCGTTATACGC GCTCAGGTGATACCTAGCAGCCGC

SacII
CGTTTACAACGTCGACTGGGAAACCCCTGGCGT TACCAACTTAATCGCCCTTGTACGCA
GCAAAATGTACGACCTACCCCTTTCGGAAGCGCAATGGGGATTAATACGGCAACGTCTG

KS2 primer
**Trouble Shooting**

**Problem**: Few or no colonies

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination in PCR product.</td>
<td>Purify DNA using column or beads to remove contamination.</td>
</tr>
<tr>
<td>DNA degraded or insufficient DNA.</td>
<td>Check DNA by gel electrophoresis. Determine DNA concentration and add the correct amount. Use the supplied positive control to test the system.</td>
</tr>
<tr>
<td>Incorrect amounts of antibiotic or wrong antibiotic was used in agar plates.</td>
<td>Check correct amount of Ampicillin or Carbenicillin was used in agar plates. Do not spread antibiotic onto the surface of agar plates.</td>
</tr>
<tr>
<td>The transformation efficiency of competent cells is too low</td>
<td>Use competent cells with high transformation efficiency. Check the transformation efficiency of competent cells.</td>
</tr>
</tbody>
</table>

**Problem**: High background of colonies that do not contain inserts.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligonucleotides or non-specific PCR products contamination in ligation.</td>
<td>Gel purify DNA before ligation.</td>
</tr>
<tr>
<td>Enzyme contamination in ligation.</td>
<td>Purify DNA using column or beads.</td>
</tr>
<tr>
<td>Incorrect amounts of antibiotic or wrong antibiotic was used in agar plates.</td>
<td>Check correct amount of Ampicillin or Carbenicillin was used for agar plates. Do not spread antibiotic onto the surface of agar plates.</td>
</tr>
</tbody>
</table>
Quality Control

- Kit components passed stringent functional quality test.
- Exonuclease and endonuclease activities are not detectable in all components.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to www.BioDynami.com for Material Safety Data Sheet of the product.

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