# [Cat. No.] K-7110, K-7120, K-7130

#### Introduction

AccuRapid<sup>™</sup> Cloning Kit is designed for directional cloning of up to 3 PCR products into linearized vectors without the need for restriction enzymes, ligase, or phosphatase. This kit contains an optimized Enzyme Mix, which fuses PCR products and linearized vectors by annealing a 18-21 bp complementary sequence located at their ends. The 18-21 bp homologue sequence is added to the extension primers for the amplification of the inserts.



Figure 1. Workflow for cloning

### Features & Benefits

- Convenient: Directional cloning of inserts into a vector is possible without the use of restriction enzymes, a ligase, or a phosphatase.
- Rapid: Clones accurately in 30 min.
- Accurate: Cloning of up to 3 inserts is possible through precise design of extension primers.

#### Components

Components	K-7110
AccuRapid™ Enzyme Mix	45 µl
2 kb pBHA Control Vector (25 ng/µl)	3 µl
750 bp Control Insert (50 ng/µl)	5 µl

\* Note: For research use only. Not for use in diagnostic or therapeutic procedures.

#### Storage

Store at -20°C.

#### **Online Resources**





Korean

Visit our product page for additional information and protocols.

## **Ordering Information**

Description	1	Cat. No.
	10 rxns x 1 ea	K-7110
AccuRapid™ Cloning Kit	10 rxns x 2 ea	K-7120
	10 rxns x 5 ea	K-7130

### Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

## Explanation of Symbols



Use-by

Date

REF Catalog Number

Manufacturer RUO Use



Temperature Limitation

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# **Experimental Procedures**

1       Image: Construction of linearized vector         2       Extension primer design         2       Extension primer design         3       Insert amplification         3       Insert amplification         4       Coning         4       Coning         5       8. Add the reaction mixture to the <i>E. coli</i> cells to be transformed.	Steps		Procedure Details						
2       2. Design extension primers which contain a 18-21 bp complementary sequence at the end or linearized vector.         2       Extension primer design         3       Insert amplification         3       Insert amplification         4       Insert amplification         5       Insert amplification         6       Note: Refer to the User's guide for additional information.         3       Insert amplification         3       Perform the PCR for the amplification of insert.         4       Insert amplification         5       Preparation of reaction mixture.         9       Preparation of reaction mixture         1011111111111111111111111111111111111	1	Preparation of linearized vector	1. Prepare a linearized vector through PCR or treatment with restriction enzymes.						
<ul> <li>3 Insert amplification</li> <li>3. Perform the PCR for the amplification of insert.</li> <li>4. Purify the samples using a PCR/Gel purification kit.</li> <li>5. Prepare the reaction mixture.</li> <li>6. Preparation of reaction mixture</li> <li>1. Perified PCR products (70-150 ng) 1 µl 1 µl 1 µl</li> <li>Purified PCR products (70-150 ng) - 1.5 µl Variable<sup>†</sup></li> <li>Distilled water 5 µl 3.5 µl Variable</li> <li>AccuRapid<sup>™</sup> Enzyme Mix 4 µl</li> <li>Total volume 10 µl</li> <li>* Note: After cloning the reaction mixture should be stored either on ice or at -20°C until transformation.</li> <li>* Purified PCR products need to be 70-150 ng/rxn totally, and 5 µl maximum in volume.</li> <li>(a) I tragment: 5 µl, 2 fragments: 2.5 µl x 2, 3 fragments: 1.7 µl x 3</li> <li>6. Mix the reaction mixture by tapping the tube gently and briefly spin down.</li> <li>7. Incubate the reaction mixture for 30 min at 50°C.</li> <li>8. Add the reaction mixture to the <i>E. coli</i> cells to be transformed.</li> </ul>	2	Extension primer design	2. Design extension primers which contain a 18-21 bp complementary sequence at the end of a linearized vector.          16-21 tp extensions         Complementary to         Source         Forward         Extension Primer         Source         Insert sequence         Source         Insert sequence         Source         Insert sequence         Insert sequence						
<ul> <li>4</li> <li>5. Prepare the reaction mixture.</li> <li>• Preparation of reaction mixture.</li> <li>• Distilled Vector (25-50 ng) 1 µl 1 µl 1 µl 1 µl</li> <li>• Purified PCR products (70-150 ng) -</li> <li>• 1.5 µl Variable<sup>†</sup></li> <li>Distilled water 5 µl 3.5 µl Variable</li> <li>AccuRapid<sup>™</sup> Enzyme Mix 4 µl</li> <li>• Total volume 10 µl</li> <li>• Note: After cloning the reaction mixture should be stored either on ice or at -20°C until transformation.</li> <li><sup>†</sup> Purified PCR products need to be 70-150 ng/rxn totally, and 5 µl maximum in volume.</li> <li>• x) 1 fragment: 5 µl 2 fragments: 2.5 µl x 2, 3 fragments: 1.7 µl x 3</li> <li>6. Mix the reaction mixture by tapping the tube gently and briefly spin down.</li> <li>7. Incubate the reaction mixture for 30 min at 50°C.</li> <li>8. Add the reaction mixture to the <i>E. coli</i> cells to be transformed.</li> </ul>	3	Insert amplification	<ol> <li>Perform the PCR for the amplification of insert.</li> <li>Purify the samples using a PCR/Gel purification kit.</li> </ol>						
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4       Components       Negative       Positive       Sample         Linearized Vector (25-50 ng)       1 µl       1 µl       1 µl       1 µl         Purified PCR products (70-150 ng)       -       1.5 µl       Variable <sup>†</sup> Distilled water       5 µl       3.5 µl       Variable         AccuRapid <sup>™</sup> Enzyme Mix       4 µl         Total volume       10 µl         * Note: After cloning the reaction mixture should be stored either on ice or at -20°C until transformation.         * Purified PCR products need to be 70-150 ng/rxn totally, and 5 µl maximum in volume.         ex) 1 fragment: 5 µl, 2 fragments: 2.5 µl x 2, 3 fragments: 1.7 µl x 3         6. Mix the reaction mixture by tapping the tube gently and briefly spin down.         7. Incubate the reaction mixture for 30 min at 50°C.         8. Add the reaction mixture to the <i>E. coli</i> cells to be transformed.		4 Cloning	Preparation of reaction mixture						
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Transformation	5	Transformation	8. Add the reaction mixture to the <i>E. coli</i> cells to be transformed.						

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