User's Guide

AccuRapid™ Cloning Kit

REF K-7110
K-7120
K-7130

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AccuRapid™ Cloning Kit

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K-7110	K-7120	K-7130
Σ 10	Σ 20	Σ 50

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Please read all the information in booklet before using the unit

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Safety warning and Precaution

AccuRapid™ Cloning Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.
Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information please consult the appropriate Material Safety Data Sheet (MSDS).

Warranty and Liability

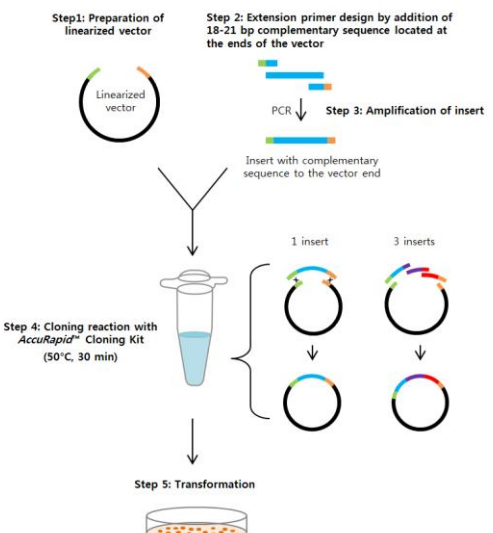
All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Contents

1. Introduction

Bioneer's *AccuRapid*™ Cloning Kit allows accurate and rapid cloning of up to three inserts (PCR products) into a linearized vector. This product recognizes and connects the 18-21 bp complementary sequence at the end of the PCR-amplified insert as well as the end of the linearized vector. This method does not require restriction enzyme treatment on PCR product/plasmid; therefore directional insertion becomes possible. Addition of 18-21 bp complementary sequence into extension primer is only required for amplification of insert.

Schematic protocol for *AccuRapid*™ Cloning Kit



*Green-colored bar: linearized vector
Blue-colored bar: target sequence for cloning
Green, yellow, purple & red-colored bar: 18-21 bp complementary sequence (designated by each color)

2. Features and Benefits

Insert can be cloned into a vector in a desired direction quickly and accurately using extension PCR instead of restriction enzyme treatment.

Multiple inserts can be cloned using the complementary sequence at the end of each fragment in any order.

3. Kit Components

AccuRapid™ Cloning Kit Components	K-7110 (10 rxns)	K-7120 (20 rxns)	K-7130 (50 rxns)
AccuRapid™ Enzyme Mix	45 µl	45 µl x 2	45 µl x 5
2 kb pBHA Control Vector (25 ng/µl)	3 µl	5 µl	5 µl
750 bp Control Insert (50 ng/µl)	5 µl	8 µl	8 µl

* All components of the kit are stored at –20℃

* Related product

Cat. No.	Product Description
S-1002	Oligo Synthesis (Primer) Service
S-2043	AccuGeneBlock Service
S-2041	Gene Synthesis Service
K-2631	*AccuPower*® *ProFi Taq* PCR PreMix
K-3111	*AccuPrep*® Nano-Plus Plasmid Mini Extraction Kit
K-3034	*AccuPrep*® PCR Purification Kit
K-3035	*AccuPrep*® Gel Purification Kit
A-2041-1N	*AllInOneCycler*™ 96 well PCR System
C-9100	Agarose, 100 g
C-9004	50X TAE, 500 ml
A-7020	*Agaro-Power*™ System

4. Cloning experiment using *AccuRapid*™ Cloning Kit

4.1. Linearized vector preparation

In order to use the *AccuRapid*™ Cloning Kit, it is important to completely linearize the vector using restriction enzyme digestion or PCR.
Experiments with perfectly linearized vectors will increase the efficiency of cloning, reducing colony formation of circular vectors without inserts as well as forming many colonies with inserts.
*** In order to increase cloning efficiency, it is recommended to check the linearized vector through electrophoresis after restriction enzyme treatment and proceed to the next experiment.**

a. The vector to be used for cloning is extracted from *E. coli* using the *AccuPrep*® Nano-Plus Plasmid Mini Extraction Kit (K-3111).

b. Linearize the vector by restriction enzyme treatment or PCR.

c. After electrophoresis, the linearized vector is separated from the circularized vector, and then purified by gel elution using *AccuPrep*® Gel Purification Kit (K-3035).

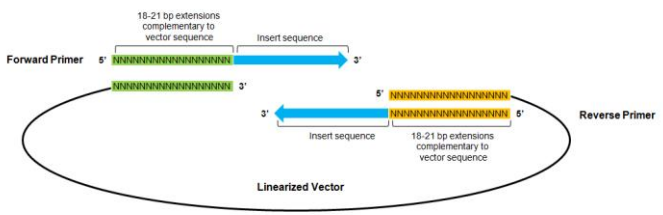
d. Prepare DNA at a concentration of 25 to 50 μg/μl.

4.2. Primer design

The primer to amplify the insert should be designed to contain 18–21 bp complementary sequence at both ends of the linearized vector.

* You can design an extension primer for easy insert amplification on the *AccuRapid*™ Cloning Kit product homepage.

* Bioneer's oligo synthesis service allows you to receive high-quality primers quickly.



ex) When cloning into *Nde*I (CATATG), *Xho*I (CTCGAG) enzyme site.

Vector sequence:

TCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAAT
AATTTTGTTTAACTTTAAGAAGGAGATATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCC
TGGTGCCGCGCGGCAGCCATATG(NdeI)GCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAAT
TCGAGCTCCGTCGACAAGCTTGCGGCCGCA(XhoI)CTCGAGCACCACCACCACCACCACTGAGATCCGGCT
GCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGG
GGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT

3' end vector sequence (Forward primer)

GTGCCGCGCGGCAGCCATATGNNNNNNNNNNNNNNNNNNNNNNNNNN

5' end insert sequence

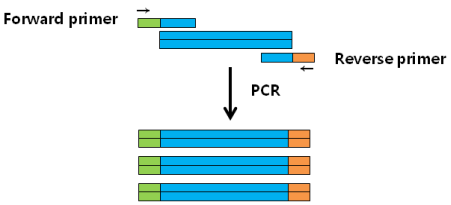
5' end vector sequence (Reverse primer)

GTGGTGGTGGTGGTGCTCGAGNNNNNNNNNNNNNNNNNNNNNNNNNN

3' end insert sequence (Reverse complement)

4.3. Insert amplification

After the primer design, PCR is performed with the synthesized extension primer to amplify the insert (With Bioneer's PCR equipment, *AllInOneCycler*™). Perform a PCR purification (*AccuPrep*® PCR Purification Kit, K-3034) or electrophoresis followed by gel purification (*AccuPrep*® Gel Purification Kit, K-3035) to obtain a purified insert.



Using an existing template

a. Use the *AllInOneCycler*™ to amplify the insert from the template.

b. *AllInOneCycler*™ amplification conditions are as follows.
Template: 1 ng/μl or more (amplification length 500 bp or less)
Primer: 10 pmole each
PCR product: *AccuPower*® *ProFi Taq* PCR PreMix (K-2631)
Cycle:
94 ℃ 1 min
94 ℃ 5 sec ┐
55 ℃ 5 sec ┘ 30 cycles
Time required: Approximately 30 minutes

c. After amplified inserts are electrophoresis, gel eluted and purified with *AccuPrep*® Gel Purification Kit (K-3035).

When using AccuGeneBlock Service

a. PCR insert up to 1 kb size can also be ordered through Bioneer's AccuGeneBlock Service (http://eng.bioneer.com/products/GeneSynthesis/AccuGeneBlockService-overview.aspx). When synthesizing an insert using that service, it is recommended to add a vector complement seqeuence (18-21 bp) at both ends.

4.4. Cloning reaction

For the cloning reaction, mix the reagents as described below, spin down, and incubate at 50°C for 30 minutes using an incubator or PCR machine. When the reaction is over, keep it in ice or at −20°C until use for transformation.

Reaction mixture	Positive control	Negative control	Cloning reaction
Linearized Vector	1 μl of 2 kb pBHA Control Vector	y μl (25–50 ng vector)	y μl (25–50 ng vector)
Purified PCR Fragment	1.5 μl of 750 bp Control Insert	0 μl	x μl* (70–150 ng insert)
D.W.	3.5 μl	(6 − y) μl	(6 − y − x) μl
AccuRapid™ Enzyme Mix	4 μl		
Total	10 μl		
Reaction Condition	50°C, 30 min		

* Purified PCR Fragment should be 70–150 ng total. Depending on the concentration, the amount to be added may vary, but up to 1.5 μl is possible. ex) 1 fragment: 5 μl, 2 fragments: 2.5 μl each, 3 fragments: 1.7 μl each

4.5. Transformation

a. Remove competent cell from deep freezer and slowly thaw on ice.

b. Mix 50–100 µl of competent cells on ice with 10 µl of *AccuRapid* ™ Cloning Kit reagent and incubate on ice for 20 minutes.

c. Heat shock at 42°C for 90 seconds.

d. Add 1 ml of LB medium and incubate for 1 hour at 37°C shaking incubator (200 rpm).

e. Centrifuge at 3,000 rpm, remove the supernatant, add 100 µl of LB medium, and gently thaw the cell pellet by tapping.

f. Transfer to a pre-prepared LB agar plate (including antibiotics) and spread to completely penetrate.

g. Incubate overnight at 37°C in an incubator.

5. Check the Result

Check the colony cultured after the transformation (Colony numbers can be different depending on the size and number of inserts, conditions of competent cells, transformation conditions, etc.).

Single colonies are inoculated into LB broth containing antibiotics and incubated overnight in a shaking incubator (200 rpm). Then use the *AccuPrep*® Nano-Plus Plasmid Mini Kit (K-3111) to extract the plasmid. Restriction enzyme treatment, PCR or sequencing can be used to determine whether cloning is efficient.

6. Quick Protocol of *AccuRapid*™ Cloning Kit

a. Prepare a linearized vector by restriction enzyme treatment or PCR.

b. Extension primer design and order (The extension primer to amplify the insert should be designed to contain 18–21 bp complementary sequence at both ends of the linearized vector).

c. Amplify the insert by PCR and purify.

d. Cloning reaction (store reaction mixture on ice or −20°C until transformation).

Reaction mixture	Positive control	Negative control	Cloning reaction
Linearized Vector	1 μl of 2 kb pBHA Control Vector	y μl (25–50 ng vector)	y μl (25–50 ng vector)
Purified PCR Fragment	1.5 μl of 750 bp Control Insert	0 μl	x μl* (70–150 ng insert)
D.W.	3.5 μl	(6 – y) μl	(6 – y – x) μl
AccuRapid™ Enzyme Mix	4 μl		
Total	10 μl		
Reaction Condition	50°C, 30 min		

* Purified PCR Fragment should be 70–150 ng total. Depending on the concentration, the amount to be added may vary, but up to 1.5 μl is possible.
ex) 1 fragment: 5 μl, 2 fragments: 2.5 μl each, 3 fragments: 1.7 μl each

7. Troubleshooting

Problem aspect	Cause	Resolution
Little or no colony produced	If the insert's extension primer design is incorrect	Check the vector sequence for the right complementary sequence, then design the extension primer again.
	Invalid vector sequence information	After confirming the correct vector sequence through sequencing, proceed.
	If the amount of vector or insert used in the reaction is small	Even if the band is well recognized on the electrophoresis after restriction enzyme treatment or PCR, insert may be contaminated or lost during gel purification or PCR purification. Therefore, after gel extraction or PCR purification, a small aliquot should be used for re-confirmation by electrophoresis then used as an appropriate concentration of reaction.
	When the transformation efficiency of competent cell is low	Validate and use the valid period and efficiency of competent cell.
	If *AccuRapid*™ Cloning Kit reaction proceeded for more than 30 minutes	Since the efficiency of cloning may be reduced in overtime, the reaction proceeds for 30 minutes. If the transformation cannot be done immediately, store the reaction at -20℃.
	Using large amounts of *AccuRapid* ™ Enzyme Mix	Use only 4 µl of *AccuRapid*™ Enzyme Mix.

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