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ExiProgen™ ProXpress PCR Template Kit generates linear template DNA for protein synthesis on Bioneer's *ExiProgen*™. [Cat . # K-7400]

1. Kit Contents and Storage Condition

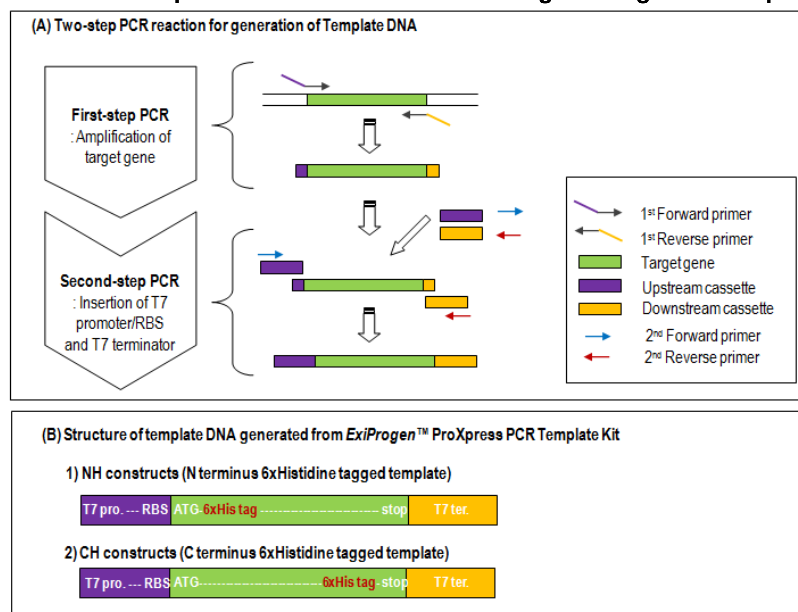
Catalog No.	K-7400	K-7401	K-7400-CP
Number of reactions	**16**	**32**	**100**
AccuPower® ProFi Taq PCR Premix	20 uL x 96 tubes	20 uL x 192 tubes	-
N terminus upstream cassette (5 ng/uL)	70 uL x 1 tubes (Green, NU)	70 uL x 2 tubes (Green, NU)	400 uL x 1 tube
N terminus downstream cassette (5 ng/uL)	70 uL x 1 tubes (Green, ND)	70 uL x 2 tubes (Green, ND)	400 uL x 1 tube
C terminus upstream cassette (5 ng/uL)	70 uL x 1 tubes (Red, CU)	70 uL x 2 tubes (Red, CU)	400 uL x 1 tube
C terminus downstream cassette (5 ng/uL)	70 uL x 1 tubes (Red, CD)	70 uL x 2 tubes (Red, CD)	400 uL x 1 tube
2nd Forward primer (10 pmoles/uL)	70 uL x 1 tubes (Black, 2F)	70 uL x 2 tubes (Black, 2F)	400 uL x 1 tube
2nd Reverse primer (10 pmoles/uL)	70 uL x 1 tubes (Black, 2R)	70 uL x 2 tubes (Black, 2R)	400 uL x 1 tube

->All components of this kit should be stored at -20℃ ~ -70℃

2. Overview

ExiProgen™ ProXpress PCR Template Kit generates template DNA for protein synthesis *via* PCR reaction, with no cloning required. It consists of a two step process that generates template DNA including the appropriate promoter, RBS (Ribosomal Binding Site) and His-tags required to express recombinant proteins; 1) Step One — PCR : Amplification of coding region through PCR from the DNA (cDNA, genomic DNA, plasmid DNA, etc.) which codes for the protein of interest, 2) Step Two — Overlapping PCR : In this step, the T7 promoter and RBS (Ribosomal binding Sequence) is joined to 5'- region of the target gene (coding region) from the first PCR. At the same time, T7 terminator sequence is joined to 3'- region. Finally, linear template for protein synthesis using *ExiProgen*™ is obtained by Gel purification of PCR products from the second step. Figure 1. shows principle of generation of template DNA and its structure.

Figure 1. Principle of generation of template DNA and its structure using *ExiProgen*™ ProXpress PCR Template Kit



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3. Experimental Protocol

3.1. First-step PCR reaction

This section describes detailed experimental protocol to amplify target gene *via* PCR from samples of cDNA, bacterial genomic DNA, or TA cloning vector.

1) Primers design

Primers for the first-step PCR, which is not included in this kit, can be ordered easily for each target gene *via* the Bioneer website (www.bioneer.com). To design the primers, use the 18-bases of DNA sequence from the end of 5'-end of the gene of interest immediately after the ATG codon (as indicated below) for the forward primer. For the reverse primer,18-bases from the 3'-end of the target gene (complementary sequence, immediately before the stop codon) is required. Overlapping sequence will be introduced automatically.

Forward Primer (5' to 3')	XXXXXXXXXXXXXXXXXXXXX-target gene forward sequences(18-mer) Overlapping on upstream cassette
Reverse Primer (5' to 3')	XXXXXXXXXXXXXXXXXXXXX -target gene reverse sequences (18-mer) Overlapping on downstream cassette

Ex) Primers designed for CAT (Chloramphenicol Acetyltransferase)

A. CAT gene sequences

```
ATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGC
ATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGT
AAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGG
AATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTC
CATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACAT
ATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTT
TTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCT
TCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA
GGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATG
AGTGGCAGGGCGGGGCG
```

B. Design of primers NH construct

Forward ; 5'- XXXXXXXXXXXXXXXXXXXXXGAGAAAAAAATCACTGGA-3
target sequences

Reverse ; 5'- XXXXXXXXXXXXXXXXXXXXXCGCCCCGCCCTGCCACTC -3'
target sequences

C. Design of primers CH construct

Forward ; 5'- XXXXXXXXXXXXXXXXXXXXXGAGAAAAAAATCACTGGA -3'
target sequences

Reverse ; 5'- XXXXXXXXXXXXXXXXXXXXXCGCCCCGCCCTGCCACTC -3'
target sequences

2) PCR reaction

A. Prepare PCR reaction mixture using AccuPower® ProFi Taq PCR Premix (20 uL) as follows.

	Negative control	Sample
Template DNA	-	X uL (ex) plasmid DNA -10 ng)
1st Forward primer (10 pmoles/uL)	1 uL	1 uL
1st Reverse primer (10 pmoles/uL)	1 uL	1 uL
DEPC DW	18 uL	(18 - X) uL
Total Volume	20 uL	20uL

B. Close the lid of each tube, thaw its contents for 1 min, then vortex briefly followed by a short spin-down in a microcentrifuge.

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C. Perform the PCR reaction under the following conditions.

Temperature	Time	Repeats
94 ℃	5 min.	-
94 ℃	30 sec.	30~33 cycles
58 ℃	30 sec.	
72 ℃	1 min/kb	
72 ℃	5 min.	-

D. Check the existence of target product with expected size by running of 1% TBE agarose gel, after PCR reaction. The size of target product should be 80-bases larger than the size of your target gene due to the first-step primers.
→ Perform PCR purification using Bioneer *AccuPrep*® PCR purification Kit (Cat. # K-3034) if the target band is the the only amplicon present. In cases where minor bands can be seen on the gel, purify the PCR product *via* gel extraction using Bioneer's *AccuPrep*® Gel Purification Kit (Cat. # K-3035)..

3.2. Second-step Overlapping PCR reaction

- This section describes the protocol to generate linear form DNA template, with T7 promoter, RBS and T7 terminator, for protein synthesis through overlapping PCR using first-step PCR product and cassette sets provided in this kit.
- *AccuPower*® ProFi Taq PCR Premix has been recommended usage of 4 tubes for the second-step PCR

1) PCR reaction

A. Prepare PCR reaction mixture using *AccuPower*® ProFi Taq PCR Premix (20 uL) as follows.

	Negative control	Sample
Template DNA (1st PCR product)	-	X uL (> 10 ng)
Upstream cassette (5 ng/uL)	1 uL	1 uL
Downstream cassette (5 ng/uL)	1 uL	1 uL
2nd Forward primer (10 pmoles/uL)	1 uL	1 uL
2nd Reverse primer (10 pmoles/uL)	1 uL	1 uL
DEPC-DW	16 uL	(16 - X) uL
Total Volume	20 uL	20uL

B. Close the lid of each tube, thaw its contents for 1 min, then vortex briefly followed by a short spin-down in a microcentrifuge.

C. Perform the PCR reaction under the following condition.

Temperature	Time	Repeats
94 ℃	5 min.	-
94 ℃	1 min.	30~33 cycles
48 ℃	1 min.	
72 ℃	1 min/kb	
72 ℃	5 min.	-

D. Check the presence of target product with expected size by running on a 1% TBE agarose gel after PCR. Purify the final product of linear template via gel extraction using Bioneer *AccuPrep*® Gel Purification Kit (Cat. # K-3035). It is recommended to run the second-step PCR product with the first-step PCR product side-by-side on the same gel: the second step PCR product will be -200 bp larger than the first-step one in size.

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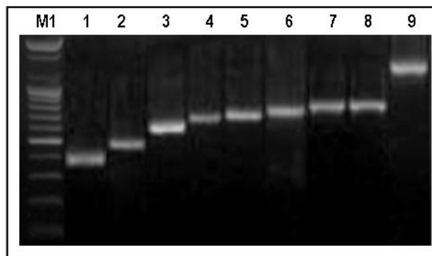
4. Experimental Data

4.1. Production of linear template DNA

< First-step PCR products >



< Second-step Overlapping PCR products >

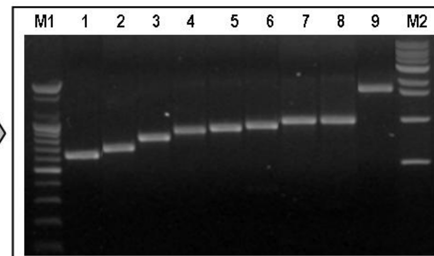


Figure 2. Synthesis of a linear template DNA by using *ExiProgen*™ ProXpress PCR Template Kit.
M1; 100bp DNA Ladder (Bioneer, Cat. # D-1030), M2; 1kb DNA Ladder (Bioneer, Cat. # D-1040)
1; SAV (Template – pT), 2; RNase H (Template – BL21(DE3) gDNA), 3; hGH (Template –pT), 4; CAT (Template - pBIVT),
5; UDG (Template - BL21(DE3) gDNA), 6; AcGFP (Template – pBIVT), 7; EVO (Template –pT), 8; RFP (Template – pIVEX),
9; Poly A polymerase (Template – pET15b),

4-2. Protein Synthesis with linear template DNA by using *ExiProgen*™ ProXpress PCR Template Kit.

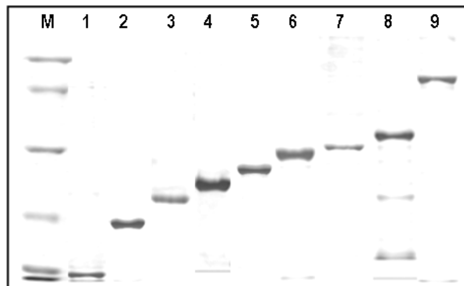


Figure 3. Synthesis of proteins in *ExiProgen*™.
Each linear template DNA was generated by *ExiProgen*™ ProXpress PCR Template Kit, and that was used as template for protein synthesis with *ExiProgen*™ EC Protein Synthesis Kit (Cat. # K-7300).
M; AccuLadder™ Protein Size Marker (Low, Bioneer, Cat. # D-2020),
1; SAV (13 kDa), 2; RNase H (20 kDa),3; hGH (23 kDa),
4; CAT (26.5 kDa), 5; UDG (28 kDa), 6; AcGFP (28 kDa), 7; EVO (30 kDa),
8; RFP (31kDa), 9; Poly A polymerase (54 kDa)

5. Note

It is recommended to use 500ng (for amplicons less than 1 kb in size), or 1 ug (for amplicons of 1-2 kb) of the final PCR product, when *ExiProgen*™ EC Protein Synthesis Kit is used. Idealy the PCR products should have $A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.5 for protein synthesis.

6. Related Products

Cat. No.	Products	Size
K-7300 ~7302	*ExiProgen*™ EC Protein Synthesis Kit	16/32/96 reactions
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit	45 uL x 24 reactions
K-2631	*AccuPower*® *ProFi* Taq PCR PreMix	20 uL x 96 tubes
K-3034	*AccuPrep*® PCR purification Kit	200 preps
K-3035	*AccuPrep*® Gel Purification Kit	200 preps
A-2040-1	*MyGenie* 96	-
A-5041	*ExiProgen*™	-