



SMOBIO

Small Bio, Smart Tool

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Product Information

GetClone™ PCR Cloning Vector II

CV1100 20 reactions

pGet II vector (25ng/μl)	23 μl
pGet-For Primer (10 μM)	100 μl
pGet-Rev Primer (10 μM)	100 μl

Storage

-20°C ≥ 24 months

Features

1. Cloning efficiency greater than 90%
2. IPTG and X-Gal are not required
3. Accepts a wide range of insert/vector ratios 0.5:1 to 12:1
4. Accepts insert size from 6 bp to 11 kb
5. The phosphorylation of PCR fragments is not required
6. Accepts **blunt end** amplicon or DNA fragment (not for sticky ends)
7. Ampicillin and kanamycin selection markers

Description

The GetClone™ PCR Cloning Vector is a positive selection system for high efficiency cloning of blunt end DNA or amplicons. This cloning vector contains a lethal gene which can be disrupted by ligation of a blunt end DNA insert into the cloning site. Only colonies with inserted vectors are able to propagate, eliminating the additional needs of IPTG and X-Gal for blue/white screening. This cloning vector includes ampicillin and kanamycin resistance genes that can meet the needs of most users.

Contents

- pGet II vector
- Sequencing Primers

pGet-For: 5'-TCGAAGTTAAAGATGATTACGG-3'

pGet-Rev: 5'-TCTCTCGATAGCATTTCCTGC-3'

Ligation Example 1 (NEB T4 DNA Ligase #M0202)

Insert (Blunt end)	X μ l (Y ng*)
pGet II (3954 bp)	1 μ l (25 ng)

Mix well then add

10X T4 DNA Ligase Buffer	2 μ l
T4 DNA Ligase	1 μ l
ddH ₂ O	to 20 μ l

Final volume 20 μ l

Mix well then incubate at 16°C or room temperature (20~25°C) for 1 hours.

Ligation Example 2 (TOYOBO Ligation High ver2 #LGK-201)

Insert (Blunt end)	X μ l (Y ng*)
pGet II (3954 bp)	1 μ l (25 ng)
ddH ₂ O	up to 7 μ l
Ligation high ver2	3.5 μ l

Final volume 10.5 μ l

Mix well then incubate at 16°C or room temperature (20~25°C) for 5~30 mins.

*For 3/1 of Insert/Vector molar ratio:

$$Y(\text{ng}) = \frac{3}{1} \times \frac{25(\text{ng}) \times \text{Insert size (kb)}}{3.954 (\text{kb})}$$

Transformation

The GetClone™ is compatible with most available competent *E. coli* cells. Apply 1 ~10 µl of ligation mixture to 10 times volume competent *E. coli* cells. Perform transformation procedures according to the instruction of the competent cell. Spread the transformed *E. coli* cells on an ampicillin (50~100 µg/ml) or Kanamycin (50 µg/ml) LB plate for colony selection.

Suggested colony PCR condition

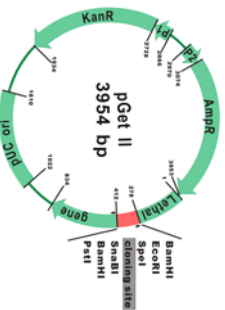
94°C	2 min	} 30~35 cycles
94°C	30 sec	
50°C	30 sec	
72°C	30 sec/kb	
72°C	1 min	

Recommended PCR enzyme or master mix

TP1200, ExcelTaq 5× PCR Master Dye Mix

TP1260, ExcelTaq 5× Fluorescent PCR Master Mix

The plasmid map and cloning sites of pGet II vector



278 **BamHI** _____ **pGet-For primer** _____

5'-GGG ATC CTC GAA GTT AAA GAT GAT TAC GGT GAA TTC AGA ATT CTA GTA GTG GCA GAA
3'-CCC TAG GAG CTT CAA TTT CTA CTA ATG CCA CCA CTT AAG TCT TAA GAT GAT CAC CGT CTT

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GCT AAA CAT CAG GGA AAG GAT _____ **SnaBI** _____ **BamHI**
CGA TTT GTA GTC CCT TTC CTA _____ **Cloning site** _____ TAG TTA CAT GCA TTA TTA CCC TAG GAT CAC

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PstI _____

GGT AAG AGA GGA GAC CAA GAT TTG ATG GCT GCA GGA AAT GCT ATC GAG AGA 3'
CCA TTC TCT CCT CTG GTT CTA AAC TAC CGA CGT TTA CGA TAG CTC TCT 5'

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_____ **pGet-Rev primer** _____

Genetic elements of pGet II vector

Element	Function	Position (bp)
Lethal gene	For screening against self-ligation	1...834
MCS	Multiple cloning site	278...412
Insertion site	The ligation site of blunt end insert	354...355
pUC-ori	Initiation of replication	1022...1610
Kan ^R	Kanamycin resistance gene	1934~2738
P1	The promoter for Kanamycin resistance	2729-2866
P2	The promoter for expressing the ampicillin resistance and lethal gene	2970...3073
Amp ^R	Ampicillin resistance gene	3074...3953

The restriction enzyme with one or two restriction sites on pGet II

Enzyme	Positions	Enzyme	Positions
AclI	3261, 3634	MscI	2518
ApaI	1353, 3191	NaeI	2098
BamHI	279, 375	NarI	2598, 2846
BanII	2239	NcoI	2164
BglII	434	NgoMIV	2096
BsaBI	2741,2765	PciI	1667, 3954
BspHI	947, 3023	PstI	418
BspMI	2328(c), 2709	PvuI	3494
BssHII	2199	RsrII	2081
BtgI	2164	Scal	3382
EagI	2690	SnaBI	368
EcoRI	315	SpeI	322
FspI	2498,3640	SphI	2199
HinCI	568 822	SspI	3058
HindIII	204	TatI	3380
HpaI	568, 822		

For more details of the sequence of the GetClone vector, visit our website www.smobio.com

Related Products

DM1100	ExcelBand 50 bp DNA Ladder, 500 μ l
DM2100	ExcelBand 100 bp DNA Ladder, 500 μ l
DM2300	ExcelBand 100 bp+3K DNA Ladder, 500 μ l
DM3100	ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 μ l
DM3200	ExcelBand 1 KB Plus (0.1-10 kb) DNA Ladder, 500 μ l
DM4100	ExcelBand XL 25 kb DNA Ladder, Broad Range (up to 25 kb), 500 μ l
DL5000	FluoroDye DNA Fluorescent Loading Dye (Green, 6 \times), 1 ml
DS1000	FluoroStain DNA Fluorescent Staining Dye (Green, 10,000 \times), 500 μ l
TF1000	ExcelTaq SMO-HiFi DNA Polymerase, 1 U/ μ l, 100 U \times 1
TP1000	ExcelTaq DNA Polymerase, 500 U \times 1
TP1200	ExcelTaq 5 \times PCR Master Dye Mix, 200 RXN
TP1260	ExcelTaq 5 \times Fluorescent PCR Master Mix Kit, 200 RXN
TP2000	ExcelTaq Blood Direct DNA Polymerase, 5 U/ μ l, 500 U \times 1
TP2100	ExcelTaq Blood Direct PCR Master Mix Kit, 200 RXN