SMOBIO®
Small Bio, Smart Tool

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Product Information GetClone™series

GetClone™ PCR Cloning Vector II

CV1100	**20 RXN**	
	pGet II Vector (25 ng/µl)	23 µl
	pGet-For Primer (10 µM)	100 µl
	pGet-Rev Primer (10 µM)	100 µl

Storage

-20°C for 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. Resistance to ampicillin and kanamycin

Description

The GetClone™ PCR Cloning Vector II is a positive selection system for high efficiency cloning of blunt end DNA or PCR products amplified by high fidelity DNA polymerase. The linearized GetClone™ pGet II Vector contains a lethal gene which can be disrupted by ligation of a blunt end DNA insert into the cloning site. After ligation and transformation, only *E.coli* clones carrying the pGet II Vector with inserted DNA at cloning site are able to propagate LB-ampicillin or LB-Kanamycin agar plates, eliminating the additional needs of IPTG and X-Gal for blue/white screening. The GetClone™ pGet II Vector includes ampicillin and kanamycin resistance genes that can meet the needs of most users.

Primers Sequence

pGet-For Primer:

5'-TCGAAGTTAAAGATGATTACGG-3'

pGet-Rev Primer:

5'-TCTCTCGATAGCATTTCCTGC-3'

Ligation Example 1 (NEB T4 DNA Ligase #M0202)

Insert (Blunt end)	X µl (Y ng*)
pGet II (3954 bp)	1 µl (25 ng)
Mix well then add	
10X T4 DNA Ligase Buffer	2 µl
T4 DNA Ligase	1 µl
ddH_2O	to 20 µl
Final volume	20 µl

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 µl (Y ng*)
pGet II (3954 bp)	1 µl (25 ng)
ddH_2O	up to 7 µl
Ligation high ver2	3.5 µl
Final volume	10.5 µl

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:

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

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 cells. Spread the transformed *E. coli* cells on an LB-ampicillin (50~100 µg/ml) or LB-Kanamycin (50 µg/ml) plate for colony selection.

Recommended colony PCR condition

(SMOBIO's TP1200 ExcelTaq™ 5X PCR Master Dye Mix is suggested)

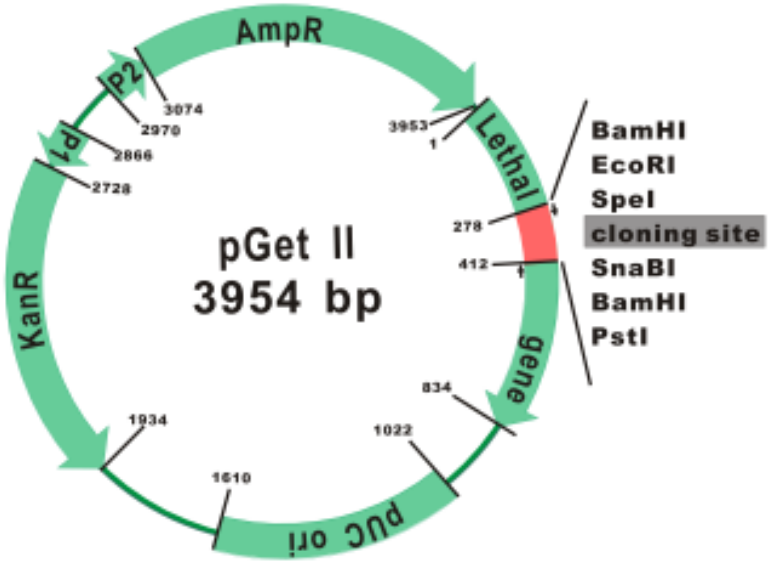
Template	Single colony
Forward primer	0.1 – 0.5 µM
Reverse primer	0.1 – 0.5 µM
5X PCR Master Dye Mix	5 µl
ddH_2O	Variable
Total volume	25 µl

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

Note: The amplicon size of the colony PCR is "insert size +152 bp" when using pGet-For Primer and pGet-Rev Primer.

P06-F11-A1

The plasmid map and cloning sites of pGet II Vector



278 BamHI pGet-For primer EcoRI SpeI

5′-GGG ATC CTC GAA GTT AAA GAT GAT TAC GGT GAA TTC AGA ATT CTA CTA GTG GCA GAA

3′-CCC TAG GAG CTT CAA TTT CTA CTA ATG CCA CTT AAG TCT TAA GAT GAT CAC CGT CTT

354 355 SnaBI BamHI

GCT AAA CAT CAG GGA AAG GAT Cloning site ATC AAT ATA CGT AAT AAT GGG ATC CTA GTG

CGA TTT GTA GTC CCT TTC CTA TAG TTA CAT GCA TTA TTA CCC TAG GAT CAC

PstI 435

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 CCT TTA CGA TAG CTC TCT-5′

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
Primer position		
pGet-For primer	Sequencing of insert, colony PCR	284-305
pGet-Rev primer	Sequencing of insert, colony PCR	415-435

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

Enzyme	Positions	Enzyme	Positions
AclI	3261, 3634	MscI	2518
ApaLI	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	ScaI	3382
EagI	2690	SnaBI	368
EcoRI	315	SpeI	322
FspI	2498,3640	SphI	2199
HincII	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

CK1000	Champion E. coli Transformation Kit
DM2300	ExcelBand 100 bp+3K DNA Ladder, 500 µl
DM3200	ExcelBand 1 KB Plus (0.1-10 kb) DNA Ladder, 500 µl
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000×), 500 µl
PM2500	ExcelBand 3-color Regular Range Protein Marker, 250 µl × 2
PM2800	ExcelBand 3-color Extra Range Protein Marker, 250 µl × 2
RP1000	ExcelRT Reverse Transcriptase, 20,000 U
TF1000	SMO-HiFi DNA Polymerase, 100 U
TF3000	G-HiFi DNA Polymerase, 100 U
TP1000	ExcelTaq DNA Polymerase, 500 U
TP5000	ExcelTaq Hot Start II DNA Polymerase, 500 U
TP1200	ExcelTaq 5× PCR Master Dye Mix, 200 RXN
VE0100	B-BOX Blue Light LED epi-illuminator, AC 100-240V, 50/60Hz
WM1000	YesBlot Western Marker I, 250 µl

The latest version of the manual can be downloaded from www.smobio.com/shop.

For Research use only
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