SMOBIO
Small Bio, Smart Tool

www.smobio.com

## Product Information

**GetClone™ PCR Cloning Vector**

| | | |
|---|---|---|
| **CV1000** | **20 RXN** | |
| | pGet1.1 Vector (50 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)

## Description

The GetClone™ PCR Cloning Vector 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™ pGet1.1 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 pGet1.1 Vector with inserted DNA at cloning site are able to propagate on LB-ampicillin agar plates, eliminating the additional needs of IPTG and X-Gal for blue/white screening.

## 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*) |
| pGet1.1 (2995 bp) | 1 µl (50 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*) |
| pGet1.1 (2995 bp) | 1 µl (50 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:

$$\mathrm{Y(ng)} = \frac{3}{1} \times \frac{50(\mathrm{ng}) \times \mathit{Insert\ size}\ (\mathrm{kb})}{2.995\ (\mathrm{kb})}$$

## 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) plate for colony selection.

## Recommended colony PCR condition

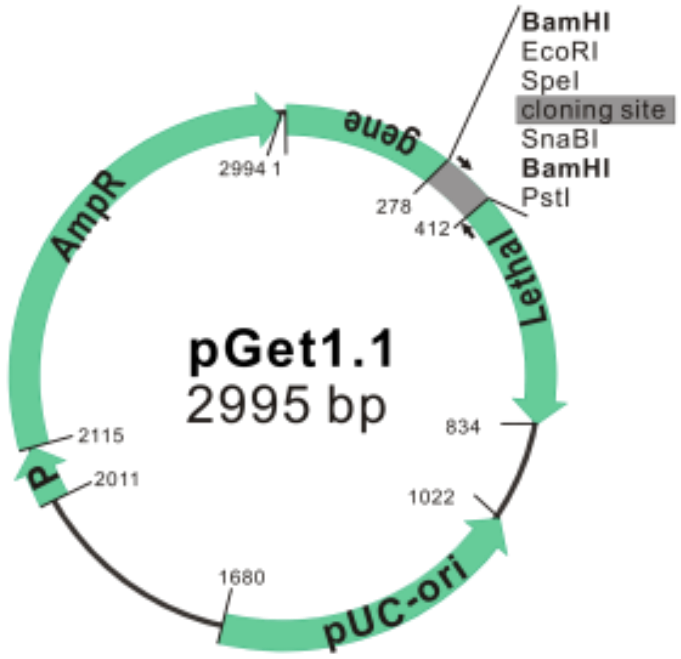
(SMOBIO's TP1200 ExcelTaq™ 5X PCR Master Dye Mix is suggested)

| | |
|---|---|
| Template | Single colony |
| Forward primer | 0.1 – 0.5 µM |
| Reverse primer | 0.1 – 0.5 µM |
| 5X PCR Master Dye Mix | 5 µl |
| dd$H_2O$ | 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.

**The plasmid map and cloning sites of pGet1.1 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 GCT AAA CAT CAG GGA AAG GAT | Cloning site | 355 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

SnaBI — BamHI

PstI

GGT AAG AGA GGA GAC CAA GAT TTG ATG GCT GCA GGA AAT GCT ATC GAG AGA 435 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 pGet1.1 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 |
| M13 Rev | M13 reverse primer | 838…854 |
| pUC-ori | Initiation of replication | 1022…1610 |
| P | The promoter for expressing the ampicillin resistance and lethal gene | 2011…2115 |
| $Amp^R$ | Ampicillin resistance gene | 2115…2973 |
| M13 For | M13 forward primer | 2975…2990 |
| 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 pGet1.1

| Enzyme | Positions | Enzyme | Positions |
|---|---|---|---|
| AclI | 2302, 2675 | FspI | 2681 |
| ApaLI | 1353, 2232 | HindIII | 204 |
| AvaII | 2540, 2762 | HpaI | 568, 822 |
| BamHI | 279, 375 | NspI | 4, 1671 |
| BanI | 1923, 2951 | PciI | 1667, 2995 |
| BglII | 434 | PssI | 1935 |
| Bme1580I | 1357, 2236 | PstI | 418 |
| BsaAI | 368, 791 | PvuI | 2535 |
| BsaHI | 2364 | PvuII | 1847 |
| BseYI | 1363 | ScaI | 2423 |
| BspHI | 947, 2064 | SnaBI | 368 |
| BsrDI | 2670, 2844(c) | SpeI | 322 |
| BsrFI | 2819 | SspI | 2099 |
| CdiI | 2351, 2652 (c) | StyI | 718 |
| EcoRI | 315 | TatI | 2421 |

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

## Related Products

| | |
|---|---|
| CK1000 | Champion E. coli Transformation Kit |
| CV1100 | GetClone PCR Cloning Vector II, 20 RXN |
| 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 |
| TF2000 | Q-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 |
| TP1260 | ExcelTaq 5× Fluorescent PCR Master Mix Kit, 200 RXN |
| VE0100 | B-BOX Blue Light LED epi-illuminator, AC 100-240V, 50/60Hz |
| WM1000 | YesBlot Western Marker I, 250 µl |