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CopyControl™ pCC1BAC™ (*Bam*H I, *Hind* III, *Eco*R I Cloning-Ready) Vector

Cat. Nos. CBAC311B, CBAC311H, and CBAC311E

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1. Introduction

The CopyControl™ pCC1BAC™ Vector* is based on an innovative technology originally developed in the laboratory of Dr. Waclaw Szybalski[1] and optimized at Epicentre.[2] The vector has two origins of replication – a single-copy *E. coli* F-factor replicon and a high-copy origin of replication called "*ori*V". Initially, replication of CopyControl clones can be controlled by the F-factor replicon so the vector is present at one copy per cell. Maintaining clones at single copy ensures insert stability and allows cloning of toxic gene products (Fig. 1).

Initiation of replication from *ori*V requires the *trfA* gene product. CopyControl Vectors use a specifically engineered *E. coli* host strain, TransforMax™ EPI300™ (available separately), which contains a mutant *trfA* gene under tight control of an inducible promoter. Addition of the CopyControl Induction Solution to the growth medium induces expression of *trfA* and subsequent amplification of the clone to high-copy number. Induction of CopyControl BAC clones from single-copy up to 10-20 copies per cell greatly improves the yield and purity of BAC DNA for sequencing, fingerprinting and other applications.

The CopyControl pCC1BAC Vector is derived from pBeloBAC113 and Epicentre's pIndigoBAC-5. The vector has been linearized at a unique restriction enzyme recognition site (*Bam*H I, *Hin*d III or *Eco*R I), dephosphorylated, and highly purified to ensure very low background. Features of the vector include:

- Chloramphenicol-resistance as an antibiotic selectable marker.
- *E. coli* F factor-based partitioning and single-copy number regulation system.
- *ori*V high-copy origin of replication.
- Primer binding sites for BAC-end sequencing
- *Not* I sites surrounding the *Bam*H I, *Hin*d III and *Eco*R I cloning sites.
- Bacteriophage P1 *lox*P site for Cre-recombinase cleavage.

2. Product Specifications

Storage: Store at –20°C.

Size: 375 ng @ 25 ng/µl (15 µl)(in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)

Quality Control: Cloning-ready preparations of the CopyControl pCC1BAC Vector yield >10^7 cfu/µg of Control Insert DNA when transformed into TransforMax EPI300 Electrocompetent *E. coli*. Greater than 95% of the colonies are recombinant clones.

Protocols: See References 4-7 for protocols on BAC cloning and working with BAC clones. Product literature for the CopyControl BAC Cloning Kits also provides thorough procedures for constructing a BAC library.

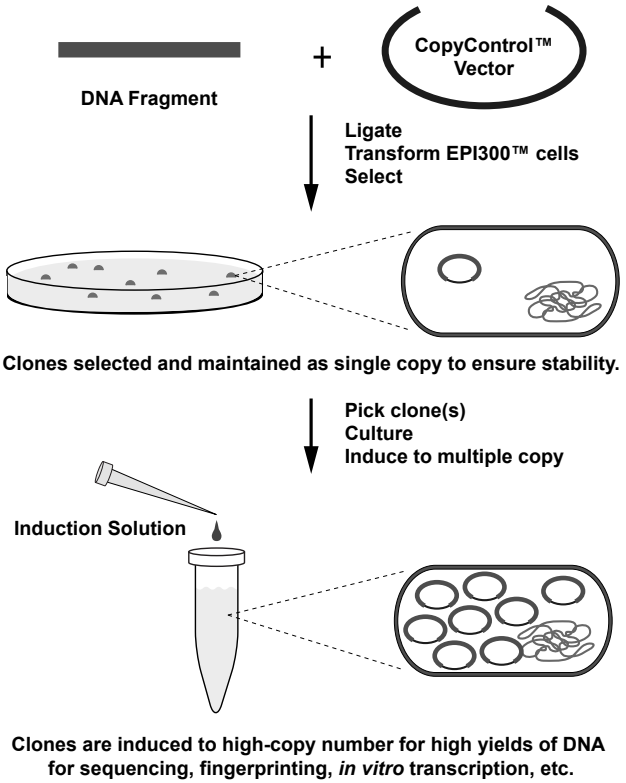
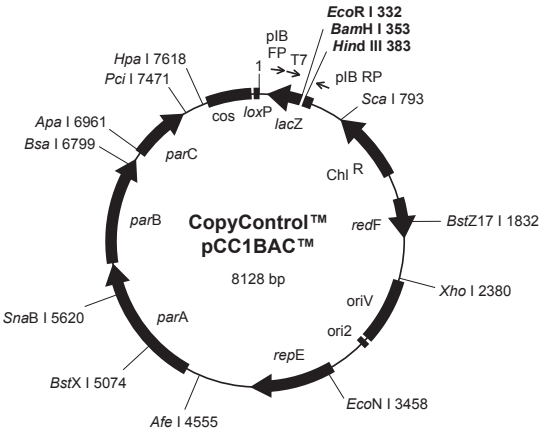


Figure 1. Overview of the CopyControl™ System.

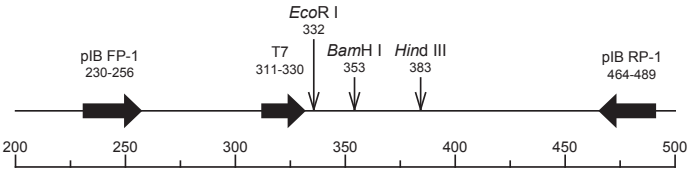
3. Related Products

The following products are also available:

- CopyControl™ BAC Cloning Kits
- TransforMax™ EPI300™ Electrocompetent and Chemically Competent *E. coli*
- TransforMax™ EPI300™-T1^{R} Electrocompetent and Chemically Competent *E. coli*
- BACMAX™ DNA Purification Kit
- Fast-Link™ DNA Ligation Kits
- Colony Fast-Screen™ Kit (Size Screen)
- EZ-Tn5™ <*ori*V/KAN-2> Insertion Kit
- GELase™ Gel-Digesting Preparation
- Plasmid-Safe™ ATP-Dependent DNase



Note: Not all restriction enzymes that cut only once are indicated above.
See page 6 for complete restriction information.
Primers are not drawn to scale.



FP = pCC1™/pEpiFOS™ Foward Sequencing Primer 5' GGATGTGCTGCAAGGCGATTAAGTTGG 3'
RP = pCC1™/pEpiFOS™ Reverse Sequencing Primer 5' CTCGTATGTTGTGTGGAATTGTGAGC 3'
T7 = T7 Promoter Primer 5' TAATACGACTCACTATAGGG 3'

Figure 2. CopyControl™ pCC1BAC™ Vector.

4. How the CopyControl Cloning System Works

1. Ligate the DNA interest into the linearized and dephosphorylated CopyControl pCC1 Cloning-Ready Vector.
2. Transform TransforMax EPI300 Electrocompetent *E. coli* and select clones on LB-chloramphenicol plates. Under these conditions, the *trfA* gene is repressed and the clones are maintained as single copy.
3. Pick individual CopyControl clones from the plate and grow in culture.
4. Add the CopyControl Induction Solution to induce expression of the *trfA* gene product and subsequent amplification of the clones to high copy number.
5. Purify plasmid DNA for sequencing, fingerprinting, subcloning or other applications.

Important: *An E. coli host carrying an inducible trfA gene (such as TransforMax EPI300 E. coli or phage T1-resistant TransforMax EPI300-T1^R E. coli) is required for amplification of the CopyControl BAC clones to high-copy number. A regulated trfA gene is not present in most lab strains of E. coli. We can not guarantee clone amplification results using any E. coli strain other than TransforMax EPI300 E. coli, which are available separately.*

5. pCC1BAC Sequencing Primers and Vector Data

pCC1 / pEpiFOS-5 Sequencing Primers

pCC1™ / pEpiFOS™ Forward Sequencing Primer ..... Cat. No. F5FP010

5′ GGATGTGCTGCAAGGCGATTAAGTTGG 3′ ..... 1 nmol supplied in TE Buffer at 50 µM

pCC1™ / pEpiFOS™ Reverse Sequencing Primer ..... Cat. No. F5RP011

5′ CTCGTATGTTGTGTGGAATTGTGAGC 3′ ..... 1 nmol supplied in TE Buffer at 50 µM

pCC1 / pEpiFOS Forward Sequencing Primer

Temperatures of Dissociation & Melting:

T_d: 79°C (nearest neighbor method)
T_m: 78°C (% G+C method)
T_m: 82°C ([2 (A+T) + 4 (G+C)] method)
T_m: 68°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

pCC1 / pEpiFOS Reverse Sequencing Primer

Temperatures of Dissociation & Melting:

T_d: 71°C (nearest neighbor method)
T_m: 75°C (% G+C method)
T_m: 76°C ([2 (A+T) + 4 (G+C)] method)
T_m: 65°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

Note: *The sequence of the pCC1/pEpiFOS Forward and Reverse Primers do not function well as IRD800-labeled sequencing primers. We recommend using the T7 and pCC1/pEpiFOS RP-2 Primers instead of the pCC1/pEpiFOS Forward and Reverse Primers respectively, for this purpose.*

pCC1™ / pEpiFOS™ RP-2 Reverse Sequencing Primer

5′ TACGCCAAGCTATTTAGGTGAGA 3′

Orientation for BAC End-Sequencing

The following is the nucleotide sequence of pCC1BAC (bases 230-489) from the pCC1/pEpiFOS Forward Sequencing Primer (230-256) to the pCC1/pEpiFOS Reverse Sequencing Primer (489-464) encompassing the T7 RNA polymerase promoter (311-330) the *Eco*R I site (332-337), the *Bam*H I site (353-358) and the *Hin*d III site (383-388).

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230 GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTTCCCAGTC
280 ACGACGTTGT AAAACGACGG CCAGTGAATT GTAATACGAC TCACTATAGG
330 GCGAATTCGA GCTCGGTACC CGGGGATCCT CTAGAGTCGA CCTGCAGGCA
380 TGCAAGCTTG AGTATTCTAT AGTCTCACCT AAATAGCTTG GCGTAATCAT
430 GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC
480 AACATACGAG
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An electronic copy of the pCC1BAC sequence is available for downloading at our Web site at http://www.epicentre.com.sequences or can be requested via e-mail (techhelp@epicentre.com) or by calling Technical Service.

Restriction Enzymes that cut the pCC1BAC Vector one to three times:

Enzyme	Sites	Location
Acc65 I	2	344, 5196
Acl I	2	1121, 5588
Afe I	1	4555
Afl II	2	6597, 6837
Afl III	3	4962, 5136, 7471
Age I	3	3816, 5046, 5939
Ahd I	1	7475
Ale I	1	6532
Apa I	1	6961
ApaB I	3	96, 1934, 7635
ApaL I	1	87
BamH I	1	353
Bbs I	3	5039, 5228, 6105
BciV I	1	2486
Bcl I	1	5787
Bgl I	3	639, 3160, 7609
Bgl II	2	3135, 5202
Blp I	1	4468
BmgB I	3	2613, 5026, 7786
Bmr I	3	268, 7007, 7136
Bpu10 I	3	1434, 3916, 5111
Bsa I	1	6799
BsaB I	2	7743, 7827
BsaH I	1	146
BseY I	3	2401, 5879, 6636
Bsm I	2	812, 1219
BsmB I	3	982, 1535, 3931
BspE I	2	1210, 5756
BspLU11 I		1 7471
BsrB I	3	464, 1648, 2270
BsrG I	1	3769
BssH II	2	5453, 5997
BssS I	3	5146, 6796, 7359
BstAP I	3	95, 1933, 7634
BstE II	1	7593
BstX I	1	5074
BstZ17 I	1	1832
Bts I	2	558, 5548
Dra III	2	1933, 7812
Eco47 III	1	4555
EcoN I	1	3458
EcoO109 I		2 1716, 7847
EcoR I	1	332
EcoR V	2	4117, 4346
Fse I	1	2478
Fsp I	3	167, 3741, 7567
Hind III	1	383
Hpa I	1	7618
Kpn I	2	348, 5200
Mfe I	1	4976
Msc I	3	943, 2623, 5407
Nar I	1	146
Nco I	2	905, 7176
Nde I	2	94, 4994
Not I	2	2, 631
Nru I	2	1632, 7663
Nsp I	3	381, 1819, 7475
PaeR7 I	1	2380
Pci I	1	7471
PflF I	1	5260
PpuM I	2	1716, 7847
Psi I	2	2915, 3111
PspOM I	1	6957
Pst I	3	375, 4014, 5555
Pvu I	2	188, 5862
Sac II	1	2472
Sal I	3	365, 645, 7651
Sap I	2	4592, 5802
Sbf I	2	375, 4014
Sca I	1	793
SexA I	1	7589
Sfi I	1	639
Sfo I	1	147
SgrA I	3	2481, 5046, 6203
Sim I	2	5160, 7847
Sma I	3	350, 639, 3482
SnaB I	1	5620
Spe I	1	6711
Sph I	1	381
Srf I	1	639
Sse8647 I	1	1716
Stu I	1	3163
Tat I	3	77, 791, 3769
Tli I	1	2380
Tth111 I	1	5260
Xba I	2	359, 3181
Xcm I	1	2676
Xho I	1	2380
Xma I	3	348, 637, 3480

Restriction Enzymes that cut the pCC1BAC Vector four or more times:

Acc I	BsmA I	Dsa I	HpyCH4 V	PspG I
Aci I	Bsp1286 I	Eae I	Mae II	Pvu II
Alu I	BspH I	Eag I	Mae III	Rsa I
Alw I	BspM I	Ear I	Mbo I	Sac I
AlwN I	Bsr I	Fau I	Mbo II	Sau3A I
Apo I	BsrD I	Fnu4H I	Mly I	Sau96 I
Ase I	BsrF I	Gdi II	Mnl I	ScrF I
Ava I	BssK I	Hae I	Mse I	SfaN I
Ava II	BstDS I	Hae II	Msl I	Sfc I
Ban I	BstF5 I	Hae III	Msp I	Sml I
Ban II	BstN I	Hha I	MspA1 I	Ssp I
Bfa I	BstU I	Hinc II	Mwo I	Sty I
BfuA I	BstY I	Hinf I	Nae I	Taq I
Bme1580 I	Btg I	HinP I	Nci I	Tfi I
BsaA I	Cac8 I	Hpa II	NgoM IV	Tse I
BsaJ I	CviJ I	Hph I	Nla III	Tsp45 I
BsaW I	Dde I	Hpy188 I	Nla IV	Tsp4C I
BsiE I	Dpn I	Hpy99 I	PflM I	Tsp509 I
BsiHKA I	Dra I	HpyCH4 III	Ple I	TspR I
Bsl I	Drd I	HpyCH4 IV	PshA I	Xmn I

Restriction Enzymes that do not cut the pCC1BAC Vector:

Aat II	BbvC I	BstB I	Nhe I	Pml I
Asc I	BfrB I	Bsu36 I	Nsi I	Rsr II
AsiS I	BsiW I	Cla I	Pac I	SanD I
Avr II	BspD I	Mlu I	Pme I	Swa I

6. References

1. Wild, J. *et al.*, (2002) *Genomic Research* **12**, 1434.
2. *Epicentre Forum* (2002) **9** (1), 1.
3. Hurowitz, E.H. *et al.*, (2000) *DNA Research* **7** (2), 1.
4. Birren, B. *et al.*, (1999) Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual*, CSH Press, New York, **v. 3**, 241.

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