

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

Cat. Nos. CBAC311B, CBAC311H, and CBAC311E

The CopyControl™ pCC1BAC™ Vector\* is based on an innovative technology originally developed in the laboratory of Dr. Waclaw Szybalski<sup>1</sup> and optimized at Epicentre.<sup>2</sup> The vector has two origins of replication – a single-copy *E. coli* F-factor replicon and a high-copy origin of replication called “*oriV*”. 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 *oriV* requires the *trfA* gene product. CopyControl Vectors use a specifically engineered *E. coli* host strain, TransformMax™ 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, *Hind* 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.
- *oriV* high-copy origin of replication.
- Primer binding sites for BAC-end sequencing
- *Not* I sites surrounding the *Bam*H I, *Hind* III and *Eco*R I cloning sites.
- Bacteriophage P1 *loxP* site for Cre-recombinase cleavage.

## 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<sup>7</sup> 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.

**Related Products:** The following products are also available:

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

## 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<sup>R</sup> *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.

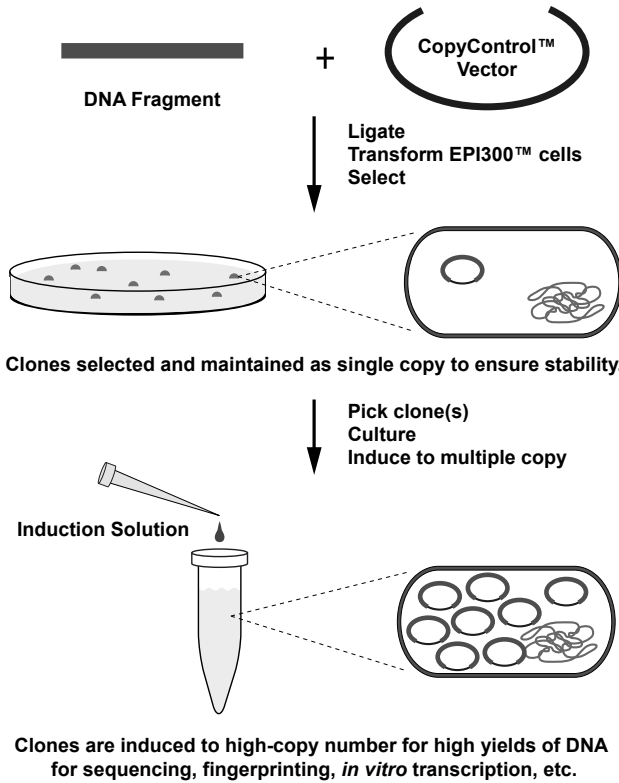


Figure 1. Overview of the CopyControl™ System.

## pCC1BAC Sequencing Primers and Vector Data

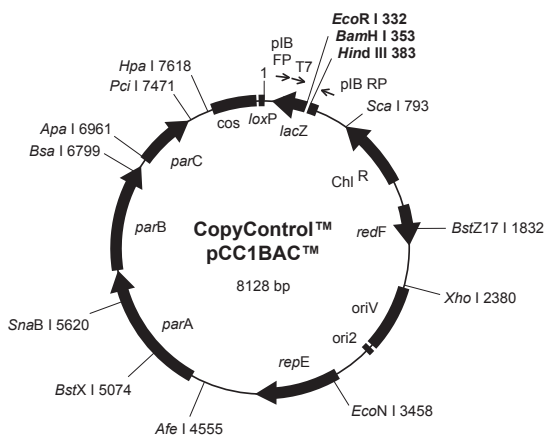
### pCC1 / pEpiFOS-5 Sequencing Primers

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

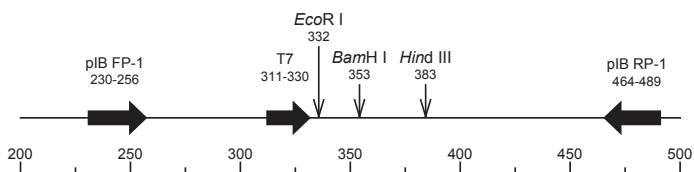
5' GGATGTGCTGCAAGGCGATTAAGTTGG 3' ..... 1 nmol supplied in TE Buffer at 50 μM

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

5' CTCGTATGTTGTGGAATTGTGAGC 3' ..... 1 nmol supplied in TE Buffer at 50 μM



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

Figure 2. CopyControl™ pCC1BAC™ Vector.

### 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<sup>+</sup>])) + ((41 (#G+C) - 500) / length) method)
- where [Na<sup>+</sup>] = 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<sup>+</sup>])) +  
 ([41 (#G+C) - 500] / length) method)  
 where [Na<sup>+</sup>] = 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 *Hind* III site (383-388).

230 GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTTCCCAGTC  
 280 ACGACGTTGT AAAACGACGG CCAGTGAATT GTAATACGAC TCACTATAGG  
 330 GCGAATTTCGA GCTCGGTACC CGGGGATCCT CTAGAGTCGA CCTGCAGGCA  
 380 TGCAAGCTTG AGTATTCTAT AGTCTCACCT AAATAGCTTG GCGTAATCAT  
 430 GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCACAC  
 480 AACATACGAG

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:**

<b>Enzyme</b>	<b>Sites</b>	<b>Location</b>	<b>Enzyme</b>	<b>Sites</b>	<b>Location</b>
Acc65 I	2	344, 5196	Fsp I	3	167, 3741, 7567
Acl I	2	1121, 5588	Hind III	1	383
Afe I	1	4555	Hpa I	1	7618
Afl II	2	6597, 6837	Kpn I	2	348, 5200
Afl III	3	4962, 5136, 7471	Mfe I	1	4976
Age I	3	3816, 5046, 5939	Msc I	3	943, 2623, 5407
Ahd I	1	7475	Nar I	1	146
Ale I	1	6532	Nco I	2	905, 7176
Apa I	1	6961	Nde I	2	94, 4994
ApaB I	3	96, 1934, 7635	Not I	2	2, 631
ApaL I	1	87	Nru I	2	1632, 7663
BamH I	1	353	Nsp I	3	381, 1819, 7475
Bbs I	3	5039, 5228, 6105	Paer7 I	1	2380
BciV I	1	2486	Pci I	1	7471
Bcl I	1	5787	PfFI	1	5260
Bgl I	3	639, 3160, 7609	PpuMI	2	1716, 7847
Bgl II	2	3135, 5202	Psi I	2	2915, 3111
Blp I	1	4468	PspOM I	1	6957
BmgB I	3	2613, 5026, 7786	Pst I	3	375, 4014, 5555
Bmr I	3	268, 7007, 7136	Pvu I	2	188, 5862
Bpu10 I	3	1434, 3916, 5111	Sac II	1	2472
Bsa I	1	6799	Sal I	3	365, 645, 7651
BsaB I	2	7743, 7827	Sap I	2	4592, 5802
BsaH I	1	146	Sbf I	2	375, 4014
BseY I	3	2401, 5879, 6636	Sca I	1	793
Bsm I	2	812, 1219	SexA I	1	7589
BsmB I	3	982, 1535, 3931	Sfi I	1	639
BspE I	2	1210, 5756	Sfo I	1	147
BspLU11 I	1	7471	SgrA I	3	2481, 5046, 6203
BsrB I	3	464, 1648, 2270	Sim I	2	5160, 7847
BsrG I	1	3769	Sma I	3	350, 639, 3482
BssH II	2	5453, 5997	SnaB I	1	5620
BssS I	3	5146, 6796, 7359	Spe I	1	6711
BstAP I	3	95, 1933, 7634	Sph I	1	381
BstE II	1	7593	Srf I	1	639
BstX I	1	5074	Sse8647 I	1	1716
BstZ17 I	1	1832	Stu I	1	3163
Bts I	2	558, 5548	Tat I	3	77, 791, 3769
Dra III	2	1933, 7812	Tli I	1	2380
Eco47 III	1	4555	Tth111 I	1	5260
EcoN I	1	3458	Xba I	2	359, 3181
EcoO109 I	2	1716, 7847	Xcm I	1	2676
EcoR I	1	332	Xho I	1	2380
EcoRV	2	4117, 4346	Xma I	3	348, 637, 3480
Fse I	1	2478			

**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

**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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