CopyControl™ pCC1BAC™ (BamH I Cloning-Ready) Vector
CopyControl™ pCC1BAC™ (Hind III Cloning-Ready) Vector
CopyControl™ pCC1BAC™ (EcoR 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[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 (Figure 1, page 2).

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 pBeloBAC11[3] 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.

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. An electronic copy is available for downloading at the following URL: http://www.epicentre.com/item.asp?id=380 and following the "protocol" hyperlink.

Related Products: The following products are also available:

- CopyControl™ BAC Cloning Kits
- Fast-Link™ DNA Ligation Kits
- Colony Fast-Screen™ Kit (Size Screen)
- BAC-Tracker™ Supercoiled DNA Ladder
- EZ::TN™ <*ori*V/KAN-2> Insertion Kit
- GELase™ Gel-Digesting Preparation
- Plasmid-Safe™ ATP-Dependent DNase

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.
5. http://www.tree.caltech.edu/protocols/BAC_lib_construction.html.
6. http://hbz.tamu.edu/bacindex.html.
7. http://www.genome.clemson.edu.

continued

How the CopyControl Cloning System Works:

1. Ligate the DNA interest into the linearized and dephosphorylated CopyControl pCC1 Cloning-Ready Vector.

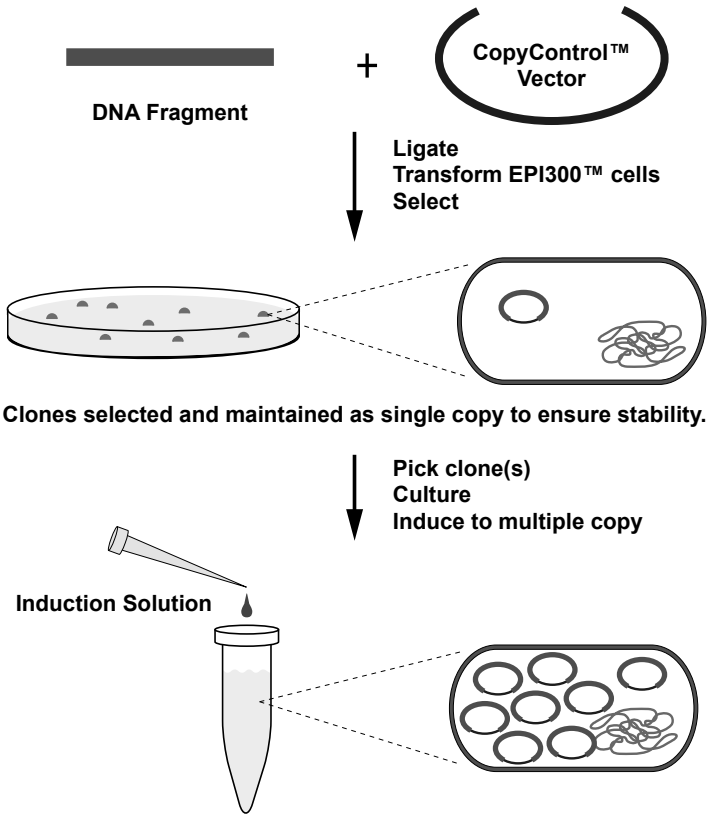
2. Transform TransforMax EPI300 Electrocompetent *E. coli* and select clones on LB-chloramphenicol plates. Under these conditions, the *trf*A gene is repressed and the clones are maintained as single copy.

3. Pick individual CopyControl clones from the plate and grow in culture.

4. Add the CopyControl Induction Solution to induce expression of the *trf*A gene product and subsequent amplification of the clones to high copy number.

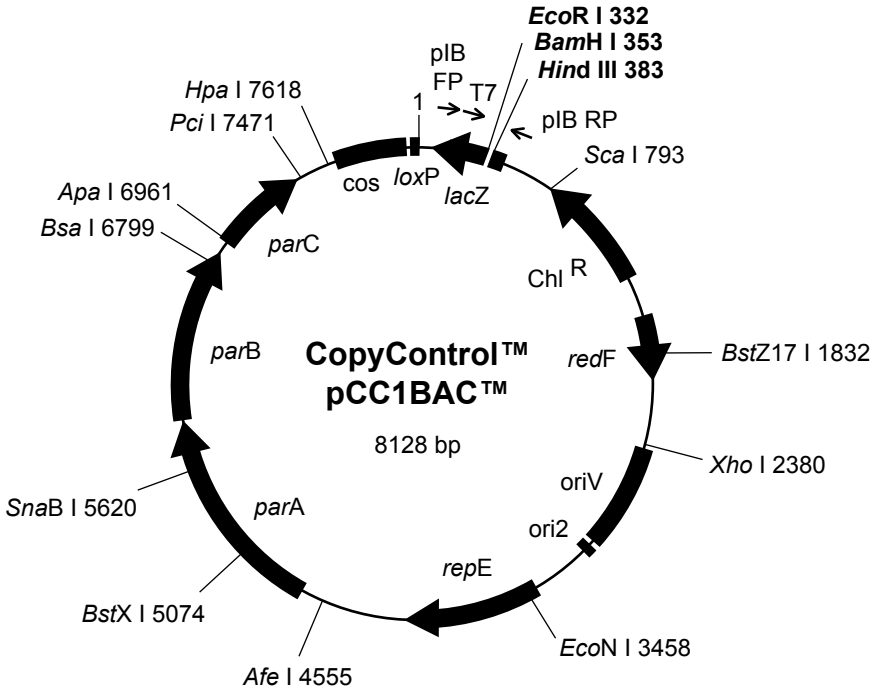
5. Purify plasmid DNA for sequencing, fingerprinting, subcloning or other applications.

Figure 1. Overview of the CopyControl™ System.

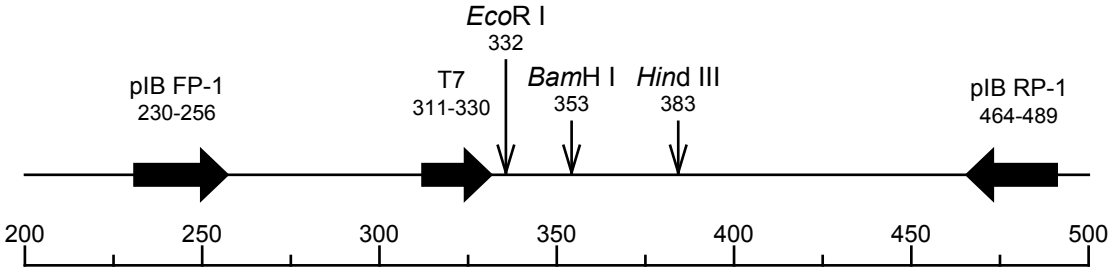


***Important*: An *E. coli* host carrying an inducible *trf*A gene (such as TransforMax EPI300 *E. coli* or phage T1-resistant TransforMax EPI300-T1^R *E. coli*) is required for amplification of the CopyControl BAC clones to high-copy number. A regulated *trf*A gene is not present in most lab strains 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 2. CopyControl™ pCC1BAC™ Vector.



Note: Not all restriction enzymes that cut only once are indicated above.
  See page 5 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'

pCC1BAC Sequencing Primers and Vector Data

pCC1 / pEpiFOS-5 Sequencing Primers

Primers are available separately:

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

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
```

An electronic copy of the pCC1BAC sequence is available for downloading at our Web site at http://www.epicentre.com/technical.htm or can be requested via e-mail (techhelp@epicentre.com) or by calling Technical Service.

CopyControl, pCC1BAC, TransforMax, EPI300, Fast-Link, Fast-Screen, BAC-Tracker, EZ::TN, GELase, Plasmid-Safe, pCC1 and pEpiFOS are trademarks of EPICENTRE, Madison, Wisconsin.

Restriction Enzymes that cut the pCC1BAC Vector 1 to 3 times:

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

Restriction Enzymes that cut the pCC1BAC Vector 4 or more times:

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

Restriction Enzymes that do not cut the pCC1BAC Vector:

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