epicentre®
an Illumina® company

CopyControl™ BAC Cloning Kit

Cat. No. CCBAC1B – (*Bam*H I)

Cat. No. CCBAC1E – (*Eco*R I)

Cat. No. CCBAC1H – (*Hind* III)

Connect with Epicentre on our blog **(epicentral.blogspot.com)**,
Facebook **(facebook.com/EpicentreBio)**, and Twitter **(@EpicentreBio)**.

1. Introduction

The CopyControl Cloning System, based on technology developed by Dr. Waclaw Szybalski [1-3] at the University of Wisconsin-Madison, provides the user with total control over the clone copy number. The system combines the clone stability afforded by single-copy cloning with the advantages of high yields of DNA obtained by "on-demand" induction of the clones to high-copy number. For example, the copy number of CopyControl BAC clones can be induced to 10-20 copies per cell and CopyControl Fosmid and PCR clones can be induced from single-copy to 10-200 copies per cell to improve DNA yields for applications including sequencing, fingerprinting, subcloning, *in vitro* transcription and other applications. CopyControl Cloning Kits for BAC (Bacterial Artificial Chromosome) cloning, fosmid library production and cDNA, Gene & PCR cloning are available.

The CopyControl Cloning System has two required components:

1. The CopyControl pCC1™ Vector contains both a single-copy and the high-copy *ori*V origin of replication. Initiation of replication from *ori*V requires the *trf*A gene product that is supplied by the second system component, the EPI300™ *E. coli* strain.

   Features of the CopyControl pCC1BAC™ Vector (Fig. 1):

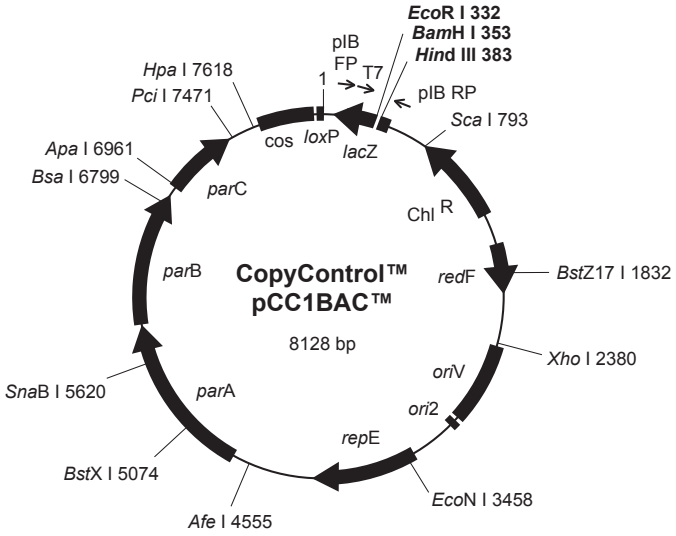
   - Chloramphenicol-resistance as an antibiotic selectable marker.
   - *E. coli* F factor-based partitioning and single-copy origin of replication.
   - *ori*V high-copy origin of replication.
   - *Not* I sites surrounding the *Bam*H I, *Hin*d III and *Eco*R I cloning sites.
   - Bacteriophage P1 loxP site for Cre-recombinase cleavage.
   - Bacteriophage T7 RNA polymerase promoter flanking the cloning site.

2. The TransforMax EPI300 *E. coli* provide a mutant *trf*A gene whose gene product is required for initiation of replication from *ori*V. The cells have been engineered so that the *trf*A gene is under tight, regulated control of an inducible promoter.

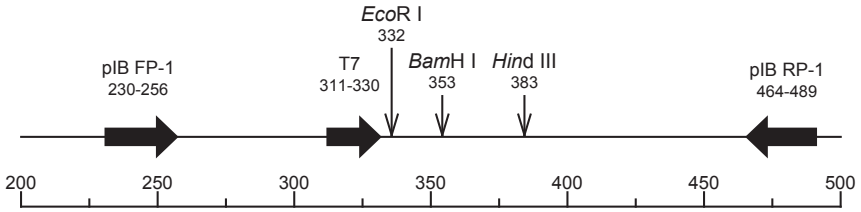
   Important: *An E. coli host carrying an inducible trfA gene (such as TransforMax EPI300 E. coli or phage T1-resistant TransforMax EPI300-T1^R E. coli) is required for amplification of the CopyControl BAC clones to high-copy number. A regulated trfA gene is not present in most lab strains of E. coli.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

How the CopyControl Cloning System Works:

1. Ligate the DNA of interest into the linearized and dephosphorylated CopyControl pCC1 Cloning-Ready vector supplied with the respective kit.
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.



Note: Not all restriction enzymes that cut only once are indicated above. See Appendix E 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 1. pCC1BAC™ Vector Map.

2. Kit Contents

Desc.	Concentration	Quantity
Reagents included in the kit are sufficient to perform 25 BAC cloning reactions.		
CopyControl™ pCC1BAC™ (*Bam*H I, *Eco*R I or *Hin*d III) Cloning-Ready Vector: (25 ng/µl) 25 µl (linearized at the unique *Bam*H I, *Eco*R I or *Hin*d III site and dephosphorylated)		
Fast-Link™ DNA Ligase	(100 U @ 2 U/µl)	50 µl
Fast-Link™ 10X Ligation Buffer	300 µl	
ATP Solution	100 mM)	25 µl
Control Insert (*Bam*H I, *Eco*R I or *Hin*d III)	(4 ng/µl)	50 µl
pCC1BAC™-145 CopyControl™ BAC DNA	(500 pg/µl)	10 µl
EpiBlue™ Solution		2.5 ml
EpiLyse™ Solution		10 ml
Sterile Water		500 µl

Note: *The EpiBlue and EpiLyse Solutions are supplied in the Colony Fast-Screen™ Kit (cat. no. FS08250) that accompanies the CopyControl BAC Cloning Kit.*

Storage: Store the EpiLyse and EpiBlue Solutions at room temperature. Store the remainder of the kit components at -20°C.

Additionally Required Reagents

In addition to the component supplied, the following reagents are required:

TransforMax™ EPI300™ Electrocompetent *E. coli*

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. Copy number amplification procedures described in this manual have been optimized using TransforMax EPI300 E. coli. We can not guarantee clone amplification results using any E. coli strain other than TransforMax EPI300 E. coli.*

LB plates + 12.5 µg/ml chloramphenicol + 40 µg/ml X-Gal + 0.4 mM IPTG

TE Buffer: (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)

Quality Control

The CopyControl BAC Cloning Kit is function-tested using the Control Insert DNA provided in the kit. Each kit must yield >10^7 cfu/µg of the Control Insert DNA when transformed into TransforMax EPI-300 Electrocompetent *E. coli*. >95% of the colonies will be white (recombinant clones).

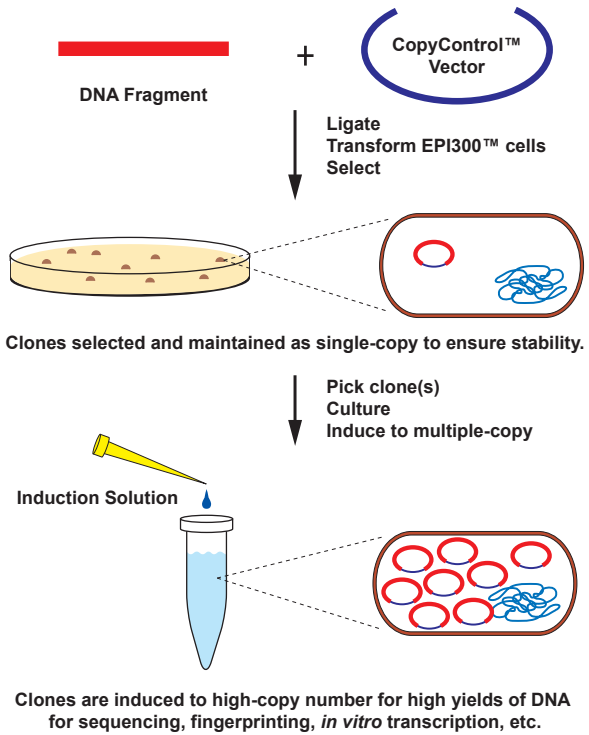


Figure 2. Overview of the CopyControl™ System.

3. The Steps Involved in the CopyControl BAC Cloning Process

1. Purify genomic DNA from the desired source (the kit does not supply materials for this step).
2. Partially digest and size select the genomic DNA (the kit does not supply materials for this step).
3. Ligate the genomic DNA into the CopyControl pCC1BAC Cloning-Ready Vector.
4. Transform TransforMax EPI300 Electrocompetent *E. coli* (available separately) with an aliquot of the ligation reaction and select on LB-chloramphenicol plates.
5. Size screen the CopyControl BAC clones to assess the quality of the library produced.
6. Repeat steps 3-4 as necessary to obtain the number of desired clones for the library.
7. Pick CopyControl BAC clones of interest and induce them to high-copy number using the CopyControl Induction Solution (provided with the TransforMax EPI300 Electrocompetent *E. coli* and available separately).
8. Purify CopyControl BAC DNA for sequencing, fingerprinting, subcloning or other applications using Epicentre's BACMAX™ DNA Purification Kit or your method of choice.

4. CopyControl BAC Cloning Protocol

General Considerations

1. ***Important!*** *Users should avoid exposing DNA to UV light. Even exposure for short periods of time can decrease the efficiency of cloning by two orders of magnitude or more.*
2. The user must prepare the genomic DNA fragments for cloning. There are many useful references describing this process. See references 4-6 on page 20 of this manual.
3. To prevent unwanted shearing of the large genomic fragments that will be cloned, use large-bore pipette tips (e.g., Molecular BioProducts, Catalog Number: MBP 200G) when transferring the DNA.
4. An *E. coli* host carrying an inducible *trf*A gene (such as TransforMax EPI300 *E. coli*) is required for amplification of the CopyControl BAC clones to high-copy number. Clone copy number induction procedures described in this manual have been optimized using TransforMax EPI300 *E. coli*. We can not guarantee clone amplification results using any *E. coli* strain other than TransforMax EPI300 *E. coli*. TransforMax EPI300 Electrocompetent *E. coli* are available separately.

A. Ligation of Genomic DNA into the CopyControl pCC1BAC Cloning-Ready Vector

Kit components used in this step: **Fast-Link DNA Ligase, Fast-Link 10X Ligation Buffer, ATP Solution, CopyControl pCC1BAC Cloning-Ready Vector, Sterile Water.**

Additionally required equipment and reagents: Water baths at 16°C, 55°C, and 65°C; agarose cone, large-bore pipette tips, microcentrifuge tubes.

1. Combine the following in a microcentrifuge tube.

   Note: *Use a large-bore pipette tip to prevent shearing the genomic DNA fragments.*

x	µl	sterile water
1	µl	CopyControl pCC1BAC Cloning-Ready Vector
100	ng	partially-digested and size-selected genomic DNA fragments
87	µl	Total reaction volume

2. Mix gently by pipetting the solution up and down 2-3 times using a large-bore pipette tip.
3. Incubate at 55°C for 10 minutes. Allow the solution to cool at room temp for 15 minutes.
4. To the cooled solution, add:

10	µl	10X Fast-Link Ligation Buffer
1	µl	100 mM ATP
2	µl	Fast-Link DNA Ligase
100	µl	Total reaction volume

5. Incubate the ligation reaction for 4 hours at 16°C.

   Note: *Longer incubation times (e.g., overnight) will not adversely affect the ligation reaction.*

6. Heat the reaction at 65°C for 15 minutes to inactivate the Fast-Link DNA Ligase.
7. Desalt the ligation reaction using an agarose cone (see Appendix B and ref. 8) on ice for 1 hour or by other desalting method of choice.

B. Transformation, Plating and Selecting the CopyControl BAC Clones

Reagents and equipment supplied by the user: TransforMax EPI300 Electrocompetent *E. coli*, electroporator, electroporation cuvettes, 14-15 ml round bottom culture tubes, large-bore pipette tips, microcentrifuge tubes, 37°C incubator shaker, LB plate + chloramphenicol (12.5 µg/ml) + X-Gal (40 µg/ml) + IPTG (0.4 mM), SOC medium.

Note: *An E. coli host carrying an inducible trfA gene (such as TransforMax EPI300 E. coli) is required for amplification of the CopyControl BAC clones to high-copy number. Copy number induction procedures described in this manual have been optimized using TransforMax EPI300 E. coli. We can not guarantee clone amplification results using any E. coli strain other than TransforMax EPI300 E. coli.*

1. Prepare 1 ml of SOC medium (do not include antibiotic in the medium) for each electroporation to be performed. This medium will be used for post-electroporation outgrowth of transformed cells. Maintain the medium at room temperature.
2. Pre-chill electroporation cuvettes and 1.5 ml tubes on ice.
3. Set-up the electroporation device according to the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
4. Thaw TransforMax EPI300 Electrocompetent *E. coli* cells on ice. Mix gently. Use the cells immediately. Unused cells can be refrozen at –70°C.

   Note: *Refrozen cells may have reduced transformation efficiency.*
5. Transfer 2 µl of the desalted ligation reaction from Part A and 50 µl of cells to a pre-chilled microcentrifuge tube.

   Note: *A smaller volume of cells can be used based on the needs and experiences of the user.*
6. Store the remainder of the ligation reaction at 4°C until the size of the BAC clones and the quality of the library is assessed in Step C.
7. Mix the cells and DNA by pipetting up and down 2-3 times or by tapping the tube gently.
8. Transfer the cell/DNA mix to the electroporation cuvette. Be sure that there are no air bubbles in the cuvette. Wipe the cuvette of any condensation. Place into the electroporator.
9. Apply the electric pulse at manufacturer's recommendations for bacterial (*E. coli*) electroporation.
10. Immediately after electroporation, add 950 µl of the room temperature SOC medium to the cuvette. Mix gently by pipetting up and down 2-3 times.
11. Transfer the cells to a 15 ml tube and incubate at 37°C with shaking at 220-230 rpm for 1 hour to recover the cells and allow expression of the antibiotic resistance marker.
12. Plate 100 µl of transformation reaction on LB + chloramphenicol (12.5 µg/ml) + X-GAL + IPTG plates. Store the remaining 900 µl at 4°C until the size of the BAC clones and the quality of the library is assessed in Part C.
13. Incubate the plates overnight at 37°C.

C. Sizing the CopyControl BAC Clones

Kit components used in this step: **EpiBlue Solution, EpiLyse Solution, BAC-Tracker Supercoiled DNA Ladder.**

Additionally required equipment and reagents: **Agarose gel apparatus, microcentrifuge tubes, 96 well plate (optional), SYBR® Gold or ethidium bromide DNA stains.**

The size of the CopyControl BAC clones can be estimated and the quality of the library assessed by the procedure described here. It is important to stain the agarose gel with a highly sensitive stain such as SYBR Gold or for a prolonged period of time with ethidium bromide.

1. Add 25 µl of EpiLyse Solution to microcentrifuge tubes or wells of a 96 well plate for each CopyControl BAC clone that will be sized.
2. Individually pick CopyControl BAC clones with a pipette tip. Because the CopyControl BAC clones are growing at single-copy number, an entire colony must be picked from the overnight plate to obtain enough BAC DNA to be seen on an agarose gel. Therefore, if you desire to retain the clones that are picked, pick the entire colony and then inoculate a second LB/chloramphenicol plate (called the "gridding plate") by lightly touching the plate with the pipette tip. Deposit the remainder of the picked colony into the EpiLyse Solution. Repeat the procedure for each colony picked from the overnight plate. Incubate the gridding plate overnight at 37°C and then store at 4°C.
3. Pipette the solutions up and down to get the entire colony into solution, then vortex the tubes or the microtiter plate vigorously to completely resuspend the cells.
4. Add 10 µl of EpiBlue Solution to each tube or each well of the microtiter plate. Vortex and then centrifuge briefly to get all the solution to the bottom of the tubes or microtiter plate wells.
5. Load 20-25 µl onto a 0.8% agarose gel.
6. Run the gel at 4.5 V/cm for 3 hours.
7. Stain the gel with SYBR Gold per manufacturer's instructions for 20 minutes or with ethidium bromide for 40 minutes. Destain an ethidium bromide stained gel to visualize the BAC DNA bands. The BAC DNA bands will be faint bands migrating above (slower than) a broad smear of DNA and RNA.
8. If the size of the inserts is acceptable, plate the remaining 900 µl of transformed cells from Part B, Step 9, transform and plate the remaining ligation reaction from Part B, Step 5 and perform additional ligation reactions as necessary to construct the desired library.
9. Once plating is complete and the entire library is constructed, pick the CopyControl BAC clones and store and maintain by your method of choice.

5. Induction of the CopyControl BAC Clones to High-Copy Number

Reagents and equipment supplied by the user: 37°C incubator and shaker, LB + chloramphenicol (12.5 µg/ml), CopyControl Induction Solution (supplied with TransforMax EPI300 *E. coli* or available separately) or CopyControl BAC Autoinduction Solution (available separately), deep-well (2 ml) 96 well plate (optional).

Once the desired CopyControl BAC clones are identified, they can be induced to high copy number for high yields of DNA for sequencing, fingerprinting or other application. Two different induction protocols can be used depending on the experimental needs of the researcher.

The CopyControl Induction Solution (also know as "standard induction") protocol requires an overnight culture to be sub-cultured prior to induction. It involves more hands-on time from the researcher, but is more flexible in its use and time requirements. The CopyControl BAC Autoinduction Solution can be supplemented into the cultures prior to inoculation and requires no sub-culturing of the bacteria. It is ideal for growing BAC clones in 96-well format or other high-throughput applications where sub-culturing is tedious and time consuming.

Either copy-number induction process can be done in any culture volume desired depending on the need of the user. Generally, a 1 ml culture will provide a sufficient amount of DNA (typically 1–2 µg) for most applications. Below we provide the standard induction procedure for amplifying the clones in 1 ml, 5 ml and 50 ml cultures and the autoinduction protocol, which is freely scalable.

Standard Induction Using the CopyControl Induction Solution

Preparation of the Clone Induction Inocula

1. Add 5 ml of LB medium + 12.5 µg/ml chloramphenicol to 15 ml tubes for each clone to be induced to high copy number.
2. Individually inoculate the media with a small portion of the desired CopyControl BAC clones grown on an overnight plate.
3. Grow the cultures overnight at 37°C with shaking. These cultures will be used as inocula for the copy number amplification procedure.

Copy Number Amplification

4. From the table on the next page, combine the appropriate volumes of fresh LB + chloramphenicol, the overnight culture and the CopyControl Induction Solution for the desired volume of induction culture. Aeration of the induction cultures is critical. Therefore, to maximize the surface area of the culture solution in the tube, perform the induction in the largest volume tubes that reasonably meets your needs and resources. For example, to induce clones to high copy number in 1 ml of culture, use 1.5 ml tubes or larger.

Total volume of clone induction culture (12.5 µg/ml)	Volume of fresh LB + chloramphenicol	Volume of overnight 5 ml culture	Volume of 1000X CopyControl Induction Solution *
1 ml	800 µl	200 µl	1 µl
5 ml	4.5 ml	500 µl	5 µl
50 ml	45 ml	5 ml	50 µl

**Mix thoroughly after thawing.*

5. Vigorously shake the tubes at 37°C for 5 hours. Aeration is critical! Shake the tubes in a manner that will maximize aeration of the cultures (for example 1.5 ml tubes can be taped horizontally to the shaking table).
6. Centrifuge the cells and purify the DNA using the BACMAX DNA Purification Kit or other standard laboratory methods.[6]

Autoinduction using the CopyControl BAC Autoinduction Solution

Note: *If the clones are to be grown in a 96 well plate, we suggest using 1.2 ml of culture in a 2 ml deep-well plate. Incubating the plate at a slight angle can improve culture aeration and provide higher DNA yields.*

1. Supplement the appropriate amount of LB medium + 12.5 µg/ml chloramphenicol with the CopyControl BAC Autoinduction Solution. Refer to the table below.

Volume of fresh LB + chloramphenicol (12.5 µg/ml)	Volume of CopyControl BAC Autoinduction Solution
1 ml	6 µl
5 ml	30 µl
50 ml	300 µl

2. Individually inoculate the media with a small portion of the desired CopyControl BAC clones grown on an overnight plate.
3. Grow the cultures overnight (17–20 hours) at 37°C with shaking. Cultures incubated for longer or shorter periods of time may not properly induce. Aeration during this incubation is critical! Purify the cultures using the BACMAX DNA Purification Kit or other standard protocol.

6. Appendix

Appendix A

Determining the Approximate Number of Clones for a BAC Library

Using the following formula[7], determine the number of fosmid clones required to reasonably ensure that any given DNA sequence is contained within the library.

$N = \ln (1-P) / \ln (1-[I/GS])$

Where *P* is the desired probability (expressed as a fraction); I is the BAC clone insert size; GS is the genome size; and N is the required number of BAC clones.

For example, the number of clones required to ensure a 99% probability of a given DNA sequence of human genome (3×10^9 bp) being contained within a BAC library with an average insert size of 100,000 bp is:

$N = \ln (1 - 0.99) / \ln (1 - [10^5 \text{ bp} / 3 \times 10^9 \text{ bp}]) = -4.61 / -3.33 \times 10^{-5} = 138{,}298$ clones

Appendix B

Preparation of Agarose Cones for Desalting the Ligation Reactions (adapted from reference 8).

Reagents and equipment supplied by the user: 1.5 ml and 0.5 ml microcentrifuge tubes, glucose solution in water, agarose, microwave oven.

1. Combine the following reagents:

0.9	g	glucose
0.5	g	agarose
50	ml	water

2. Heat in a microwave oven to dissolve the glucose and agarose.
3. Cool the solution to 50°C.
4. Dispense 800 µl of the solution into 1.5 ml microcentrifuge tubes for each ligation reaction to desalt.
5. Place a 0.5 ml microcentrifuge tube on top of the molten agarose-glucose solution. Press down lightly. Allow the agarose-glucose to solidify.
6. Remove the 0.5 ml tube from the solidified agarose-glucose using a gentle twisting action. After removing the 0.5 ml tube, there should be a concave "pit" in the solidified agarose-glucose.
7. Transfer the ligation reaction using a large-bore pipette tip into the "pit" on top of the agarose-glucose. Incubate on ice for 1 hour. The salt from the ligation reaction will diffuse into the agarose-glucose.
8. Using a large-bore pipette tip, transfer the desalted ligation reaction into a microcentrifuge tube and store at 4°C or on ice. Discard the agarose cone.

Appendix C

Construction of a CopyControl BAC Library Using the Control Insert

Kit components used in this step: **Fast-Link DNA Ligase, Fast-Link 10X Ligation Buffer, ATP Solution, CopyControl pCC1BAC Cloning-Ready Vector, Sterile Water.**

Additionally required equipment and reagents: Water baths at 16°C, 55°C, and 65°C; agarose cone, large-bore pipette tips, microcentrifuge tubes.

The approximate size of the Control Inserts supplied with the respective kits are:

*Bam*H I = 20 Kb

*Eco*R I = 30 Kb

*Hin*d III = 33 Kb

1. Combine the following in a microcentrifuge tube.

   Note: *Use a large-bore pipette tip to prevent shearing the genomic DNA fragments.*

61	µl	sterile water
1	µl	CopyControl pCC1BAC Cloning-Ready Vector (25 ng)
25	µl	Control Insert DNA (100 ng)
87	µl	Total reaction volume

2. Mix gently by pipetting the solution up and down 2-3 times using a large-bore pipette tip.
3. Incubate at 55°C for 10 minutes. Allow the solution to cool at room temp for 15 minutes.
4. To the cooled solution, add:

10	µl	10X Fast-Link Ligation Buffer
1	µl	100 mM ATP
2	µl	Fast-Link DNA Ligase
100	µl	Total reaction volume

5. Incubate the ligation reaction for 4 hours at 16°C.

   Note: *Longer incubation times (e.g., overnight) will not adversely affect the ligation reaction.*

6. Heat the reaction at 65°C for 15 minutes to inactivate the Fast-Link DNA Ligase.
7. Transform TransforMax EPI300 Electrocompetent *E. coli*, plate and select clones according to the procedure described on page 8.
8. Pick white colonies and induce the clones to high-copy number according to the procedure described on page 9.

Appendix D

Transformation of the pCC1BAC-145 CopyControl BAC DNA

Kit components used in this step: **pCC1BAC-145 Control BAC DNA.**

Reagents and equipment supplied by the user: TransforMax EPI300 Electrocompetent *E. coli*, electroporator, electroporation cuvettes, 14-15 ml round bottom culture tubes, large-bore pipette tips, microcentrifuge tubes, 37°C incubator shaker, LB plate + chloramphenicol (12.5 µg/ml) + X-Gal (40 µg/ml) + IPTG (0.4 mM), SOC medium.

Note: *An E. coli host carrying an inducible trfA gene (such as TransforMax EPI300 E. coli) is required for amplification of the CopyControl BAC clones to high-copy number. Copy number induction procedures described in this manual have been optimized using TransforMax EPI300 E. coli. We can not guarantee clone amplification results using any E. coli strain other than TransforMax EPI300 E. coli.*

1. Prepare 1 ml of SOC medium (do not include antibiotic in the medium) for each electroporation to be performed. This medium will be used for post-electroporation outgrowth of transformed cells. Maintain the medium at room temperature.
2. Pre-chill electroporation cuvettes and 1.5 ml tubes on ice.
3. Set-up the electroporation device according to the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
4. Thaw TransforMax EPI300 Electrocompetent *E. coli* cells on ice. Mix gently. Use the cells immediately. Unused cells can be refrozen at –70°C.

   Note: *Refrozen cells may have reduced transformation efficiency.*
5. Transfer 1 µl (500 pg) of the pCC1BAC-145 Control CopyControl BAC DNA and 50 µl of cells to a pre-chilled microcentrifuge tube.

   Note: *a smaller volume of cells can be used based on the needs and experiences of the user.*
6. Mix the cells and DNA by pipetting up and down 2-3 times or by tapping the tube gently.
7. Transfer the cell/DNA mix to the electroporation cuvette. Be sure that there are no air bubbles in the cuvette. Wipe the cuvette of any condensation. Place into the electroporator.
8. Apply the electric pulse at manufacturer's recommendations for bacterial (*E. coli*) electroporation.
9. Immediately after electroporation, add 950 µl of the room temperature SOC medium to the cuvette. Mix gently by pipetting up and down 2-3 times.
10. Transfer the cells to a 15 ml tube and incubate at 37°C with shaking at 220-230 rpm for 1 hour to recover the cells and allow expression of the antibiotic resistance marker.
11. Dilute the transformation outgrowth 1:10 in SOC and plate 100 µl of the cells on LB/chloramphenicol (12.5 µg/ml) plates. The remaining cell outgrowth can be stored at 4°C in the event additional cell dilutions are plated.
12. The transformation efficiency will be $>10^7$ cfu/µg of the pCC1BAC-145 Control BAC DNA.

    Note: *the pCC1BAC-145 BAC DNA provided in solution may give lower transformation efficiency over time. However, just a single colony is needed to perform the amplification step.*

Induction of the pCC1BAC-145 Control CopyControl BAC Clones in 1.5 ml tubes

1. Dispense 1 ml of LB + chloramphenicol (12.5 µg/ml) into 1.5 ml tubes. Inoculate each tube with an isolated single white colony (BAC clone) from an overnight plate.
2. Incubate the cultures at 37°C overnight **without** shaking.
3. Following overnight incubation, mix each tube and then aspirate off 800 µl of culture medium from each and discard.
4. Add 800 µl of fresh LB + chloramphenicol (12.5 µg/ml) to the remaining 200 µl of the overnight culture. Mix by vortexing.
5. Incubate the tubes for 30 minutes at 37°C with shaking at 250 rpm. After 30 minutes, the $O.D_{600}$ will be at 0.4 - 0.6.
6. Thaw the CopyControl Induction Solution and mix thoroughly. Add 1 µl of 1000X CopyControl Induction Solution (to a 1X final concentration) to each tube. Incubate each for 2 hour at 37°C with vigorous shaking. Aeration is critical! Shake the tubes in a manner that will maximize aeration of the cultures (for example 1.5 ml tubes can be taped horizontally to the shaking table).
7. Isolate DNA from the induced culture by your method of choice.

Appendix E

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).

```
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
```

The transposon sequence can be downloaded at www.epibio.com/sequences.

Restriction Analysis of the pCC1BAC CopyControl Vector

Restriction Enzymes that cut pCC1BAC one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
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	PflF I	1	5260
Bgl I	3	639, 3160, 7609	PpuM I	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
EcoR V	2	4117, 4346	Xma I	3	348, 637, 3480
Fse I	1	2478			

Restriction Enzymes that cut pCC1BAC 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 pCC1BAC:

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

7. Related Products

BACMAX™ DNA Purification Kit

BMAX044 1 kit

The BACMAX DNA Purification Kit uses a modified alkaline lysis procedure to obtain high yields of intact BAC DNA without expensive columns or organic solvents.

BACMAX96™ DNA Purification Kit

Liquid reagents and plastic consumables

BAC96116 384 purifications

The BACMAX96 DNA Purification Kit optimizes the FosmidMAX protocol for use in 96-well format. The BACMAX96 Kit can purify 384 BACs, Fosmids, or other large DNA clones in 2.5 hours without robotics or automation. Contaminating RNA is completely removed using the included RNase Blend; contaminating genomic DNA is removed with the included Plasmid-Safe™ DNase. Please inquire regarding bulk pricing.

CopyControl™ BAC Autoinduction Solution

AIS107B 150 ml

CopyControl BAC Autoinduction Solution can be used in the place of the CopyControl Induction Solution. The autoinduction process is simpler than the induction protocol and can provide superior DNA yields during BAC purification.

Plasmid-Safe™ ATP-Dependent DNase

E3101K 1000 units

E3105K 5000 units

E3110K 10000 units

Plasmid-Safe ATP-Dependent DNase removes contaminating genomic DNA from BAC and fosmid DNA preps. The enzyme works by selectively and processively degrading linear DNA molecules from the ends. Circular DNAs, such as BACs, fosmids and plasmids do not contain ends, and are therefore not degraded by the enzyme.

Fast-Link™ DNA Ligation Kit

LK11025 25 rxns

LK0750H 50 rxns

LK6201H 100 rxns

The Fast-Link DNA Ligation Kit ligates cohesive-ended DNA in 5 minutes, and blunt-ended DNA in 15 minutes at room temperature. The optimized ligase in the kit is compatible with any application that uses T4 DNA ligase.

TransforMAX™ EPI300™ Electrocompetent *E. coli*

EC300105 5 x 100 µl

EC300110 10 x 100 µl

EC300150 50 x 100 µl

TransforMAX™ EPI300™-T1^{R} Electrocompetent *E. coli*

EC02T15 5 x 100 µl

EC02T110 10 x 100 µl

TransforMax EPI300 Electrocompetent *E. coli* cells carry the inducible *trf*A gene needed to support the inducible features of CopyControl vectors.

GELase™ Agarose Gel-Digesting Preparation

G09050	50 units
G09100	100 units
G09200	200 units

GELase Agarose Gel-Digesting Preparation aids in the recovery of high molecular weight DNA for BAC libraries from low melting point agarose.

8. References

1. Hradecna, Z., Wild, J., and Szybalski, W. (1998) *Microbial. And Comp. Genomics* **3**, 58.
2. Wild, J., Hradecna, Z., and Szybalski, W. (2001) *Plasmid* **45**, 142.
3. Wild, J. *et al*., (2002) *Genomic Research* **12**, 1434.
4. Peterson, D.G. *et al*., Construction of Plant Bacterial Artificial Chromosome (BAC) Libraries: An illustrated Guide. www.plantgenome.uga.edu/dgp/hub/pdf_files/bacman2.pdf.
5. Osoegawa, K. *et al*., (1998) *Genomics* **52**, 1.
6. Shizuya, H. *et al*., (1992) *ProC. Natl. Acad. Sci. USA* **89**, 8794.
7. Clarke, L. and Carbon, J. (1976) *Cell* **9**, 91 (see also Sambrook, J. *et al*., eds. [2001] in: *Molecular Cloning: A Laboratory Manual* [3rd ed.], CSH Laboratory Press, New York, pg. 4.6.
8. Atrazhev, A. M. and Elliott, J.F. (1996) *BioTechniques* **21**, 1024.

Visit our technical blog: **epicentral.blogspot.com**