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CopyControl™ Fosmid Library Production Kit with pCC1FOS™ Vector

with pCC1FOS™ Vector and
Phage T-1 Resistant EPI300™-T1^R *E. coli* Plating Strain

Cat. No. CCFOS110

CopyControl™ HTP Fosmid Library Production Kit with pCC2FOS™ Vector

with pCC2FOS™ Vector and
Phage T-1 Resistant EPI300™-T1^R *E. coli* Plating Strain

Cat. No. CCFOS059

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1. Introduction

The CopyControl Cloning System,* based on technology developed by Dr. Waclaw Szybalski [1-3] at the University of Wisconsin-Madison, 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, CopyControl BAC (Bacterial Artificial Chromosome) 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 sequencing, fingerprinting, subcloning, *in vitro* transcription, and other applications. CopyControl Cloning Kits for BAC cloning, fosmid library production, and cDNA, Gene & PCR cloning are available.

The CopyControl Cloning System Has Two Required Components

1. Each CopyControl Vector contains both a single-copy origin 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™-T1^R *E. coli* strain.*
2. The EPI300 *E. coli* provides 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. Phage T1-resistant EPI300-T1^R cells are provided with the kits.

Quality Control

The CopyControl Fosmid Library Production Kits are function-tested using the Fosmid Control DNA provided in the kit. Each kit must yield >10^7 cfu/µg (>2.5 x 10^6 cfu/ml) with the Fosmid Control DNA. The MaxPlax Lambda Packaging Extracts have been tested to give >10^8 pfu/µg with Ligated Lambda Control DNA.

Features of the CopyControl pCC1FOS™ and pCC2FOS™ Vectors

- 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.
- Bacteriophage lambda *cos* site for lambda packaging or lambda-terminase cleavage.
- Bacteriophage P1 *lox*P site for Cre-recombinase cleavage.
- Bacteriophage T7 RNA polymerase promoter flanking the cloning site.

2. Kit Contents

Desc.	Concentration	Quantity

The CopyControl Fosmid Library Production Kit contains the pCC1FOS Vector
CCFOS110

The CopyControl HTP Fosmid Library Production Kit contains the pCC2FOS Vector
CCFOS059

Reagents included in each kit are sufficient to construct 10 fosmid libraries.

CopyControl™ pCC1FOS™ or pCC2FOS™ Fosmid Vector (Cloning-Ready; linearized at the unique Eco72 I site and dephosphorylated)	(0.5 µg/µl)	20 µl
End-Repair 10X Buffer (330 mM Tris-acetate [pH 7.5], 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM DTT)		100 µl
dNTP Mix: (2.5 mM each of dATP, dCTP, dGTP, dTTP)		100 µl
End-Repair Enzyme Mix		50 µl
ATP Solution	(10 mM)	100 µl
Fast-Link™ DNA Ligase	(40 U @ 2 U/µl)	20 µl
Fast-Link™ 10X Ligation Buffer		100 µl
GELase™ Enzyme Preparation	(25 U @ 1 U/µl)	25 µl
GELase™ 50X Buffer (2.0 M Bis-Tris [pH 6.0], 2.0 M NaCl)		100 µl
Fosmid Control DNA	(100 ng/µl)	50 µl
EPI300™-T1R Phage T1-resistant *E. coli* Plating strain: glycerol stock 2 [F– mcrA Δ(*mrr-hsd*RMS-*mcr*BC) (StrR) φ80dl*ac*ZΔM15 Δl*ac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ– *rps*L *nup*G *trf*A *ton*A *dhfr*]		50 µl
MaxPlax™ Lambda Packaging Extracts		10 Extracts
Ligated Lambda Control DNA: (λc1857 *Sam*7)	(1 µg @ 0.02 µg/µl)	50 µl
LE392MP E. coli Control strain: glycerol stock [F– e14–(McrA–) Δ(*mcr*C-*mrr*) (TetR) *hsd*R514 *sup*E44 *sup*F58 *lac*Y1 or Δ(*lac*IZY)6 galK2 galT22 *met*B1 *trp*R55 λ–]		250 µl
Fosmid Autoinduction Solution	(500X concentrate)	2 x 1 ml

Note: *MaxPlax Lambda Packaging Extracts are supplied as freeze-thaw/sonicate extracts in unlabeled single tubes. The extracts, Ligated Lambda Control DNA, and LE392MP Control Plating Strain are packaged together in a CO2-impermeable foil pouch.*

Storage: Store the EPI300-T1^R Plating Strain and MaxPlax Lambda Packaging Extracts at –70°C. Exposure to higher temperatures will greatly compromise packaging extract efficiency. Once the MaxPlax Packaging Extracts are opened, do not expose them to dry ice. Store the remainder of the kit components at –20°C. After thawing, store the Ligated Lambda Control DNA at 4°C.

Additional Required Reagents

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

- **LB broth + 10 mM $MgSO_4$ + 0.2% Maltose**
- **Low-melting-point (LMP) agarose**
- **Ethanol** (100% and 70%)
- **3 M Sodium Acetate** (pH 7.0)
- **Phage Dilution Buffer** (10 mM Tris-HCl [pH 8.3], 100 mM NaCl, 10 mM $MgCl_2$)
- **TE Buffer** (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)

Additional Features of the pCC2FOS Vector

The CopyControl HTP Fosmid Library Production Kit contains the pCC2FOS vector (Fig. 2). The pCC2FOS vector, a modification of the pCC1FOS (Fig. 1) vector, contains a primer cassette that optimizes end-sequencing results, especially in a high-throughput setting.[4] The pCC2FOS primer cassette eliminates wasteful vector-derived sequencing reads by having the 3′ terminus of the forward and reverse sequencing primers anneal three nucleotides from the cloning site. In addition, the seven-base sequence at the 3′ end of each primer was specifically designed to minimize mispriming from any contaminating *E. coli* DNA present after template purification.

How the CopyControl Cloning System Works (Figs. 3, 4)

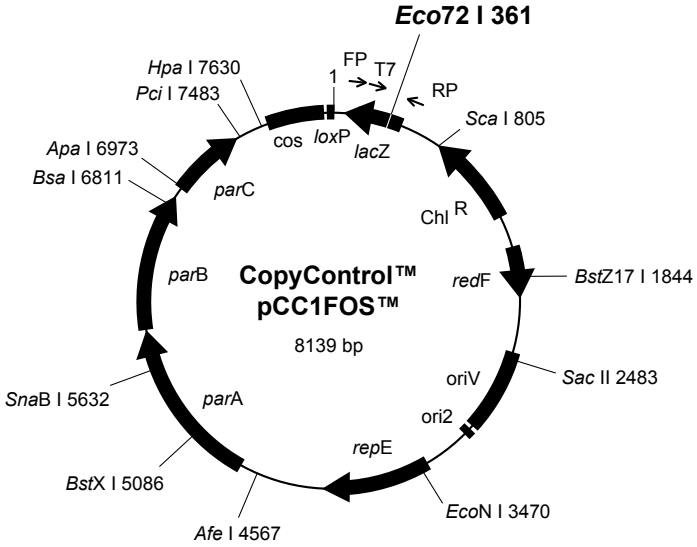
1. Ligate the DNA of interest into the linearized and dephosphorylated CopyControl Cloning-Ready Vector supplied with the respective kit.
2. Package the ligated DNA into the lambda phage and infect EPI300-T1^R *E. coli* and select on LB-chloramphenicol plates. Under these conditions, the *trf*A gene is not expressed and the clones are maintained at single-copy.
3. Pick individual CopyControl clones from the plate and grow in culture.
4. Add the CopyControl Fosmid Autoinduction Solution (included) or CopyControl Induction Solution (available separately) 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.

3. Overview of the CopyControl Fosmid Library Production Process

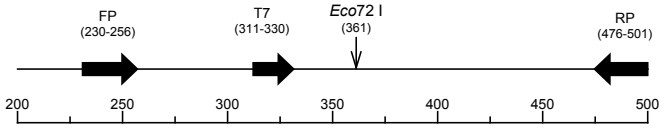
The CopyControl Fosmid Library Production Kits will produce a complete and unbiased primary fosmid library in about 2 days. The kit utilizes a novel strategy of cloning randomly sheared, end-repaired DNA. Shearing the DNA leads to the generation of highly random DNA fragments in contrast to more biased libraries that result from fragmenting the DNA by partial restriction digests.

The steps involved (protocols for steps 2-8 are included in this manual):

1. Purify DNA from the desired source (the kit does not supply materials for this step).
2. Shear the DNA to approximately 40-kb fragments.
3. End-repair the sheared DNA to blunt, 5′-phosphorylated ends.

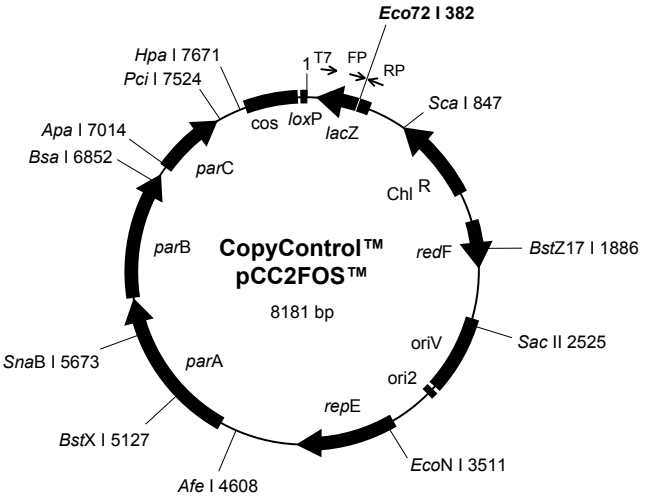


Note: Not all restriction enzymes that cut only once are indicated above.
See Appendix E for complete restriction information.
Primers are not drawn to scale.

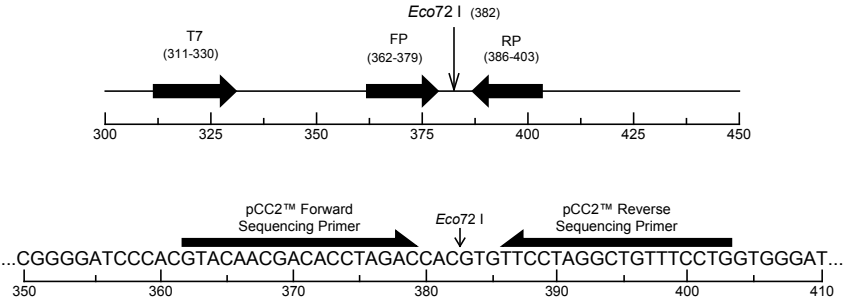


FP = pCC1™/pEpiFOS™ Foward Sequencing Primer	5' GGATGTGCTGCAAGGCGATTAAGTTGG 3'
RP = pCC1™/pEpiFOS™ Reverse Sequencing Primer	5' CTCGTATGTTGTGTGGAATTGTGAGC 3'
T7 = T7 Promoter Primer	5' TAATACGACTCACTATAGGG 3'

Figure 1. pCC1FOS™ Vector Map.



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



FP = pCC2™ Foward Sequencing Primer	5' GTACAACGACACCTAGAC 3'
RP = pCC2™ Reverse Sequencing Primer	5' CAGGAAACAGCCTAGGAA 3'
T7 = T7 Promoter Primer	5' TAATACGACTCACTATAGGG 3'

Figure 2. pCC2FOS™ Vector Map.

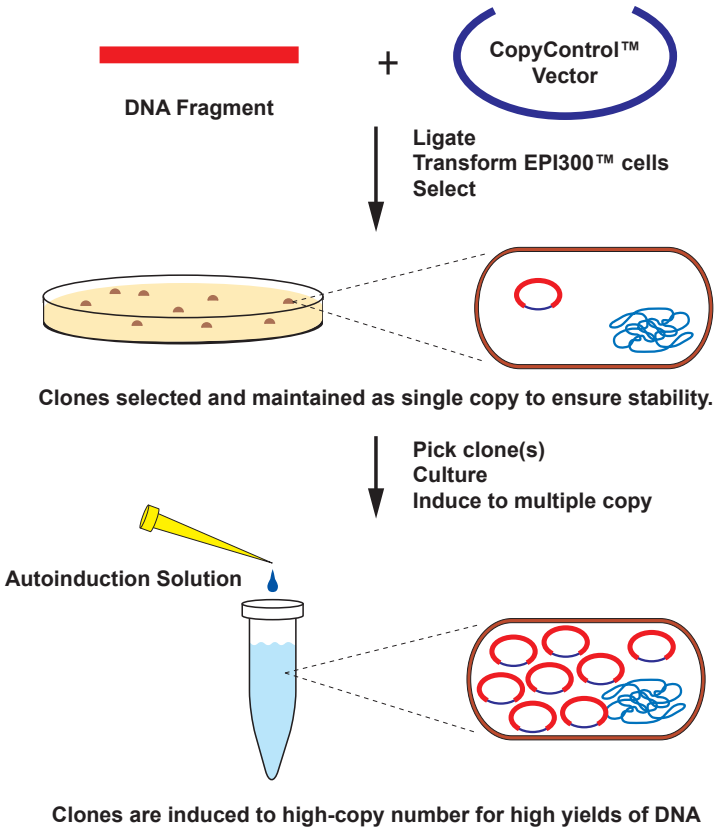


Figure 3. Overview of the CopyControl™ System.

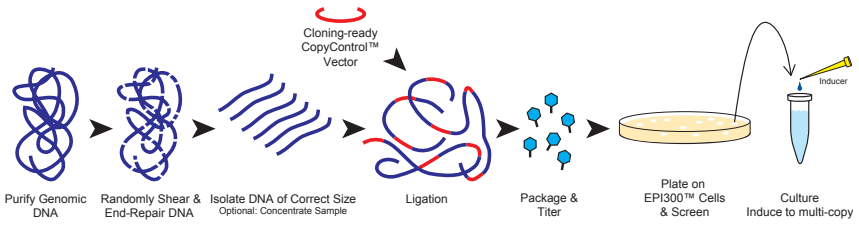


Figure 4. Production of a CopyControl™ Fosmid library and subsequent induction of clones to high-copy number.

4. Isolate the desired size range of end-repaired DNA by LMP agarose gel electrophoresis.
5. Purify the blunt-ended DNA from the LMP agarose gel.
6. Ligate the blunt-ended DNA to the Cloning-Ready CopyControl pCC1FOS or pCC2FOS Vector.
7. Package the ligated DNA and plate on EPI300-T1^R plating cells. Grow clones overnight.
8. Pick CopyControl Fosmid clones of interest and induce them to high-copy number using the CopyControl Fosmid Autoinduction Solution.
9. Purify DNA for sequencing, fingerprinting, subcloning, or other applications. The kit does not supply materials for this step. See Related Products.

4. CopyControl Fosmid Library Production 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 **Fosmid Control Insert** for the CopyControl Fosmid library Production Kit is an approximately 42 kb piece of DNA of the human X-chromosome. It is to be used for two purposes:
   1) As a ligation/packaging control that is used for library construction quality assurance
   2) As a size marker for the gel size selection step

   The insert also contains a kanamycin selection marker. This marker is useful as a positive selection for Fosmid control clones that confirms that the insert DNA in the control testing is actually the control DNA. Selection for the control clones can be performed using 12.5 µg/ml chloramphenicol and 50 µg/ml Kanamycin (see Appendix B).
3. The **Ligated Lambda Control DNA** (λ*c*1857 *Sam*7) and the Control Strain LE392MP are used to test the efficiency of the MaxPlax Lambda Packaging Extracts (see Appendix C).

Preparation

1. Prepare high-molecular-weight genomic DNA from the organism using the MasterPure™ DNA Purification Kit or standard methods.[5] Resuspend the DNA in TE buffer at a concentration of 0.5 µg/µl. This DNA will be referred to as the "insert DNA" throughout this manual.
2. The EPI300-T1^R Plating strain is supplied as a glycerol stock. Prior to beginning the CopyControl Fosmid Library Production procedure, streak out the EPI300-T1^R cells on an LB plate. Do not include any antibiotic in the medium. Grow the cells at 37°C overnight, and then seal and store the plate at 4°C. The day before the Lambda Packaging reaction (Part F), inoculate 50 ml of LB broth + 10 mM $MgSO_4$ + 0.2% Maltose with a single colony of EPI300-T1^R cells and shake the flask overnight at 37°C.

A. Shearing the Insert DNA

Kit component used in this step: **Fosmid Control DNA**.

Shearing the DNA into approximately 40-kb fragments leads to the highly random generation of DNA fragments in contrast to more biased libraries that result from partial restriction endonuclease digestion. Frequently, genomic DNA is sufficiently sheared as a result of the purification process, and additional shearing is not necessary. Test the extent of shearing of the DNA by first analyzing a small amount of it by pulse field gel electrophoresis (PFGE) with voltage and ramp times recommended by the manufacturer for separation of 10- to 100-kb DNA. If a PFGE apparatus is not available, run the sample on a 20-cm long, 1% standard agarose gel at 30-35 V overnight. Load 100 ng of the Fosmid Control DNA in an adjacent gel lane as a control. Do not include ethidium bromide in the gel or running buffer. Stain the gel with ethidium bromide or SYBR® Gold (Invitrogen) after the run is complete and visualize the gel.

If 10% or more of the genomic DNA migrates with the Fosmid Control DNA, then proceed to Part B. If the genomic DNA migrates slower (higher MW) than the Fosmid Control DNA, then the DNA needs to be sheared further as described below. If the genomic DNA migrates faster than the Fosmid Control DNA (lower MW) then it has been sheared too much and should be reisolated.

If shearing is required, we recommend that at least 2.5 µg (at a concentration of 500 ng/µl) of DNA be used. Randomly shear the DNA by passing it through a 200-µl small-bore pipette tip. Aspirate and expel the DNA from the pipette tip 50-100 times. Examine 1-2 µl of the DNA on a 20-cm agarose gel using the Fosmid Control DNA as a size marker. If 10% or more of the genomic DNA migrates with the Fosmid Control DNA, then proceed to Part B. If >90% of the sheared DNA comigrates with the Fosmid Control DNA and appears as a relatively tight band (as in Fig. 5, lane 3), gel size-selection may not be necessary; you may skip the gel-sizing step and proceed directly with ligation of the DNA to the vector (Part E). If the DNA is still too large, aspirate and expel the DNA from the pipette tip an additional 50 times. Examine 1-2 µl of this DNA by agarose gel electrophoresis as described previously.

B. End-Repair of the Insert DNA

Kit components used in this step: **End-Repair Enzyme Mix, 10X Buffer, dNTPs, ATP**.

This step generates blunt-ended, 5′-phosphorylated DNA. The end-repair reaction can be scaled as dictated by the amount of DNA available.

1. Thaw and thoroughly mix all of the reagents listed below before dispensing; place on ice. Combine the following on ice:

x	µl	sterile water
8	µl	10X End-Repair Buffer
8	µl	2.5 mM dNTP Mix
8	µl	10 mM ATP
up to 20	µg	sheared insert DNA (approximately 0.5 µg/µl)
4	µl	End-Repair Enzyme Mix
80	µl	Total reaction volume

2. Incubate at room temperature for 45 minutes.
3. Add gel loading buffer and incubate at 70°C for 10 minutes to inactivate the End-Repair Enzyme Mix. Proceed with **Size Selection of the End-Repaired DNA** in Part C.

C. Size Selection of the End-Repaired DNA

Kit components used in this step: **Fosmid Control DNA**.

If the DNA to be used in the cloning process appears as a long smear (Fig. 5, Lanes 1 and 2), size-select the end-repaired DNA by LMP agarose gel electrophoresis. Ideally, use PFGE with voltage and ramp times recommended by the manufacturer for separation of 10- to 100-kb DNA. If a PFGE apparatus is not available, analyze the sample on a 20-cm long, 1% LMP agarose gel at 30-35 V overnight. **Minigels (e.g., 10 cm) do not provide sufficient resolution of DNA in the 20- to 60-kb size range.**

Fractionate the DNA on an LMP agarose gel. **It is important to perform this electrophoresis in the absence of ethidium bromide (do not add ethidium bromide to the gel). The DNA that will be cloned should not be exposed to UV light under any circumstances.** This can decrease the cloning efficiency by 100-fold or more. A diagram of the recommended method is shown in Fig. 5.

Note 1: *Even 30 seconds of exposure to 302-nm UV light will cause a 100- to 200-fold drop in ligation and cloning efficiency.*

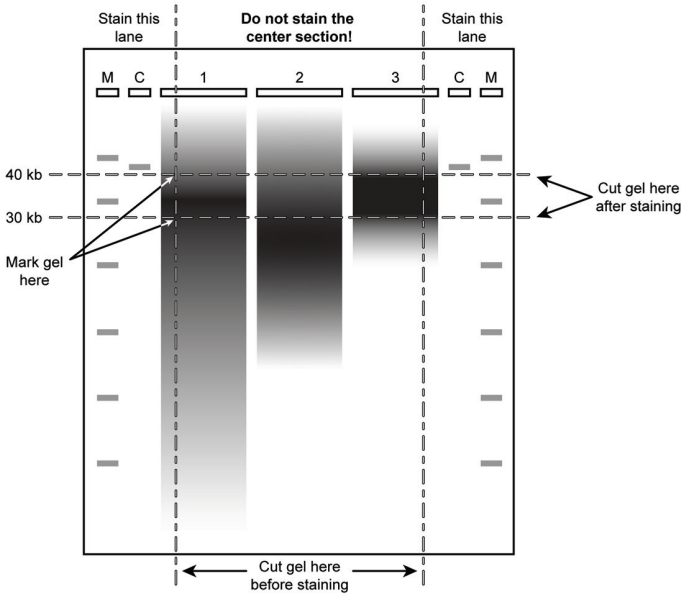


Figure 5. Gel purification of DNA: Keeping ethidium and UV away from your DNA.

Note 2: *The protocol below is designed for use with Epicentre's GELase™ Agarose Gel-Digesting Preparation, and thus requires LMP agarose. Standard high-melt agarose can also be used and the DNA extracted from the gel slices by other methods.*

1. Prepare a 1% **LMP** agarose gel in 1X TAE or 1X TBE buffer. Use a wide comb as needed to be able to load sufficient DNA into the gel (see Fig. 5).

   Note: *Do not include ethidium bromide in the gel solution.*

2. Load DNA size markers into each of the outside lanes of the gel. Load 100 ng of Fosmid Control DNA into each of the inner adjacent lanes of the gel. Load the end-repaired insert DNA in the lane(s) between the Fosmid Control DNA lanes.
3. Resolve the samples by gel electrophoresis at room temperature overnight at a constant voltage of 30-35 V. Do not include any DNA stain in the gel or in the gel running buffer during electrophoresis.
4. Following electrophoresis, cut off the outer lanes of the gel containing the DNA size markers, the Fosmid Control DNA, and a small portion of the next lane that contains your random sheared end-repaired genomic DNA (see Fig. 5).
5. Stain the cut-off sides of the gel with ethidium bromide or SYBR Gold (Invitrogen), which is more sensitive than ethidium bromide, and visualize the DNA with UV light. Mark the position of the desired size DNA in the gel using a pipet tip or a razor blade.

   Note: *Do not expose the sample DNA to UV! Even short-duration UV exposure can decrease cloning efficiencies by 100- to 1,000-fold.*

6. Reassemble the gel and excise a gel slice that is 2- to 4-mm below the position of the Fosmid Control DNA.

   Caution: *Be sure to cut the gel slice so that the DNA recovered is ≥25 kb. Cloning DNA smaller than ~25 kb may result in unwanted chimeric clones.*

   Note: *Prior to reassembly, without breaking the gel, carefully rinse the stained gel with distilled water to remove excess stain from the gel pieces. This will prevent the gel pieces containing the sample DNA from being exposed to stain.*

7. Transfer the gel slice to a tared, sterile, screw-cap tube for extraction, either by using the GELase method, or other desired method for isolating DNA from agarose gels. The size of the tube to be used will be dictated by the size and number of gel slices being digested with GELase enzyme.
8. Proceed with **Recovery of the Size-Fractionated DNA** in Part D or store the gel slice at 4°C to –20°C for up to 1 year.

D. Recovery of the Size-Fractionated DNA

Kit components used in this step: **GELase 50X Buffer, GELase Enzyme Preparation**.

Before beginning this step, prepare a 70°C and a 45°C water bath or other temperature-regulated apparatus.

1. Weigh the tared tubes to determine the weight of the gel slice(s). Assume 1 mg of solidified agarose will yield 1 µl of molten agarose upon melting.

2. Warm the GELase 50X Buffer to 45°C. Melt the LMP agarose by incubating the tube at 70°C for 10-15 minutes. Quickly transfer the tube to 45°C.
3. Add the appropriate volume of warmed GELase 50X Buffer to 1X final concentration. Carefully add 1 U (1 µl) of GELase Enzyme Preparation to the tube for each 100 µl of melted agarose. Keep the melted agarose solution at 45°C and gently mix the solution. Incubate the solution at 45°C for at least 1 hour (overnight incubation is acceptable, if desired).
4. Transfer the reaction to 70°C for 10 minutes to inactivate the GELase enzyme.
5. Remove 500-µl aliquots of the solution into sterile, 1.5-ml microfuge tube(s).
6. Chill the tube(s) in an ice bath for 5 minutes. Centrifuge the tubes in a microcentrifuge at maximum speed (>10,000 x g) for 20 minutes to pellet any insoluble oligosaccharides. Any "pellet" will be gelatinous, and translucent to opaque. Carefully remove the upper 90%-95% of the supernatant, **which contains the DNA**, to a sterile 1.5-ml tube. Be careful to avoid the gelatinous pellet.
7. Precipitate the DNA.
   a) Add 1/10 volume of 3 M sodium acetate (pH 7.0) and mix gently.
   b) Add 2.5 volumes of ethanol. Cap the tube and mix by gentle inversion.
   c) Allow precipitation to proceed for 10 minutes at room temperature.
   d) Centrifuge the precipitated DNA for 20 minutes in a microcentrifuge, at top speed (>10,000 x g).
   e) Carefully aspirate the supernatant from the pelleted DNA.
   f) Wash the pellet twice with cold, 70% ethanol, repeating steps d) and e), using care not to disrupt the DNA pellet.
   g) After the second 70% ethanol wash, carefully invert the tube and allow the pellet to air-dry for 5-10 minutes (longer dry times will make resuspension of the DNA difficult).
   h) Gently resuspend the DNA pellet in TE Buffer.

   Note: *A 10-µl ligation reaction volume allows a maximum 6 µl of input DNA.*
8. Determine the DNA concentration by fluorimetry. Alternatively, estimate the concentration of the DNA by running an aliquot of the DNA on an agarose gel using dilutions of known amounts of the Fosmid Control DNA as standard.

   Note: *Measuring the DNA concentration by spectrophotometry (A_{260}) is not recommended because the DNA concentration will not be high enough to be measured accurately.*

   Note: *If desired, the reactions can now be frozen and stored overnight at –20°C.*

E. Ligation Reaction

Kit components used in this step: **Fast-Link 10X Ligation Buffer, Fast-Link DNA Ligase, ATP, CopyControl pCC1FOS or pCC2FOS Cloning-Ready Vector**.

1. Please refer to Appendix A to determine the approximate number of CopyControl Fosmid clones that you will need for your library. A single ligation reaction will produce 10^3-10^6 clones, depending on the quality of the insert DNA. Based on this information, calculate the number of ligation reactions that you will need to perform. The ligation reaction can be scaled as needed.
2. Combine the following reagents in the order listed and mix thoroughly after each addition.

   A 10:1 molar ratio of CopyControl pCC1FOS or pCC2FOS Vector to insert DNA is optimal.

   0.5 µg CopyControl pCC1FOS or pCC2FOS Vector ≈ 0.09 pmol vector

   0.25 µg of ≈40-Kb insert DNA ≈ 0.009 pmol insert DNA

x	µl	sterile water
1	µl	10X Fast-Link Ligation Buffer
1	µl	10 mM ATP
1	µl	CopyControl pCC1FOS or pCC2FOS Vector (0.5 µg/µl)
x	µl	concentrated insert DNA (0.25 µg of ≈40-kb DNA)
1	µl	Fast-Link DNA Ligase
10	µl	Total reaction volume

3. Incubate at room temperature for 4 hours.

   Note: *Overnight ligation reactions at 16°C may be performed but should not be necessary. Transfer the reaction to 70°C for 10 minutes to inactivate the Fast-Link DNA Ligase. Proceed to Part F or, if desired, the reactions can now be frozen and stored overnight at –20°C.*

F. Packaging the CopyControl Fosmid Clones

Kit components used in this step: **MaxPlax Lambda Packaging Extracts, EPI300-T1^R Plating Strain**.

1. On the day of the packaging reactions, inoculate 50 ml of LB broth + 10 mM $MgSO_4$ + 0.2% Maltose with 0.5 ml of the EPI300-T1^R overnight culture from the **Preparation** step on page 9. Shake the flask at 37°C to an A_{600} of 0.8-1.0 (~2 hours). Store the cells at 4°C until needed (Part G). The cells may be stored for up to 72 hours at 4°C if necessary.
2. Thaw, on ice, one tube of the MaxPlax Lambda Packaging Extracts for every ligation reaction performed in Part E. For example, thaw one tube of the MaxPlax Lambda Packaging Extracts if the standard 10-µl ligation reaction was done. Thaw two tubes if the ligation reaction was scaled up to 20 µl, etc.
3. When the extracts are thawed, **immediately** transfer 25 µl (one-half) of each to a second 1.5-ml microfuge tube and place on ice. Return the remaining 25 µl of the MaxPlax Packaging Extract to a –70°C freezer for use in Part F, Step 7.

Note: *Do not expose the MaxPlax Packaging Extracts to dry ice or other CO_2 source.*

4. Add 10 µl of the ligation reaction from Part E to each 25 µl of the thawed extracts being held on ice.
5. Mix by pipetting the solutions several times. Avoid the introduction of air bubbles. Briefly centrifuge the tubes to get all liquid to the bottom.
6. Incubate the packaging reactions at 30°C for 2 hours.
7. After the 2-hour packaging reaction is complete, add the remaining 25 µl of MaxPlax Lambda Packaging Extract from Part F, Step 3 to each tube.
8. Incubate the reactions for an additional 2 hours at 30°C.
9. At the end of the second incubation, add Phage Dilution Buffer (PDB) to **1 ml final volume** in each tube and mix gently. Add 25 µl of chloroform to each. Mix gently and store at 4°C. A viscous precipitate may form after addition of the chloroform. This precipitate will not interfere with library production. Determine the titer of the phage particles (packaged fosmid clones) in Part G, and then plate the fosmid library in
Part H. Or, store the phage particles as described in Appendix D.

   Note: *In the construction of metagenomic fosmid libraries from environmental water or soil microbes, the amount of PDB to be added to the packaged phage may require some adjustment depending on the starting amount of DNA. If the DNA used in ligation is lower than the protocol recommends, then the addition of 0.5 ml of the PDB may be needed.*

G. Titering the Packaged CopyControl Fosmid Clones

Kit components used in this step: **EPI300-T1^R Plating Strain from Part F, Step 1.**

Before plating the library, we recommend that you determine the titer of the phage particles (packaged CopyControl Fosmid clones). This will aid in determining the number of plates and dilutions required to obtain a library that meets your needs.

1. Make serial dilutions of the 1 ml of packaged phage particles from Part F, Step 9 into Phage Dilution Buffer (PDB) in sterile microfuge tubes.

   A) 1:10^1 Dilute 10 µl of packaged phage into 90 µl of PDB.

   B) 1:10^2 Dilute 100 µl of the 1:10^1 dilution into 900 µl of PDB.

   C) 1:10^3 Dilute 100 µl of the 1:10^2 dilution into 900 µl of PDB.
2. Add 10 µl of each above dilution, and 10 µl of the undiluted phage, individually, to 100 µl of the prepared EPI300-T1^R host cells from Part F, Step 1 above. Incubate each tube for 1 hour at 37°C.
3. Spread the infected EPI300-T1^R cells on an LB plate + 12.5 µg/ml chloramphenicol and incubate at 37°C overnight to select for the CopyControl Fosmid clones.
4. Count colonies and calculate the titer of the packaged phage particles from Part F, Step 9.

Sample Calculation:

If there were 200 colonies on the plate streaked with the 1:10^3 dilution, then the titer in cfu/ml, (where cfu represents colony forming units) of this reaction would be:

$$\frac{(\text{\# of colonies})\ (\text{dilution factor})\ (1{,}000\ \mu l/ml)}{(\text{volume of phage plated [}\mu l\text{]})}\ \textbf{OR}\ \frac{(200\ \text{cfu})\ (10^3)\ (1{,}000\ \mu l/ml)}{(10\ \mu l)} = 2 \times 10^7\ \text{cfu/ml}$$

H. Plating and Selecting the CopyControl Fosmid Library

Based on the titer of the packaged CopyControl Fosmid clones and the estimated number of clones required (see Appendix A), calculate the volume of the packaged fosmid clones that will be needed to prepare the CopyControl Fosmid library.

1. Based on the titer of the phage particles determined in Part G, dilute the phage particles from Part F, Step 9 with Phage Dilution Buffer to obtain the desired number of clones and clone density on the plate. Proceed to the next step or store the diluted phage particles as described in Appendix D.
2. Mix the diluted phage particles from Part H, Step 1 with EPI300-T1^R cells prepared in Part F, Step 1 in the ratio of 100 µl of cells for every 10 µl of diluted phage particles.
3. Incubate the tubes at 37°C for 1 hour.
4. Spread the infected bacteria on an LB plate + 12.5 µg/ml chloramphenicol and incubate at 37°C overnight to select for the CopyControl Fosmid clones.
5. We recommend plating as much of the library as possible. Storage of the phage library for more than 72 hours at 4°C will result in a severe loss of phage viability and the plating efficiency will be severely compromised. We recommend storing the phage as an amplified library (see Appendix D, Method C) for best results.

Induction of the CopyControl Fosmid Clones to High-Copy Number

Once the desired CopyControl Fosmid clones are identified, they can be induced to high-copy number for high yields of DNA for sequencing, fingerprinting, or other applications.

The CopyControl Fosmid Autoinduction Solution can be supplemented into the cultures prior to inoculation and requires no subculturing of the bacteria. It is ideal for growing fosmid clones in any culture volume, including 96-well format or other high-throughput applications where subculturing is tedious and time-consuming.

The copy-number induction process can be done in any culture volume desired, depending on your needs. Generally, a 1-ml culture will provide sufficient DNA (typically 1-2 µg) for most applications. Below, we provide the standard autoinduction procedure for amplifying the clones in 200-µl, 1-ml, 2-ml, and 50-ml cultures, and the autoinduction protocol, which is freely scalable.

Autoinduction using the CopyControl Fosmid 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. Alternatively, it the clones can be grown in as little as 200 µl of culture in a 1-ml 96-well plate.*

1. Supplement the appropriate amount of LB medium + 12.5 µg/ml chloramphenicol with the 500X CopyControl Fosmid Autoinduction Solution. Refer to the table below.

Volume of fresh LB + chloramphenicol (12.5 µg/ml)	Volume of 500X CopyControl Fosmid Autoinduction Solution*	Vessel size recommended for optimum aeration
200 µl	0.4 µl	1-ml 96-well plate
1 ml	2 µl	2-ml 96-well plate
2 ml	4 µl	14-ml Falcon tube
50 ml	100 µl	250-ml EM flask

** Mix thoroughly after thawing.*

2. Individually inoculate the media with a small portion of the desired CopyControl Fosmid clones grown on an overnight plate.
3. Grow the cultures overnight (16-20 hours) at 37°C with shaking (225-250 rpm). Cultures incubated for longer or shorter periods of time may not properly induce. **Aeration during this incubation is critical!**
4. Centrifuge the cells and purify the DNA using the FosmidMAX™ DNA Purification Kit (Epicentre) or other standard laboratory methods.[6] For 1-ml 96-well plate preps, use the BACMAX96™ DNA Purification Kit (Epicentre) or for 200-µl 96-well plate preps, use the Direct Lysis Fosmid96 DNA Purification Kit (Epicentre).

5. Appendix

Appendix A

Determining the Approximate Number of Clones for a Complete Fosmid Library

Using the following formula,[6] determine the number of fosmid clones required to reasonably ensure that any given DNA sequence is contained within the library.

$$N = \ln (1\text{-}P) / \ln (1\text{-}f)$$

Where P is the desired probability (expressed as a fraction); f is the proportion of the genome contained in a single clone; and N is the required number of fosmid clones.

For example, the number of clones required to ensure a 99% probability of a given DNA sequence of *E. coli* (genome = 4.7 Mb) being contained within a fosmid library composed of 40-kb inserts is:

$$N = \ln (1 - 0.99) / \ln (1 - [4 \times 10^4 \text{ bases} / 4.7 \times 10^6 \text{ bases}]) = -4.61 / -0.01 = 461 \text{ clones}$$

Appendix B

Control Fosmid Library Production

The Fosmid Control DNA provided in the kit can be used to familiarize yourself with all the steps involved in producing a CopyControl Fosmid Library. We recommend that new CopyControl Fosmid Kit users perform the control ligation and packaging steps to familiarize themselves with the protocol.

The Fosmid Control DNA, as provided in the kit, is purified, blunt-ended, and ready for ligation to the Cloning-Ready pCC1FOS or pCC2FOS Vector. If desired, the Control DNA can be put through the end-repair and gel purification steps (Parts B, C, D) of the CopyControl Fosmid Library Production procedure.

1. Prepare EPI300-T1^R host cells as described in Part F, Step 1.
2. Ligate the Fosmid Control DNA to the CopyControl pCC1FOS or pCC2FOS Vector.

   Combine the following reagents in the order listed and mix after each addition.

3.5	µl	sterile water
1	µl	10X Fast-Link Ligation Buffer
1	µl	10 mM ATP
1	µl	CopyControl pCC1FOS or pCC2FOS Vector (0.5 µg/µl)
2.5	µl	Fosmid Control DNA (100 ng/µl) (See General Considerations)
1	µl	Fast-Link DNA Ligase
10	µl	Total reaction volume

3. Incubate at room temperature for 4 hours.
4. Transfer the reaction to 70°C for 10 minutes to inactivate the Fast-Link DNA Ligase.
5. Package the entire 10-µl ligation reaction as directed in Part F, Steps 2-9.
6. Titer the packaged control clones by making a 1:1,000 dilution of the packaged phage extract in Phage Dilution Buffer. Add 10 µl of the diluted packaged phage to 100 µl of EPI300-T1^R host cells. Incubate the tube at 37°C for 1 hour.
7. Spread the infected EPI300-T1^R cells on LB medium + 12.5 µg/ml chloramphenicol. Incubate the plate overnight at 37°C to select for the control CopyControl Fosmid clones.
8. Count the colonies and determine the titer, cfu/ml of the reaction (refer to Part G, Step 4). You should expect a titer of >1 x 10^7 cfu/ml; this corresponds to a packaging efficiency of >10^7 cfu/µg of the Fosmid Control DNA.
9. The single-copy CopyControl Fosmid clones produced can be induced to high-copy number by following the procedure on page 16.

Appendix C

Testing the Efficiency of the MaxPlax Packaging Extracts

Kit components used in this step: **Ligated Lambda Control DNA, MaxPlax Lambda Packaging Extracts, LE392MP Plating Strain**.

Additionally required:

- **LB Plates without antibiotic**
- **LB Top Agar** (LB broth containing 0.7% [w/v] Bacto-agar supplemented with 10 mM $MgSO_4$)
- **Phage Dilution Buffer** (10 mM Tris-HCl [pH 8.3], 100 mM NaCl, and 10 mM $MgCl_2$)

Please see the product literature for the MaxPlax Lambda Packaging Extracts, that was included with the CopyControl Fosmid Library Production Kits, for details on how to test the efficiency of the extracts.

Appendix D

Amplification and Storage of the Fosmid Library

Short-Term Storage: After dilution of the packaging reaction and addition of chloroform, the packaged fosmid library can be stored at 4°C for several days. For longer-term storage, see recommendations below.

Long-Term Storage: For longer-term storage we recommend storage of the packaged DNA as a primary library, or storage of the library in the EPI300-T1^R Phage T1-resistant *E. coli* plating strain using one of the methods described below.

Method A - Storage of Packaged DNA

1. To the packaged fosmid library, add sterile glycerol to a final concentration of 20%, mix, and store at –70°C.

Method B - Storage of Infected Cells

1. Infect the bacterial cells (see Part H).
2. Based on the expected titer, resuspend the cells in an appropriate volume of liquid media.
3. Transfer the final resuspension to a sterile tube and add sterile glycerol to a final concentration of 20%. Mix the solution and store aliquots (which would each constitute a library of the desired coverage) at –70°C.

Method C - Storage of Amplified Library (preferred method)

1. Infect the bacterial cells (see Part H).
2. Spread an appropriate volume of infected bacteria onto a plate(s) with the appropriate antibiotic and incubate at 37°C overnight.
3. Add ~2 ml of liquid media (e.g., LB) to a plate and resuspend all of the bacterial cells.
4. Transfer the resuspended cells and media to the next plate (if more than one overnight plate was used) and repeat resuspension process. Do this for as many plates as desired.
5. Transfer the final resuspension to a sterile tube and add sterile glycerol to a final concentration of 20%. Mix the solution and store aliquots (which would each constitute a library of the desired coverage) at –70°C.

Appendix E

pCC1FOS Sequencing Primers and Vector Data

pCC1/pEpiFOS-5 Sequencing Primers

Primers are available separately: 1 nmol supplied in TE Buffer at 50 µM

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

5′ - GGATGTGCTGCAAGGCGATTAAGTTGG - 3′

Length: 27 nucleotides

G+C content: 14

Molecular Weight: 8,409 daltons

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, Cat. No. F5RP011

5′ - CTCGTATGTTGTGTGGAATTGTGAGC - 3′

Length: 26 nucleotides

G+C content: 12

Molecular Weight: 8,038 daltons

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 Fosmid End-Sequencing

The following is the nucleotide sequence of pCC1FOS (bases 230-501) from the pCC1/pEpiFOS Forward Sequencing Primer (230-256) to the pCC1/pEpiFOS Reverse Sequencing Primer (501-476) encompassing the T7 RNA polymerase promoter (311-330) and the *Eco*72 I site (359-364).

230 GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTTCCCAGTC
280 ACGACGTTGT AAAACGACGG CCAGTGAATT GTAATACGAC TCACTATAGG
330 GCGAATTCGA GCTCGGTACC CGGGGATCCC AC------**Cloned Insert**------
----------**Cloned Insert**---------- GTGGGATC CTCTAGAGTC
380 GACCTGCAGG CATGCAAGCT TGAGTATTCT ATAGTCTCAC CTAAATAGCT
430 TGGCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAATTG TTATCCGCTC
480 ACAATTCCAC ACAACATACG AG

Appendix F

pCC2FOS Sequencing Primers and Vector Data

pCC2 Sequencing Primers

Primers are available separately: 1 nmol supplied in TE Buffer at 50 µM

pCC2FOS Forward Sequencing Primer, Cat. No. HTFP061

5′ - GTACAACGACACCTAGAC - 3′

Length: 18 nucleotides

G+C content: 9

Molecular Weight: 5,462 daltons

Temperatures of Dissociation & Melting:

T_d: 48°C (nearest neighbor method)

T_m: 64°C (% G+C method)

T_m: 54°C ([2 (A+T) + 4 (G+C)] method)

T_m: 58°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

pCC2FOS Reverse Sequencing Primer, Cat. No. HTRP062

5′ - CAGGAAACAGCCTAGGAA - 3′

Length: 18 nucleotides

G+C content: 9

Molecular Weight: 5,551 daltons

Temperatures of Dissociation & Melting:

T_d: 57°C (nearest neighbor method)

T_m: 64°C (% G+C method)

T_m: 54°C ([2 (A+T) + 4 (G+C)] method)

T_m: 58°C ((81.5 + 16.6 (log $[Na^+]$)) + ([41 (#G+C) - 500] / length) method)
where $[Na^+] = 0.1$ M

Orientation for Fosmid End-Sequencing

The following is the nucleotide sequence of pCC2FOS (bases 360-409) from the pCC2FOS Forward Sequencing Primer (362-379) to the pCC2FOS Reverse Sequencing Primer (403-386) encompassing the *Eco*72 I site (380-385).

360 ACGTACAACG ACACCTAGAC CAC **- - Cloned Insert - -** GTGTTCC

390 TAGGCTGTTT CCTGGTGGGA

An electronic copy of the pCC2FOS sequence is available for downloading at www.epibio.com/sequences.

Restriction Analysis of the pCC1FOS CopyControl Vector

Restriction Enzymes that cut the pCC1FOS Vector one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Acc65 I	2	344, 5207	EcoR V	2	4128, 4357
Acl I	2	1133, 5599	Fse I	1	2489
Afe I	1	4566	Fsp I	3	167, 3752, 7578
Afl II	2	6608, 6848	Hind III	1	395
Afl III	3	4973, 5147, 7482	Hpa I	1	7629
Age I	3	3827, 5057, 5950	Kpn I	2	348, 5211
Ahd I	1	7486	Mfe I	1	4987
Ale I	1	6543	Msc I	3	955, 2634, 5418
Apa I	1	6972	Nar I	1	146
ApaB I	3	96, 1946, 7646	Nco I	2	917, 7187
ApaL I	1	87	Nde I	2	94, 5005
BamH I	2	353, 365	Not I	2	2, 643
Bbs I	3	5050, 5239, 6116	Nru I	2	1644, 7674
BciV I	1	2497	Nsp I	3	393, 1831, 7486
Bcl I	1	5798	Pci I	1	7482
Bgl I	3	651, 3171, 7620	PflF I	1	5271
Bgl II	2	3146, 5213	Pml I	1	361
Blp I	1	4479	PpuM I	2	1728, 7858
BmgB I	3	2624, 5037, 7797	Psi I	2	2926, 3122
Bmr I	3	268, 7018, 7147	PspOM I	1	6968
Bpu10 I	3	1446, 3927, 5122	Pst I	3	387, 4025, 5566
Bsa I	1	6810	Pvu I	2	188, 5873
BsaB I	2	7754, 7838	Sac II	1	2483
BsaH I	1	146	Sal I	3	377, 657, 7662
BseY I	3	2412, 5890, 6647	Sap I	2	4603, 5813
Bsm I	2	824, 1231	Sbf I	2	387, 4025
BsmB I	3	994, 1547, 3942	Sca I	1	805
BspE I	2	1222, 5767	SexA I	1	7600
BspLU11 I	1	7482	Sfi I	1	651
BsrB I	3	476, 1660, 2282	Sfo I	1	147
BsrG I	1	3780	SgrA I	3	2492, 5057, 6214
BssH II	2	5464, 6008	Sim I	2	5171, 7858
BssS I	3	5157, 6807, 7370	Sma I	3	350, 651, 3493
BstAP I	3	95, 1945, 7645	SnaB I	1	5631
BstE II	1	7604	Spe I	1	6722
BstX I	1	5085	Sph I	1	393
BstZ17 I	1	1844	Srf I	1	651
Bts I	2	570, 5559	Sse8647 I	1	1728
Dra III	2	1945, 7823	Stu I	1	3174
Eco47 III	1	4566	Tat I	3	77, 803, 3780
Eco72 I	1	361	Tth111 I	1	5271
EcoN I	1	3469	Xba I	2	371, 3192
EcoO109 I	2	1728, 7858	Xcm I	1	2687
EcoR I	1	332	Xma I	3	348, 649, 3491

Restriction Enzymes that cut the pCC1FOS 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	MnI 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 pCC1FOS Vector:

Aat II	BfrB I	Cla I	PaeR7 I	Tli I
Asc I	BsiW I	Mlu I	Pme I	Xho I
AsiS I	BspD I	Nhe I	Rsr II	
Avr II	BstB I	Nsi I	SanD I	
BbvC I	Bsu36 I	Pac I	Swa I	

An electronic copy of the pCC1FOS sequence is available for downloading at www.epibio.com/sequences.

Restriction Analysis of the pCC2FOS CopyControl Vector

Restriction Enzymes that cut the pCC2FOS Vector one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Acc65 I	2	344, 5249	Fse I	1	2531
Acl I	2	1175, 5641	Fsp I	3	167, 3794, 7620
Afe I	1	4608	Hind III	1	437
Afl II	2	6650, 6890	Hpa I	1	7671
Age I	3	3869, 5099, 5992	Kpn I	2	348, 5253
Ahd I	1	7528	Mfe I	1	5029
Ale I	1	6585	Msc I	3	997, 2676, 5460
Apa I	1	7014	Nar I	1	146
ApaB I	3	96, 1988, 7688	Nco I	2	959, 7229
ApaL I	1	87	Nde I	2	94, 5047
Avr II	1	388	Not I	2	2, 685
BamH I	2	353, 407	Nru I	2	1686, 7716
Bau I	3	5199, 6849, 7412	Nsp I	3	435, 1873, 7528
Bbs I	3	5092, 5281, 6158	Pas I	3	1029, 1608, 5219
BciV I	1	2539	Pci I	1	7524
Bcl I	1	5840	PflF I	1	5313
Bgl I	3	693, 3213, 7662	Pfo I	1	6793
Bgl II	2	3188, 5255	Pml I	1	382
Blp I	1	4521	PpuM I	2	1770, 7900
BmgB I	3	2666, 5079, 7839	Psi I	2	2968, 3164
Bmr I	3	268, 7060, 7189	PspOM I	1	7010
Bpu10 I	3	1488, 3969, 5164	Pst I	3	429, 4067, 5608
Bsa I	1	6852	Pvu I	2	188, 5915
BsaB I	2	7796, 7880	Sac II	1	2525
BsaH I	1	146	Sal I	3	419, 699, 7704
BseY I	3	2454, 5932, 6689	Sap I	2	4645, 5855
Bsm I	2	866, 1273	Sbf I	2	429, 4067
BsmB I	3	1036, 1589, 3984	Sca I	1	847
BspE I	2	1264, 5809	SexA I	1	7642
BspLU11 I	1	7524	Sfi I	1	693
BsrB I	3	518, 1702, 2324	Sfo I	1	147
BsrG I	1	3822	SgrA I	3	2543, 5099, 6256
BssH II	2	5506, 6050	Sim I	2	5213, 7900
BssS I	3	5199, 6849, 7412	Sma I	3	350, 693, 3535
BstAP I	3	95, 1987, 7687	SnaB I	1	5673
BstE II	1	7646	Spe I	1	6764
BstX I	1	5127	Sph I	1	435
BstZ17 I	1	1886	Srf I	1	693
Bts I	2	612, 5601	Sse8647 I	1	1770
Dra III	2	1987, 7865	Stu I	1	3216
Eco47 III	1	4608	Tat I	3	77, 845, 3822
Eco72 I	1	382	Tth111 I	1	5313
EcoN I	1	3511	Xba I	2	413, 3234
EcoO109 I	2	1770, 7900	Xcm I	1	2729
EcoR I	1	332	Xma I	3	348, 691, 3533
EcoR V	2	4170, 4399			

Restriction Enzymes that cut the pCC2FOS Vector four or more times:

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

Restriction Enzymes that do not cut the pCC2FOS Vector:

Aat II	Bmt I	Cla I	PaeR7 I	Swa I
Asc I	BsiW I	Mlu I	Pme I	Tli I
AsiS I	BspD I	Nhe I	PspX I	Xho I
BbvC I	BstB I	Nsi I	Rsr II	Zra I
BfrB I	Bsu36 I	Pac I	SanD I	

6. Related Products

CopyControl™ Fosmid Autoinduction Solution

AIS107F 50 ml

The CopyControl Fosmid Autoinduction Solution provided in the CopyControl Fosmid Library Production Kits is also available as a stand-alone product for those researchers who will be screening many more clones than is possible with the volume of the CopyControl AutoInduction Solution in the CopyControl Fosmid Library kit.

FosmidMAX™ DNA Purification Kit

FMAX046 1 kit

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

BACMAX96™ DNA Purification Kit

BAC96116 Liquid reagents and plastic consumables for 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.

Direct Lysis Fosmid96 DNA Purification Kit

FOS84596 Liquid reagents and plastic consumables for 480 purifications

The Direct Lysis Fosmid96 DNA Purification Kit eliminates the need for centrifugation of the culture and resuspension of the cell pellet during Fosmid purification. The kit is designed for rapid preparation of sequencing templates in a 96-well format from fosmid clones. The typical DNA yield from a 200-µl overnight autoinduced culture of a CopyControl fosmid is sufficient for six to eight end-sequencing reactions. The kit includes reagents and plastics for high-throughput DNA extraction, and is suitable for robotic automation procedures.

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 are 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™-T1R Electrocompetent *E. coli*

EC02T15	5 x 100µl
EC02T110	10 x 100µl

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

MasterPure™ DNA Purification Kit

MCD85201	200 Purifications

The MasterPure DNA Purification Kit can produce DNA suitable for fosmid library construction from a variety of sources.

MaxPlax™ Lambda Packaging Extracts Protocol

(This protocol is also available as product literature #65 and is provided with Cat. Nos. MP5105, MP5110, and MP5120)

1. Introduction

MaxPlax™ Lambda Packaging Extracts are a convenient, high-efficiency system designed for in vitro lambda packaging reactions. MaxPlax Lambda Packaging Extracts are supplied as predispensed single-tube reactions that have been optimized for packaging of methylated and unmethylated DNA. The packaging extracts routinely yield packaging efficiencies of >1 x 10^9 pfu/µg of Control λ DNA. The extracts can be used in the construction of representative cDNA libraries and genomic cloning of highly modified (methylated) DNA into λ-phage or cosmid vectors.

Traditional packaging extracts are derived from two complementary lysogenic *E. coli* strains, BHB2690 and BHB2688, as described by Hohn (1979).[5] The MaxPlax extracts utilize a new packaging strain, NM759*, reported by Gunther, Murray and Glazer (1993).[6] This strain, which replaces strain BHB2690 in the preparation of the sonication extract, is a restriction-free K12-derived strain deficient in the production of λ-phage capsid protein D. When combined with the complementary freeze-thaw extract from strain BHB2688**,[5] deficient in the production of λ-phage capsid protein E, an extremely high-efficiency of packaging for λ DNA is obtained. Moreover, the ability to package λ DNA bearing the mammalian methylation pattern is greatly enhanced, as evidenced by the high efficiency of λ-vector rescue from transgenic mouse DNA.[6] The lack of restriction activity has been shown to be crucial for the high efficiency rescue of lambda shuttle vectors from transgenic mouse DNA.[6,7]

**NM759: [W3110 recA56, Δ(mcrA) e14, Δ(mrr-hsd-mcr), (λimm434, clts, b2, red3, Dam15, Sam7)/λ]*

***BHB2688: [N205 recA⁻, (λimm434 clts, b2, red3, Eam4, Sam7)/λ]*

Store the MaxPlax Lambda Packaging Extracts at –70°C or below. Exposure to higher temperature will decrease packaging efficiencies.

2. Product Specifications

Storage: Store the control host bacteria and the MaxPlax Lambda Packaging Extracts at –70°C. Exposure to higher temperatures will greatly compromise packaging extract efficiency. Avoid long term storage of product in the presence of dry ice. Once removed from the foil package, avoid any exposure to dry ice. Store the remainder of the kit components at –20°C. After thawing, store the Control DNA at 4°C.

Storage Buffers: MaxPlax Lambda Packaging Extracts are supplied as unlabeled single tubes of freeze-thaw/sonicate extracts. Control plating bacteria are supplied as a glycerol stock. Control ligated λ DNA is supplied in 1X Ligation Buffer.

Guaranteed Stability: MaxPlax Lambda Packaging Extracts are guaranteed to maintain a packaging efficiency of >1.0 x 10^9 pfu/µg of control λ DNA, when stored as directed for one year from the date of purchase.

3. Related Products

The following products are also available:

– pWEB-TNC™ Cosmid Cloning Kit
– pWEB™ Cosmid Cloning Kit
– EpiFOS™ Fosmid Library Production Kit
– Lambda Terminase

4. Protocol for Packaging Lambda Control DNA

This protocol can be used for the positive control reaction as well as for experimental reactions. The positive control reactions must be plated on the control host bacterial strain (LE392MP) included with the MaxPlax Extracts. The proper bacterial plating strain for the experimental reactions will vary depending on the cloning vector used. See the vector manufacturer's recommendations for the proper strain and plating media requirements. Ligation reactions may be added directly to the packaging extracts. When doing so, it is important to: a) add a volume of 10 µl or less to the packaging reaction, and b) heat inactivate the ligase (i.e., treatment at 65°C for 15 minutes) as active DNA ligase will decrease packaging efficiencies.

Solutions:

Phage Dilution Buffer
10 mM Tris-HCl (pH 8.3)
100 mM NaCl
10 mM $MgCl_2$

LB Broth (1 Liter)
10 g Bacto-tryptone
5 g Bacto-yeast extract
10 g NaCl
Adjust pH to 7.0 with NaOH

LB Plates
LB Broth with 1.5% (w/v) Bacto-agar

LB Top Agar
LB Broth with 0.7% (w/v) Bacto-agar

Plating Bacteria Preparation:

1. The day before performing the packaging reactions, inoculate 50 ml of supplemented (10 mM $MgSO_4$) LB broth with a single colony of the plating bacterial strain and shake overnight at 37°C.
2. The day of the packaging reactions, inoculate 50 ml of supplemented (10 mM $MgSO_4$ + 0.2% maltose) LB broth with 5 ml of the overnight culture and shake at 37°C to an OD_{600} = 0.8-1.0. Store the cells at 4°C until needed; cells may be stored for up to 72 hours.

Packaging Reactions:

1. Thaw the appropriate number of packaging extracts at room temperature. For every two packaging reactions, thaw one extract then place on ice.
2. When thawed, immediately transfer half (25 µl) of each packaging extract to a second 1.5-ml tube and place on ice.
3. Add the substrate DNA (10 µl [0.2 µg] of the control DNA) to a tube containing 25 µl of extract. If performing an odd number of packaging reactions, the remaining 25 µl of extract can be refrozen at –70°C.
4. Mix by pipetting several times; avoid the introduction of air bubbles. Return all of the contents to the bottom of the tube by brief centrifugation if necessary.
5. Incubate the reaction(s) at 30°C for 90 minutes.
6. At the end of this incubation, add the additional 25 µl of thawed extract to each reaction tube at 30°C (If performing two packaging reactions, thaw another tube of extract and add 25 µl to each tube.) and incubate the reaction(s) for an additional 90 minutes at 30°C.
7. Add 500 µl of phage dilution buffer and mix by gentle vortexing.
8. Add 25 µl of chloroform and mix by gentle vortexing (store at 4°C).
9. Assay the packaged phage by titering on the appropriate bacterial strain (LE392MP for the control).

Titering Phage Extracts:

1. Make serial dilutions of the packaged phage in phage dilution buffer. Use 10^{-5} and 10^{-6} dilutions for the control reactions.

   10^{-2} dilution is 10 µl of packaged phage particles into 990 µl of phage dilution buffer; vortex mix.

   10^{-4} dilution is 10 µl of 10^{-2} dilution into 990 µl phage dilution buffer; vortex mix.

   10^{-5} dilution is 100 µl of 10^{-4} dilution into 900 µl phage dilution buffer; vortex mix.

   10^{-6} dilution is 10 µl of 10^{-4} dilution into 990 µl phage dilution buffer; vortex mix.
2. Add 100 µl of the appropriate serial dilutions to 100 µl of prepared plating bacteria (use LE392MP for the control reactions) and incubate for 15 minutes at 37°C.
3. Add 3.0 ml of melted supplemented (10 mM $MgSO_4$) LB top agar (cooled to ~48°C). Vortex gently and pour onto pre-warmed (37°C) LB plates. Allow the top agar to solidify and then incubate overnight at 37°C.
4. Count the plaques and determine the titer (pfu/ml) and packaging efficiency (See sample calculations).

Sample Calculations:

If there were 110 plaques on a 10^{-6} dilution plate, then the titer, pfu/ml, (where pfu represents plaque forming units) of this reaction would be:

$$\frac{\text{(\# of plaques) (dilution factor) (1000 µl/ml)}}{\text{(volume of phage plated [µl])}} \textbf{ OR } \frac{\text{(110 pfu) (}10^6\text{) (1000 µl/ml)}}{\text{(100 µl)}} = 1.1 \times 10^9 \text{ pfu/ml}$$

The packaging efficiency (pfu/µg DNA) of this reaction would be:

$$\frac{\text{(\# of plaques) (dilution factor) (total reaction vol.)}}{\text{(vol. of dilution plated) (amount of DNA packaged)}} \textbf{ OR } \frac{\text{(110 pfu) (}10^6\text{) (550 µl)}}{\text{(100 µl) (0.2 µg)}} = 3 \times 10^9 \text{ pfu/µg}$$

5. References

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