Manual

CopyControl Fosmid Library Production Kit with pCC1FOS Vector

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

CopyControl HTP Fosmid Library Production Kit with pCC2FOS Vector

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

For Research Use Only. Not for use in diagnostic procedures.

CopyControl™ is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

CopyControl Fosmid and HTP Fosmid Library Production Kit

Contents

Manual

CopyControl Fosmid and HTP Fosmid Library Production Kit

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. CopyControl Fosmid clones can be induced from single-copy to 10-20 copies per cell to improve DNA yields for sequencing, fingerprinting, subcloning, *in vitro* transcription, and other applications.

The CopyControl Cloning System has two required components

1. Each CopyControl Vector contains both a single-copy origin and the high-copy *oriV* origin of replication. Initiation of replication from *oriV* 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 × 10^{6} cfu/mL) with the Fosmid Control DNA. Each lot of MaxPlax™ Lambda Packaging Extracts is also tested individually, and is guaranteed to maintain a packaging efficiency of >10^{7} cfu/µg of Fosmid Control DNA when stored as directed for one year from date of purchase.

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. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
CopyControl Fosmid Library Production Kit	1 Kit	CCFOS110	End-It™ Enzyme Mix	E0025-D1	50 µL
			End-It 10X Buffer	SS000272-D1	100 µL
			dNTP Mix (2.5 mM each)	SS000055-D1	100 µL
			ATP (10 mM)	SS000391-D1	100 µL
			Fast-Link™ DNA Ligase (2 U/µL)	E0077-2D1	20 µL
			Fast-Link 10X Ligation Buffer	SS000272-D2	100 µL
			GELase™ Enzyme Preparation (1 U/µL)	E0032-1D	25 µL
			GELase 50X Buffer	SS00087-D1	100 µL
			Fosmid Control DNA (100 ng/µL)	SS000485-D	50 µL
			pCC1FOS Fosmid Vector (0.5 µg/µL)	SS000483-D	20 µL
			CopyControl Fosmid Autoinduction Solution (500X)	SS000728-D2	2 × 1 mL
			Phage T1 Resistant EPI300 $T1^R$ Glycerol Stock	SS001002-D	250 µL
			MaxPlax Lambda Packaging Extract	SS000437-D	10 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL

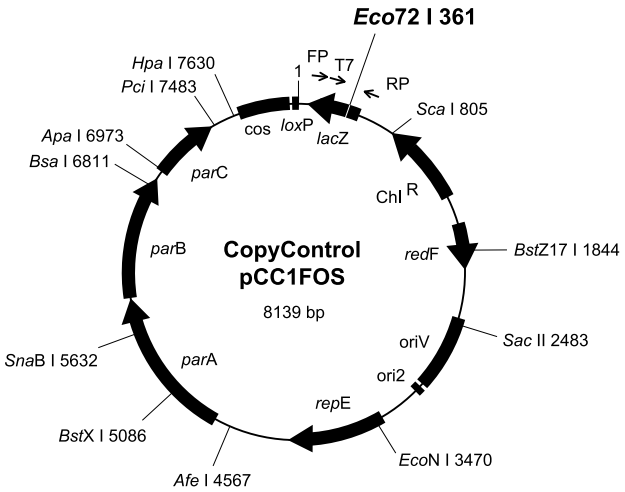
Manual

CopyControl Fosmid and HTP Fosmid Library Production Kit

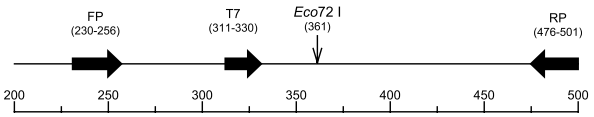
Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
CopyControl HTP Fosmid Library Production Kit	1 Kit	CCFOS059	End-It Enzyme Mix	E0025-D1	50 µL
			End-It 10X Buffer	SS000272-D1	100 µL
			dNTP Mix (2.5 mM each)	SS000055-D1	100 µL
			ATP (10 mM)	SS000391-D1	100 µL
			Fast-Link DNA Ligase (2 U/µL)	E0077-2D1	20 µL
			Fast-Link 10X Ligation Buffer	SS000272-D2	100 µL
			GELase Enzyme Preparation (1 U/µL)	E0032-1D	25 µL
			GELase 50X Buffer	SS00087-D1	100 µL
			Fosmid Control DNA (100 ng/µL)	SS000485-D	50 µL
			pCC2FOS Fosmid Vector (0.5 µg/µL)	SS000700-D	20 µL
			CopyControl Fosmid Autoinduction Solution (500X)	SS000728-D2	2 × 1 mL
			Phage T1 Resistant EPI300 $T1^R$ Glycerol Stock	SS001002-D	250 µL
			MaxPlax Lambda Packaging Extract	SS000437-D	10 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL

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 CO_2-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.



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.



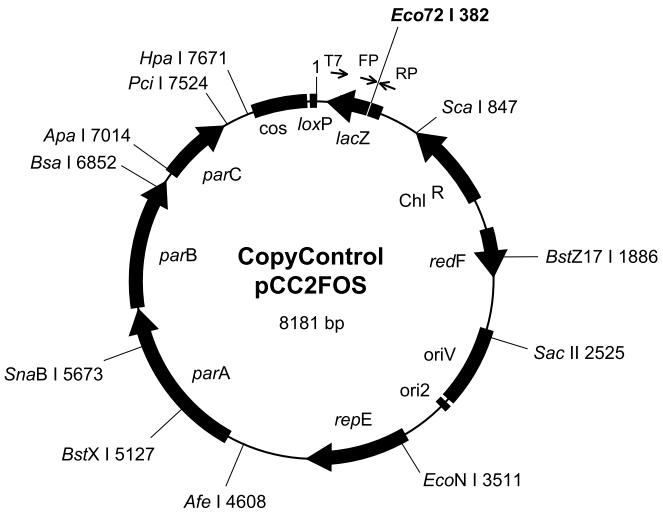
FP = pCC1™ Foward Sequencing Primer 5' GGATGTGCTGCAAGGCGATTAAGTTGG 3'
RP = pCC1™ Reverse Sequencing Primer 5' CTCGTATGTTGTGTGGAATTGTGAGC 3'
T7 = T7 Promoter Primer 5' TAATACGACTCACTATAGGG 3'

Figure 1. pCC1FOS Vector Map.

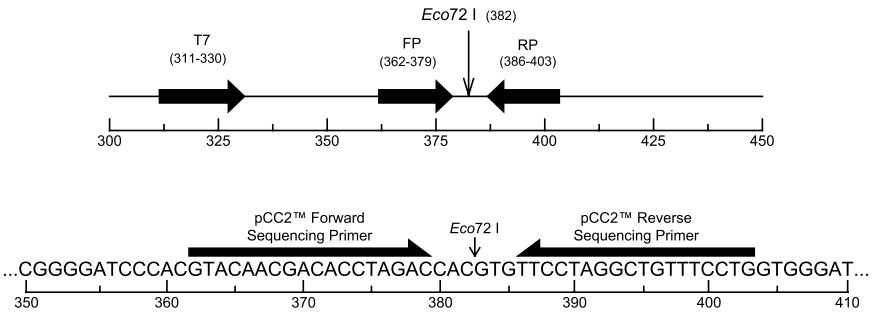
Additional required reagents

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)



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

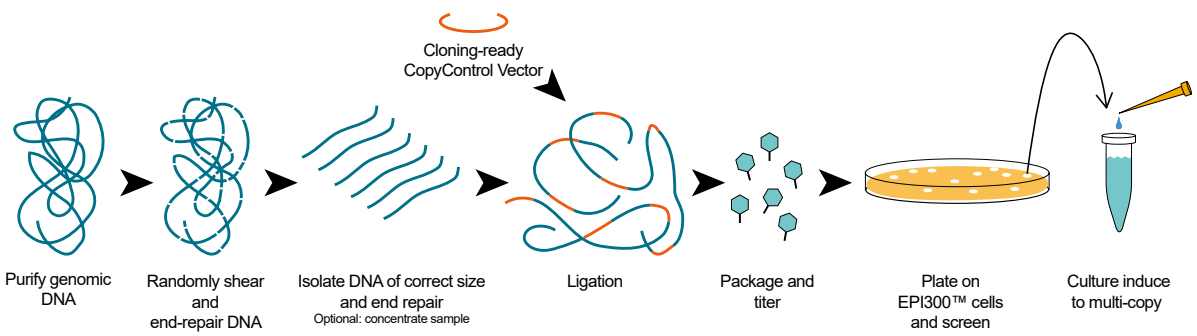


Figure 3. Production of a CopyControl Fosmid library and subsequent induction of clones to high-copy number.

Additional features of the pCC2FOS Vector

The CopyControl HTP Fosmid Library Production Kit contains the pCC2FOS Vector (Figure 2). The pCC2FOS Vector, a modification of the pCC1FOS (Figure 1) vector, contains a primer cassette that optimises 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 minimise mispriming from any contaminating *E. coli* DNA present after template purification.

How the CopyControl Cloning System works (Figure 3)

1. Ligate the DNA of interest into the linearised 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 *trfA* 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 *trfA* 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 utilises 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.
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.

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** (*λc1857 Sam7*) 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 (Epicentre) or other standard methods or kits.[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 analysing 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 visualise 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. 4, 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.

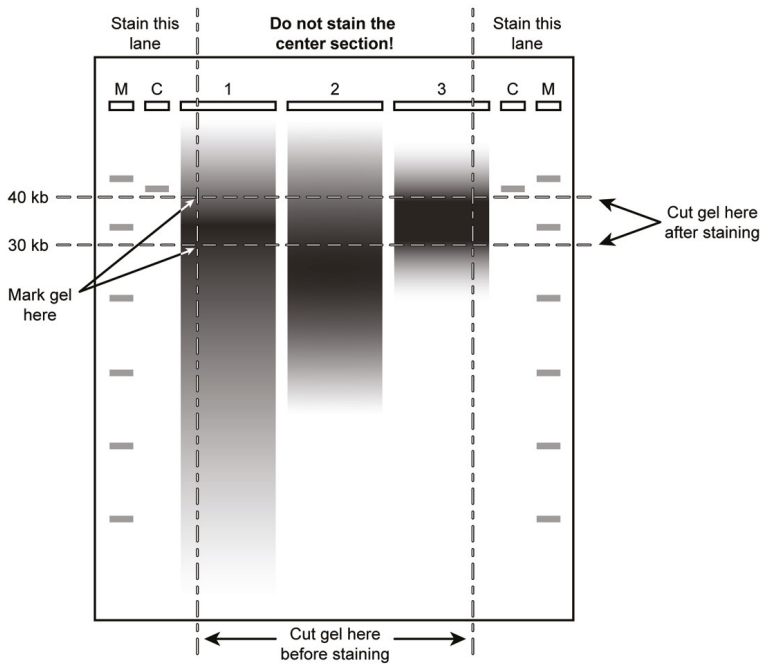


Figure 4. Gel purification of DNA: Keeping ethidium and UV away from your DNA.

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. 4, 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, analyse 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. 4.

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.

Note 2: The protocol below is designed for use with GELase Agarose Gel-Digesting Preparation (kit component), 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 Figure 4).
   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 Figure 4).
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 600 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 (A260) 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 103-106 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 A600 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) 1:101 Dilute 10 µL of packaged phage into 90 µL of PDB.
    B) 1:102 Dilute 100 µL of the 1:101 dilution into 900 µL of PDB.
    C) 1:103 Dilute 100 µL of the 1:102 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 1:103 dilution, then the titer in cfu/mL, (where cfu represents colony forming units) of this reaction would be:

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

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 or other standard laboratory methods.[6]

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.

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

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 familiarise 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 familiarise 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 1:1000 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-T1R 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 × 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 Sequencing Primers

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

pCC1 Forward Sequencing Primer

5′ - GGATGTGCTGCAAGGCGATTAAGTTGG - 3′

Length: 27 nucleotides

G+C content: 14

Molecular weight: 8,409 daltons

Temperatures of dissociation and 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 Reverse Sequencing Primer

5′ - CTCGTATGTTGTGTGGAATTGTGAGC - 3′

Length: 26 nucleotides

G+C content: 12

Molecular weight: 8,038 daltons

Temperatures of dissociation and 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 Forward and Reverse Primers do not function well as IRD800-labeled sequencing primers. We recommend using the T7 and pCC1 Primers instead of the pCC1 Forward and Reverse Primers respectively, for this purpose.

pCC1 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/ Forward Sequencing Primer (230-256) to the pCC1 Reverse Sequencing Primer (501-476) encompassing the T7 RNA polymerase promoter (311-330) and the Eco72 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
5′ - GTACAACGACACCTAGAC - 3′

Length: 18 nucleotides
G+C content: 9
Molecular weight: 5,462 daltons
Temperatures of dissociation and 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
5′ - CAGGAAACAGCCTAGGAA - 3′

Length: 18 nucleotides
G+C content: 9
Molecular weight: 5,551 daltons
Temperatures of dissociation and 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
```

The pCC2FOS sequence is available at lucigen.com/sequences.

Restriction analysis of the pCC1FOS CopyControl Vector

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

Enzyme	Sites	Location
Acc65 I	2	344, 5249
Acl I	2	1175, 5641
Afe I	1	4608
Afl II	2	6650, 6890
Age I	3	3869, 5099, 5992
Ahd I	1	7528
Ale I	1	6585
Apa I	1	7014
ApaB I	3	96, 1988, 7688
ApaL I	1	87
Avr II	1	388
BamH I	2	353, 407
Bau I	3	5199, 6849, 7412
Bbs I	3	5092, 5281, 6158
BciV I	1	2539
Bcl I	1	5840
Bgl I	3	693, 3213, 7662
Bgl II	2	3188, 5255
Blp I	1	4521
BmgB I	3	2666, 5079, 7839
Bmr I	3	268, 7060, 7189
Bpu10 I	3	1488, 3969, 5164
Bsa I	1	6852
BsaB I	2	7796, 7880
BsaH I	1	146
BseY I	3	2454, 5932, 6689
Bsm I	2	866, 1273
BsmB I	3	1036, 1589, 3984
BspE I	2	1264, 5809
BspLU11 I	1	7524
BsrB I	3	518, 1702, 2324
BsrG I	1	3822
BssH II	2	5506, 6050
BssS I	3	5199, 6849, 7412
BstAP I	3	95, 1987, 7687
BstE II	1	7646
BstX I	1	5127
BstZ17 I	1	1886
Bts I	2	612, 5601
Dra III	2	1987, 7865
Eco47 III	1	4608
Eco72 I	1	382
EcoN I	1	3511
EcoO109 I	2	1770, 7900
EcoR I	1	332
EcoR V	2	4170, 4399

Enzyme	Sites	Location
Fse I	1	2531
Fsp I	3	167, 3794, 7620
Hind III	1	437
Hpa I	1	7671
Kpn I	2	348, 5253
Mfe I	1	5029
Msc I	3	997, 2676, 5460
Nar I	1	146
Nco I	2	959, 7229
Nde I	2	94, 5047
Not I	2	2, 685
Nru I	2	1686, 7716
Nsp I	3	435, 1873, 7528
Pas I	3	1029, 1608, 5219
Pci I	1	7524
PflF I	1	5313
Pfo I	1	6793
Pml I	1	382
PpuM I	2	1770, 7900
Psi I	2	2968, 3164
PspOM I	1	7010
Pst I	3	429, 4067, 5608
Pvu I	2	188, 5915
Sac II	1	2525
Sal I	3	419, 699, 7704
Sap I	2	4645, 5855
Sbf I	2	429, 4067
Sca I	1	847
SexA I	1	7642
Sfi I	1	693
Sfo I	1	147
SgrA I	3	2543, 5099, 6256
Sim I	2	5213, 7900
Sma I	3	350, 693, 3535
SnaB I	1	5673
Spe I	1	6764
Sph I	1	435
Srf I	1	693
Sse8647 I	1	1770
Stu I	1	3216
Tat I	3	77, 845, 3822
Tth111 I	1	5313
Xba I	2	413, 3234
Xcm I	1	2729
Xma I	3	348, 691, 3533

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	Mnl I	ScrF I
Ava I	BssK I	Hae I	Mse I	SfaN I
Ava II	BstDS I	Hae II	Msl I	Sfc I
Ban I	BstF5 I	Hae III	Msp I	Sml I
Ban II	BstN I	Hha I	MspA1 I	Ssp I
Bfa I	BstU I	Hinc II	Mwo I	Sty I
BfuA I	BstY I	Hinf I	Nae I	Taq I
Bme1580 I	Btg I	HinP I	Nci I	Tfi I
BsaA I	Cac8 I	Hpa II	NgoM IV	Tse I
BsaJ I	CviJ I	Hph I	Nla III	Tsp45 I
BsaW I	Dde I	Hpy188 I	Nla IV	Tsp4C I
BsiE I	Dpn I	Hpy99 I	PflM I	Tsp509 I
BsiHKA I	Dra I	HpyCH4 III	Ple I	TspR I
Bsl I	Drd I	HpyCH4 IV	PshA I	Xmn I

Restriction enzymes that do not cut the 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	

The pCC1FOS sequence is available at lucigen.com/sequences.

Restriction analysis of the pCC2FOS CopyControl Vector

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

Enzyme	Sites	Location
Acc65 I	2	344, 5249
Acl I	2	1175, 5641
Afe I	1	4608
Afl II	2	6650, 6890
Age I	3	3869, 5099, 5992
Ahd I	1	7528
Ale I	1	6585
Apa I	1	7014
ApaB I	3	96, 1988, 7688
ApaL I	1	87
Avr II	1	388
BamH I	2	353, 407
Bau I	3	5199, 6849, 7412
Bbs I	3	5092, 5281, 6158
BciV I	1	2539
Bcl I	1	5840
Bgl I	3	693, 3213, 7662
Bgl II	2	3188, 5255
Blp I	1	4521
BmgB I	3	2666, 5079, 7839
Bmr I	3	268, 7060, 7189
Bpu10 I	3	1488, 3969, 5164
Bsa I	1	6852
BsaB I	2	7796, 7880
BsaH I	1	146
BseY I	3	2454, 5932, 6689
Bsm I	2	866, 1273
BsmB I	3	1036, 1589, 3984
BspE I	2	1264, 5809
BspLU11 I	1	7524
BsrB I	3	518, 1702, 2324
BsrG I	1	3822
BssH II	2	5506, 6050
BssS I	3	5199, 6849, 7412
BstAP I	3	95, 1987, 7687
BstE II	1	7646
BstX I	1	5127
BstZ17 I	1	1886
Bts I	2	612, 5601
Dra III	2	1987, 7865
Eco47 III	1	4608
Eco72 I	1	382
EcoN I	1	3511
EcoO109 I	2	1770, 7900
EcoR I	1	332
EcoR V	2	4170, 4399

Enzyme	Sites	Location
Fse I	1	2531
Fsp I	3	167, 3794, 7620
Hind III	1	437
Hpa I	1	7671
Kpn I	2	348, 5253
Mfe I	1	5029
Msc I	3	997, 2676, 5460
Nar I	1	146
Nco I	2	959, 7229
Nde I	2	94, 5047
Not I	2	2, 685
Nru I	2	1686, 7716
Nsp I	3	435, 1873, 7528
Pas I	3	1029, 1608, 5219
Pci I	1	7524
PflF I	1	5313
Pfo I	1	6793
Pml I	1	382
PpuM I	2	1770, 7900
Psi I	2	2968, 3164
PspOM I	1	7010
Pst I	3	429, 4067, 5608
Pvu I	2	188, 5915
Sac II	1	2525
Sal I	3	419, 699, 7704
Sap I	2	4645, 5855
Sbf I	2	429, 4067
Sca I	1	847
SexA I	1	7642
Sfi I	1	693
Sfo I	1	147
SgrA I	3	2543, 5099, 6256
Sim I	2	5213, 7900
Sma I	3	350, 693, 3535
SnaB I	1	5673
Spe I	1	6764
Sph I	1	435
Srf I	1	693
Sse8647 I	1	1770
Stu I	1	3216
Tat I	3	77, 845, 3822
Tth111 I	1	5313
Xba I	2	413, 3234
Xcm I	1	2729
Xma I	3	348, 691, 3533

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	Mnl 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 pCC1FOS 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. 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. David, R. et al., (2006) Epicentre Forum **13** (1), 17.
5. DiLella, A.G. and Woo, S.L.C. (1987) Meth. Enzymol. **152**, 199.
6. Sambrook, J. et al., (1989) in: Molecular Cloning: A Laboratory Manual (2nd ed.), CSH Laboratory Press, New York.
7. Hohn, E.G. (1979) Methods Enzymol. **68**, 299.

7. Further Support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Integrated tools.
Accelerated science.

@LGCBiosearch

lucigen.com
biosearchtech.com

GEN/762/MW/0720

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

MaxPlax Lambda Packaging Extracts

For Research Use Only. Not for use in diagnostic procedures.

MaxPlax™ is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents, which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

MaxPlax Lambda Packaging Extracts

Contents

Manual

MaxPlax Lambda Packaging Extracts

1. Introduction

MaxPlax Lambda Packaging Extracts are a convenient, high-efficiency transduction system designed for in vitro lambda packaging reactions. MaxPlax Lambda Packaging Extracts are supplied as predispensed single-tube reactions that have been optimised for packaging of methylated and unmethylated DNA. The packaging extracts routinely yield packaging efficiencies of >1 × 10^9 pfu/µg of Ligated Lambda Control 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).[1] The MaxPlax extracts utilise a new packaging strain, NM759*, reported by Gunther, Murray and Glazer (1993).[2] 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**,[1] 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.[2] The lack of restriction activity has been shown to be crucial for the high efficiency rescue of lambda shuttle vectors from transgenic mouse DNA.[2,3]

*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]

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
MaxPlax Lambda Packaging Extracts	5 extracts	MP5105	MaxPlax Lambda Packaging Extract	SS000437-D	5 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL
MaxPlax Lambda Packaging Extracts	10 extracts	MP5110	MaxPlax Lambda Packaging Extract	SS000437-D	10 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL
MaxPlax Lambda Packaging Extracts	20 extracts	MP5120	MaxPlax Lambda Packaging Extract	SS000437-D	20 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL

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 CO_2-impermeable foil pouch.

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

***E. coli* strain LE392MP Genotype:**
[F– e14–(McrA–) Δ(*mcrC-mrr*) (Tet^R) *hsd*R514 *sup*E44 *sup*F58 *lac*Y1 or Δ(*lac*IZY)6 *gal*K2 *gal*T22 *met*B1 *trp*R55 λ–]

3. Product specifications

Storage: Store the LE392MP Control Plating Strain Glycerol Stock and the MaxPlax Lambda Packaging Extract 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 Ligated Lambda Control DNA 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. LE392MP Control Plating Strain is supplied as a glycerol stock. Ligated Lambda Control DNA is supplied in 1X Ligation Buffer.

Guaranteed Stability: MaxPlax Lambda Packaging Extracts are quality tested by packaging a ligation reaction containing a fosmid vector backbone and a 42 kb control insert DNA from the human X chromosome. MaxPlax Lambda Packaging Extracts are guaranteed to maintain a packaging efficiency of $>1.0 \times 10^7$ cfu/µg of control insert DNA, when stored as directed for 1 year from the date of purchase.

4. Example protocol

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 (that is, 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.

Plating bacteria preparation:

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 inverted 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:

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

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

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

5. References

1. Hohn, E.G. (1979) *Methods Enzymol.* **68,** 299.
2. Gunther, E.G. *et al.,* (1993) *Nucl. Acids Res.* **21,** 3903.
3. Kohler, S.W. *et al.,* (1990) *Nucl. Acids Res.* **18,** 3007.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Integrated tools.
Accelerated science.

@LGCBiosearch

lucigen.com
biosearchtech.com

GEN/717/MW/1119

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC