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Direct Lysis Fosmid96 DNA Purification Kit

Cat. No. FOS84596

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1. Introduction

The Direct Lysis Fosmid96 DNA Purification Kit is designed for the fast purification of DNA from 40-kb fosmid clones in a 96-well format for use in DNA sequencing reactions. The kit uses direct lysis of a 200-µl overnight culture, eliminating the need for centrifugation of the culture and resuspension of the bacterial pellet, steps that are cumbersome to perform in a 96-well format. The kit procedure results in an average yield of 800 ng of DNA from an autoinduced fosmid. This is sufficient for six to eight fosmid-end sequencing reactions.

The kit is ideally suited for use with the Copy-Control™ Fosmid System (Epicentre) in conjunction with our CopyControl Fosmid Autoinduction Solution. This system uses an inducible "*oriV*" origin of replication, a copy-number induction feature that allows an "on-demand" induction of the fosmid copy-number from a single copy per cell (uninduced) to approximately 50 copies per cell (induced) when the growth medium is supplemented with the CopyControl Autoinduction Solution. The kit protocol can also be used with non-CopyControl fosmids that contain an inducible *oriV* origin of replication. A host strain carrying the *trf*A gene is required for initiation of replication from *oriV* (e.g. Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli* [Epicentre]).

This kit can also be used for high-copy-number plasmid DNA purification with an approximate yield of 1 µg of DNA per reaction (see Part B, Step 1).

2. Kit Contents

Cat. #	Quantity
Direct Lysis Fosmid96 DNA Purification Kit Contents The Direct Lysis Fosmid96 DNA Purification Kit contains sufficient reagents to perform five 96-well preps.	
PF96 Solution 1*	55 ml
PF96 Solution 2	85 ml
PF96 RNase Blend	3.4 ml
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	28 ml
CopyControl™ Fosmid Autoinduction Solution (500X)	250 µl
1.1 ml Sterile Culture Plates	5
Fritted Filter Plates	5
Round-Bottom Collection Plates	5
Gas-Permeable Plate Sealers	5
1 Pad of Standard Sealers	25 seals

**PF96 Solution 1 may form a precipitate during storage. If this occurs, heat the bottle at 37°C until the precipitate dissolves. Mix thoroughly and cool to room temperature.*

Product Specifications

Storage: Upon kit arrival, store the PF96 RNase Blend at –20°C in a freezer without a defrost cycle. Store the remainder of the kit components at room temperature.

Quality Control: The Direct Lysis Fosmid96 DNA Purification Kit is function-tested by purifying a 40-kb autoinduced fosmid clone. DNA quality and yield are assayed by gel electrophoresis and fluorimetry.

3. Related Products

The following products are also available:

- CopyControl™ Fosmid Library Production Kit
- CopyControl™ Fosmid Autoinduction Solution
- CopyControl™ HTP Fosmid Library Production Kit
- Plasmid-Safe™ ATP-Dependent DNase
- Direct Lysis Plasmid96 DNA Purification Kit
- Fast-Link™ DNA Ligation and Screening Kit
- FosmidMAX™ DNA Purification Kit
- BACMAX96™ DNA Purification Kit
- CopyControl™ BAC Cloning Kit
- TAQXpedite™ PCR System (FAST End-Point)
- End-It™ DNA End-Repair Kit
- Phage T1-Resistant TransforMax™ EPI300™-T1^{R} Electrocompetent *E. coli*

4. Direct Lysis Fosmid96 DNA Purification Protocols

The following equipment is not supplied with the Direct Lysis Fosmid96 kit:

100% (Absolute) Ethanol	Boxes of appropriate pipette tips
70% Ethanol	Centrifuge with microplate carrier
Reagent reservoir trays	Vortex mixer with microplate adaptor
Multi-channel pipette	96-well growth plate or tubes for preculture
	LB or similar bacterial growth media

A. Before Starting

1. The table below lists the recommended volumes of solutions for purifications from single or multiple 96-well plates.

Number of Plates	PF96 Solution 1*	PF96 Solution 2	PF96 RNase Blend (add to PF96 Solution 2)
1	11 ml	17 ml	663 µl
2	22 ml	34 ml	1.33 ml
3	33 ml	51 ml	1.99 ml
4	44 ml	68 ml	2.65 ml
5	55 ml	85 ml	3.32 ml
Storage	Room Temp.	Ice	Ice

2. Just prior to use: Add 39 µl of PF96 RNase Blend per 1 ml of PF96 Solution 2 to be used.
3. A preculture is recommended for the purification of good quality DNA. To start a preculture, inoculate a 96-well growth plate (not included in the kit) containing LB growth media plus the appropriate antibiotic (DO NOT add Autoinduction Solution) with an isolated colony from a freshly streaked plate or from the glycerol stock, and grow overnight at 37°C. Use this preculture for Part B, Step 2. Inoculation of cultures directly from a long-term stored glycerol stock may result in poor or no yield.

B. Growing 96-Well Bacterial Cultures (for ~40kb single-copy fosmids containing inducible *oriV*)

1. Dispense 200 µl of LB medium containing the appropriate antibiotic and CopyControl Fosmid Autoinduction Solution (2 µl/ml medium; if using an *oriV*-inducible system) into each well of a 1.1-ml 96-well culture plate. If the fosmid contains a chloramphenicol resistance marker, use 12.5 µg/ml of chloramphenicol.

   Note: *Make sure to use a culture plate and NOT a collection plate.*
2. Inoculate each well from a glycerol stock using a 96-pin device or add 2 µl of preculture to each well with a multi-channel pipette or other method of choice.
3. Seal the plate using an air-permeable plate sealer. Incubate cultures in an incubator/air shaker for 16-17 hours at 37°C with constant shaking at 250 rpm.
4. Proceed to Part C.

B1. Growing 96-Well Bacterial Cultures (for high copy-number plasmids)

1. Dispense 200 µl of LB medium containing the appropriate antibiotic into each well of a 1.1-ml 96-well culture plate.

   Note: *Make sure to use a culture plate and NOT a collection plate.*
2. Inoculate each well from a glycerol stock using a 96-pin device or add 2 µl of preculture to each well with a multi-channel pipette or other method of choice.
3. Seal the plate using an air-permeable plate sealer. Incubate cultures in an incubator/air shaker for 16-17 hours at 37°C with constant shaking at 250 rpm.
4. Proceed to Part C.

C. Purification of Fosmid DNA

1. Remove the plate from the 37°C incubator and cool it to room temperature. Remove the plate sealer and discard it.
2. Add 100 µl of PF96 Solution 1 to the culture in each well of the culture plate. Cover the culture plate using an adhesive plate sealer and press firmly with your fingertips to ensure the wells are tightly sealed.
3. Mix by inverting the plates 2-3 times very gently. Remove the plate sealer and discard it. Using a stack of paper towels, lightly blot the wells of the plate to prevent cross contamination between the wells.

   Note: *Do not allow the lysis to exceed 5 minutes.*

4. Add 150 µl of chilled PF96 Solution 2 (containing the PF96 RNase Blend) to each well of the culture plate. Seal the plate securely using a new plate sealer.
5. Mix by inverting the plates 2-3 times very gently, then incubate on for ice 5 minutes.

   Note: *Excessive, vigorous mixing may result in shearing of the DNA.*
6. Place the filter plate on top of the collection plate. Transfer the entire lysate volume from the bottom of each well of the culture plate to the corresponding wells of the filter plate. Centrifuge the block at 1,000 x g for 2 minutes at 4°C and collect the lysate in the collection plate OR use a vacuum filtration method. Follow manufacturer's recommended protocol for this step. Discard the filter plate.
7. Add 1 volume (~400 µl) of absolute ethanol (200 proof) to each well containing the cleared lysate. Cover the collection plate securely using a new plate sealer. Mix thoroughly by inverting the plates 6-8 times.
8. Centrifuge the plate at 1,400 x g for 10 minutes at 4°C to precipitate the DNA.
9. Carefully remove the plate sealer and discard. Pour off the ethanol and remove any excess liquid by briefly placing the plate upside down on a clean stack of paper towels.
10. Add 500 µl of freshly prepared 70% ethanol to each well. Cover the plate with a fresh plate sealer.

    Note: *Do not shake or mix, and centrifuge the plate at 1,400 x g for 5 minutes at 4°C.*
11. Remove the plate sealer and pour off the ethanol. Remove any excess liquid by placing the plate upside down on a clean stack of paper towels and tapping gently several times. Turn the plate right side up and air-dry at room temperature for 5-8 minutes.

    Note: *Drying time may vary depending on the air temperature and humidity. Do NOT over-dry or it will be difficult to resuspend the pellet; however, it is important that the ethanol is completely removed.*
12. Add 25 µl of TE Buffer or sterile water to each well. Place the plate on a rotary shaker and shake the plate gently for 2-3 minutes. Centrifuge the plate briefly (quick spin) to collect any remaining liquid. The DNA is now ready for use. We suggest storage at 4°C if the DNA is to be used in the next 48 hours, otherwise store the DNA at –20°C.

    Optional: The fosmid DNA may be treated with Plasmid-Safe ATP-Dependent DNase (available separately from Epicentre) to remove any low chromosomal background DNA (see protocol below).

D. Plasmid-Safe DNase Treatment of Fosmid DNA (optional)

1. Prepare Plasmid-Safe Master Mix: The table below lists the reagent volumes required to make enough master mix for single to multiple 96-well plate preps.

Number of Plates	Plasmid-Safe 10X Buffer	100 mM ATP	Plasmid-Safe DNase	Sterile Water
1	252 µl	25 µl	75 µl	70 µl
2	504 µl	50 µl	150 µl	140 µl
3	756 µl	75 µl	225 µl	210 µl
4	1008 µl	100 µl	300 µl	280 µl
5	1260 µl	125 µl	375 µl	350 µl

2. Remove and save the plate sealer. Add 4 µl of the Plasmid-Safe Master Mix to each well and cover the receiver plate securely using the saved plate sealer. Mix by swirling the plate or place the plate on a rotary shaker and shake very gently (lowest speed) for 2-3 minutes.
3. Centrifuge the plate at 1,400 x g for 1 minute at 4°C.
4. Incubate the plate at 37°C for 20 minutes.
5. Incubate the plate at 65°C for 15 minutes to inactivate the enzyme.
6. Cool the plate to room temperature. Centrifuge the plate at 1,400 x g for 1 minute at 4°C to collect any condensation that may have formed.
7. The DNA is now ready for use. We suggest storage at 4°C if the DNA is to be used in the next 48 hours, otherwise store the DNA at –20°C.

Troubleshooting Direct Lysis Fosmid96 DNA Purifications

Low DNA yield

1) **Fosmids need to be maintained in a compatible host carrying the *trf*A gene.** Only *trf*A-containing *E. coli* host strains are amenable to fosmid copy-number amplification using the *ori*V origin of replication.
2) **Fosmid Autoinduction Solution was not added to the fosmid culture.** Be sure to add Fosmid Autoinduction Solution (FAIS) to the culture medium prior to inoculation of the fosmid clone. Omitting FAIS from the culture will result in single-copy-number fosmid production and will consequently result in low fosmid DNA yields.
3) **Growth was not optimal.** Do not overgrow the cultures. A growth period of 16-17 hours is ideal for growing fosmids in LB medium. Follow the bacterial growth conditions in Part B.
4) **Insufficient lysis.** Cells lysed insufficiently will result in a low DNA yield. Overgrowth of cells will result in incomplete lysis because it can overload the lysis reagents.
5) **Use of a different culture medium.** LB medium is the recommended medium to use in this protocol for growing the cultures. The use of rich medium may result in a higher density of cells and thus will result in insufficient lysis.
6) **Poor culture growth.** Low fosmid DNA yields will result from poorly grown cultures. Follow the preculture method for inoculation in Part A, Step 3.

Low DNA quality

1) **RNA in eluates.** Be sure to add the PF96 RNase Blend to the PF96 Solution 2 prior to starting.
2) **Chromosomal contamination in eluates.** Vigorous mixing after adding PF96 Solutions 1 and 2 may result in shearing of chromosomal DNA into fragments that can easily be copurified with the fosmid DNA, resulting in contaminants in the fosmid DNA prep. Gentle mixing is recommended after addition of PF96 Solutions 1 and 2 to the culture. Lysis should not exceed 5 minutes. Longer lysis may also result in irreversible denaturation of the DNA.

No DNA

1) **DNA did not precipitate.** Be sure to precipitate the DNA as recommended in the protocol. The centrifugation speed and time used must be the same or higher as that recommended in the protocol.
2) **DNA pellet lost.** During the washing step, care should be taken not to dislodge the DNA pellet when adding the 70% ethanol. No mixing or vortexing is necessary. The plate should be tapped gently on the paper towel stack to get rid of most of the excess ethanol. The remaining ethanol should be removed by air-drying the pellet.
3) **DNA did not dissolve.** The DNA pellet should not be over-dried since this will result in difficulties in resuspension of the DNA.

Poor DNA sequence quality

1) **Washing the pellet.** Be sure to perform the wash step with fresh 70% ethanol for the removal of residual salts.
2) **Removal of ethanol.** Be sure to air-dry the pellets completely to ensure complete removal of the 70% ethanol after the wash step. Do not over-dry the pellet.

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