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BACMAX96™ DNA Purification Kit

(with consumable plastics)

Cat. No. BAC96116

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1. Introduction

The BACMAX96™ DNA Purification Kit is (with consumable plastics) designed for easy, reliable isolation of high-quality BAC and fosmid DNA in a 96-well format. Smaller batches of cultures can be prepped using smaller portions of the 96-well plates.

The protocol is based on a modified alkaline-lysis procedure that incorporates proprietary enzyme technologies into an easy-to-use format. Purification starts with cell pellets obtained by centrifugation of 1.2 ml *E. coli* cultures grown in a 96-well plate. Lysates are cleared in the presence of the BACMAX96 RNase Blend which efficiently removes unwanted RNA. Following filtration and selective precipitation steps, Plasmid-Safe™ ATP-Dependent DNase is used to remove any remaining bacterial genomic DNA.

2. Kit Contents

Desc.	Quantity
The BACMAX96™ DNA Purification Kit contains sufficient reagents to perform four 96-well plate purifications.	
Cat. No. BAC96116	
BACMAX96™ Solution 1	50 ml
BACMAX96™ Solution 2*	100 ml
BACMAX96™ Solution 3	80 ml
BACMAX96™ RNase Blend	1.35 ml
Plasmid-Safe™ ATP-Dependent DNase Plasmid-Safe™ 10X Buffer	300 µl
100 mM ATP Solution	1.1 ml
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	100 µl
BAC Autoinduction Solution	8 ml
Fosmid Autoinduction Solution	3.1 ml
2 ml Culture Plates	1.1 ml
Fritted Filter Plates	4
Round-bottom Collection Plates	4
Gas-permeable Plate Sealers	4
1 pad of Standard Sealers	4
	25 seals

*BACMAX96™ Solution 2 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.

To maximize flexibility, culture blocks and filtration plates are supplied. Filtration steps can therefore be performed via vacuum or centrifugation, making the kit easy to use with standard laboratory equipment. The kit is also amenable to semi- or fully automated purification systems.

The BACMAX96 Kit consistently yields up to 900 ng of DNA from a single-copy BAC clone and up to 2.5 µg of DNA from CopyControl™ BAC clones[1] induced to high-copy number. The unprecedented yields from CopyControl clones is due in part to an autoinduction protocol that was specifically designed for a high-throughput format. In this "hands-off" approach, the Autoinduction Solution is added to the growth media prior to inoculation and copy-number remains suppressed until shortly before the cells are harvested.

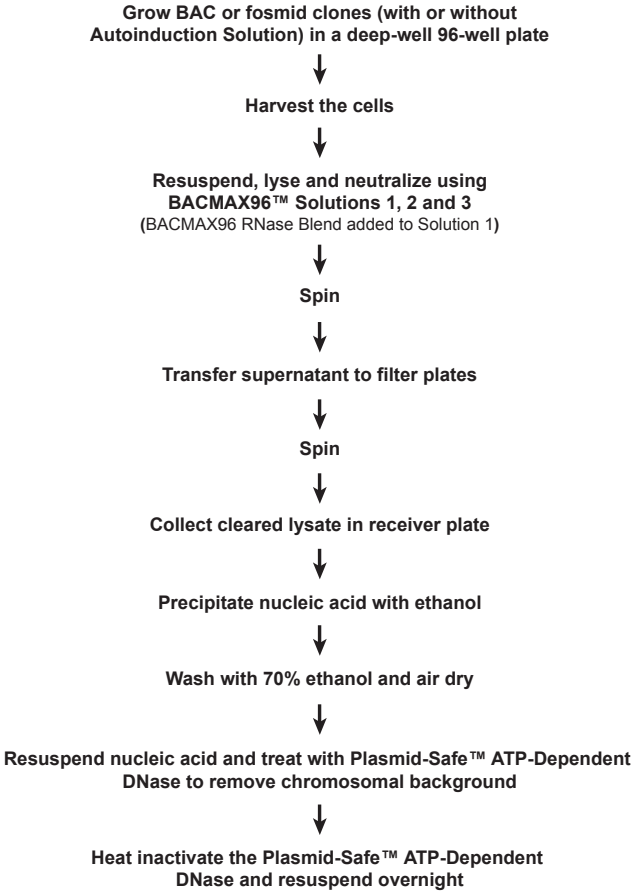

Figure 1. An overview of the BACMAX96™ DNA Purification Kit protocol.

3. Product Specifications

Storage: Store the BACMAX96 Solutions 1, 2, and 3, and consumable plastics at room temperature. Store the remainder of the kit components at –20°C in a freezer without a defrost cycle.

Quality Control: The BACMAX96 DNA Purification Kit is function-tested by purifying a 130 kb BAC. DNA quality and yield are assayed by gel electrophoresis, fluorimetry and restriction enzyme digestion.

4. Related Products

The following products are also available:

- CopyControl™ BAC Cloning Kits
- CopyControl™ Fosmid Library Production Kit
- BACMAX™ DNA Purification Kit
- Fast-Link™ DNA Ligation Kits
- End-It™ DNA End-Repair Kit

5. BACMAX96 DNA Purification Protocols

The following equipment is not supplied:

100% (Absolute) Ethanol	Multi-channel pipette
70% Ethanol	Boxes of appropriate pipette tips
Reagent reservoir trays	LB or similar bacterial growth media

A. Growing 96-Well Bacterial Cultures

1. *Single-copy BAC and fosmid clones:* Dispense 1.2 ml of LB containing the appropriate antibiotic into each well of a 2 ml deep well culture plate.

   CopyControl BAC clones: Dispense 1.2 ml of LB containing chloramphenicol (12.5 µg/ml) and BAC Autoinduction Solution (6 µl/ml media) into each well of a 2 ml deep well culture plate.

   CopyControl Fosmid clones: Dispense 1.2 ml of LB containing chloramphenicol (12.5 µg/ml) and Fosmid Autoinduction Solution (2 µl/ml media) into each well of a 2 ml deep well culture plate.
2. Inoculate each well with an isolated colony from a freshly streaked plate using a toothpick or from a glycerol stock using a 96-pin device or multi-channel pipette.
3. Seal the plate using an air permeable plate seal. Incubate cultures in an incubator/air shaker for approximately 17 hours at 37°C with constant shaking at 250 rpm.

B. Before Starting

1. Listed items are supplied in the BAC96116 Kit.
   Culture plate (1)
   Round-bottom collection plate (1)
   Air permeable sealer (1)
   Filter plate (1)
   Plate sealers (6)

   In addition, 6 reservoir trays will be needed for each time this protocol is used, regardless of the number of purifications performed.
2. The table on the following page lists the recommended volumes of BACMAX96 Solutions 1, 2 and 3 for purifications from single or multiple 96-well plates. Keep BACMAX96 Solutions 1 and 3 on ice and BACMAX96 Solution 2 at room temperature.
3. Just prior to use: add 30 µl of BACMAX96 RNase Blend per 1 ml of BACMAX96 Solution 1 to be used.

Number of Plates	BACMAX96 Solution 1 with RNase Blend	BACMAX96 Solution 2	BACMAX96 Solution 3
1	11 ml	22 ml	17 ml
2	22 ml	44 ml	34 ml
3	33 ml	66 ml	51 ml
4	44 ml	88 ml	68 ml
Storage	Ice	Room Temp.	Ice

C. BAC/Fosmid Purification Protocol

1. Pellet the cells at 1,000 x g for 10 minutes at 4°C in a table top centrifuge. Discard the plate sealer and carefully decant the media. Turn the plate upside down on a stack of paper towels to drain residual media.
2. Add 100 µl of chilled BACMAX96 Solution 1 to 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 vortexing the plate at maximum speed. Make sure the pellets are completely resuspended before proceeding.
4. Carefully remove the plate sealer and discard. Add 200 µl of room temperature BACMAX96 Solution 2 to each well of the culture plate. Cover the culture plate securely using a new plate sealer. Mix by inverting the plates 2-3 times very gently.
5. Incubate at room temperature for 4 minutes.

   Note: *The lysis reaction should not exceed 5 minutes. Carefully remove the plate sealer and discard. To ensure there will be no cross contamination between adjacent wells, remove any excess liquid by blotting the plate with a paper towel.*

6. Add 150 µl of chilled BACMAX96 Solution 3 to each well of the culture plate. Cover the culture plate securely using a new plate sealer. Mix by inverting the plates 2-3 times very gently.
7. Incubate on ice for 10 minutes.
8. Centrifuge the block at ≥1,400 x g for 10 minutes at 4°C.
9. Prior to transferring the lysate, place the filter plate on top of the collection plate. Transfer 300 µl of the lysate from the culture plate to the corresponding wells of the filter plate. When filtering the lysate via centrifugation, spin the filter and collection plate assembly at 1,000 x g for 5 minutes at 4°C. If you are using a vacuum manifold, follow the manufacturer's recommendations.
10. Add 2 volumes of absolute ethanol (200 proof) to the recovered lysate. Cover the receiver plate securely using a new plate sealer. Mix by inverting the plates 5-6 times.
11. Centrifuge the plate at ≥1,400 x g for 20 minutes at 4°C to precipitate the DNA.
12. Carefully remove the plate sealer and discard. Pour off the ethanol and remove any excess liquid by placing the plate upside down on a clean stack of paper towels.
13. Add 600 µl of freshly prepared 70% ethanol to each well. Cover the plate with a fresh plate sealer, and centrifuge the plate at ≥1,400 x g for 10 minutes at 4°C.
14. 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 several times. Turn the plate right side up and air dry at room temperature for 30 minutes.
15. During this drying step, prepare the appropriate amount of Plasmid-Safe Master Mix (use the table in Step 16).
16. Add 20 µl of TE Buffer (sterile deionized water or Tris-buffer can also be used). Cover the receiver plate securely using a new plate sealer. Place the plate on a rotary shaker and shake very gently (lowest speed) for 5 minutes. Give the plate a 1 minute quick spin to collect any remaining liquid.

Plasmid-Safe Master Mix: The table below lists the reagent volumes required to make enough Master Mix for single to multiple 96-well plates.

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

17. 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.
18. Centrifuge the plate at ≥1,400 x g for 1 minute at 4°C.
19. Incubate the plate at 37°C for 20 minutes.
20. Incubate the plate at 65°C for 15 minutes to inactivate the enzyme.
21. Cool the plate at room temperature. Centrifuge the plate at ≥1,400 x g for 1 minute at 4°C to collect any condensation that may have formed.
22. 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.

D. Expected Yields

Single-copy BAC and fosmid clones: Yields range from 500-900 ng of DNA depending on the growth characteristics and size of the construct. We recommend 8 µl of sample for each end sequencing reaction or restriction enzyme digest.

CopyControl BAC and fosmid clones: Yields range from 2.1-2.5 µg of DNA depending on the growth characteristics and size of the construct. As little as 1 µl of sample can be used for each end sequencing reaction or restriction enzyme digest.

6. Reference

1. *Epicentre Forum* (2002) **9** (1), 4.

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