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FosmidMAX™ DNA Purification Kit

Cat. No. FMAX046

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1. Introduction

The FosmidMAX™ DNA Purification Kit was developed for easy, reliable isolation of high-quality fosmid DNA. The scalable protocol is based on a modified alkaline-lysis procedure that can be used with 1.5–100 ml of culture. Consistent yields of up to 0.6, 15 or 25 µg of fosmid DNA are obtained from 1.5, 40 or 100 ml cultures of a single-copy fosmid, respectively. Up to 4 µg of fosmid DNA can be isolated from 1.5 ml of an induced CopyControl™ fosmid clone (see below). Selective precipitation steps and the incorporation of Epicentre's RiboShredder™ RNase Blend effectively remove

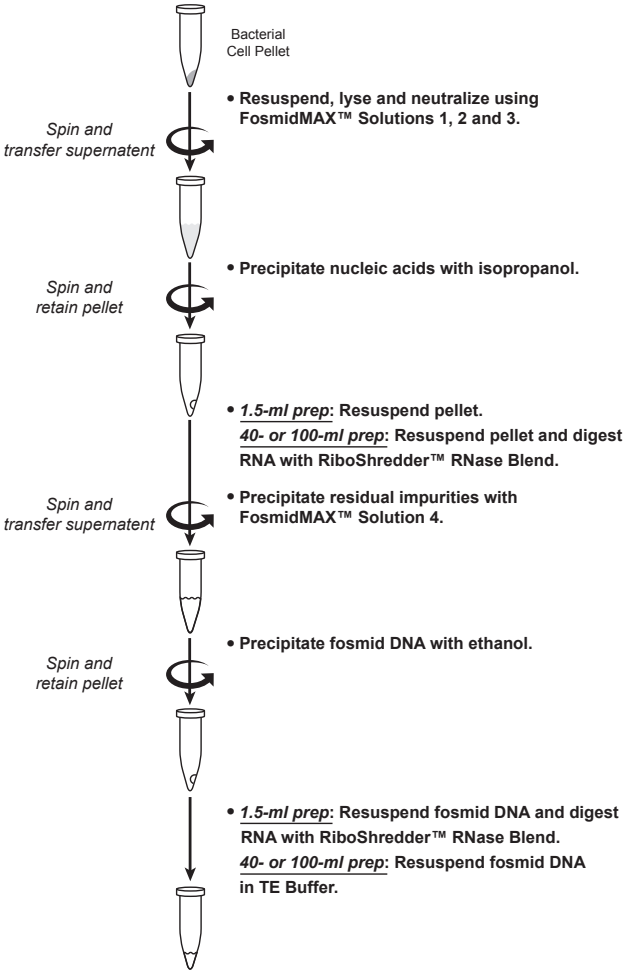

Figure 1. An overview of the FosmidMAX™ DNA Purification Kit protocol.

contaminants that degrade DNA and interfere with downstream applications. There is no need for columns, resins or organic extractions. The exceptionally pure fosmid DNA can be used for many applications, including sequencing, fingerprinting, PCR and preparation of shotgun libraries. The FosmidMAX Kit can also be used to isolate cosmid DNA.

Epicentre also offers the CopyControl Fosmid Library Production Kit.[1] This kit utilizes a strategy of cloning blunt-ended DNA fragments, generated by random shearing of DNA, to produce complete and unbiased genomic libraries. Greater than 10^6 clones are produced in a single experiment. The unique CopyControl cloning technology enables the user to grow the clones at single copy to ensure insert stability and cloning of toxic gene products and then induce the clones to high-copy number for high yields of DNA.

2. Product Specifications

Storage: Store the RiboShredder 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 FosmidMAX DNA Purification Kit is function-tested by purifying a single-copy fosmid clone. DNA quality and yield are assayed by gel electrophoresis, fluorimetry and restriction enzyme digestion.

3. Kit Contents

Desc.	Quantity
The FosmidMAX™ DNA Purification Kit contains sufficient reagents to perform 150 x 1.5 ml, 10 x 40 ml or 5 x 100 ml purifications.	
FosmidMAX™ Solution 1	30 ml
FosmidMAX™ Solution 2*	60 ml
FosmidMAX™ Solution 3	45 ml
FosmidMAX™ Solution 4	38 ml
RiboShredder™ RNase Blend	200 µl
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	42 ml

**FosmidMAX™ 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.`*

4. Related Products

The following products are also available:

- CopyControl™ Fosmid Library Production Kit
- EpiFOS™ Fosmid Library Production Kit
- pWEB™ Cosmid Cloning Kit
- CopyControl™ BAC Cloning Kits
- Fast-Link™ DNA Ligation Kits
- End-It™ DNA End-Repair Kit

The recommended protocol for 1.5-purifications is shown here and described in Section A. Yields are maximized by including the RNA digestion step at the end of the protocol. This enables the RNA to act as a carrier which facilitates DNA precipitation and visualizing the sample pellet. In our experience, downstream applications, like fingerprinting, sequencing and shotgun library construction, are not inhibited by the small amount of residual free nucleotides or the RiboShredder Blend. However, the alternative protocol described in Section B includes an RNA digestion step earlier in the purification process so these residual contaminants are eliminated.

5. General Considerations

1. **Optimal cell density:** Harvest cells at an OD_{600} of 3 to 4 to maximize yields of fosmid DNA. Growing cells for more than 16 hours is not recommended.
2. **Avoid shearing:** Fosmid DNA, because of its large size, is prone to shearing. Do **NOT** vortex, shake or pipet the cells after adding FosmidMAX Solution 2 and 3 during the lysis and neutralization steps. The lysis reaction should not exceed 5 minutes. Mix with gentle inversion and use a wide-orifice pipet where noted in the protocol.
3. **Proper storage conditions:** Store fosmid DNA at –20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

6. Fosmid DNA Purification Protocols

A.Purification from 1.5 ml of Culture

Note: *The protocol given below maximizes yields by including the RNA digestion step at the end of the procedure. This enables the RNA to act as a carrier which facilitates DNA precipitation and visualizing the sample pellet. In our experience, downstream applications, like fingerprinting, sequencing and shotgun library construction, are not inhibited by the small amount of residual free nucleotides or the RiboShredder Blend. However, the alternative protocol described in Section B includes an RNA digestion step earlier in the purification process so these residual contaminants are eliminated.*

Growing the culture: Prepare 2 ml of LB medium containing the appropriate antibiotic in a 14 ml snap cap culture tube. Inoculate with an isolated colony from a freshly streaked plate and shake (~250 rpm) at 37°C for 12-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

Dilute RiboShredder RNase Blend 1:4 in TE Buffer. Keep on ice until needed.

1. Transfer 1.5 ml of the overnight culture to a 1.7 ml microcentrifuge tube. Pellet the cells by centrifuging at 15,000 x g or maximum speed for 1-3 minutes. Discard the supernatant.
2. Add 200 µl of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.
3. Add 400 µl of FosmidMAX Solution 2. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

   Note: *The lysis reaction should not exceed 5 minutes.*

4. Add 300 µl of chilled FosmidMAX Solution 3. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.
5. Incubate on ice for 15 minutes.
6. Centrifuge at 15,000 x g or maximum speed for 15 minutes at 4°C to pellet cellular debris.
7. Transfer the supernatant to a microcentrifuge tube.

   Note: *To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*
8. Add 540 µl or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.
9. Precipitate the nucleic acids by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.
10. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
11. Resuspend the pellet in 250 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.
12. Add 250 µl of chilled FosmidMAX Solution 4 to the tube. Mix thoroughly by tapping the tube and incubate on ice for 15 minutes.
13. Centrifuge the tube at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully transfer the supernatant to a microcentrifuge tube without disrupting the pellet.
14. Add 1 ml of absolute ethanol (200 proof) to the recovered supernatant. Mix gently by inverting the tube 4-6 times.
15. Precipitate the DNA by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully pipette off the ethanol without disrupting the pellet. Centrifuge briefly and pipette off any residual ethanol.
16. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
17. Add 25 µl of TE Buffer to the tube (sterile deionized water or Tris-buffer can also be used) by tapping the tube and leave at room temperature for 10 minutes.
18. Add 1 µl of diluted RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.
19. Quantitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.
20. Store the fosmid DNA at –20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

B. Alternative Protocol for Purification from 1.5 ml of Culture

Growing the culture: Prepare 2 ml of LB medium containing the appropriate antibiotic in a 14 ml snap cap culture tube. Inoculate with an isolated colony from a freshly streaked plate and shake (~250 rpm) at 37°C for 12-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

Steps 1-11 are as described in Section A.

12. Add 1 µl of undiluted RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.
13. Add 250 µl of chilled FosmidMAX Solution 4. Mix gently by tapping the tube and incubate on ice for 15 minutes.
14. Centrifuge the tube at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully transfer the supernatant to a microcentrifuge tube without disrupting the pellet.

    Note: *Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*
15. Add 1 ml of absolute ethanol (200 proof) to the recovered supernatant. Mix gently by inverting the tube 4-6 times.
16. Precipitate the DNA by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully pipette off the ethanol without disrupting the pellet. Centrifuge briefly and pipette off any residual ethanol.
17. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
18. Resuspend the pellet in 25 µl of TE Buffer (sterile deionized water or Tris-buffer can also be used) by tapping and swirling the tube.
19. Quanitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.
20. Store the fosmid DNA at –20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

C. Purification from 40 ml of Culture

Growing the culture: Prepare 50 ml of LB medium containing the appropriate antibiotic in a 250 ml flask. Inoculate with an isolated colony from a freshly streaked plate and shake vigorously (~250 rpm) at 37°C for 14-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

1. Transfer 40 ml of the overnight culture to a 40 ml Oakridge-style centrifuge tube. Pellet the cells by centrifuging at 5,000 x g for 8 minutes at 4°C. Discard the supernatant.
2. Add 3 ml of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.

3. Add 6 ml of FosmidMAX Solution 2. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

   Note: *The lysis reaction should not exceed 5 minutes.*

4. Add 4.5 ml of chilled FosmidMAX Solution 3. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.
5. Incubate on ice for 15 minutes.
6. Centrifuge at ≥15,000 x g for 15 minutes at 4°C to pellet cellular debris.
7. Transfer the supernatant to a 40 ml Oakridge-style centrifuge tube using a 10 ml pipet.

   Note: *To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*

8. Add 8.1 ml or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.
9. Precipitate the nucleic acids by centrifugation at ≥15,000 x g for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.
10. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
11. Resuspend the pellet in 500 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.
12. Add 18 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes. Add an additional 500 µl of TE Buffer and mix by tapping the tube.
13. Add 1 ml of chilled FosmidMAX Solution 4. Mix gently by tapping the tube and incubate on ice for 15 minutes.
14. Centrifuge the tube at ≥15,000 x g for 15 minutes at 4°C. Carefully transfer the supernatant to a 40 ml Oakridge-style centrifuge tube without disrupting the pellet.

    Note: *Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

15. Add 4 ml of absolute ethanol (200 proof) to the recovered supernatant in each tube. Mix gently by inverting the tube 4-6 times.
16. Precipitate the DNA by centrifugation at ≥15,000 x g for 15 minutes at 4°C. Carefully decant the ethanol. Centrifuge briefly and pipette off any residual ethanol without disrupting the pellet.

    Note: *Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

17. Air-dry the pellet at room temperature for 5-7 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
18. Add 200 µl of TE Buffer to each tube (sterile deionized water or Tris-buffer can also be used). Resuspend the pellet by tapping the tube and leave the DNA overnight at 4°C to dissolve completely.

19. Quantitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.

D. Purification from 100 ml of Culture

Growing the culture: Prepare 100 ml of LB medium containing the appropriate antibiotic in a 500 ml flask. Inoculate with an isolated colony from a freshly streaked plate and shake vigorously (~250 rpm) at 37°C for 14-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

1. Transfer 100 ml of the overnight culture to a 250 ml centrifuge bottle. Pellet the cells by centrifuging at 5,000 x g for 8 minutes at 4°C. Discard the supernatant.
2. Add 6 ml of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.
3. Transfer equal volumes of the cell suspension (3 ml) to two 40 ml Oakridge-style centrifuge tubes.
4. Add 6 ml of FosmidMAX Solution 2 to each tube. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

   Note: *The lysis reaction should not exceed 5 minutes.*
5. Add 4.5 ml of chilled FosmidMAX Solution 3 to each tube. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.
6. Incubate on ice for 15 minutes.
7. Centrifuge at ≥ 15,000 x g for 15 minutes at 4°C to pellet cellular debris.
8. Transfer the supernatant to a 40 ml Oakridge-style centrifuge tube using a 10 ml pipet.

   Note: *To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*
9. Add 8.1 ml or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.
10. Precipitate the nucleic acids by centrifugation at ≥15,000 x g for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.
11. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
12. Resuspend the pellet in 500 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.
13. Add 20 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes. Add an additional 500 µl of TE Buffer and mix by tapping the tube.

14. Add 1 ml of chilled FosmidMAX Solution 4 to each tube. Mix gently by tapping the tube and incubate on ice for 15 minutes.
15. Centrifuge the tube at ≥15,000 x g for 15 minutes at 4°C. Carefully transfer the supernatant to a 40 ml Oakridge-style centrifuge tube without disrupting the pellet.

    Note: *Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*
16. Add 4 ml of absolute ethanol (200 proof) to the recovered supernatant in each tube. Mix gently by inverting the tube 4-6 times.
17. Precipitate the DNA by centrifugation at ≥15,000 x g for 15 minutes at 4°C. Carefully decant the ethanol. Centrifuge briefly and pipette off any residual ethanol without disrupting the pellet.

    Note: *Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*
18. Air-dry the pellet at room temperature for 5-7 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.
19. Add 200 µl of TE Buffer to each tube (sterile deionized water or Tris-buffer can also be used). Resuspend the pellet by tapping the tube and leave the DNA overnight at 4°C to dissolve completely.
20. Quantitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.

7. References

1. *Epicentre Forum* (2002) **9** (1), 3.

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