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BACMAX™ DNA Purification Kit

Cat. No. BMAX044

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1. introduction

The BACMAX™ DNA Purification Kit was developed for easy, reliable isolation of high-quality BAC DNA.1 The scalable protocol is based on a modified alkaline-lysis procedure that can be used with 1.5 to 100 ml of culture. Consistent yields of up to 0.6, 4, 11, or 25 µg of BAC DNA are obtained from 1.5-, 10-, 40-, or 100-ml cultures of a single-copy BAC, respectively. Selective precipitation steps and the incorporation of Epicentre's RiboShredder™ RNase Blend effectively remove contaminants that degrade DNA and interfere with downstream applications. There is no need for columns, resins, or organic extractions. The exceptionally pure BAC DNA can be used for many applications, including sequencing, fingerprinting, PCR and preparation of shotgun libraries. The BACMAX Kit can also be used to isolate PAC DNA.

Epicentre also offers CopyControl™ BAC Cloning Kits. Each kit includes a linearized, dephosphorylated, highly-purified pCC1BAC™ Vector, which increases cloning efficiencies and significantly lowers background. Combining this vector with other time-saving components, like Fast-Link™ DNA Ligase, these kits significantly reduce the amount of time and effort needed to make a BAC library. Moreover, the CopyControl BAC Kits offer researchers the unique opportunity to induce single-copy BAC clones to high-copy number.2

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 BACMAX DNA Purification Kit is function-tested by purifying a 165-kb BAC. DNA quality and yield are assayed by gel electrophoresis, fluorimetry, and restriction enzyme digestion.

3. Kit Contents

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

**BACMAX™ 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™ BAC Cloning Kits
- CopyControl™ pCC1BAC™ Cloning–Ready Vectors
- pIndigoBAC-5 Cloning-Ready Vectors
- CopyControl™ Fosmid Library Production Kit
- EpiFOS™ Fosmid Library Production Kit
- Fast-Link™ DNA Ligation Kits
- End-It™ DNA End-Repair Kit

5. General Considerations

1. **Optimal cell density:** Harvest cells at an A_{600} of 3 to 4 to maximize yields of BAC DNA. Growing cells for more than 16 hours is not recommended.
2. **Avoid shearing:** BAC DNA, because of its large size, is prone to shearing. Do **not** vortex, shake, or pipet the cells after adding BACMAX 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 BAC DNA at –20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

6. BAC DNA Purification Protocols

A. 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 A_{600} of 3 to 4.

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

1. Transfer 1.5 ml of the overnight culture to a 1.7-ml microcentrifuge tube. Pellet the cells by centrifugation at 15,000 x g or maximum speed for 1-3 minutes. Discard the supernatant.
2. Add 200 µl of chilled BACMAX 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 BACMAX Solution 2. Mix by inverting the tube 2-3 times *very gently*. To avoid shearing the BAC DNA, do **not** vortex, shake, or pipet the lysate.

   Note: *The lysis reaction time should not exceed 5 minutes.*
4. Add 300 µl of chilled BACMAX Solution 3. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the BAC DNA do **not** vortex, shake, or pipet the lysate.
5. Incubate on ice for 15 minutes.

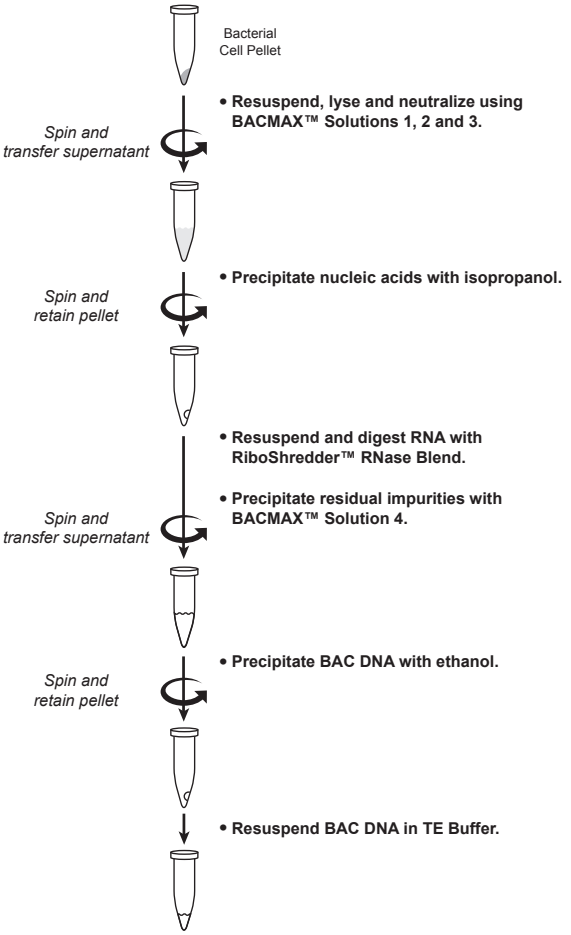

Figure 1. An overview of the BACMAX™ DNA Purification Kit protocol.

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 using a 1-ml large-orifice pipet tip to minimize shearing.

   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 1 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.
    Cool the tube at room temperature.
13. Add 250 µl of chilled BACMAX Solution 4 to the tube. Mix thoroughly 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 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 3-5 minutes. Do **not** over-dry or it will be difficult to resuspend the pellet.
18. Add 25 µl of TE Buffer to the tube (sterile deionized water or Tris buffer can also be used). Resuspend the pellet by tapping the tube and leave at room temperature for 10 minutes.
19. Quantitate the yield of BAC DNA by fluorimetry using a DNA-specific dye (e.g., PicoGreen® or bis-benzimide [Hoechst dye 33258]), or by agarose gel electrophoresis.

B. Purification from 10 ml of Culture

Growing the culture: Prepare 10 ml of LB medium containing the appropriate antibiotic in a 50-ml 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 A_{600} of 3 to 4.

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

1. Transfer 10 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 1 ml of chilled BACMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.
3. Add 2 ml of BACMAX Solution 2. Mix by inverting the tube 2-3 times *very gently*. To avoid shearing the BAC DNA do **not** vortex, shake or pipet the lysate.

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

4. Add 1.5 ml of chilled BACMAX Solution 3. Mix by inverting the tube 2-3 times *very gently*. A white precipitate will form in the tube. To avoid shearing the BAC 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 5-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 2.7 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 250 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.
12. Add 6 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.
    Add an additional 250 µl of TE Buffer and mix by tapping the tube.
13. Transfer equal volumes of the suspension (250 µl) to two 1.7-ml microcentrifuge tubes.
14. Add 250 µl of chilled BACMAX Solution 4 to each tube. Mix thoroughly by tapping the tube and incubate on ice for 15 minutes.
15. 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.*
16. Add 1 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 or maximum speed 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 3-5 minutes. Do **not** over-dry or it will be difficult to resuspend the pellet.
19. Add 50 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 BAC DNA by fluorimetry using a DNA-specific dye (e.g., PicoGreen or bis-benzimide [Hoechst dye 33258]), or by agarose gel electrophoresis.

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 A_{600} of 3 to 4.

Prior to starting: Chill BACMAX 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 BACMAX 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 BACMAX Solution 2. Mix by inverting the tube 2-3 times *very gently*. To avoid shearing the BAC DNA do **not** vortex, shake or pipet the lysate.

   Note: *The lysis reaction time should not exceed 5 minutes.*
4. Add 4.5 ml of chilled BACMAX Solution 3. Mix by inverting the tube 2-3 times *very gently*. A white precipitate will form in the tube. To avoid shearing the BAC 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 BACMAX 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 3-5 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 BAC 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 A_{600} of 3 to 4.

Prior to starting: Chill BACMAX 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 BACMAX 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 BACMAX Solution 2 to each tube. Mix by inverting the tube 2-3 times *very gently*. To avoid shearing the BAC DNA do **not** vortex, shake or pipet the lysate.

    Note: *The lysis reaction time should not exceed 5 minutes.*
5. Add 4.5 ml of chilled BACMAX 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 BAC 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 BACMAX 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 3-5 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 BAC 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. Begum, D. (2004) *Epicentre Forum* **11** (3), 4
2. *Epicentre Forum* (2002) **9** (1), 4.

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