BAC/PAC Isolation Mini Kit

Important Notes:

Expected yield: The yield of BAC is around 0.6 µg from 2 mL LB culture and 1 µg from 5 mL culture. If cultured in TB, the yield is about 1 µg from 1.5 mL culture and 3 µg from 5 mL culture

Culture volume: Use a flask or tube with a volume at 4 times the culture medium to secure optimal condition for bacteria growth. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.

Storage and Stability

All EZgene™ BAC/PAC DNA Isolation Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Buffer X1/RNase A mixture at 4°C, and all other materials at 22-25°C.

Introduction

The EZgene high-capacity-plasmid DNA Isolation Kit is designed for rapid purification of BAC, PAC, cosmid and P1 from small volume of bacterial cultures. It is based on a modified alkaline lysis procedure that is specially adapted for spin column. The procedure associated with this kit has been tested using a variety of low copy cosmid, BAC, PAC and P1 in different E. coli strains. In addition, this kit can also be used for high copy plasmid isolation.

Before start

- ☑ Add whole vial of RNase A to Buffer X1 and Store at 4°C.
- ☑ Dilute DNA Wash Buffer with **absolute ethanol** as follows,
  - **PD1311-01** Add 48 mL ethanol to the bottle.
  - **PD1311-02** Add 200 mL ethanol to each bottle.
- ☑ Dilute BAC Binding Buffer with **isopropanol** as follows,
  - **PD1311-01** Add 25 mL isopropanol to the bottle.
  - **PD1311-02** Add 125 mL isopropanol to each bottle.
- ☑ It's strongly recommended to use 2 x YT media for the cultivation of cosmids, BACs, PACs, and P1s.
- ☑ Buffer X2 should be kept at room temperature. Check for SDS precipitation before use. If necessary re-dissolve SDS precipitate by warming. Close the Buffer X2 bottle immediately after use to avoid acidification that may result from air CO2.
- ☑ Chill Buffer X3 for precipitation enhancement.

Prewarm dd H2O or elution buffer at 60°C before elution.

Use 4°C microcentrifuge for step 6.

Kit Contents

Catalog #	PD1311-01	PD1311-02
Preps	50	250
ezBind™ Columns	50	250
Buffer X1	20 mL	90 mL
Buffer X2	20 mL	90 mL
Buffer X3	20 mL	90 mL
BAC Binding Buffer	2 mL	10 mL
Elution Buffer	10 mL	30 mL
DNA Wash Buffer	12 mL	50 mL
RNase A	100 µL	500 µL
User Manual	1	1

Materials Supplied by User

- ☑ Microcentrifuge capable of at least 12,000 x g
- ☑ Microcentrifuge set at 4°C capable of at least 12,000 x g
- ☑ Sterile Deionized Water
- ☑ Sterile 1.5 mL and 2.0 mL Centrifuge Tubes
- ☑ 10-15 mL Culture Tubes
- ☑ 96-100% Ethanol
- ☑ 96%-100% Isopropanol

BAC, PAC, and P1 purification protocol

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a starter culture of 2-5 mL LB or 2 mL TB medium containing the appropriate selective antibiotic. Incubate for 20-24 hr at 37°C with vigorous shaking (~ 300 rpm). Use a flask with a volume at least 4 times the volume of the culture.

2. Pellet 1.5-5 mL bacteria by centrifugation at 12,000 x g for 2 min at room temperature. Decant or aspirate medium and discard.

3. Resuspend the bacterial pellet by adding 400 µL Buffer X1/RNase A solution, and vortexing. Complete resuspension of cell pellet is vital for obtaining good yields. Transfer the resuspended bacterial into a 2 mL tube.

4. Add 400 µL Buffer X2 and mix gently but thoroughly by inverting 5-10 times to obtain a clear lysate. Incubate at room temperature for 5 min. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. The lysate should appear viscous. Do not incubate more than 5 min. (Store Buffer X2 tightly capped).

5. Add 400 µL Buffer X3 (chilled) and gently but thoroughly mix the sample by inverting 10-15 times until a flocculent white precipitate forms. Incubate on ice for 5 min.

6. Centrifuge at 12,000 x g for 10 min at 4°C. Promptly proceed to the next step.

7. Carefully transfer the clear supernatant to a new 2 mL tube.
   Add 450 µL BAC binding buffer.
   Note: Add 25 mL of isopropanol to BAC binding buffer before use.

8. Transfer 700 µL of the sample to the DNA column. Centrifuge at
   12,000 x g for 15 seconds at room temperature. Discard the flow-through. Transfer the remaining sample to the column and
   centrifuge at 12,000 x g for 30 seconds at room temperature. Discard the flow-through.

9. Add 700 µL DNA Wash Buffer. Centrifuge at 12,000 x g for 30 seconds at room temperature. Discard the flow-through.

10. Place the DNA column, with the lid open, back into the collection tube and centrifuge at 12,000 x g for 1 min to remove residual ethanol.

11. Place the DNA column into a clean 1.5 mL centrifuge tube, add 35-50 µL pre-warmed (60°C) Elution Buffer (10mM Tris-HCl, pH 8.5) or ddH_2O onto the center of the membrane. Incubate 5 min at 60°C.

12. Centrifuge at 12,000 x g for 1 min to elute the DNA. Add the eluted DNA back to the column and centrifuge at 12,000 x g for 1 min to elute the DNA.

    Note: Pre-warm elution buffer or ddH_20 at 60°C and incubate the column at 65°C for 5 min after adding elution buffer or ddH_20 will increase the DNA yield.
    Note: The first elution normally yields 60-70% of the DNA. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA.

13. Store the eluted DNA at -20°C.

Limited liability and Warranty

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 603-3219 or visit our website at www.biomiga.com

Trouble Shooting Guide

Problem	Possible Reason	Solutions
Low DNA Yield	Poor Cell lysis	Only use LB or TB medium containing ampicillin. Do not use more than 5 mL culture with the basic protocol.
		Cells may not have been dispersed adequately prior to the addition of Buffer X2. Make sure to vortex cell suspension to completely disperse.
		Continue inverting vials after adding Buffer X2 to obtain a clear lysate.
		If not tightly closed, Buffer X2 may need to be replaced. Prepare as follows: 0.2 M NaOH, 1% SDS.
	Bacterial Clone is not fresh	Use fresh glycerol cultures and avoid repeated freezing/thawing cycles of clones. Always make enough replica plates and use fresh cultures for inoculation. Any remaining cultures can be used to set up fresh glycerol stocks.
No DNA Eluted	Lysate prepared incorrectly	Check the stock of buffers and age of the buffers. Make sure that the correct volume of buffer has been added to the samples.
	Buffer X2 precipitated	Warm up the Buffer X2 to dissolve the precipitate.
	Cells are not completely resuspended	Pelleted cells should be completely resuspended with Buffer X1. Do not add Buffer X2 until an even cell suspension is obtained.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of X2	Do not vortex or mix aggressively after adding Buffer X2.
	Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.
DNA degraded after the storage	High levels of endonuclease activity.	Perform the heat inactivation step.
RNA visible on agarose gel	RNase A not added to Buffer X1.	Add 1 vial of RNase to each bottle of Buffer X1.
	DNA floats out of well while loading agarose gel.	Air dry the DNA pellet before re-dissolving the DNA.