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Introduction

The EZgene™ lines of products are innovative system that radically simplifies the extraction of nucleic acids from a variety of sources. Key to the system is Biomiga's proprietary ezBind matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The 96-Well ezFilter Yeast Plasmid Isolation Kit is designed for rapid high-throughput purification of plasmid from yeast cultures grown and processed in a 96-well Plate format. The purification process may be performed by centrifugation or vacuum protocol. One 96-well plate can be processed manually within 60 minutes. The procedure has been developed and tested using a variety of yeast plasmids and low copy plasmids.

This protocol has been successfully used to isolate autonomous plasmids from *S. cerevisiae*. As a modified alkaline lysis procedure, genomic DNA is virtually eliminated from the preparation. Note that all centrifugation steps are to be performed at room temperature.

Storage and Stability

All 96-Well ezFilter Yeast Plasmid Kit components are guaranteed for at least 12 months from the date of production when stored as follows: The mixture of YP1/RNase A at 4 °C; all other materials at 22-25 °C. Store YP II tightly capped.

Kit Contents

Catalog#	YD1291-S	YD1291-01	YD1291-02
Preps	1	4	20
96-Well DNA Plate	1	4	20
Deep Well Plate	1	4	20
Collection Plate (300µL)	1	4	20
96-Well Lysate Clearance Plate	1	4	20
Buffer YP I	30 mL	110 mL	2 x300 mL
Buffer YP II	30 mL	110 mL	2 x300 mL
Buffer YPIII	40 mL	160 mL	800 mL
Lyticase	300 U	12,000 U	60,000 U
Buffer SE	50 mL	200 mL	1000 mL
Glass Beads	5.4 g	22 g	110 g
DNA Wash Buffer	54 mL (Add 162 mL ethanol before use)	2×1 00 mL (Add 300 mL ethanol before use)	7×1 25 mL (Add 325 mL ethanol before use)
Elution Buffer	15 mL	60 mL	300 mL
RNase A Concentrate	140 µL	560 µL	2 x 1500 µL
Instruction Booklet	1	1	1

* The 2 mL 96-Well Collection Plates are reusable.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

Important

- ☼ Add vial of RNase A to bottle of Buffer YP I and Store at 4 ºC
- ☼ Dissolve the Lyticase to 150U/mL using SE Buffer
- ☼ DNA Wash Buffer has to be diluted with absolute Ethanol (~96-100%) as follows:
  YD1291-S: Add 150 mL ~96%-100% ethanol
  YD1291-01: Add 300mL ~96%-100% ethanol
  YD1291-02: Add 325 mL ~96%-100% ethanol

Materials Supplied By User

- ☼ Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g
- ☼ Adapter for 96-well Collection Plate
- ☼ 2.2 mL 96 deep-well Plate for culture bacteria
- ☼ Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inches Hg
- ☼ Standard vacuum manifold (i.e: Biomiga Product #VAC- 03)
- ☼ Vacuum oven or incubator preset to 70 ºC
- ☼ Isopropanol
- ☼ Sealing film

Vacuum Manifold Protocol

1. Inoculate ***5 mL YDP*** medium placed in a 10-20 mL culture tube with yeast carrying desired plasmid and grow at 30 °C with agitation for 16-24 h.
2. Pellet ***1-3 mL yeast culture*** (use < 2 x 10^7 cells) in a 2.2 mL deep-well plate by centrifugation at 4,000 × g for 10 min at room temperature.
3. Discard supernatant and resuspend yeast cells in ***480 µL Buffer SE/2-mercaptoethanol*** and ***20 µL lyticase solution***. Resuspend the pellet by pipetting or vortexing (a sealing film should be used if using vortexing to resuspend the pellet). Complete resuspension of cell pellet is vital of obtaining good yields. Incubate at 30 °C for at least 30 min.

   Note: Remember to add 10 µL of 2-mercaptoethanol per 1 mL of Buffer SE before use. This mixture can be made and stored at room temperature for one week.
4. Pellet spheroblasts by centrifuging at 4,000 x g for 5 min at room temperature. Discard the supernatant completely. Resuspend the spheroblasts pellet with ***250 µL Buffer YP I*** by pipetting or vortexing the plate.

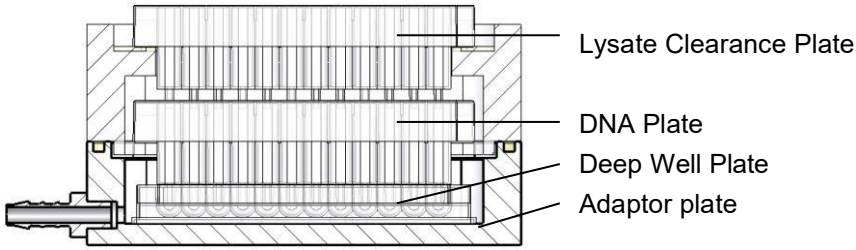
   Ensure *that RNase A has been added into Buffer YP I before use.*

   Note: No cell pellet clumps should be observed after resuspension.
5. **Optional:** Add ***50 mg glass beads*** to each well, seal the plate with the film, and vortex at max speed for 5 min. Let stand to allow the beads to settle. Transfer the supernatant to a new 2.0 mL deep-well plate (not supplied).

   Note: This beads beating step is to help the completely removal of the yeast cell wall. This step can be skipped if the cell wall can be efficiently removed by lyticase digestion.

6. **Add *250 µL Buffer YP II* and gently mix by rotating/shaking the plate 5-10 times till a homogeneously colored suspension is achieved**. Incubation at room temperature for 3-5 min. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. **Note:** Continue mixing the solution if brownish cell clumps are still visible. This is critical for optimized yield.
7. **Add *350 µL Buffer YP III* to each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by rotating/shaking the plate several times or vortexing briefly until a flocculent white precipitate forms. Peel the film off carefully to avoid cross contamination from well to well.

8. Assemble the vacuum manifold: **1).** Place 96-well Lysate Clearance Plate on top of the Top Block of the manifold; **2).** Place one 96 Well adaptor plate and one deep well plate (supplied) inside the manifold Base Block; **3).** Place the 96-well DNA Plate on top of the collection plate **4).** Place the Top Block of manifold over the Base Block, the Deep Well Plate now should be positioned under the 96-well DNA Plate. The DNA Plate should be positioned under the Lysate Clearance Plate. Seal the unused wells of 96-well Lysate Clearance Plate with sealing film.



9. Carefully transfer the lysate from the culture plate into the wells of 96-well Lysate Clearance Plate. Allow the lysate to stand for 8-10 min. The white precipitate should float to the top.
10. Turn on the vacuum pump and apply the vacuum by switching vacuum valve to the opening position at low vacuum force. Vacuum until all the liquid passes through the membrane of 96-well Lysate Clearance Plate.
    Note: Low vacuum force sets a stead filtration and prevents the clogging of filter membranes.
11. Turn off the vacuum by slowly turning the vacuum valve to the close position. Allow the vacuum manifold pressure to equalize to ambient pressure.

Note: If some wells of the Lysate Clearance Plate clog, cover Plate with a sealing film while the vacuum is been drawn. Cover the Plate with sealing film will increase the vacuum pressure. Some appearance of the white flocculent material in wells is normal and should not be mistaken for clogging. No more than 5 min should be spent in trying to get clogged wells to clear through the Lysate Clearance Plate.

12. Discard the Lysate Clearance Plate. Place a waste collection tray inside the vacuum manifold and place the 96-well DNA Plate on top of the Top Block. The lysate is now in the 96-well DNA Plate
13. Turn on the vacuum pump and apply the vacuum by switching vacuum valve to the opening position to let the lysate pass through the DNA Plate.
14. Turn off the vacuum, wash the wells by adding ***800 µL DNA Wash Buffer*** diluted with absolute ethanol to each well of the 96-well DNA Plate. Apply the vacuum until all liquid passes through.

    Ensure that the 100% ethanol has been added to DNA Wash Buffer according to instructions before use.
15. Turn off the vacuum, wash the wells by adding ***800 µL DNA Wash Buffer*** diluted with absolute ethanol to each well of the 96-well DNA Plate. Apply the vacuum until all liquid passes through.
16. After all liquid pass through the membrane, dry the membrane by applying maximum vacuum for another 10-12 min.
17. Remove the 96-well DNA Plate from the vacuum manifold, then vigorously tap the Plate on a stack of absorbent paper towels until no drops come out. Remove any residual moisture from the tip ends of the DNA Plate with clean absorbent paper towels.
18. Place the 96-well DNA Plate back to the vacuum manifold and apply the maximum vacuum for another 5 min. This step will ensure to evaporate any remaining ethanol from membrane.

19. **Elution with provided 96-Well Plate:** Assemble the vacuum manifold by place a deep well collection plate inside the Base Block and place the new 300 µL Collection Plate (provided) on top of the deep well plate. Place the 96-well DNA Plate on top of the Top Base.
    Note: The collection plate should be positioned well under the DNA Plate. Adjust the plate with adaptor plate if needed.
20. **Add *60 µL Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water*** to the center of well of the 96-well DNA Plate, let stand for 5 min. Apply maximum vacuum for 5-10 min to elute DNA from the Plate. Turn off the vacuum and ventilate the manifold slowly.
21. **Add another *60 µL Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water*** to each the center of well of the 96-well DNA Plate, let stand for 5 min. Apply maximum vacuum for 5-10 min to elute DNA from the Plate. Turn off the vacuum and ventilate the manifold slowly.
22. Remove the Collection Plate containing eluted DNA and sealwith film. Store sample at -20 oC.

Note: When maximum yield and concentration is required, use the optional elution method

Centrifugation Protocol

Materials Supplied By User:

- ☼ **Centrifuge with swinging-bucket rotor at room temperature capable of 3000 x g (such as Eppendorf 5810 with MTP rotor)**
- ☼ **Adapter for 96-well Collection Plate**
- ☼ **Deep well culture Plates for Yeast culture**
- ☼ **Absolute (96%-100%) ethanol**
- ☼ **Vacuum oven or incubator preset to 70 ℃**
- ☼ **Isopropanol**
- ☼ **Sealing film**

1. Inoculate ***5 mL YDP*** medium placed in a 10-20 mL culture tube with yeast carrying desired plasmid and grow at 30 °C with agitation for 16-24 h.
2. Pellet ***1-3 mL yeast culture*** (use < 2 x 10^7 cells) in a 2.2 mL deep-well Plate by centrifugation at 4,000 × g for 10 min at room temperature.
3. Discard medium and resuspend yeast cells in ***480 µL Buffer SE/2-mercaptoethanol*** *and* ***20 µL lyticase solution***. Resuspend the pellet by vortexing at maxi speed for 2-3 minute. Complete resuspension of cell pellet is vital of obtaining good yields. Incubate at 30 °C for at least 30 min.

   Note: Remember to add 10 µL of 2-mercaptoethanol per 1 mL of Buffer SE before use. This mixture can be made and stored at room temperature for one week.
4. Pellet spheroblasts by centrifuging at 4,000 x g for 5 min at room temperature. Discard the supernatant completely. Resuspend the spheroblasts pellet with 250 µ L Buffer YP I by pipetting or vortexing the plate.

Ensure that RNase A has been added into Buffer YP I before use.

Note: No cell pellet clumps should be observed after resuspension.

5. Optional: Add ***50 mg glass beads*** to each well, seal the plate with the film, and vortex at max speed for 5 min. Let stand to allow the beads to settle. Transfer the supernatant to a new 2.2 mL deep-well plate (not supplied).

   Note: This beads beating step is to help the completely removal of the yeast cell wall. This step can be skipped if the cell wall can be efficiently removed by lyticase digestion.

6. **Add *250 µL Buffer YP II* and gently mix by rotating plate 5-10 times till a homogeneously colored suspension is achieved**. Incubation at room temperature for 3-5 min. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity.

   Note: Continue mixing the solution if brownish cell clumps are still visible. This is critical for optimized yield.

7. **Add *350 µL Buffer YP III* to each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by gently inverting the plate for 5-6 times until a flocculent white precipitate forms. Let the plate stand by for 10 min at room temperature.

8. Place the ***96 well Lysate Clearance Plate*** onto a new 2.2 mL Deep Well Collection Plate (supplied). Transfer the entire cell lysate into the 96-WELL Lysate Clarence Plate. Centrifuge at 3000 x g for 5 min at room temperature. **Discard the 96-Well Lysate Clearance Plate.**

9. Place a new ***96-well DNA Plate*** on top of a 96-well deep well Plate (e.g., 2.0 mL deep well Plate supplied with kit) and transfer the entire lysate from previous step into 96-well DNA Plate.

10. Centrifuge the plate at 3,000 x g for 5 min. Discard the flow-through liquid and re-use the deep well Collection Plate for next step.

11. **Add *800 µL DNA Wash Buffer* to each well**. Centrifuge at 3000 x g for 15 min. Discard the flow-through and re-use the Collection Plate for next step.
    Ensure that the 100% ethanol has been added to DNA Wash Buffer according to instructions before use.

12. Remove the 96-well DNA Plate, tap the Plate on a stack of absorbent paper until no drops come out, clean the nozzles of the DNA Plate with clean absorbent paper

13. **Elution:** Place the 96-well DNA Plate on top of a ***clean 300µL Collection Plate (supplied).***

14. **Add *50 µL Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile water* to each well of the 96-well DNA Plate.** Let stand for 5 min.

15. Centrifuge the 96-well DNA Plate/Collection Plate at 3000 x g for 10 min.

16. Apply second ***100 µL Elution Buffer*** (10 mM Tris-HCl, pH 8.5) or sterile water to center of each well of the 96-Well DNA Plate.

17. Centrifuge the 96-well DNA Plate/Collection Plate at 3000 x g for 5 min.

18. Remove the Collection Plate containing eluted DNA and seal with film. Store sample at -20 ℃.

Alternative Elution Method

The following optional elution method can be used when higher yield and concentration are required. An additional drying step is required following elution to reduce the final volume of elute and concentrate the DNA. Start this procedure after the drying step (step 18 of the standard protocols).

1. Add ***80 µL of the Elution Buffer*** to each well of the 96-well DNA Plate. Ensure that the Elution Buffer was applied on the center of the membrane. Do not apply the Elution Buffer on the side of the well.
2. **Incubate for 5 min.**
3. Turn on the vacuum pump and apply the vacuum by switching vacuum valve to the opening position.
4. Turn off the vacuum by slowly turn the vacuum valve to the close position. Allow the vacuum manifold pressure to equalize to ambient pressure.
5. **Add *a second 80 µL Elution Buffer* (10 mM Tris-HCl, pH 8.5) or *sterile water*** to each the center of well of the 96-Well DNA Plate, let stand for 5 min. Apply maximum vacuum for 5-10 min to elute DNA from the Plate. Turn off the vacuum and ventilate the manifold slowly. Allow the vacuum manifold pressure to equalize to ambient pressure.
6. **Add *a third 80 µL Elution Buffer* (10 mM Tris-HCl, pH 8.5) or *sterile water*** to each the center of well of the 96-Well DNA Plate, let stand for 5 min. Apply maximum vacuum for 5-10 min to elute DNA from the Plate. Turn off the vacuum and ventilate the manifold slowly. Allow the vacuum manifold pressure to equalize to ambient pressure.
7. Dry the eluted DNA sample in the Collection Plate for 2-3 hours at 60 °C in a vacuum chamber. If the vacuum chamber is not accessible, the sample can be dried down in an incubate at 37 °C for overnight.

Cleaning of 2 mL 96-Well Collection Plates

The 2 mL 96-well Collection Plates are reusable. To avoid cross-contamination, rinse the Plates throughly with tap water after each user. Rinse with ***0.5 M HCl*** for 5min, then rinse throughly with distilled water. 2mL 96-well Collection Plates can also be autoclaved after washing.

Troubles Shooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff.

Problem	Possible reason	Suggestions
Low DNA yields	Poor cell lysis or cell growth Condition	Only use the growth condition outline in the protocol. If other media are used, the growth time may have to be Adjusted Cells may not have been dispersed adequately prior to the addition of Buffer YP II. Make sure to vortex cell suspension to completely disperse. Increase incubation time with YP III to obtain a clear lysate. Buffer YP II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS
	Yeast clone is overgrown or not fresh.	Do not incubate cultures for more than 30 hrs at 30° C. Storage of cultures for extended periods prior to DNA isolation is detrimental.
	Low elution efficiency	The pH of Elution Buffer or water must be ≥8.0
	Poor Yeast Plasmid replication during host cell growth	Some yeast clones do not replicate efficiently within their host cells. Even with lower yield, it is still very possible to obtain good sequencing reading.
	Elution Buffer is added improperly to the membrane	Apply the Elution Buffer to the center of the membrane. Do not apply Elution Buffer to the side of wells, which will cause poor elution.

Problem	Possible reason	Suggestions
No DNA Eluted	DNA Wash Buffer is not diluted with 96-100% ETOH	Prepare DNA Wash Buffer according to instructions
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of YP II Buffer.	Do not vortex or mix aggressively after adding Buffer YP II.
RNA visible on agarose gel	RNase A not added to Solution	Check that RNase A provided with the kit has been used. If Solution I is more than 6 months old, add more RNase A.
DNA floats out of well while loading agarose gel	Ethanol has not completely been removed from column following wash steps.	Centrifuge column as instructed to dry the column before elution.
Poor DNA sequencing	Poor cell growth condition	Proper cultivation of cell clone is essential to obtain optimal good quality of template for DNA sequencing. Use proper inoculation and growth condition to grow the bacteria clone.
	Poor sequencing primer design	Re-design the sequencing primers
	Ethanol contamination in the eluted DNA sample	Completely dry the 96-WELL DNA Plate before the elution. If the Eluted DNA contains ethanol residue from the DNA Wash Buffer, it could interfere the cycle sequencing reactions, resulting in short sequencing read length. Longer drying time (by either centrifugation or vacuum) may be necessary. Remove any liquid residue from drips and inside of each well.

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