EasyPrep™ Yeast Genomic DNA Miniprep Manual

Catalog#: GD05-01, GD05-02

Bioland

For research use only

(March 2015)

Table of Contents

Introduction

The EasyPrep™ Yeast gDNA Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 mL of log-phase culture (OD_{600} of 1.0 in YPD medium) can be processed. The system combines the reversible nucleic acid-binding properties of DNA-binding matrix with the speed and versatility of spin column technology to yield approximately 15-30 µg of DNA with an A_{260}/A_{280} ratio of 1.7-1.9. The kit will isolate all cellular DNA, including plasmid DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the EasyPrep™ Yeast gDNA Kit for the first time, please read this manual before beginning the procedure. Yeast cells are grown to log-phase and speroblasts are subsequently prepared. Following lysis, binding conditions are adjusted and the sample applied to an ezBind DNA Mini column. Two rapid wash steps remove trace salt and protein contaminants and finally DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the EasyPrep™ Yeast gDNA Kit, except the Proteinase K, RNase A and Lyticase can be stored at 22 -25 °C and are guaranteed for at least 24 months from the dated of purchase. Once reconstituted in water, Proteinase K and Lyticase must be stored at –20°C. Store RNase A at 4°C. Under cool ambient conditions, a precipitate may form in the Buffer YBL/YTL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer YTL/ YBL at room temperature.

Kit Content

Catalog#	GD05-01	GD05-02
DNA Mini Columns	50	250
Buffer YTL	20 mL	100 mL
Buffer YBL	20 mL	100 mL
Buffer KB	28 mL	135 mL
DNA Wash Buffer	12 mL	54 mL
Glass Beads	3 g	15 g
Elution Buffer	15 mL	70 mL
Buffer SE	30 mL	135 mL
PK storage Buffer	5 mL	10 mL
Lyticase (Units)	150	750
Proteinase K	30 mg	150 mg
RNase A	100 µL	500 µL
User Manual	1	1

*Buffer YBL contains a chaotropic salt.

Before Starting

1. Prepare a stock solution of Proteinase K (provided) as follows and aliquot into adequate portions. Store aliquots at -20 °C.

GD05-01	Dissolve with 1.35 mL of PK Storage Buffer
GD05-02	Dissolve with 7 mL of PK Storage Buffer

2. Prepare a lyticase stock solution at 100 unit/mL and aliquot into adequate portions. Store each aliquot at -20 °C and thaw before use. Each sample will require 28 µL of this solution.

GD05-01	Dissolve with 1.35 mL of Buffer SE
GD05-02	Dissolve with 7 mL of Buffer SE

3. Equilibrate Elution Buffer provided to 65 °C Carry out all of centrifugation step at room temperature. Dilute DNA Wash Buffer with ethanol as follows and store at room temperature:

GD05-01	Add 48mL absolute (96%-100%) ethanol
GD05-02	Add 216 mL absolute (96%-1 00%) ethanol

Yeast gDNA Kit Spin Protocol

Materials to Be Provided by User

- Tabletop microcentrifuge and nuclease-free 1.5 mL tubes
- Water bath set to 30 °C
- Shaking water bath set to 55 °C
- Incubator or waterbath set to 65 °C
- Absolute ethanol (96%-100%) - Do not use other alcohols

This method allows genomic DNA isolation from up to 3 mL yeast culture (< 2 x 10^7 cells)

1. Grow yeast culture in YPD medium to an OD_{600} of 1.0

2. Harvest no more than 3 mL culture (< 2 x 10^7) by centrifugation at 4,000 x g for 10 min at room temperature.

3. Discard medium and resuspend cells in 480 µL Buffer SE and 25 µL lyticase solution. Incubate at 30 °C for at least 30 min.

4. Pellet spheroblasts by centrifuging 10 min at 4000 x g at room temperature.

5. Resuspend cells in 200 µL Buffer YTL. Add 50 mg Glass beads and vortex for 5 min.

6. Add 25 µL Proteinase K solution and vortex to mix well. Incubate at 55 °C in a shaking water bath to complete lysis. Usually no more than 1 h is required for cell lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 min.

7. Add 5 µL RNase A to samples and invert tube several times to mix. Incubate at room temperature for 5 min. Centrifuge at 13,000 rpm for 5 min to pellet insoluble debris. Carefully aspirate the supernatant and transfer to a sterile micro-centrifuge tube leaving behind any insoluble pellet.

8. Add 220 µL Buffer YBL and vortex to mix at maxi speed for 15 s. Incubate at 65 °C for 10 min. A wispy precipitate may form upon addition of Buffer YBL; it does not interfere with DNA recovery.

9. Add 220 µL *absolute ethanol* (96-100%) and mix thoroughly by vortexing at maxi speed for 20 s. If any precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.

10. Transfer the entire sample from Step 10 into the column, including any precipitate that may have formed. Centrifuge at 13,000 rpm for 1 min to bind DNA. Discard the collection tube and flow-through.

11. Place the column into a second 2 mL tube and wash by adding 500 µL Buffer KB. Centrifuge at 13,000 rpm for 1 min. Discard flow-through and reuse the collection tube.

12. Place the column into the same collection tube and wash by adding 700 µL DNA Wash Buffer. Centrifuge at 13,000 rpm for 1 min. Discard flow-through and reuse the collection tube.

    NOTE: *DNA Wash Buffer* is supplied as a concentrate and must be diluted with absolute ethanol according to the instructions on Page 3, under "Before Starting".

13. Wash the column with another 600 µL DNA Wash Buffer and centrifuge as above. Discard flow-through and reuse the collection tube.

14. Using the same 2 mL collection tube, centrifuge DNA Mini Column, with the lid open, at 13,000 rpm for 2 min to dry the column. This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications.

15. Place the column into a nuclease-free 1.5 mL microfuge tube and add 50-100 µL of preheated (65°C) Elution Buffer to DNA Mini column matrix. Allow columns to incubate for 3 to 5 min at room temperature after addition of Elution Buffer.

    NOTE: Incubating the DNA column at 65 °C rather than at room temperature prior to centrifugation will give a modest increase in DNA yield per elution.

16. To elute DNA from the column, centrifuge at 13,000 rpm for 1 min.

17. Repeat the elution with a second 50-100 µL Elution Buffer.

    NOTE: Each 50-100 µL elution typically yields 60-70% of the DNA bound to the column. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70 °C (rather than at room temperature) upon addition of Elution Buffer. The expected yield from a 3 mL culture sample is 15-30 µg DNA depending on bacterial strain, medium, and growth phase.

Yeast gDNA Vacuum/Spin Protocol

NOTE: Please read through previous section of this manual before using this protocol.

1. Prepare samples and column by following the standard Protocol in previous section (Steps 1-11).
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.
3. Load the sample/YDL/Ethanol mixture to the column. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
4. Wash the column by adding 500 µL Buffer KB, draw the Buffer KB through the column by turning on the vacuum source.
5. Wash the column by adding 600 µL DNA Wash Buffer, draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 600 µL DNA Wash Buffer.
6. Proceed step 15-17 of Yeast gDNA Spin Protocol on page 6.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water or Tris-HCl buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg /µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity. The expected yield from a 3 mL culture sample is 15-30 µg DNA depending on yeast strain, medium, and growth phase. If DNA is eluted with ddH_2O rather than Tris-HCL buffer, store the sample at -20°C to prevent degradation.

Trouble Shooting

Problem	Cause	Possible Solution
Clogged column	Incomplete lysis	Add the correct volume of Buffer YTL and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 30 min.
	Sample too large	Do not use greater than 3 mL culture at OD_{600} 1.0 or 2 x 10^7 cell per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall	Add more lyticase or extend the incubation time. It may be necessary to increase incubation by 60 min.
Low DNA Yield	Clogged column	See above.
	Poor elution	Repeat elution or increase elution volume (see note on page 6). Incubation of column at 65°C for 5 min after addition of Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (96%-100%) ethanol
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	Incomplete mixing with Buffer YBL	Repeat the procedure, this time making sure to vortex the sample with Buffer YBL immediately and completely.
	Insufficient incubation.	Increase incubation time with Buffer YTL. Ensure that no visible cell clumps remain.
No DNA eluted	Poor cell lysis	Mix thoroughly with Buffer YBL and incubate at 70 °C prior to adding ethanol.
	Incomplete spheroblasting	Add more lyticase or extend the incubation time. It maybe necessary to increase incubation by 60 min.
	No ethanol added to Wash Buffer	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human. This product is warranted to perform as described in its labeling and in Bioland'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 Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland 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 visit our website at www.bioland-sci.com or contact us at (562)602-8882.

Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (877) 603-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

Visit our web at **www.bioland-sci.com** and learn more about Bioland products