G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Yeast-Geno-DNA-Template™

(Cat. # 786-134)

INTRODUCTION

The Yeast Geno-DNA-Template DNA Extraction Kit isolates high quality genomic DNA from yeast. On an average, the DNA isolated is 100kb in size. This kit is suitable for 100 preps (1.5 ml culture / each).

ITEM(S) SUPPLIED (Cat. # 786-134)

Description	Size
Yeast Suspension Buffer	15ml
Genomic Lysis Buffer	100ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
TE Buffer	10ml
LongLife™ Zymolyase	0.5ml
β -Mercaptoethanol	100µl
LongLife™ Proteinase K (5mg/ml)	2 x 0.5ml
LongLife™ RNase	0.5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store components at 4°C, except β-Mercaptoethanol at RT and LongLife™ enzymes at -20°C. When stored and used properly, the reagents are stable for 1 year.

ADDITIONAL ITEMS REQUIRED

- Chloroform
- Isopropyl alcohol
- 70% Ethanol
- 1.5 ml and 2 ml microfuge tubes
- 2-mercaptoethanol

PREPARATION BEFORE USE

If precipitate develops in Lysis Buffer & DNA Stripping Solution during cold storage, allow solution to warm to room temperature or until precipitate dissolves.

PROTOCOL

NOTE: *Unless otherwise indicated, all steps should be carried out at room temperature.*

1. Grow yeast cells at 30°C in YPD broth or selective medium. Aliquot 1.5ml of the fully-grown yeast cells into 1.5-2ml microfuge tubes.
2. Spin down the cells at 1,000xg for 2 minutes. Discard the supernatant.
3. Centrifuge again for 10 seconds and remove and discard any remaining supernatant.
4. Add 150µl Yeast Suspension Buffer to each pellet.
5. Briefly vortex the LongLife™ Zymolyase® and add 5µl LongLife™ Zymolyase® to each pellet.
6. Add 1µl β-mercaptoethanol to each tube.
7. Resuspend the pellet by gently vortexing the tube.
8. Incubate at 37°C for 30-60 minutes, with periodic mixing.
9. Centrifuge the tube for 5 minutes at 1,000xg for 2 minutes. Remove and discard the supernatant.
10. Add 50µl pure water into the tube and gently vortexing to suspend the pellet.
11. Add 400µl Lysis Buffer and mix by inverting the tube several times. Do NOT vortex the tube.
12. Mix the provided LongLife™ Proteinase K solution and add 5µl to the sample. Incubate at 60°C for 1-3h. During incubation, invert the tube several times.
    NOTE: *Any solid mass or clumps dissolves during incubation. If solid or clumps remain at the end of incubation the DNA yield will decrease.*
13. Allow the sample to cool to room temperature. Add 200µl chloroform to each sample and mix by inverting the tube several times.
14. Centrifuge the samples for 5 minutes in a microcentrifuge, or until a tight pellet is formed. Carefully, with a wide bore pipette, remove the upper phase and transfer to a clean tube.
15. Add 60µl DNA Stripping Solution to the sample and invert several times to mix.
16. Incubate the sample for 5-10 minutes at 60°C.
17. Allow the sample to cool to room temperature then add 120µl Precipitation Solution. Mix by inverting the tube several times.
    NOTE: *The sample should become cloudy or milky with precipitate. If precipitate dissolves or a massive precipitation is not observed, add additional volume (30-60µl) of Precipitation Solution to encourage precipitation or until massive precipitation is observed.*
18. Centrifuge for 5-6 minutes at 14,000g. Transfer the supernatant to a clean tube.
19. Add 700µl isopropanol (or a volume equal to total volume) to the sample. Invert 10-20 times to precipitate the DNA.
20. Centrifuge at 14,000g for 5 minutes to pellet DNA. A white, almost translucent pellet should be present at the bottom of the tube. Decant or pipette off the ethanol.

21. Add 1ml 70% ethanol to the tube and invert several times to wash the DNA pellet. Centrifuge for 40-60 seconds at 14,000g to pellet the DNA.
    NOTE: *In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.*
22. Decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not allow the pellet to dry completely or the pellet will be difficult to re-hydrate.
23. Add 50-100µl TE buffer to the pellet.
    OPTIONAL: *Add 1µl LongLife™ RNase*.
24. Let the DNA rehydrate for at least 15 minutes. Some samples may take several hours to overnight for full hydration. Increasing the volume of TE Buffer and/or incubation at 37-50°C will aid DNA hydration.

TYPICAL YIELD

400-600µg DNA/ 10^9 cells (*Pichia pastoris*)

APPLICATION NOTES

1. If DNA recovery and quality is poor, increase the incubation time in Steps 8 & 12. Make sure there is no clumps or solid pellet mass at the end of Proteinase K treatment.
2. For isolation of >100 kilobases genomic DNA, we recommend using MegaLong™ DNA isolation kit (Cat # 786-146, 786-147). The kit involves isolation of nuclei under mild conditions. The isolated nuclei are transferred into a Tube-O-DIALYZER™, where the nuclei are digested with protease followed by dialysis to remove protein and other contaminants.

RELATED PRODUCTS

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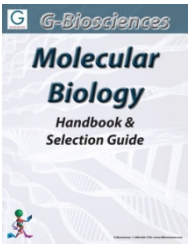

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