

For Research Use

TAKARA

**Dr. GenTLE™ (from Yeast)
High Recovery**

Product Manual

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I. Description

Dr. GenTLE (from Yeast) High Recovery is designed to efficiently extract and purify genomic DNA from yeast cells by using both a cell wall digestive enzyme and salting-out for DNA purification. The procedure for genomic DNA purification using this kit involves recovery of yeast cells by centrifugation, digestion of the yeast cell wall using GenTLE Yeast Solution A (which contains the cell wall lytic enzyme Zymolyase-100T), cell lysis using GenTLE Yeast Solution B, salting-out proteins by adding GenTLE Yeast Solution C and centrifuging, and DNA recovery by isopropanol precipitation. This kit may be used to isolate genomic DNA for common molecular biology applications, such as Southern blotting, PCR, or restriction enzyme treatment, from the various species of yeast listed in rows A and B of Table 1 below. For a standard experiment, 4 - 10 μ g of genomic DNA (35 - 200 kb) can be obtained from 1 - 2 x 10⁸ yeast cells.

Table 1. Yeast useful in this kit.

A	Yeast from which DNA can be prepared	<i>Ashbya, Candida, Debaryomyces, Endomyces, Eremothecium, Hanseniaspora, Hansenula, Kloeckera, Kluyveromyces, Lipomyces, Metschikowia, Pullularia, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schizosaccharomyces, Selenozyma, Trigonopsis, Wickerhamia</i>
B	Yeast from which DNA can be prepared (strain-dependent)	<i>Bretanomyces, Cryptococcus, Nadsonia, Pichia, Rodosporidium, Schwanniomyces, Stephnoascus, Torulopsis</i>
C	Yeast from which DNA cannot be prepared	<i>Bullera, Pityrosporum, Rhosotorula, Sporidiobolus, Sporobolomyces, Stetigmatomyces, Trichosporon</i>

II. Components (For 30 reactions)

- | | |
|---|-------|
| 1. GenTLE Yeast Solution A* ^{1, 2} | 15 ml |
| 2. GenTLE Yeast Solution B* ³ | 3 ml |
| 3. GenTLE Yeast Solution C | 6 ml |
| 4. TE Buffer | 6 ml |

*1 GenTLE Yeast Solution A contains an enzyme. Avoid vigorous mixing or other manipulations which may inactivate the enzyme.

2 It has been reported that Zymolyase-100T, the yeast cell wall digestive enzyme in GenTLE Yeast Solution A, contains a small amount of fungal DNA.
* *Jpn J Med Mycol.* (2009) **50**: No. 4. 259-262.

*3 If GenTLE Yeast Solution B contains any precipitates, heat the solution to 37°C to dissolve them before use.

III. Materials Required but Not Provided

- Centrifuge
- Isopropanol
- 70% ethanol
- Tubes
- Thermostatic bath (for incubation at 37°C)
- Heating block (for heating at 70°C)

IV. Storage

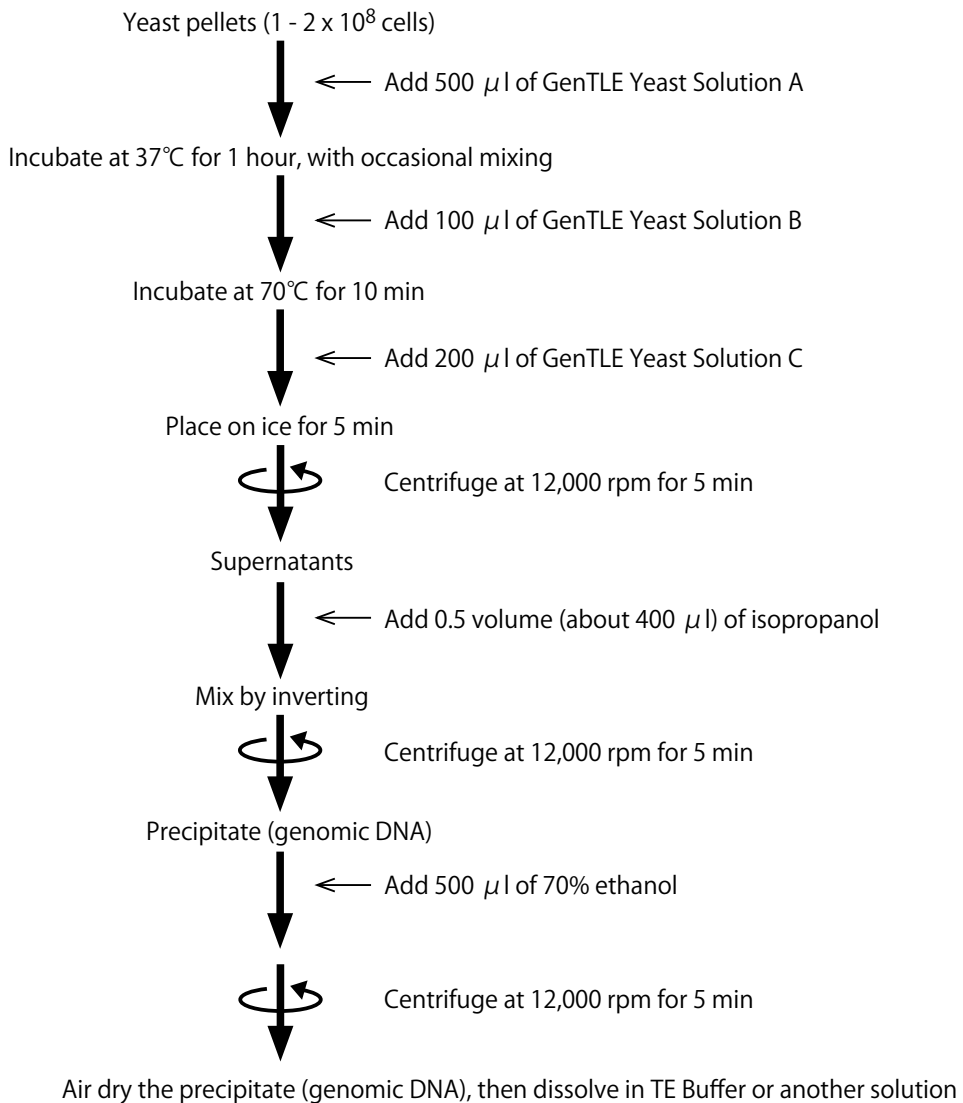
4°C

Only GenTLE Yeast Solution B can be stored at room temperature. If it is stored at a lower temperature, precipitates may appear, which should be dissolved completely by heating at 37°C before use.

V. Protocol

1. Add culture medium containing $1 - 2 \times 10^8$ yeast cells to a microcentrifuge tube, and centrifuge at 12,000 rpm for 1 minute.
2. Thoroughly remove the supernatant using a micropipette.
3. Add 500 μ l of GenTLE Yeast Solution A to the yeast pellet. Resuspend the cells by vortexing and incubate for 1 hour at 37°C, with occasional mixing.
4. Add 100 μ l of GenTLE Yeast Solution B. Mix gently by vortexing and heat at 70°C for 10 minutes.
5. Add 200 μ l of GenTLE Yeast Solution C. Mix gently by vortexing and place the tube on ice for 5 minutes.
6. Centrifuge at 12,000 rpm for 5 minutes at 4°C.
7. Transfer the supernatant to a new microcentrifuge tube, being careful to avoid picking up any of the precipitate. Add half the supernatant volume (about 400 μ l) of isopropanol, and mix well by inverting the tube several times.
8. Centrifuge at 12,000 rpm for 5 minutes at 4°C and discard the supernatant.
9. Wash the DNA pellet with 500 μ l of cold 70% ethanol and centrifuge at 12,000 rpm for 5 minutes at 4°C.
10. Thoroughly remove the supernatant using a micropipette and air dry the DNA pellet.
11. Resuspend the DNA pellet in a volume of TE Buffer (or other solution) that is appropriate for your experiment.

VI. Protocol Overview



VII. Experimental Example

Genomic DNA preparation from *Saccharomyces cerevisiae* (Y187 strain, AH109 strain)

[Method]

Yeast pellets were collected from 1, 2, or 3 ml of *S. cerevisiae* (Y187, AH109) culture suspensions (YPD medium, OD₆₀₀~3) by centrifugation. Genomic DNA was prepared according to the kit protocol.

[Result]

Genomic DNA with an OD₂₆₀/OD₂₈₀>1.6 was efficiently prepared in amounts that were proportional to the starting amount of yeast.

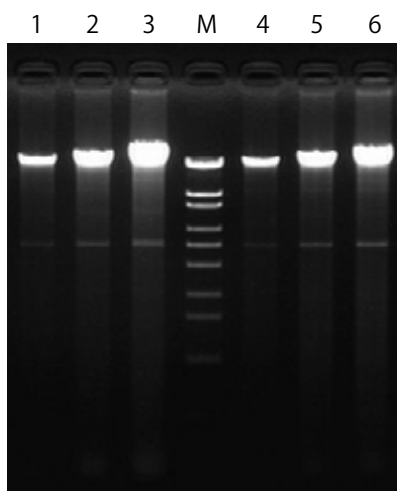


Figure 1. Genomic DNA extracted from *S. cerevisiae* (Y187, AH109)

1/20 volume of the recovered DNA was analyzed using agarose gel electrophoresis.

M: λ -*Eco*T14 I digest 50 ng

Lanes 1, 2, 3: 1, 2, or 3 ml of Y187 culture

Lanes 4, 5, 6: 1, 2, or 3 ml of AH109 culture

VIII. Troubleshooting

1. Low yield or no yield of DNA

- Starting amount of yeast is too high
When more than 2×10^8 yeast cells are used, the cell wall is not sufficiently degraded by GenTLE Yeast Solution A, and the amount of DNA isolated may decrease. If it is necessary to prepare DNA using more than 2×10^8 yeast cells, scale up the procedure in "V. Protocol" in proportion to the starting amount of yeast.
- Starting amount of yeast is too low
When genomic DNA is prepared from significantly fewer than 1×10^8 yeast cells, less DNA will be recovered relative to the starting amount of yeast. To purify consistent amounts of DNA, prepare DNA using the recommended amounts of yeast cells.
- DNA is prepared from yeast which is not suited for this kit
This kit extracts genomic DNA by dissolving the yeast cell wall with an enzyme contained in GenTLE Yeast Solution A. Therefore, if DNA is prepared from a yeast strain whose cell wall cannot be dissolved with this enzyme, the amount of DNA isolated may be significantly lower. Check the above table to determine if your yeast strain is suitable for preparing DNA using this kit.
- DNA pellet is not completely dissolved
If air-dried DNA is difficult to dissolve after isopropanol precipitation, add TE Buffer and dissolve gently by mixing overnight at room temperature, or by heating for 1 hour at 65°C.

2. Isolated DNA contains RNA

In general, RNA can be removed by performing this protocol. However, there are cases in which RNA cannot be removed completely, depending on the type of yeast used, or the culture conditions. In such cases, perform RNase treatment as necessary.

IX. Related Product

NucleoSpin Tissue (Cat. #740952.50/.250)

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