CYDN20-CHANGYU Yeast Genomic DNA Mini Kit (Spin Column)

Catalog number	Package
CYDN2001	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Lyticase and proteinase K should be stored at -20 °C.

Description

This kit combines a DNA spin column with a unique solution system and is suitable for rapid and simple extraction of genomic DNA from yeast cultures from various sources. About 3 ml of yeast culture in exponential growth phase typically yields 10-15 μg of high-quality genomic DNA in a single extraction. The purified DNA can be used directly for PCR, restriction digestion, hybridization and similar experiments. After yeast cells are treated with Lyticase to remove the cell wall, a unique Binding Buffer/proteinase K combination rapidly lyses the cells and inactivates cellular nucleases. Genomic DNA then binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. A series of rapid wash and centrifugation steps with Inhibitor Removal Buffer IR and Wash Buffer WB removes cell metabolites, proteins and other impurities, and purified genomic DNA is eluted from the membrane with low-salt Elution Buffer EB.

Kit components

Component	Storage	50 preps (CYDN2001)
Buffer YB	15-25 °C	20 ml
Binding Buffer CB	15-25 °C	11 ml
Inhibitor Removal Buffer IR	15-25 °C	25 ml
Wash Buffer WB	15-25 °C	15 ml
Elution Buffer EB	15-25 °C	15 ml
Lyticase 10 U/μl	-20 °C	2500 U
Proteinase K powder (optional) 20 mg/ml	-20 °C	20 mg
Spin Column AC	15-25 °C	50
Collection Tube (2 ml)	15-25 °C	50

Before first use, add 60 ml absolute ethanol to the 15 ml Wash Buffer WB, mix thoroughly and tick the box on the label to mark that ethanol has been added. The kit is stable for 12 months at room temperature.

Storage notes

Binding Buffer CB or Inhibitor Removal Buffer IR may precipitate at low temperature. Warm in a 37 °C water bath for a few minutes to redissolve; after the buffer becomes clear, cool to room temperature before use.

Proteinase K is supplied as a lyophilized powder (20 mg) to preserve activity and facilitate shipping. After a brief centrifugation, dissolve the powder in 1 ml sterile water to prepare a 20 mg/ml solution. Repeated freeze-thaw cycles may reduce activity; aliquot immediately after dissolution and store at -20 °C.

Lyticase (2500 U) is supplied as a lyophilized powder to preserve activity and facilitate shipping. After a brief centrifugation, dissolve the powder in 0.25 ml sterile water to prepare a 10 U/μl solution. Repeated freeze-thaw cycles may reduce activity; aliquot immediately after dissolution and store at -20 °C.

Avoid prolonged exposure of reagents to air, which causes evaporation, oxidation and pH changes. Keep all solutions tightly capped after use.

Features

The silica membrane in the spin columns is a specially manufactured adsorption membrane; adsorption varies very little from column to column and reproducibility is good.

No toxic reagents such as phenol are required, and no ethanol precipitation step is needed.

Fast and simple: after sample lysis, a single sample is usually processed within 30 minutes.

Repeated column washes ensure high purity. Typical OD260/OD280 is 1.7-1.9; the DNA is suitable for PCR, Southern-blot and restriction digestion.

Notes before starting

Note: All centrifugation steps are carried out at room temperature in a conventional benchtop centrifuge reaching 13,000 rpm, such as an Eppendorf 5415C or equivalent.

Note: The user must supply ethanol, isopropanol and β-mercaptoethanol.

Note: Preheat the required water baths to 37 °C and 70 °C before starting.

Note: Binding Buffer CB and Inhibitor Removal Buffer IR contain irritants. Wear latex gloves and avoid contact with skin, eyes and clothing. If skin, eyes or clothing are contaminated, rinse with plenty of water or saline.

Note: The user must supply Sorbitol buffer (1 M sorbitol, 0.1 M Na2EDTA, 14 mM β-mercaptoethanol). Preparation: dissolve 182.2 g sorbitol in 600 ml deionized water, add 200 ml 0.5 M Na2EDTA (pH 8.0), do not adjust the pH, make up to 1 L and store at 4 °C. Add 0.1% β-mercaptoethanol immediately before use (commercial β-mercaptoethanol is generally about 14 M).

Note: At OD600 = 1, a Saccharomyces cerevisiae culture contains about 1-2 × 10^7 cells/ml. Because strains and spectrophotometers differ, the OD value for the same cell number may vary considerably; this value is for reference only.

Note: Elution Buffer EB contains no EDTA and does not interfere with downstream digestion or ligation. Water may also be used for elution if its pH is above 7.5; a low pH reduces elution efficiency, and DNA eluted with water should be stored at -20 °C. For long-term storage, DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), but EDTA may affect downstream restriction digestion, so the eluate may need to be diluted before use.

❖ Procedure

Read the notes before starting. Before first use, add 60 ml absolute ethanol to the 15 ml Wash Buffer WB, mix thoroughly and tick the box on the label.

1. Take 1-3 ml yeast culture (no more than 3 × 10^7 cells, preferably in early logarithmic phase), centrifuge at 12,000 rpm for 30 seconds and aspirate the supernatant as completely as possible to collect the cells.

2. If more than 1.5 ml of culture is to be collected, centrifuge, discard the supernatant, add more culture to the same 1.5 ml tube and repeat step 1 until enough cells have been collected.

3. Add 600 µl Sorbitol buffer and resuspend the cells gently by pipetting. Add Lyticase at 20-50 U per 1 × 10^7 cells (3 ml of culture may require up to 150 U), mix thoroughly by inversion and incubate at 37 °C for at least 30 minutes to digest the cell wall, inverting several times during incubation to help digestion.

Note: If cell wall digestion is poor and DNA yield is low, increase the amount of Lyticase to raise the working concentration, or extend the digestion time. For yeasts that are not suitable for Lyticase, use Zymolyase or another method such as glass-bead vortexing, boiling or repeated freeze-thaw.

4. Centrifuge at 13,000 rpm for 1 minute, aspirate the supernatant as completely as possible and resuspend the cell pellet in 180 µl Buffer YB.

5. Add 20 µl proteinase K solution (20 mg/ml) and vortex immediately and thoroughly.

6. Place the mixture in a 55 °C water bath and digest until complete, shaking gently several times to help lysis.

Note: The digestion time depends on the number, species and growth state of the yeast; generally 15 minutes is sufficient, but digestion overnight is also acceptable if convenient.

Optional step (generally not required): if residual RNA must be removed, add 20 µl RNase A (25 mg/ml) after step 5, vortex to mix and leave at room temperature for 5-10 minutes.

7. Add 200 µl Binding Buffer CB, vortex immediately and mix thoroughly, and place at 70 °C for 10 minutes.

8. After cooling, add 100 µl isopropanol and vortex immediately and thoroughly; a flocculent precipitate may appear.

9. Load the mixture from the previous step, including any precipitate, onto Spin Column AC placed in a collection tube. Centrifuge at 13,000 rpm for 30-60 seconds and discard the flow-through.

10. Add 500 µl Inhibitor Removal Buffer IR, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

11. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

12. Add 600 µl Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

13. Return Spin Column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

14. Place Spin Column AC in a clean centrifuge tube. Apply 100 µl Elution Buffer EB (prewarmed in a 65-70 °C water bath for best results) to the centre of the membrane, leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency. For a higher DNA concentration the elution volume may be reduced, but it should not be less than 50 µl; too small a volume reduces elution efficiency and DNA yield.

Store the DNA at 2-8 °C; for long-term storage, keep at -20 °C.

Troubleshooting

Problem	Comments and suggestions
Low DNA yield	Some yeast species are difficult to lyse - read step 2 carefully and confirm that the yeast species can be lysed with Lyticase; other lysis methods such as Zymolyase, glass-bead vortexing, boiling or repeated freeze-thaw may be considered. Lyticase should preferably be aliquoted and frozen according to the amount used to ensure activity, and yeast in early logarithmic phase should be used. Inactive proteinase K - aliquot and freeze according to the amount used, avoid repeated freeze-thaw and extend the treatment time. Incomplete lysis or incomplete mixing with isopropanol - pipette or vortex immediately after adding Binding Buffer and proteinase K, and immediately after adding isopropanol before loading the column; if the lysate is very viscous, vortex for 15 seconds.
DNA degraded	Nuclease activity in the sample caused degradation - store samples properly at -20 °C before processing and do not overload the sample.

No DNA obtained	Absolute ethanol was forgotten in Wash Buffer WB - add the indicated volume of absolute ethanol to WB before the first experiment.
Low DNA yield in the eluate	Residual ethanol in the spin column or ethanol at the bottom of the tube - make sure step 12 was performed, otherwise residual ethanol reduces elution efficiency. Water or another non-optimal liquid was used instead of Elution Buffer - read note 7 and step 13 carefully and elute only with Elution Buffer EB.
Abnormally high A260	Silica membrane components co-eluted and interfered with absorbance - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant.
DNA cannot be cut or is only partially digested by restriction enzymes	Silica membrane components co-eluted and inhibited digestion - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant. Residual ethanol in the spin column or at the bottom of the tube inhibited digestion - make sure step 12 was performed, then air-dry for a few minutes to let residual ethanol evaporate.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.