FISHER MOLECULAR BIOLOGY

FUNGI /YEAST GENOMIC DNA EXTRACTION MINI KIT DE-046

Bashing Beads

The Fungi/Yeast Genomic DNA Extraction Mini Kit is designed for efficient extraction of high quality DNA from fungi ,yeast , and other samples through the technology based on the spin columns and bashing beads.

CAT.N°	DE-046s 4-preps-sample	DE-046 50 preps	DE-046-1 100 preps
Bashing Beads Tube	4 pcs	50 pcs	100 pcs
FA Buffer	5 ml	60 ml	120 ml
FB Buffer	2,7 ml	32 ml	65 ml
TG1 Buffer	2 ml	22 ml	45 ml
TG2 Buffer	2 ml	15 ml	30 ml
Wash Buffer 1[a] (concentrate)	1.3 ml	22 ml	44 ml
Wash Buffer 2[b] (concentrate)	1 ml	10 ml	20 ml
Elution Buffer	0.5 ml	7 ml	15 ml
Lyticase solution	250 ul	550 µl x 5	550 µl x 10
Proteinase K (liquid)	100 µl	1050 µl	1050 µl x 2
TG Mini Column	4 pcs	10 pcs x 5	10 pcs x10
Collection Tube	8 pcs	100 pcs	100 pcs x 2
Elution Tube	4 pcs	50 pcs	100 pcs

Preparation of W1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%) and **Store at RT.**			
Ethanol volume for Wash Buffer 1 [a]	0.5 ml	8 ml	16 ml
Ethanol volume for Wash Buffer 2 [b]	4 ml	40 ml	80 ml

Specifications

Technology: mini spin column (silica matrix) - including Bashing Beads
Sample size:1~ 5 x10^7 cell culture fungal/ yeast cells - up to 50 mg fungi/yeast
Operation time:~ 60 minutes
Binding capacity:60 µg/ column
Column applicability: Vacuum/ Centrifugation

Typical DNA yield from Yeast /filamentous fungus

Saccaromyces Cerevisiae	2-8 µg / 5x10^7 cells
Auricularia auricula-judae	0.5-2 µg/ 50 mg (wet weight
Fusarium	1-2 µg/ 50 mg (wet weight)

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Store the Lyticase can be shipped at room temperature , for long term store the solution at -20 °C on arrival.
3. Caution: Lyticase solution and FB Buffer containing 14 mM of ß-mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical fume hood.
4. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer 2 when first open. Store the solution at room temperature.
5. Prepare a heating block or a water bath to 37 °C for step 4 and to 55 °C for step 9 before operation

Additional Requirements:

1. Microcentrifuge capable of speed at ~18,000 x g
2. 1.5 ml microcentrifuge tube
3. 96~100 % ethanol
4. Vortex
5. Heating block or water bath

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General Protocol:
Please Read Important Notes Before Starting Following Steps.

1. Transfer 1~ 5 x10^6 of cultures (fungal/ yeast cells) to a 1.5 ml microcentrifuge tube. (not provided)
2. Add 1 ml of **FA Buffer** to the cells and resuspend the cells by pipetting.
3. Descend the cells by centrifuging at 5,000 x g for 2 min and discard the supernatant completely.
4. Resuspend the cells in **550 µl of FB buffer and add 50 µl of lyticase solution, mix well by vortexing**. Incubat the sample at 37 °C for 30 min.
   Caution: Lyticase solution and FB Buffer containing 14 mM of ß-mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical fume hood.
5. **(Optional)** If RNA-free genomic DNA is required, add 8 µl of 50 mg/ml **RNase A** (not provided) and incubate for 2 min at room temperature.
6. Descend the cells by centrifuging at 5,000 x g for 10 min. Remove the supernatant completely.
7. Add 450 µl **TG1 Buffer** and mix well by pipetting. Transfer the sample mixture into a **bashing bead tube**. (provided)
8. **Mix well by Plus-vortexing for 5 minutes.**
   Extend the pulse-vortexing time to 15-30 min if sample cells are hard to be broken.
9. Add 20 µl of **Proteinase K** (10 mg/ml) **and mix well by vortexing.** Incubate at 55 °C for 15 min**,** vortex 30 seconds for every 5 minutes incubation.
10. Centrifuge the sample mixture at 5,000 x g for 1 min and transfer 200 µl of supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
11. Add 200 µl of **TG2 Buffer** and mix well by pipetting.
12. Add 200 µl of **ethanol** (96-100%) and mix well by pulse-vortexing for 10 seconds.
13. Place a **TG Mini Column** in **Collection Tube**. Transfer the sample mixture (including any precipitate) carefully to **TG Mini Column**. Centrifuge at 11,000 x g for 30 second **then place the TG Mini Column to a new Collection Tube.**
14. Add 400 µl of **Wash Buffe**r **1** to the **TG Mini Column.** Centrifuge at 11,000 x g for 30 seconds and discard the flow-through. Place the TG Mini Column back to the Collection Tube.
    --Make sure ethanol has been added into Wash Buffer1 when first use.
15. .Add 750 µl of **Wash Buffer 2** to the **TG Mini Column.** Centrifuge at 11,000 x g for 30 seconds and discard the flow-through. Place the TG Mini Column back to the Collection Tube.
    --Make sure ethanol has been added into Wash Buffer 2 when first use.
16. Centrifuge at full speed (~ 18,000 x g) for an additional 3 min to dry the column.
    Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
17. Place the **TG Mini Column** to **Elution Tube**.
18. Add 50 ~100 µl of **Elution Buffer or ddH$_2$O** to the membrane center of the **TG Mini Column**. Stand **TG Mini Column** for 3 min.
    Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
19. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute total DNA.
20. Store total DNA at 4°C or -20°C.

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