Technical Note: TNCFGLYNXMITOCH 1012

Isolation of Mitochondria Using the Thermo Scientific Sorvall LYNX 6000 Superspeed Centrifuge

Key Words
Mitochondria Isolation, Discontinuous Sucrose Gradient, Large Volume Pelleting, *Saccharomyces cerevisiae*, Superspeed Centrifuges, Carbon Fiber Rotors

Introduction

Mitochondrial isolations are required for studies of a wide range of biological questions. The purity of the mitochondrial preparation is extremely important when studying the functional assembly of mitochondrial DNA. This procedure describes the isolation of mitochondria from large volume cultures of *Saccharomyces cerevisiae* using the Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge. In addition, this procedure can be readily adapted to other cell types, including bacteria, insects, or mammalian cells.

The following protocols were developed based on References 1–4.

Procedures

STEP 1: Pelleting the Mitochondria

1. Grow overnight a pre-culture of *Saccharomyces cerevisiae* at 30 °C in Difco YM.
2. Transfer 8 mL of pre-culture into four flasks containing 2 L of Difco YM and grow at 30 °C overnight to OD_{600}.
3. Place the culture into Thermo Scientific Nalgene 1 L superspeed bottles. Perform centrifugation in a Thermo Scientific Fiberlite F9-6x1000 LEX or F10-4x1000 LEX rotor and a Sorvall® LYNX 6000 superspeed centrifuge with the following parameters: 2,000 x g for 15 min at 4 °C.
4. Wash the pellet with ice-cold 18 ΩM H_20 and perform centrifugation with the following parameters: 2,000 x g for 15 min at 4 °C.
5. Re-suspend the pelleted cells in 0.1 M Tris-HCl, 1% 2-mercaptoethanol (pH 9.3), and incubate at 32 °C for 10 min.
6. Re-pellet cells using 0.01 M Tris- HCl, 0.5 M KCl (pH 7.0).

Figure 1. Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge with Fiberlite F9-6x1000 LEX rotor.

7. Re-suspend the cells in 0.01 M citrate-phosphate buffer (pH 5.8), 1 mM EDTA, 1.35 M sorbitol.
8. Add 1 mg/mL Zymolyase 20T (ICN Biomedicals) and gently shake for 90 min at 32 °C in N_2 atmosphere to digest the cell wall for spheroplast release.
9. Pellet the spheroplasts by performing centrifugation in the Thermo Scientific Fiberlite F20-12x50 LEX rotor and the Sorvall LYNX 6000 superspeeed centrifuge with the following parameters: 3,000 x g for 10 min at 4 °C.

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Figure 2. The Thermo Scientific Fiberlite F20-12x50 LEX rotor.

10. Discard the supernatant and suspend the pellet in 40 mL of 0.01 M Tris-maleate, 0.75 sorbitol, 0.4 M mannitol 2 mM EDTA, 0.1% BSA, pH 6.8.
11. Perform centrifugation with the following parameters: 3,000 x g for 10 min at 4 °C. Repeat 1X.
12. Re-suspend the final pellet in 80 mL of 0.01 M Tris-maleate, 0.6 M mannitol, 2 mM EDTA, 0.2% BSA, pH 6.8, at 4 °C and mix thoroughly with a glass rod.
13. Perform centrifugation with the following parameters: 1,000 x g for 10 min at 4 °C. Collect the supernatant into clean tubes.
14. Repeat steps 10-12 and combine supernatants.
15. Perform centrifugation with the following parameters: 17,000 x g for 10 min at 4 °C. Collect the mitochondrial pellet.

STEP 2: Sucrose Gradient Purification of Mitochondria

Perform the entire purification at 4 °C.

1. Prepare a discontinuous sucrose gradient (30% w/w, 40% w/w, 50% w/w, 60% w/w), using cold sucrose solutions containing 1 mM EDTA, 0.1% BSA, 10 mM Tris-HCl (pH 7.5)[5].
2. Re-suspend a mitochondrial pellet in ~ 0.5 mL X number of centrifuge of 0.5 M sucrose.
3. Gently layer the mitochondrial suspension on the sucrose gradient and perform centrifugation in the Fiberlite F20-12x50 LEX rotor with the following parameters: 51,000 x g for 2.5 hr at 4 °C.

   Note: The intact mitochondria form a brown band at 1.19 g/mL sucrose density (approximately the center of the tube). One or more impurity bands may be seen below the mitochondria band.
4. Remove the mitochondria band and dilute it with 2 volumes of 1 mM EDTA, 10 mM Tris HCl (pH 7.4). Pellet the mitochondria by performing centrifugation with the following parameters: 26,000 x g for 10 min at 4 °C.
5. Collect the mitochondrial pellet and re-suspend in pH 7.5 buffer. Add 1% SDS and 50 mg/mL proteinase K. Incubate for 3 to 3.5 h at 37 °C with gently shaking. Solution can be stored for use in further studies.

Conclusion

This technical note describes a process for the purification of mitochondria from yeast cells using reliable Thermo Scientific Fiberlite carbon fiber rotors with certified biocontainment and dependable leakproof Thermo Scientific Nalgene large volume superspeed bottles. This combination, along with the Thermo Scientific Sorvall LYNX superspeed centrifuge, offers the total solution for efficient and safe processing of all your samples. This protocol can be adapted to retrieve mitochondria from other cell types such as bacterial, mammalian, or insect cells.

References

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