

Materials List for:

# Isolation of mRNAs Associated with Yeast Mitochondria to Study Mechanisms of Localized Translation

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## Materials

Name	Company	Catalog Number	Comments
Yeast Extract			
BactoPeptone			
Galactose			Do not autoclave Galactose
Growth medium			For mitochondrial enrichment, you should use any nonfermentable carbon source, such as Galactose-based growth medium sterilized 1% Yeast Extract, 2% BactoPeptone, 2% Galactose
0.1 M Tris-HCl, pH 9.4			
30 mM Tris-HCl, pH 7.6			
Dithiothreitol (DTT)			
10 mM DTT Buffer			0.1 M TrisHCl pH 9.4, 10 mM Dithiothreitol (DTT). Make fresh every time
1.2 M Sorbitol			
4 mM KH <sub>2</sub> PO <sub>4</sub>			
16 mM K <sub>2</sub> HPO <sub>4</sub>			
0.2 µm filter			
Zymolyase Buffer			1.2 M Sorbitol, 4 mM KH <sub>2</sub> PO <sub>4</sub> , 16 mM K <sub>2</sub> HPO <sub>4</sub> . Filter this buffer (0.2 µm) and keep at room temperature for future use
Zymolyase 20T			20,000 U/g
Recovery medium			Galactose-based growth medium supplemented with 1 M Sorbitol
0.1 mg/ml Cycloheximide (CHX)			
0.6 M Mannitol			
5 mM MgAc			
100 mM KCl			
0.5 mg/ml Heparin			
1 mM Phenylmethanesulfonylfluoride (PMSF)			
Mannitol Buffer			0.6 M Mannitol, 30 mM Tris-HCl pH7.6, 5 mM MgAc, 100 mM KCl. Add freshly: 0.5 mg/ml Heparin, 0.1 mg/ml CHX and 1mM (PMSF). Filter this buffer and use it ice-cold

8 M Guanidinium-HCl			
100% and 70% Ethanol (EtOH)			
3 M Sodium Acetate, pH 5.2			
10 M LiCl stock solution			
250 mM Tris HCl pH 6.8			
SDS			
Glycerol			
$\beta$ -Mercaptoethanol			
Bromophenol blue			
4x LSB			250 mM Tris HCl pH 6.8, 8% SDS, 40% Glycerol, 20% $\beta$ -mercaptoethanol, and 0.02% Bromophenol blue
Dounce homogenizer of 15 ml capacity equipped with tight fitting pestle			