

Materials List for:

# Growth-based Determination and Biochemical Confirmation of Genetic Requirements for Protein Degradation in *Saccharomyces cerevisiae*

Sheldon G. Watts<sup>\*1</sup>, Justin J. Crowder<sup>\*1</sup>, Samuel Z. Coffey<sup>1,2</sup>, Eric M. Rubenstein<sup>1</sup>

<sup>1</sup>Department of Biology, Ball State University

<sup>2</sup>Division of Nephrology, Cincinnati Children's Hospital

\*These authors contributed equally

Correspondence to: Eric M. Rubenstein at [emrubenstein@bsu.edu](mailto:emrubenstein@bsu.edu)

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## Materials

Name	Company	Catalog Number	Comments
Desired yeast strains, plasmids, standard medium and buffer components			Yeast strains with desired mutations may be generated in the investigator's laboratory. Wild-type yeast and a variety of mutants are also commercially available (e.g. from GE Healthcare). Plasmids encoding fusion proteins may be generated in the investigator's laboratory.
3-amino-1 <i>H</i> -1,2,4-triazole	Fisher Scientific	AC264571000	Competitive inhibitor of His3 enzyme. May be included in medium to increase stringency of growth assay using His3 reporter constructs.
Endoglycosidase H (recombinant form from <i>Streptomyces plicatus</i> )	Roche	11088726001	May be used to assess N-glycosylation of proteins; compatible with SDS and beta-mercaptoethanol concentrations found in 1x Laemmli sample buffer.
Disposable borosilicate glass tubes	Fisher Scientific	14-961-32	Available from a variety of manufacturers
Temperature-regulated incubator (e.g. Heratherm Incubator Model IMH180)	Dot Scientific	51028068	Available from a variety of manufacturers
New Brunswick Interchangeable Drum for 18 mm tubes (tube roller)	New Brunswick	M1053-0450	Tube roller is recommended to maintain overnight yeast starter cultures of yeast cells in suspension. A platform shaker or tube roller may be used to maintain larger cultures in suspension.
New Brunswick TC-7 Roller Drum 120V 50/60 H	New Brunswick	M1053-4004	For use with tube roller
SmartSpec Plus Spectrophotometer	Bio-Rad	170-2525	Available from a variety of manufacturers
Sterile 96-well flat bottom microtest plates with lid individually wrapped	Sarstedt	82.1581.001	Available from a variety of manufacturers
Pipetman Neo P8x20N, 2-20 µl	Gilson	F14401	Available from a variety of manufacturers
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Pipetman Neo P8x200N, 20-200 µl	Gilson	F14403	Single-channel and multichannel pipettors are used at various stages of the protocol. While multichannel pipettors reduce the pipetting burden at several steps, single-channel pipettors may be used throughout the entire protocol. Available from a variety of manufacturers.
Centrifuge 5430	Eppendorf	5427 000.216	Rotor that is sold with unit holds 1.5 and 2.0 ml microcentrifuge tubes. Rotor may be swapped for one that holds 15 ml and 50 ml conical tubes.
Plate imaging system (e.g. Gel Doc XR+ System)	Bio-Rad	170-8195	A variety of systems may be used to image plates, including sophisticated imaging systems, computer scanners, and camera phones.
Fixed-Angle Rotor F-35-6-30 with Lid and Adapters for Centrifuge Model 5430/R, 15/50 ml Conical Tubes, 6-Place	Eppendorf	F-35-6-30	
15 ml screen printed screw cap tube 17 x 20 mm conical, polypropylene	Sarstedt	62.554.205	Available from a variety of manufacturers
1.5 ml flex-tube, PCR clean, Natural microcentrifuge tubes	Eppendorf	22364120	Available from a variety of manufacturers
Analog Dri-Bath Heater	Fisher Scientific	1172011AQ	Boiling water bath with hot plate may also be used to denature proteins
SDS-PAGE running and transfer apparatuses, power supplies, and imaging equipment or darkrooms for SDS-PAGE and transfer to membrane			Will vary by lab and application
Western blot imaging system (e.g. Li-Cor Odyssey CLx scanner and Image Studio Software)	Li-Cor	9140-01	Will vary by lab and application
EMD Millipore Immobilon PVDF Transfer Membranes	Fisher Scientific	IPFL00010	Will vary by lab and application
Primary antibodies (e.g. Phosphoglycerate Kinase (Pgk1) Monoclonal antibody, mouse (clone 22C5D8))	Life Technologies	459250	Will vary by lab and application
Secondary antibodies (e.g. Alexa-Fluor 680 Rabbit Anti-Mouse IgG (H+L))	Life Technologies	A-21065	Will vary by lab and application