

Materials List for:

Measuring mRNA Levels Over Time During the Yeast *S. cerevisiae* Hypoxic Response

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Materials

Name	Company	Catalog Number	Comments
Enclosed dry incubator	Thermo Scientific	MaxQ 4000	Set at 30 °C. Modify the door to allow entry of one Tygon tube. Alternatively, use the New Brunswick G25 incubator, which contains a tube port. Do not use an open-air water shaker, as condensation will collect in the tubes between flasks, possibly cross-contaminating cultures.
Micro-analysis Filter Holder	Millipore	XX1002530	25mm diameter, stainless steel support, no. 5 perforated silicone stopper mounts in standard 125 mL filtering flask
Strong vacuum	Edwards	E-LAB 2	The "house" vacuum may be too weak. Alternatively, use an electric-power portable vacuum pump like the one listed here.
1,000 mL flask			To act as vacuum trap.
~2 foot lengths of Heavy Wall Vacuum Tubing, inner diameter 3/8 in, outer diameter 7/8 in	Tygon	38TT	Two pieces: the first connects vacuum to trap, and the second connects trap to filter system.
High-pressure N ₂ gas tank			99.999% purity, >1,000 psi, with a regulator and gas flow controller
Autoclaved 500 mL flask			Opening covered with aluminum foil. One for each yeast strain.
Autoclaved 250 mL flasks			Openings covered with aluminum foil. One for each time point plus two for water traps.
Flask stoppers (size 6, two holes with 5 mm diameter)			Sterilized with 70% ethanol. One for each flask.
Glass tubing, length 9 cm or 17 cm, inner diameter 2 mm, outer diameter 5 mm			Sterilized with 70% ethanol. Two tubes for each flask. Place into the holes of each stopper. See Figure 1 for placement of 9- vs 17- cm tubes.
~25 cm lengths of plastic tubing, inner diameter 5 mm	Tygon	E-3603	One piece for each flask. Sterilized with 70% ethanol.
Sterile filter discs	Millipore	HAWP02500	25 mm diameter, 0.45 µm pore size, one for each time point
Sterile dH ₂ O (~100 mL)			
1 mL cuvettes			For measuring OD600 (i.e., cell concentration)
50 mL sterile centrifuge tubes			One for each time point

Clean and sterile tweezers			
liquid nitrogen			For freezing cells
acid-washed beads	Sigma	G8772	Keep at 4 °C for lysing cells
Qiagen RNeasy Mini Kit	Qiagen	74104	For RNA column purification
Qiagen RLT buffer			Prepare by adding 10 µL of β-Mercaptoethanol per 1 mL of RLT buffer, keep at 4 °C.
2 mL collection tubes	Qiagen		included in the Qiagen Rneasy Mini Kit
Buffer RPE	Qiagen		included in the Qiagen Rneasy Mini Kit
Buffer RW1	Qiagen		included in the Qiagen Rneasy Mini Kit
DNase I stock and working solutions	Qiagen	79254	The DNase I enzyme comes as lyophilized powder in a glass vial. Using a sterile needle and syringe, inject 550 µL of RNase-free water (provided in Qiagen kit) into the vial. Mix by gently inverting the bottle. To avoid denaturing the enzyme, do not vortex. Using a pipet, remove this stock solution from the vial and store in freezer (-20 °C) in single-use aliquots (80 µL each). The stock solution should not be thawed and refrozen.
Buffer RDD	Qiagen		included in the Qiagen DNase Kit
Ice cold 2 mL screw-cap tubes			For lysing cells during RNA extraction
bead mill homogenizer	Biospec Mini-Beadbeater-24	112011	Keep in cold room
Bacto Peptone	BD	DF0118	for liquid YPD media
Bacto Yeast Extract	BD	DF0886	for liquid YPD media
glucose	Fisher	D16	for liquid YPD media
Qubit assay tubes	Thermo Fisher	Q32856	for measuring nucleic acid concentration
Quant-IT™ dsDNA BR Assay Kit	Thermo Fisher	Q32853	for measuring nucleic acid concentration
Quant-IT™ RNA Assay Kit	Thermo Fisher	Q32855	for measuring nucleic acid concentration
Qubit Fluorometer	Thermo Fisher	Q33216	for measuring nucleic acid concentration
Commercial electrophoresis system	Agilent	Bioanalyzer 2100	for measuring nucleic acid quality
Next-generation sequencer	Illumina	HiSeq 2500	for sequencing libraries
automated liquid handling system	Wafergen	Apollo 324	for creating sequencing libraries
PrepX PolyA mRNA Isolation Kit	Wafergen	400047	for isolating mRNA from total RNA
PrepX RNA SEQ for Illumina Library Kit	Wafergen	400039	for creating strand-specific sequencing libraries from total RNA
Barcode Splitter			https://toolshed.g2.bx.psu.edu/repository?repository_id=7119c4f7a89efa57&changeset_
Samtools, which includes the gzip command			http://www.htslib.org/download/

Trimmomatic			http://www.usadellab.org/cms/?page=trimmomatic
Bowtie2 (installed before TopHat)			http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
TopHat			https://ccb.jhu.edu/software/tophat/index.shtml
HTSeq			http://www-huber.embl.de/HTSeq/doc/overview.html
R (installed before R Studio)			https://cran.rstudio.com
R Studio (free version)			https://www.rstudio.com/products/rstudio/download/