Protocols

Protocol for Protocols

Wiki Markup Code for the Menu Bar

An excellent source of yeast protocols can be found here: http://cshprotocols.cshlp.org/cgi/collection/yeast

A primer on using yeast by Fred Sherman is here: Sherman_Starting_with_yeast.pdf

A more general molecular biology protocols are here: http://cshprotocols.cshlp.org/site/misc/subject.xhtml

Media & Recipes

- Common Yeast Media (YPD, SC, and the like)
- 1000x Metals
- 1000x Vitamins
- 10L of 10x Nitrogen limited salts
- 1L of 10X Phosphate Limitation Salts
- 1L of 10x Carbon Limitation Salts
- 100mM Nitrogen Stocks
- Glucose Limiting Media
- Nitrogen Limiting Media
- Phosphate Limiting Media
- Leucine and Phosphate Limiting Media
- Leucine and Uracil Limiting Media
- Nitrogen Agarose Plates
- Variable Nitrogen Source Limitation Carboy
- Denhardts Media
- D-His/D-Ser Plates

Growth and Growth Assays

- Coulter Counter
- Colony Counter
- Sixfors Chemostat
- Chemostat Protocols
- SYTO9 & PI FACS Viability Assay
- FACS-based analysis for competition experiments
- FUN-1 Metabolic Activity Assay

Yeast Cytometry

Fixing

- Ethanol fix
- Filter & PFA fix, lyticase digest, etOH permabilization
- Old-school (field standard) fix, digest, permeabilization for immuno or FISH applications

Probing/Staining
• DNA content flow cytometry with Sytox Green
• Amine/sulfhydryl staining (protein content proxy) with FITC
• polyA staining using singly-labeled FISH
• mRNA single molecule FISH with Stellaris-style probes
• FISH for FACS applications, using Quantigene probes (BFF)
• RNA content flow cytometry with RNAtoSelect

RNA (and Expression Analysis)

**Extracts, purifications, and enrichments:**

• Yeast RNA Extraction (growing)
• RNA extraction from yeast, a different version (2016)
• RNA extraction from stationary phase yeast (thicker cell wall)
• Proteinase K-mediated extraction of RNA from yeast
• DNase treatment of RNA
• polyA selection
• Ribominus selection
• ecoli RNA extraction

**cDNA for expression analysis:**

• Making cDNA for Transcriptome Analysis - primarily microarray
• cDNA synthesis with M-Mulv RT - primarily for qPCR
• RT qPCR pre 2015
• RT qPCR workflow - from 2015

**RNAseq**

• RNA-Seq (directional) rnaseq RNAseq
• Nextera Based RNASeq using ds cDNA from polyDT primers
• Nextera Based RNASeq using ds cDNA from Random Hexamers

**4tU labeling related methods**

• Making spike-ins, linearizing and in-vitro transcription
• HPDP Biotinylation of 4tU labeled RNA
• Streptavidin Pull-down of Biotinylated-HPDP-4tU RNA
• Dot Blot Assay

**Analysis**

• Separation of RNA by electrophoresis or Denaturing gel (formaldehyde) or Non-denaturing RNA gel
• Transfer of Denatured RNA to positively charged nylon membrane
• Preparation of an Exemplary RNAlater- RNA Preservation Medium

**DNA**

• Quick yeast gDNA extraction for PCR-based applications
• High Throughput DNA extraction with PureLinkTM Pro 96
• Hoffman Winston DNA Prep
• Southern Blot Analysis
• Bar-seq Barseq (high-throughput analysis of competing mutants, see Robinson, Chen, Storey, and Gresham 2014)
• low-input barseq, aka SoBaSeq for amplicon-sequencing of dead sorted cells
• DNA fragmentation
• Ethanol precipitation/concentration of DNA

**DNAseq**

• DNA Library Preparation Using Nextera tagmentation
• DNA Library Preparation For Illumina Sequencing (Update 05/2013 - Naomi Ziv)
• DNA Libray Prepartaion For Amiicon Miseq Sequencing (Updated 04/2014 - Jungeui Hong)

**DNA Microarrays - for cDNA from RNA, see above section**
• Hybridization Mix
• Affymetrix Tiling Arrays
• Slide Stripping Protocol Agilent Yeast Arrays
• Agilent Custom Mutation Detection Tiling Microarrays

• qPCR with SybrGreen
• using the tapestation

**Molecular Biology**

• Measuring DNA using SYBR Green
• Biobricking Protocol Overview
• Bioanalyzer protocol links, info
• TAP reagents
• TAP protocol
• DIG 3’-end labeling
• Detection of DIG labeled nucleic acid
• Annealing Oligonucleotides
• Non-denaturing polyacrylamide gel electrophoresis (PAGE gel)
• E. coli transformation
• Messing about with vectors, using PCR and NEB HiFi assembly

**Yeast Techniques**

• PCR-based Yeast allele replacement methods
• Colony PCR
• Dapi Staining and Morphology
• Sporulation / tetrad dissection
• Mating / mating type halo assay
• Using the Pinner to transfer the Yeast Deletion Collection to new plates
• Sonicator
• High Efficiency Transformation Protocol
• Density Fractionation and Trehalose & Glycogen Assay

**Experimental Evolution**

• Experimental evolution in chemostats

**DGseq sequencing analysis**

• DGseq sequencing adapter information
• Deduplicating a bam file using umi-tools
• DGseq demultiplexing
• DGseq removal of PCR duplicates reads

**RATE-Seq**

• RATE-Seq Protocol
• RATE-seq Bioinformatic Analysis

**GitHub**
Theme Songs, Chants, Incantations

- I'm GlycoBlue
- Qubit Song
- RiboZero Song
- Lost My Controls Again
- All the single labels