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 RNAsytoSelect

RNA (and Expression Analysis)

**Extractions, 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 RNAsseq
- 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 PureLink™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 Amilicon 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