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 RNA SytoSelect

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 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 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 Library Preparation 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