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 permeabilization
- 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 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
Git Data Transport Commands

http://osteele.com

commit -m
add (-u) commit
push
pull or rebase
fetch
checkout HEAD
checkout
diff HEAD
diff

Git / GitHub

How to "Pull Request"

1. Your PC 2. add 3. commit 4. push 7. merge

1. clone 2. fork 7. pull request

1. Partner’s PC 2. add 3. commit 4. push

Partner’s GitHub
Theme Songs, Chants, Incantations

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