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](http://cshprotocols.cshlp.org/cgi/collection/yeast)

A primer on using yeast by Fred Sherman is here: [Sherman_Starting_with_yeast.pdf](http://cshprotocols.cshlp.org/cgi/collection/yeast)

A more general molecular biology protocols are here: [http://cshprotocols.cshlp.org/site/misc/subject.xhtml](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 Limitation 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
- Soft Agarose
- YPG(YEPG OR YEP-GLYVEROL)

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
- Benomyl Assay
- Competitions in the ministats

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 (deprecated)
- RT qPCR workflow

**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 Illumina Miseq Sequencing (Updated 04/2014 - Jungeui Hong)
• Barcoded Library PCR for Illumina sequencing

**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 HIHi assembly
• Glucose Assay
• Gibson 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

Submitting stuff to the SRA
GitHub

Git Data Transport Commands

http://osteele.com

commit -a
add (-u)
commit
push
pull or rebase
Fetch
checkout HEAD
check out
checkout
diff HEAD
diff

Git / GitHub How to “Pull Request”

1 Your PC
2 add
3 commit
4 push
5 merge
6 Partner’s PC
7 pull request

Your GitHub

1 fork
2 clone
3 add
4 commit
5 push
6 Partner’s GitHub

designless.net

Statistics
Theme Songs, Chants, Incantations

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