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/s...

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 & Recipies

- **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**
- **Denhardt’s 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 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

DNAsseq

• DNA Library Preparation Using Nextera tagmentation
**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
- 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
### Theme Songs, Chants, Incantations

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