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
- 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 RNAsq
- Nextera Based RNAsq using ds cDNA from polyDT primers
- Nextera Based RNAsq 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 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
GitHub

**Git Data Transport Commands**

commit -a
add (-u) → commit → push
workspace → index → local repository
remote repository

- pull or rebase
- checkout HEAD → fetch
- checkout
- diff HEAD
- diff

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**Git / GitHub**

**How to “Pull Request”**

1. Your PC
2. add
3. commit
4. push
5. fork
6. pull request
7. merge

Your GitHub

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

Partner’s GitHub

designless.net
Theme Songs, Chants, Incantations

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