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
http://osteele.com

```
commit -a
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
5. merge
6. pull request
7. Your GitHub

```
clone
fork
partner's PC
3. add
4. commit
5. push
6. Partner's GitHub
```

Statistics
Theme Songs, Chants, Incantations

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