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 & 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**
- **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 Amilicon 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 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
Git Data Transport Commands

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

GitHub

Git / GitHub

How to "Pull Request"

Your PC
1. \[\text{Your PC}\]
2. add
3. commit
4. push
5. merge

Your GitHub
6. \[\text{Your GitHub}\]
7. pull request

Partner’s PC
1. fork
2. clone

Partner’s GitHub
3. \[\text{Partner’s GitHub}\]
4. add
5. commit
6. push

Statistics
Theme Songs, Chants, Incantations

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