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/site/misc/subject.xhtml).

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 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
- DNA content flow cytometry with PI
- 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
Other/Misc

- Submitting stuff to the SRA
- Transposon Saturation using Hermes and Hygromycin Resistance

GitHub

Git Data Transport Commands
http://csteele.com

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

Git / GitHub
How to "Pull Request"

1. fork
2. clone
3. add
4. commit
5. push
6. pull request
7. merge
Statistics

Theme Songs, Chants, Incantations

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