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 & Recipies

- Common Yeast Media (YPD, SC, and the like)
- 1000x Metals
- 1000x Vitamins
- 1L 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
• 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 Libray Prepartation 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://c Steele.com

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