Biotechnology in the Post-Genomic Age

STARS Inservice on Biotechnology

February 4, 2006

Joel M. Goodman, Ph.D.

Supporting Cast

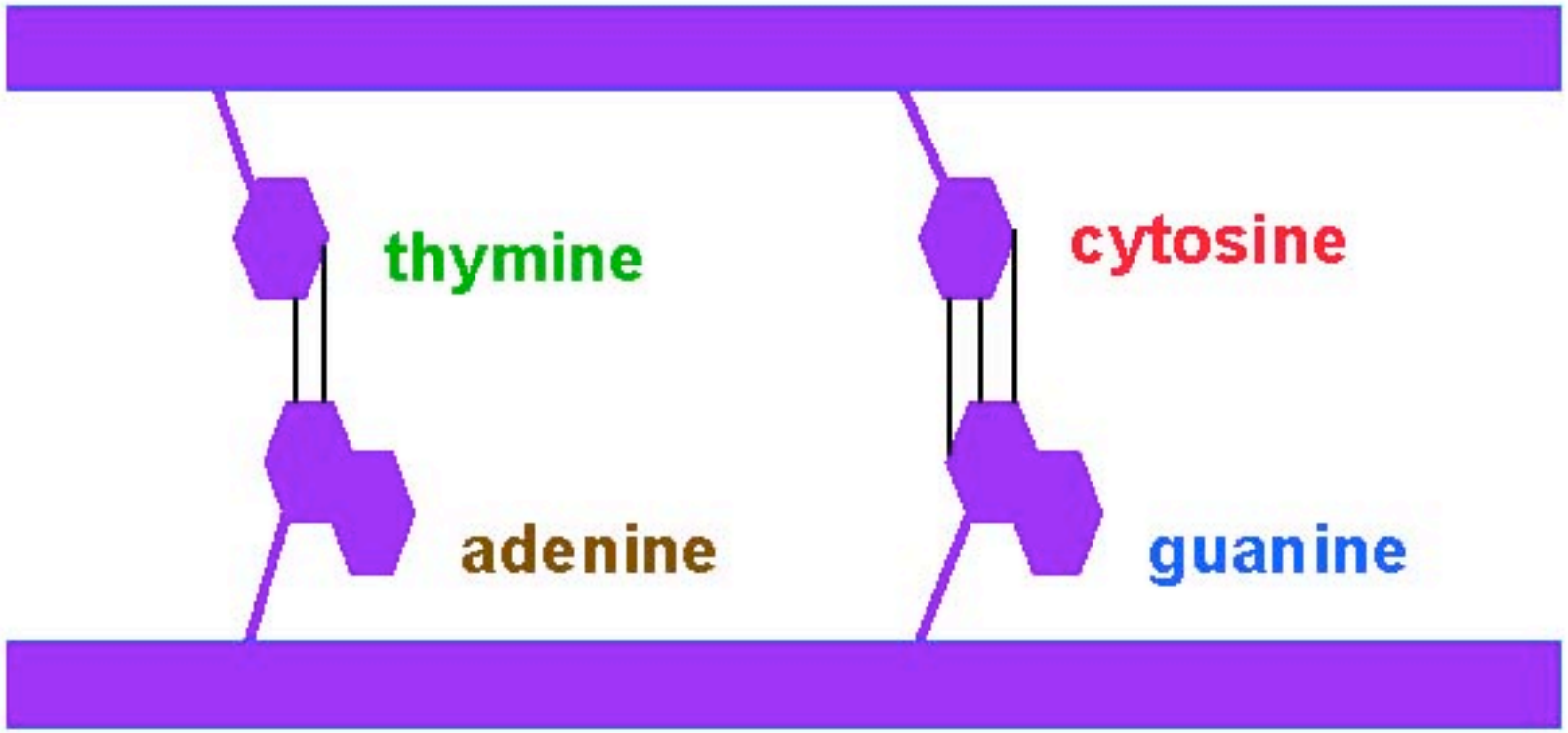
- STARS support
  - Ms. Jeannie Han
  - Ms. Traydell Beard
- Goodman lab support
  - Dr. Derk Binns
  - Ms. Kimberly Szymanski

Activity 1: PCR

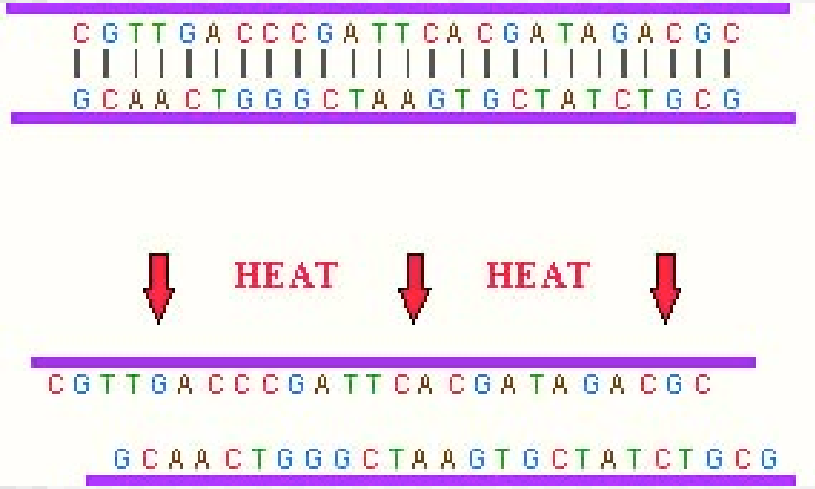
- We'll start this now, talk more later
- You will be amplifying a yeast gene (one of five different genes) using the polymerase chain reaction.
- 6 groups, 4 teachers to a group
- By the end of the day, you will identify which gene you have amplified

DNA

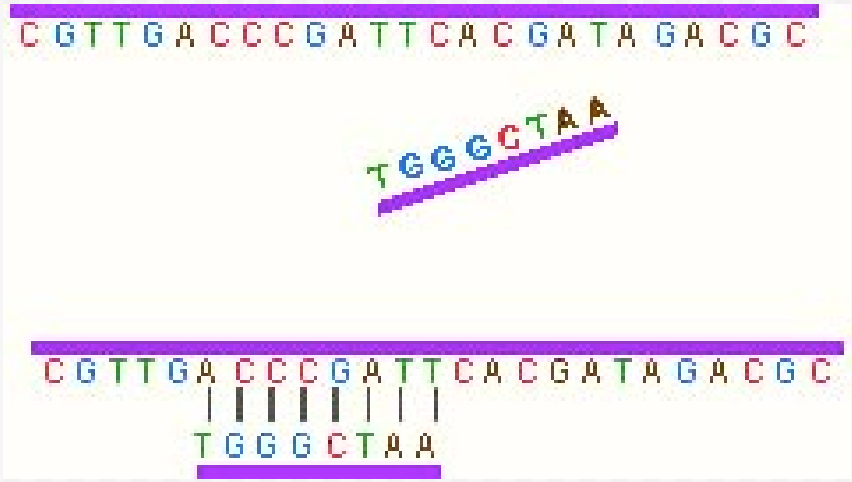
PCR figs from
http://www.lshtm.ac.uk/pmbu/staff/rmcnerney/homepage/basicstext.html



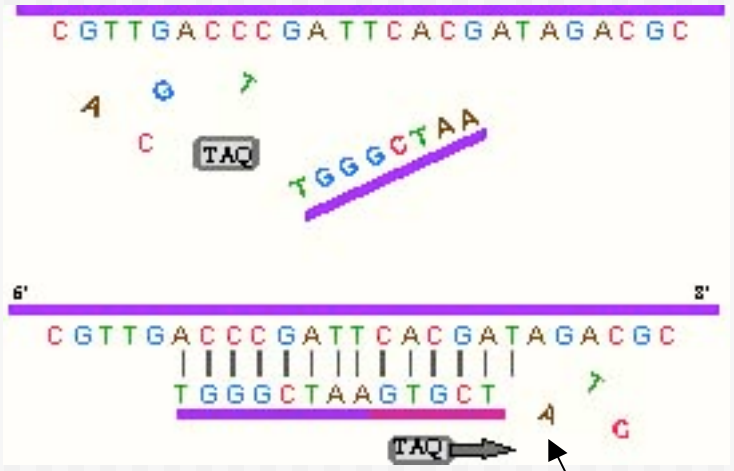
DNA melting



Annealing of “oligos”

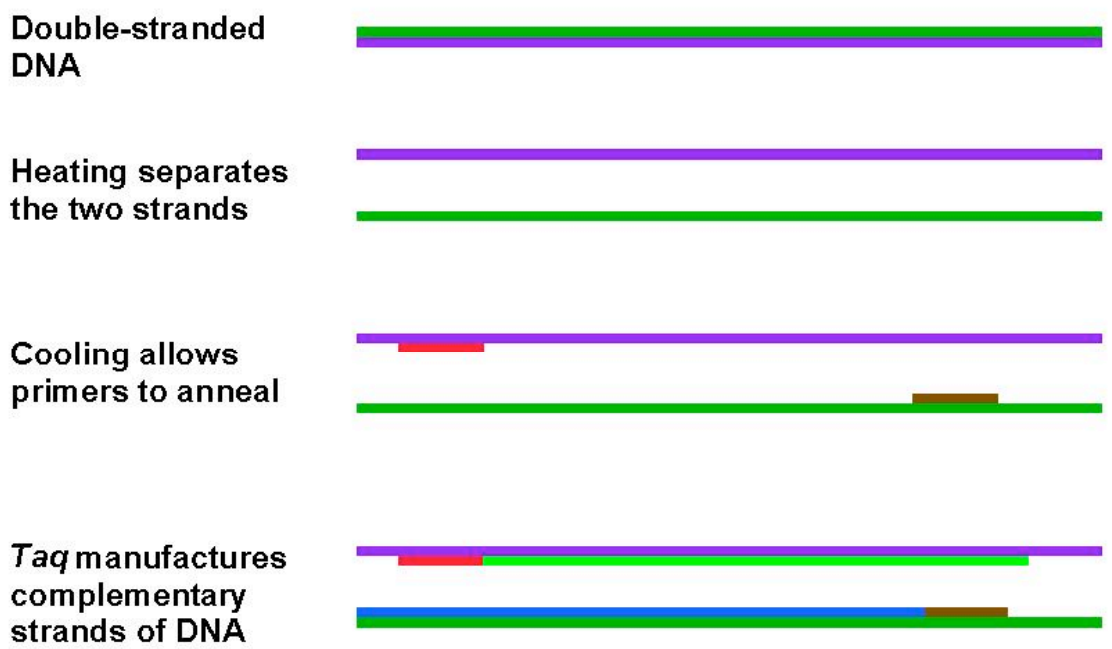


Strand elongation



"A" is really Deoxyadenosine triphosphate, or dATP

PCR cycle 1

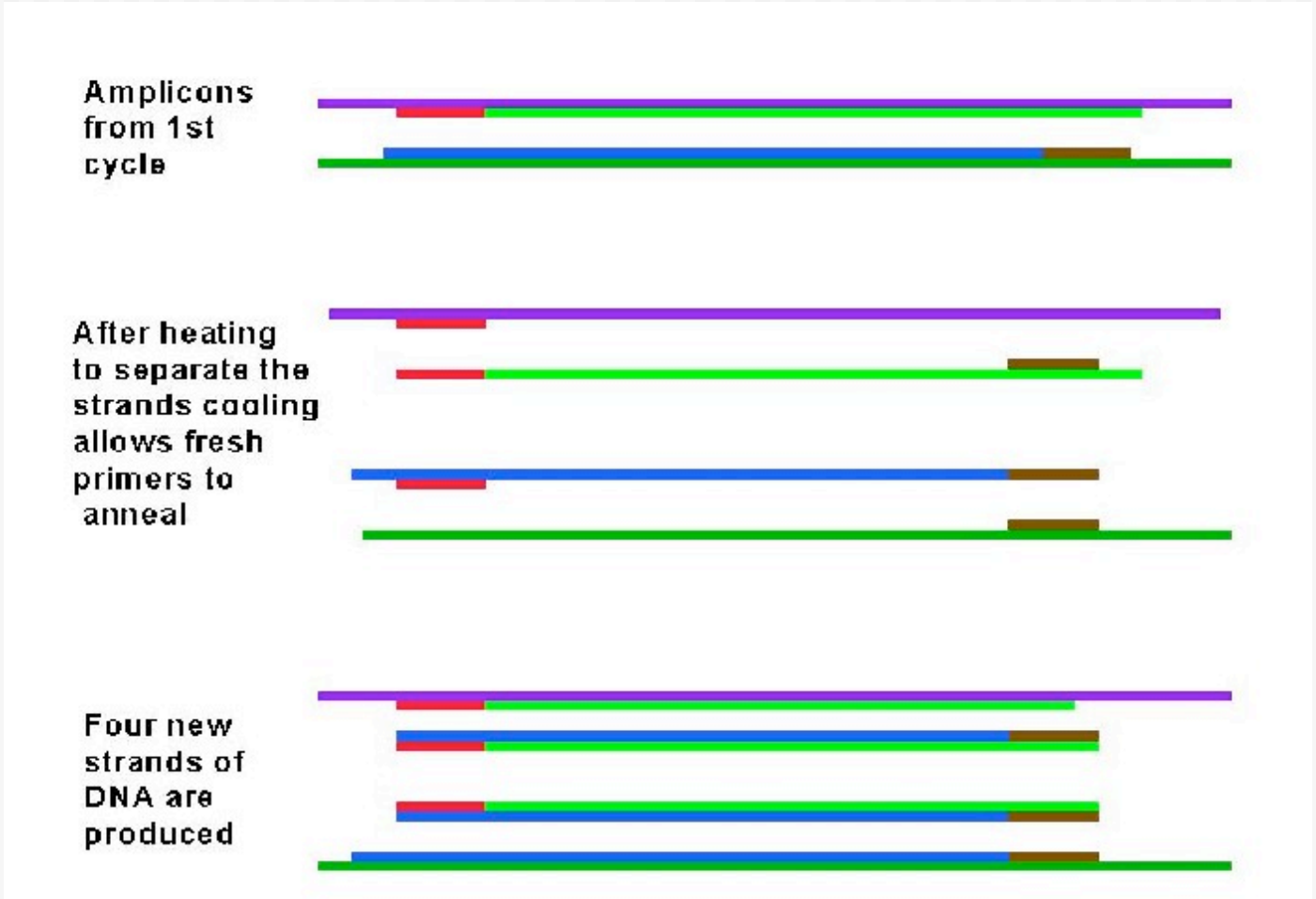


"template" (genomic DNA)

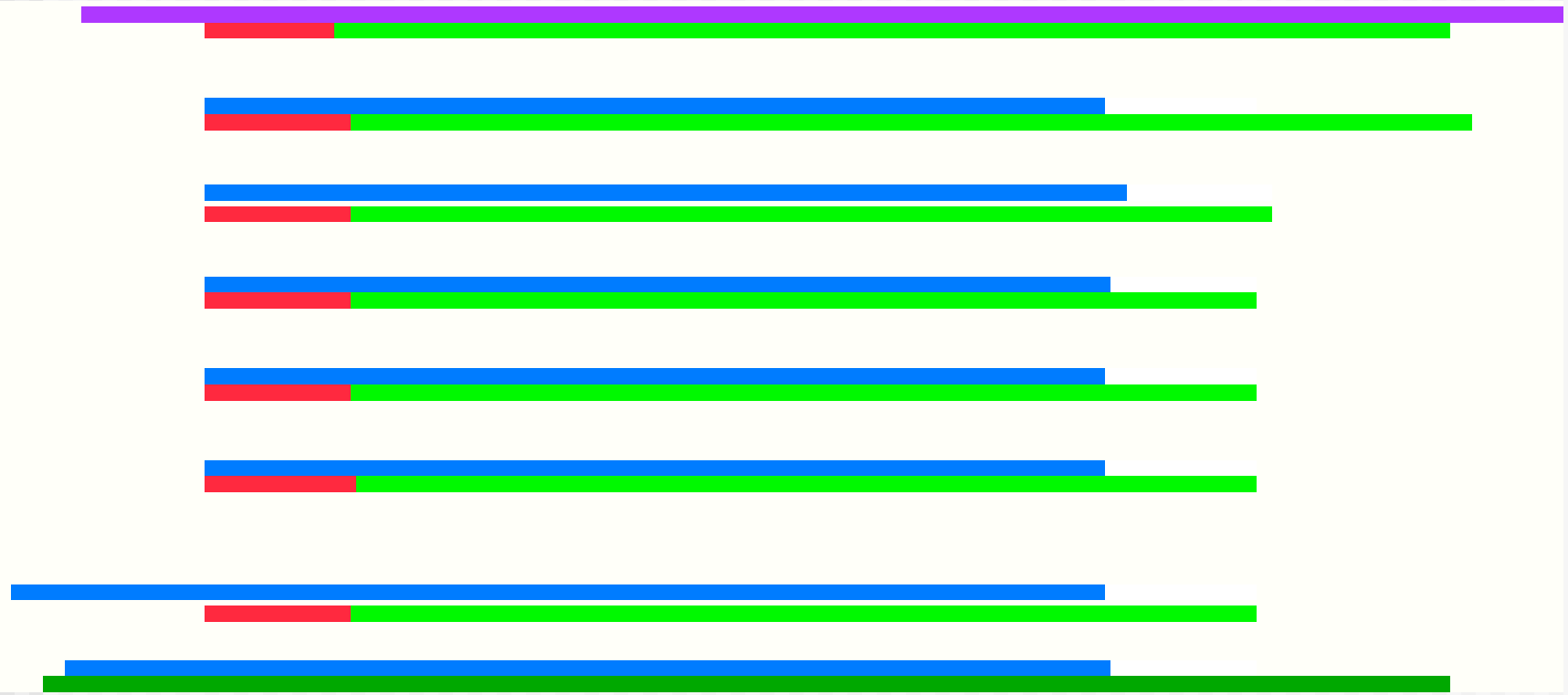
Primers used

dXTPs, Polymerase used

PCR cycle 2


Amplicons from 1st cycle
After heating to separate the strands cooling allows fresh primers to anneal
Four new strands of DNA are produced

PCR cycle 3



PCR exercise

- You will amplify your gene through 30 cycles of primer annealing, elongation, and melting
- Reagents:
  - Template: yeast DNA
  - Primers: ends of genes, ~20 bases
  - Heat-stable Polymerase (Vent ®)
  - Nucleotides (building blocks for polymerase)
  - Buffer

Today's Aims

- You will focus on five genes from bakers' yeast (*Saccharomyces cerevisiae*)
- You will be assigned one of these, the identity unknown to you
- You will identify it based on its PCR product and its phenotype

Activities

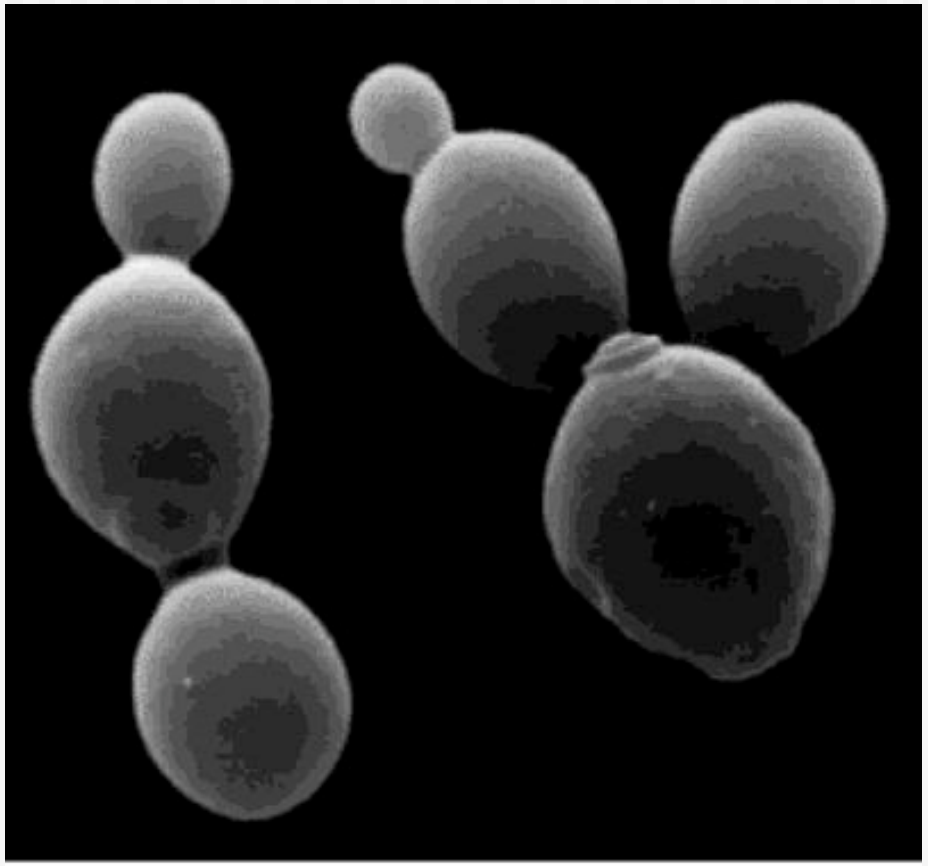
- Perform PCR to amplify your gene
- Become familiar with the *Saccharomyces cerevisiae* Genomic Database, SGD (www.yeastgenome.org)
- Learn about the six genes and phenotypes of the corresponding gene disruptions
- Based on PCR and phenotype, identify your gene

Schedule

- Morning
  - Prepare PCR reaction, start cycling
  - Introduction to SGD and strain library
  - Sleuthing SGD of 6 genes
- Lunch
- Afternoon
  - Loading PCR products on gel
  - Innoculating plates with yeast strains
  - Determining migrations of PCR products
  - Determining your mystery gene

Saccharomyces cerevisiae

- Bakers yeast
- Budding yeast
- 7 μm diameter
- Haploid or diploid
- Cell wall
- Contains 6600 genes

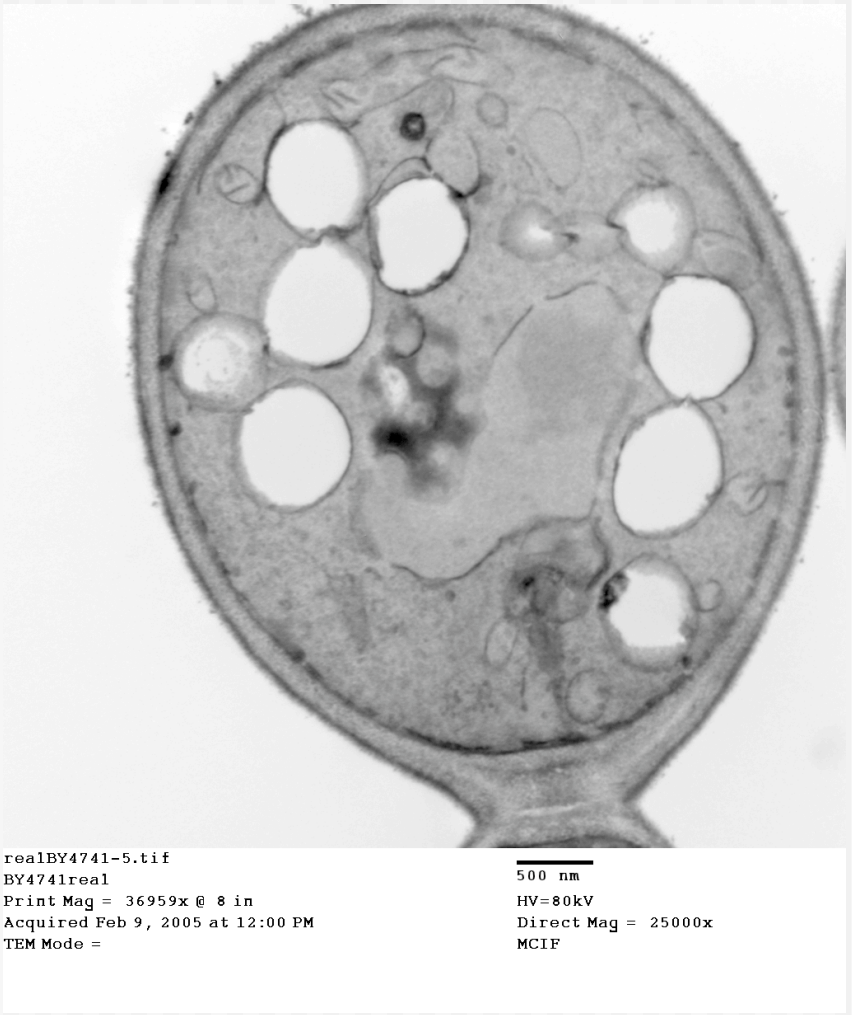


Yeast (*Saccharomyces cerevisiae*)

- wallhttp://www.sb-roscoff.fr/CyCell/Images/yeast1.jpg

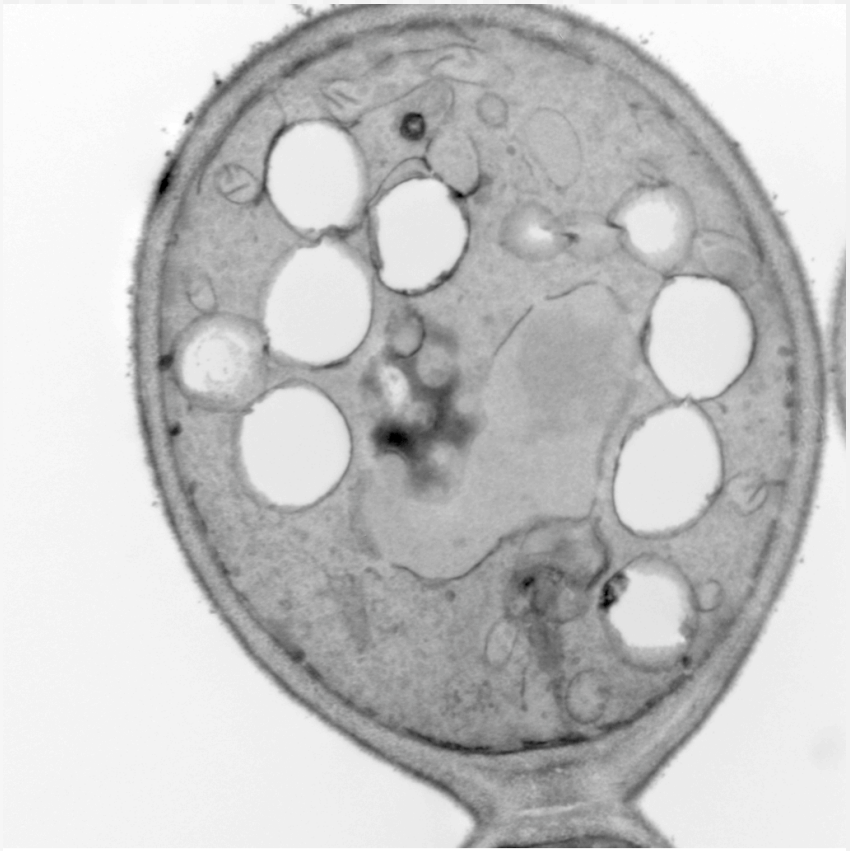
Yeast as a Model Organism

- Cell Biology - simpler than mammalian cells
- Signal Transduction
- Molecular Genetics - DNA/RNA metabolism
- Finding mammalian orthologs in disease
- Study of mutants easy

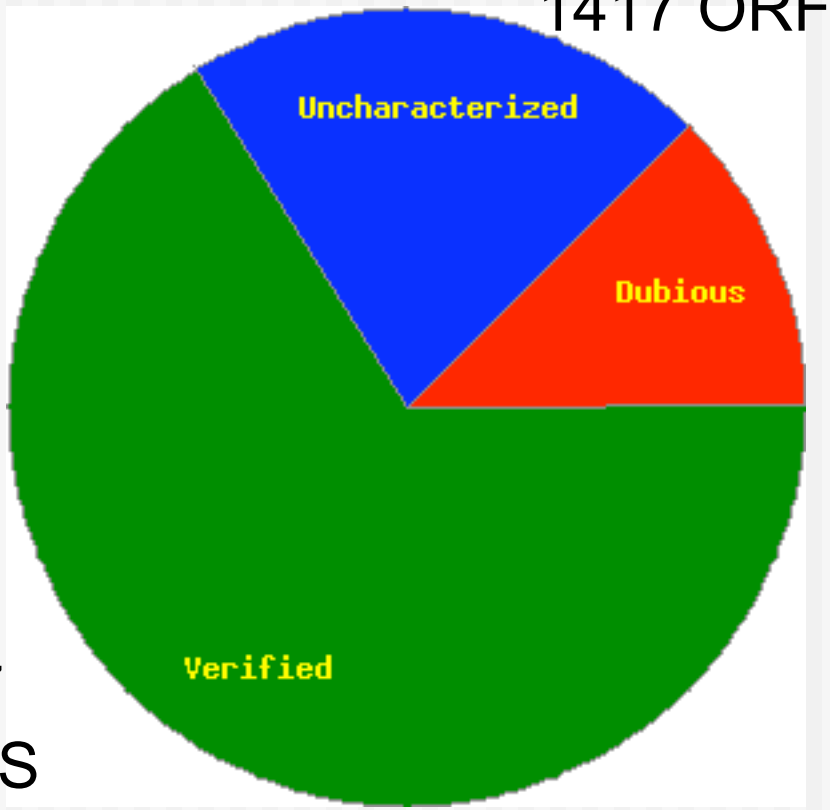


Yeast is NOT good for

- Many cell-cell interactions
- Cell specializations
- Developmental biology
- Stem cell research



The yeast genome: 6604 ORFS



1417 ORFS

820 ORFS

4367 ORFS

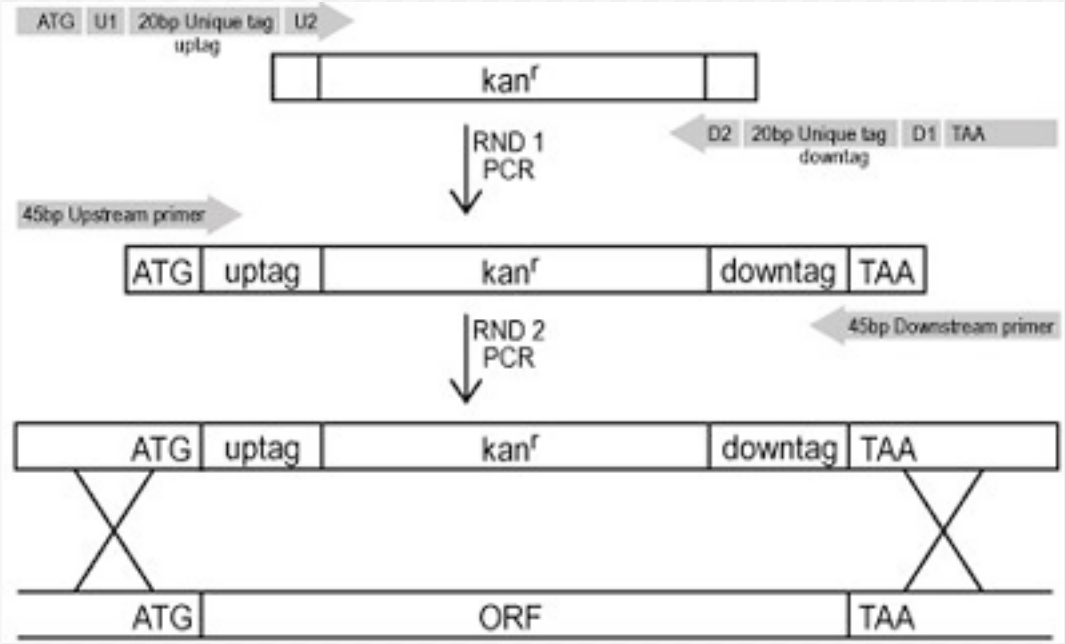
ORF =
Open Reading Frame

What does gene X do?

- You are studying a human gene. Cells difficult to manipulate. Find yeast ortholog and mutate
- What genes are involved with process X?
- What genes interact with your favorite gene?

Yeast deletion libraries

- By PCR, and homologous recombination, every gene has been “knocked out.”



Homologous recombination

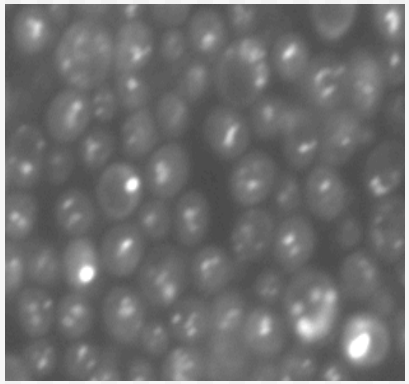
Openbiosystems.com

Yeast deletion libraries

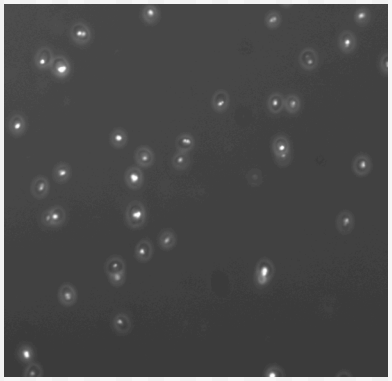
- By PCR, and homologous recombination, every gene has been “knocked out.”
- You can buy a “knock-out strain” for about $50 each.
- You can buy an ENTIRE library of strains for $1500.
- Only non-essential genes can be knocked-out in haploid cells; essential genes can be knocked out in diploids (one normal copy still present)

Lipid body phenotypes

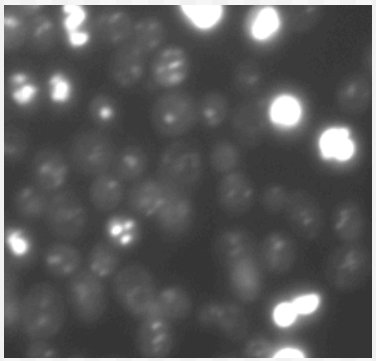
Normal Phenotype



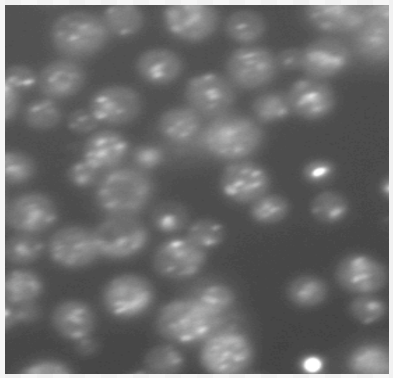
"Starry Night"



Big Lipid Bodies



Small Lipid Bodies



Kim Szymanski

First step: PCR

- You will be assigned a deletion strain, missing one of 5 genes
- You will be given oligos to your assigned gene
- You will run a PCR reaction
- In the afternoon, you will analyze the PCR product by agarose gel electrophoresis against standards.

Second Step: yeastgenome.org

- The yeast genome was the first eukaryotic genome to be sequenced (~1993)
- Stanford University assembled this database
- Constantly being improved and updated
- Comparatively easy to navigate

Sleuthing SGD for clues

Gene	*ADE2*	*CKB1*	*GAL1*	*SIW14*	*TRP1*
Function; which pathway?					
Phenotype of null mutant					
Which chromosome?					
Watson or Crick strand?					
# of bases					
Location of protein					
Why named?					
Other interesting stuff					

Third step: Phenotypes

- The phenotype of null mutants is very useful to obtaining clues as to function of the gene
  - Cell morphology, growth, organelle movement and morphology protein trafficking, etc.
- You will be given agar plates to innoculate with your mutant strain
- You will determine the extent of growth on those plates and choose which of the five genes is missing.

Translation to the Classroom

- Exercises with a genome database
  - Sleuth for information on gene and protein
- Polymerase chain reaction (PCR)
  - Many schools have PCR machines, STARS has one available
  - PCR kits, PCR not required
- Deletion library
  - Phenotype observations - Plates easy to make, students can observe different behaviors and morphologies, relate them to gene deletions