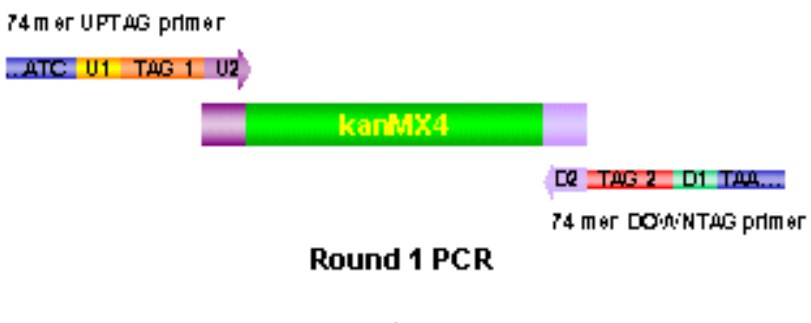
Primers

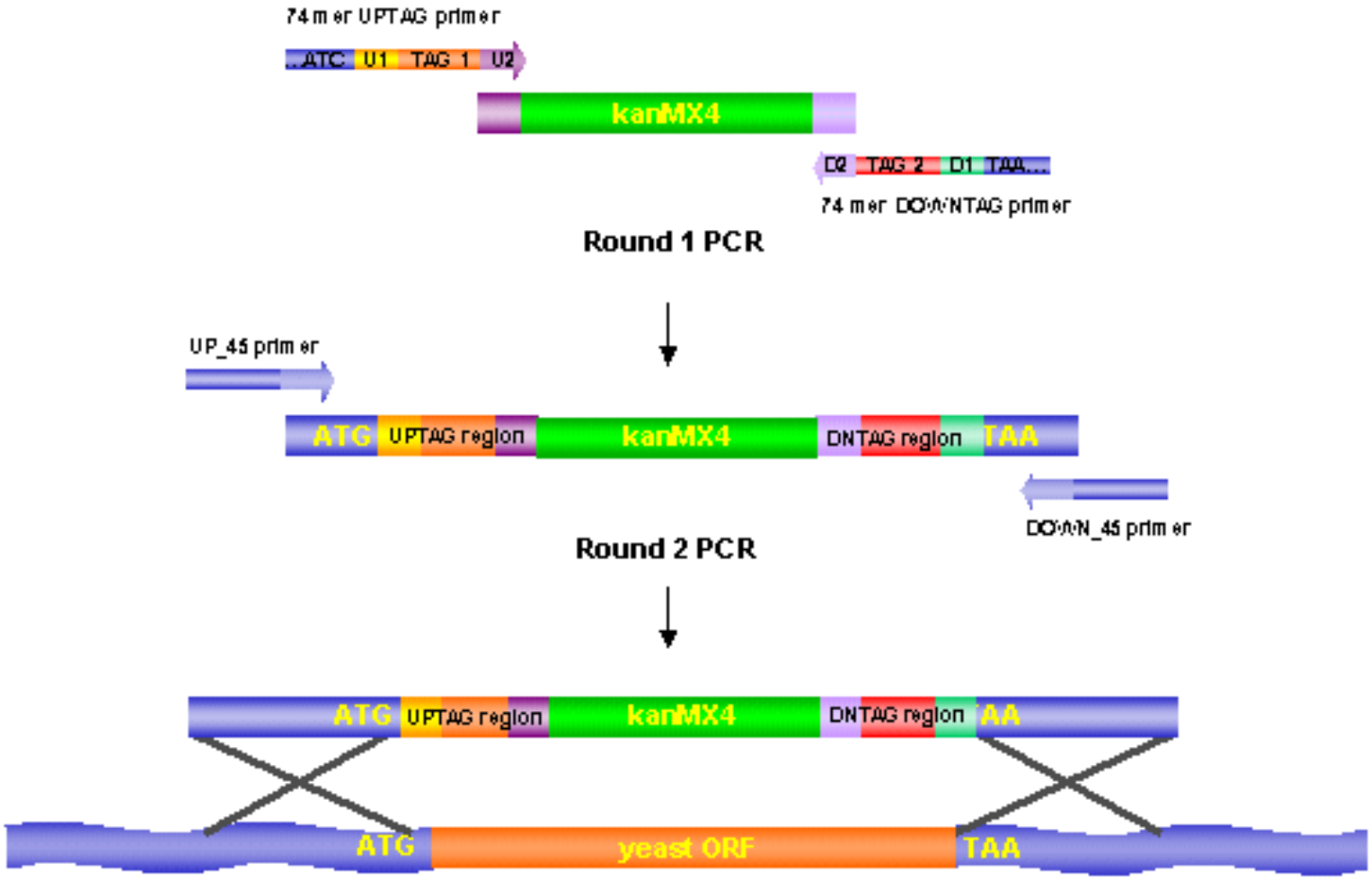
- 8~10 different primers/ORF
- High-throughput primer synthesis
  - Primer-picking scripts
    - Input:
      - ORF data + UPTAG/DOWN list
    - Output:
      - Primer sequences
  - Automated Multiplex Oligonucleotide Synthesizer



- Homology to ORF upstream
- Common tag priming site (U1)
- UPTAG (20 bases)
- Common tag priming site (U2) homologous to 5' to the Kan gene

PCR-based Gene Deletion

- Deletion strategy



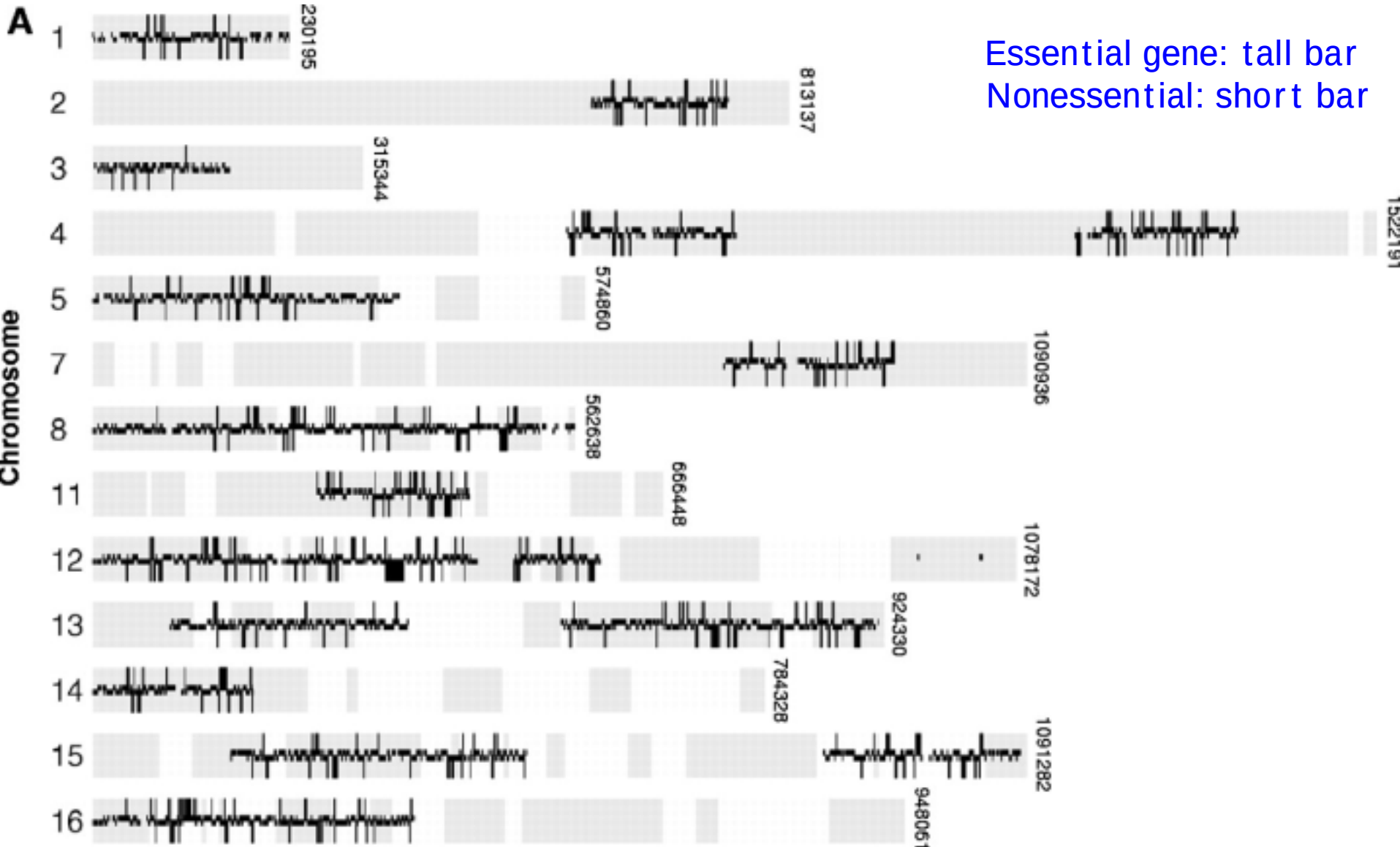
Chromosomal integration by homologous recombination

http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html

Results

- 6925 deletion strains of yeast constructed…
  - Essential for viability
  - Lack of human homologs
  - Targets for antifungal drugs
- Screen for genes essential for viability
  - Spores from 2026 ORF (1/3 of the genome) heterozygous strains on YPD media at 30°C
    - 356 haploid deletants could not be recovered
    - 1620 ORFs not essential for viability in yeast
      - Construct one additional homozygous and two haploid deletants
- Statistics
  - 8.5% of the identified non-essential ORFs in the yeast genome have a closely related homolog elsewhere (redundancy)
  - 1% of the essential gene have homologs

Genomic locations



Competitive growth assays

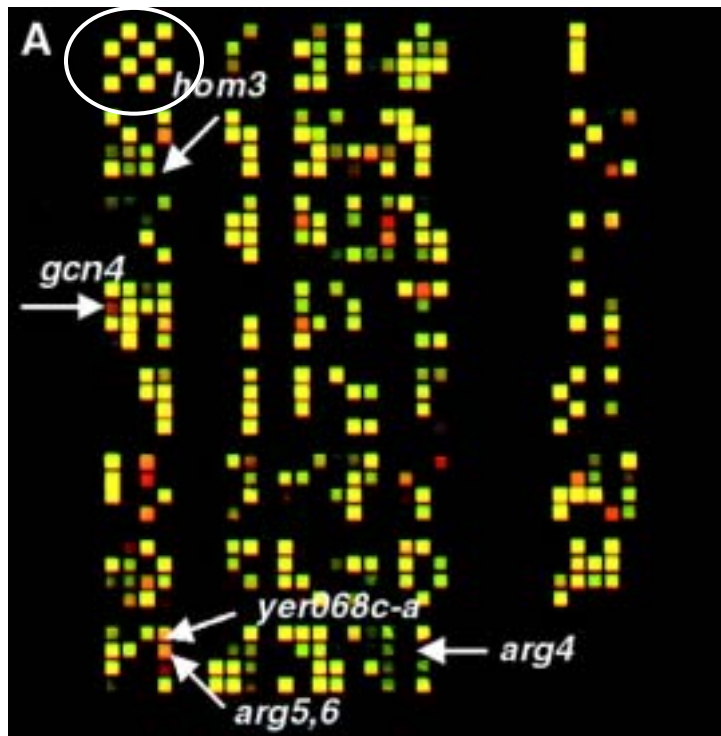
- How to characterize the genes nonessential for viability?
- Pooled functional assay
  - 558 homozygous deletion strains were pooled
  - Grow in rich and minimal media for ~60 generations
  - Remove aliquots from the two pools at various time points
  - Tags were amplified and hybridized to DNA array

Red: grown for 0 hr
Green: grown for 6 hr

Normal growth (expression)

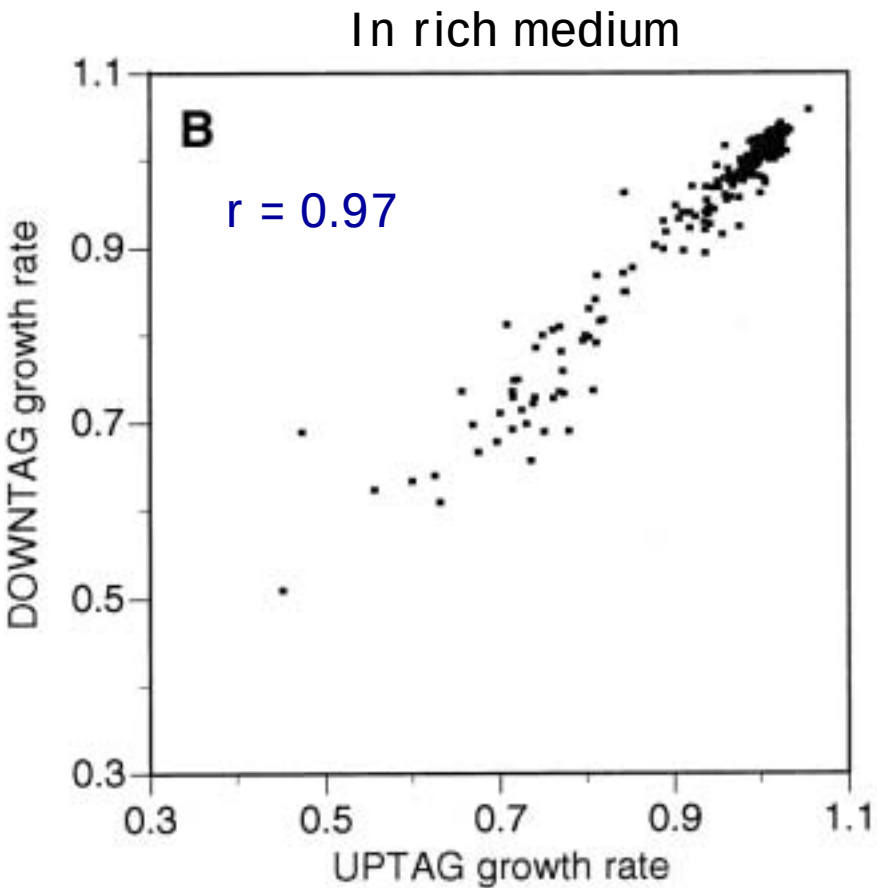
Grow slow (reduced expression)

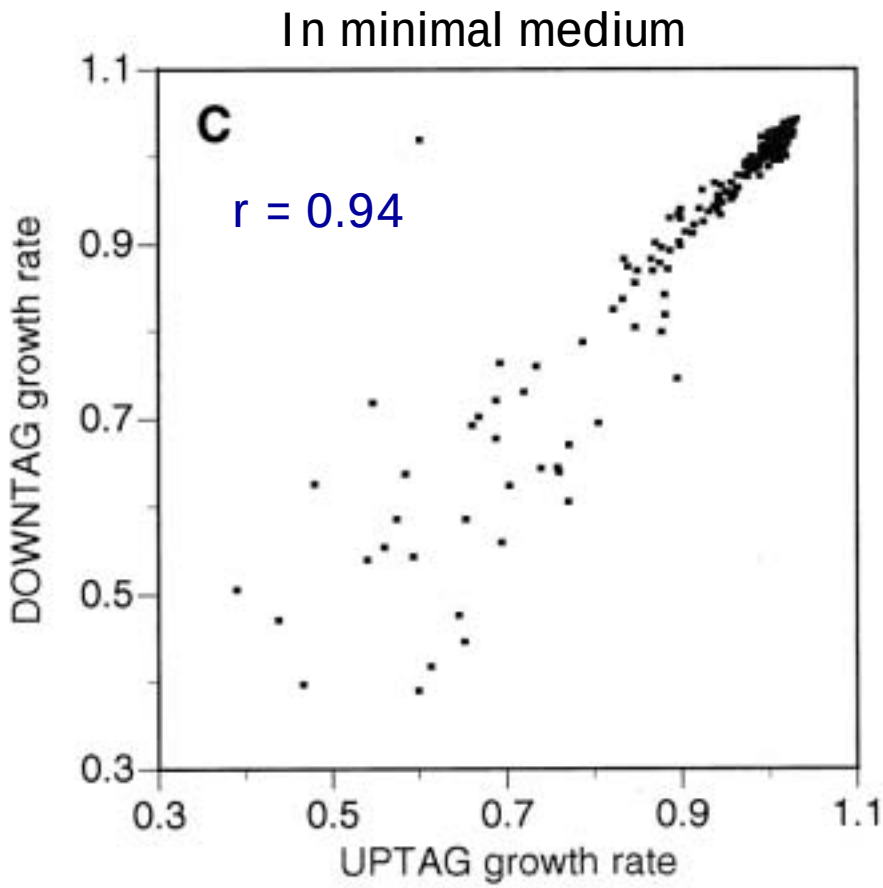
Grow fast (enhanced expression)



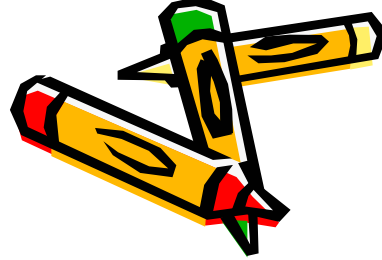
Correlation of growth rate

- Where is the wild type ?





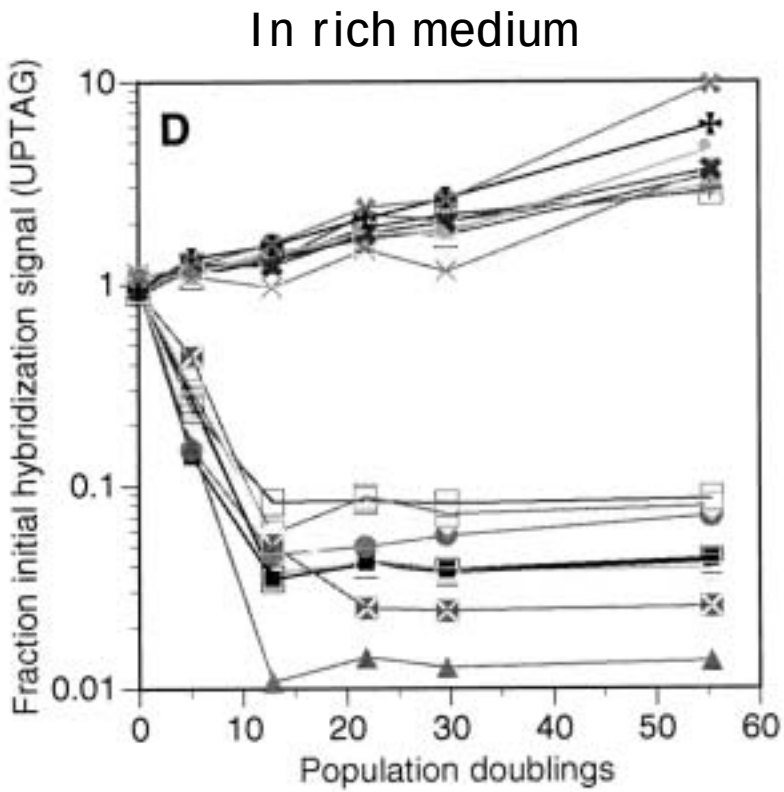
Normalized growth rate



- Hybridization intensity = growth rate
  - Normal growth = 1
  - Grown fast (abundant) > 1
  - Grown slow (fewer) < 1

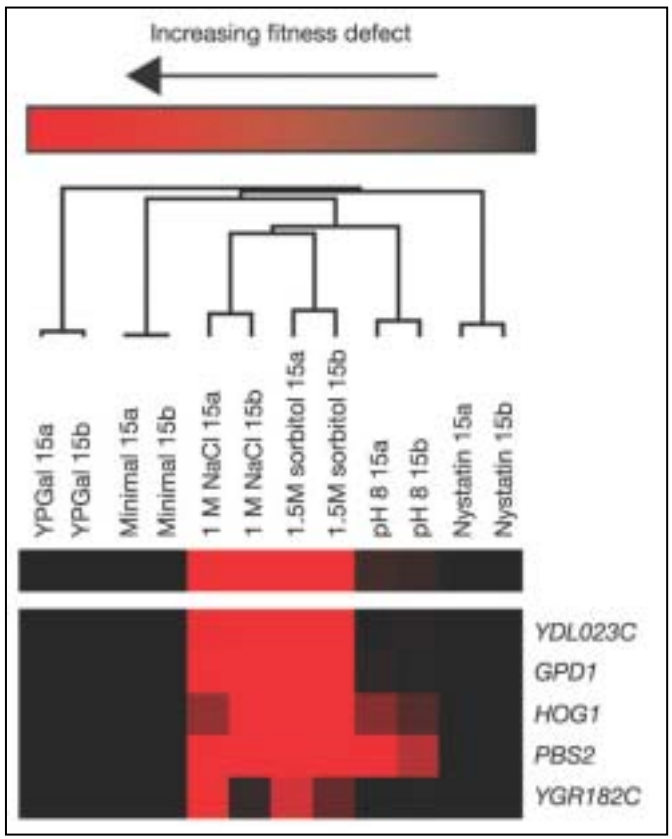
Q:

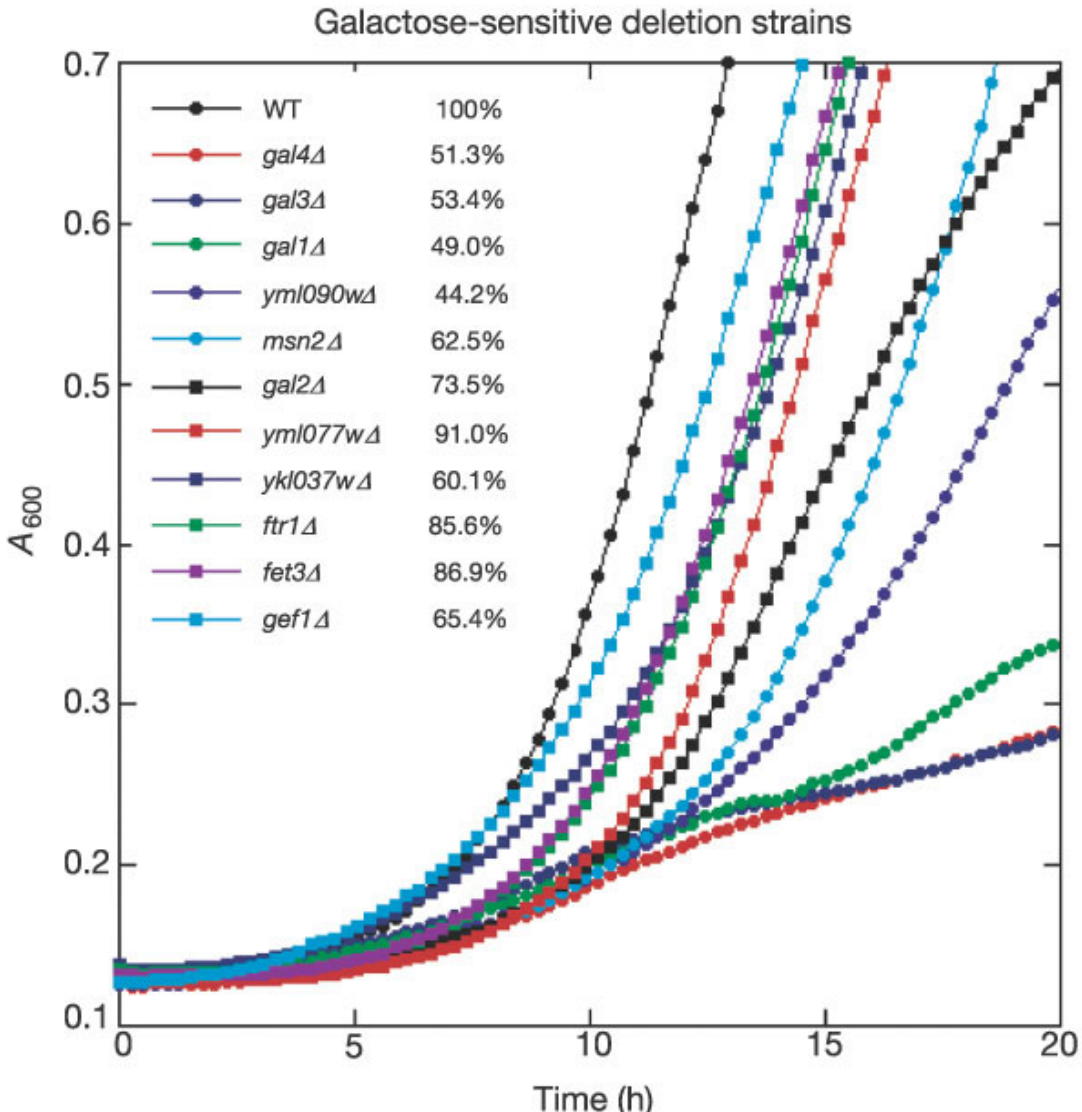
Predict what might happen if only the slowest growing strains were incubated together.



Whole-genome parallel analysis

- Fitness profiling
  - C source
- Clustering
  - Osmoregulation





Nature 2002 418, 387-391.

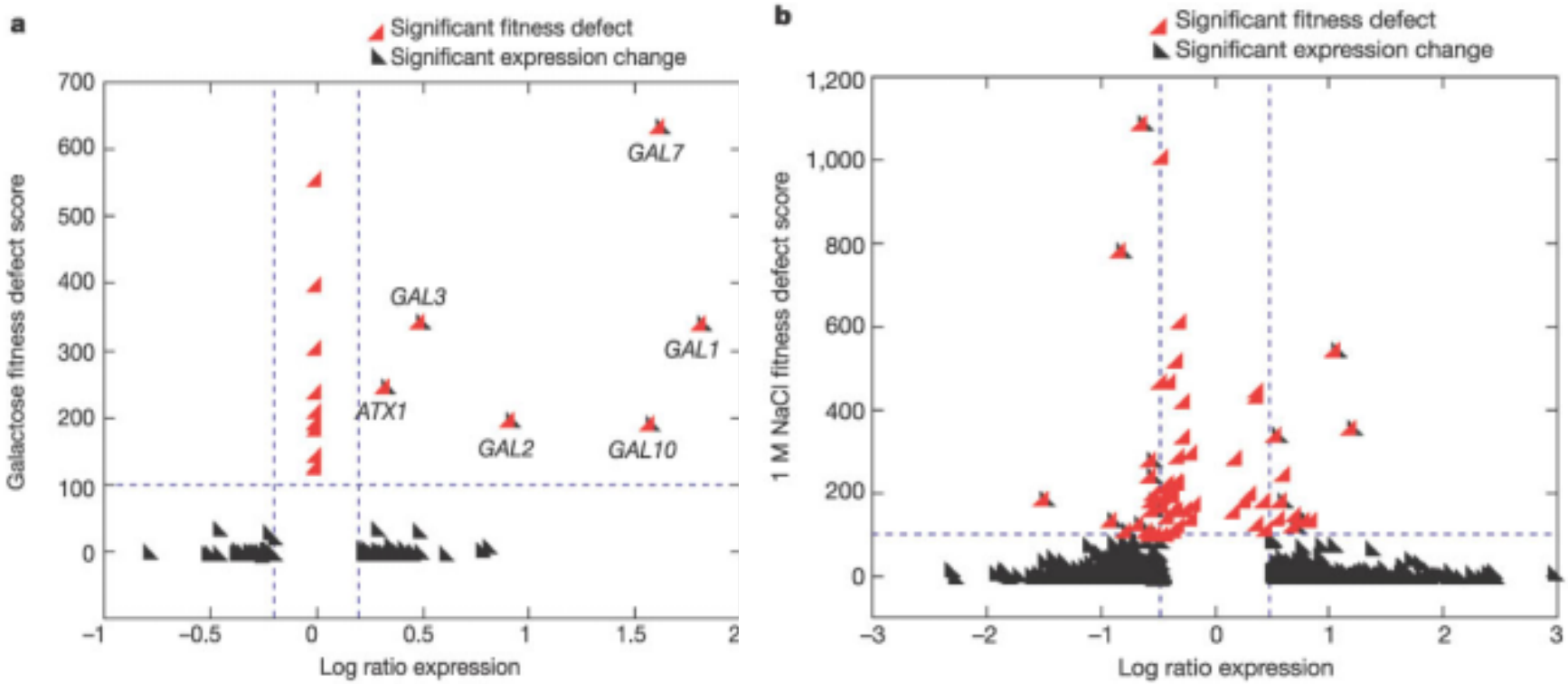
Fitness vs. Expression profiling

- H_o: in a given condition, if a gene expression ↑, then growth ↑.
  - Good fitness → good expression
  - Good expression → good fitness

Condition	Measured genes	Up*-regulated	Down*-regulated	% Up*-reg. & Fitness defect*	% Down*-reg. & Fitness defect*
Galactose	4682	99	84	6.06	0.00
Alkali	4711	434	464	3.00	3.23
1M NaCl	4711	679	1047	0.88	1.15
1.5 M Sorbitol	4711	588	1024	0.34	0.0

Nature 2002 418, 387-391.

Fitness vs. Expression



Q: (1) Fitness ↑, no Δ expression.
(2) Expression ↑, no Δ fitness.

Nature 2002 418, 387-391.

Comparison

- mTn method
- Pros:
- Cons:

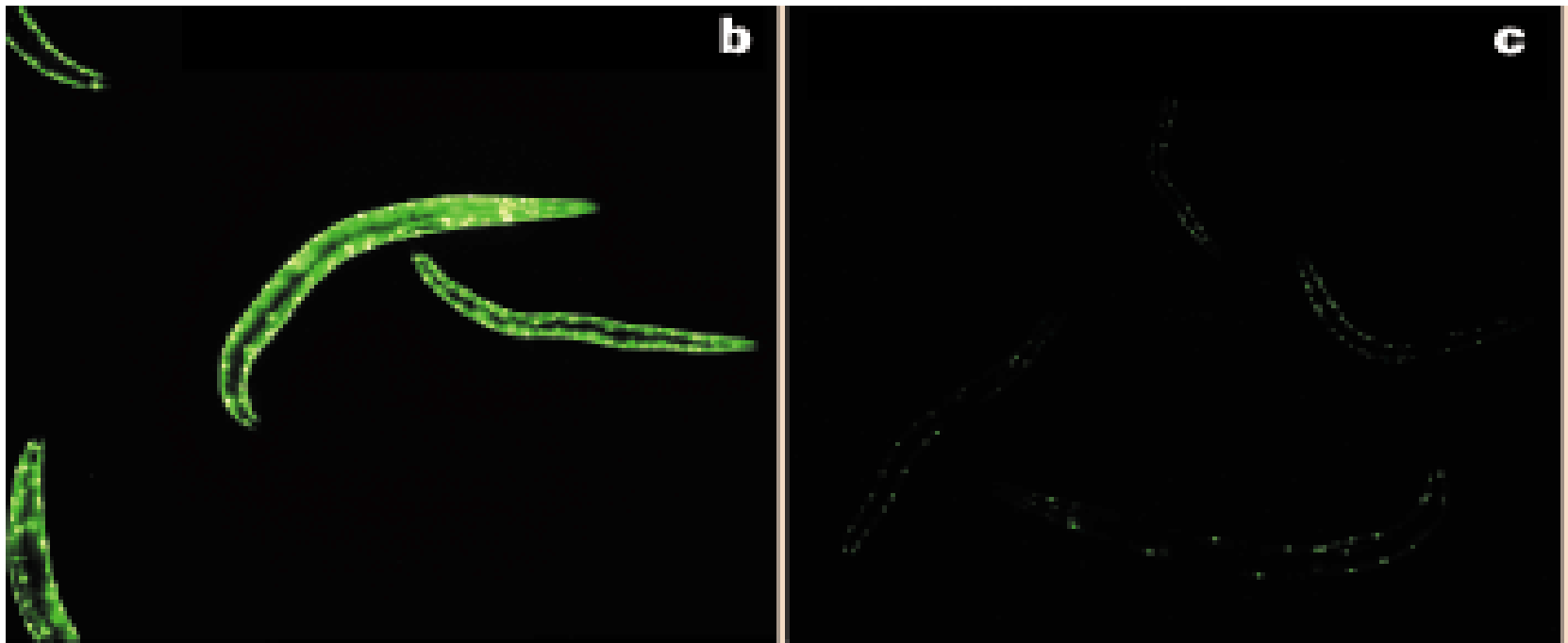
- PCR based method
  - bar code
- Pros:
- Cons:

Completed genome

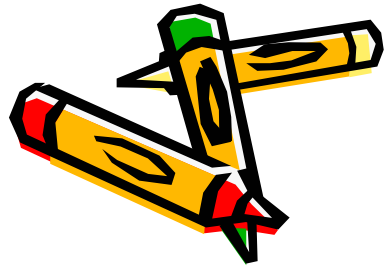
- Unicellular eukaryotes
  - Budding yeast, *Saccharomyces cerevisiae*
- Multicellular eukaryotes
  - Nematode, *Caenorhabditis elegans*
  - Fruit fly, *Drosophila melanogaster*

RNAi

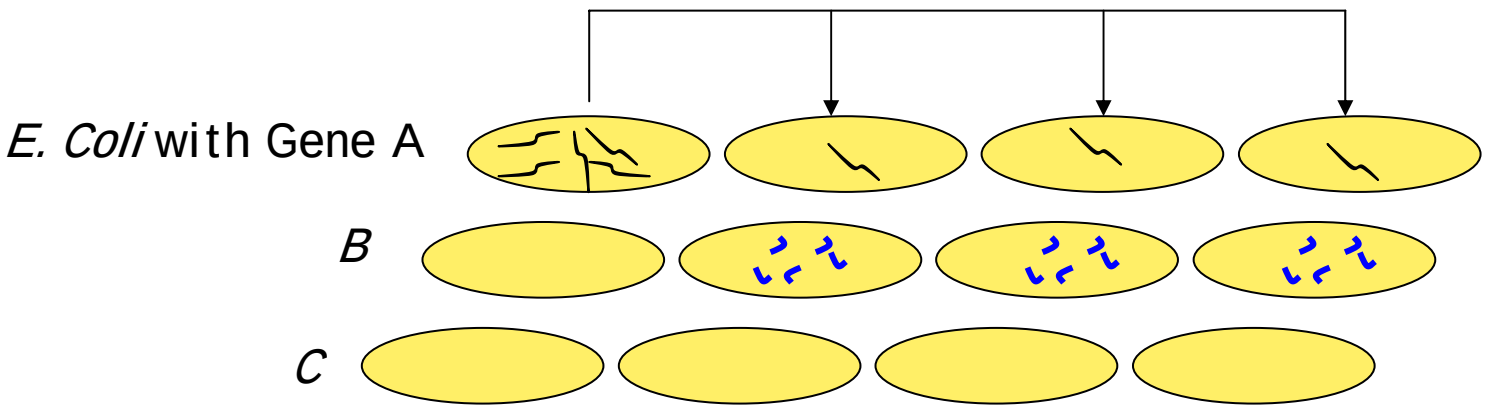
- RNA interference by Andy Fire, 1998
- RNAi transiently inhibits the activity of a target gene with a dsRNA



RNAi and *C. elegans*

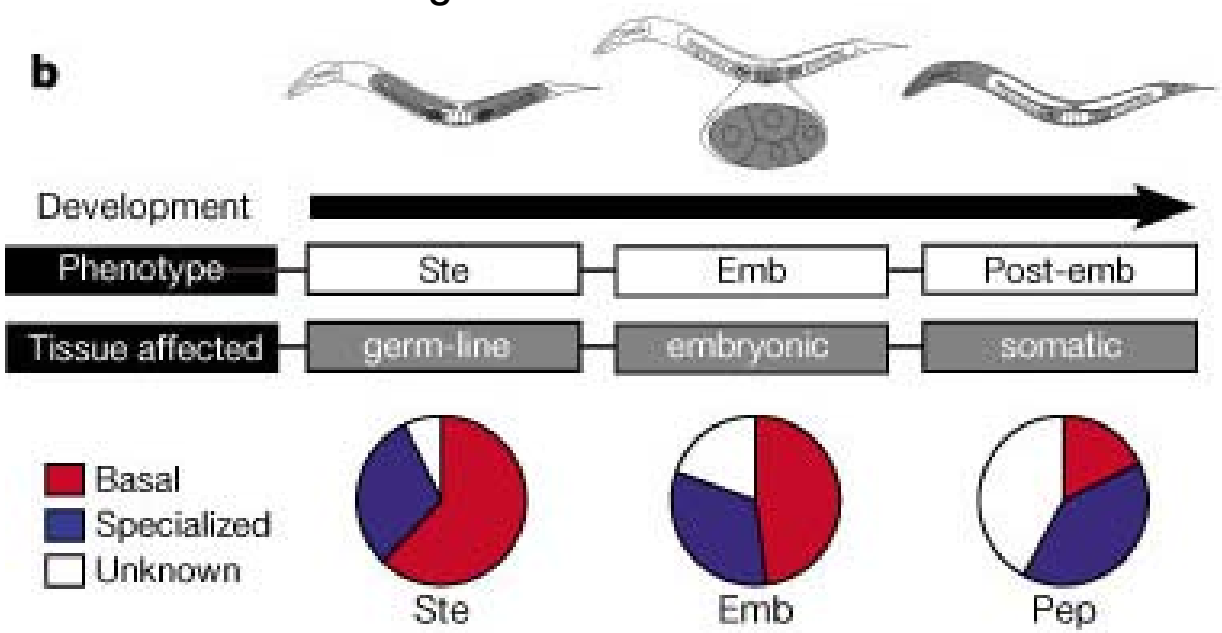


- *C. elegans* eats *E. coli* expressing specific dsRNA
- Observe phenotypes of adult and embryo development



Functional Distribution

- Genes on chromosome I of C. elegans
  - Ste: sterile
  - Emb: embryonic phenotype
  - Pep: post-embryonic phenotype
- Basal metabolic process vs. Specialized functions
  - Germline function/embryonic viability
  - Later developmental process
  - Fractions of unknown genes



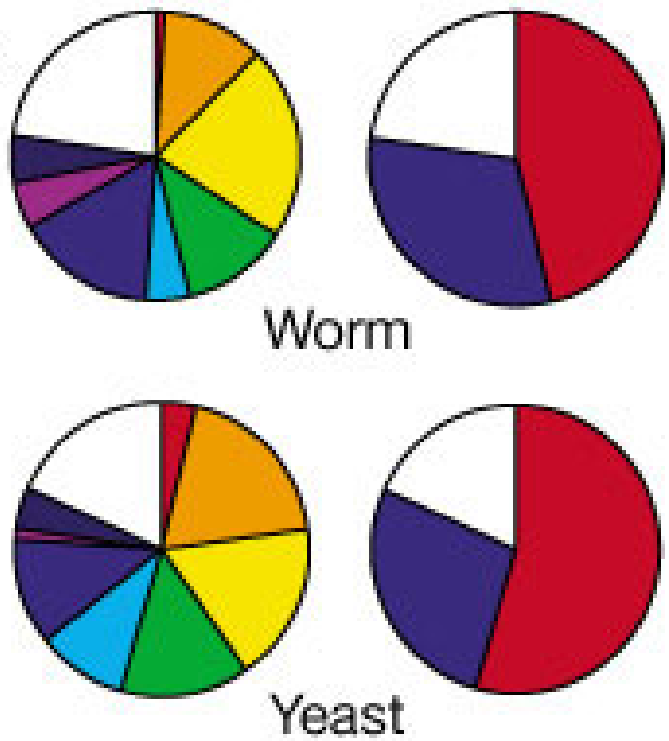
Fraser et al., 2000 *Nature* 408, 325-330.

Fraser et al., 2000 *Nature* 408, 325-330.

Worm vs. Yeast

- Genes important for viability
  - Similar distribution within the different functional classes

	Ste	Emb	Unc	Pep
DNA synthesis	1.2	1.3	0.0	2.2
RNA metabolism	4.8	11.8	4.7	8.9
Protein metabolism	44.6	22.3	7.8	4.4
Energy/metabolism	10.8	10.9	6.3	2.2
Chrme dynamics/cell cycle	0.0	6.1	0.0	2.2
Cell structure/organisation	18.1	15.7	26.6	15.6
Specific transcription	7.2	4.8	12.5	13.3
Signalling	4.8	4.8	9.4	8.9
Unknown	7.2	18.8	31.3	42.2



Comparisons

- The British group
  - Chromosome I
  - Bacterial expressed dsRNA
  - By feeding
  - Viability, and observable phenotypes
- The German group
  - Chromosome III (cell division process)
  - PCR amplified, in vitro transcription ssRNA, annealed to generate dsRNA
  - By microinjection
  - Cell-division process (time-lapse differential interference contrast microscopy)
- RNAi strategy
  - Pros
  - Cons