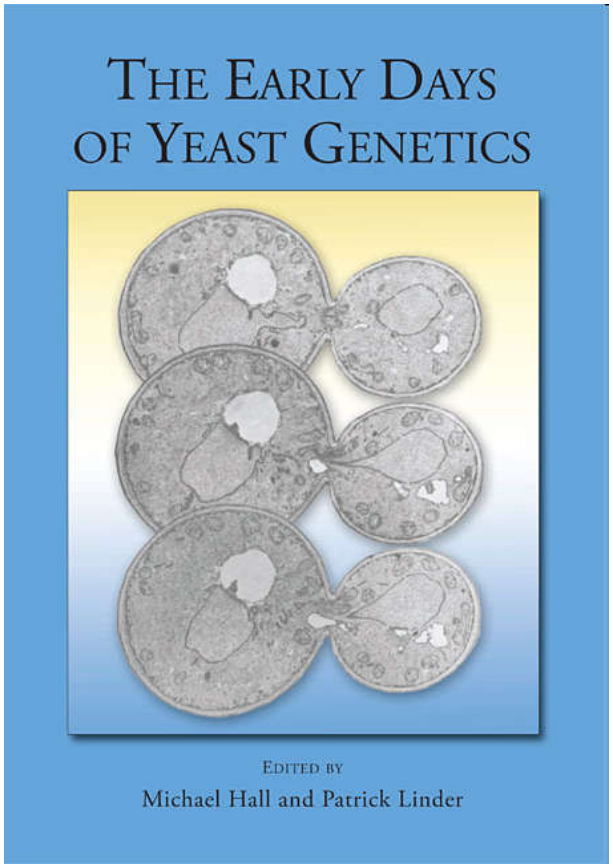
Uso de *S. cerevisiae* como organismo modelo

Mario H. Barros -

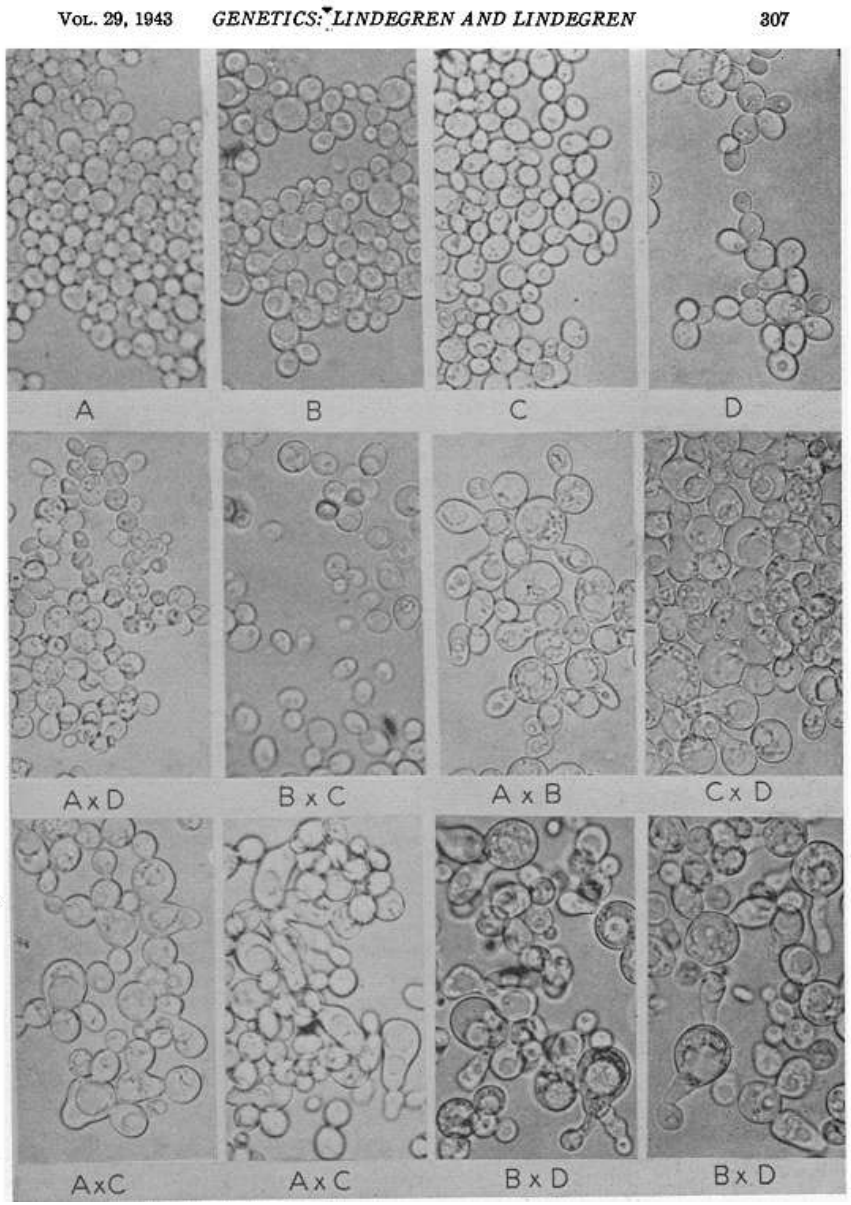
Alguns livros/textos referências para a disciplina

Livros (Cold Spring Harbor Laboratory press):

The Early Days of Yeast Genetics Edited By Michael N. Hall, *Biozentrum der Universität Basel*



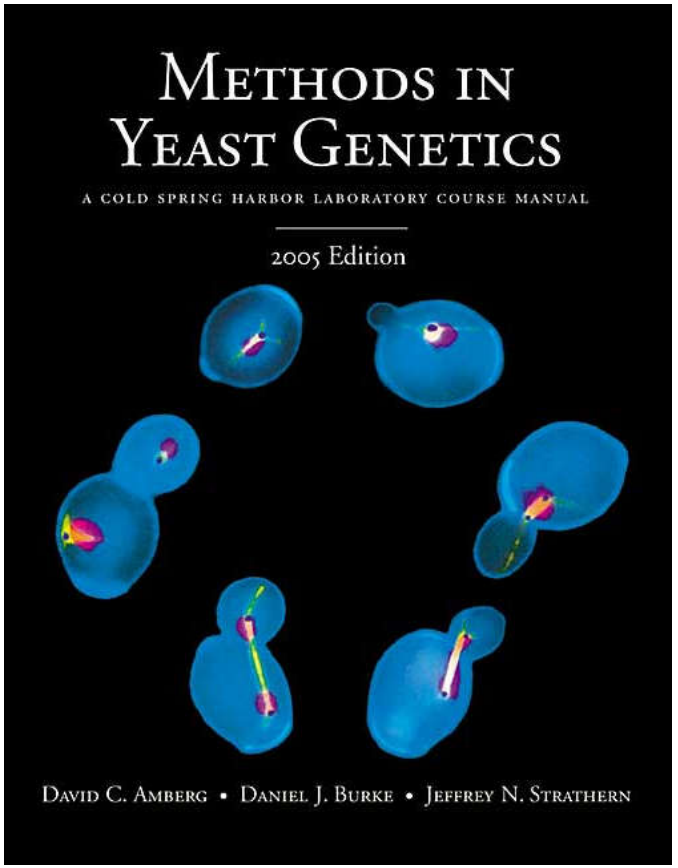
Lindegren e os primeiros cruzamentos:



Proc Natl Acad Sci U S A. **29**: 306-308 (1943)

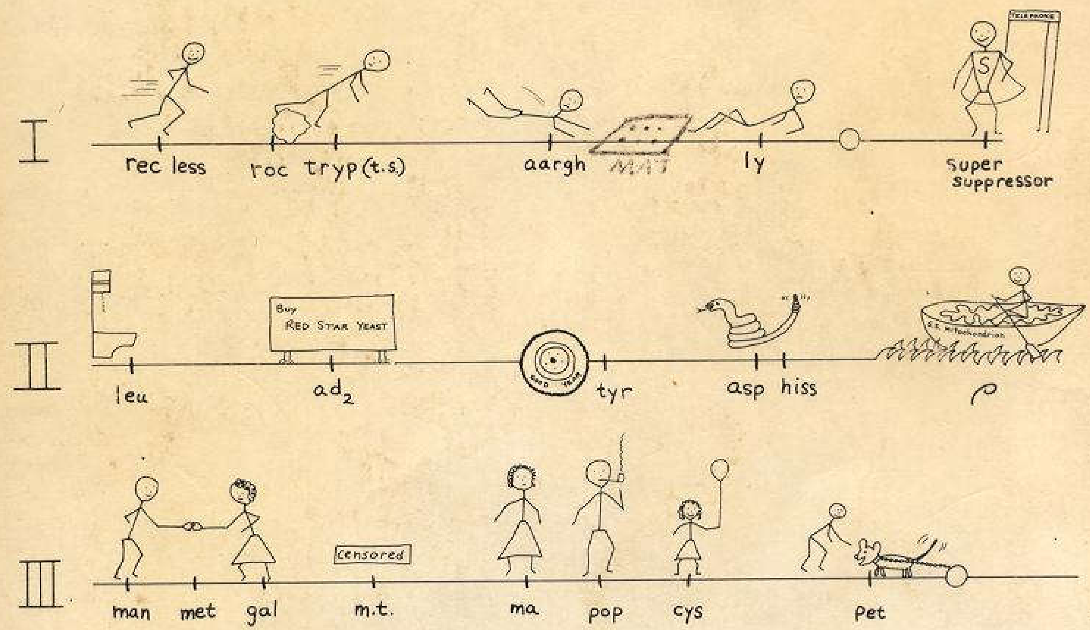
Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 2005 Edition

David C. Amberg, *Upstate Medical University, Syracuse;* Daniel J. Burke, *University of Virginia Medical Center, Charlottesville;* Jeffrey N. Strathern, *National Cancer Institute*



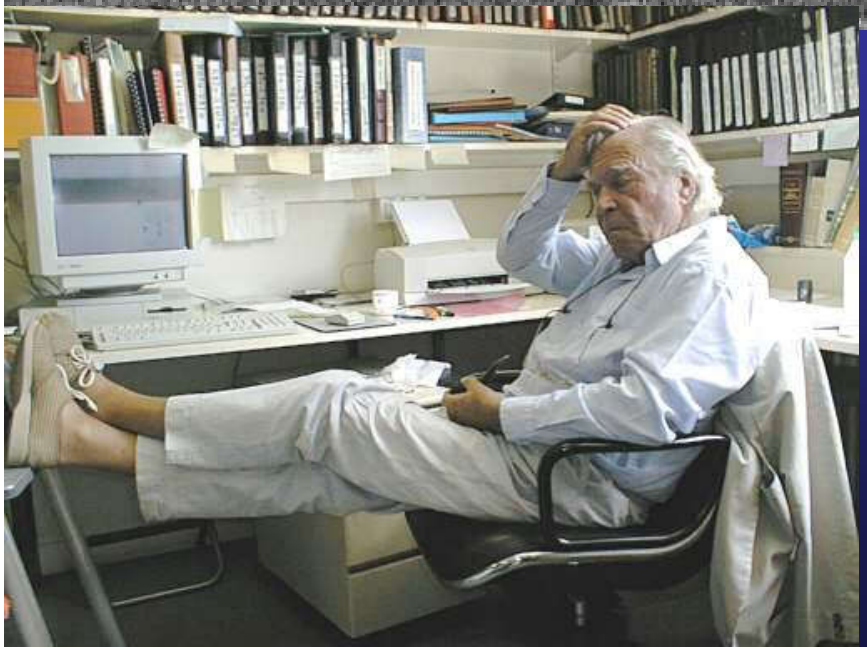
August 11–August 16, 1981

YEAST GENETICS 1970



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Yeast people



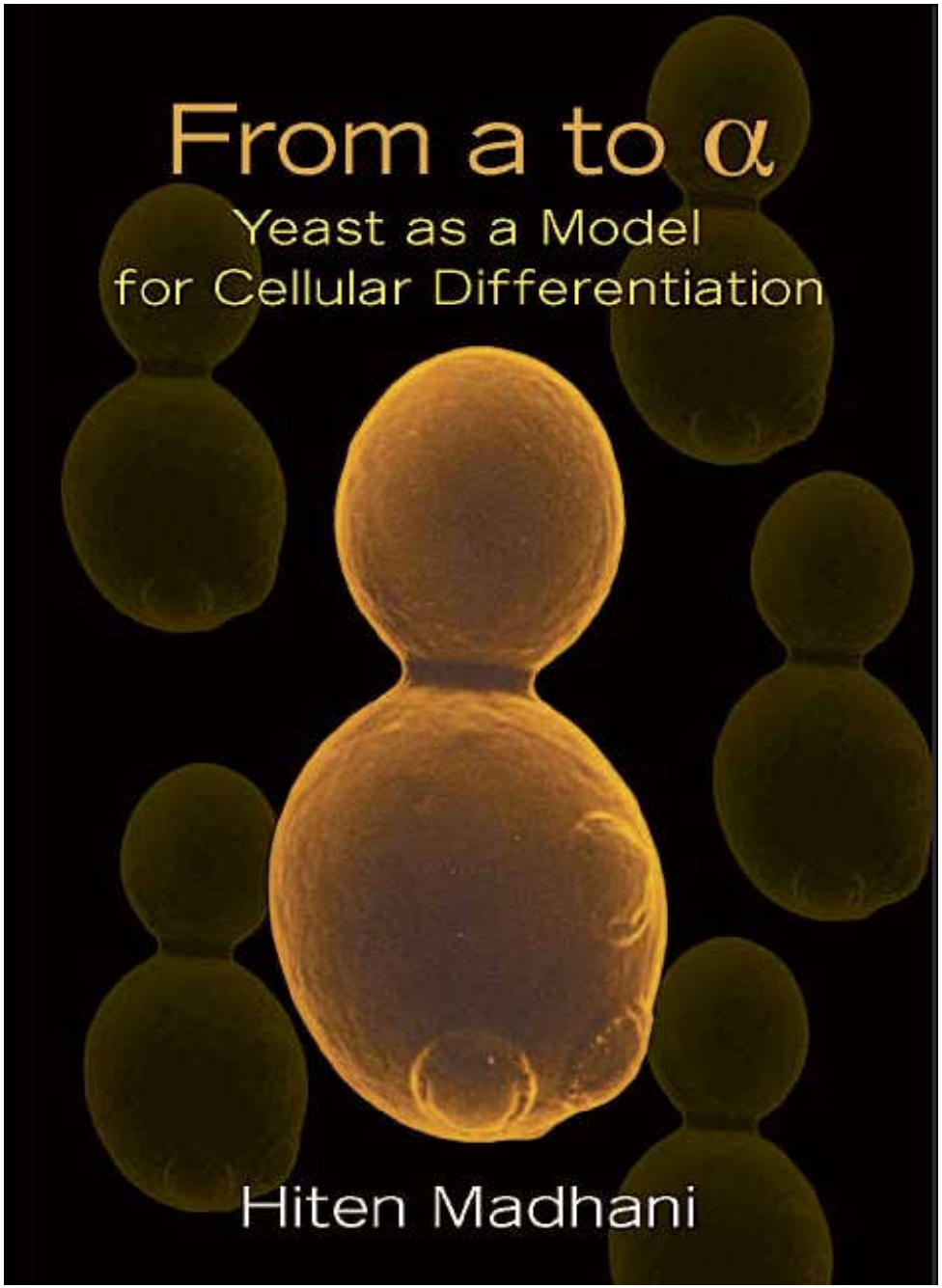
Yeast Genetics - CSHL - 1997

An Introduction to the Genetics and Molecular Biology of the Yeast *Saccharomyces cerevisiae*

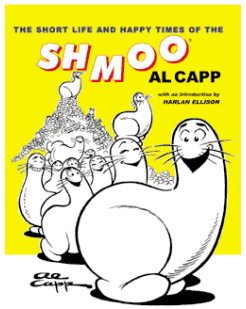
FRED SHERMAN
Department of Biochemistry and Biophysics
University of Rochester Medical School, Rochester, NY 14642

• 1998 •

The yeast *Saccharomyces cerevisiae* is clearly the most ideal eukaryotic microorganism for biological studies. The "awesome power of yeast genetics" has become legendary and is the envy of those who work with higher eukaryotes. The complete sequence of its genome has proved to be extremely useful as a reference towards the sequences of human and other higher eukaryotic genes. Furthermore, the ease of genetic manipulation of yeast allows its use for conveniently analyzing and functionally dissecting gene products from other eukaryotes.

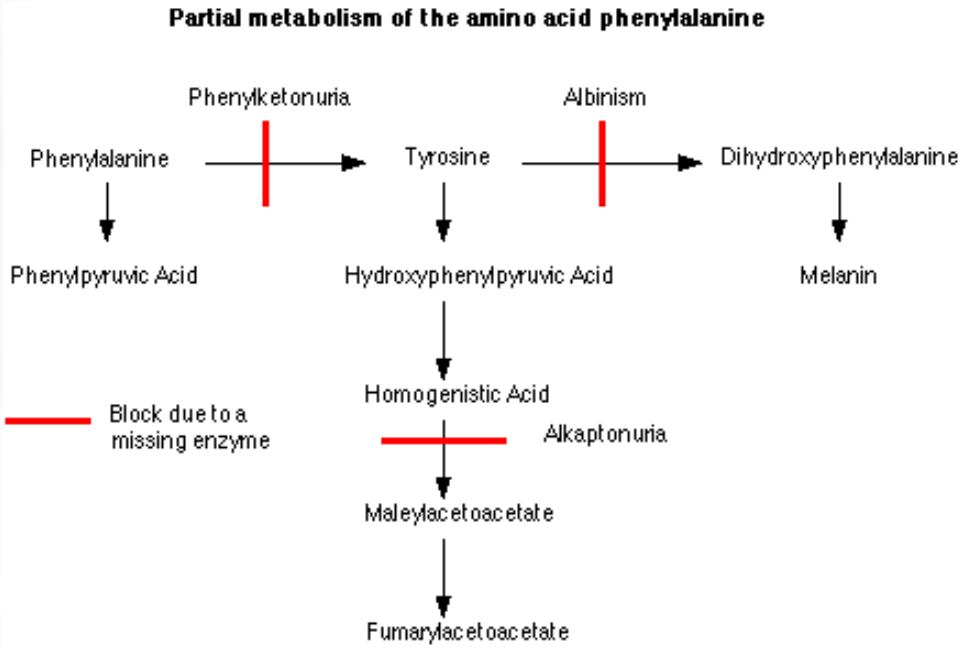

From a to α
Yeast as a Model
for Cellular Differentiation
Hiten Madhani

Saccharomyces cerevisiae



Ira Herskowitz

Archibald E. Garrod: escreveu em 1909 sobre erros inatos do metabolismo –

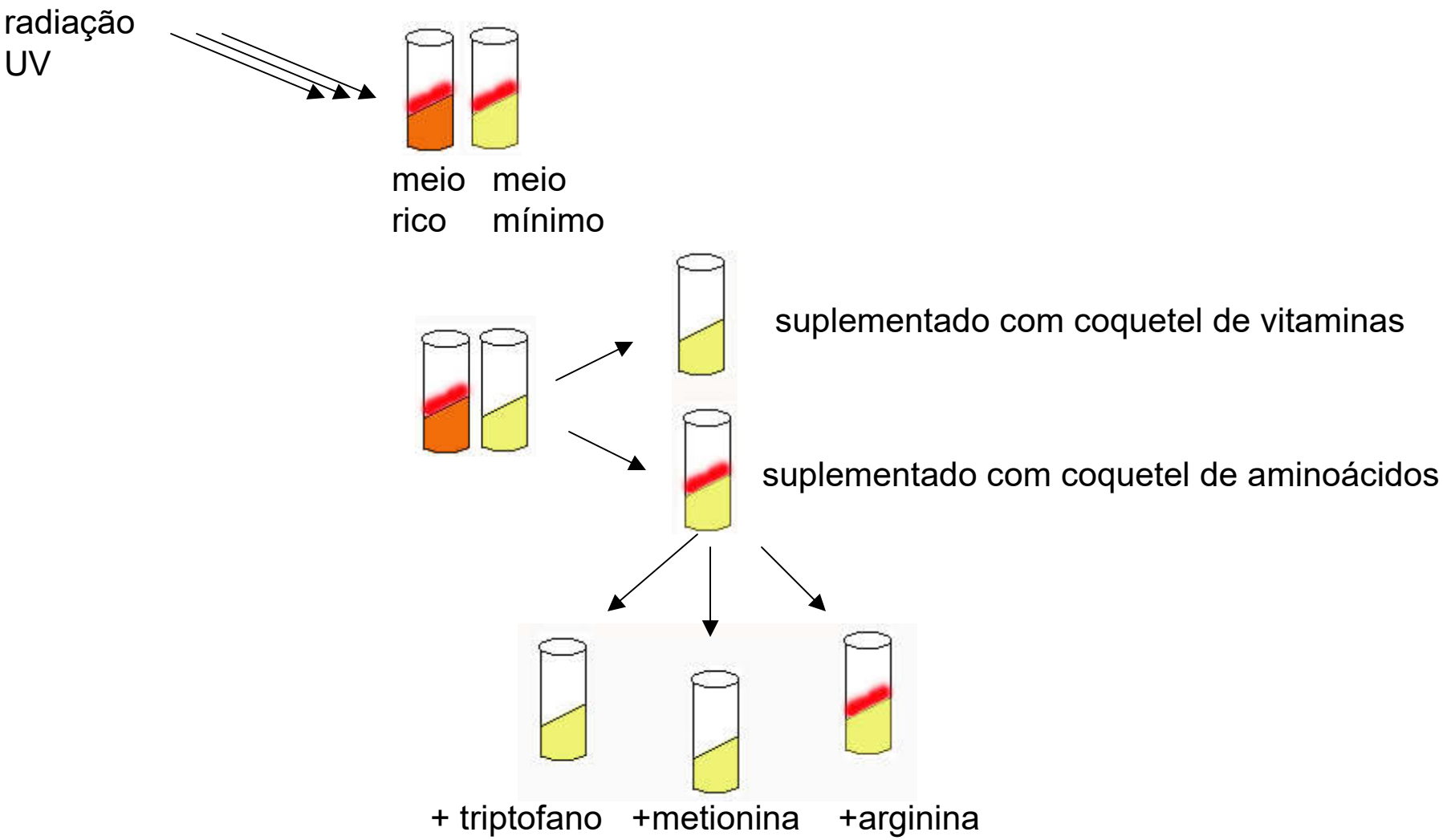


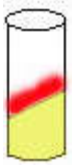
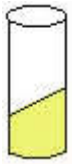
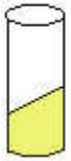
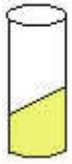
George Beadle e Edward Tatum foram condecorados com o prêmio Nobel de medicina em 1958 pelos estudos com *Neurospora* que confirmaram as hipóteses de Garrod sobre o modo de ação dos genes através das enzimas

George Wells Beadle

Edward Lawrie Tatum

Experimentos de Beadle e Tatum com *Neurospora crassa* (bolor vermelho do pão)



Mutantes	+ Arginina	+ Citrulina	+ Ornitina
A			
B			
C			

Nature Reviews | Genetics

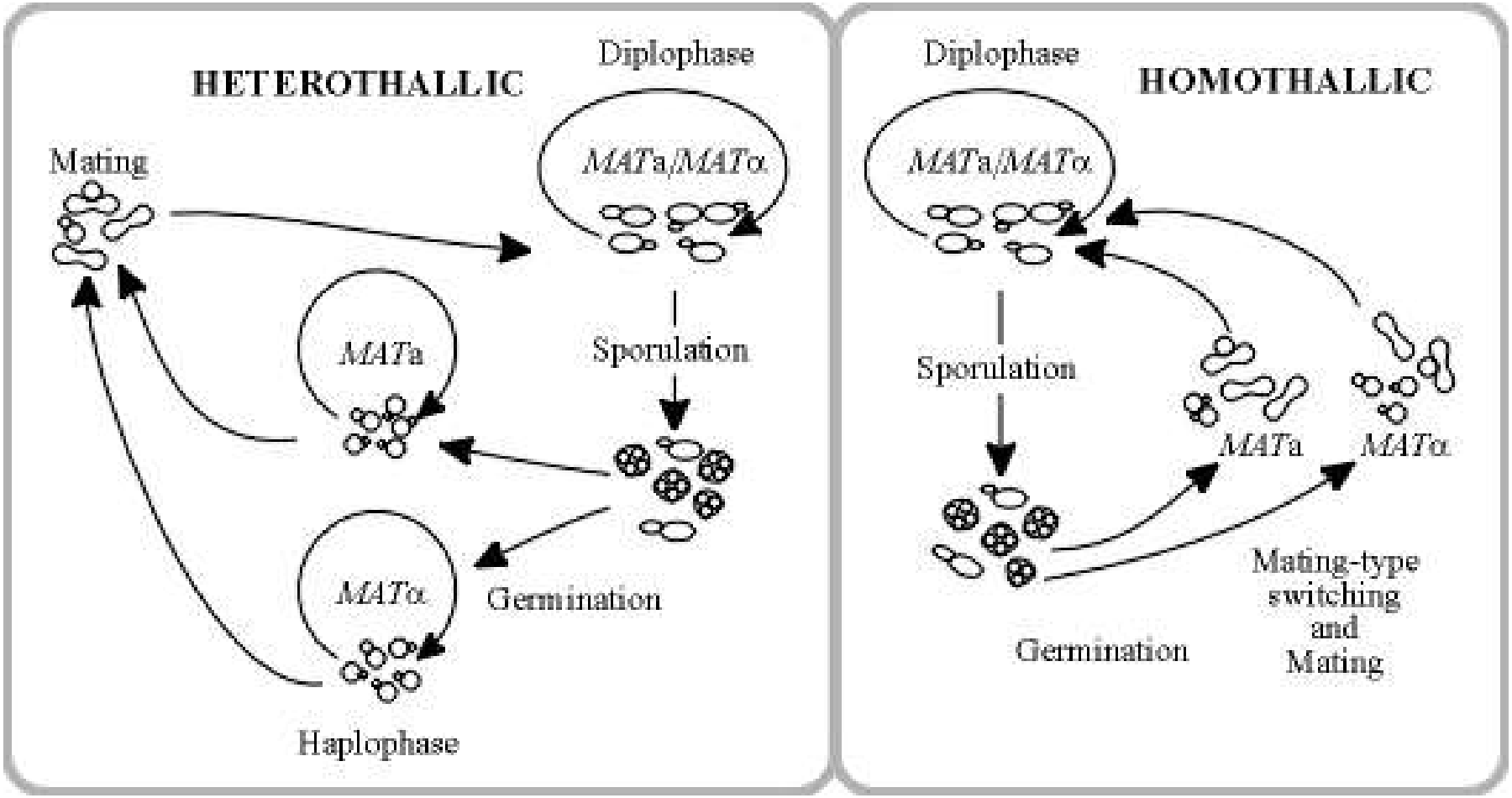
Precursor → Ornitina → Citrulina → Arginina

Enzima A Enzima B Enzima C

Yeast and Nobel Laureates

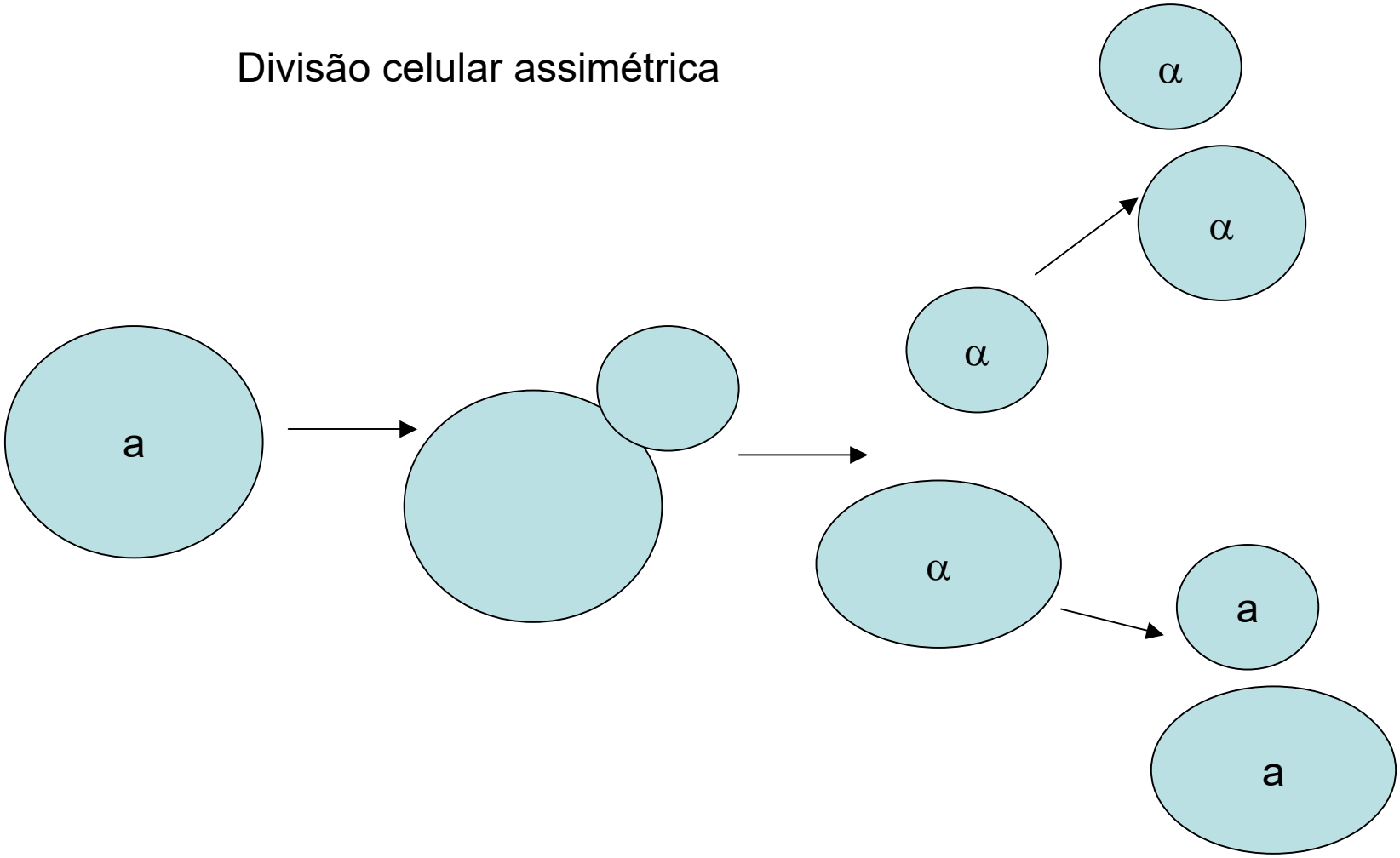
1907- Eduard Buchner	Cell Free Fermentation	(Chemistry)
.		
.		
.		
2001- Leland H. Hartwell	Key regulators of cell cycle	(Medicine)
2004 – Avram Hershko	Ubiquitin-mediated protein degradation	(Chemistry)
2006 – Rober Kornberg	Eukaryotic transcription	(Chemistry)
2009 – Elizabeth H. Blackburn	Telomeres and telomerases	(Medicine)
2013 – Randy Schekman	Secretory Pathway	(Medicine)
2016 – Yoshinori Ohsumi	Mechanisms for Autophagy	(Medicine)

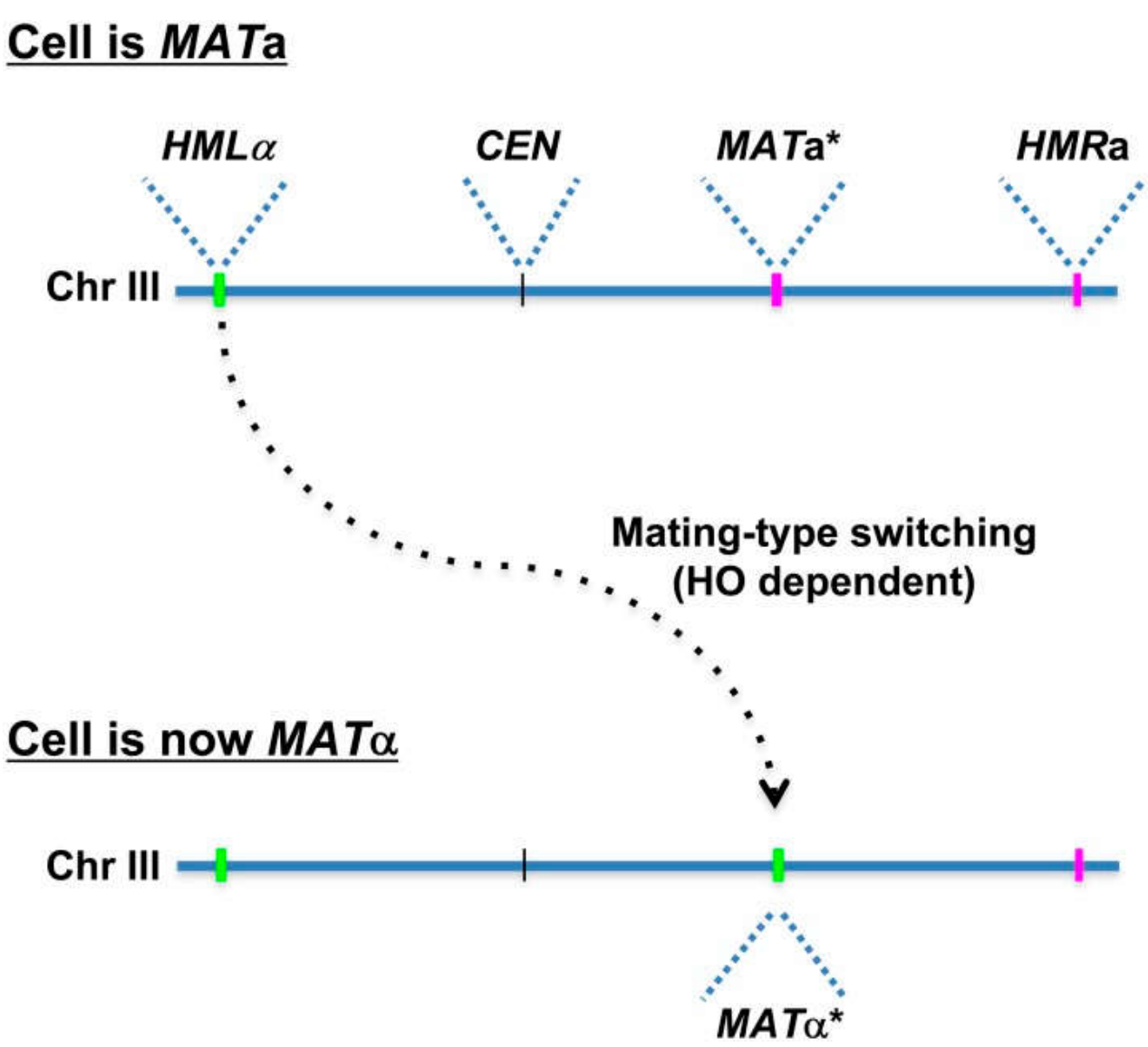
Comportamento homotálico vs heterotálico



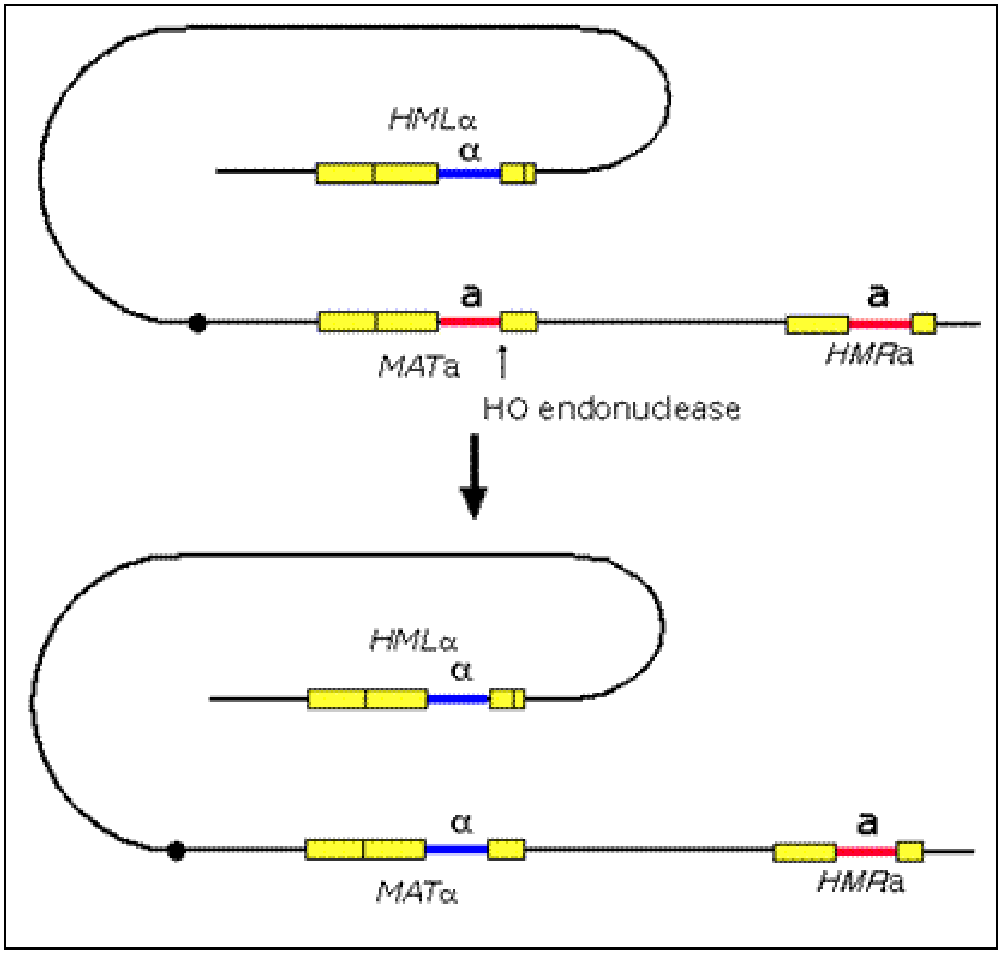
Promiscuidades de *S. cerevisiae* - o gene homotálico

Divisão celular assimétrica





Genetics, Vol. 197, 33–48 May 2014

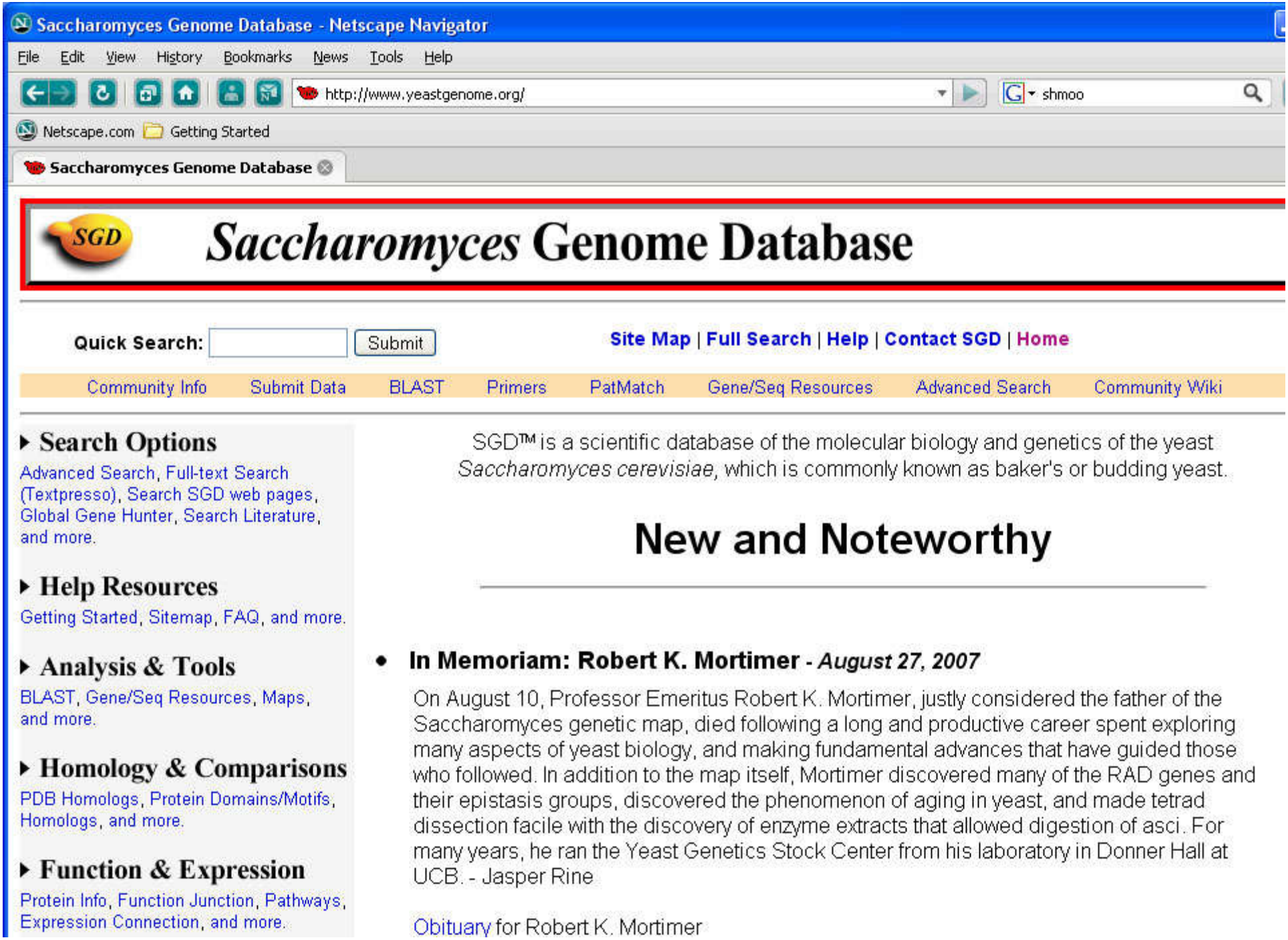

HMLα
α
a
a
MATa
HMRa
HO endonuclease
HMLα
α
α
a
MATα
HMRa

Manipulação *S. cerevisiae* vs agências e manuais de biossegurança

FDA – classifica *S. cerevisiae* como “Generally recognized as safe”

NIH – isenta maioria das manipulações de *S. cerevisiae* do seu manual de instruções

EPA – isenta *S. cerevisiae* “Toxic Substance control act”


Saccharomyces Genome Database - Netscape Navigator
File Edit View History Bookmarks News Tools Help
http://www.yeastgenome.org/
shmoo
Netscape.com Getting Started
Saccharomyces Genome Database
SGD
Saccharomyces Genome Database
Quick Search: Submit
Site Map | Full Search | Help | Contact SGD | Home
Community Info Submit Data BLAST Primers PatMatch Gene/Seq Resources Advanced Search Community Wiki
Search Options
Advanced Search, Full-text Search (Textpresso), Search SGD web pages, Global Gene Hunter, Search Literature, and more.
Help Resources
Getting Started, Sitemap, FAQ, and more.
Analysis & Tools
BLAST, Gene/Seq Resources, Maps, and more.
Homology & Comparisons
PDB Homologs, Protein Domains/Motifs, Homologs, and more.
Function & Expression
Protein Info, Function Junction, Pathways, Expression Connection, and more.
SGD™ is a scientific database of the molecular biology and genetics of the yeast Saccharomyces cerevisiae, which is commonly known as baker's or budding yeast.
New and Noteworthy
In Memoriam: Robert K. Mortimer - August 27, 2007
On August 10, Professor Emeritus Robert K. Mortimer, justly considered the father of the Saccharomyces genetic map, died following a long and productive career spent exploring many aspects of yeast biology, and making fundamental advances that have guided those who followed. In addition to the map itself, Mortimer discovered many of the RAD genes and their epistasis groups, discovered the phenomenon of aging in yeast, and made tetrad dissection facile with the discovery of enzyme extracts that allowed digestion of asci. For many years, he ran the Yeast Genetics Stock Center from his laboratory in Donner Hall at UCB. - Jasper Rine
Obituary for Robert K. Mortimer

Características das Linhagens de *S. cerevisiae*

As linhagens "selvagens" de laboratório carregam caracteres ditos "mutantes".

São geneticamente incompatíveis com linhagens domesticadas, e as utilizadas na fermetação cerveja , pão.

Umas são impróprias para um determinado estudo , enquanto outras linhagens não servem para outros estudos.

many freely interbreeding species of the budding yeast *Saccharomyces* and to the fission yeast *Schizosaccharomyces pombe*. Although "*Saccharomyces cerevisiae*" is commonly used to designate many of the laboratory stocks of *Saccharomyces* used throughout the world, it should be pointed out that most of these strains originated from the interbred stocks of Winge, Lindegren, and others who employed fermentation markers not only from *S. cerevisiae* but also from *S. bayanus, S. carlsbergensis, S. chevalieri, S. chodati, S. diastaticus*, etc. Nevertheless, it is still recommended that the interbreeding laboratory stocks of *Saccharomyces* be denoted as *S. cerevisiae*, in order to conveniently distinguish them from the more distantly related species of *Saccharomyces*.

Care should be taken in choosing strains for genetic and biochemical studies. Unfortunately there are no truly wild-type *Saccharomyces* strains that are commonly employed in genetic studies. Also, most domesticated strains of brewers' yeast and probably many strains of bakers' yeast and true wild-type strains of *S. cerevisiae* are not genetically compatible with laboratory

Table 4.1. Size and composition of yeast cells

Characteristic	Haploid cell	Diploid cell
Volume (μm^3)	70	120
Composition (10^{-12} g)		
Wet weight	60	80
Dry weight	15	20
DNA	0.017	0.034
RNA	1.2	1.9
Protein	6	8

Genoma Levedura:

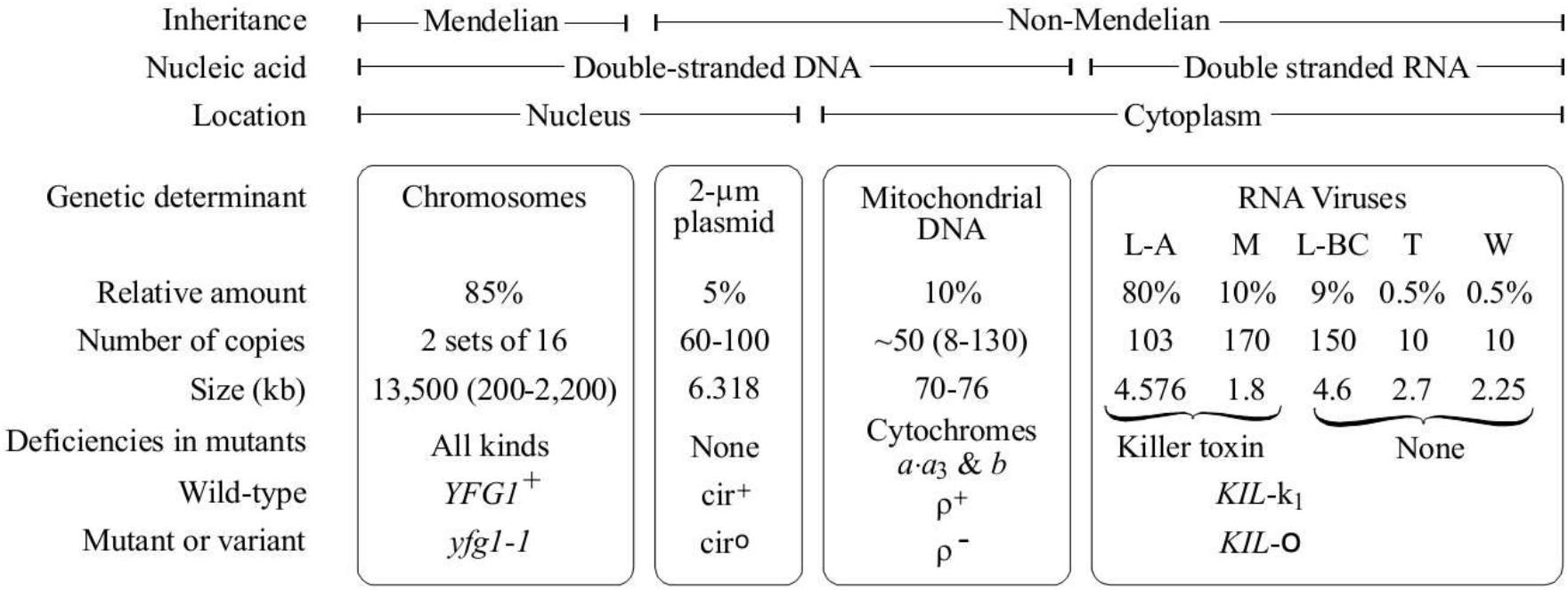


Figure 2. The genome of a diploid cell of *S. cerevisiae* (see the text). A wild-type chromosomal gene is depicted as *YFG1*$^+$ (Your Favorite Gene) and the mutation as *yfg1-1*.

12.052 kb - 72% seq ORFs

3,8% ORFs com introns

Table 6.1. Genetic nomenclature, using *ARG2* as an example

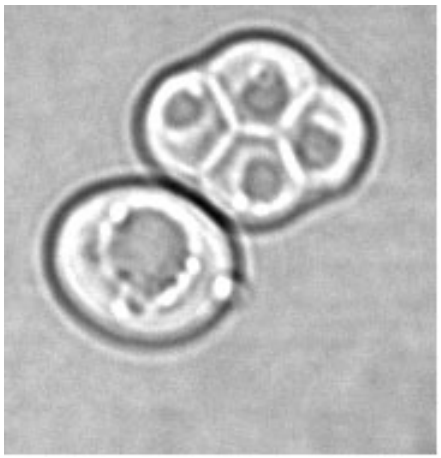
Gene symbol	Definition
ARG+	All wild-type alleles controlling arginine requirement
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
arg2-	Any arg2 allele confering an arginine requirement
ARG2+	The wild-type allele
arg2-9	A specific allele or mutation
Arg+	A strain not requiring arginine
Arg-	A strain requiring arginine
Arg2p	The protein encoded by ARG2
Arg2 protein	The protein encoded by ARG2
ARG2 mRNA	The mRNA transcribed from ARG2
arg2-D1	A specific complete or partial deletion of ARG2
ARG2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *ARG2* remains functional and dominant
arg2::*LEU2*	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *arg2* is or became nonfunctional
arg2-10::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and the specified arg2-10 allele which is nonfunctional
cyc1-arg2	A fusion between the *CYC1* and *ARG2* genes, where both are nonfunctional
PCYC1-ARG2	A fusion between the *CYC1* promoter and *ARG2*, where the *ARG2* gene is functional

Table 6.4. Nomenclature of presumptive prions exhibiting non-Mendelian inhertance

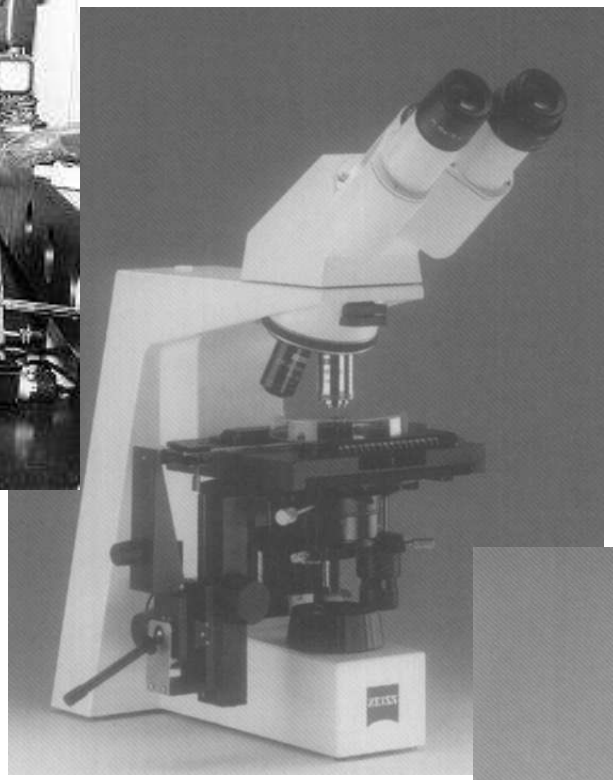
Prion state		Putative gene	
Positive	Negative	product	Phenotype of negative state
ψ^+	ψ^-	Sup35p	Decreased efficiency of certain suppression
ξ^+	ξ^-	Sup35p	Decreased efficiency of certain suppression
[*URE3*]	[*ure3*$^-$]	Ure2p	Deficiency in ureidosuccinate utilization

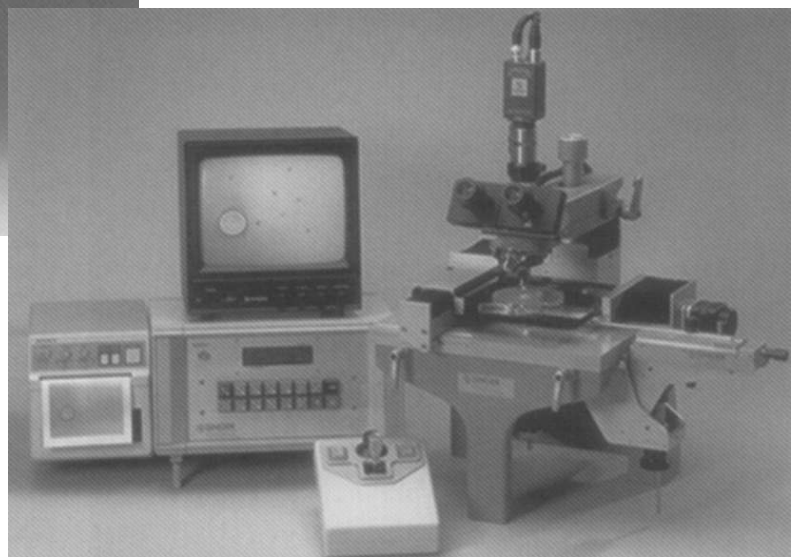
The awesome power of yeast genetics

Micromanipulador de tetrades

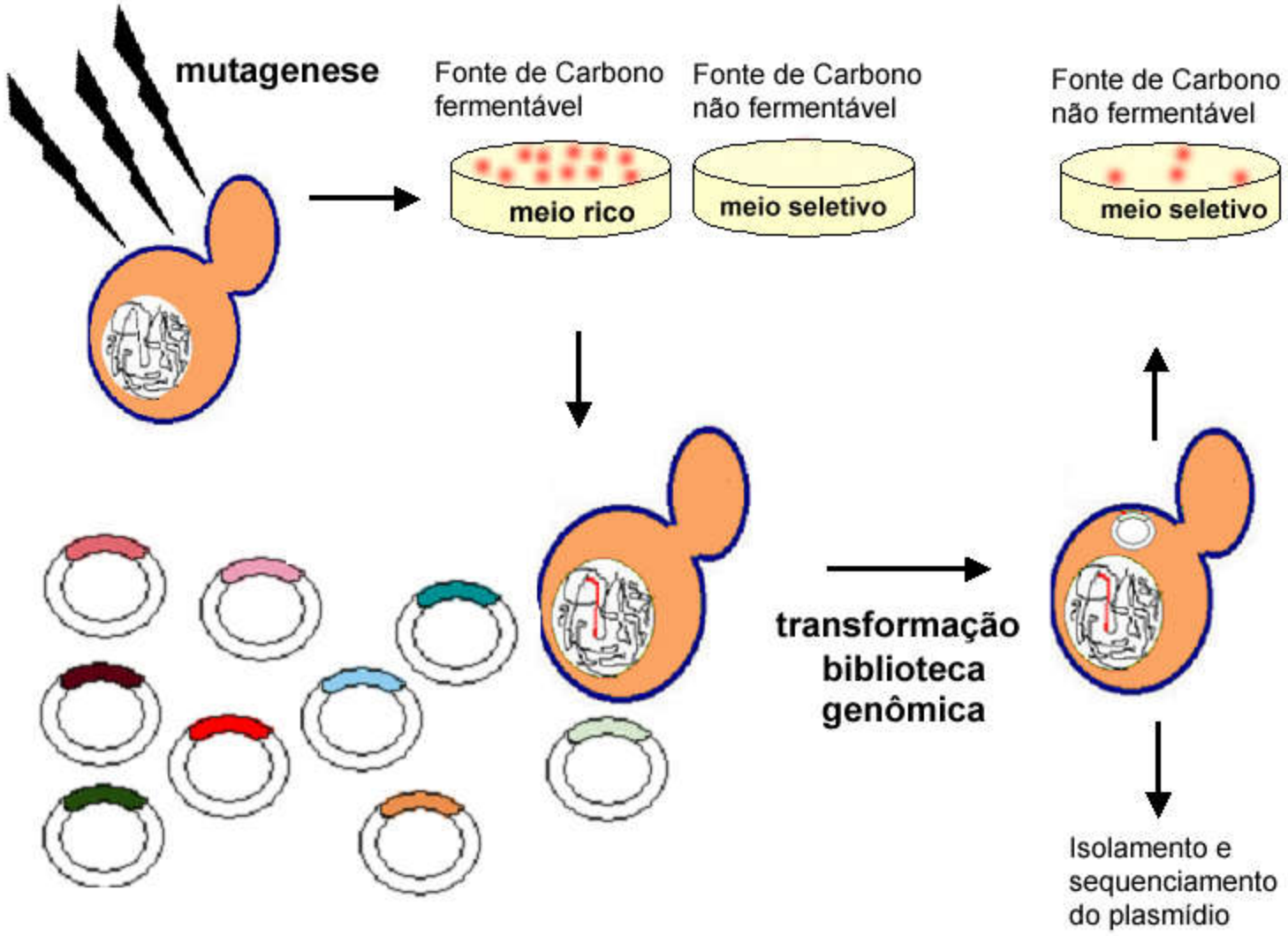


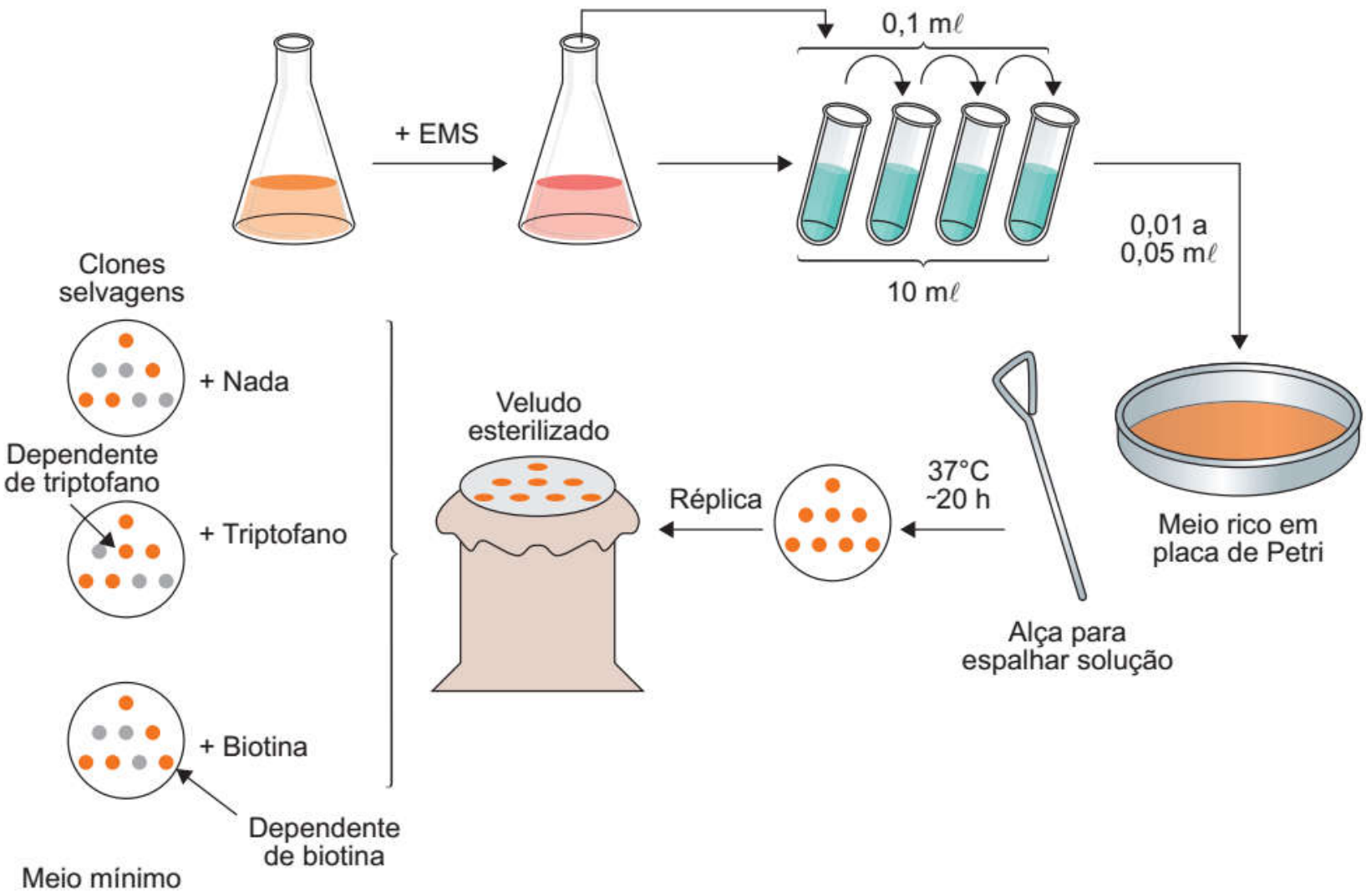
Amar Klar





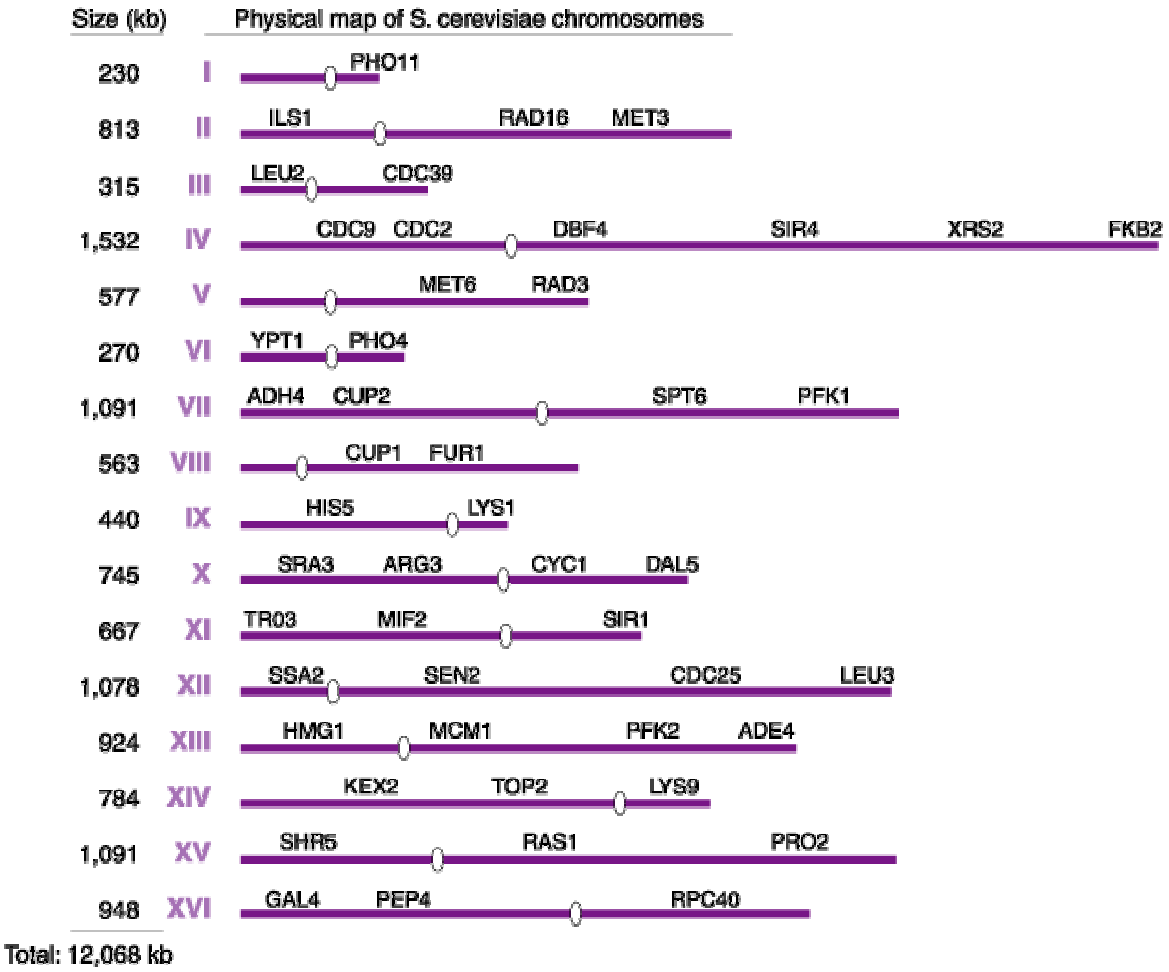
A manipulação genética é favorável e de fácil execução, como no isolamento de mutantes com deficiência respiratória




+ EMS
0,1 mℓ
10 mℓ
0,01 a
0,05 mℓ
Meio rico em
placa de Petri
Alça para
espalhar solução
37°C
~20 h
Réplica
Veludo
esterilizado
Clones
selvagens
+ Nada
Dependente
de triptofano
+ Triptofano
+ Biotina
Dependente
de biotina
Meio mínimo

1º Eucarioto a ter seu genoma sequenciado (1996)

Transcriptômica , Proteômica, Metabolômica

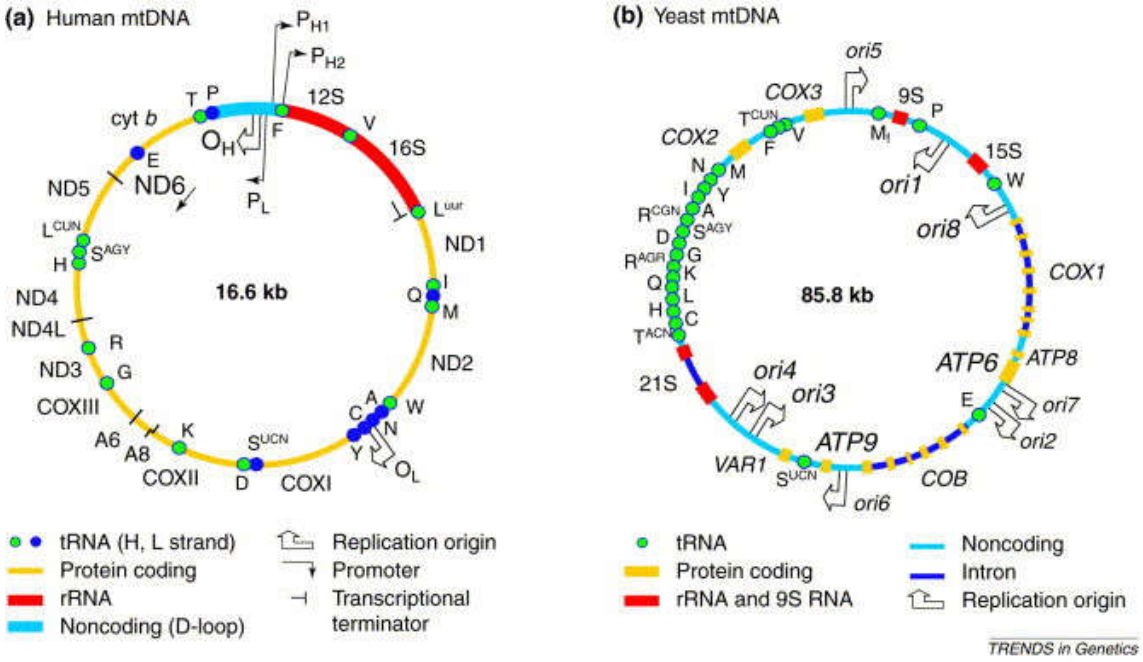


Hemiascomycetous yeast genomes are intron-poor.

	S. cerevisiae	*S. servazzii*	*S. kluyveri*	*K. marxianus*	*C. tropicalis*	*D. hansenii*	*P. angusta*	*Y. lipolytica*
Number of detected introns	260	23	36	19	12	14	30	19
Total number of genes	5261	1410	1406	1301	1130	1119	2320	1078
Number of genes with introns	252	22	36	17	11	12	27	19
Proportion of genes with introns [a]	4.8	1.6	2.6	1.3	1.0	1.1	1.2	1.7

[a] Expressed in percentages

Bon *et al.*, 2003

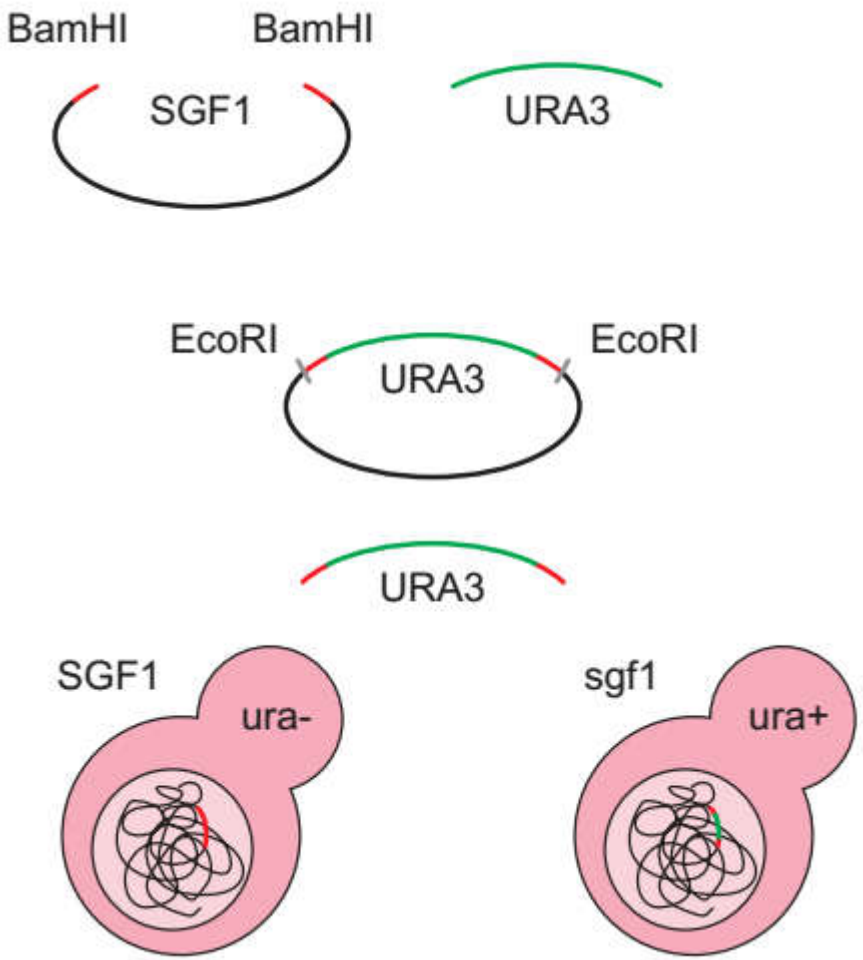


Disponibilidade de mutantes nulos para cada uma das 6000 ORFs (desde 1999)

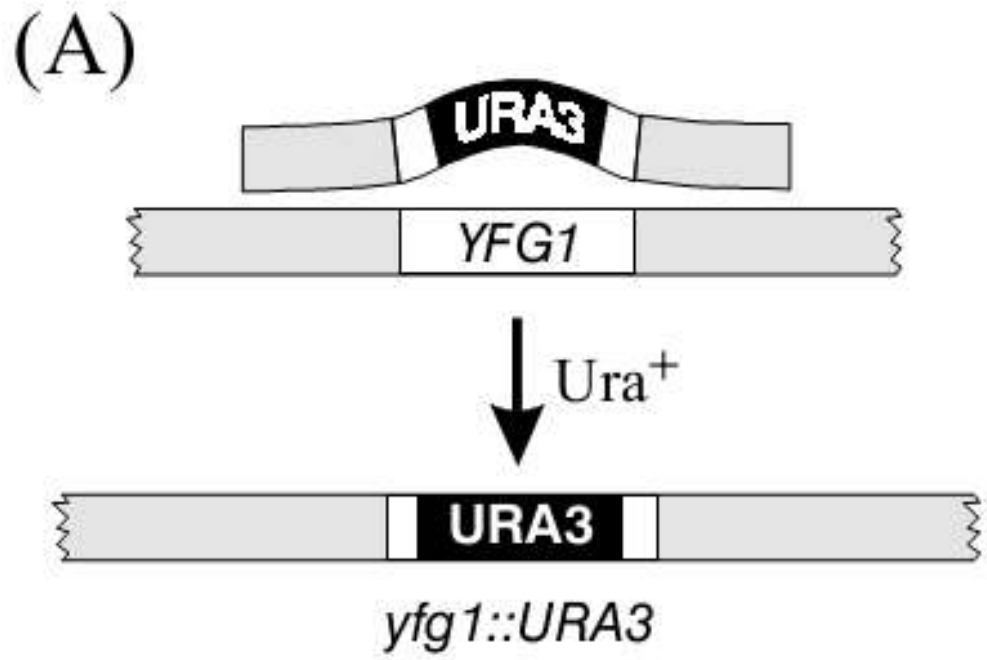
Genética clássica	Genética reversa
Fenótipo mutante ↓	Sequência DNA ↓
Alelo mutante	Alelo mutante
Sequência DNA ↓	Fenótipo mutante ↓

Esquema para inativação
Do gene SGF1

Emprego da Genética Reversa



Necessidade de pareamento homólogo entre segmentos de DNA para recombinação ocorrer



Modified from: F. Sherman, Yeast genetics. •
The Encyclopedia of Molecular Biology and Molecular Medicine, •
pp. 302-325, Vol. 6. Edited by R. A. Meyers, VCH Publisher, Weinheim, Germany, 1997.•

Uso de plasmídios tal qual em *Escherichia coli*

Tipos de Plasmídios:
Multicópias
Integrativos
Centroméricos

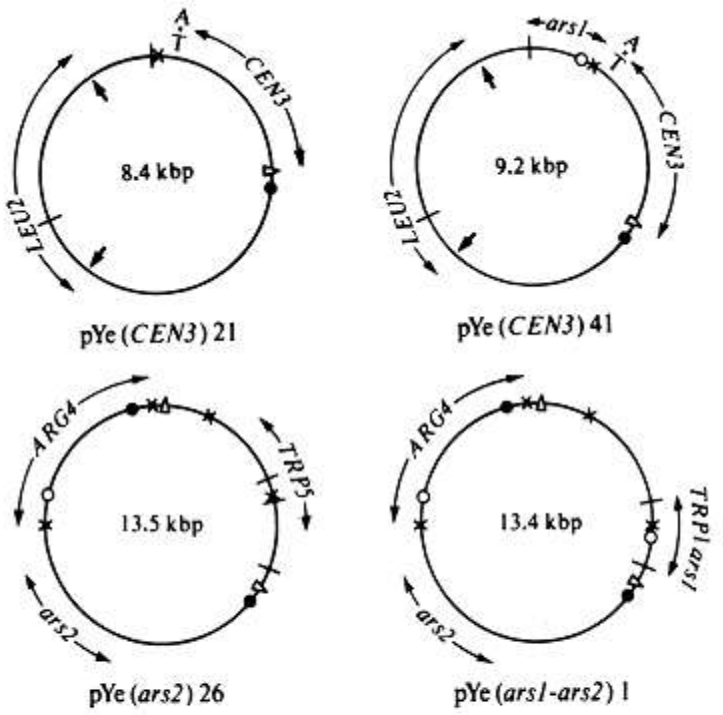


Fig. 2 Physical maps of various plasmid DNAs. The maps show the location of *Eco*RI (–|–), *Hin*dIII (–×–), *Bam*HI (–△–), *Pst*I (–↑–), *Sal*I (–●–) and *Bgl*II (–○–) sites in the DNAs and indicate approximate locations of pertinent replicators and genes. The construction and use of these plasmids is described in the text. Their sizes are indicated in kilobase pairs (kbp).

Nature. Vol 287, No. 5782, pp. 504-509, October 9 1980

Características dos plasmídios de levedura

Table 5. Components of common yeast plasmid vectors

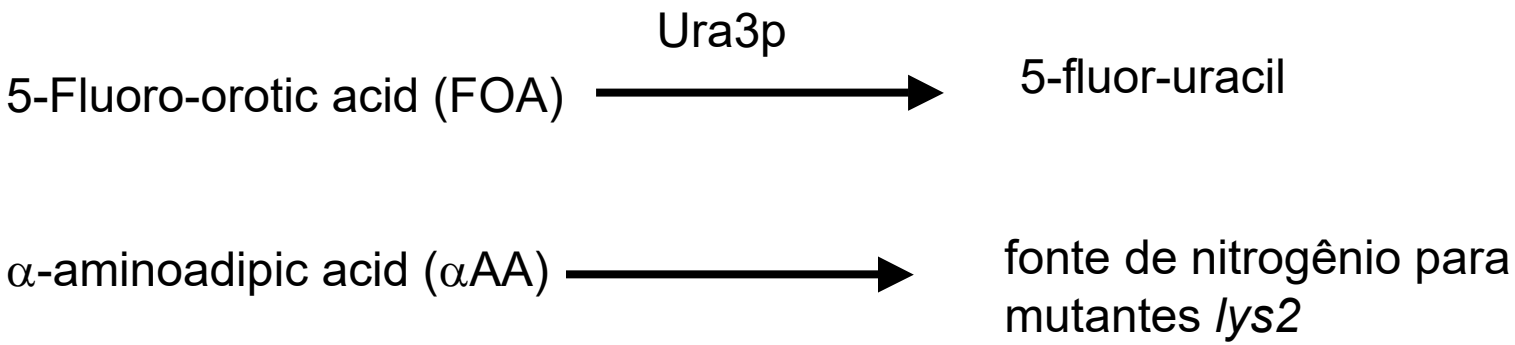
	YIp	YEp	YRp	YCp
Plasmid				
E. coli genes or segments				
ori, *bla*; *tet*	+	+	+	+
Yeast genes or segments				
URA3; *HIS3*; *LEU2*; *TRP1*; *LYS2*; *etc.*	+	+	+	+
leu2-d	0	+	+	0
2 μm; 2 μm-*ori REP3*;	0	+	0	0
ARS1; *ARS2*; *ARS3*; *etc.*	0	0	+	+
CEN3; *CEN4*; *CEN11*; *etc.*	0	0	0	+
Host (yeast) markers				
ura3-52; *his3-Δ1*; *leu2-Δ1*; *trp1-Δ1*; *lys2-201*; *etc.*	+	+	+	+
Stability	++	+	±	+

Modified from: F. Sherman, Yeast genetics. •
The Encyclopedia of Molecular Biology and Molecular Medicine, •
pp. 302-325, Vol. 6. Edited by R. A. Meyers, VCH Publisher, Weinheim, Germany, 1997.•

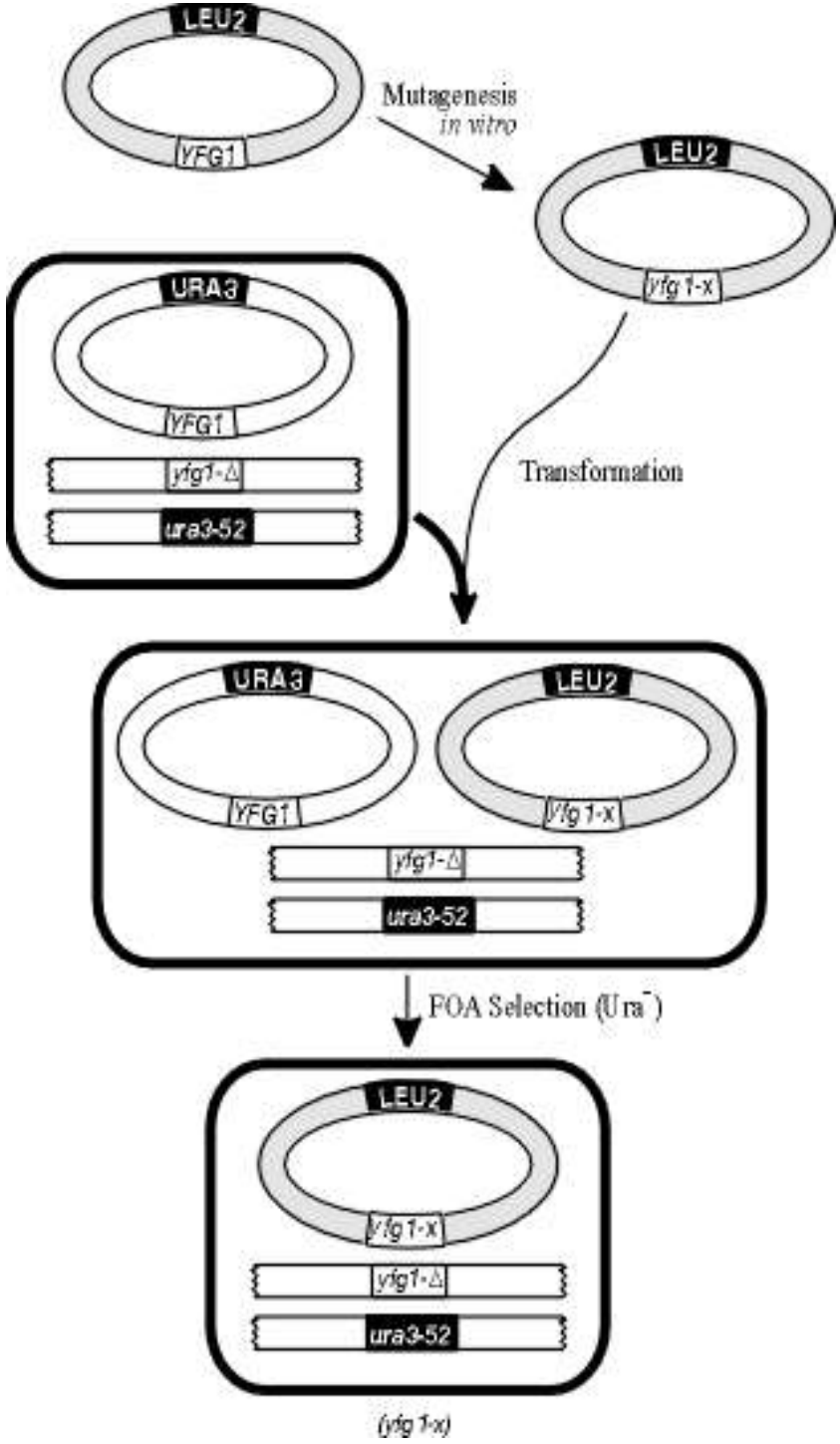
Marcas de seleção mais comuns em plasmídios/linhagens de levedura

GENE	ENZIMA	SELEÇÃO
HIS3	Imidazol glicerolfosfato desidratase	Histidina
LEU2	β-Isopropilmalato desidrogenase	Leucina
LYS2	α-Aminoadipato redutase	Lisina
TRP1	N-(5'-fosforibosil)- antranilato isomerase	Triptofano
URA3	Orotidina-5'fosfato decarboxilase	Uracil

Seleção Positiva e negativa:
válida para os genes *URA3 e LYS2*:



Troca de plasmídios



Estudo de Mutantes:

→Mutantes nulos (genética reversa)

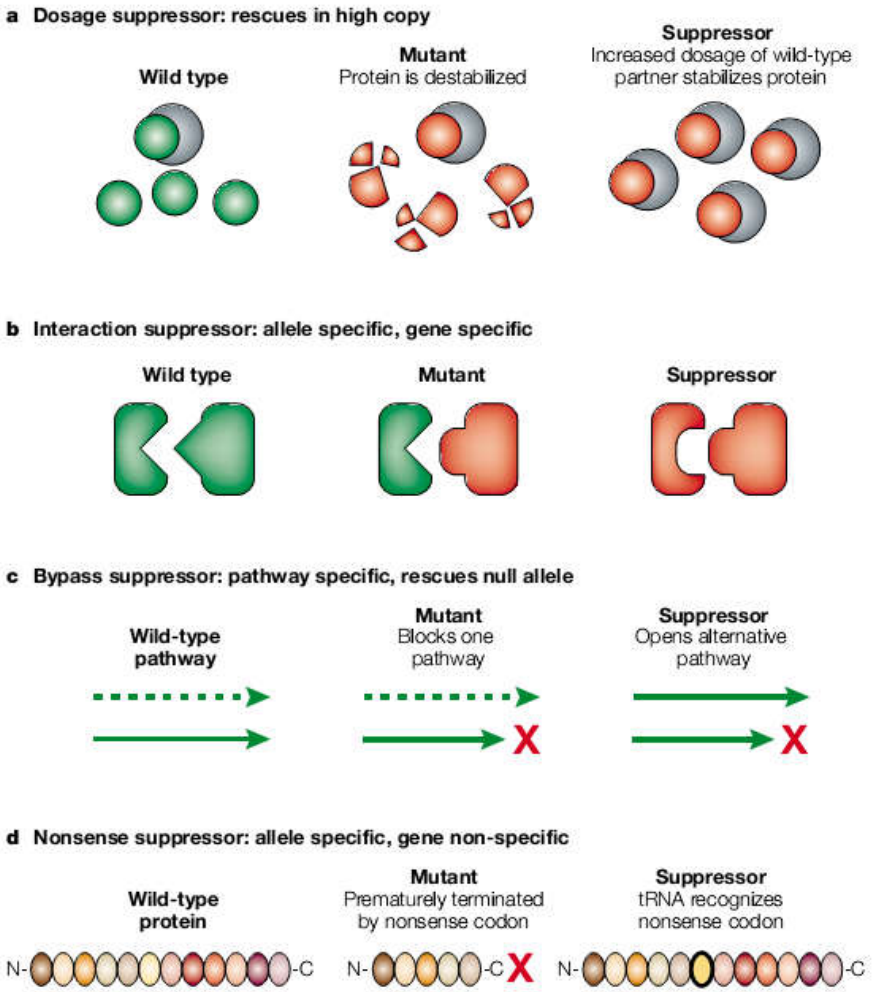
→Mutantes condicionais (sensível a temperatura)

→Mutações dominantes (como determinar se um mutante hisX é dominante?)

→Mutações Supressoras (por epistasia – por excesso – por desvio de via)

→Mutações Letais (como definir? – sintéticos letais?)

Tipos de supressão genética

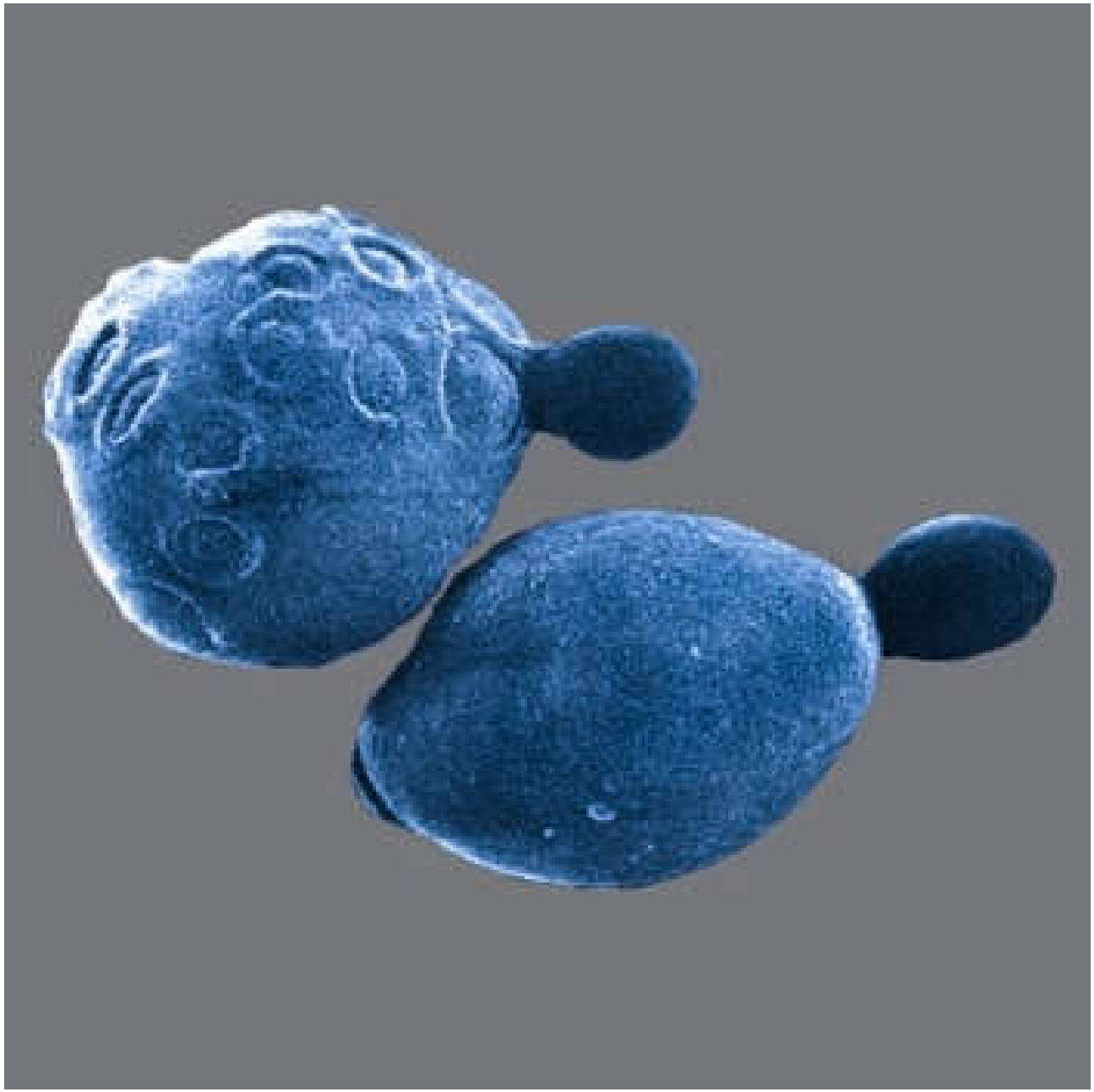


NATURE REVIEWS | GENETICS
VOLUME 2 | SEPTEMBER 2001 | 663

Figure 3 | **Suppressor mechanisms.** Depending on the allele and gene specificity associated with suppressors, mechanisms can be inferred, as shown. **a** | Dosage suppressors encode proteins that stabilize the mutant product when they are expressed at high levels. **b** | An interaction suppressor restores the interaction between the mutant product and its partner(s). **c** | A bypass suppressor activates an alternative pathway to the wild-type pathway. **d** | A nonsense suppressor encodes a tRNA molecule that recognizes a premature termination codon and inserts an amino acid at that position.

Algumas aplicações

Estudo do envelhecimento



Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Cell Biology

A mother's sacrifice: what is she keeping for herself?

Kiersten A Henderson and Daniel E Gottschling

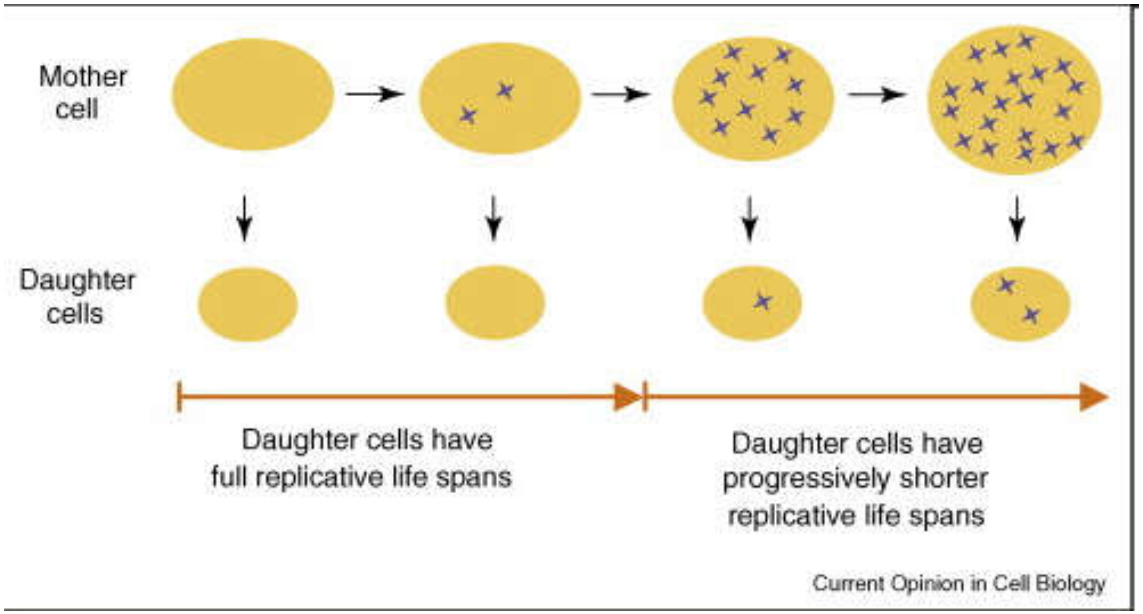
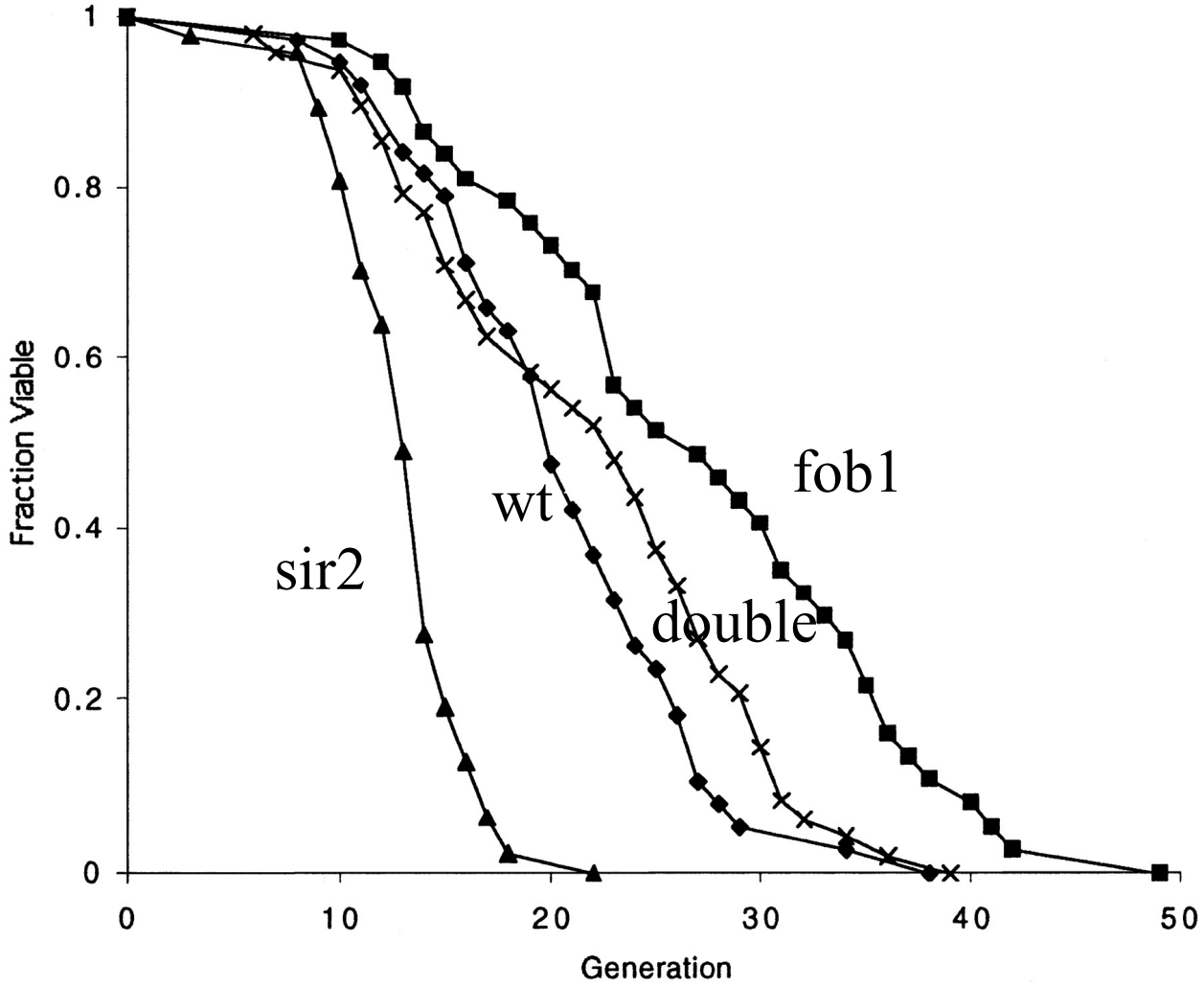
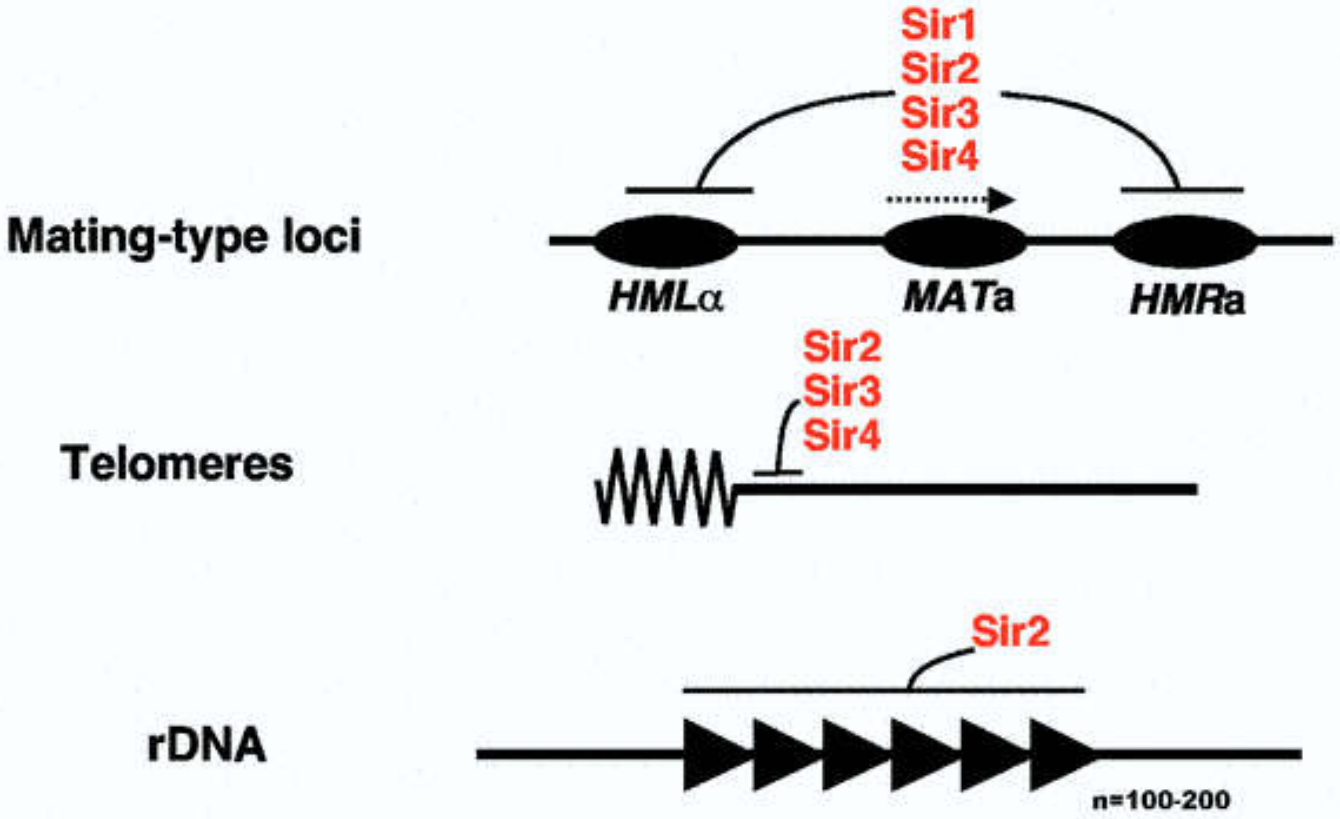


Figure 6. Mutation of fob1 suppresses the life span defect of a sir2 mutant



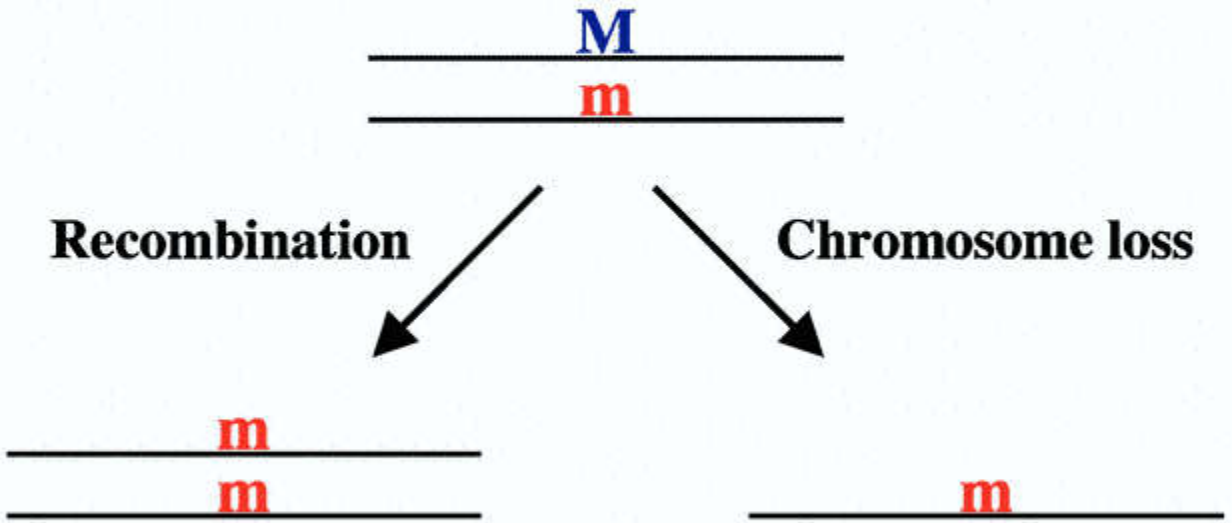
Matt Kaeberlein et al. Genes Dev. 1999; 13: 2570-2580

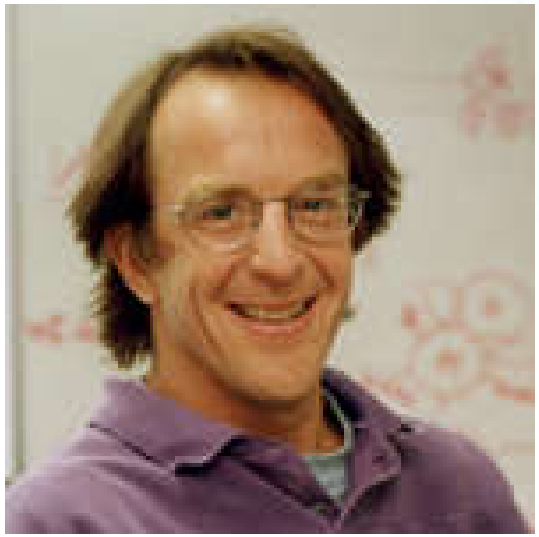
Gene Silencing in *S. cerevisiae*



(~10% of the yeast genome is silenced)

Loss of Heterozygosity





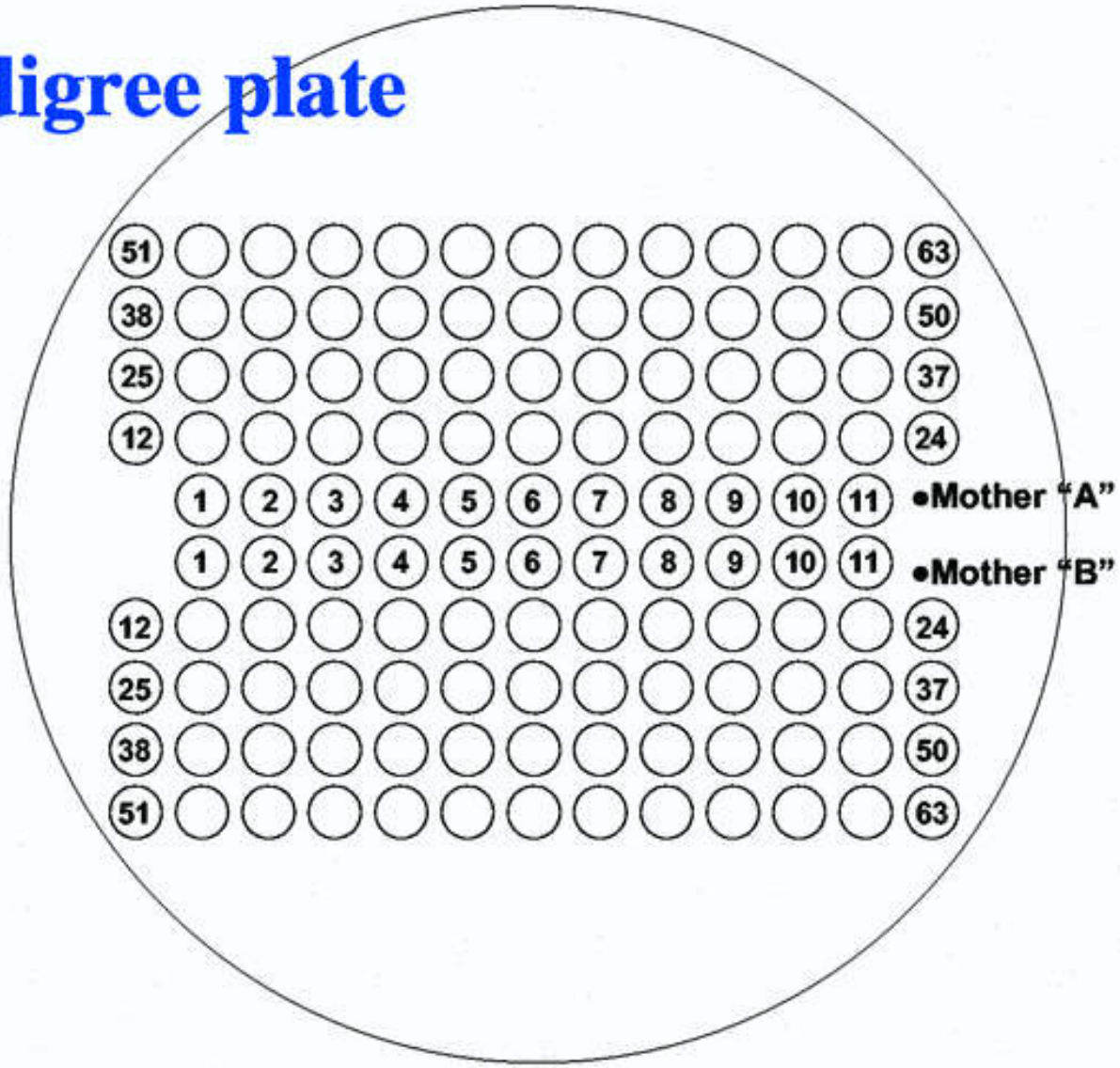
Dan Gottschling

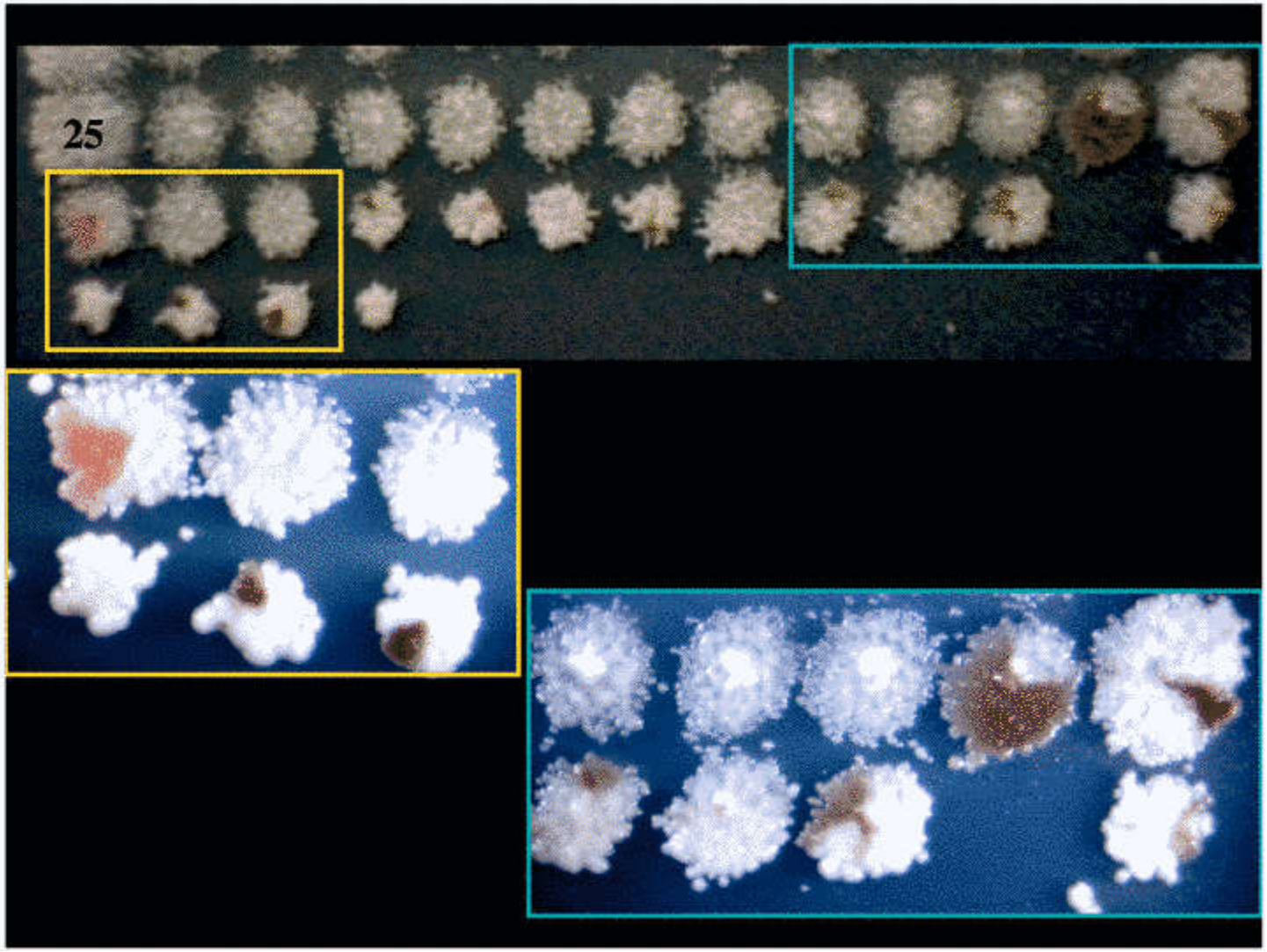
Color Assays for LOH

MET15/met15 ---> **met15/met15**

ADE2/ade2 ---> **ade2/ade2**

Pedigree plate




25

Produção de glicoproteínas humanizadas em levedura

THE HUMANIZATION OF *N*-GLYCOSYLATION PATHWAYS IN YEAST

Stefan Wildt and Tilllman U. Gerngross‡*

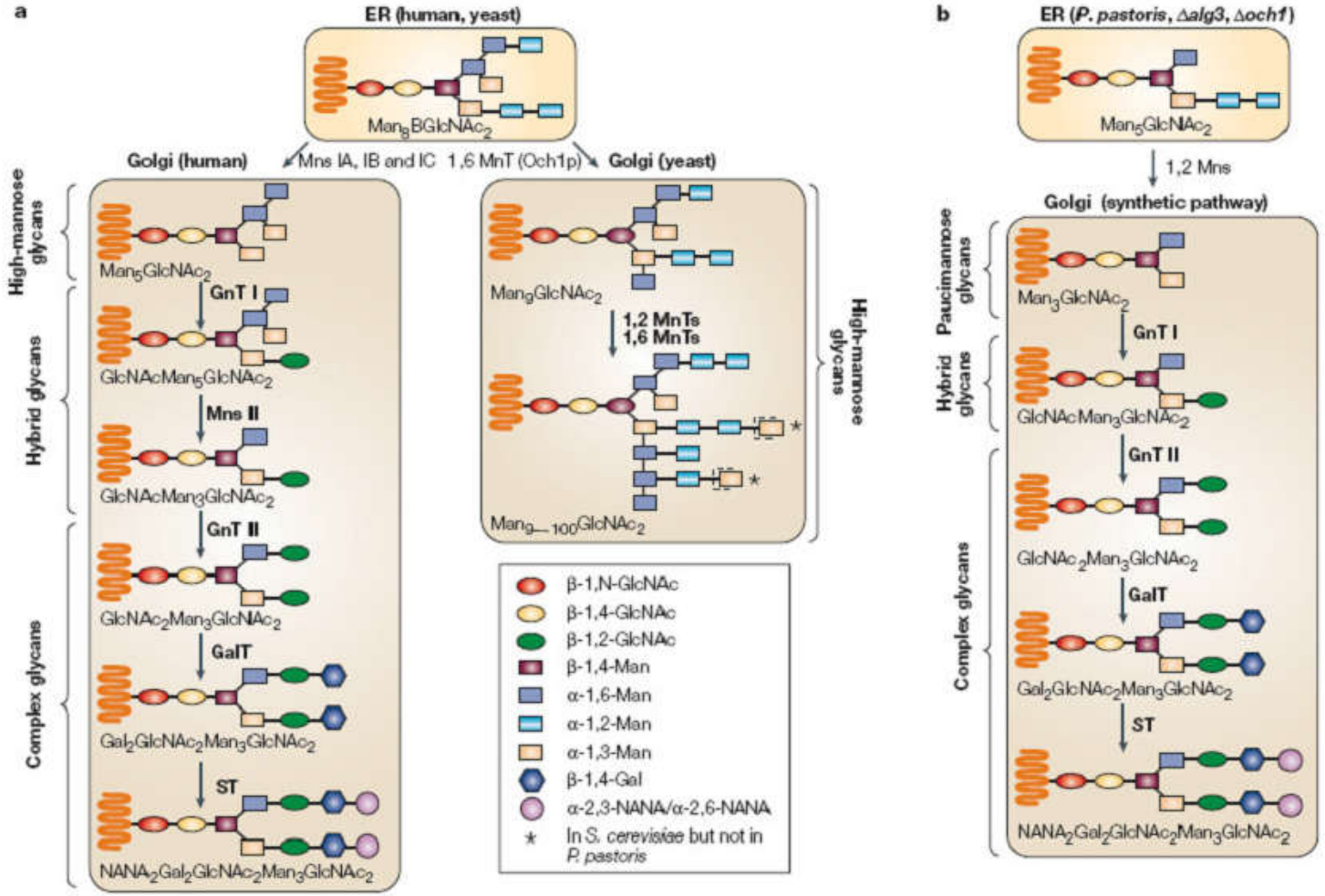
Glycosylation engineering in yeast: the advent of fully humanized yeast

Stephen R Hamilton[1] and Tillman U Gerngross[1,2]

Production of humanized glycoproteins in bacteria and yeasts

Yasunori Chiba[1,2] and Yoshifumi Jigami[1]

Principais vias de N-glicosilação em humanos e levedura



Wildt and Gerngross (2005) *Nat Rev Microbiol* 3: 119-128

Table 1 Therapeutic proteins produced in the yeasts *S. cerevisiae* and *P. pastoris*

Products on the market

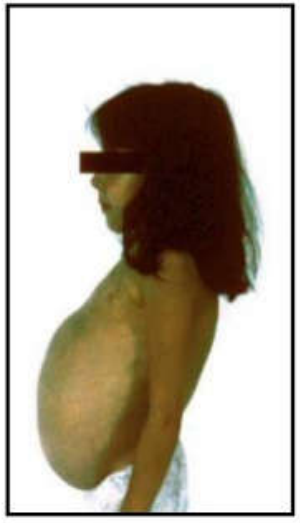
Commercial name	Recombinant protein	Company	Expression system
Actrapid	Insulin	NovoNordisk	*S. cerevisiae*
Ambirix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Comvax	Hepatitis B surface antigen	Merck	*S. cerevisiae*
Elitex	Urate oxidase	Sanofi-Synthelabo	*S. cerevisiae*
Glucagen	Glucagon	Novo Nordisk	*S. cerevisiae*
HBVAXPRO	Hepatitis B surface antigen	Aventis Pharma	*S. cerevisiae*
Hexavac	Hepatitis B surface antigen	Aventis Pasteur	*S. cerevisiae*
Infanrix-Penta	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Leukine	Granulocyte-macrophage colony stimulating factor	Berlex	*S. cerevisiae*
Novolog	Insulin	Novo Nordisk	*S. cerevisiae*
Pediarix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Procomvax	Hepatitis B surface antigen	Aventis Pasteur	*S. cerevisiae*
Refuldan	Hirudin/lepirudin	Hoechst	*S. cerevisiae*
Regranex rh	Platelet-derived growth factor	Ortho-McNeil Phama (US), Janssen-Cilag (EU)	*S. cerevisiae*
Revasc	Hirudin/desirudin	Aventis	*S. cerevisiae*
Twinrix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*

Wildt and Gerngross (2005) *Nat Rev Microbiol* 3: 119-128

Doença de Gaucher - afeta lisossomo tratamento com Glucocerobridase → ainda não produzida em levedura!

Cerezyme→ cada dose USD 432,97
No Brasil 726 pacientes – USD 153.000.000

Response to Enzyme Therapy

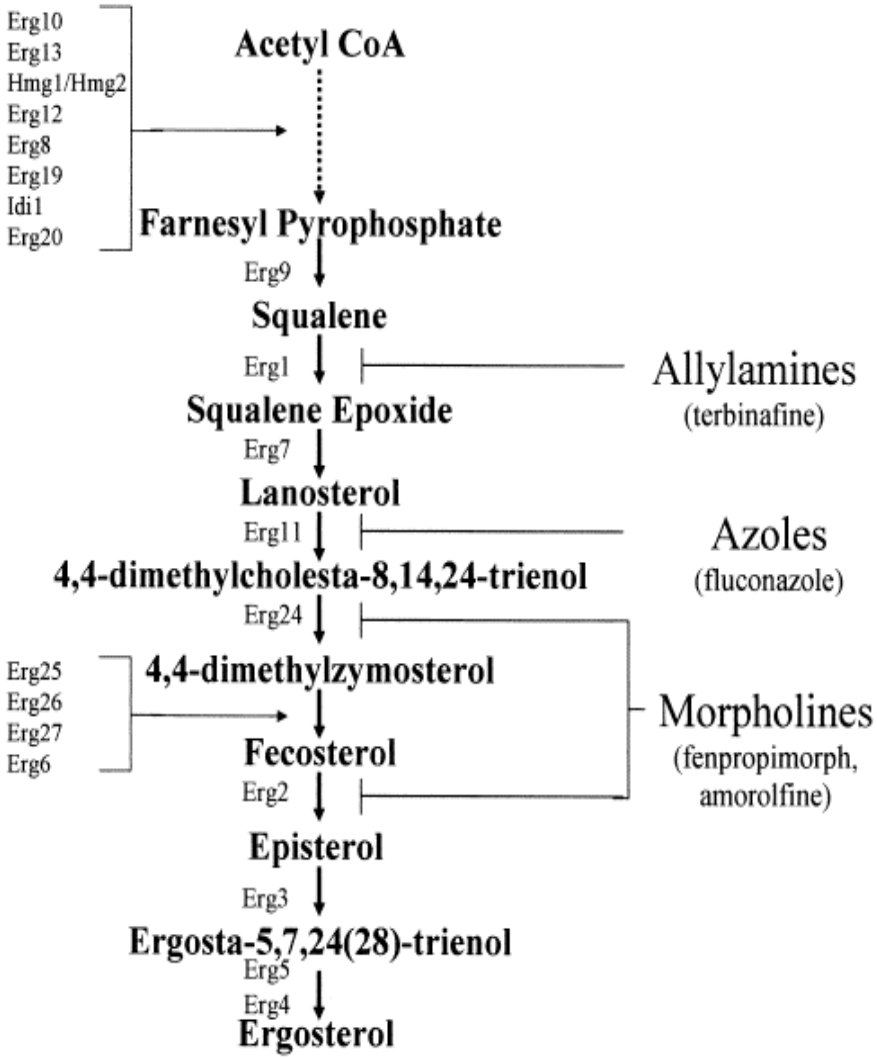


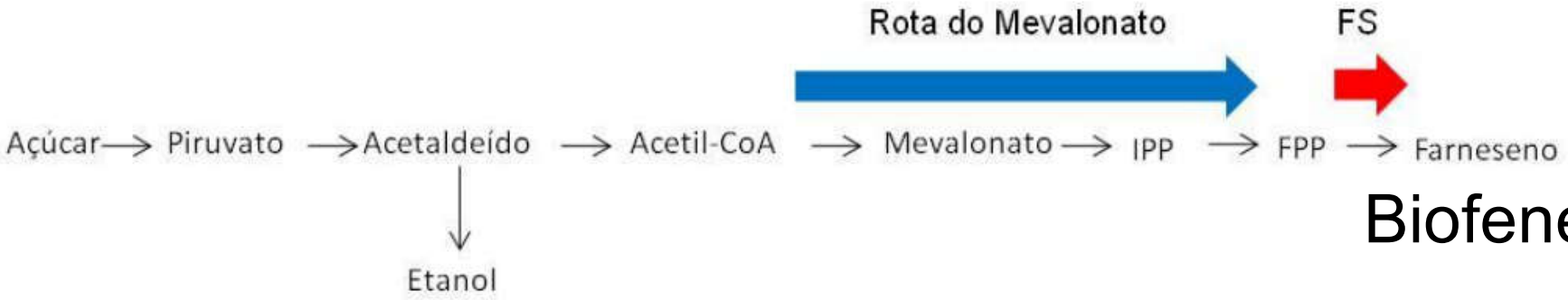
Pretreatment
Age 8 Years, 8 Months

Post-treatment
Age 10 Years, 10 Months

Nos processos biotecnológicos

Por exemplo:
Alteração da via do mevalonato para produção de diesel

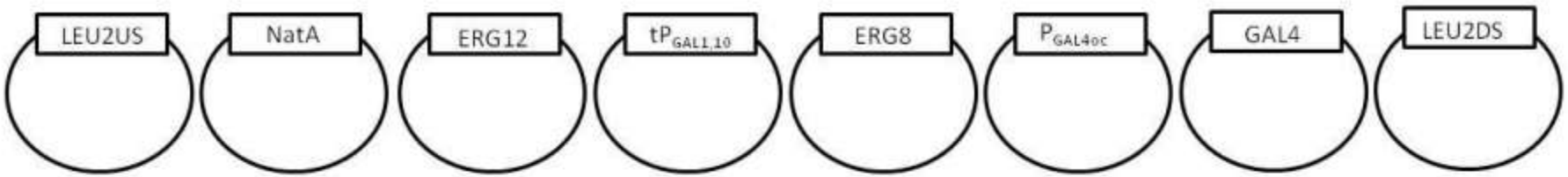




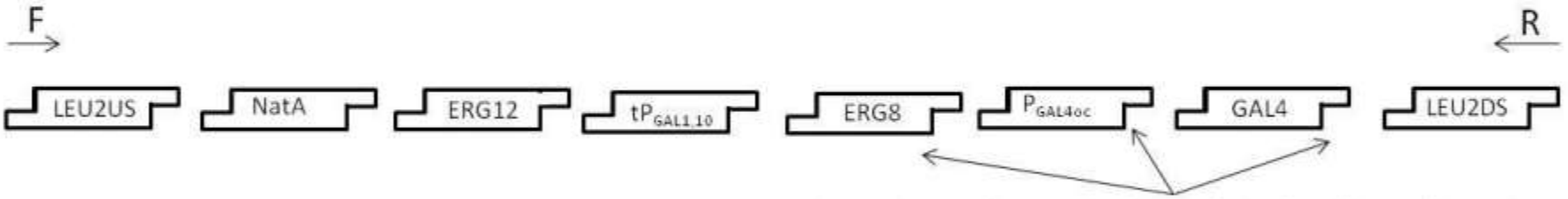
Biofene

AMYRIS

1. Seleção dos elementos genéticos da biblioteca plasmidial e ligação das regiões correspondentes para facilitar a montagem do cassete de integração específico



2. Liberação de cada elemento genético por digestão de restrição

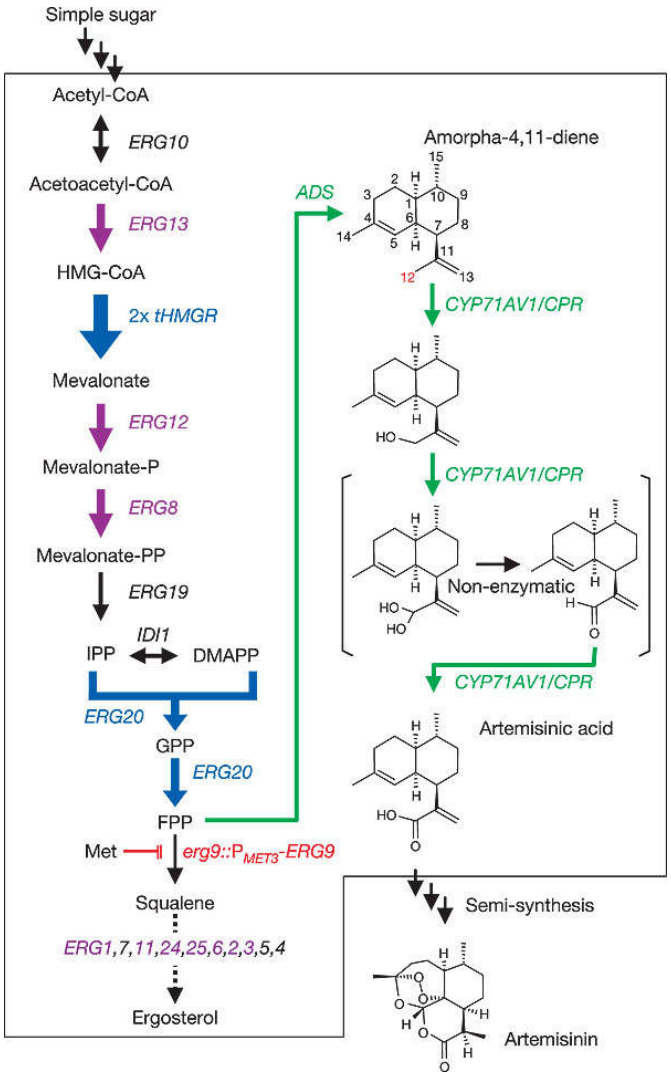


Fragmentos contínuos contendo sequências homólogas sobrepostas

3. Montagem do cassete de integração pela PCR
4. Amplificação do cassete pela PCR usando os iniciadores externos (F, R)

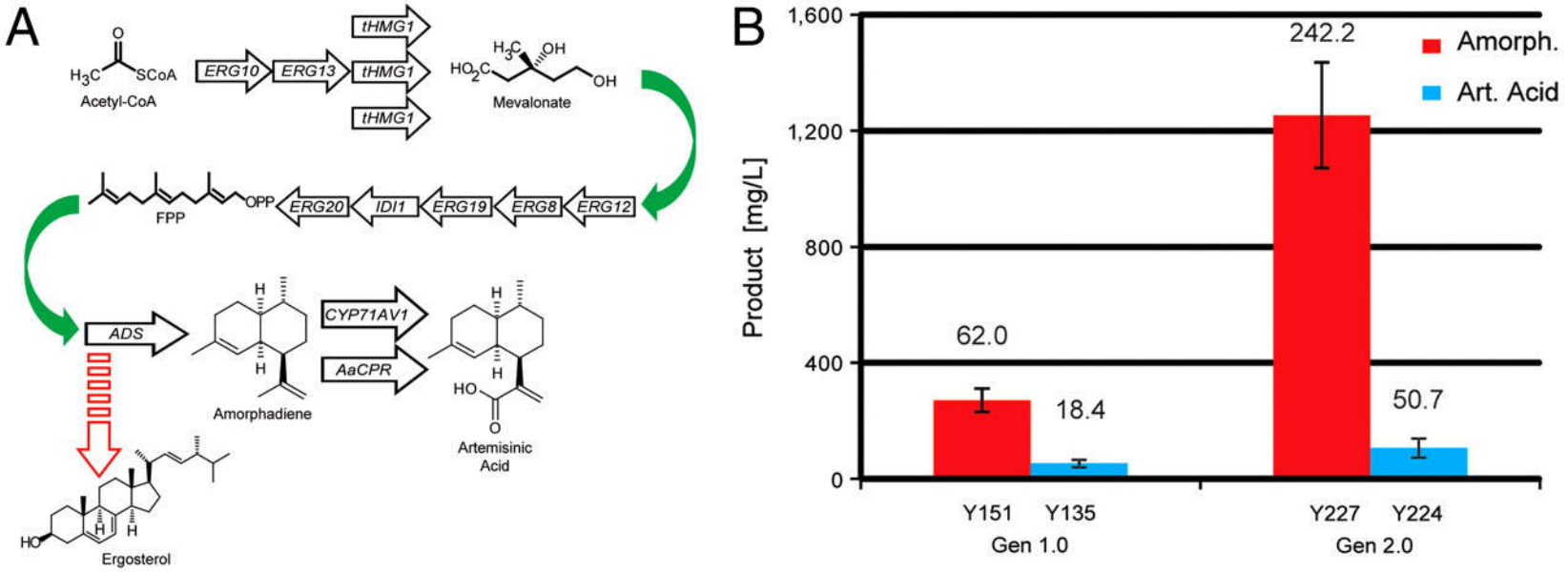
5. Transformação das células de levedura. Selecão para o evento de integração.

Síntese artemisinic acid – anti-malária



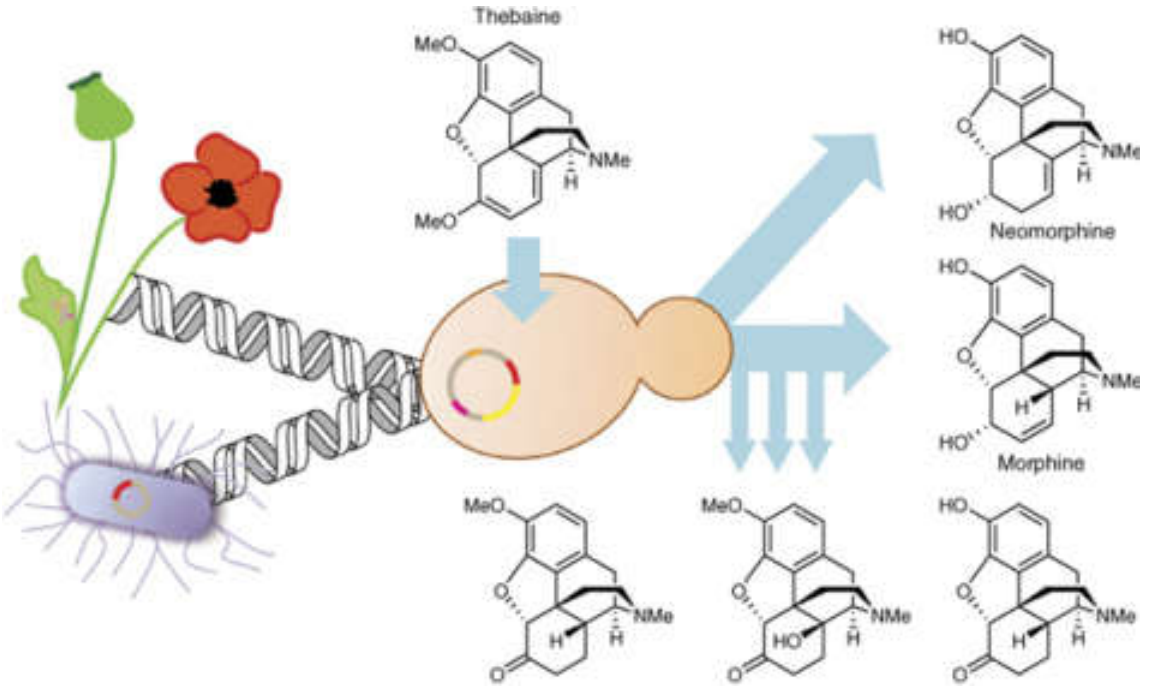
Nature 440(7086):940-3 ·

Construction of CEN.PK2 Gen 2.0 and comparison of production by Gen 1.0 and 2.0 strains.

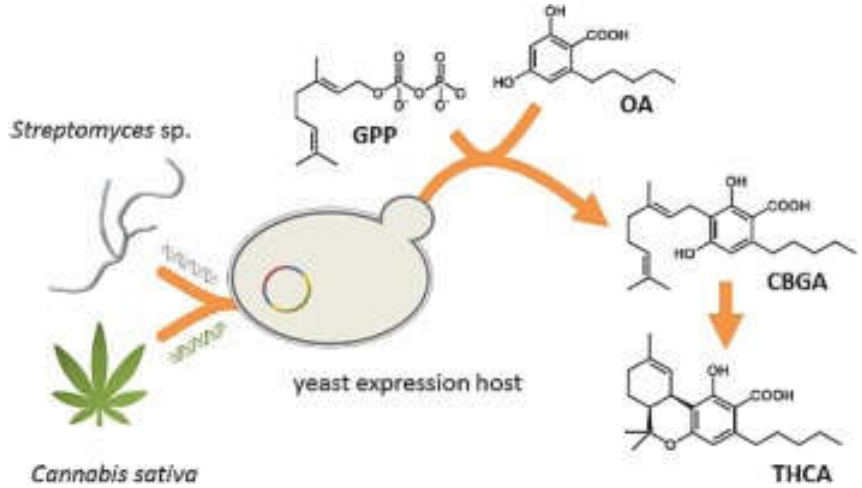


Patrick J. Westfall et al. PNAS 2012;109:E111-E118

Yeast breaking bad



Nature Chemical Biology DOI: 10.1038/nchembio.1613

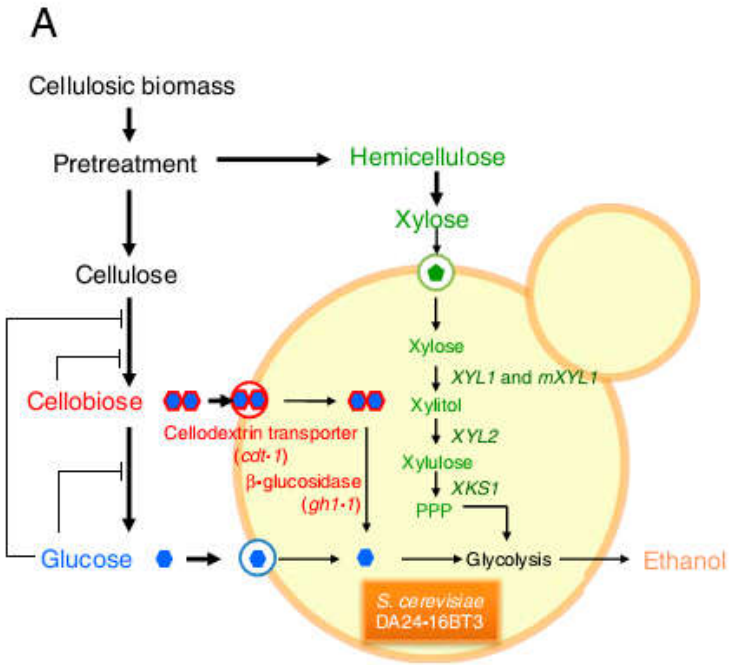


J Biotechnol. 2017 Jul 8. pii: S0168-1656(17)31520-1. doi: 10.1016/j.jbiotec.2017.07.008.

Na produção de etanol

Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation

Suk-Jin Ha[a,b,1], Jonathan M. Galazka[c,1], Soo Rin Kim[a,b], Jin-Ho Choi[a,b], Xiaomin Yang[d], Jin-Ho Seo[e], N. Louise Glass[f], Jamie H. D. Cate[c,g,2], and Yong-Su Jin[a,b,2]



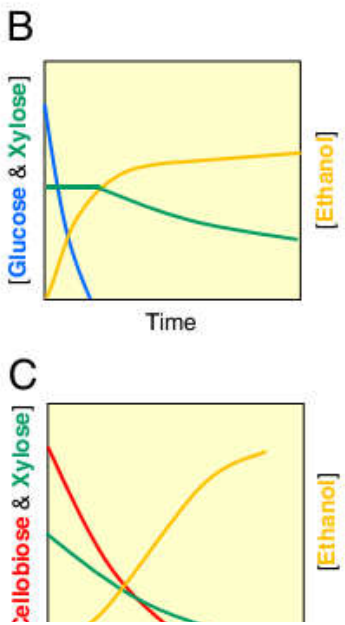


Fig. 1. Strategy for simultaneous cofermentation of cellobiose and xylose without glucose repression. (*A*) A strain improvement strategy to engineer yeast capable of fermenting two nonmetabolizable sugars (cellobiose and xylose). The cellodextrin assimilation pathway consists of a cellodextrin transporter (*cdt-1*) and an intracellular β-glucosidase (*gh1-1*) from the filamentous fungus *N. crassa*. The modified xylose metabolic pathway utilizes xylose reductase isoenzymes (wild-type XR and a mutant XR^{R276H}), xylitol dehydrogenase (*XYL2*), and xylulokinase (*XKS1*) from the xylose-fermenting yeast *P. stipitis*. (*B*) Schematic fermentation profile of a sugar mixture containing glucose and xylose by the engineered *S. cerevisiae*. Glucose fermentation represses xylose fermentation completely so that xylose fermentation begins only after glucose depletion (analogous fermentation result shown in Fig. 5*A*). (*C*) Schematic fermentation profile of a sugar mixture containing cellobiose and xylose by the engineered *S. cerevisiae*. Cellobiose [illegible]neously utilized, as neither consumption of the other (analogous fermentation result shown in Fig. 5*B*).

Yeast Idiosyncrasies

ade1* and *ade2 cultures turn red. The intensity depends on the amount of adenine in the medium. YPD is not rich in adenine, so cultures turn red in 2-3 days. Petites don't turn red.

asp5 requires asparagine added to YPD ascospore dissection plate. Extra asp needed only for germination; cultures grow normally on YPD. *asp5* turns red in aged cultures; not as fast or intense as *ade2* or *ade1*.

gcn4 has an arginine requirement (partial?) *canr* (arginine permease) makes *gcn4* grow very slowly.

trp slows growth, especially at lower temperatures. Extra trp in medium helps, but does not restore wildtype growth. Different mutants may show a range of phenotypes.

Petites may be rho minus or rho zero. Colonies are smaller and white, and their size can vary over a wide range in one culture.

Minimal Media *E. coli* minimal vs. yeast minimal: wild-type E. coli grow equally well on complete (LB) and minimal (M9). Wild-type yeast grows slower on minimal (YNB=SD) than on complete (YPD). Wild-type yeast seems to grow equally well on complete (YPD) and on synthetic complete (SC), however.

Doubling time *S. cerevisiae* haploid in YPD liquid is 90 min. at 30°C. Stationary phase has a cell density of $2X10^8$ per ml. In YNB (SD) it is less, about $5X10^7$ per ml.

Psi Factor Zygote viability and possibly sporulation and spore viability may fail due to Ψ (psi), a cytoplasmic factor, that can arise and be lost spontaneously. Ψ enhances the effect of suppressors which can act as dominant (semi-) lethals in diploids when introduced from one parent into the Ψ+ cytoplasm brought in by the other parent. See Protocol: Curing cytoplasm of Ψ+, C. Styles 1981 (Blue Protocols Notebook).

Sporulation Quality and efficiency is sensitive to many factors (genotypes and conditions). Sometimes, simply repeating the procedure (GNA 2 days at 30°C, SPO 1 day at 23°C, then 1-4 days at 30°C) improves results. A second sporulation medium (NGS) occasionally gives better results. Sometimes the opposite is true.

Tetrad storage Storage on sporulation medium should be stored at 4°C after one week of incubation. They remain reasonably viable for a couple of months. Longer incubation above 4°C toughens the diploid wall, requiring increased digestion for dissection. Asci of wild strains are harder to digest than those of laboratory strains.

Inositol Inositol is present in trace amounts in Difco Yeast Nitrogen Base. The medium supports partial growth of *ino* mutants, making it difficult to distinguish the mutant phenotype from wild type. We regularly supplement YNB preparations with inositol to allow *ino* mutants to grow like wild type. Medium lacking inositol is made "from scratch," using stock solutions stored over chloroform in a cold room according to the Cold Spring Harbor Manual formula. Difco Bacto agar should be used to make solid medium.

Agar Agar is a crudely refined natural product and varies greatly from one processor to another. More highly refined agar is more expensive, has fewer impurities, and usually makes weaker gels. We are using Difco Bacto agar for routine purposes. Less refined agar should be tested carefully. Some agars support *ura* mutants and some have a water-soluble substance toxic to wild type. Agar can be washed to avoid these problems if necessary.

ade 3 Requires both adenine and histidine.

hom 3 Requires methionine and threonine.

TRP *TRP* cultures leak tryptophan into the medium. After 2-3 days, the edges of trp patches in prints to trp-plates start to grow if they are adjacent to TRP patches.

URA Does the same as *TRP* cultures, but the cross-feeding is not seen until after 7-9 days.

Leucine Leucine (L+) contains trace amounts of methionine as an impurity virtually impossible to entirely eliminate in its manufacture. Biochemically, the leucine is "essentially methionine-free," but in a growth test, methionine auxotrophs appear to be "leaky" or "disappear" when grown on medium containing leucine, especially in larger quantities as in drop-out. If your having difficulty tracking a methionine auxotroph, try to use strains which don't require leucine and omit it from the medium, or do a complementation test using Leu+ testers.