２００６年７月１２日（水）
応用分子生命科学基礎科目Ⅱ
「遺伝子とゲノムの操作法」

応用分子生命科学
赤田倫治

遺伝学

遺伝子とゲノム

応用分子生命科学

まず遺伝学


メンデル

1866年　明治維新1868年

分離の法則，独立の法則

Dominant	優性
Recessive	劣性
Phenotype	形質
Genotype	遺伝子型
Allele	対立遺伝子
Locus	遺伝子座

1866	メンデル
1902	染色体と遺伝子
1910-1920	ハエの部屋
1941	一遺伝子一酵素
1944	DNAが遺伝子
1940-1950	ファージグループ
1953	DNA構造
1950-1960	DNA複製，転写，翻訳
1973	組換えDNA分子
1970-1990	分子生物学，遺伝子工学
1995-	ゲノム

The Nobel Prize in Physiology or Medicine 2001

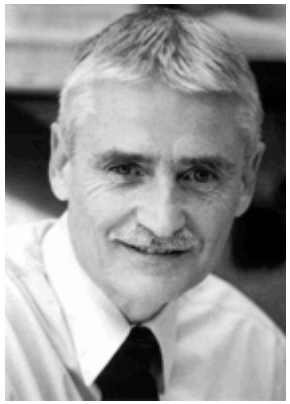
"for their discoveries of key regulators of the cell cycle"

Leland H. Hartwell

R. Timothy Hunt

Sir Paul M. Nurse

（野依良治の年）



Banquet Speech

Leland H. Hartwell's speech at the Nobel Banquet, December 10, 2001

Your Majesties, Your Royal Highnesses, Honoured Laureates, Ladies and Gentlemen,

The goal of science, as we all know, is to discover simplicity in the midst of complexity. Yet when Paul Nurse, Tim Hunt and I and our students and colleagues began studying how cells divide, any sensible scientist should have expected to find only hopeless complexity. If you think of cell division as a symphony, we knew that the symphony had to be performed by thousands of musicians each playing a different instrument. **So - our research can only be described as motivated by a kind of foolish optimist**. **Sometimes nature rewards foolish optimism.** Continuing with the metaphor of cell division as a symphony, our research paths led each of us, independently and by great luck, smack into the conductor of the symphony. And, it turned out that the same conductor performed this symphony in all types of cells - yeast, fruit flies, sea urchins, frogs and humans. **I really have no idea how often nature rewards such foolish optimism, but I am pleased to report that the Nobel committee is rather fond of foolish optimism**.

出芽酵母

Saccharomyces cerevisiae

パン酵母
清酒酵母
ワイン酵母
焼酎酵母
ビール酵母

真核生物で最初のゲノム
シークエンス

1996年　12,068Kb

600人以上の研究者によ
る国際プロジェクト

ちなみに昨年は

The Nobel Prize in Physiology or Medicine 2005

"for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease"

Barry J. Marshall and J. Robin Warren

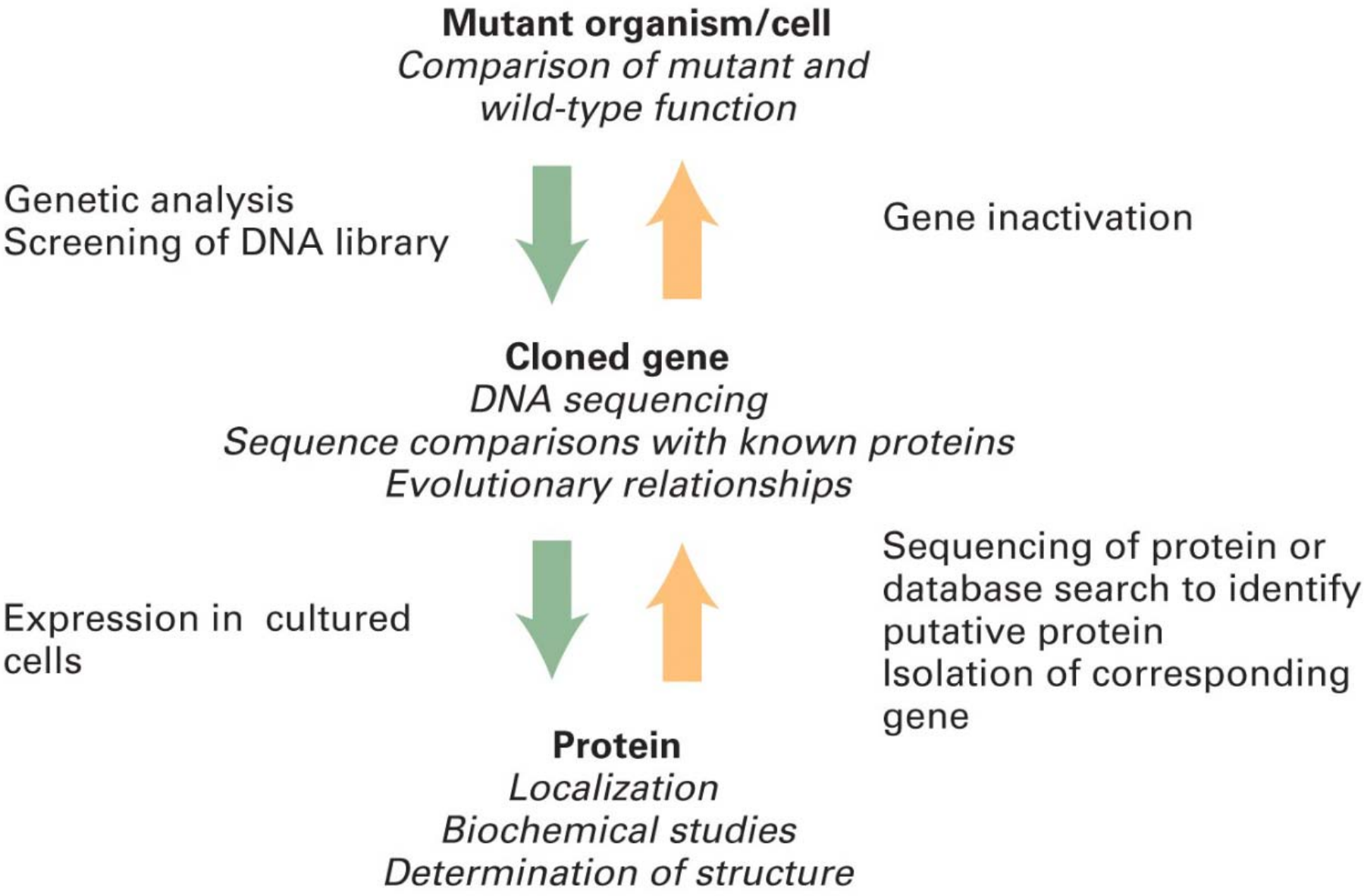
ヘリコバクターピロリ菌とその胃炎，消化性潰瘍への関与の発見

gastritis 胃炎, peptic ulcer 消化性潰瘍

実験手法

遺伝学⇒変異と形質

遺伝学で何ができるか


Mutant organism/cell
Comparison of mutant and wild-type function
Genetic analysis
Screening of DNA library
Gene inactivation
Cloned gene
DNA sequencing
Sequence comparisons with known proteins
Evolutionary relationships
Expression in cultured cells
Sequencing of protein or database search to identify putative protein
Isolation of corresponding gene
Protein
Localization
Biochemical studies
Determination of structure

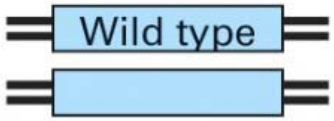
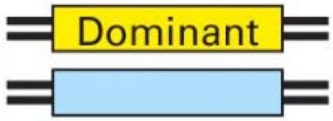
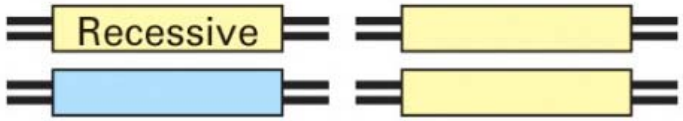
遺伝子型－形質の関連

DNA配列の変化が何を示すか

遺伝子変異の分類

Loss of function mutations: 機能不全
Gain of function mutations: 能力異常

配列よりもその効果が重要

DIPLOID GENOTYPE				
DIPLOID PHENOTYPE	Wild type	Mutant	Wild type	Mutant

The main classes of mutation 変異の分類

Deletions　欠損 1 bpからmegabaseまで
Insertions　挿入，重複
Single base substitutions　一塩基置換
　　missense mutations　他のアミノ酸に置換
　　nonsense mutations　ストップコドンに置換
　　splice site mutations　スプライスサイトの変化
Frameshifts　フレームシフト（欠損，挿入，スプライシングエラー）
Dynamic mutations　タンデムリピートのサイズ変化

Nomenclature 変異遺伝子の効果による命名

Null allele: 変異が何も作らない。完全にない状態
Hypomorph: 変異が活性低下を示す。
Hypermorph: 変異が量的または活性上昇を示す。
Neomorph: 変異が新規な活性を示す。
Antimorph: 変異が正常産物と拮抗する

Loss of functionにもいろいろ

2倍体には遺伝子が2セット
１つが壊れて，もう一つが正常遺伝子なら普通形質は正常

１つが壊れて（５０％レベル）も形質を示す変異
⇒**haploinsufficiency**（半数体不十分）

機能不全の変異タンパクが正常タンパクを阻害する場合。
⇒**dominant negative** (antimorph)

Delete	α-thalassemia mutations
	60% of Duchenne muscular dystrophy
Disruption	Duchenne muscular dystrophy
Prevent correct splicing	
	PAX3
	SMN2
	LGMD2A
	CFTR
Haploinsufficiency	
	Alagille syndrome JAG1
	Multiple exostoses EXT1
	Tomaculous neuropathy PMP22
	Supravalvular aortic stenosis ELN
	Tricho-rhino-phalangeal syndrome TRPS1
	Waardenburg syndrome Type1 PAX3

Gain of function mutations

Overexpression	PMP22	Charot-Marie-Tooth disease
Receptor permanently ‘on’	GNAS	McCune-Albright disease
Acquire new substrate	PI	Antitrypsin deficiency
Ion channel inappropriately open	SCN4A	Paramyotonia congenita
Structurally abnormal multimers	COL2A1	Osteogenesis imperfecta
Protein aggregation	HD	Huntington disease
Chimeric gene	BCR-ABL	Chroic myeloid leukemia

結局は遺伝子の正常機能と病気を起す異常機能の解明

遺伝学を利用した細胞周期の解析

"for their discoveries of key regulators of the cell cycle"

生きるか死ぬか
増殖するかしないか

生きるのに大事な遺伝子
↓
壊れたら死ぬ。
↓
死んだら調べられない。

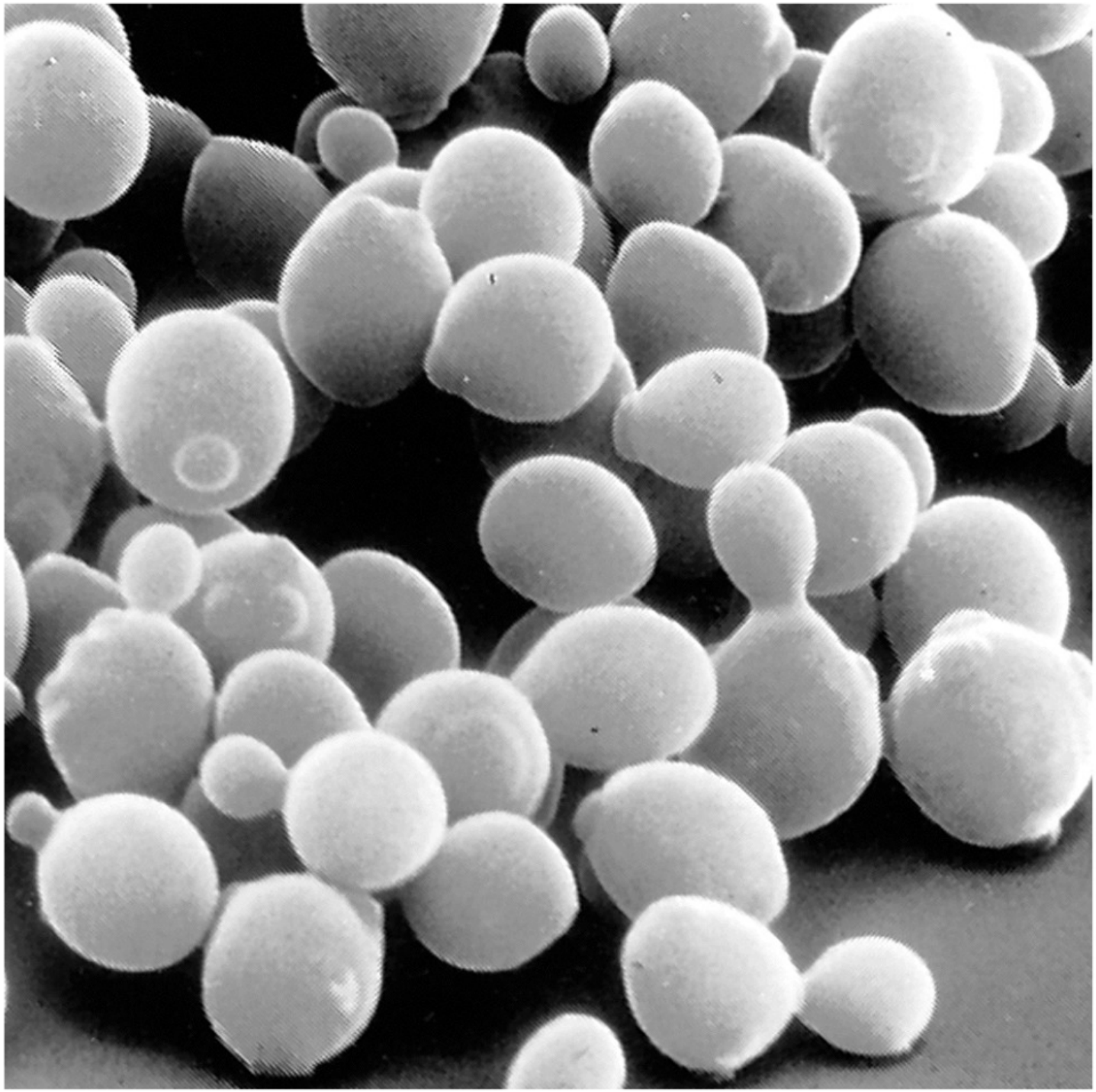
どうする？⇒条件致死（温度感受性変異など）
Temperature sensitive mutation
ts変異

変異は自然に起こる（10^{-8}）し，誘導することもできる（10^{-5}）

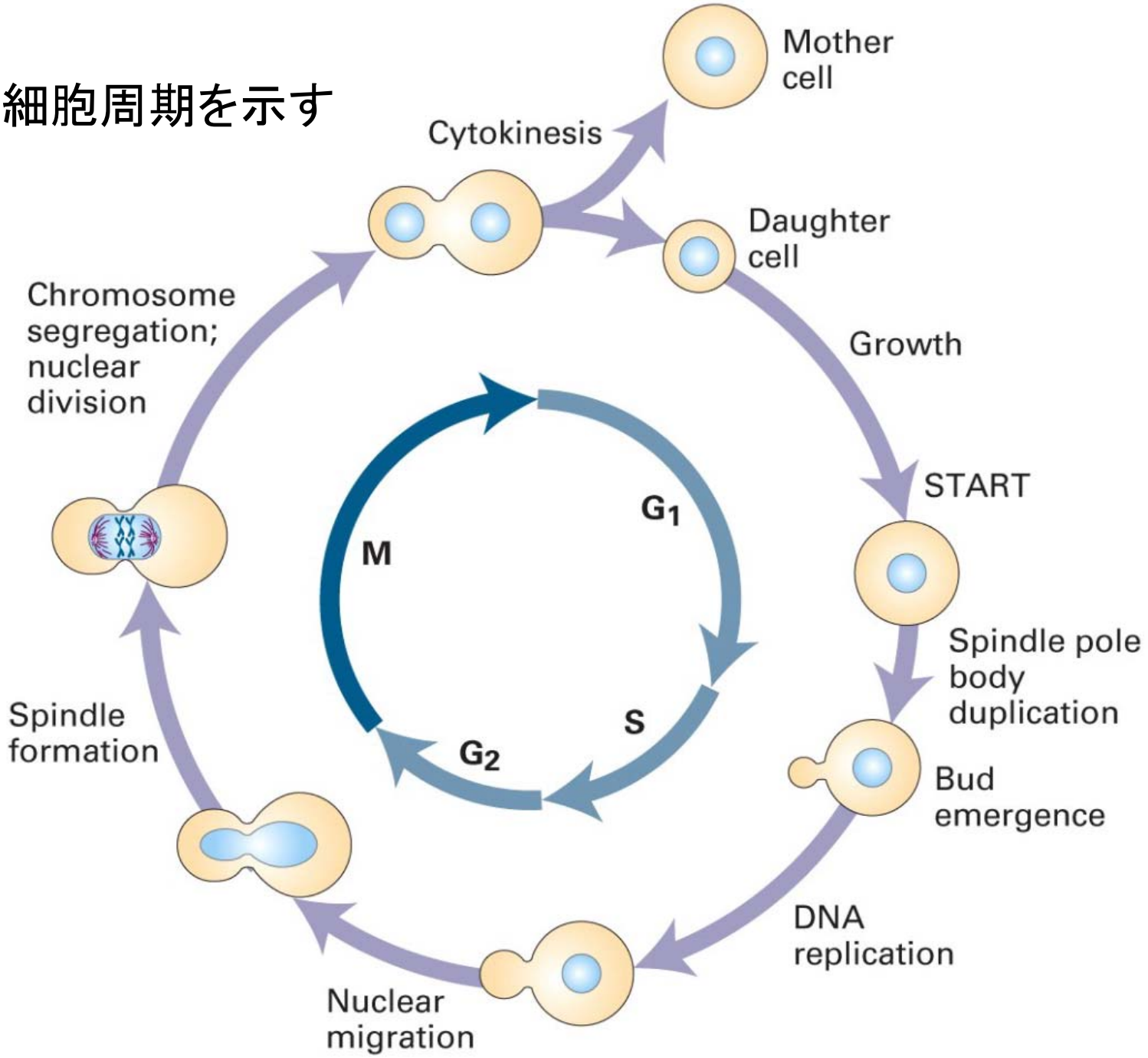
Leland H. Hartwell

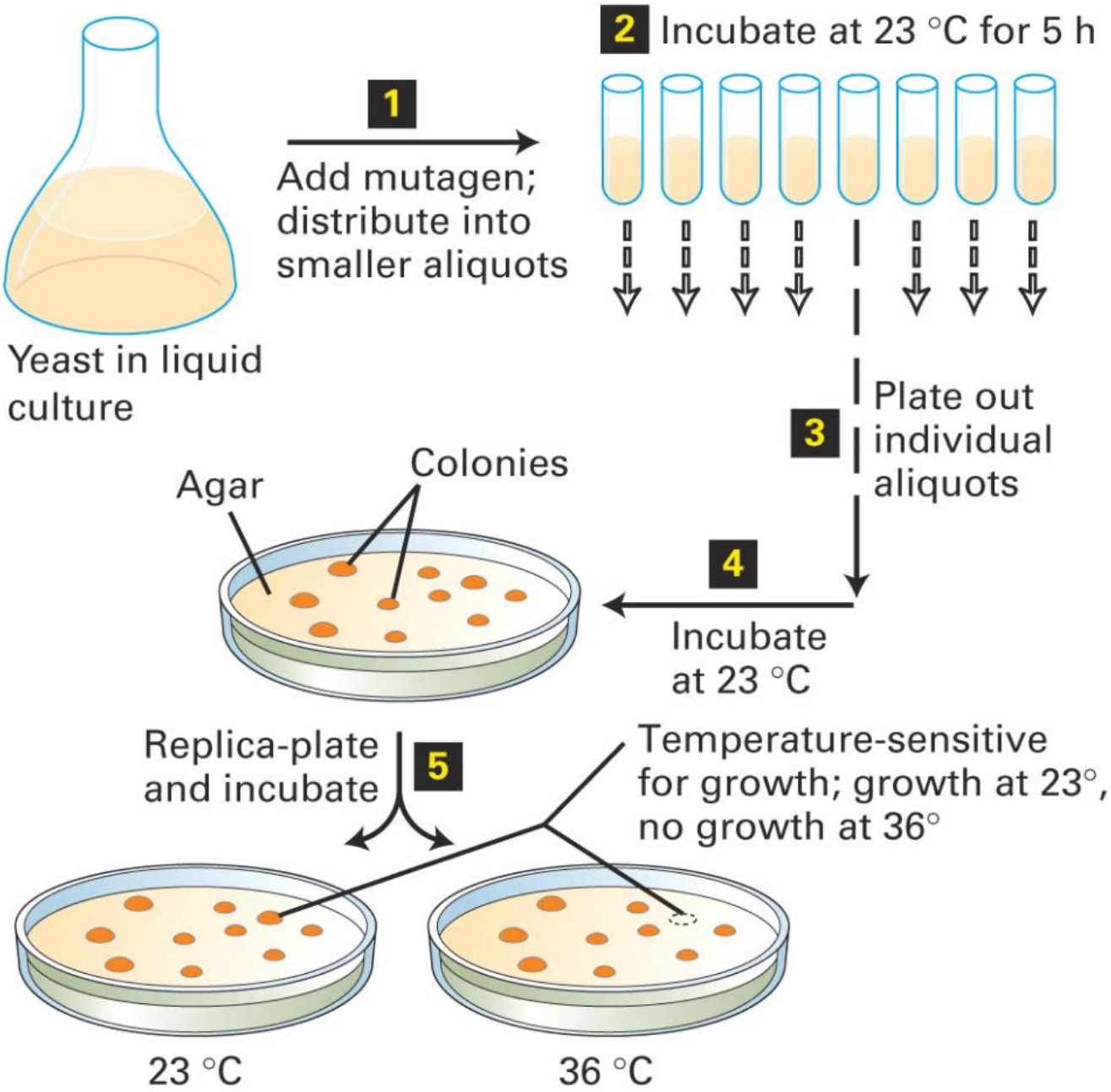
Posdoc. Renato Dulbecco Lab. Cell culture system 細胞分裂研究⇒全部失敗
1965　Assistant Prof. Univ. California at Irvine　T4 phage genetics
研究費 : Control of DNA replication in mammalian cells
機器を注文したが設置まで2, 3ヶ月かかる⇒することがない。
そこで,
Genetic approachができる新しいモデル
Neurospora and Yeast

1967　400 ts mutant温度感受性変異株. J Bacteriol 93:1662
1968　Washington University
Screening ts mutants. Photographically 夏の間中を使ってJ Bacteriol 104: 1280, 1970
1973　148 cell cycle mutants in 32 genes. Genetics 74:267
大学生Brian Reidとcdc変異株をスクリーニング

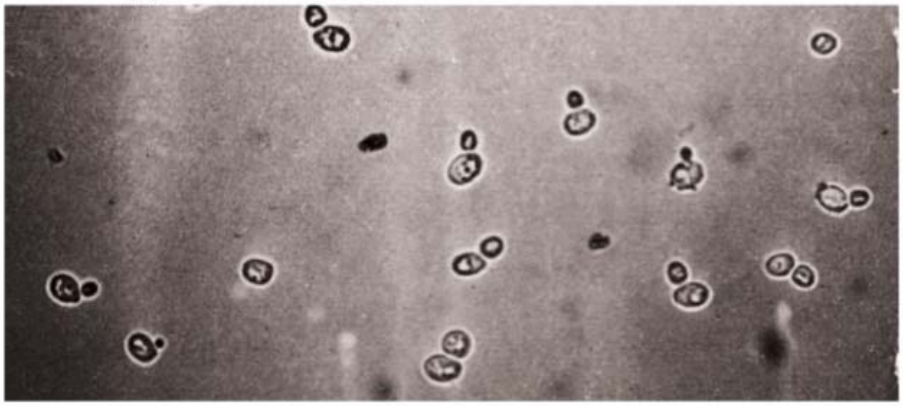


形が細胞周期を示す

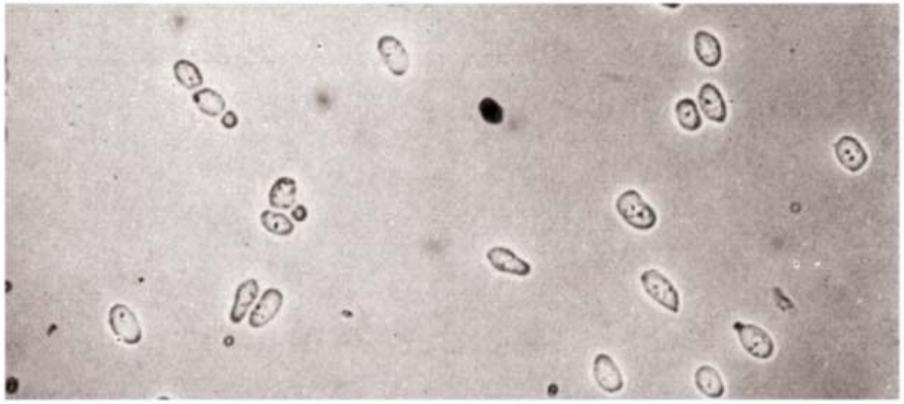

Mother cell
Cytokinesis
Daughter cell
Growth
START
Spindle pole body duplication
Bud emergence
DNA replication
Nuclear migration
Spindle formation
Chromosome segregation; nuclear division
M
G1
S
G2


Yeast in liquid culture
1
Add mutagen; distribute into smaller aliquots
2 Incubate at 23 °C for 5 h
3
Plate out individual aliquots
Agar
Colonies
4
Incubate at 23 °C
Replica-plate and incubate
5
Temperature-sensitive for growth; growth at 23°, no growth at 36°
23 °C
36 °C

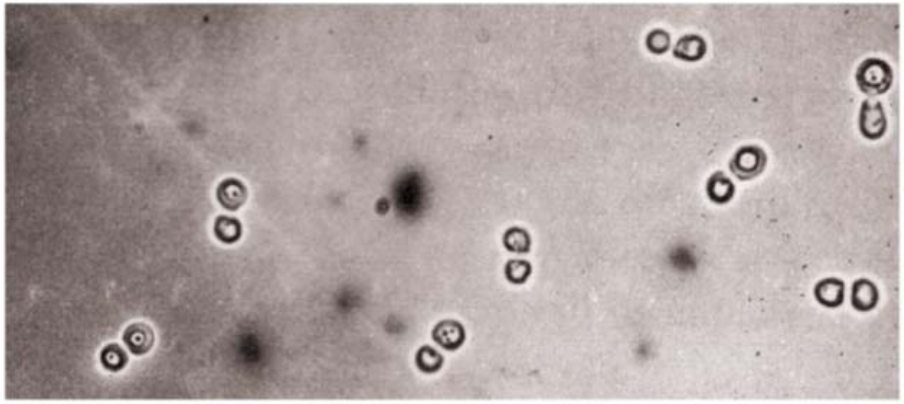
Wild type



cdc28 mutants



cdc7 mutants



制限温度（37℃）で一定の形態で増殖が停止する変異株を取得した。
（通常は形態はばらばらで止まる）

これがcdc (cell division cycle)細胞周期変異株

S. cerevisiae

CDC28
150のcdc変異株の一つだけが出芽しない，単一核できれいに停止
↓
cdc28+cdc1のダブル変異株だった（ラッキー）。
↓
cdc28だけにすると異常形態を示す。
（cell cycle mutant形質ではない！）

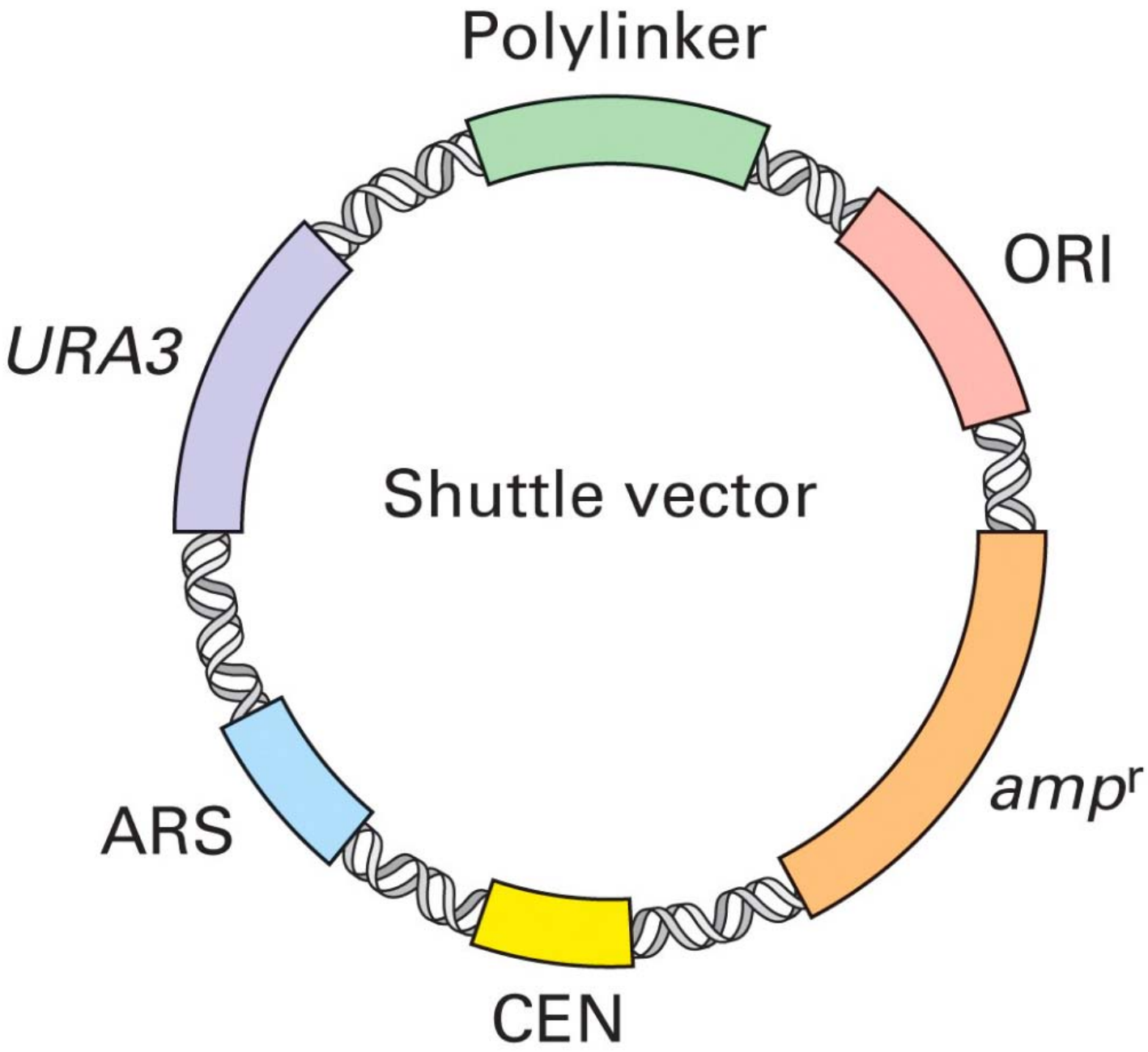
S. pombe
CDC2=CDC28 of S. cerevisiae
25 small cell cycle mutants
24= wee1
1=cdc2

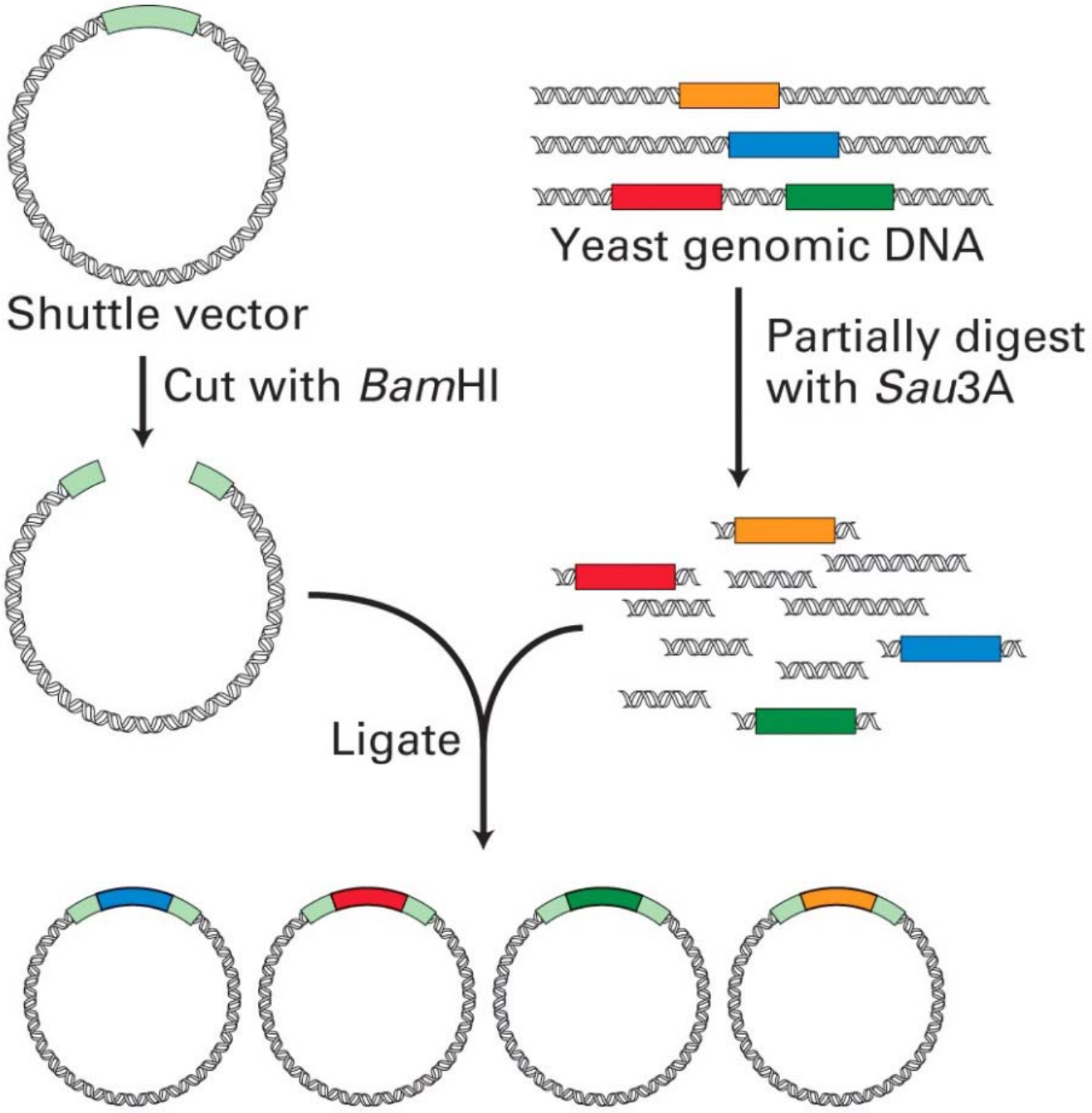
重要な遺伝子がたまたま取れていた

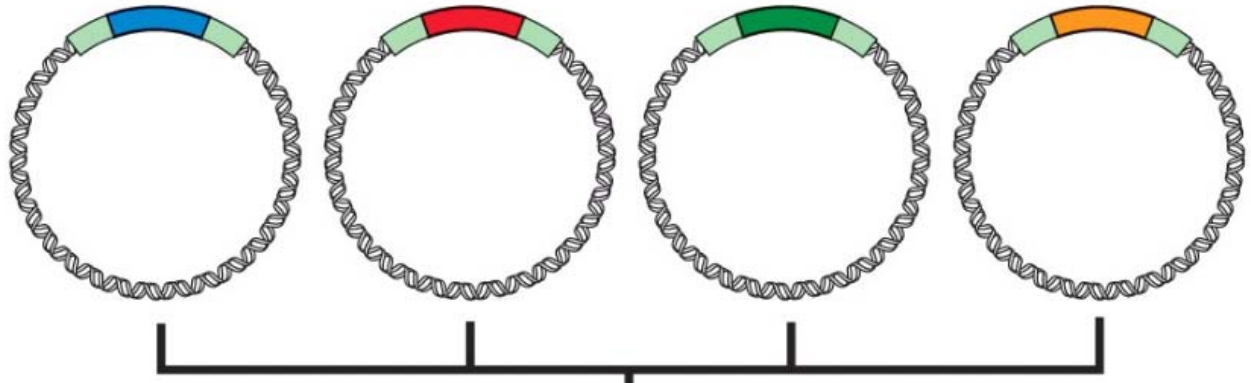
遺伝子のクローニング

酵母の変異株を相補するクローニング

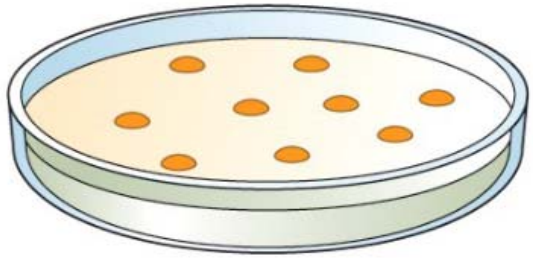
相補性クローニング（Complementation cloning)


Polylinker
ORI
*amp*r
CEN
ARS
URA3
Shuttle vector


Shuttle vector
Cut with *Bam*HI
Yeast genomic DNA
Partially digest
with *Sau*3A
Ligate

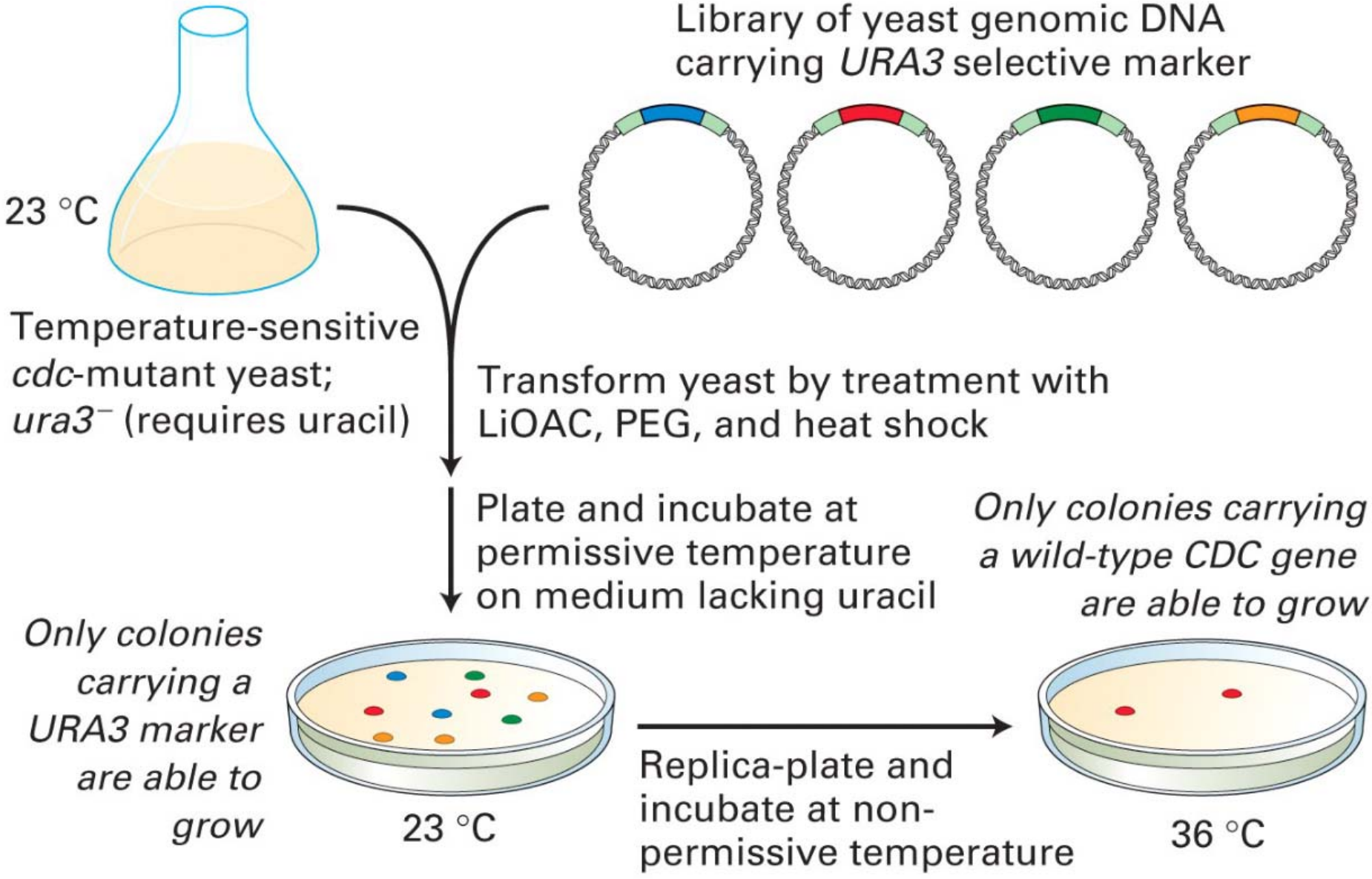


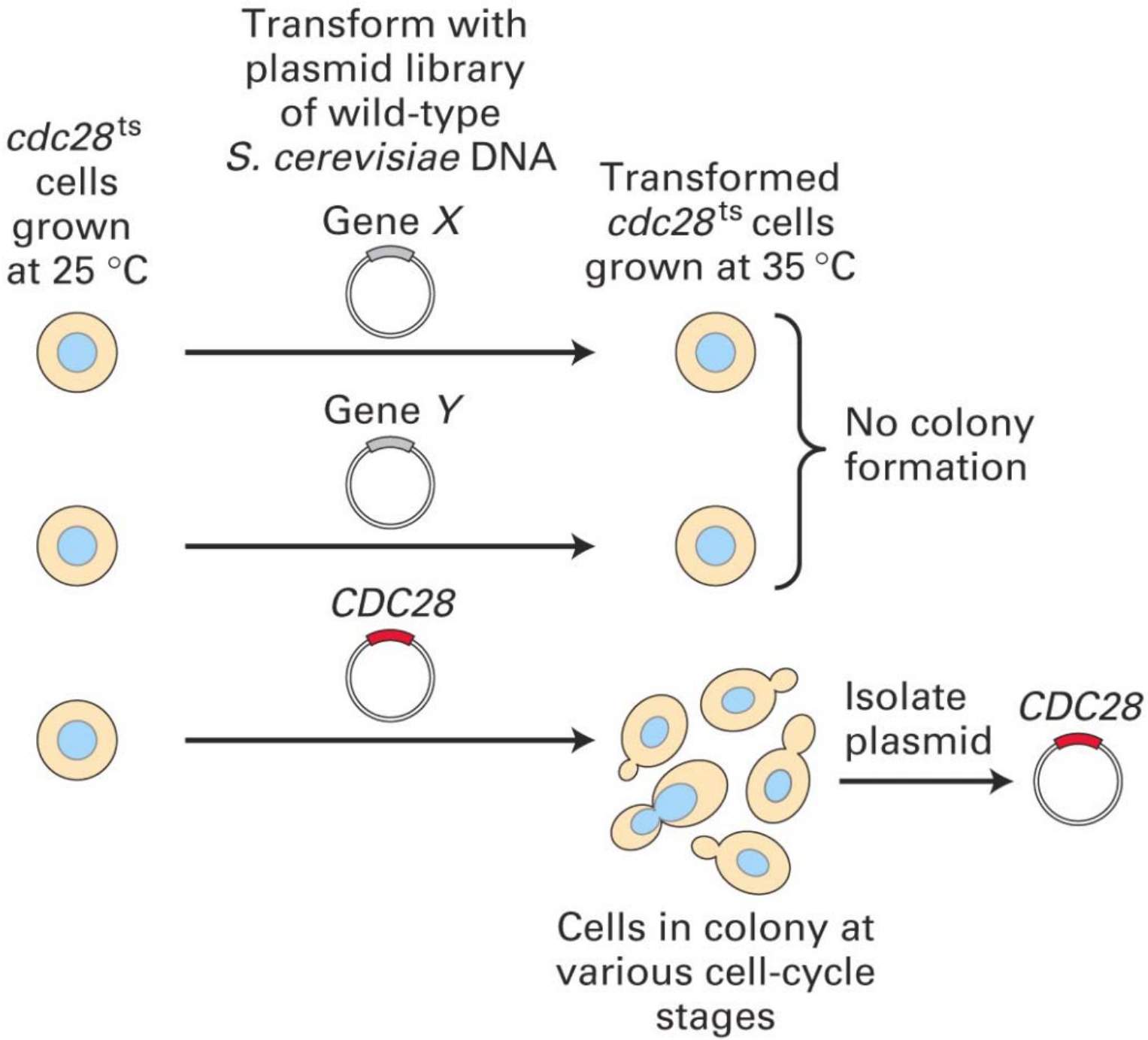
Transform *E. coli*
Screen for ampicillin resistance

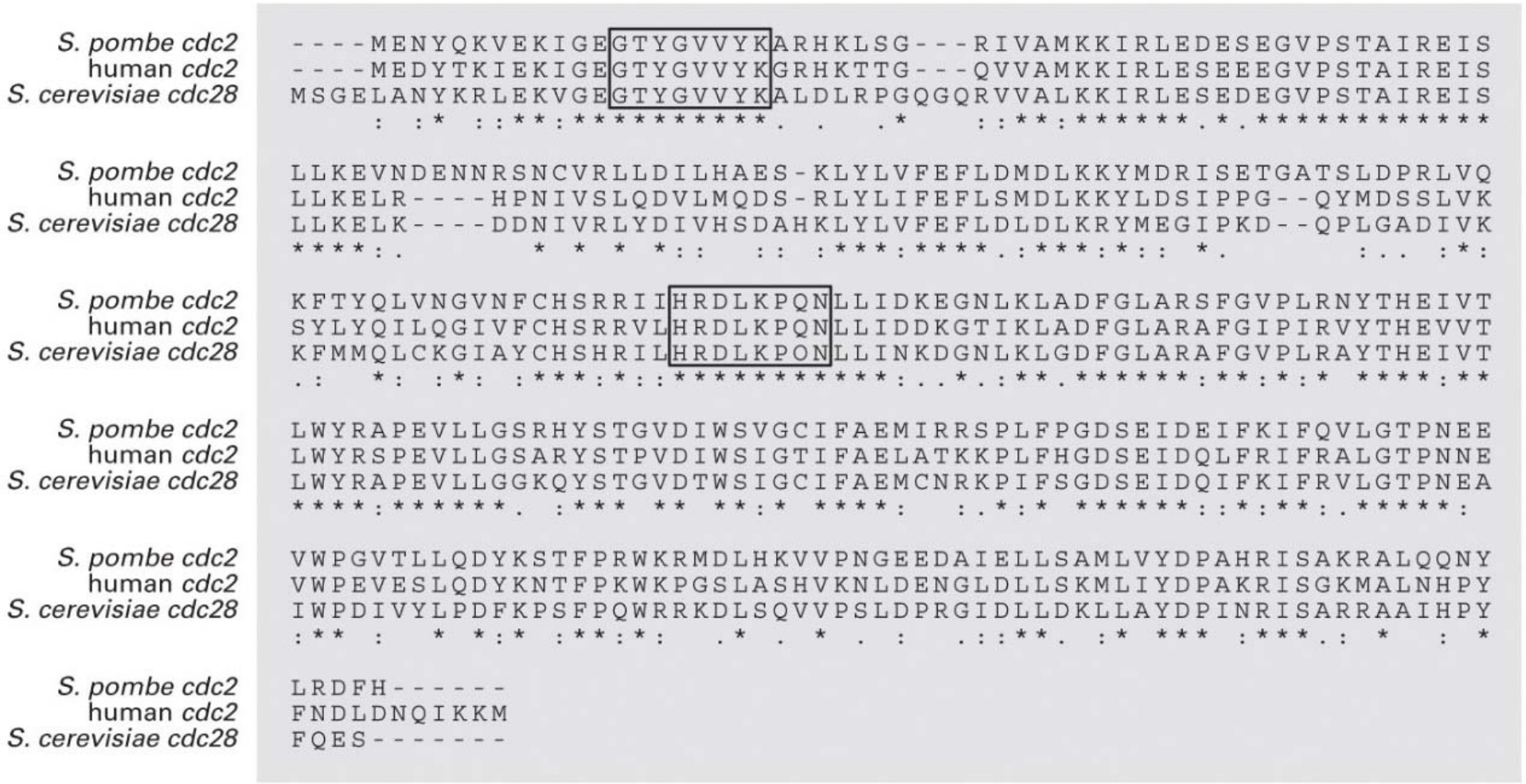


Isolate and pool recombinant plasmids from 10^5 transformed *E. coli* colonies

Assay yeast genomic library by functional complementation


Library of yeast genomic DNA carrying *URA3* selective marker
23 °C
Temperature-sensitive *cdc*-mutant yeast; *ura3*⁻ (requires uracil)
Transform yeast by treatment with LiOAC, PEG, and heat shock
Plate and incubate at permissive temperature on medium lacking uracil
Only colonies carrying a wild-type CDC gene are able to grow
Only colonies carrying a URA3 marker are able to grow
23 °C
Replica-plate and incubate at non-permissive temperature
36 °C


Transform with plasmid library of wild-type *S. cerevisiae* DNA
*cdc28*ts cells grown at 25 °C
Gene *X*
Transformed *cdc28*ts cells grown at 35 °C
Gene *Y*
No colony formation
CDC28
Isolate plasmid
CDC28
Cells in colony at various cell-cycle stages


S. pombe cdc2
human cdc2
S. cerevisiae cdc28
----MENYQKVEKIGEGTYGVVYKARHKLSG---RIVAMKKIRLEDESEGVPSTAIREIS
----MEDYTKIEKIGEGTYGVVYKGRHKTTG---QVVAMKKIRLESEEEGVPSTAIREIS
MSGELANYKRLEKVGEGTYGVVYKALDLRPGQGQRVVALKKIRLESEDEGVPSTAIREIS
LLKEVNDENNRSNCVRLLDILHAES-KLYLVFEFLDMDLKKYMDRISETGATSLDPRLVQ
LLKELR----HPNIVSLQDVLMQDS-RLYLIFEFLSMDLKKYLDSIPPG--QYMDSSLVK
LLKELK----DDNIVRLYDIVHSDAHKLYLVFEFLDLDLKRYMEGIPKD--QPLGADIVK
KFTYQLVNGVNFCHSRRIIHRDLKPQNLLIDKEGNLKLADFGLARSFGVPLRNYTHEIVT
SYLYQILQGIVFCHSRRVLHRDLKPQNLLIDDKGTIKLADFGLARAFGIPIRVYTHEVVT
KFMMQLCKGIAYCHSHRILHRDLKPQNLLINKDGNLKLGDFGLARAFGVPLRAYTHEIVT
LWYRAPEVLLGSRHYSTGVDIWSVGCIFAEMIRRSPLFPGDSEIDEIFKIFQVLGTPNEE
LWYRSPEVLLGSARYSTPVDIWSIGTIFAELATKKPLFHGDSEIDQLFRIFRALGTPNNE
LWYRAPEVLLGGKQYSTGVDTWSIGCIFAEMCNRKPIFSGDSEIDQIFKIFRVLGTPNEA
VWPGVTLLQDYKSTFPRWKRMDLHKVVPNGEEDAIELLSAMLVYDPAHRISAKRALQQNY
VWPEVESLQDYKNTFPKWKPGSLASHVKNLDENGLDLLSKMLIYDPAKRISGKMALNHPY
IWPDIVYLPDFKPSFPQWRRKDLSQVVPSLDPRGIDLLDKLLAYDPINRISARRAAIHPY
LRDFH------
FNDLDNQIKKM
FQES-------

Nature 1987, 327:31-35

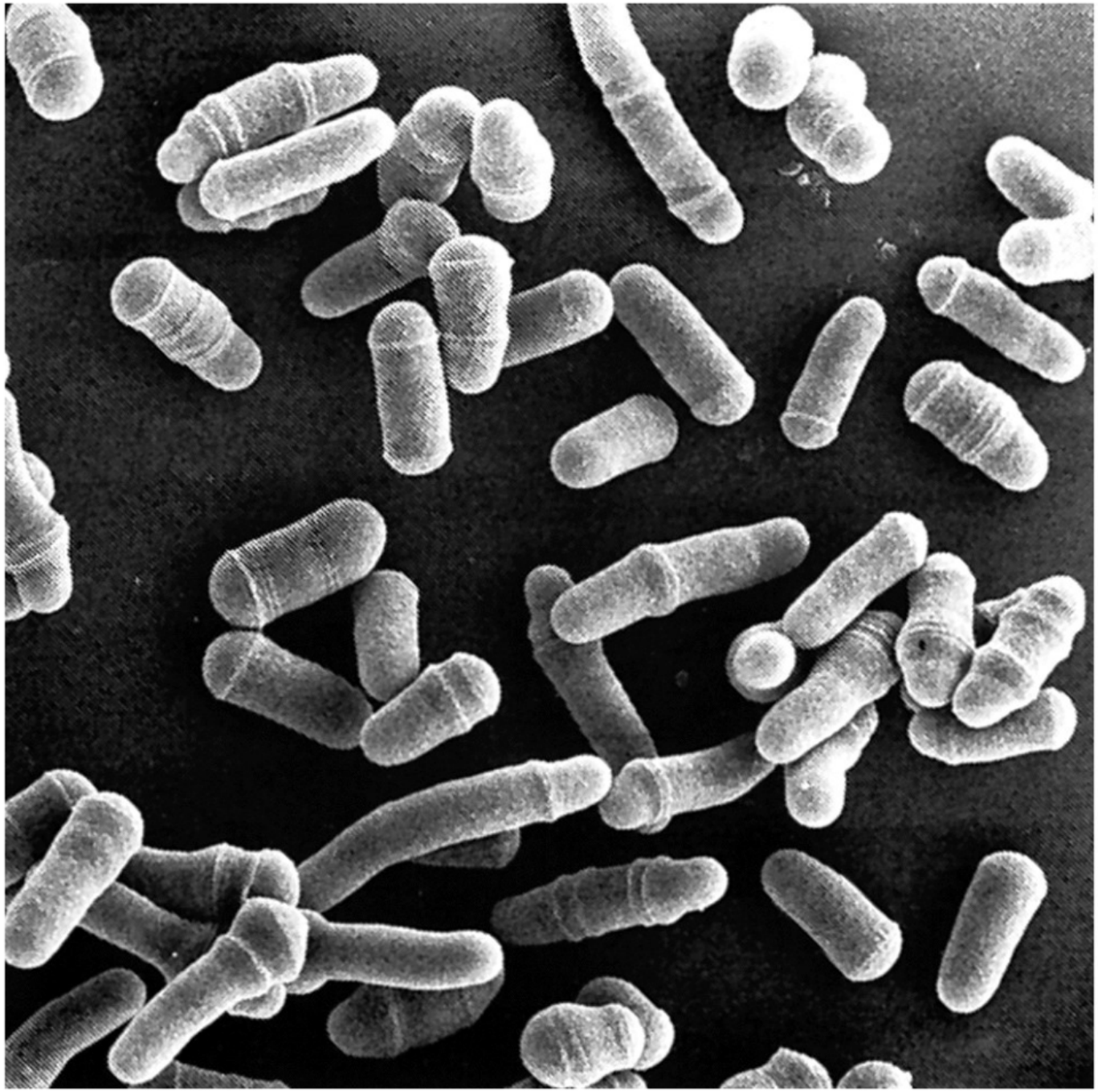
Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.

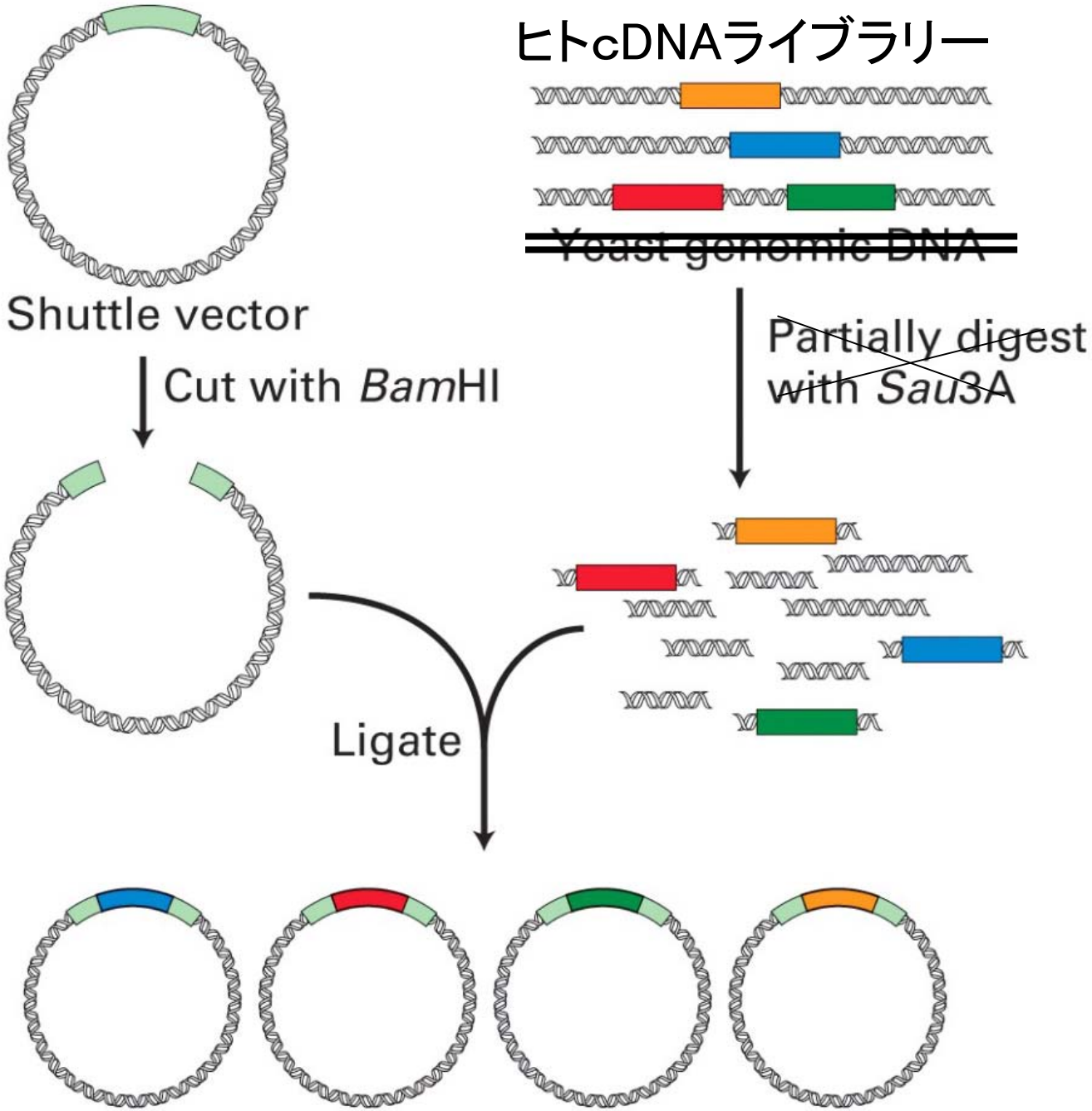
Lee MG, Nurse P.

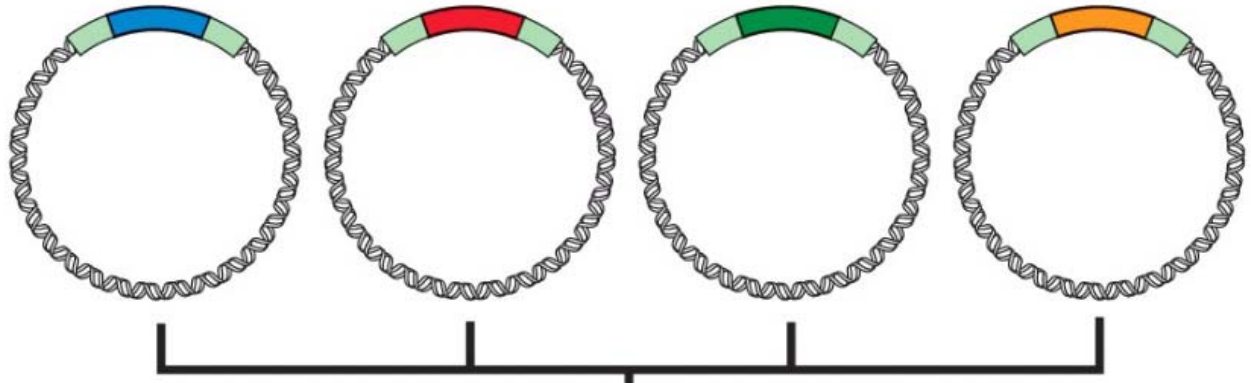
A human homologue of the cdc2 gene has been cloned by expressing a human cDNA library in fission yeast and selecting for clones that can complement a mutant of cdc2. The predicted protein sequence of the human homologue is very similar to that of the yeast cdc2 gene. These data indicate that elements of the mechanism by which the cell cycle is controlled are likely to be conserved between yeast and humans.

（P. Nurse: 2001年ノーベル賞）

ヒトのCdc2遺伝子を分裂酵母のcdc変異株を相補させることでクローニングした




ヒトcDNAライブラリー
Yeast genomic DNA
Shuttle vector
Cut with *Bam*HI
Partially digest
with *Sau*3A
Ligate



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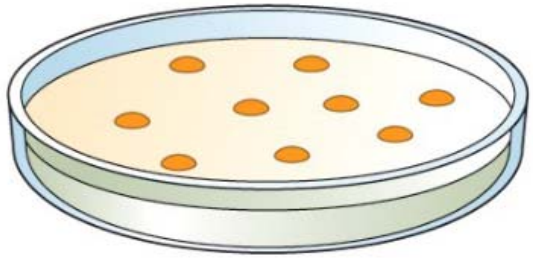
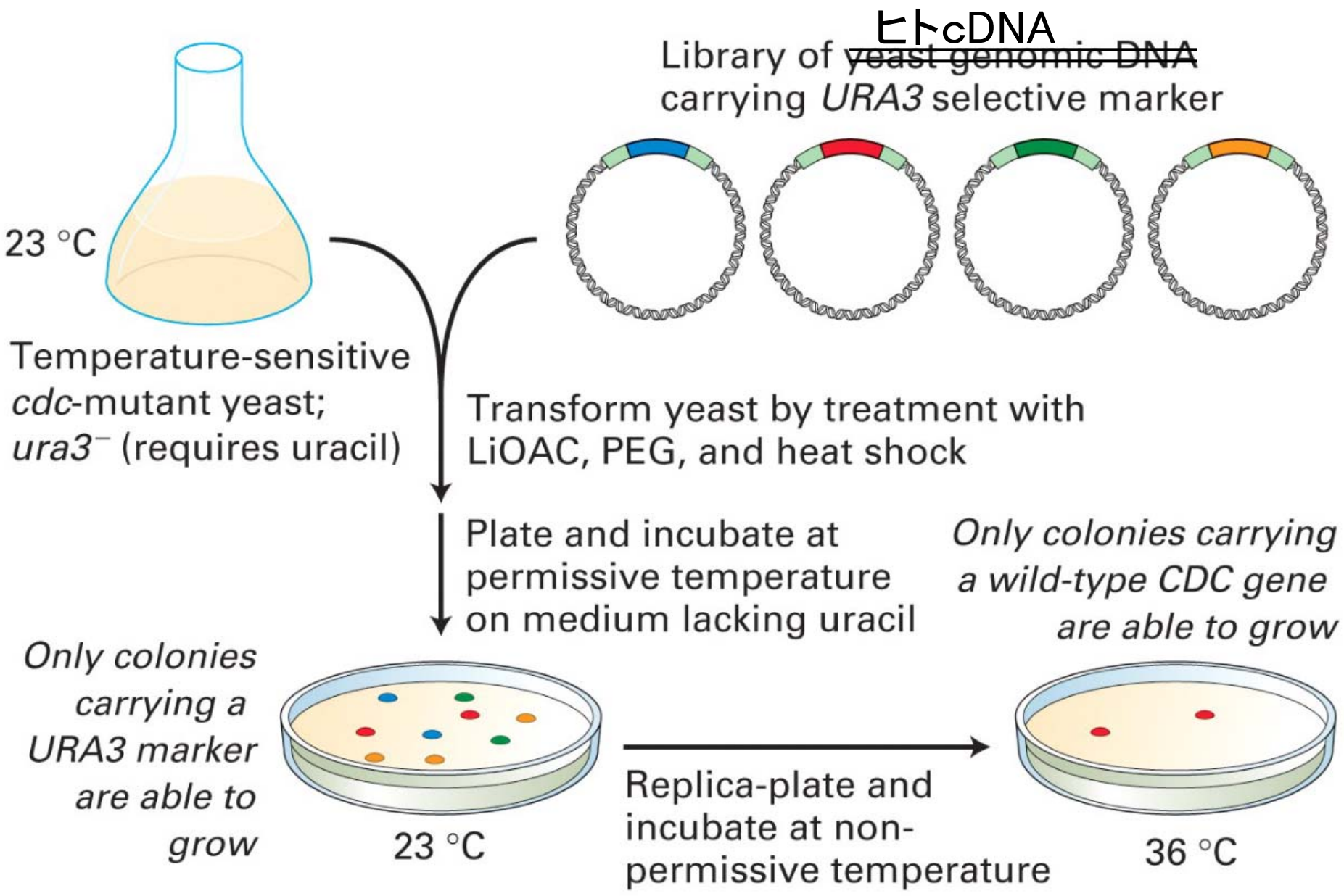
Screen for ampicillin resistance



Isolate and pool recombinant plasmids from 10^5 transformed *E. coli* colonies

Assay yeast genomic library by functional complementation


인간cDNA
Library of ~~yeast genomic DNA~~
carrying *URA3* selective marker
23 °C
Temperature-sensitive
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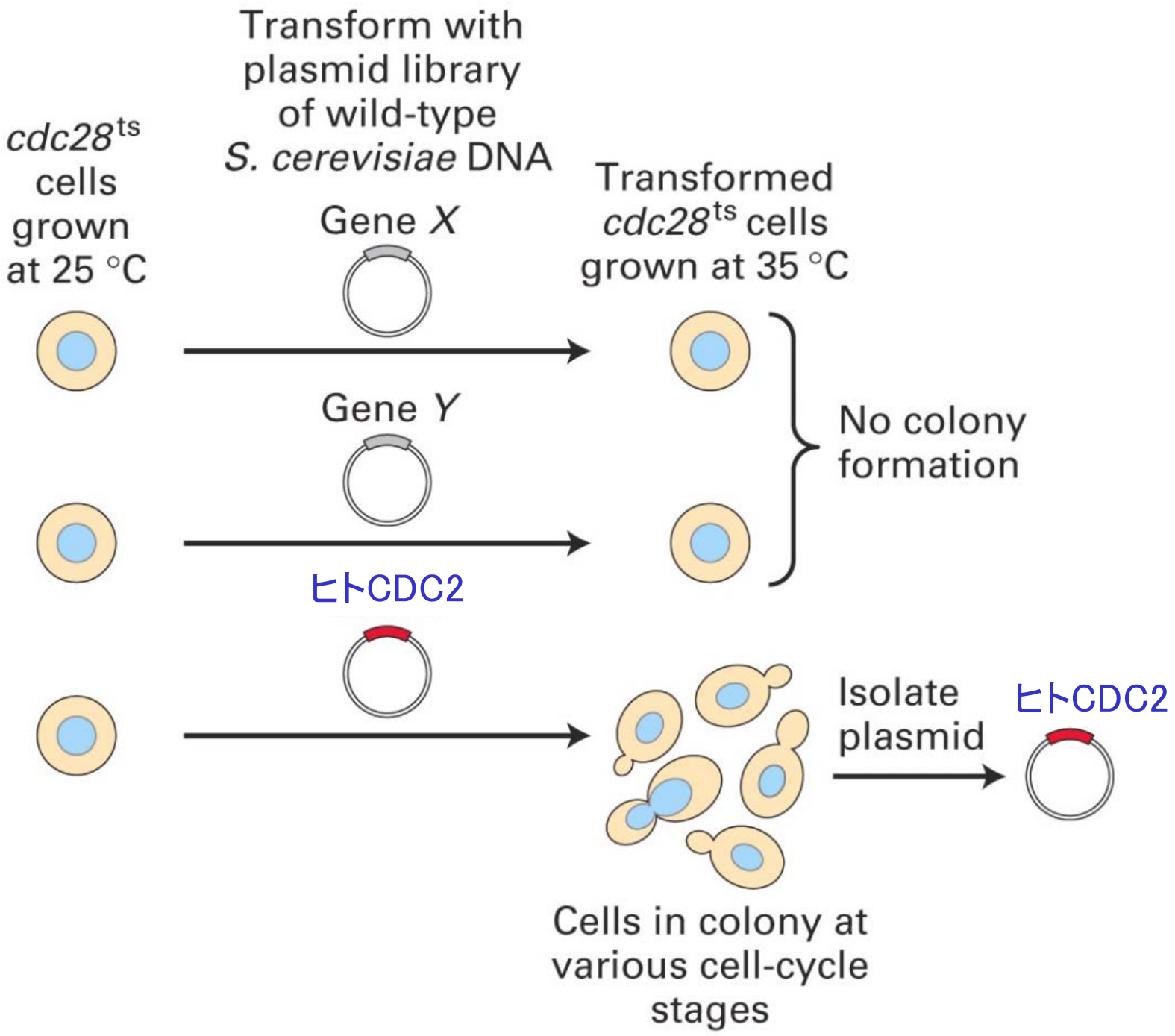
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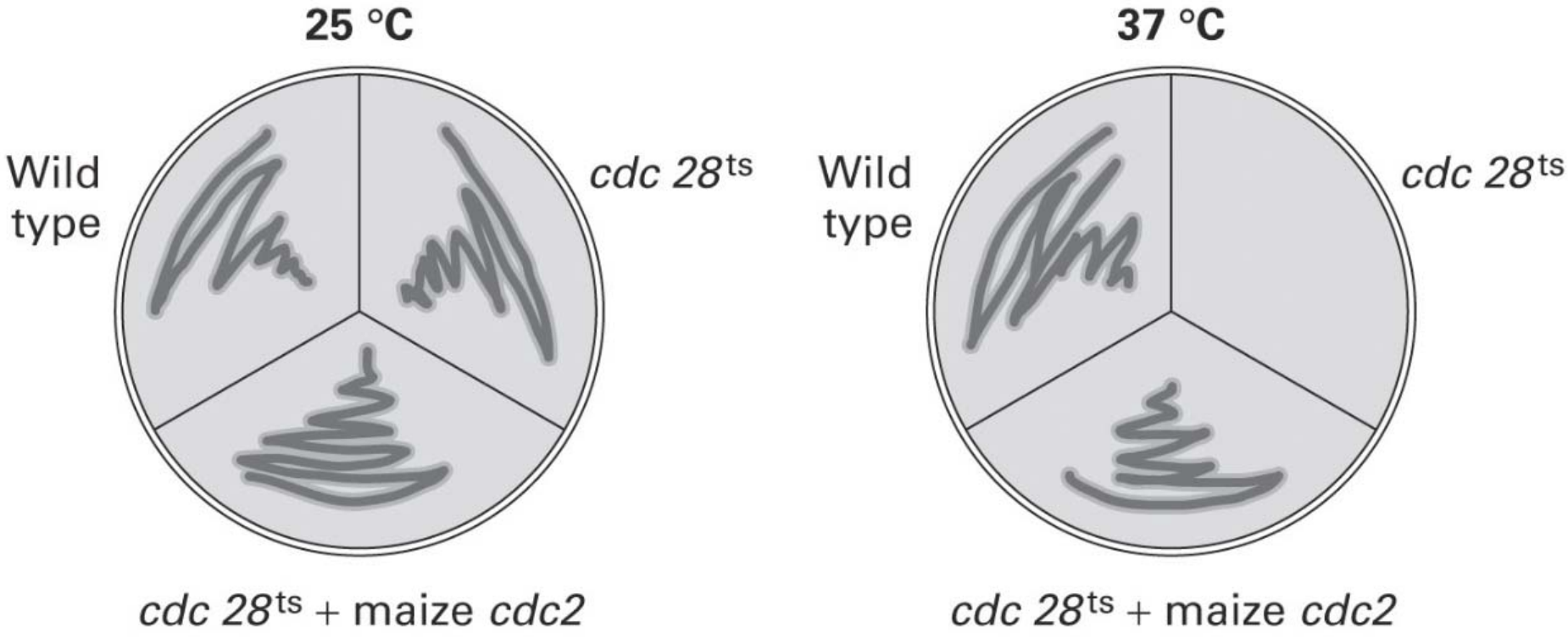
Gene X
Transformed cdc28ts cells grown at 35 °C
Gene Y
No colony formation
ヒトCDC2
ヒトCDC2
Isolate plasmid
Cells in colony at various cell-cycle stages

Functional complementation



ヒトCDC2遺伝子でも

次はCdc28と相互作用する遺伝子を知りたい

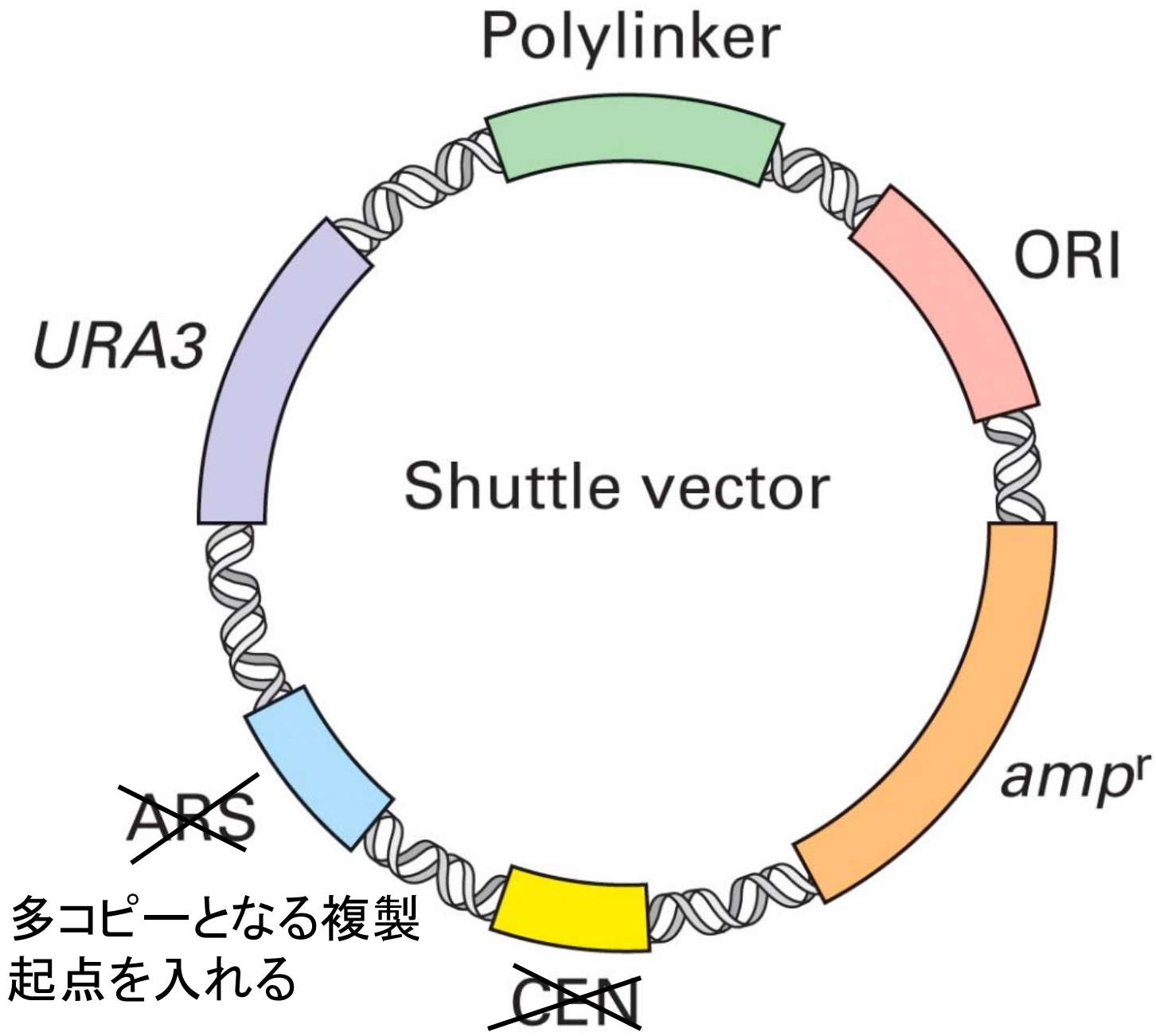
Cdc28と相互作用するタンパクを探す

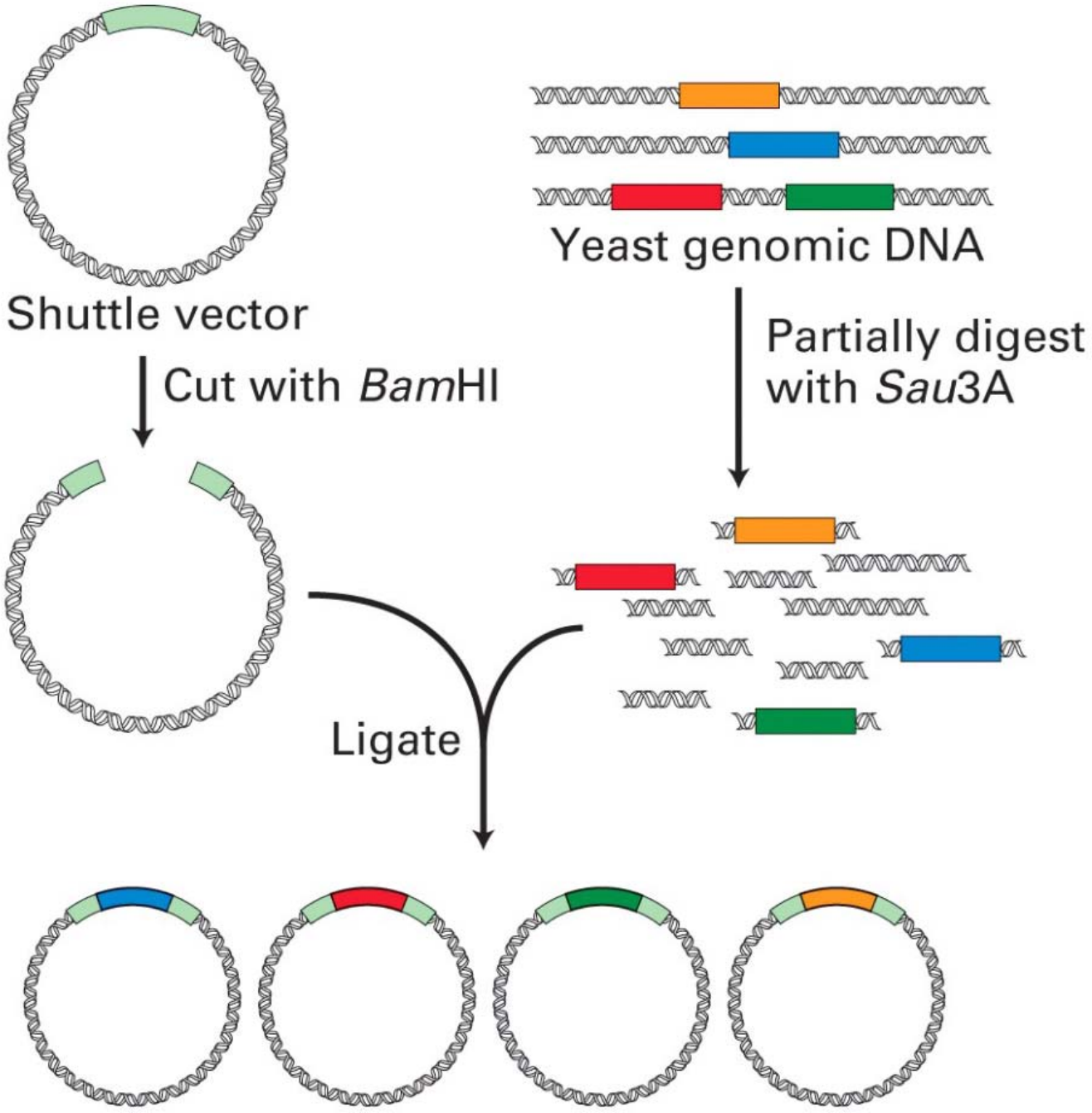
$cdc28^{ts}$　　このタンパク質の能力を向上させるものはもちろん重要

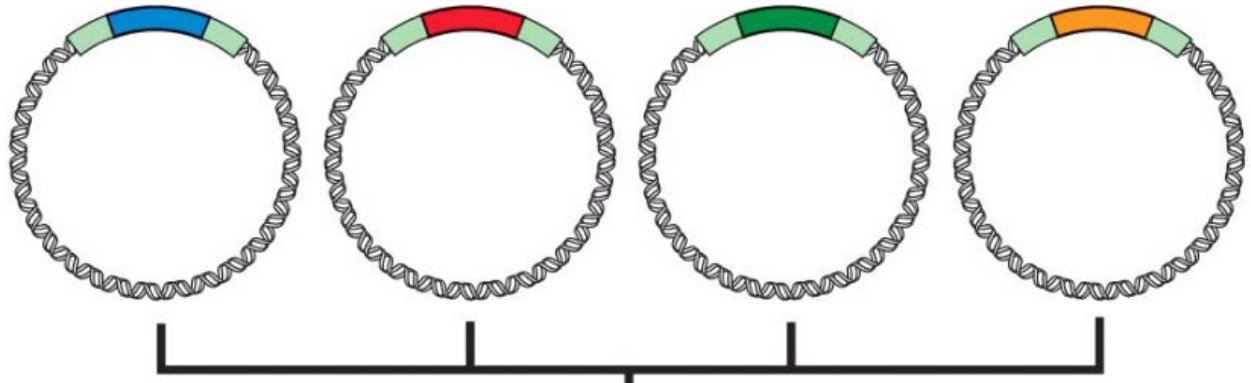
37℃で能力低下で死ぬのだから37℃でも生きる変異（遺伝子）を探せばよい⇒サプレッサー（抑圧）

・変異サプレッサー
・過剰発現サプレッサー

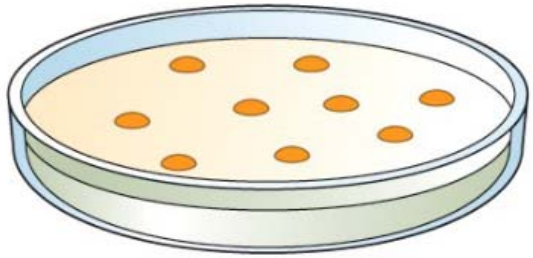
いろんな遺伝子過剰発現させてサプレッサーを探す
⇒取れたものはCLN（サイクリン）＝カエルのCLNと相同


Polylinker
ORI
URA3
Shuttle vector
ampr
ARS
CEN
多コピーとなる複製
起点を入れる


Shuttle vector
Cut with *Bam*HI
Yeast genomic DNA
Partially digest
with *Sau*3A
Ligate

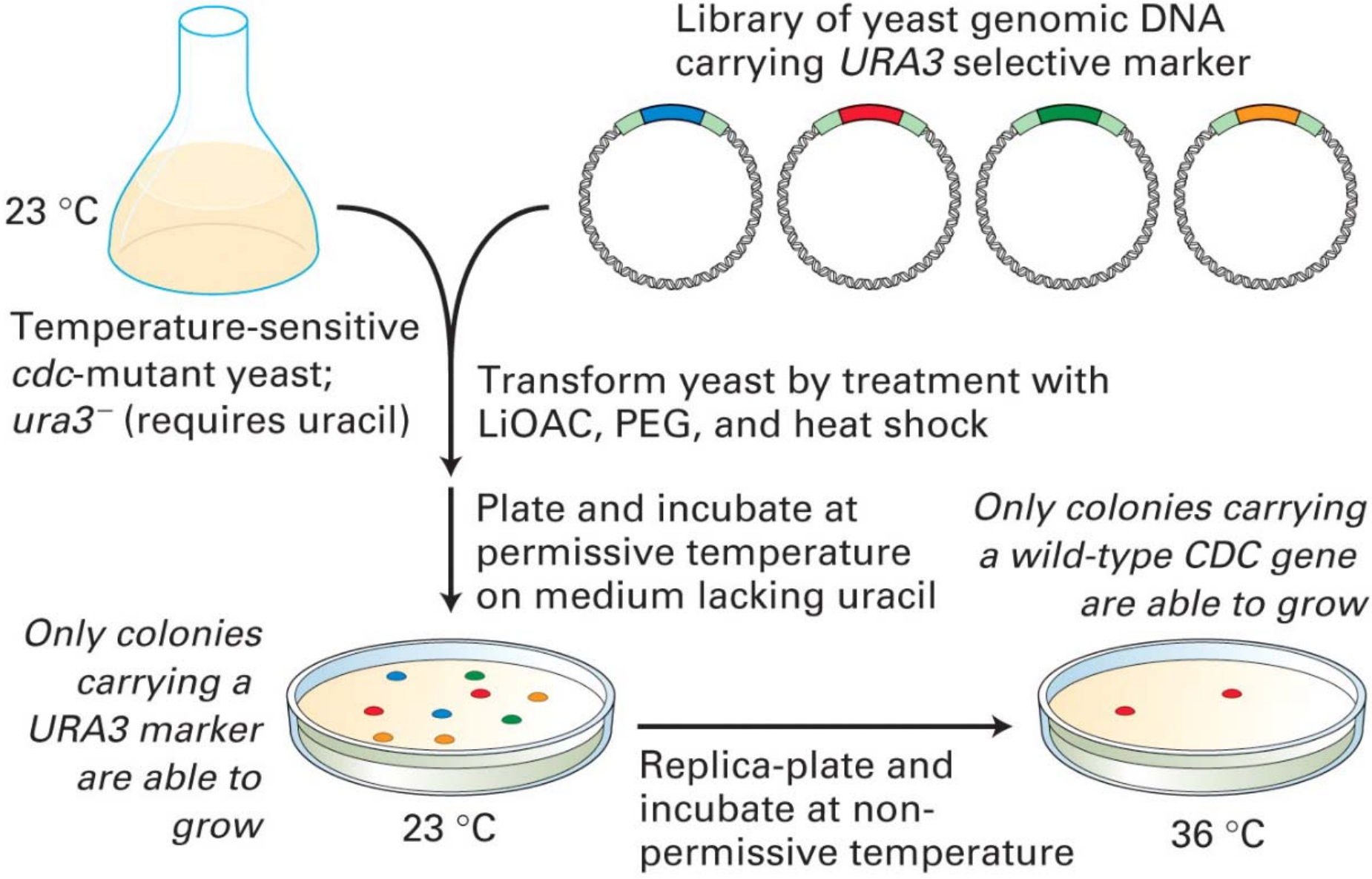


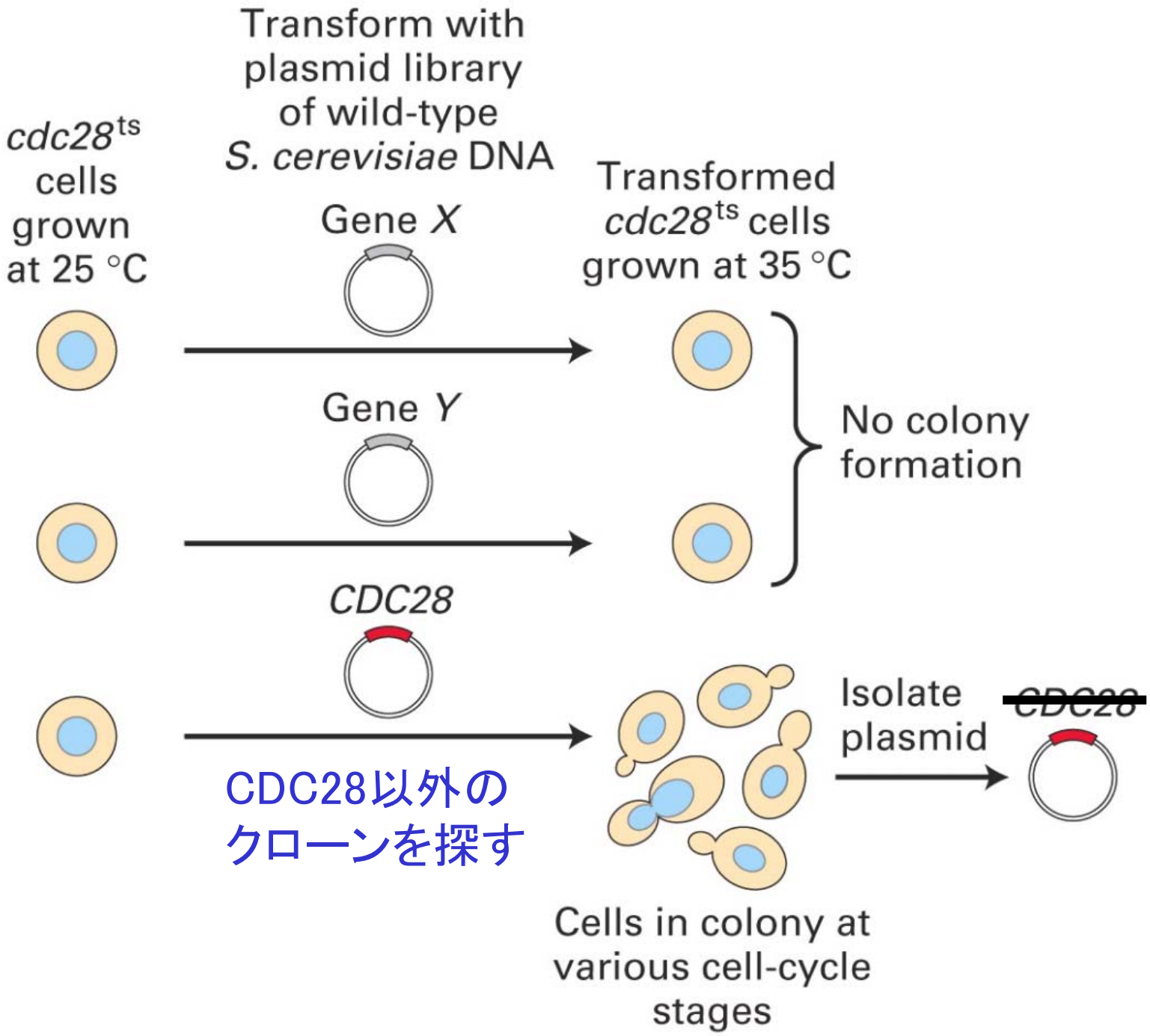
Transform *E. coli*
Screen for ampicillin resistance

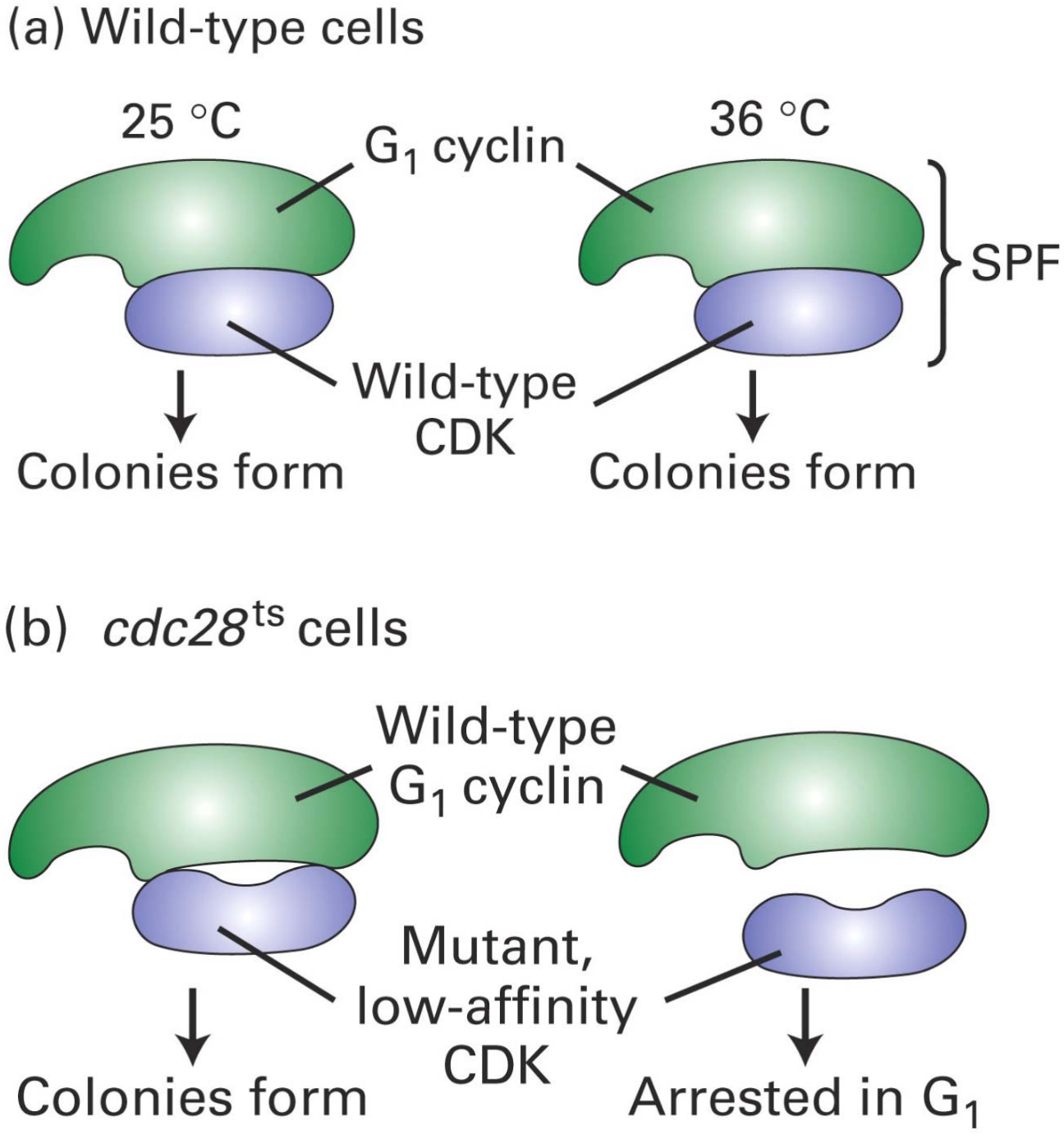


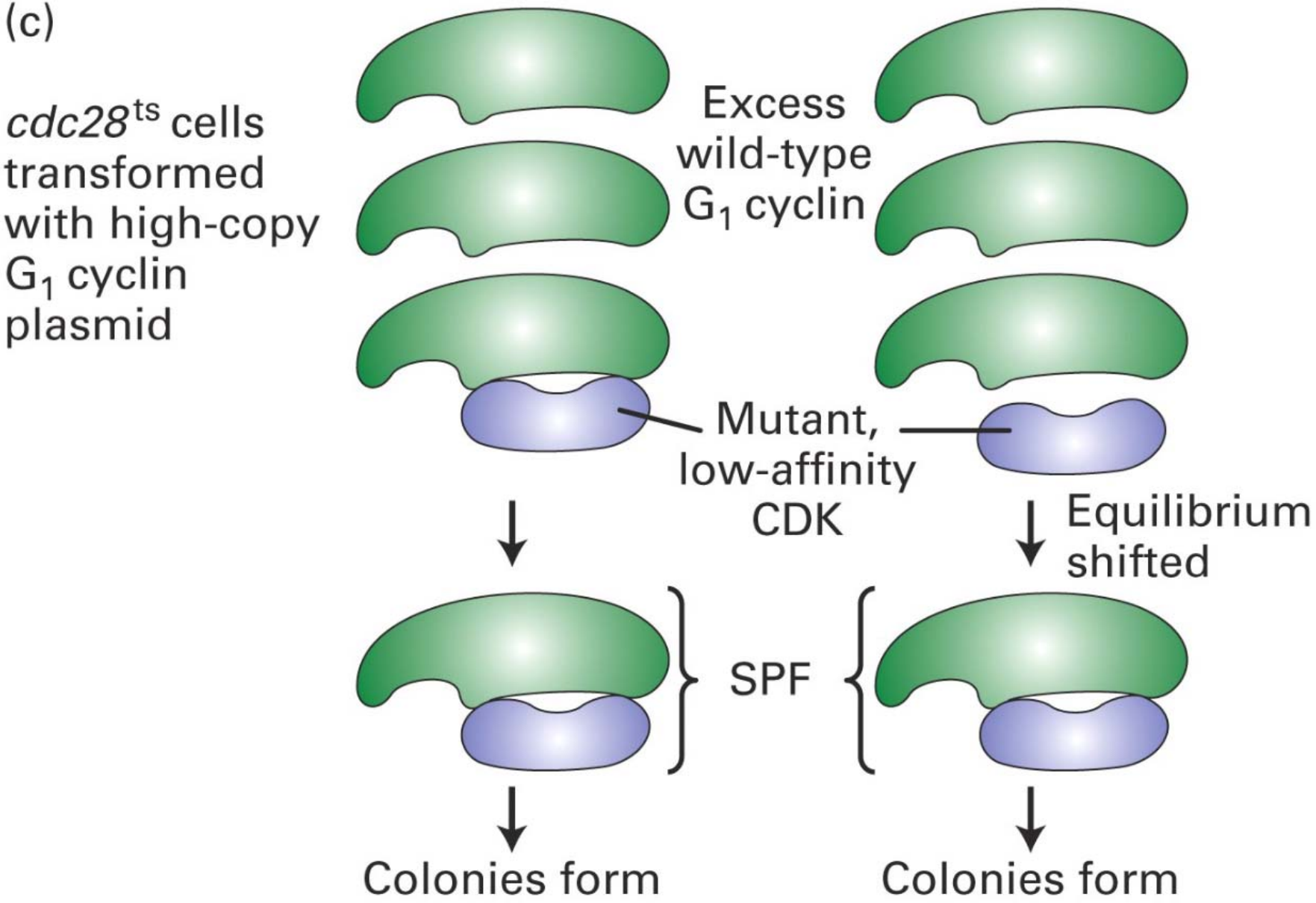
Isolate and pool recombinant plasmids from 10^5 transformed *E. coli* colonies

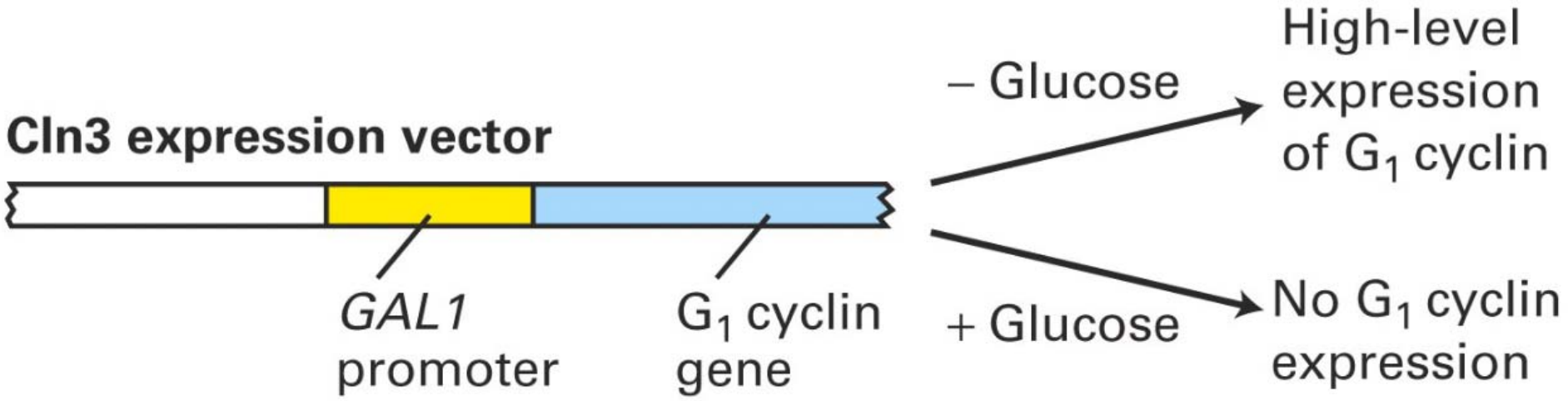
Assay yeast genomic library by functional complementation


Library of yeast genomic DNA carrying *URA3* selective marker
23 °C
Temperature-sensitive *cdc*-mutant yeast; *ura3*⁻ (requires uracil)
Transform yeast by treatment with LiOAC, PEG, and heat shock
Plate and incubate at permissive temperature on medium lacking uracil
Only colonies carrying a wild-type CDC gene are able to grow
Only colonies carrying a URA3 marker are able to grow
23 °C
Replica-plate and incubate at non-permissive temperature
36 °C

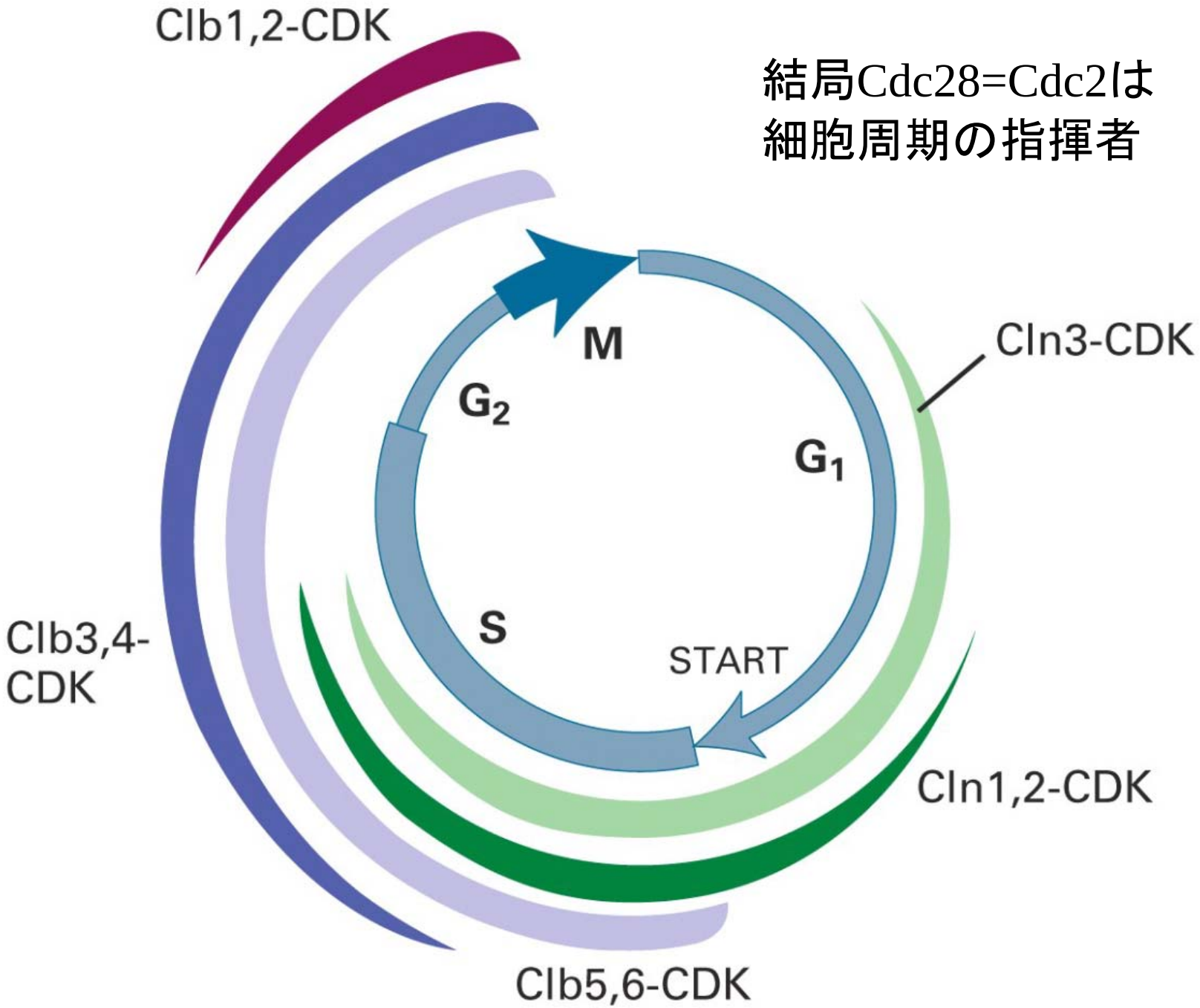

Transform with plasmid library of wild-type *S. cerevisiae* DNA
*cdc28*ts cells grown at 25 °C
Gene *X*
Gene *Y*
CDC28
Transformed *cdc28*ts cells grown at 35 °C
No colony formation
CDC28以外のクローンを探す
Cells in colony at various cell-cycle stages
Isolate plasmid
~~*CDC28*~~


(a) Wild-type cells
25 °C
G_1 cyclin
36 °C
SPF
Wild-type
CDK
Colonies form
Colonies form
(b) *cdc28*^{ts} cells
Wild-type
G_1 cyclin
Mutant,
low-affinity
CDK
Colonies form
Arrested in G_1


(c)
cdc28ts cells transformed with high-copy G1 cyclin plasmid
Excess wild-type G1 cyclin
Mutant, low-affinity CDK
Equilibrium shifted
SPF
Colonies form
Colonies form



過剰発現の別の方法
ガラクトース誘導性プロモーターを使う



結局Cdc28=Cdc2は
細胞周期の指揮者

酵母の遺伝子機能解析

クローニング
サプレッサー
過剰発現
エピスタティックテスト（上流下流試験）
Two hybrid system（タンパク結合試験）
遺伝子破壊
遺伝子置換
シンセティックリーサル(Synthetic lethal)
ゲノムワイドスクリーニング

酵母の自由自在な遺伝子操作が応用分子生命科学に必要

すべては形質転換をベースに

入れ方

1)Hinnen et al., 1978
　　スフェロプラスト法，又はプロトプラスト法

2)Ito et al., 1983
　　酢酸リチウム法

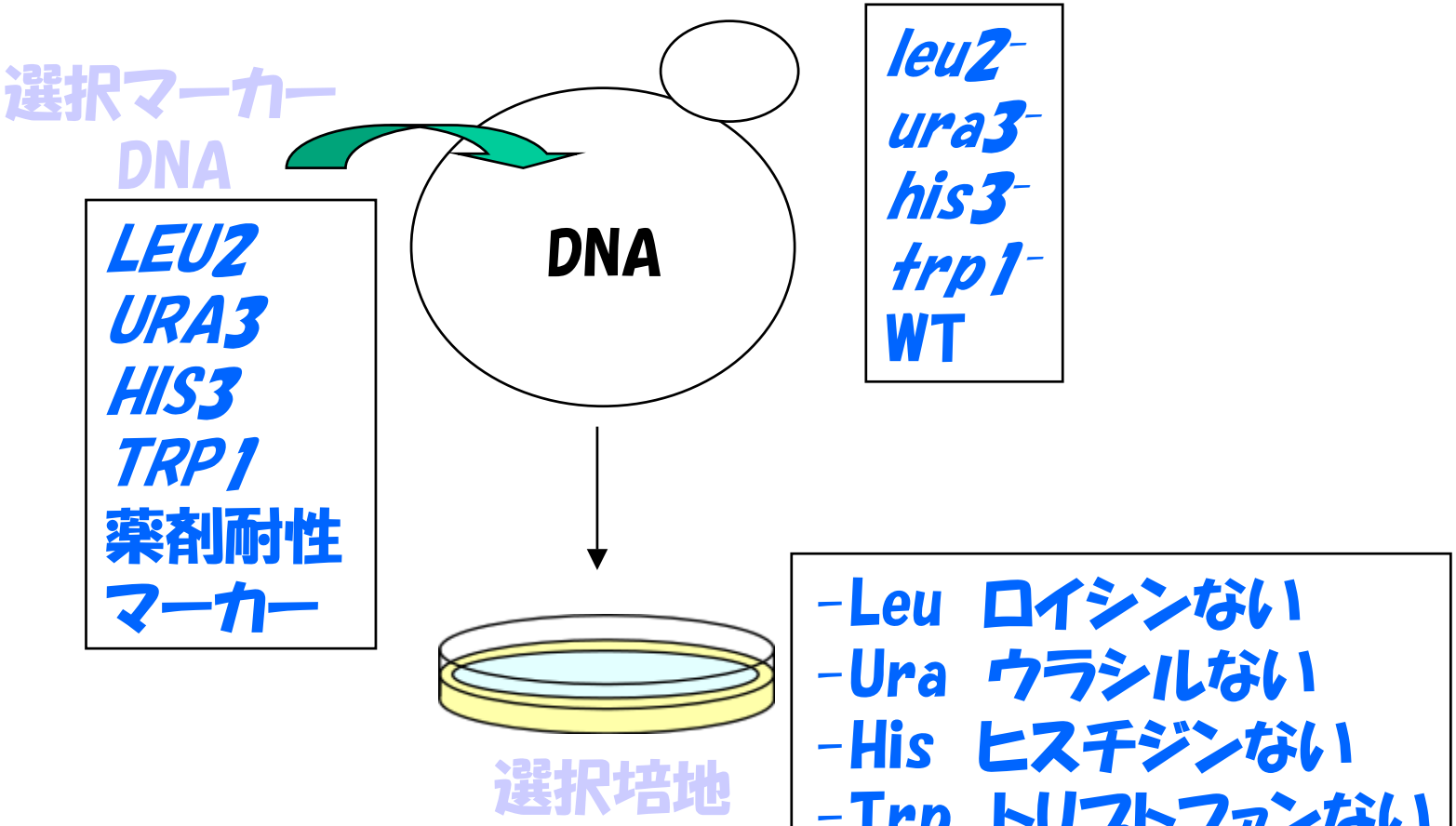
3)Hashimoto et al., 1985
　　エレクトロポレーション法

4)Johnston et al., 1988
　　バイオリスティックデリバリー（遺伝子銃）

5)Heinemann and Sprague, 1989
　　細菌と酵母の直接伝達

入ったものの選び方

選択マーカー



ベクター系
マーカーが載っているプラスミドのこと

1. 自律複製タイプ
    YEp：もともといたプラスミド2μm複製起点持つ
        多コピー　30-50個/細胞
    YRp：染色体複製起点(ARS)を持つ
        多コピー，不安定といわれている
    YCp：染色体複製起点とセントロメア(CEN)を持つ
        単コピー，CEN/ARS

2. 染色体挿入タイプ
    YIp：複製起点を持たないプラスミド，マーカーのみ

3. DNA断片を直接入れることもよくやる

Sikorski and Hieter, 1989.

YIp	YCp	YEp	Marker
pRS303	pRS313	pRS413	HIS3
pRS304	pRS314	pRS414	TRP1
pRS305	pRS315	pRS415	LEU2
pRS306	pRS316	pRS416	URA3

入った状態

・自律複製タイプは環状DNAのまま複製

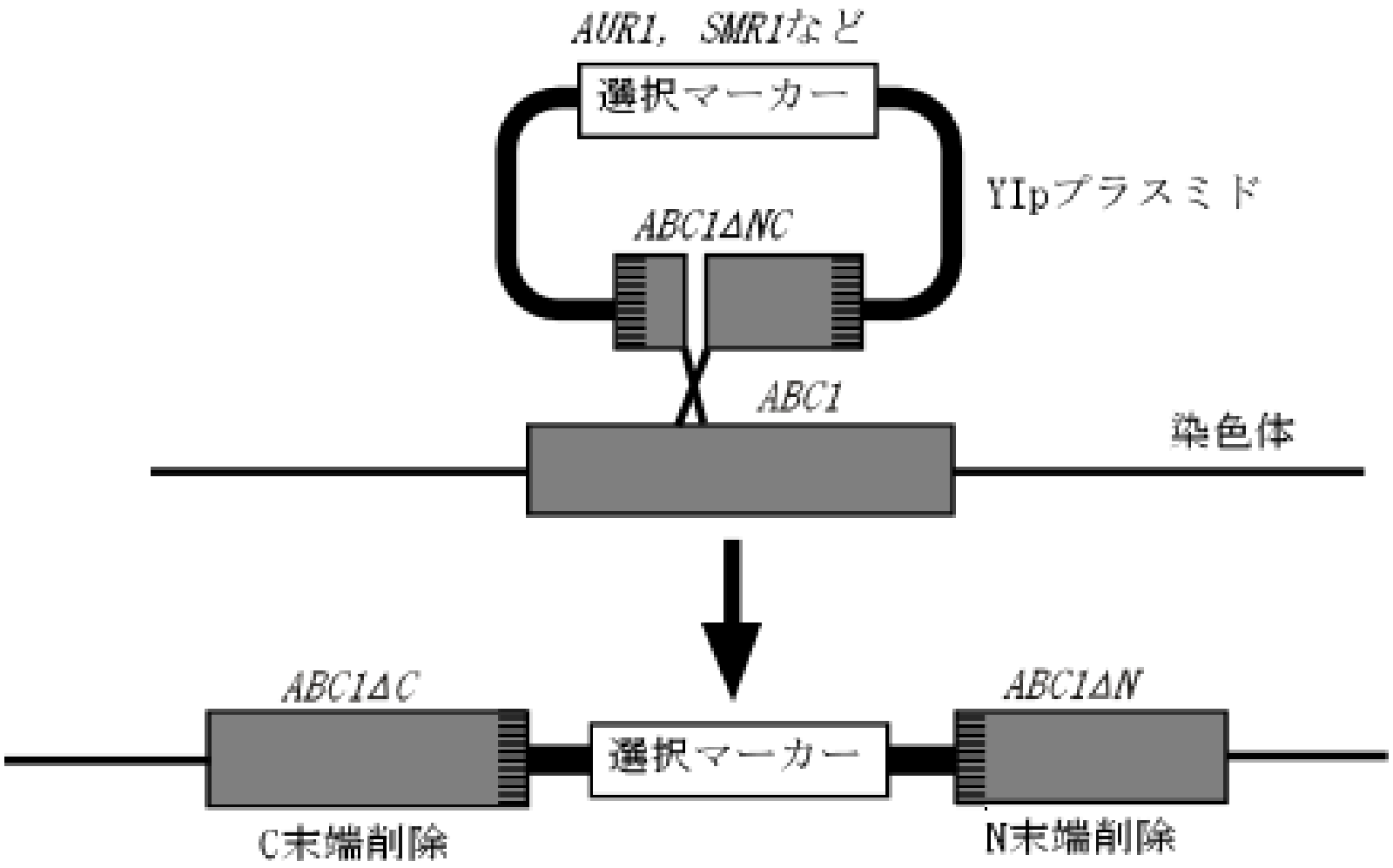
・染色体導入タイプ

Integration（挿入）

One-step replacement（1段階置換）

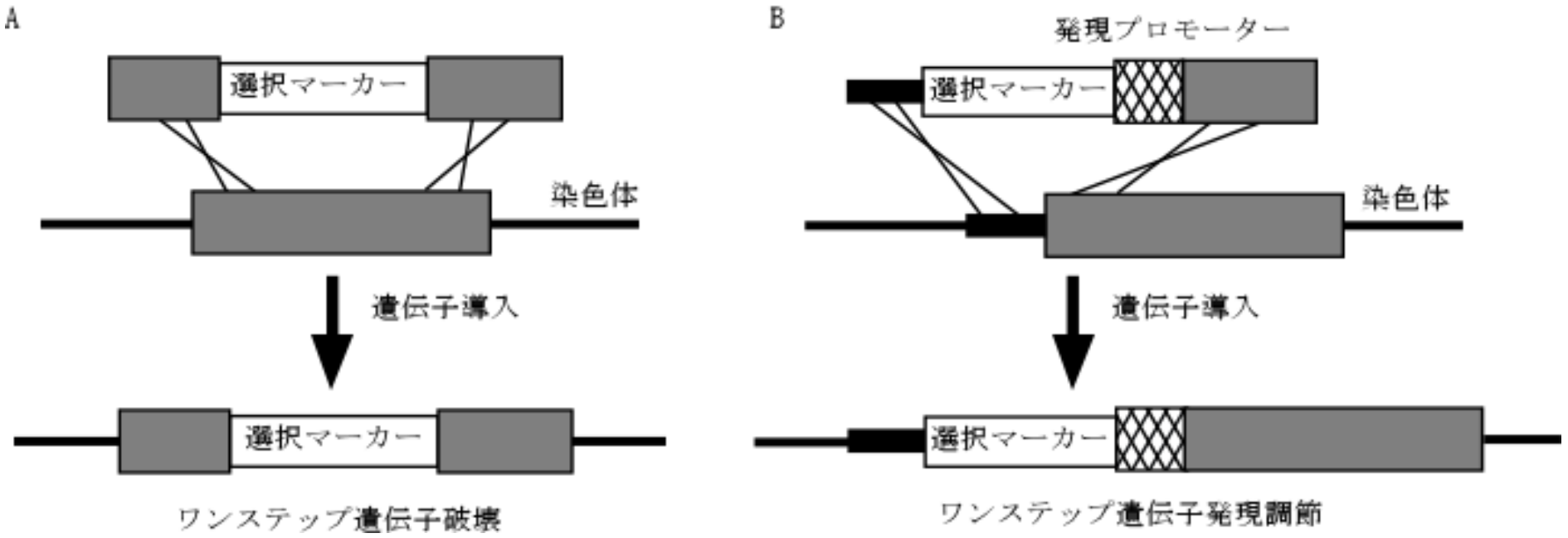
Two-step replacement（2段階置換）

染色体挿入型プラスミドの形質転換

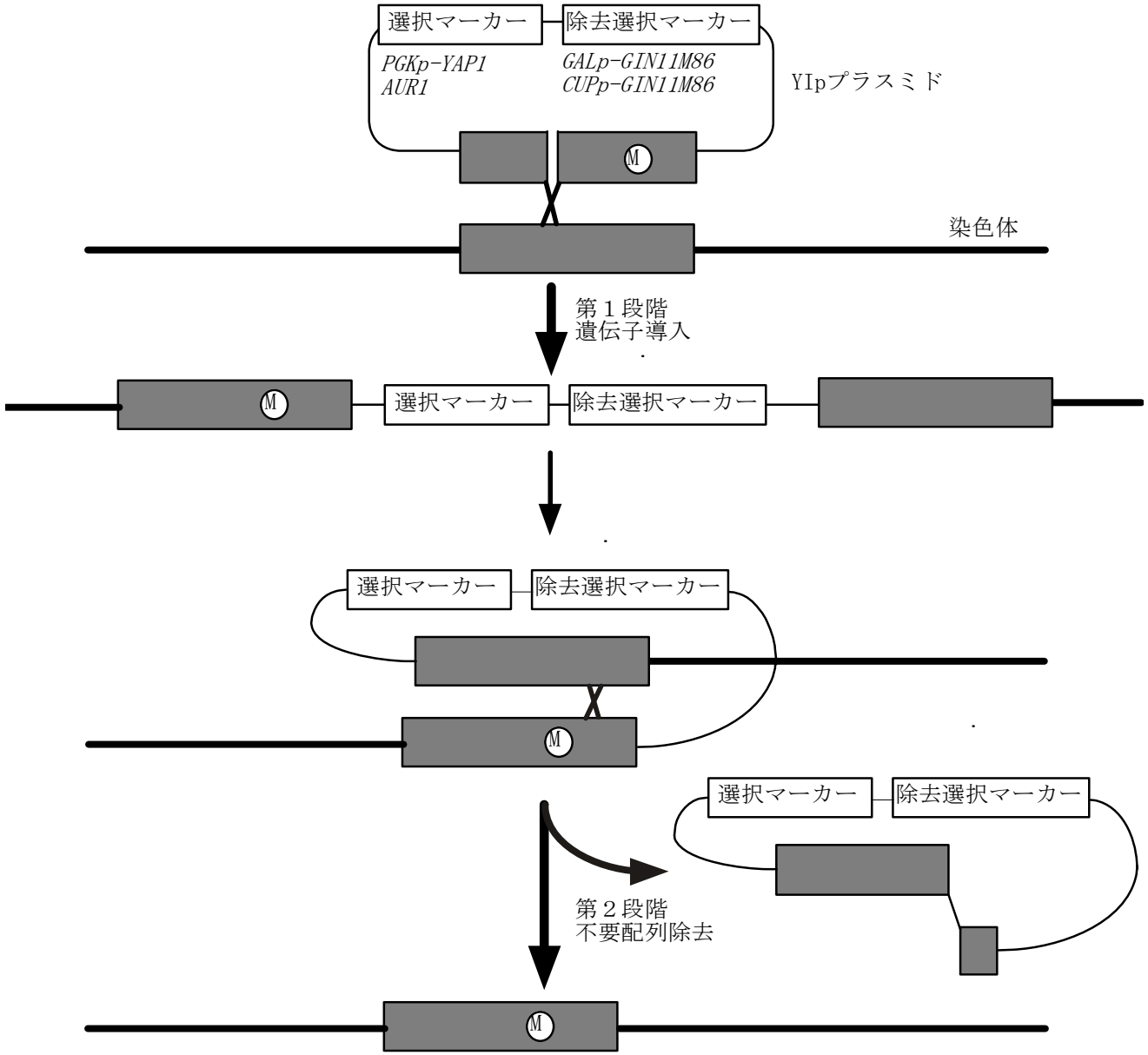


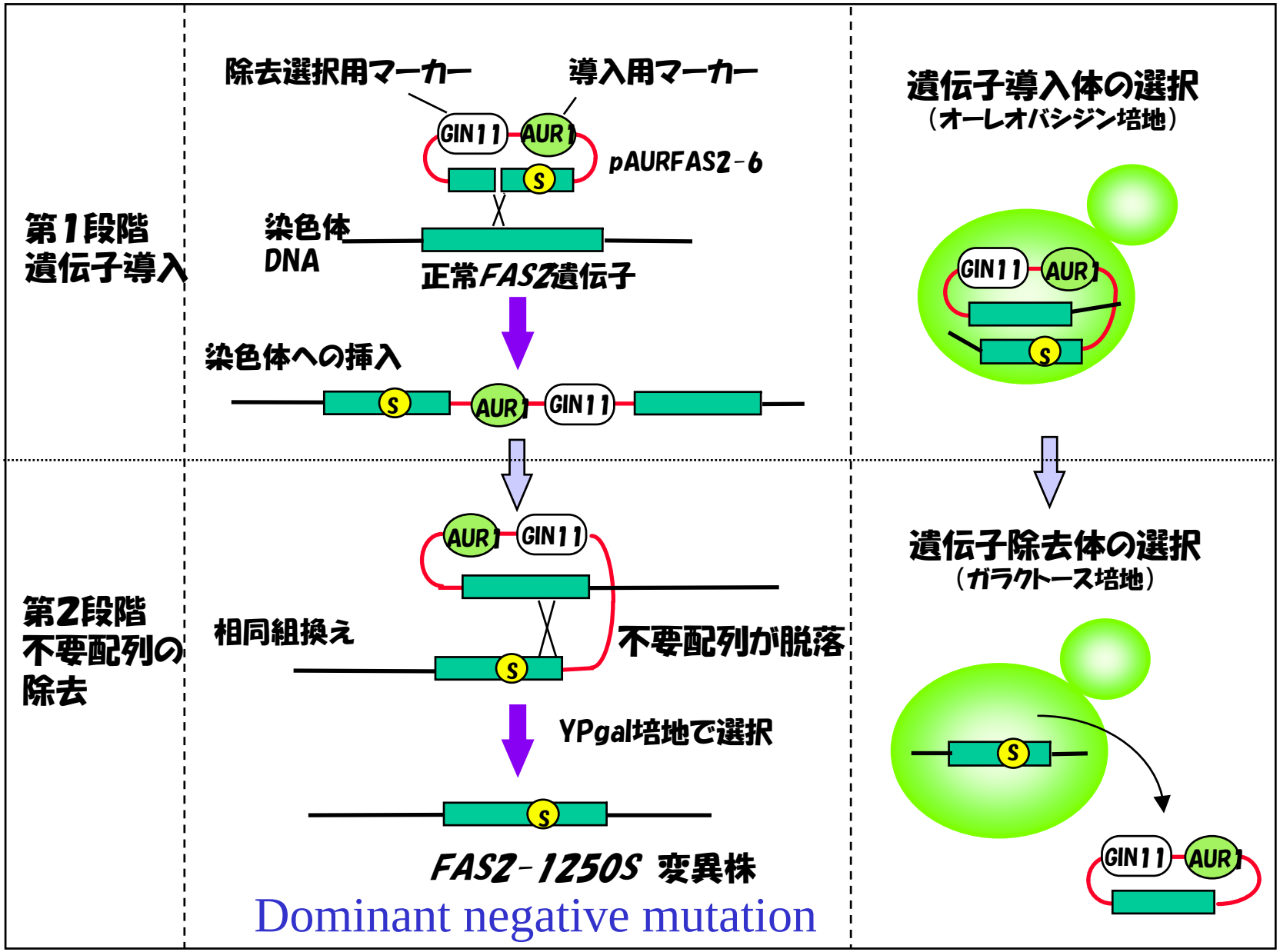
1段階置換

ワンステップ遺伝子置換または挿入



2段階遺伝子置換


選択マーカー
除去選択マーカー
PGKp-YAP1
AUR1
GALp-GIN11M86
CUPp-GIN11M86
YIpプラスミド
M
染色体
第１段階
遺伝子導入
選択マーカー
除去選択マーカー
第２段階
不要配列除去



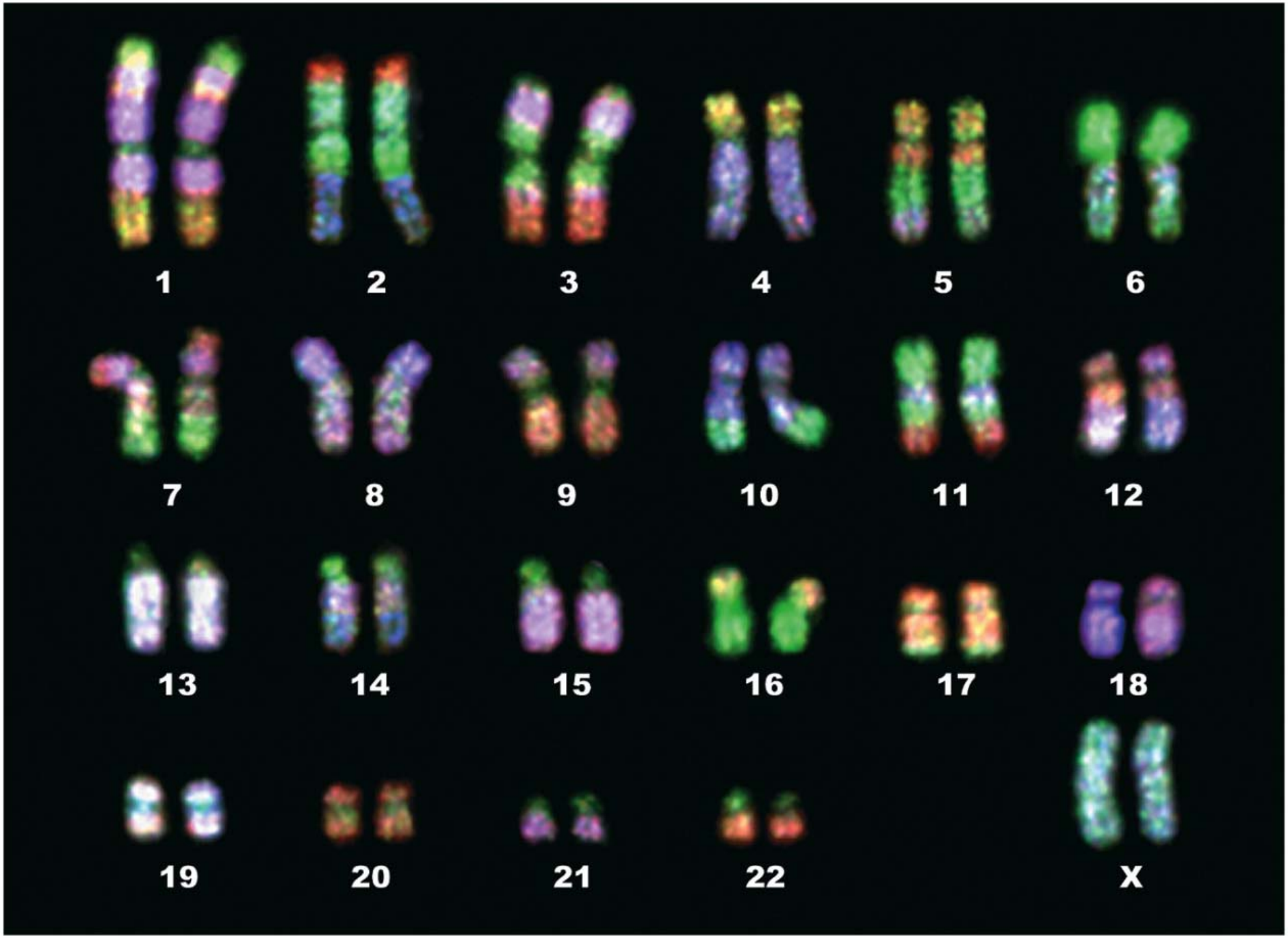
不要DNA配列を完全に除去する２段階遺伝子置換法による醸造酵母の育種

2003年春

たぶん世界最初に市販された遺伝子組換え酵母のお酒

Dominant negative typeの香り遺伝子
を2段階置換した酵母のお酒

GENOMES


1
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13
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16
17
18
19
20
21
22
X

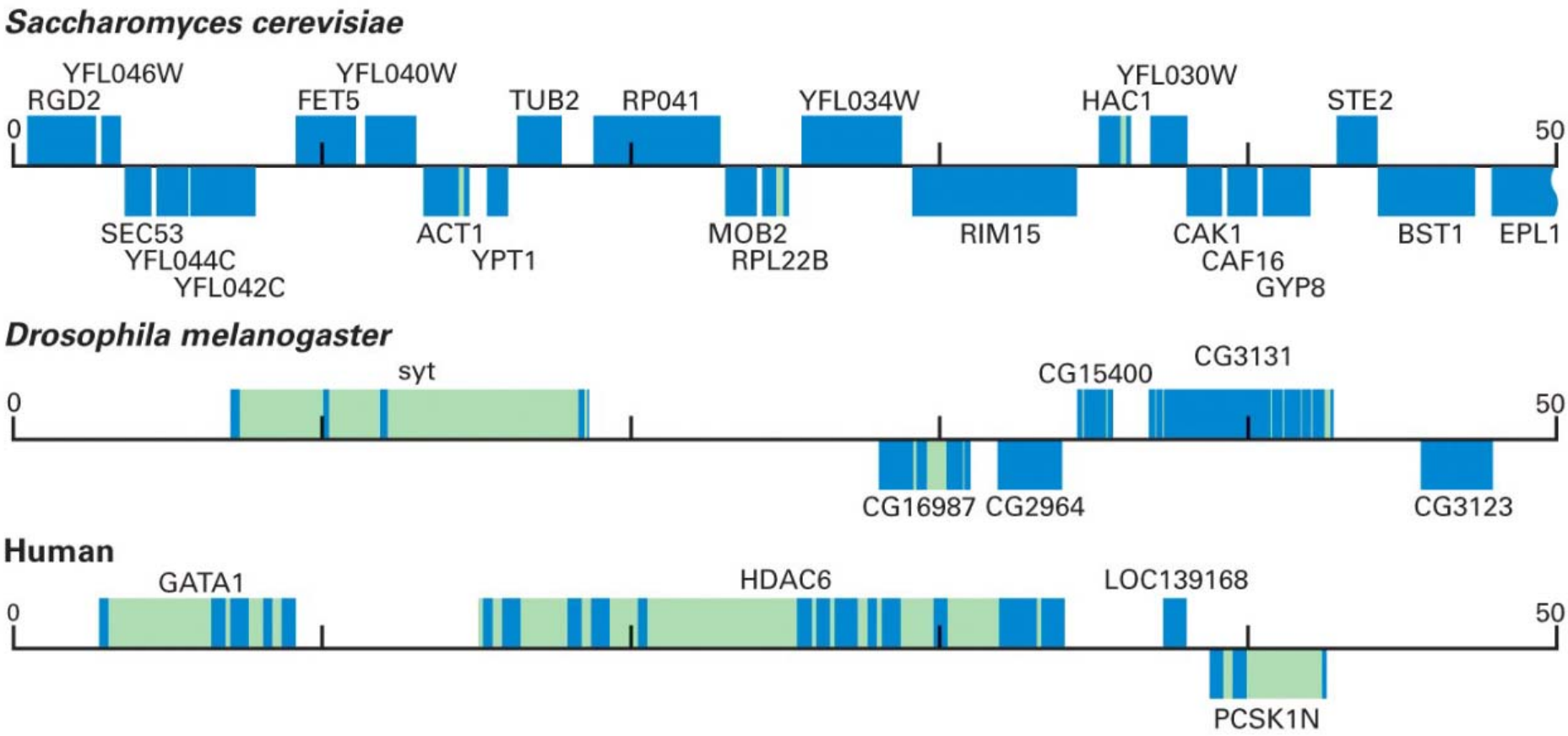
Microbial genomes

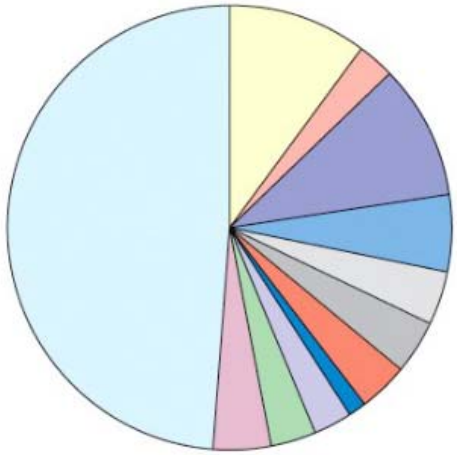
Acinetobacter sp. ADP1
Aeropyrum pernix K1
Agrobacterium tumefaciens str. C58
Agrobacterium tumefaciens str. C58
Anaplasma marginale str. St. Maries
Aquifex aeolicus VF5
Archaeoglobus fulgidus DSM 4304
Azoarcus sp. EbN1
Bacillus anthracis str. 'Ames Ancestor'
Bacillus anthracis str. A2012
Bacillus anthracis str. Ames
Bacillus anthracis str. Sterne
Bacillus cereus ATCC 10987
Bacillus cereus ATCC 14579
Bacillus cereus ZK
Bacillus halodurans C-125
Bacillus licheniformis ATCC 14580
Bacillus licheniformis ATCC 14580
Bacillus subtilis subsp. subtilis str. 168
Bacillus thuringiensis serovar konkukian str. 97-27
Bacteroides fragilis YCH46
Bacteroides thetaiotaomicron VPI-5482
Bartonella henselae str. Houston-1
Bartonella quintana str. Toulouse
Bdellovibrio bacteriovorus HD100
Bifidobacterium longum NCC2705
Bordetella bronchiseptica RB50
Bordetella parapertussis 12822
Bordetella pertussis Tohama I
Borrelia burgdorferi B31
Borrelia garinii PBi
Bradyrhizobium japonicum USDA 110
Brucella melitensis 16M
Brucella suis 1330
Buchnera aphidicola str. APS
Buchnera aphidicola str. Bp
Buchnera aphidicola str. Sg
Burkholderia mallei ATCC 23344
Burkholderia pseudomallei K96243
Campylobacter jejuni subsp. jejuni NCTC 11168
Candidatus Blochmannia floridanus
Caulobacter crescentus CB15
Chlamydia muridarum
Chlamydia trachomatis D/UW-3/CX
Chlamydophila caviae GPIC
Chlamydophila pneumoniae AR39
Chlamydophila pneumoniae CWL029
Chlamydophila pneumoniae J138
Chlamydophila pneumoniae TW-183
Chlorobium tepidum TLS
Chromobacterium violaceum ATCC 12472
Clostridium acetobutylicum ATCC 824
Clostridium perfringens str. 13
Clostridium tetani E88
Corynebacterium diphtheriae NCTC 13129
Corynebacterium efficiens YS-314
Corynebacterium glutamicum ATCC 13032
Coxiella burnetii RSA 493
Deinococcus radiodurans R1
Desulfotalea psychrophila LSv54
Desulfovibrio vulgaris subsp. vulgaris str.
Enterococcus faecalis V583
Erwinia carotovora subsp. atroseptica SCRI1043
Escherichia coli CFT073
Escherichia coli K12
Escherichia coli O157:H7
Escherichia coli O157:H7 EDL933
Francisella tularensis subsp. tularensis
Fusobacterium nucleatum subsp. nucleatum
Geobacillus kaustophilus HTA426
Geobacter sulfurreducens PCA
Gloeobacter violaceus PCC 7421
Haemophilus ducreyi 35000HP
Haemophilus influenzae Rd KW20
Haloarcula marismortui ATCC 43049
Halobacterium salinarum NRC-1
Helicobacter hepaticus ATCC 51449
Helicobacter pylori 26695
Helicobacter pylori J99
Idiomarina loihiensis L2TR
Lactobacillus johnsonii NCC 533
Lactobacillus plantarum WCFS1
Lactococcus lactis subsp. lactis Il1403
Legionella pneumophila str. Lens
Legionella pneumophila str. Paris
Legionella pneumophila subsp. pneumophila
Leifsonia xyli subsp. xyli str. CTCB07
Leptospira interrogans serovar Copenhageni
Leptospira interrogans serovar Lai str. 56601
Listeria innocua Clip11262
Listeria monocytogenes EGD-e
Listeria monocytogenes str. 4b F2365
Mannheimia succiniciproducens MBEL55E
Mesoplasma florum L1
Mesorhizobium loti MAFF303099
Methanocaldococcus jannaschii DSM 2661
Methanococcus maripaludis S2
Methanopyrus kandleri AV19
Methanosarcina acetivorans C2A
Methanosarcina mazei Go1
Methanothermobacter thermautotrophicus str. Delta H
Methylococcus capsulatus str. Bath
Mycobacterium avium subsp. paratuberculosis str. k10
Mycobacterium bovis AF2122/97
Mycobacterium leprae TN
Mycobacterium tuberculosis CDC1551
Mycobacterium tuberculosis H37Rv
Mycoplasma gallisepticum R
Mycoplasma genitalium G-37
Mycoplasma hyopneumoniae 232
Mycoplasma mobile 163K
Mycoplasma mycoides subsp. mycoides SC str. PG1
Mycoplasma penetrans HF-2
Mycoplasma pneumoniae M129
Mycoplasma pulmonis UAB CTIP
Nanoarchaeum equitans Kin4-M
Neisseria meningitidis MC58
Neisseria meningitidis Z2491
Nitrosomonas europaea ATCC 19718
Nocardia farcinica IFM 10152
Nostoc sp. PCC 7120
Oceanobacillus iheyensis HTE831
Onion yellows phytoplasma OY-M
Parachlamydia sp. UWE25
Pasteurella multocida subsp. multocida str. Pm70
Photobacterium profundum SS9
Photorhabdus luminescens subsp. laumondii TTO1
Picrophilus torridus DSM 9790
Porphyromonas gingivalis W83
Prochlorococcus marinus str. MIT 9313
Prochlorococcus marinus subsp. marinus str. CCMP1375
Prochlorococcus marinus subsp. pastoris str. CCMP1986
Propionibacterium acnes KPA171202
Pseudomonas aeruginosa PAO1
Pseudomonas putida KT2440
Pseudomonas syringae pv. tomato str. DC3000
Pyrobaculum aerophilum str. IM2
Pyrococcus abyssi GE5
Pyrococcus furiosus DSM 3638
Pyrococcus horikoshii OT3
Ralstonia solanacearum GMI1000
Rhodopirellula baltica SH 1
Rhodopseudomonas palustris CGA009
Rickettsia conorii str. Malish 7
Rickettsia prowazekii str. Madrid E
Rickettsia typhi str. Wilmington
Salmonella enterica subsp. enterica serovar Paratypi A str.
Salmonella enterica subsp. enterica serovar Typhi Ty2
Salmonella enterica subsp. enterica serovar Typhi str.
Salmonella typhimurium LT2
Shewanella oneidensis MR-1
Shigella flexneri 2a str. 2457T
Shigella flexneri 2a str. 301
Silicibacter pomeroyi DSS-3
Sinorhizobium meliloti 1021
Staphylococcus aureus subsp. aureus MRSA252
Staphylococcus aureus subsp. aureus MSSA476
Staphylococcus aureus subsp. aureus MW2
Staphylococcus aureus subsp. aureus Mu50
Staphylococcus aureus subsp. aureus N315
Staphylococcus epidermidis ATCC 12228
Streptococcus agalactiae 2603V/R
Streptococcus agalactiae NEM316
Streptococcus mutans UA159
Streptococcus pneumoniae R6
Streptococcus pneumoniae TIGR4
Streptococcus pyogenes M1 GAS
Streptococcus pyogenes MGAS10394
Streptococcus pyogenes MGAS315
Streptococcus pyogenes MGAS8232
Streptococcus pyogenes SSI-1
Streptococcus thermophilus CNRZ1066
Streptococcus thermophilus LMG 18311
Streptomyces avermitilis MA-4680
Streptomyces coelicolor A3(2)
Sulfolobus solfataricus P2
Sulfolobus tokodaii str. 7
Symbiobacterium thermophilum IAM 14863
Synechococcus sp. WH 8102
Synechocystis sp. PCC 6803
Thermoanaerobacter tengcongensis MB4
Thermoplasma acidophilum DSM 1728
Thermoplasma volcanium GSS1
Thermosynechococcus elongatus BP-1
Thermotoga maritima MSB8
Thermus thermophilus HB27
Thermus thermophilus HB8
Treponema denticola ATCC 35405
Treponema pallidum subsp. pallidum str. Nichols
Tropheryma whipplei TW08/27
Tropheryma whipplei str. Twist
Ureaplasma parvum serovar 3 str. ATCC 700970
Vibrio cholerae O1 biovar eltor str. N16961
Vibrio parahaemolyticus RIMD 2210633
Vibrio vulnificus CMCP6
Vibrio vulnificus YJ016
Wigglesworthia glossinidia endosymbiont of Glossina
Wolbachia endosymbiont of Drosophila melanogaster
Wolinella succinogenes DSM 1740
Xanthomonas axonopodis pv. citri str. 306
Xanthomonas campestris pv. campestris str. ATCC 33
Xylella fastidiosa 9a5c
Xylella fastidiosa Temecula1
Yersinia pestis CO92

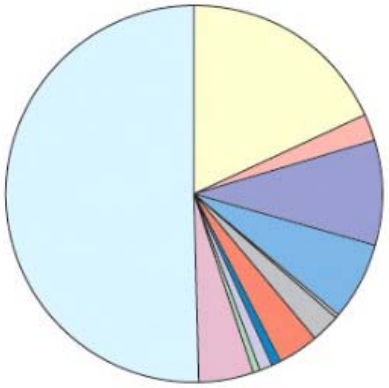
Eukaryotic microbes

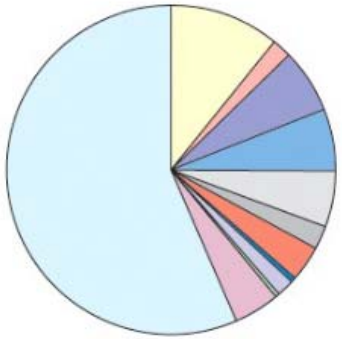
Cryptosporidium hominis
Cryptosporidium parvum
Plasmodium berghei strain ANKA
Plasmodium falciparum 3D7
Plasmodium yoelii yoelii
Theileria annulata
Toxoplasma gondii
Giardia lamblia ATCC 50803
Entamoeba histolytica
Entamoeba histolytica HM-1:IMSS
Aspergillus fumigatus
Aspergillus nidulans FGSC A4
Aspergillus terreus ATCC 20542
Coccidioides immitis RS
Coccidioides posadasii C735
Gibberella zeae PH-1
Magnaporthe grisea 70-15
Neurospora crassa
Candida albicans SC5314
Candida glabrata CBS138
Debaryomyces hansenii CBS767
Eremothecium gossypii
Kluyveromyces lactis NRRL Y-1140
Kluyveromyces waltii NCYC 2644
Naumovia castellii NRRL Y-12630
Saccharomyces bayanus 623-6C
Saccharomyces bayanus MCYC 623
Saccharomyces cerevisiae
Saccharomyces kluyveri NRRL Y-12651
Saccharomyces kudriavzevii IFO 1802
Saccharomyces mikatae IFO 1815
Saccharomyces paradoxus NRRL Y-17217
Yarrowia lipolytica CLIB99
Schizosaccharomyces pombe
Coprinopsis cinerea okayama7#130
Cryptococcus neoformans var. grubii H99
Cryptococcus neoformans var. neoformans B-3501A
Cryptococcus neoformans var. neoformans JEC21
Phanerochaete chrysosporium RP-78
Ustilago maydis 521

Genome
Transcriptome
Proteome
Interactorome
Metabolome
Phenome

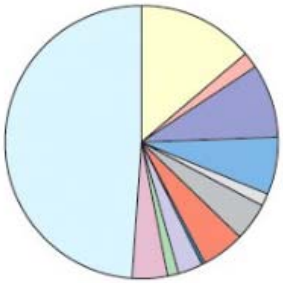

Saccharomyces cerevisiae
YFL046W
RGD2
YFL040W
FET5
TUB2
RP041
YFL034W
YFL030W
HAC1
STE2
0
50
SEC53
YFL044C
YFL042C
ACT1
YPT1
MOB2
RPL22B
RIM15
CAK1
CAF16
GYP8
BST1
EPL1
Drosophila melanogaster
syt
CG15400
CG3131
0
50
CG16987
CG2964
CG3123
Human
GATA1
HDAC6
LOC139168
0
50
PCSK1N

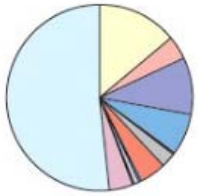






Organism	Human	*Arabidopsis* (plant)	*C. elegans* (roundworm)
Genes	~32,000	25,706	18,266



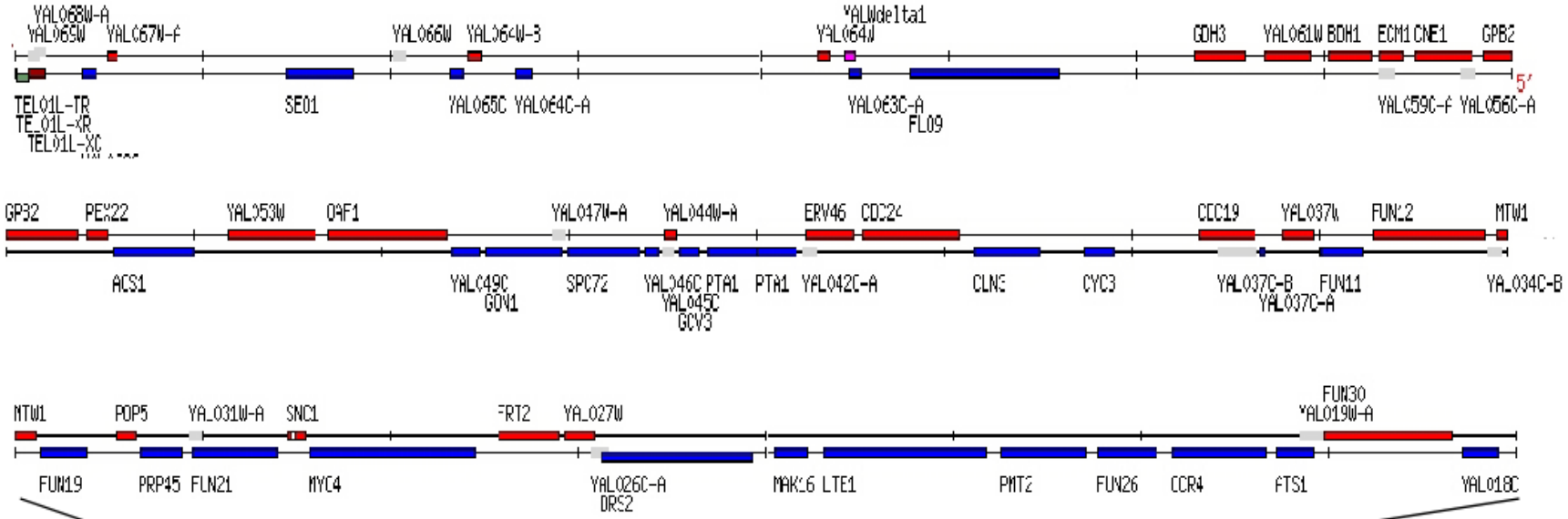


Organism	*Drosophila* (fly)	*Saccharomyces* (yeast)
Genes	13,338	~6000

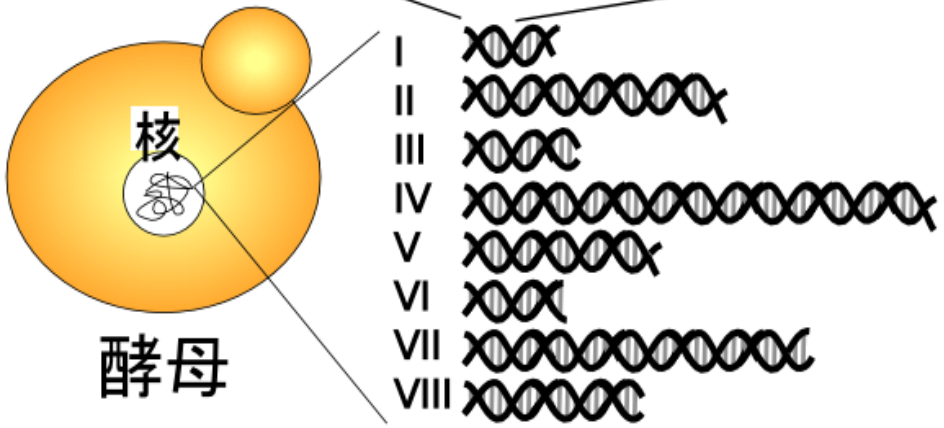
- Metabolism
- DNA replication/modification
- Transcription/translation
- Intracellular signaling
- Cell-cell communication
- Protein folding and degradation
- Transport
- Multifunctional proteins
- Cytoskeleton/structure
- Defense and immunity
- Miscellaneous function
- Unknown

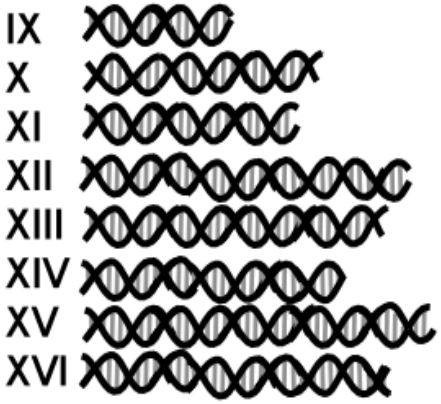
酵母ゲノム

全塩基数12,070,542 bp



全配列の約1/100





酵母遺伝子数　約6000個

内訳

1200個の必須遺伝子－－－破壊すると死ぬ

4800個の非必須遺伝子－－破壊しても生きている

Functional profiling of the *Saccharomyces cerevisiae* genome

Guri Giaever[1], Angela M. Chu[2], Li Ni[3], Carla Connelly[4], Linda Riles[5], Steeve Véronneau[6], Sally Dow[7], Ankuta Lucau-Danila[8], Keith Anderson[1], Bruno André[9], Adam P. Arkin[10], Anna Astromoff[2], Mohamed El Bakkoury[11], Rhonda Bangham[3], Rocio Benito[12], Sophie Brachat[13], Stefano Campanaro[14], Matt Curtiss[5], Karen Davis[1], Adam Deutschbauer[2], Karl-Dieter Entian[15], Patrick Flaherty[10,16], Francoise Foury[8], David J. Garfinkel[17], Mark Gerstein[18], Deanna Gotte[17], Ulrich Güldener[19], Johannes H. Hegemann[19], Svenja Hempel[15], Zelek Herman[1], Daniel F. Jaramillo[1], Diane E. Kelly[20], Steven L. Kelly[20], Peter Kötter[15], Darlene LaBonte[3], David C. Lamb[20], Ning Lan[18], Hong Liang[2], Hong Liao[3], Lucy Liu[3], Chuanyun Luo[3], Marc Lussier[6], Rong Mao[4], Patrice Menard[6], Siew Loon Ooi[4], Jose L. Revuelta[12], Christopher J. Roberts[7], Matthias Rose[15], Petra Ross-Macdonald[3], Bart Scherens[11], Greg Schimmack[7], Brenda Shafer[17], Daniel D. Shoemaker[2], Sharon Sookhai-Mahadeo[4], Reginald K. Storms[21], Jeffrey N. Strathern[17], Giorgio Valle[14], Marleen Voet[22], Guido Volckaert[22], Ching-yun Wang[17], Teresa R. Ward[7], Julie Wilhelmy[5], Elizabeth A. Winzeler[2], Yonghong Yang[3], Grace Yen[2], Elaine Youngman[4], Kexin Yu[4], Howard Bussey[6], Jef D. Boeke[4], Michael Snyder[3], Peter Philippsen[13], Ronald W. Davis[1,2] & Mark Johnston[5]

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[11]*IRMW, Université Libre de Bruxelles, B-1070 Brussels, Belgium*
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[15]*EUROSCARF, Johann Wolfgang Goethe-Universität, Institute of Microbiology, D-60439 Frankfurt/Main, Germany*
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[21]*Department of Biology, Concordia University, Montreal, Québec H3G 1M8, Canada*
[22]*Katholieke Universiteit Leuven, Laboratory of Gene Technology, B-3001 Leuven, Belgium*

Determining the effect of gene deletion is a fundamental approach to understanding gene function. Conventional genetic screens exhibit biases, and genes contributing to a phenotype are often missed. We systematically constructed a nearly complete collection of gene-deletion mutants (96% of annotated open reading frames, or ORFs) of the yeast *Saccharomyces cerevisiae*. DNA sequences dubbed 'molecular bar codes' uniquely identify each strain, enabling their growth to be analysed in parallel and the fitness contribution of each gene to be quantitatively assessed by hybridization to high-density oligonucleotide arrays. We show that previously known and new genes are necessary for optimal growth under six well-studied conditions: high salt, sorbitol, galactose, pH 8, minimal medium and nystatin treatment. Less than 7% of genes that exhibit a significant increase in messenger RNA expression are also required for optimal growth in four of the tested conditions. Our results validate the yeast gene-deletion collection as a valuable resource for functional genomics.

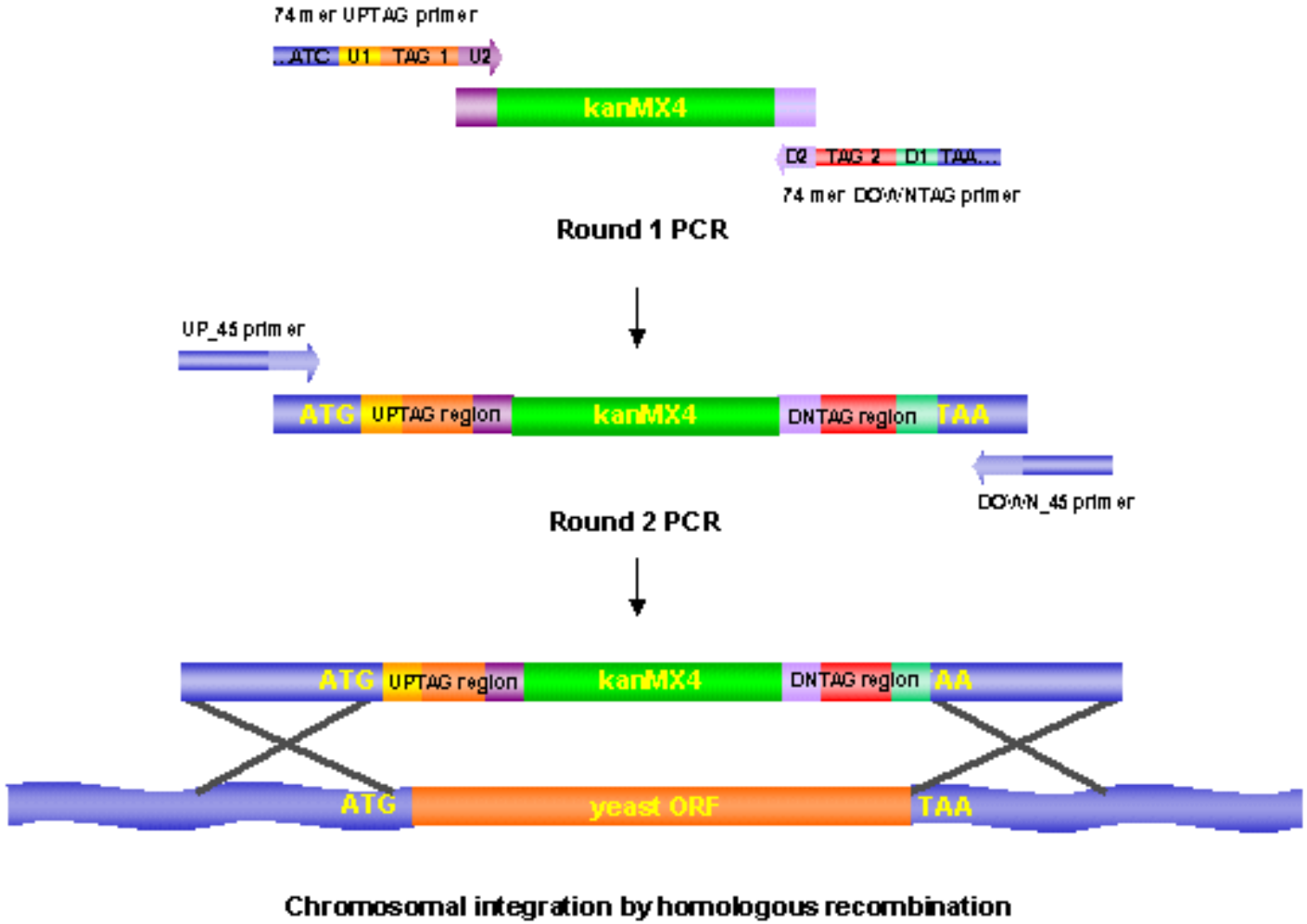
Gene disruption is a fundamental tool of the molecular geneticist and allows the consequence of loss of gene function to be determined. For organisms with facile genetic methods and known genome sequence, it is possible to systematically inactivate each gene[1–8]. Here we present the construction and initial characterization of the nearly complete set (96% of all annotated ORFs) of gene-disruption mutants in the yeast *Saccharomyces cerevisiae*. This directed approach provides major advantages over classical random mutagenesis and screening. First, the mutant phenotype reflects a complete loss of function of the gene. Second, as a 'reverse genetic' approach, the previously laborious task of identifying the gene responsible for the mutant phenotype is accomplished beforehand. Moreover, in contrast to random mutagenesis, where genes often elude detection even when a large number of mutants are screened, mutant 'saturation' of the genome is assured.

Deletion strategy

Each gene was precisely deleted from the start to stop codon (non-inclusive) and replaced by mitotic recombination with the *KanMX* deletion 'cassette' shown in Fig. 1 (ref. 9). The *KanMX* gene in each resulting mutant is flanked by two distinct 20-nucleotide sequences that serve as 'molecular bar codes' to uniquely identify each deletion mutant (see Methods for details of the design and construction of these sequence tags). Each deletion was verified by several polymerase chain reactions (PCRs), as described in Supplementary Information. In total, we deleted 5,916 genes (96.5% of total

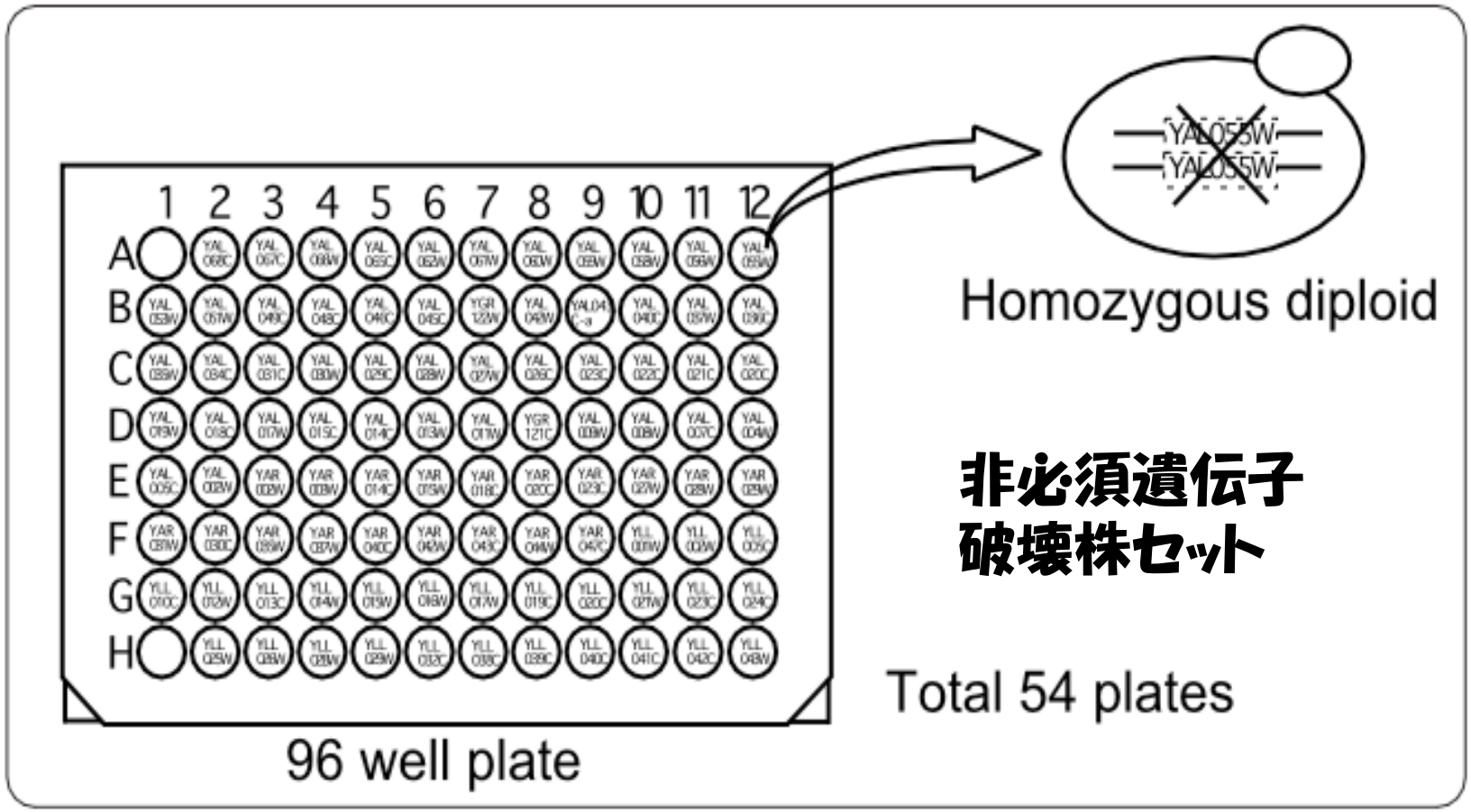
2002年
すべての遺伝子
の破壊株セット
完成

PCR産物の形質転換による相同組換えで遺伝子破壊株ができる



Chromosomal integration by homologous recombination

4,787 Yeast Deletion Strains

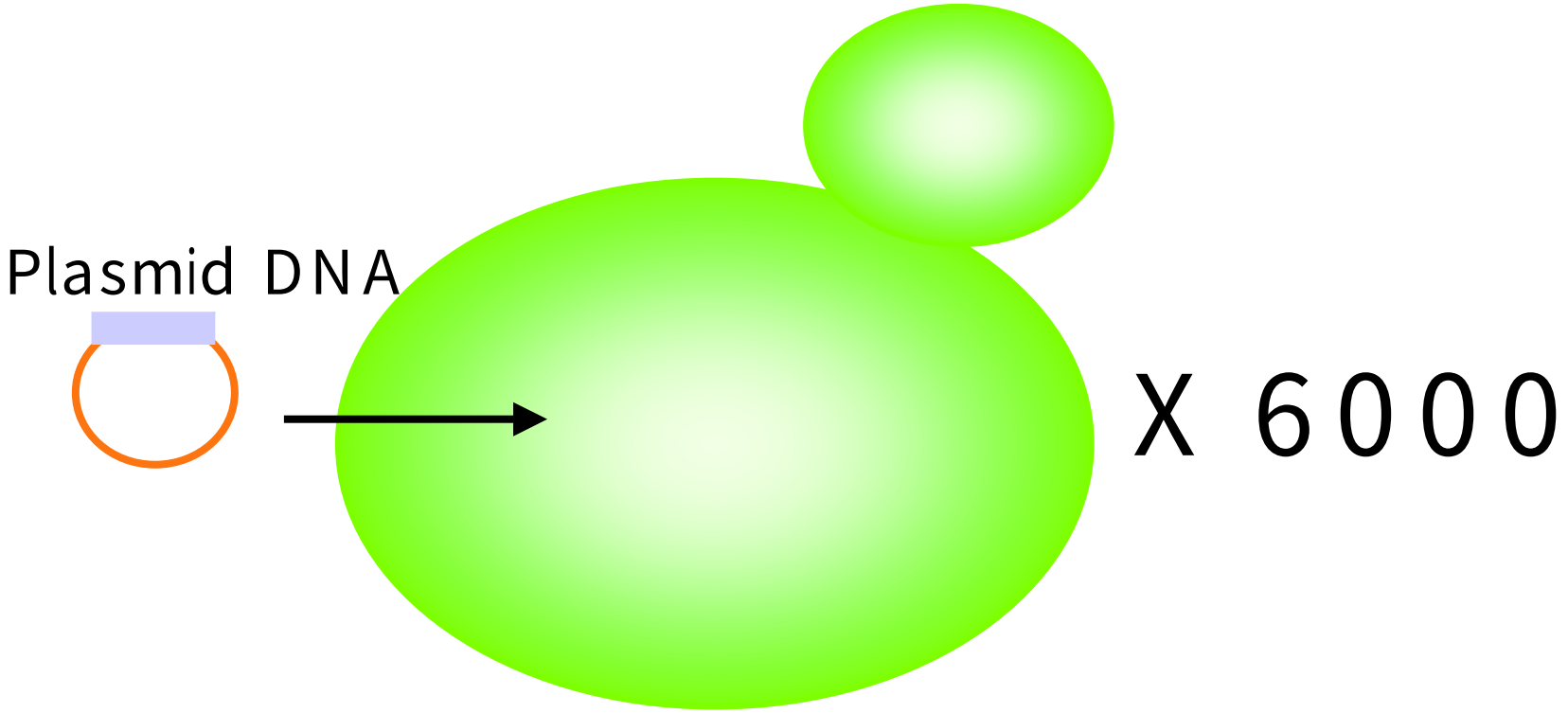


もし，調べたい遺伝子を破壊株セットに入れることができたら

１）優性遺伝子　　---サプレッサー解析
２）マーカー遺伝子　---遺伝子発現解析
３）外来遺伝子　　---タンパク質生産機能解析
４）ＧＦＰ融合遺伝子　---タンパク質局在解析

ゲノムワイド解析が可能となる。

High-throughput transformation

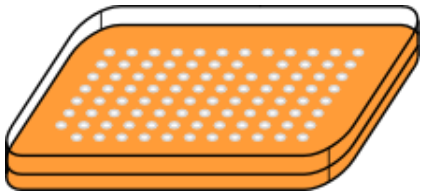


大量処理型の簡単遺伝子導入法の開発

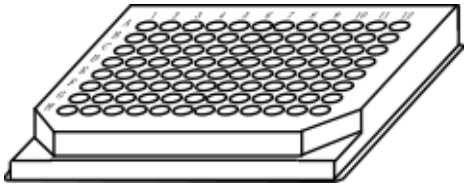
High-throughput transformation

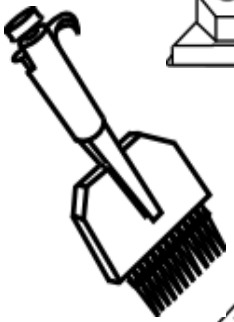
Pick

96-well picker

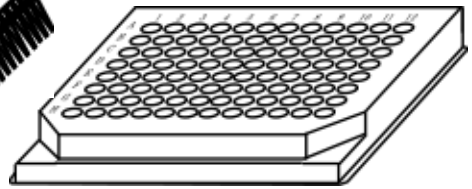


Grow

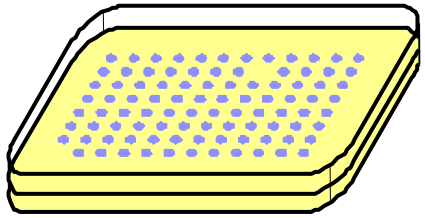




Mix



42°C, 2 hr

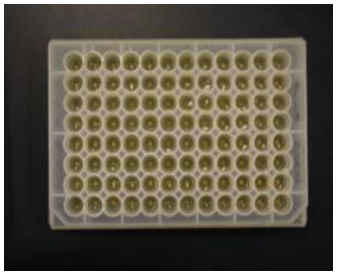


Spot

HT station 500

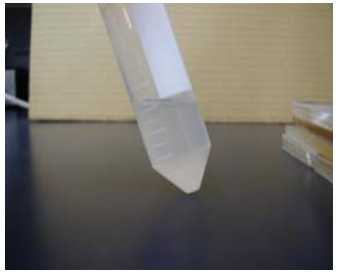
Selection plate

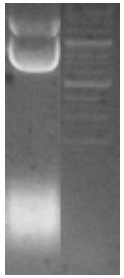
混ぜるだけで遺伝子導入ができる方法の開発



YPD 25 μl culture at 28°C for 24 hr

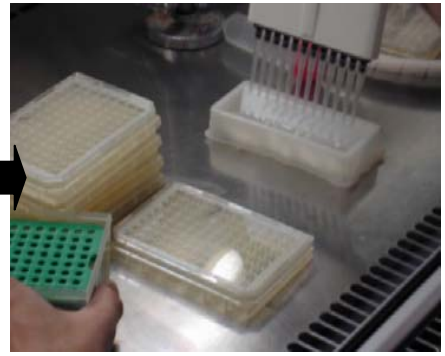
+





Alkali-SDS preparation containing *E. coli* RNA

HT transformation solution mixed with plasmid DNA

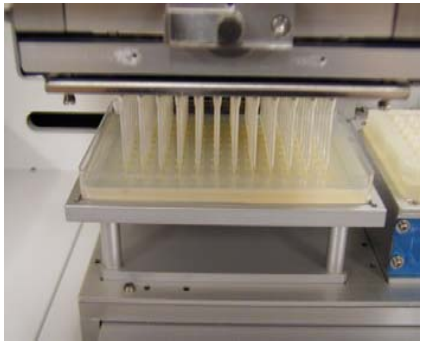


Mix by 12-channel pipet

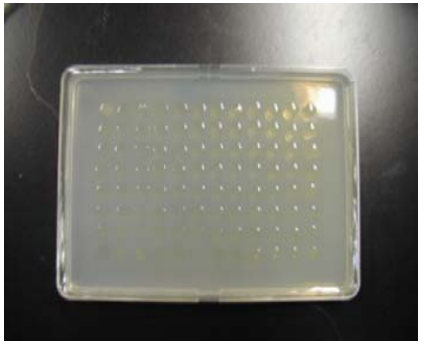
Vortexing

42°C, 2 hr

Mix by pipetting

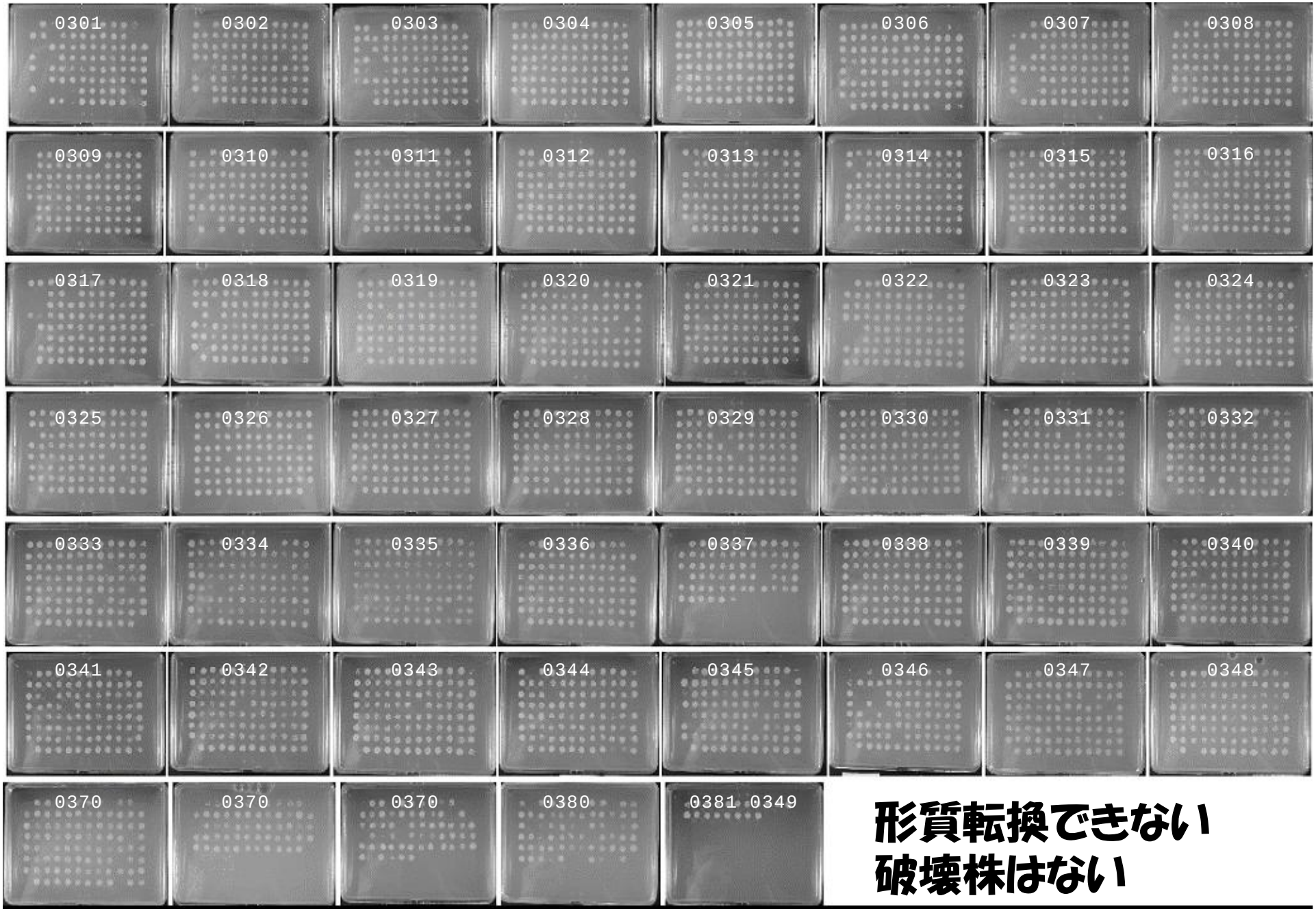


Spot 10 μl



Spot on selection plate

Result of high-throughput transformation



遺伝子を破壊株セットに入れることができるので

1)遺伝子の相互作用:

サプレッサーや超感受性となる遺伝子破壊株

酵母遺伝子	BNI1, GIN11, STE11
病原性遺伝子	CdtB, CagA, YopE, YpkA, YopM
高等動物遺伝子	p53, Bax

2)外来酵素遺伝子:

発現量が増大または減少する破壊株

麹菌分泌酵素	*Aspergillus* α-amylase
耐熱性酵素	*Bacillus* α-amylase
担子菌分泌酵素	*Pycnoporus* laccase


医療

パーキンソン病

神経変性疾患
日本10万人に50人
無動
筋固縮
静止時振戦
姿勢反射異常
自律神経症状

ドーパミン作動性神経の変性
いくつかは遺伝性，いくつかは後天的
Autosomal dominant gene= α synuclein

The Nobel Prize in Physiology or Medicine 2005

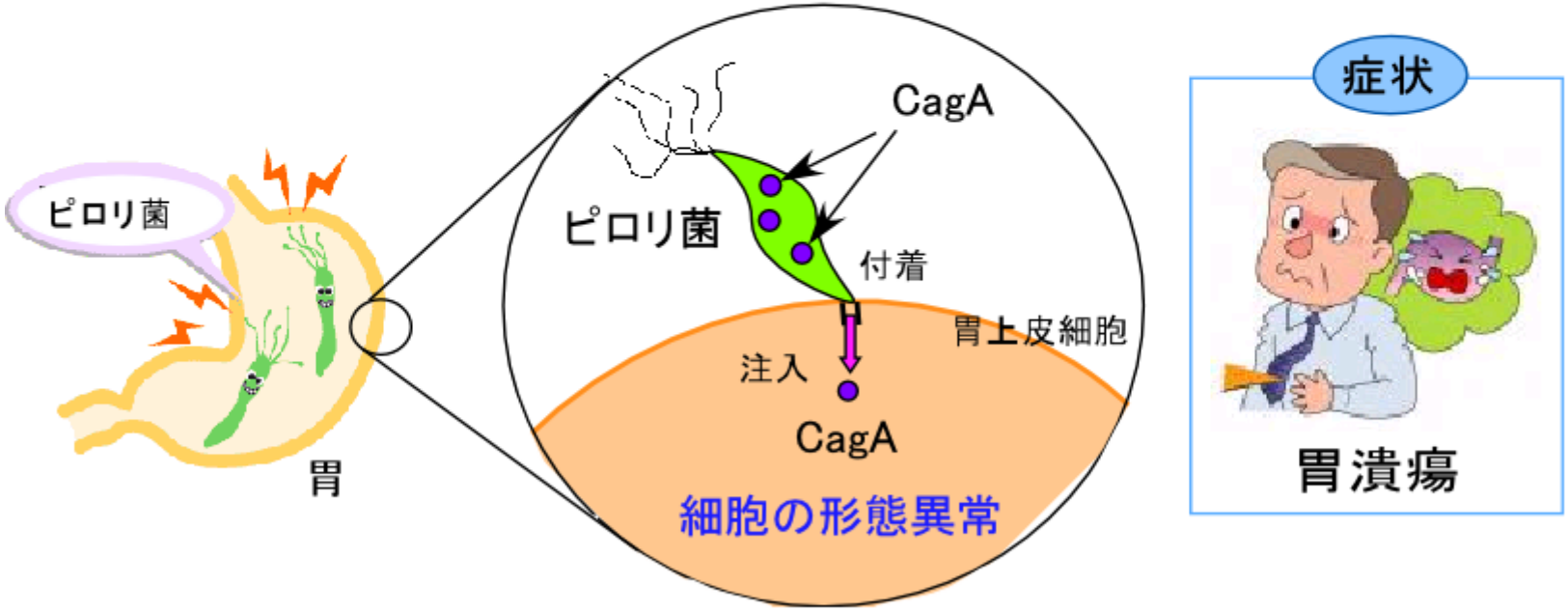
"for their discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease"

Barry J. Marshall

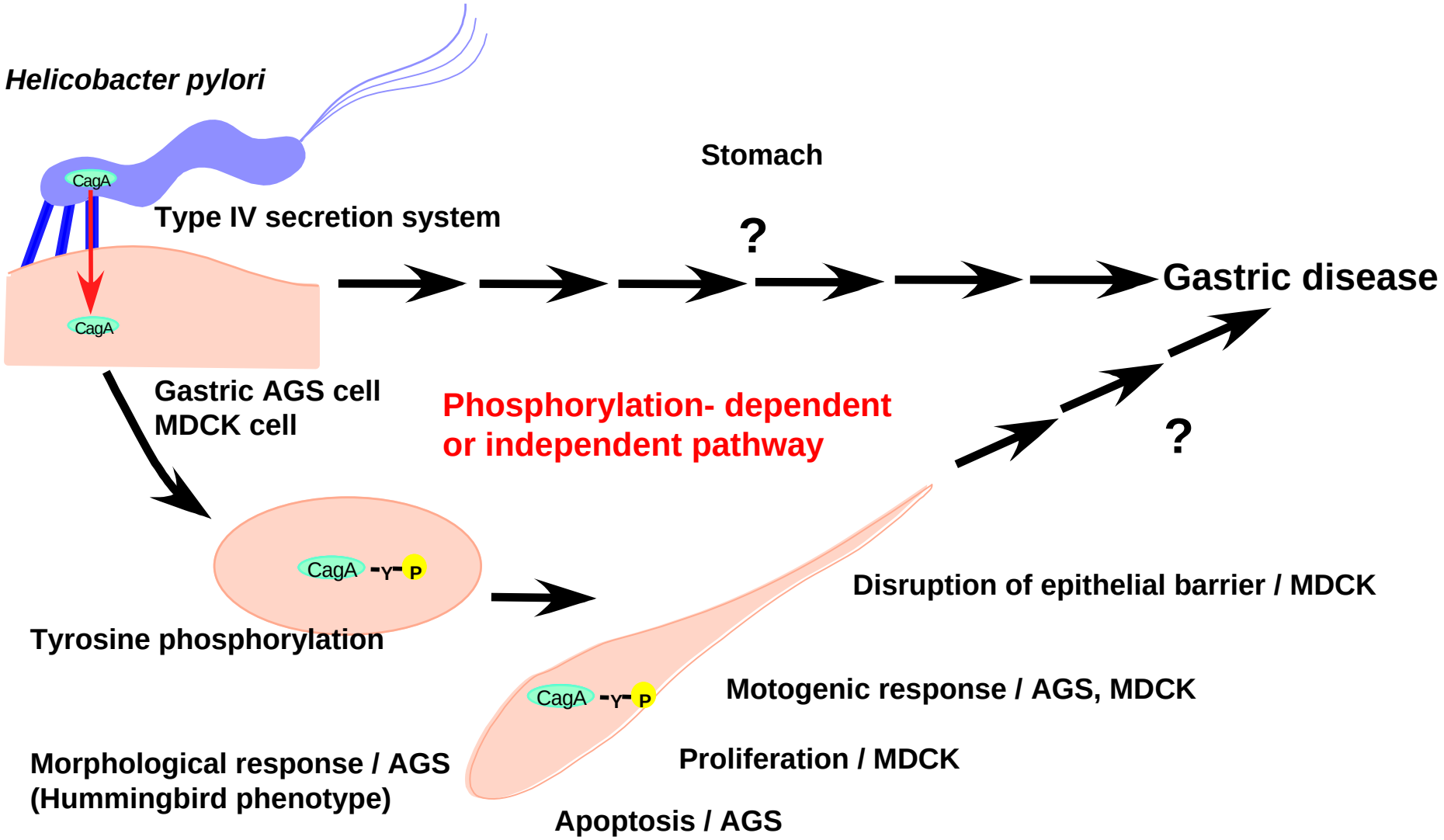
Australia

ヘリコバクター・ピロリのCagA

go-to-100.seesaa.net/archives/200504.html

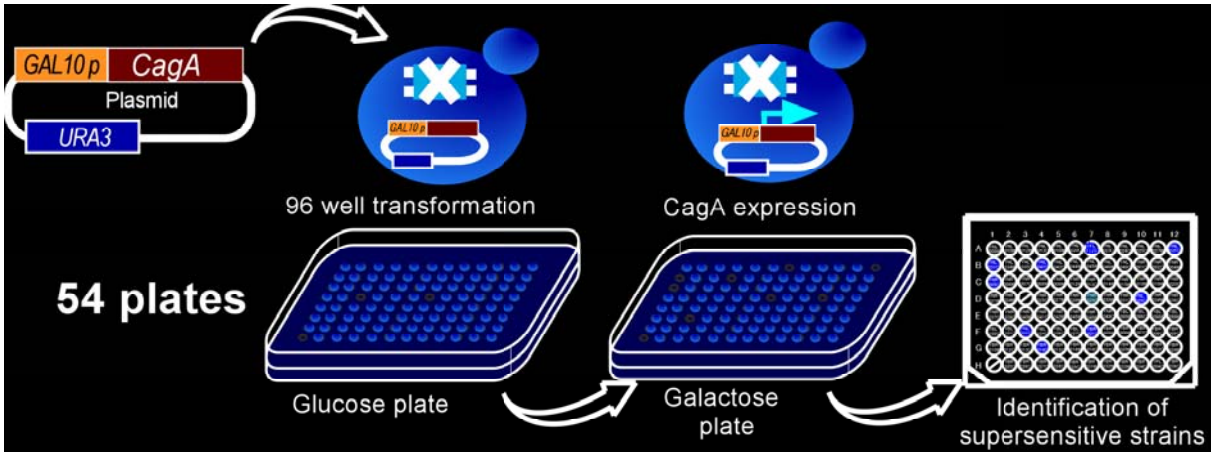


CagA protein of *Helicobacter pylori*



Step 1: Genome-wide screening

A set of 4792 non-essential gene deletion strains were transformed by a high-throughput transformation method. **124 CagA sensitive strains** were selected.



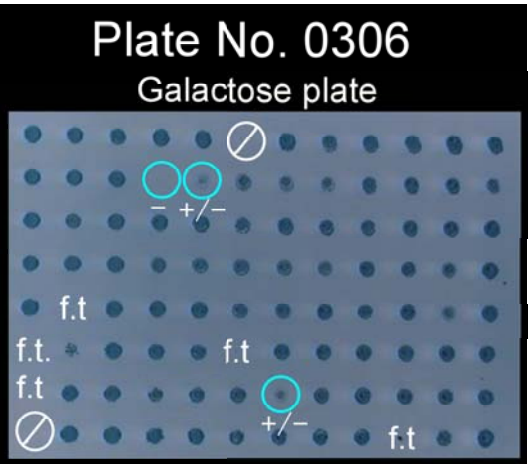
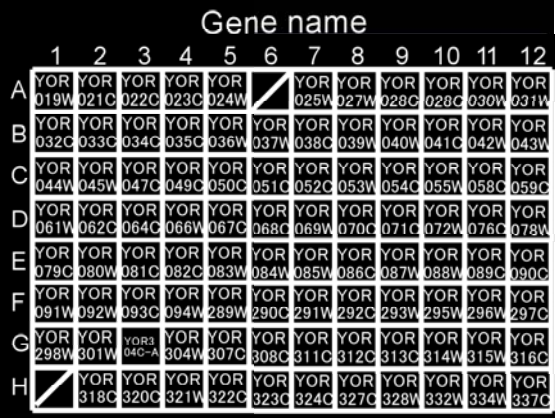
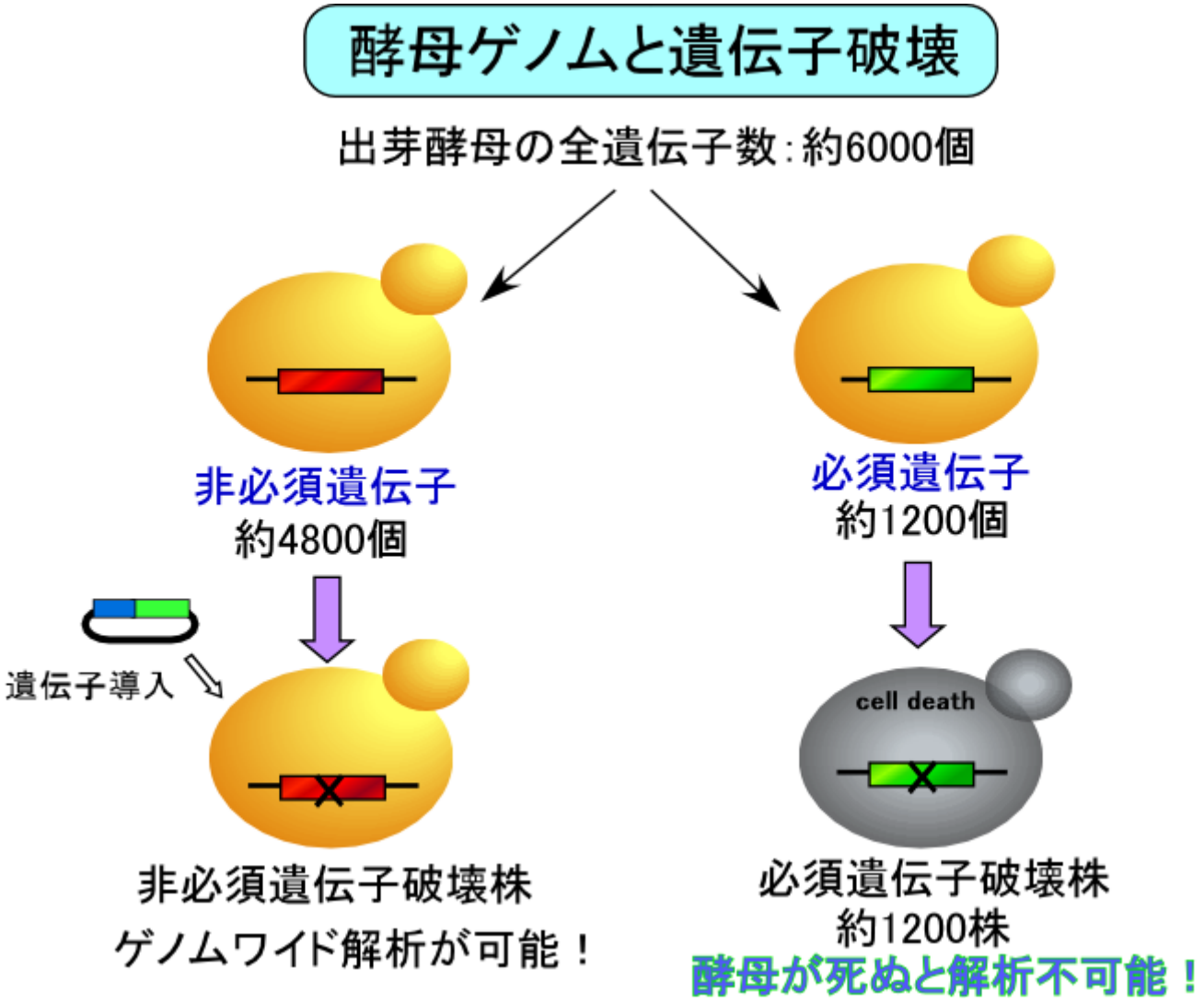


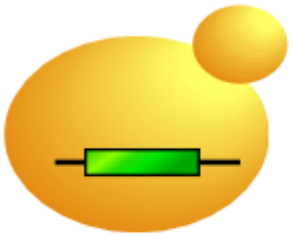
Table 1. Summary of 1st screening

Transformation performed	4,792
Transformed	4,757
Not transformed	34
No growth	63
Slow growth	61
1st selection	124

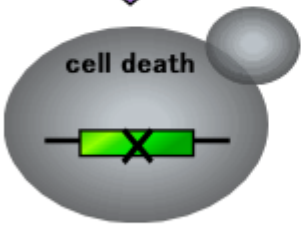



酵母ゲノムと遺伝子破壊
出芽酵母の全遺伝子数：約6000個
非必須遺伝子
約4800個
必須遺伝子
約1200個
遺伝子導入
cell death
非必須遺伝子破壊株
ゲノムワイド解析が可能！
必須遺伝子破壊株
約1200株
酵母が死ぬと解析不可能！

必須遺伝子破壊株を使うには？!

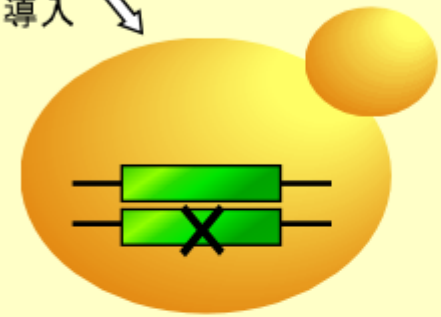


必須遺伝子
約1200個



必須遺伝子破壊株
約1200株

遺伝子導入



必須遺伝子のヘテロ破壊株
約1200株

ヘテロ破壊株とは･･･

2本の染色体のうち、片方の染色体上にある遺伝子のみを破壊した株のこと。そのため、遺伝子の機能力も半減する。

Banquet Speech

Barry J. Marshall's speech at the Nobel Banquet, December 10, 2005

Let me clarify here, while it is true that MacFarlane Burnet injected himself with the rabbit myxoma virus, and I did actually infect myself with *Helicobacter pylori*, I don't suggest to other aspiring Aussie scientists that this process will guarantee a Nobel Prize. **But to young people listening tonight I would say, find passion in your work - whatever it is**. If, like me, you are working in the area of science, I can promise you that it can be the most exciting and rewarding of careers.

So work hard, keep balance in your life and, just in case, always be nice to Swedish people.