Integrating Undergraduates into Educationally Meaningful Research Programs and Courses

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Challenge

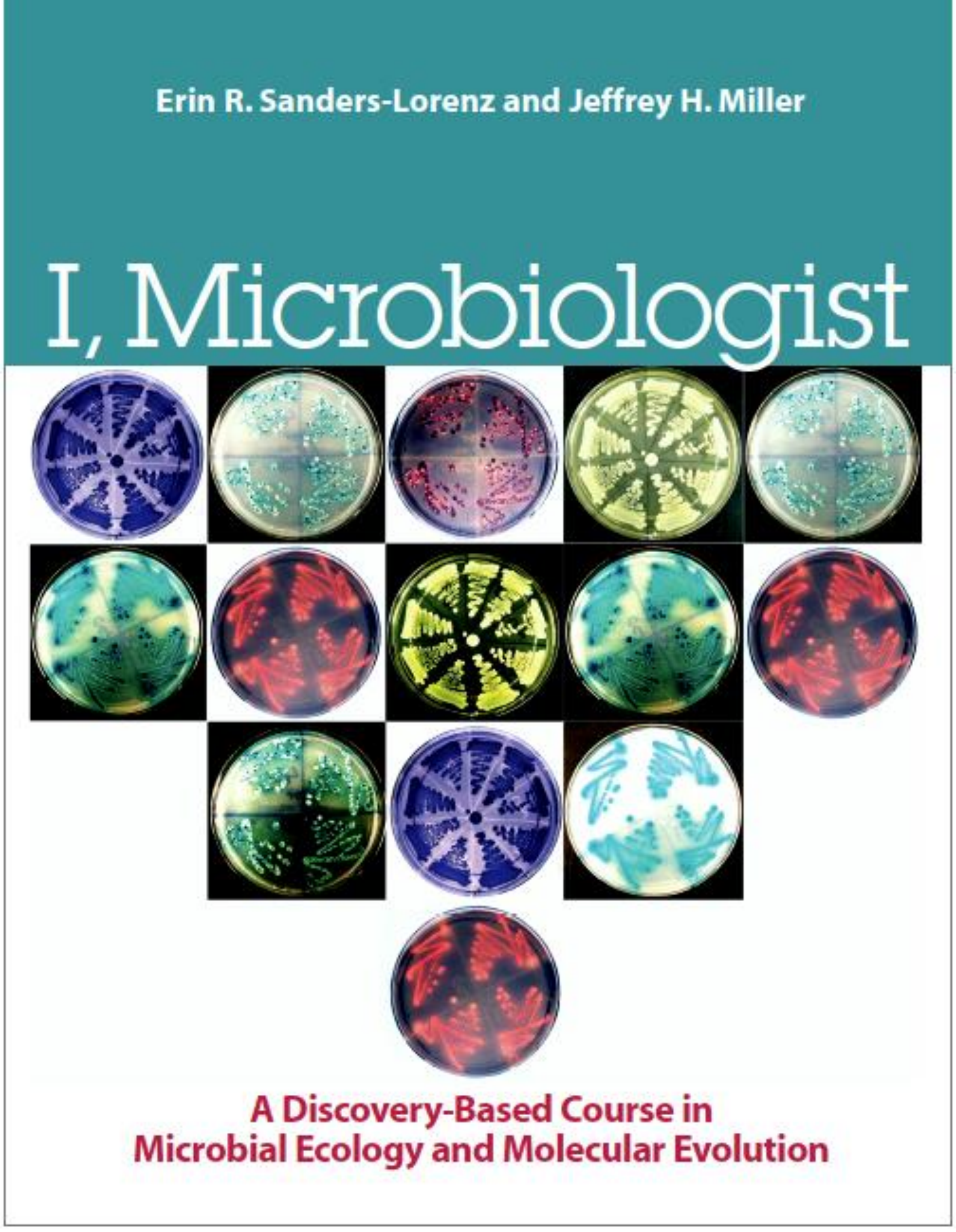
Involve Students in Research

Goals

1. Transmit the excitement of tackling the unknown
2. Have rapid rewards instead of long apprenticeship
3. Present an intellectual process
4. Student has ownership of a project and results, not a “cog in the wheel”
5. Understand the practical importance of the relevant area of research

Requirements

- Commitment from mentor
- Commitment from student
- Projects that allow goals to be achieved



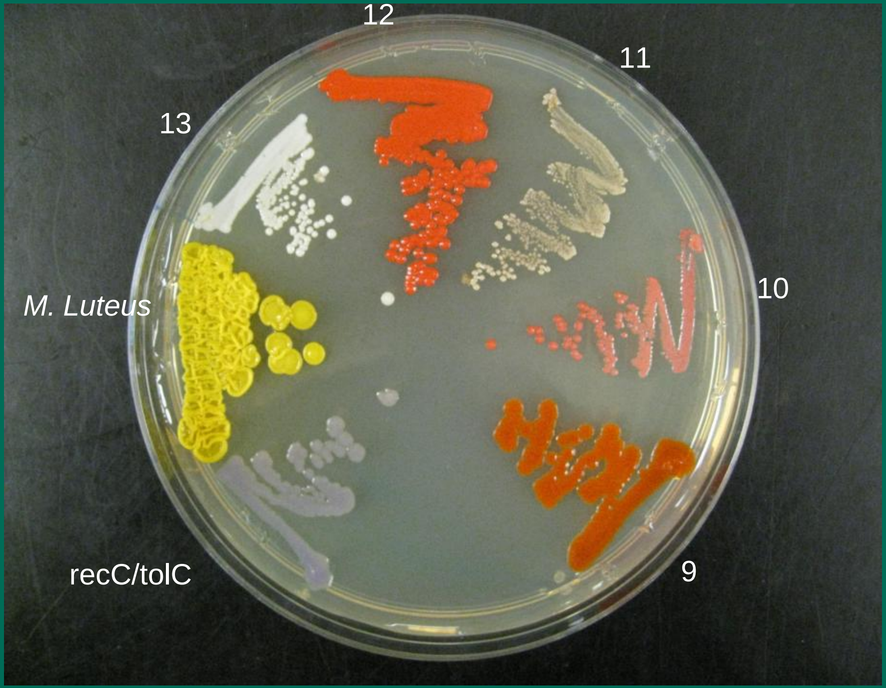
Erin R. Sanders-Lorenz and Jeffrey H. Miller

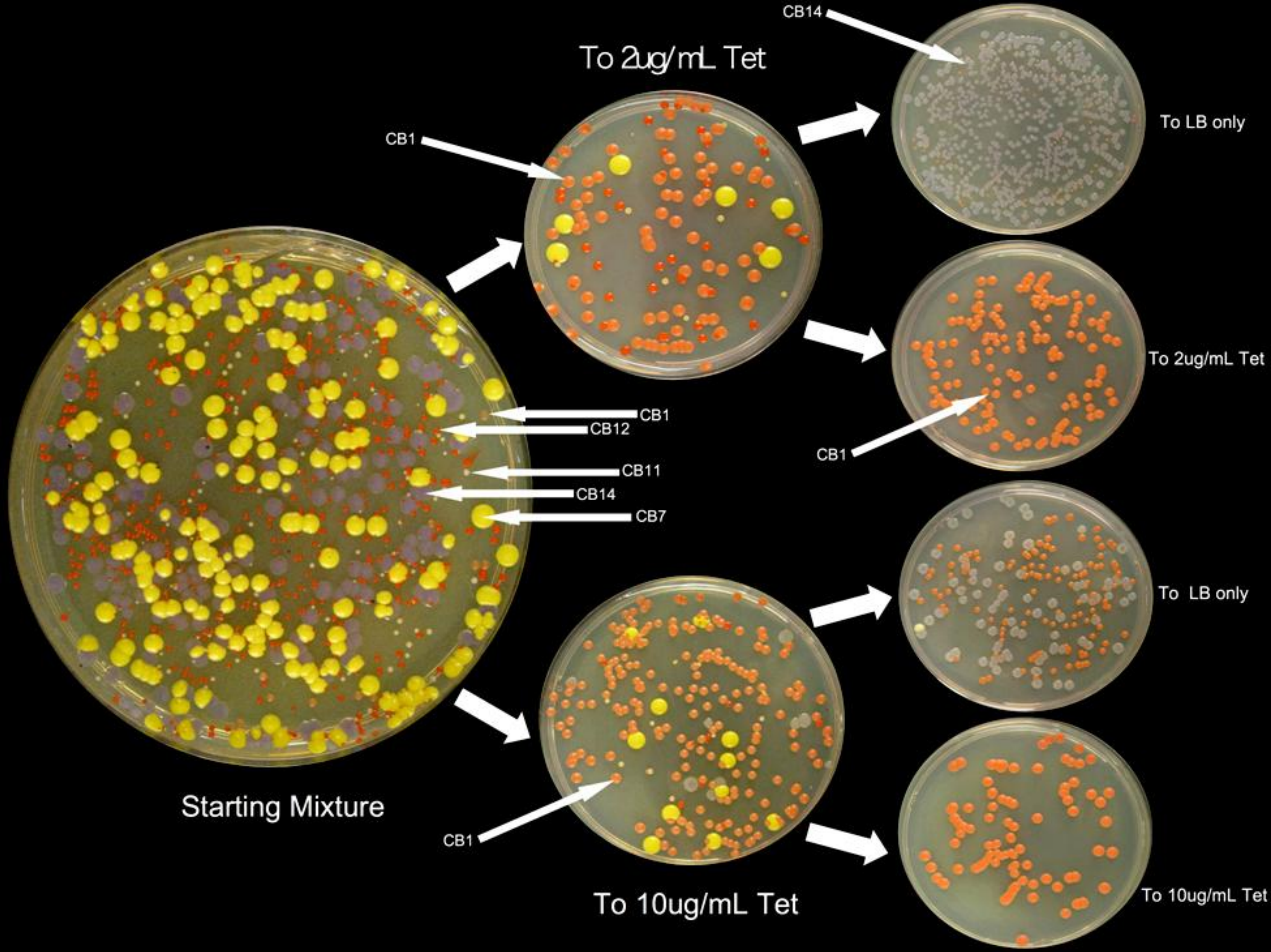
I, Microbiologist

A Discovery-Based Course in Microbial Ecology and Molecular Evolution

Environmental Samples Collected and Source:

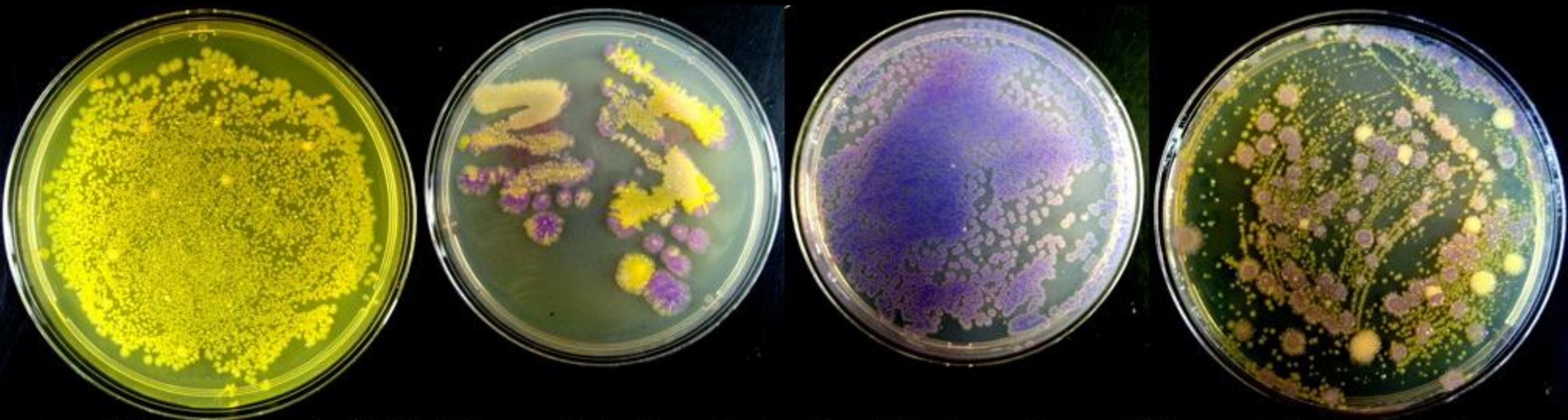
Strain	Species	Color	Source
CB001	*E. coli* strain	Pinkish red	Lab contaminant
CB002	*Staphylococcus* strain	White	Amie Fong finger
CB003	*Bacillus cereus*	Salmon	Soil
CB004	*Bacillus cereus*	Pale yellow	Soil
CB005	*Bacillus cereus*	Yellow orange	Soil
CB006	*Bacillus cereus*	Orange	Soil
CB007	*Bacillus cereus*	Yellow	Amie Fong finger
CB008	*Bacillus cereus*	Neon red	Amie Fong hair
CB009	*E. fergusonii* (potentially)	Bloody red/orange	South campus dumpster
CB010	*Bacillus cereus*	Baby pink	UCLA campus
CB011	*Staphylococcus hominis*	Tan	Arrowhead water jug from CHS
CB012	*Bacillus cereus*	Bright red	Orthopaedic hospital bathroom handle
CB013	*Staphylococcus* strain	White #2	Orthopaedic hospital bathroom handle




CB14
To 2ug/mL Tet
CB1
To LB only
To 2ug/mL Tet
CB1
CB1
CB12
CB11
CB14
CB7
To LB only
Starting Mixture
CB1
To 10ug/mL Tet
To 10ug/mL Tet

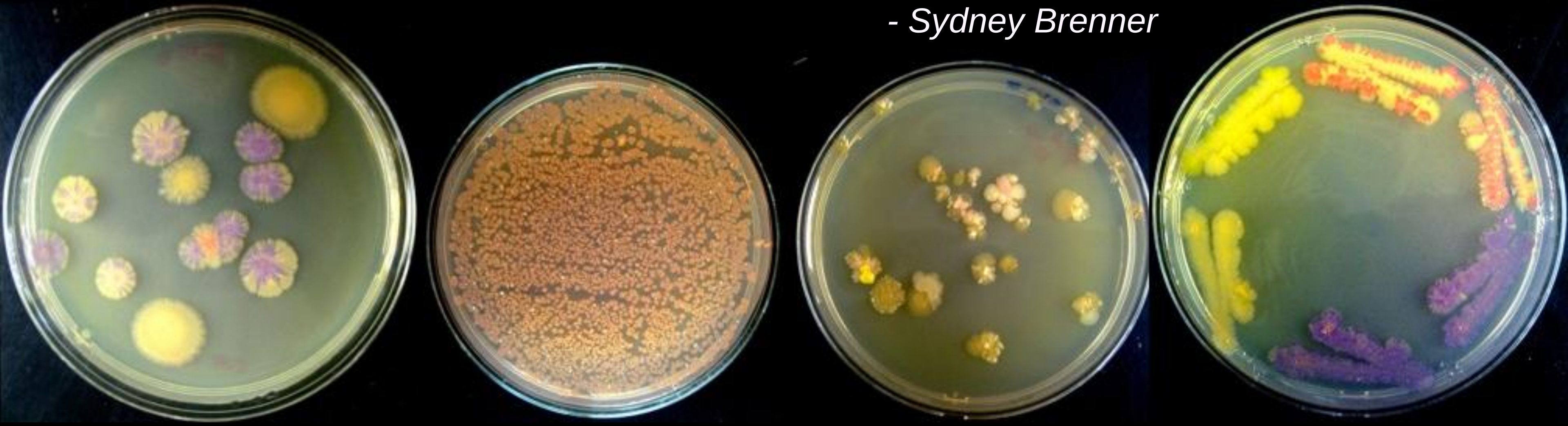
Bacterial Art

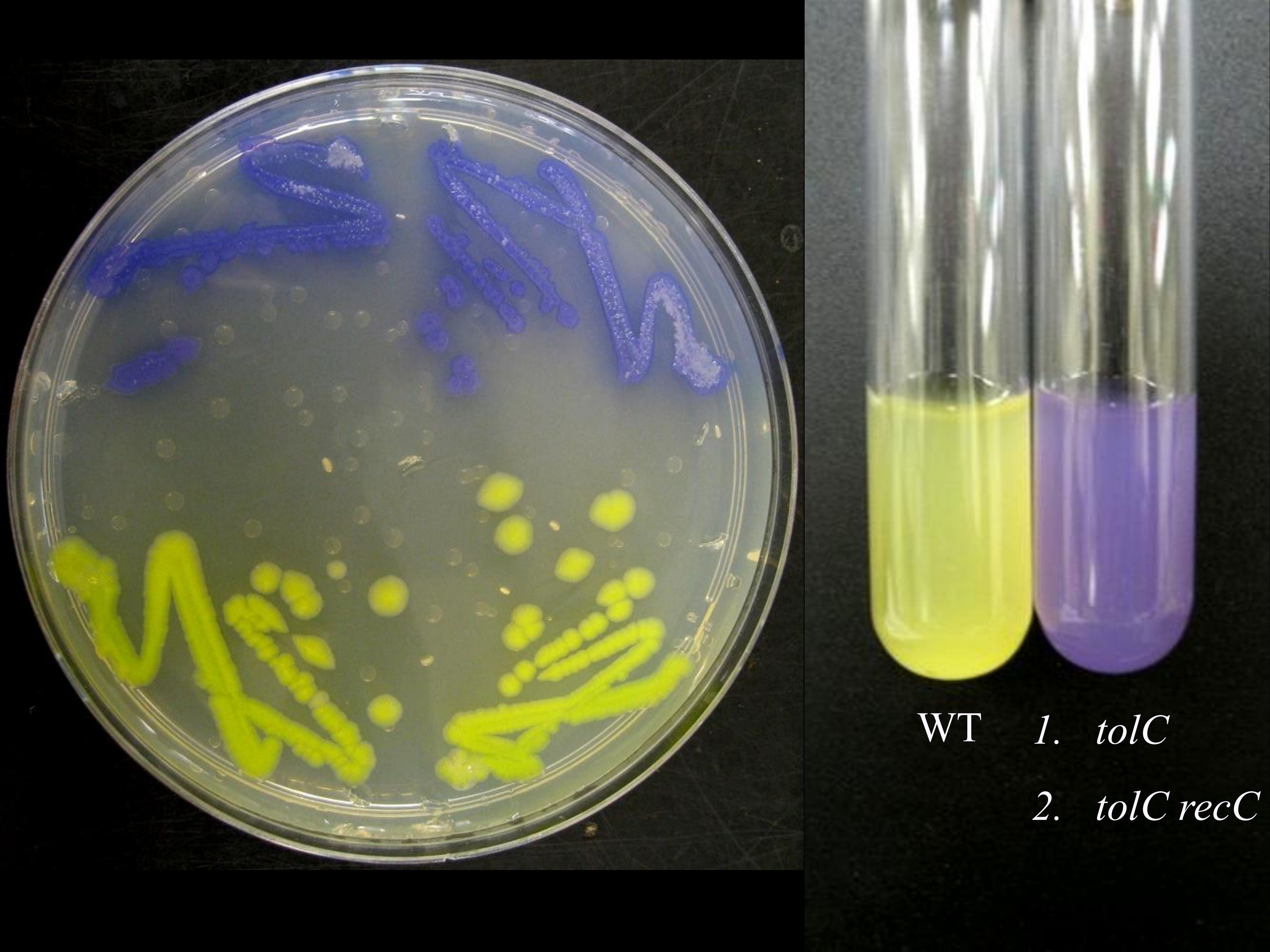

愛
Love
Sesame
Street



It Takes 1,000 Nanobiologists to Make One Microbiologist

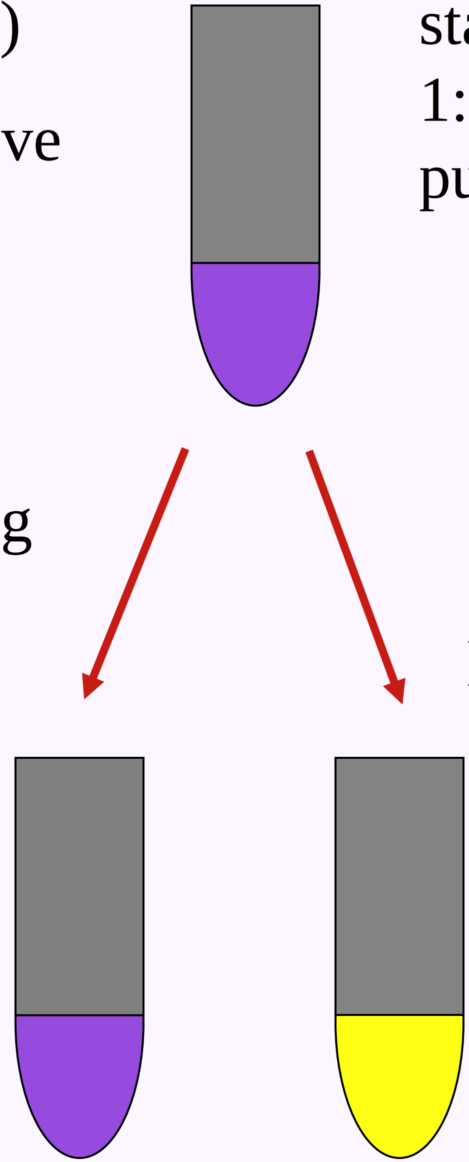
- Sydney Brenner




WT
1. tolC
2. tolC recC

Strain A=WT (yellow)

Strain B=Drug sensitive mutant (purple)



starting mixture
1:20 Strain A: Strain B
purple

growth without drug

growth in presence of drug: strain B inhibited by low concentration of drug


LB
Cipro .05
Cipro .1
Cipro .2
Cipro .5
Cipro 1
WT:*tolCrecC*
ng/ml

What is the minimum concentration of an antibiotic that is significant?

- Current thought holds that evolution of antibiotic resistance occurs at the MIC and above

- Reservoirs of antibiotic resistance already exist

- What is the minimum concentration that selects for resistance? (MSC)

Competition Experiments

Lac$^-$ CIP^S : Lac$^+$ CIP^R

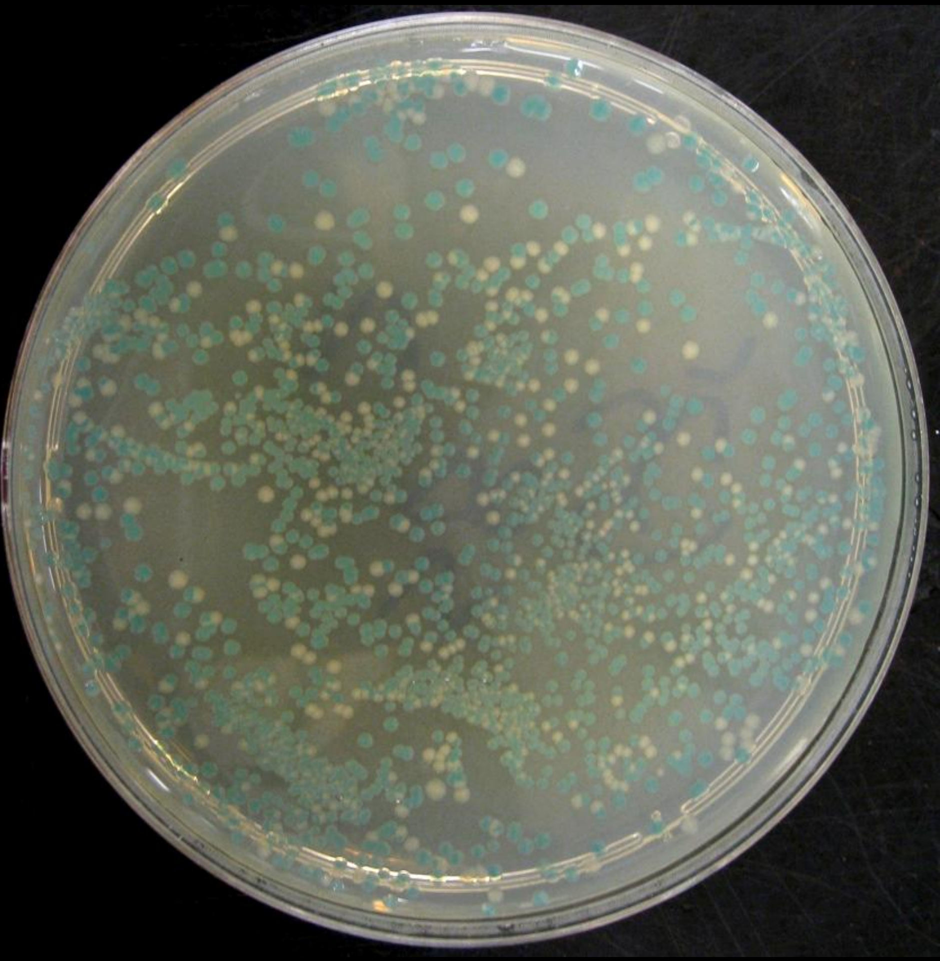
10 : 1

Grow

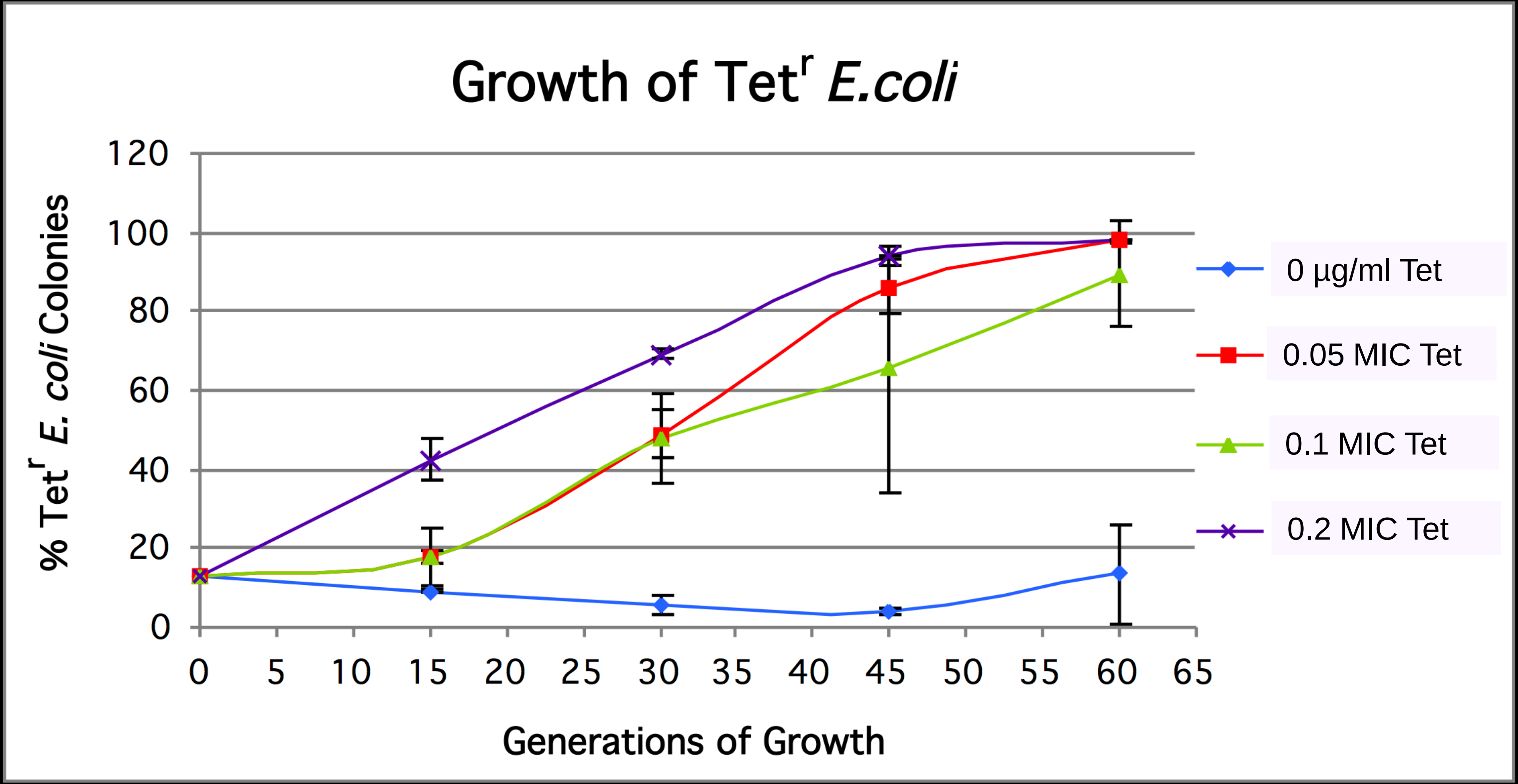
0, 0.2, 0.5, … ng/ml CIP

24 Generations

CIP 0 ng/ml



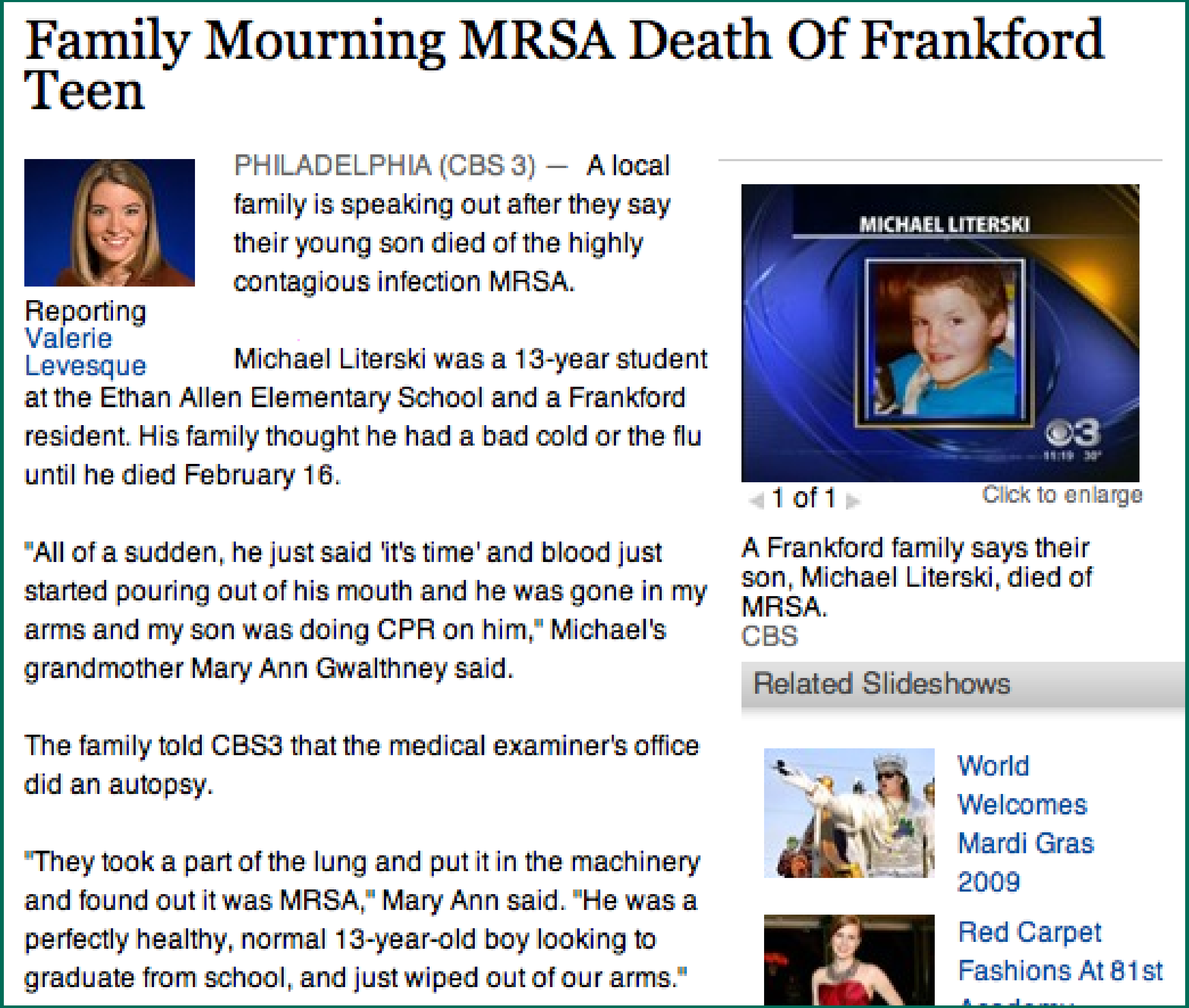
CIP <0.2 MIC


Growth of Tet^r *E.coli*
% Tet^r *E. coli* Colonies
0
20
40
60
80
100
120
Generations of Growth
0
5
10
15
20
25
30
35
40
45
50
55
60
65
0 µg/ml Tet
0.05 MIC Tet
0.1 MIC Tet
0.2 MIC Tet

Brazilian amputee model dead at 20


NO ROCK
OCTAVIO BASTOS/ WWW.NOROCK.COM.BR

- **STORY HIGHLIGHTS**
- **NEW:** Brazilian amputee model Mariana Bridi da Costa died early Saturday
- Da Costa's hands, feet were amputated after she contracted septicemia
- Da Costa placed sixth in the Miss Bikini International competition in China



Family Mourning MRSA Death Of Frankford Teen

Reporting
Valerie Levesque

PHILADELPHIA (CBS 3) — A local family is speaking out after they say their young son died of the highly contagious infection MRSA.

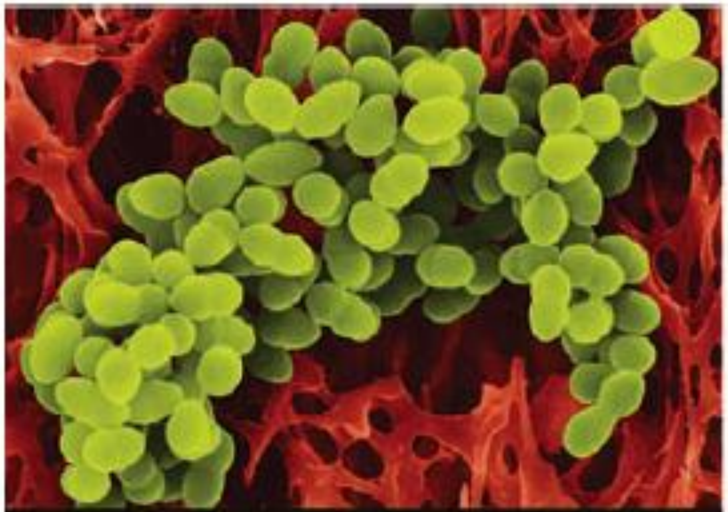
Michael Literski was a 13-year student at the Ethan Allen Elementary School and a Frankford resident. His family thought he had a bad cold or the flu until he died February 16.

"All of a sudden, he just said 'it's time' and blood just started pouring out of his mouth and he was gone in my arms and my son was doing CPR on him," Michael's grandmother Mary Ann Gwalthney said.

The family told CBS3 that the medical examiner's office did an autopsy.

"They took a part of the lung and put it in the machinery and found out it was MRSA," Mary Ann said. "He was a perfectly healthy, normal 13-year-old boy looking to graduate from school, and just wiped out of our arms."

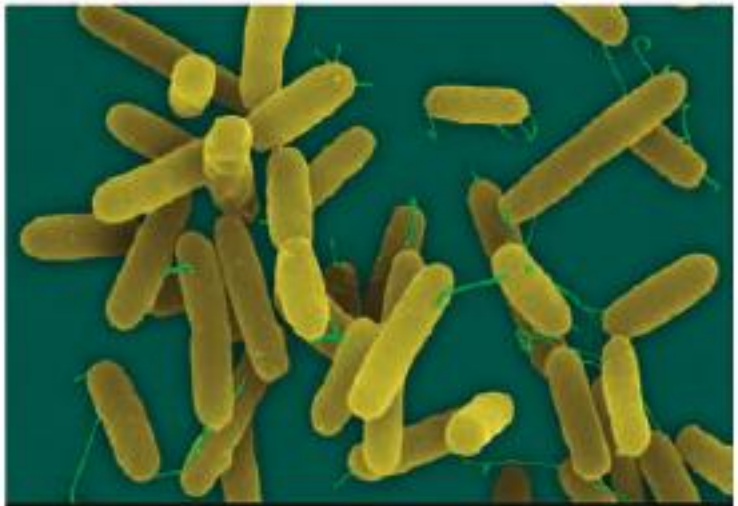
A Frankford family says their son, Michael Literski, died of MRSA.
CBS



Staphylococcus aureus

Mycobacterium tuberculosis

Acinetobacter baumannii



Pseudomonas aeruginosa

Strategy

Screen KEIO collection of ~ 4,000 single gene knockout strains in *E. coli*

Antibiotic

Find hypersensitive mutants

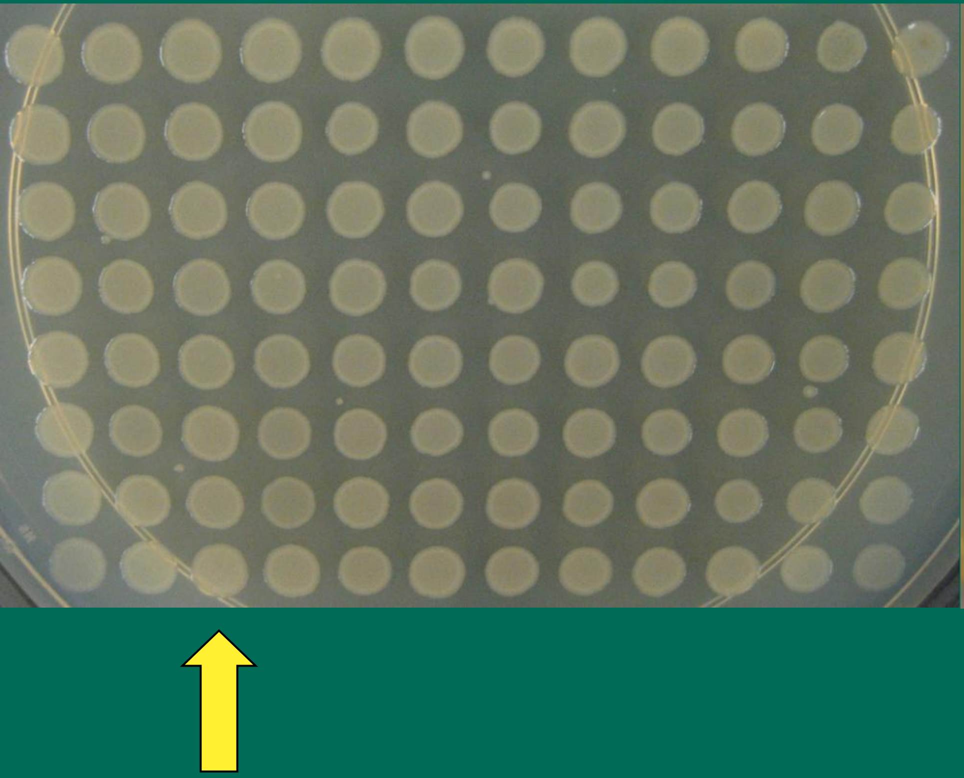
Test counterparts in other bacteria

Determine pathways involved (networks)

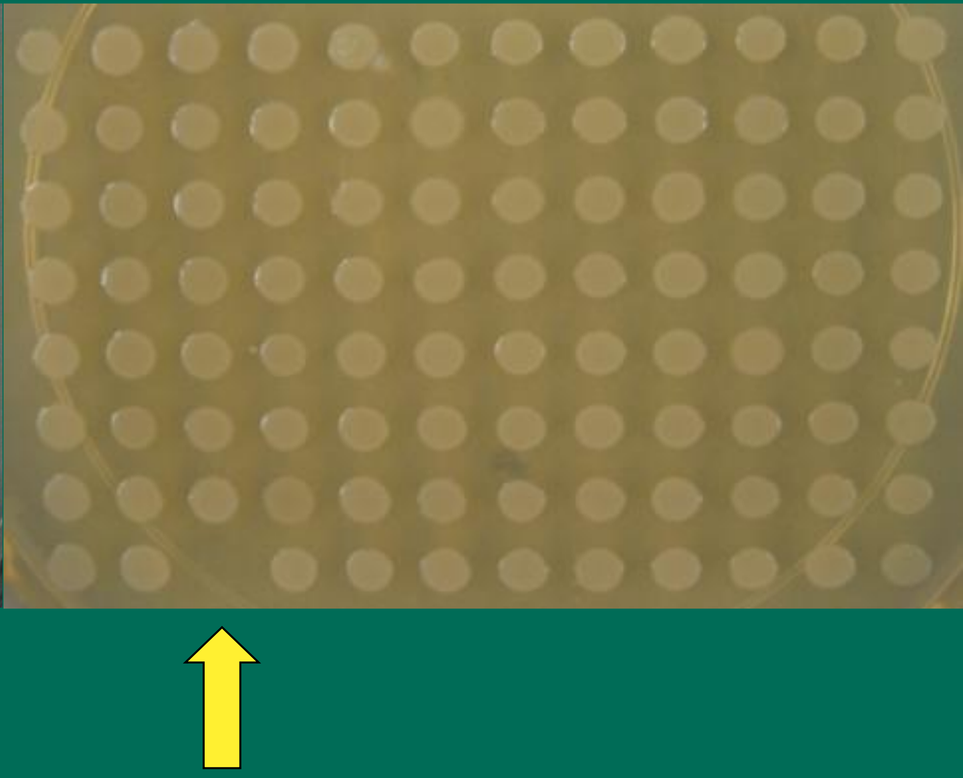
Find small molecule inhibitors of proteins involved

Use as co-drugs or potentiators

Ciprofloxacin 0 ng/ml



Ciprofloxacin 7.5 ng/ml



Cipro MIC (ng/ml)

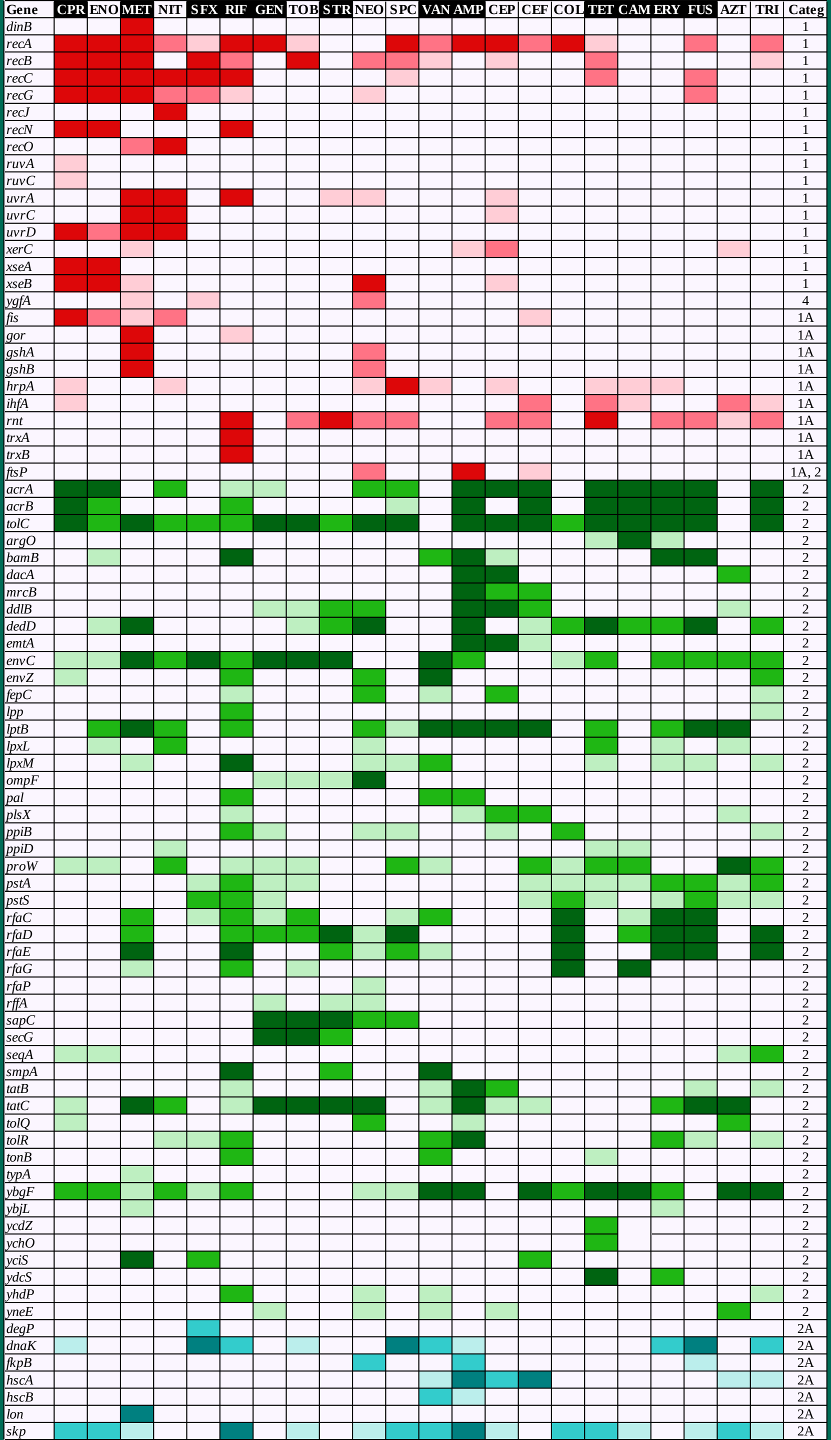
WT	16-20	*recA*	2
fis	6	*fis recA*	1
ruvC	8	*xseA*	6
fis ruvC	2	*recC*	8
tolC	5	*xseA recC*	1.3
fis tolC	0.2	*recC tolC*	0.3

Cipro Resistant Mutant Gyrase A S83L

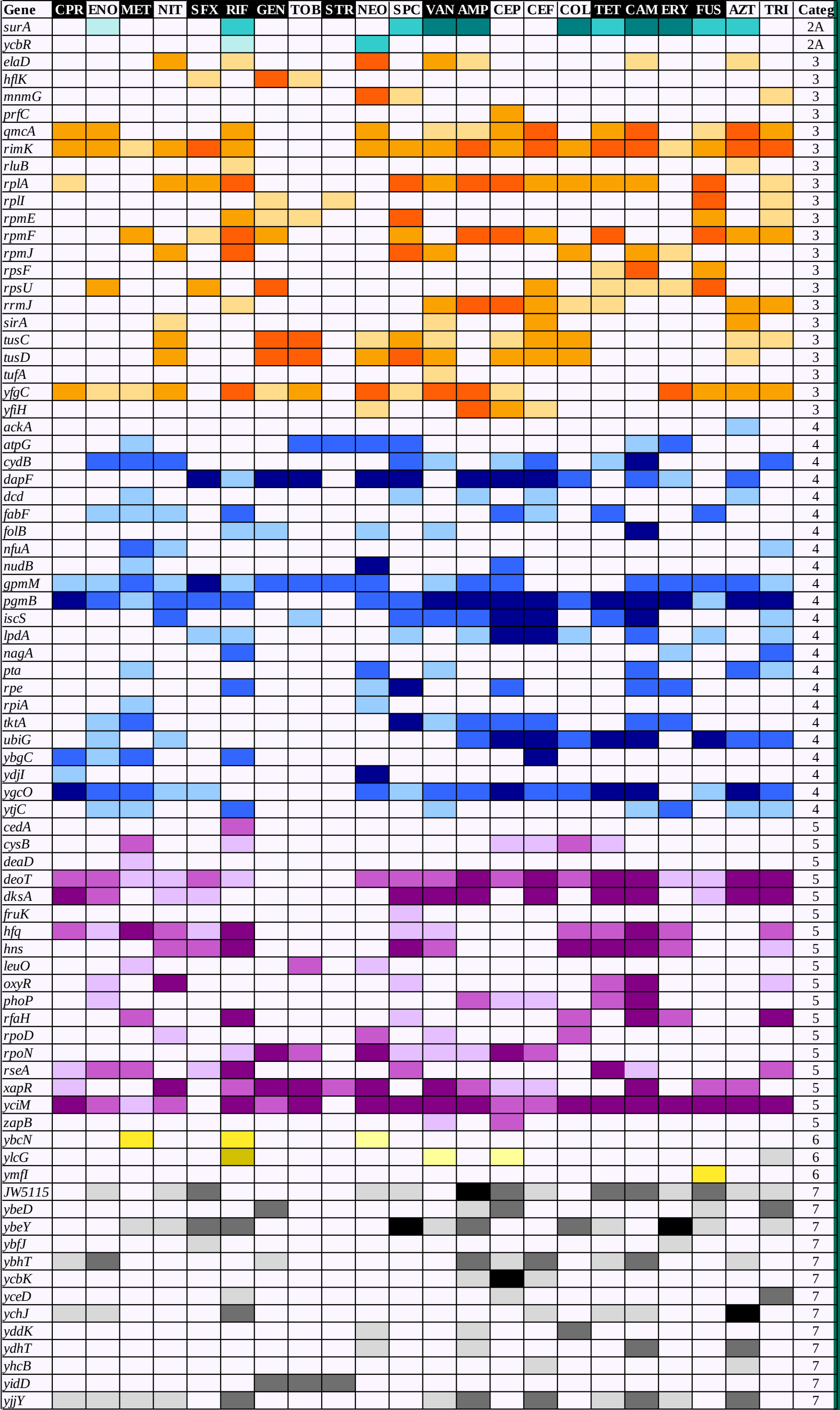
Background	MIC (ng/ml)
WT	750
recC	500
fis	300
xseA	250
recA	125
tolC	64
tolC recC	20

Vancomycin MIC (μg/ml)

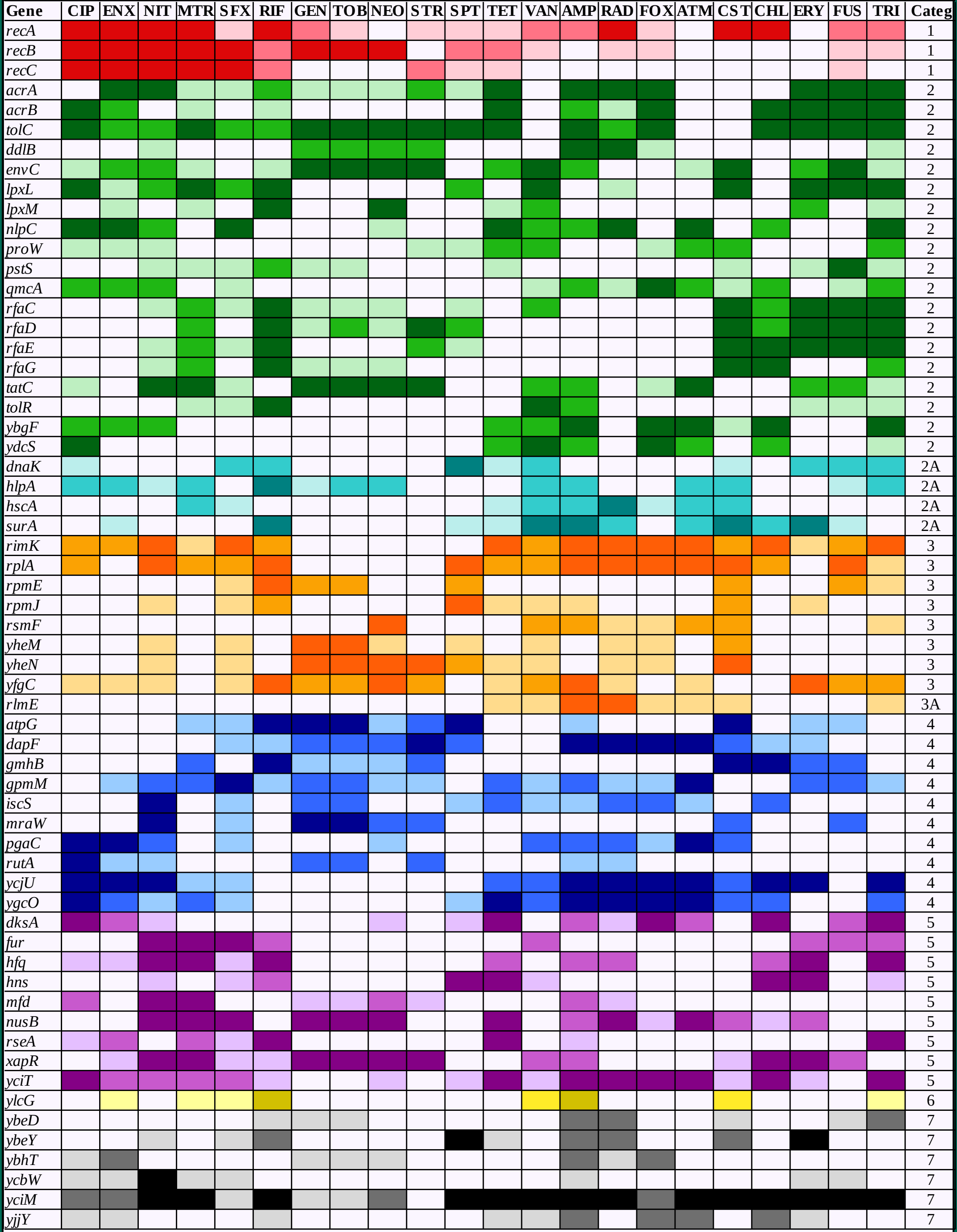
WT	500
surA	4
smpA	70
surA smpA	1.5



Gene	Categ
dinB	1
recA	1
recB	1
recC	1
recG	1
recJ	1
recN	1
recO	1
ruvA	1
ruvC	1
uvrA	1
uvrC	1
uvrD	1
xerC	1
xseA	1
xseB	1
ygfA	4
fis	1A
gor	1A
gshA	1A
gshB	1A
hrpA	1A
ihfA	1A
rnt	1A
trxA	1A
trxB	1A
ftsP	1A, 2
acrA	2
acrB	2
tolC	2
argO	2
bamB	2
dacA	2
mrcB	2
ddlB	2
dedD	2
emtA	2
envC	2
envZ	2
fepC	2
lpp	2
lptB	2
lpxL	2
lpxM	2
ompF	2
pal	2
plsX	2
ppiB	2
ppiD	2
proW	2
pstA	2
pstS	2
rfaC	2
rfaD	2
rfaE	2
rfaG	2
rfaP	2
rffA	2
sapC	2
secG	2
seqA	2
smpA	2
tatB	2
tatC	2
tolQ	2
tolR	2
tonB	2
typA	2
ybgF	2
ybjL	2
ycdZ	2
ychO	2
yciS	2
ydcS	2
yhdP	2
yneE	2
degP	2A
dnaK	2A
fkpB	2A
hscA	2A
hscB	2A
lon	2A
skp	2A

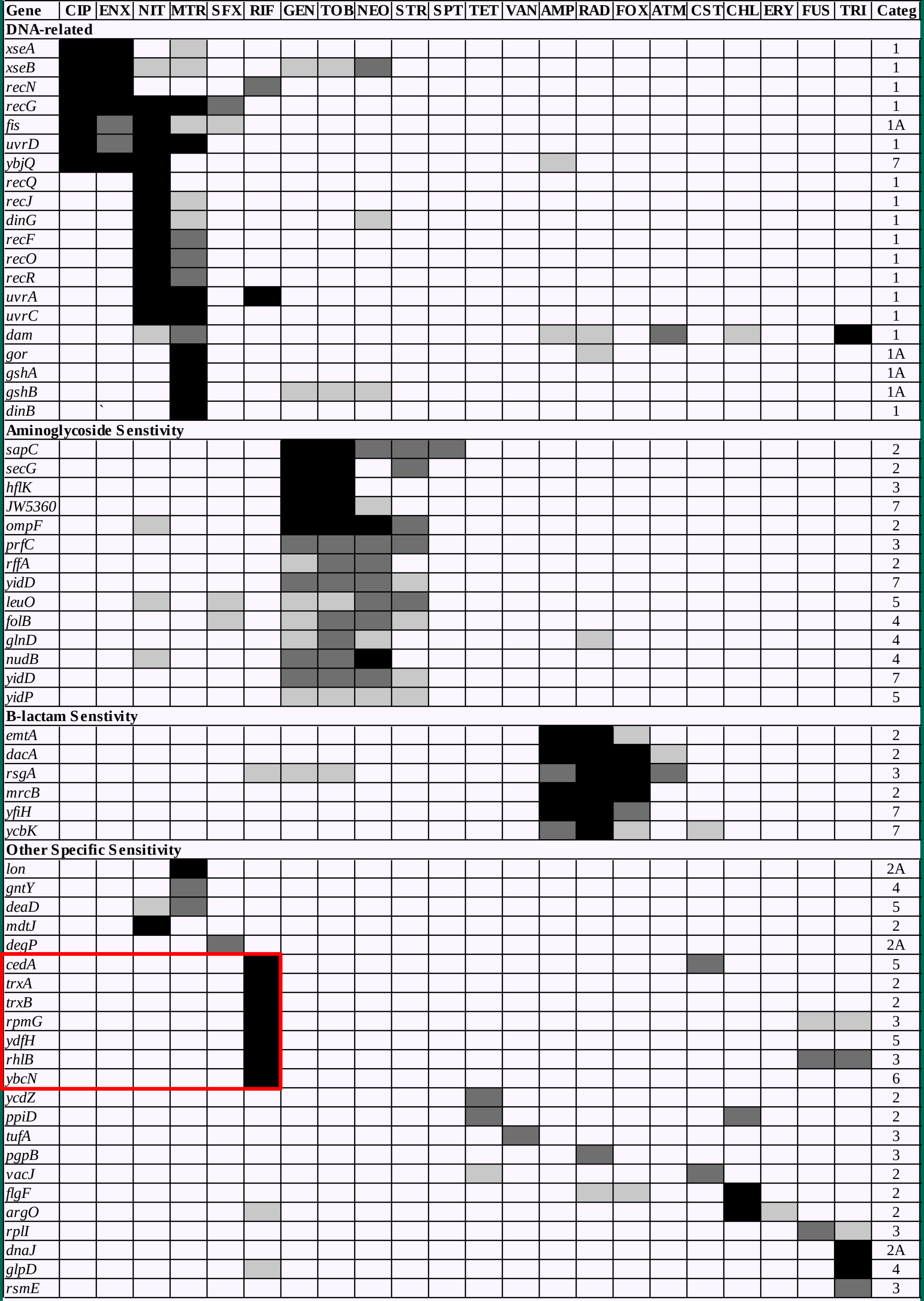


Gene	Categ
surA	2A
ycbR	2A
elaD	3
hflK	3
mnmG	3
prfC	3
qmcA	3
rimK	3
rluB	3
rplA	3
rplI	3
rpmE	3
rpmF	3
rpmJ	3
rpsF	3
rpsU	3
rrmJ	3
sirA	3
tusC	3
tusD	3
tufA	3
yfgC	3
yfiH	3
ackA	4
atpG	4
cydB	4
dapF	4
dcd	4
fabF	4
folB	4
nfuA	4
nudB	4
gpmM	4
pgmB	4
iscS	4
lpdA	4
nagA	4
pta	4
rpe	4
rpiA	4
tktA	4
ubiG	4
ybgC	4
ydjI	4
ygcO	4
ytjC	4
cedA	5
cysB	5
deaD	5
deoT	5
dksA	5
fruK	5
hfq	5
hns	5
leuO	5
oxyR	5
phoP	5
rfaH	5
rpoD	5
rpoN	5
rseA	5
xapR	5
yciM	5
zapB	5
ybcN	6
ylcG	6
ymfI	6
JW5115	7
ybeD	7
ybeY	7
ybfJ	7
ybhT	7
ycbK	7
yceD	7
ychJ	7
yddK	7
ydhT	7
yhcB	7
yidD	7
yjjY	7



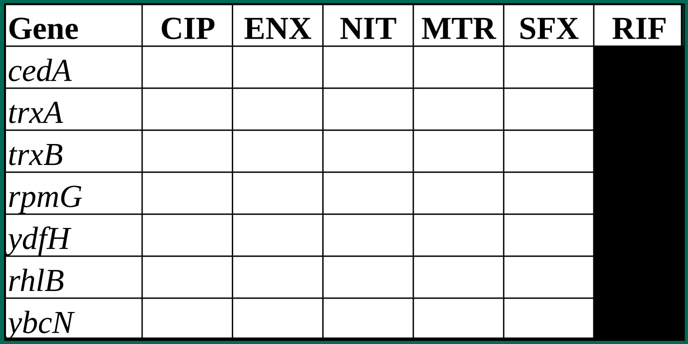
Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ
recA																							1
recB																							1
recC																							1
acrA																							2
acrB																							2
tolC																							2
ddlB																							2
envC																							2
lpxL																							2
lpxM																							2
nlpC																							2
proW																							2
pstS																							2
qmcA																							2
rfaC																							2
rfaD																							2
rfaE																							2
rfaG																							2
tatC																							2
tolR																							2
ybgF																							2
ydcS																							2
dnaK																							2A
hlpA																							2A
hscA																							2A
surA																							2A
rimK																							3
rplA																							3
rpmE																							3
rpmJ																							3
rsmF																							3
yheM																							3
yheN																							3
yfgC																							3
rlmE																							3A
atpG																							4
dapF																							4
gmhB																							4
gpmM																							4
iscS																							4
mraW																							4
pgaC																							4
rutA																							4
ycjU																							4
ygcO																							4
dksA																							5
fur																							5
hfq																							5
hns																							5
mfd																							5
nusB																							5
rseA																							5
xapR																							5
yciT																							5
ylcG																							6
ybeD																							7
ybeY																							7
ybhT																							7
ycbW																							7
yciM																							7
yjjY																							7

FIG. 3. Sixty-one strains with sensitivity to eight or more of the twenty-two antibiotics.



Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ
DNA-related																							
xseA	■	■		░																			1
xseB	■	■	░	░			░	░	▨														1
recN	■	■				▨																	1
recG	■	■	■	■	▨																		1
fis	■	▨	■	░	░																		1A
uvrD	■	▨	■	■																			1
ybjQ	■	■	■											░									7
recQ			■																				1
recJ			■	░																			1
dinG			■	░					░														1
recF			■	▨																			1
recO			■	▨																			1
recR			■	▨																			1
uvrA			■	■		■																	1
uvrC			■	■																			1
dam			░	▨										░	░		▨		░			■	1
gor				■											░								1A
gshA				■																			1A
gshB				■			░	░	░														1A
dinB		`		■																			1
Aminoglycoside Senstivity																							
sapC							■	■	▨	▨	▨												2
secG							■	■		▨													2
hflK							■	■															3
JW5360							■	■	░														7
ompF			░				■	■	■	▨													2
prfC							▨	▨	▨	▨													3
rffA							░	▨	▨														2
yidD							▨	▨	▨	░													7
leuO			░		░		░	░	▨	▨													5
folB					░		░	▨	▨	░													4
glnD							░	▨	░						░								4
nudB			░				▨	▨	■														4
yidD							▨	▨	▨	░													7
yidP							░	░	░	░													5
B-lactam Senstivity																							
emtA														■	■	░							2
dacA														■	■	■	░						2
rsgA						░	░	░						▨	■	■	▨						3
mrcB														■	■	■							2
yfiH														■	■	▨							7
ycbK														▨	■	░		░					7
Other Specific Sensitivity																							
lon				■																			2A
gntY				▨																			4
deaD			░	▨																			5
mdtJ			■																				2
degP					▨																		2A
cedA						■												▨					5
trxA						■																	2
trxB						■																	2
rpmG						■															░	░	3
ydfH						■																	5
rhlB						■															▨	▨	3
ybcN						■																	6
ycdZ												▨											2
ppiD												▨							▨				2
tufA													▨										3
pgpB															▨								3
vacJ												░						▨					2
flgF															░	░			■				2
argO						░													■	░			2
rplI																					▨	░	3
dnaJ																						■	2A
glpD						░																■	4
rsmE																						▨	3

FIG. 4. Strains with sensitivities unique to a particular class of antibiotics and strains with specific sensitivities.



Gene	CIP	ENX	NIT	MTR	SFX	RIF
cedA						■
trxA						■
trxB						■
rpmG						■
ydfH						■
rhlB						■
ybcN						■

Gene	**Rif**	**Categ**	**MIC µg/ml**
BW25113			16
bamB	■	2	1.5
yciM	■	7	1.5
rfaD	■	2	3
rfaE	■	2	3
hlpA	■	2A	3
surA	■	2A	3
rpmF	■	3	3
ylcG	■	6	3
recA	■	1	5
rfaC	■	2	5
tolR	■	2	5
trxA	■	2	5
trxB	■	2	5

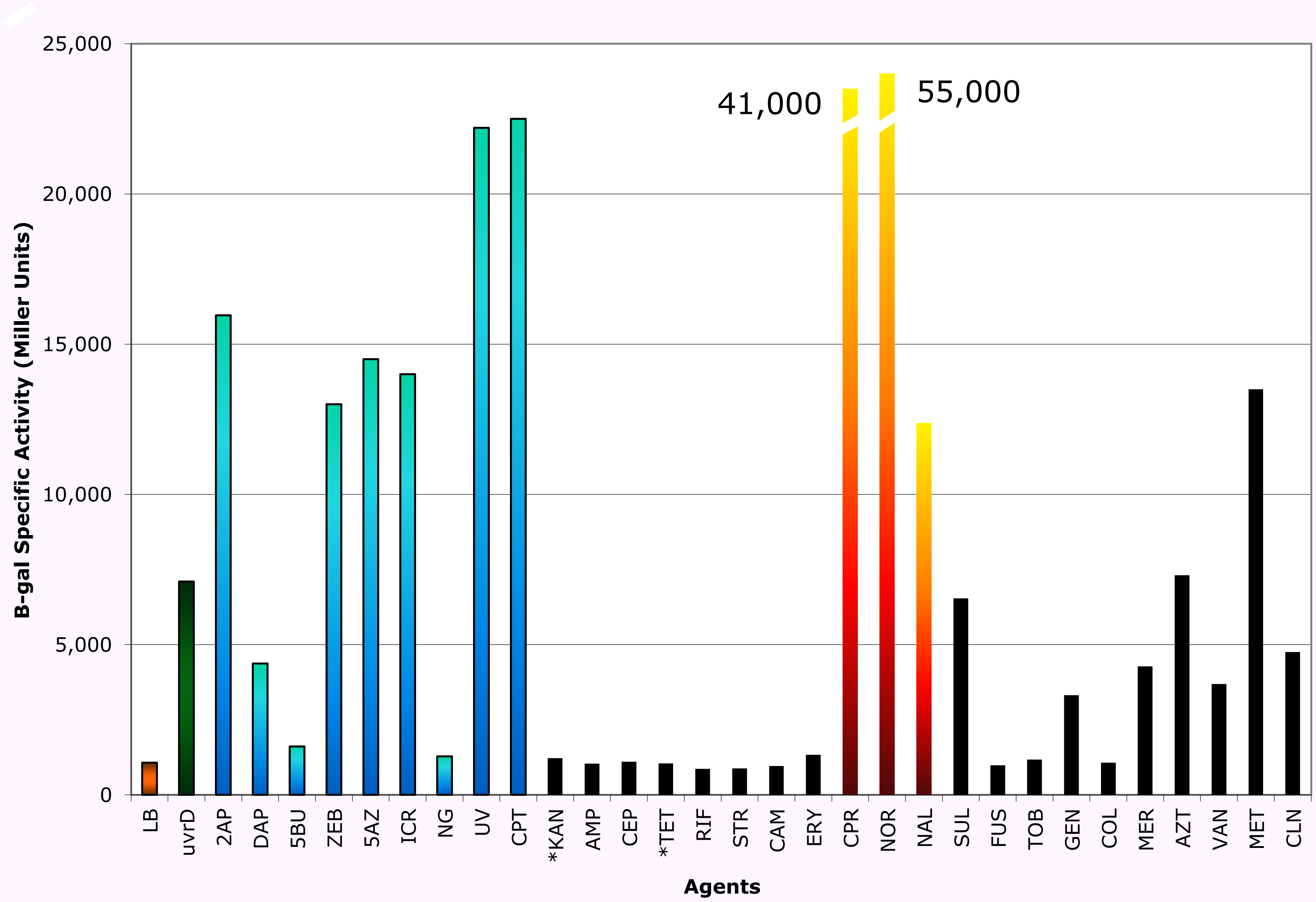
Gene	**MTR**	**Categ**	**MIC µg/ml**
BW25113			1000
gshA	■	1A	100

Applications

- Targets for co-drugs

- Typing of antibiotics

- Identify mutants for antibiotic detection


25,000
20,000
15,000
10,000
5,000
0
B-gal Specific Activity (Miller Units)
41,000
55,000
LB
uvrD
2AP
DAP
5BU
ZEB
5AZ
ICR
NG
UV
CPT
*KAN
AMP
CEP
*TET
RIF
STR
CAM
ERY
CPR
NOR
NAL
SUL
FUS
TOB
GEN
COL
MER
AZT
VAN
MET
CLN
Agents

Rif^r Mutagenic Potency

(ratio to untreated WT)

EMS	5800
5BdU	5100
UV	400
2AP	160
mutS	120
CPR	1-3
WT	1

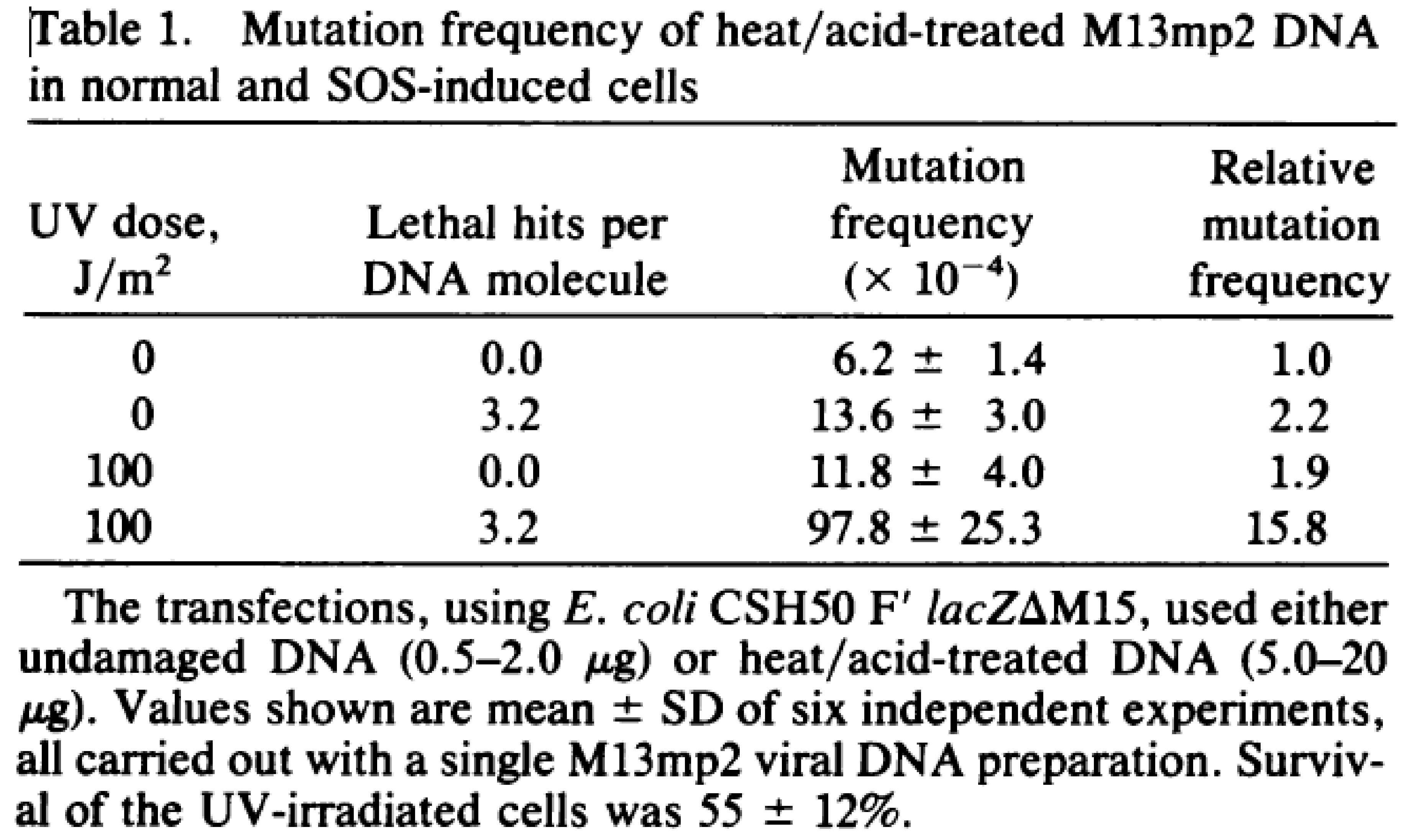


Table 1. Mutation frequency of heat/acid-treated M13mp2 DNA in normal and SOS-induced cells

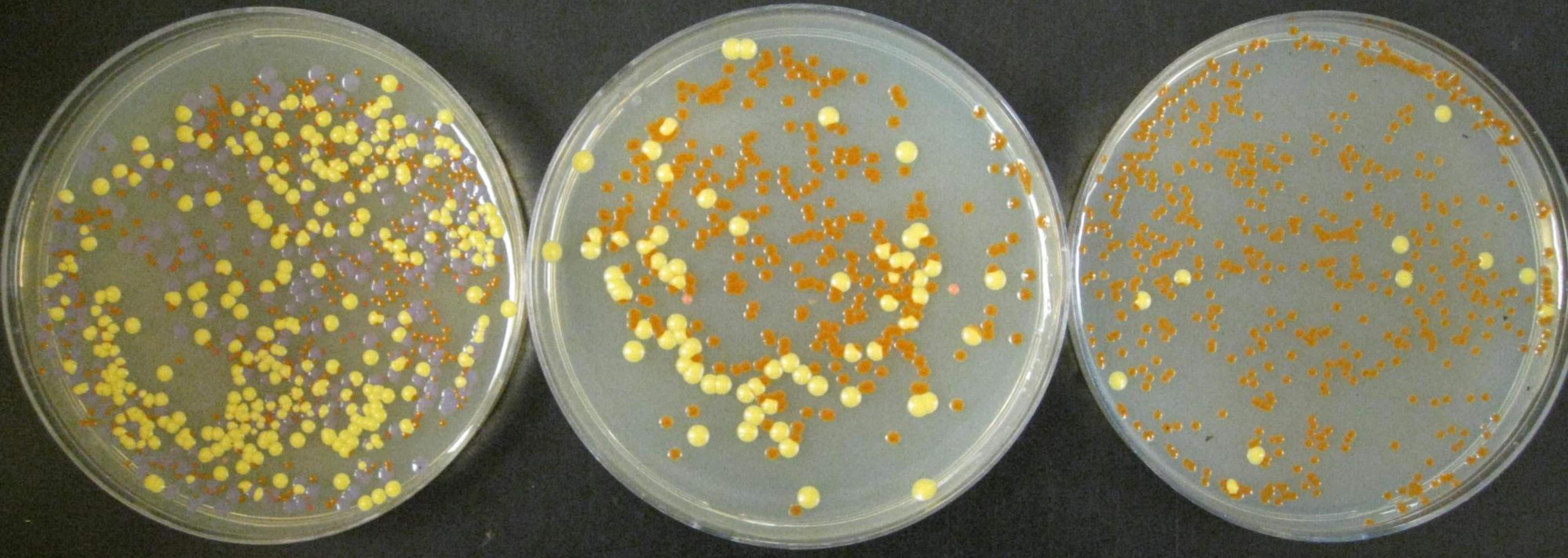
UV dose, J/m²	Lethal hits per DNA molecule	Mutation frequency (× 10^{-4})	Relative mutation frequency
0	0.0	6.2 ± 1.4	1.0
0	3.2	13.6 ± 3.0	2.2
100	0.0	11.8 ± 4.0	1.9
100	3.2	97.8 ± 25.3	15.8

The transfections, using *E. coli* CSH50 F′ *lac*ZΔM15, used either undamaged DNA (0.5–2.0 μg) or heat/acid-treated DNA (5.0–20 μg). Values shown are mean ± *SD* of six independent experiments, all carried out with a single M13mp2 viral DNA preparation. Survival of the UV-irradiated cells was 55 ± 12%.

LB Cipro .05 Cipro .1 Cipro .2 Cipro .5 Cipro 1

WT:*tolCrecC*

ng/ml



Starting Mix   Day 2 in 15 ng/ml cipro   Day 3 in 15 ng/ml cipro

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Amie Fong*
Kevin Frew*
Lucia Guisado*
Jeeyoon Hong
Katherine Kim
Catherine Lee

Kim Lee
Anne Liu
Maria Maiz*
Leah Medrano*
Eunice Park
Lillian Tran
Daniel Sitz
Jessica Yuan*
Christine Wahba

Bicyclomycin

Max E. Gottesman
Robert S. Washburn

Columbia University
Medical Center
New York, NY

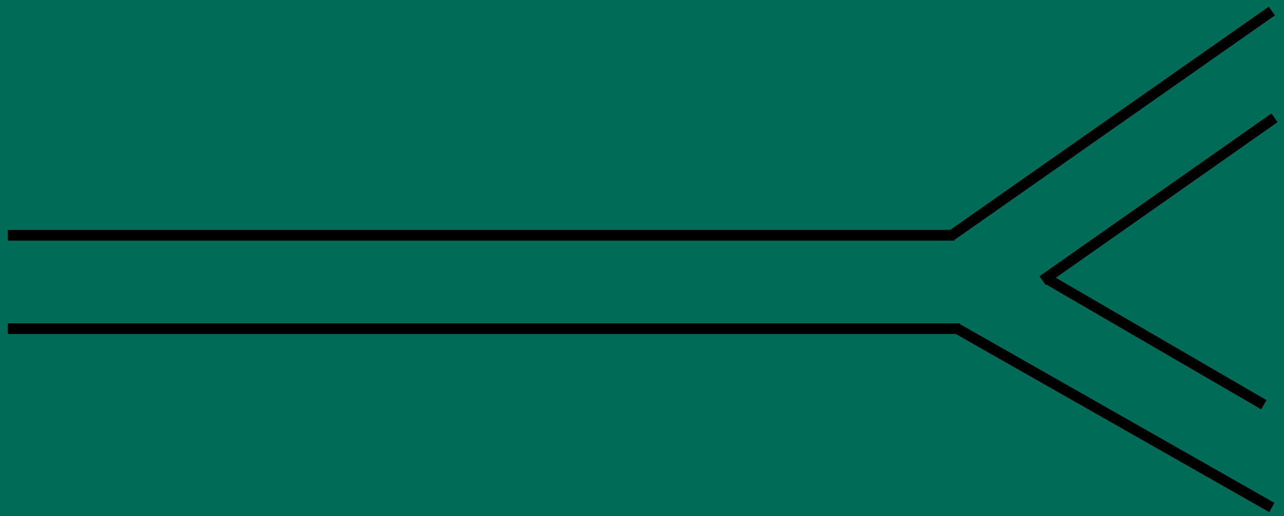
Bioluminescent Proteins

Robert M. Hoffman

Anticancer, Inc.
San Diego, CA

Anya Salih

University of
Western Sydney
New South Wales,
Australia



Damage Avoidance

- Detoxification

DNA Damage

- Direct Reversal
- Excision Repair
- Oxidative Damage Repair
- Glycosylases

DNA Replication

- Pool size
- Polymerases
- Proofreading
- Induction of error-prone polymerases

Post Replication

- Mismatch repair
- Recombination repair


LOVE

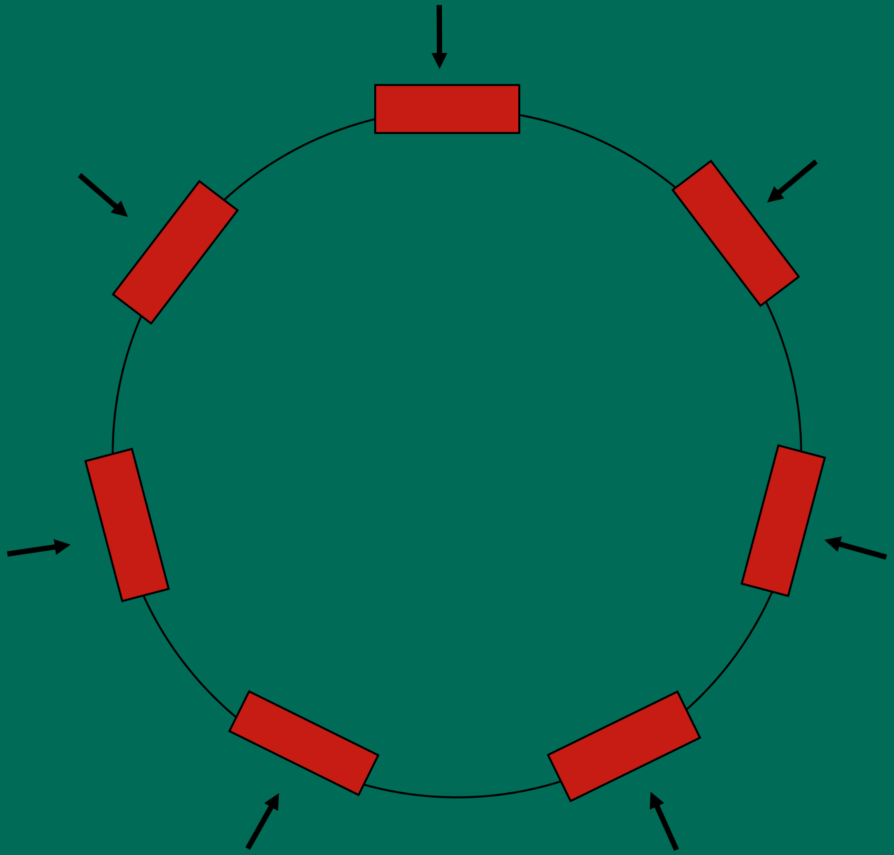
LB Cipro 1 Cipro 2 Cipro 3 Cipro 4 Cipro 5

WT:*tolC* ng/ml

Plate Test

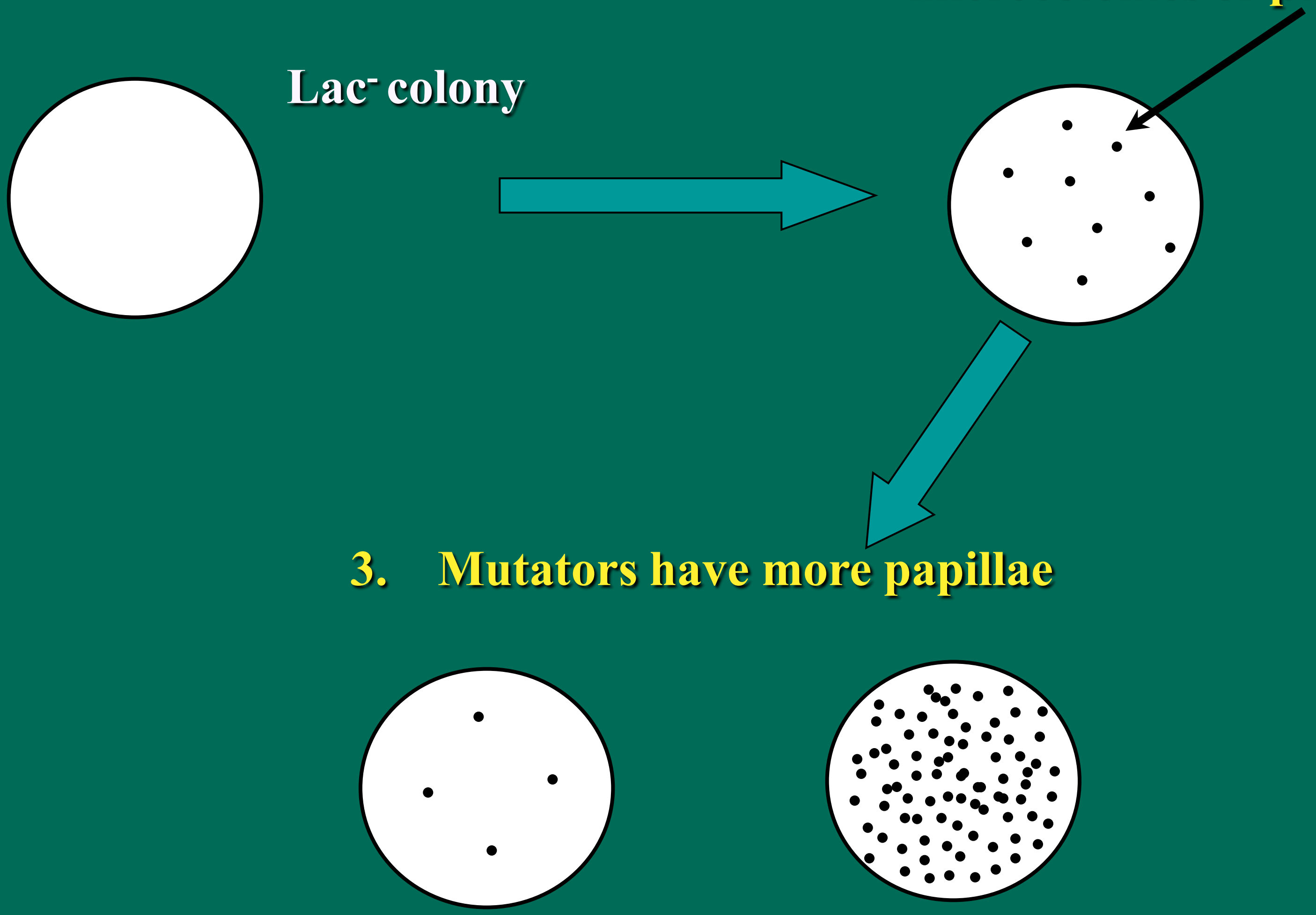
- Mixture of wild-type and sensitive mutant
- Antibiotic applied to center of plate
- Concentration gradient, killing in center
- No killing on outer rim of plate
- Selective killing at low concentration generates ring of wild-type cell color

Previous Approach - Inactivate Genes



- Knockout repair or other system
- Found many repair genes
- Many as mutators

Finding mutators by papillation



1. Rich medium + lactose

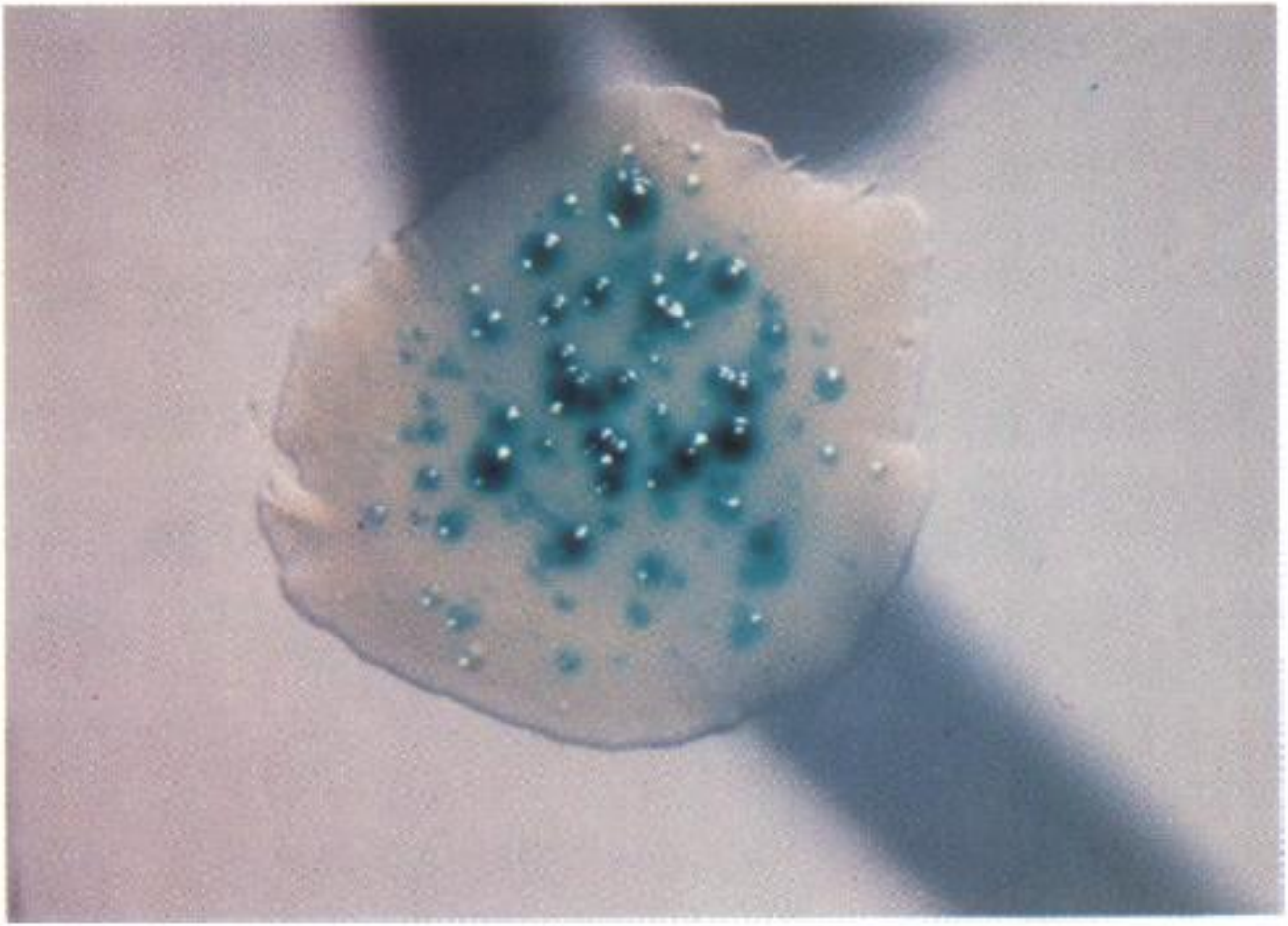
Lac$^-$ colony

2. Revertants to Lac$^+$ make new microcolonies or papillae

3. Mutators have more papillae

WT

mutator



Look for specific base changes in *lacZ*

Glu — GAG — Lac$^+$

↓

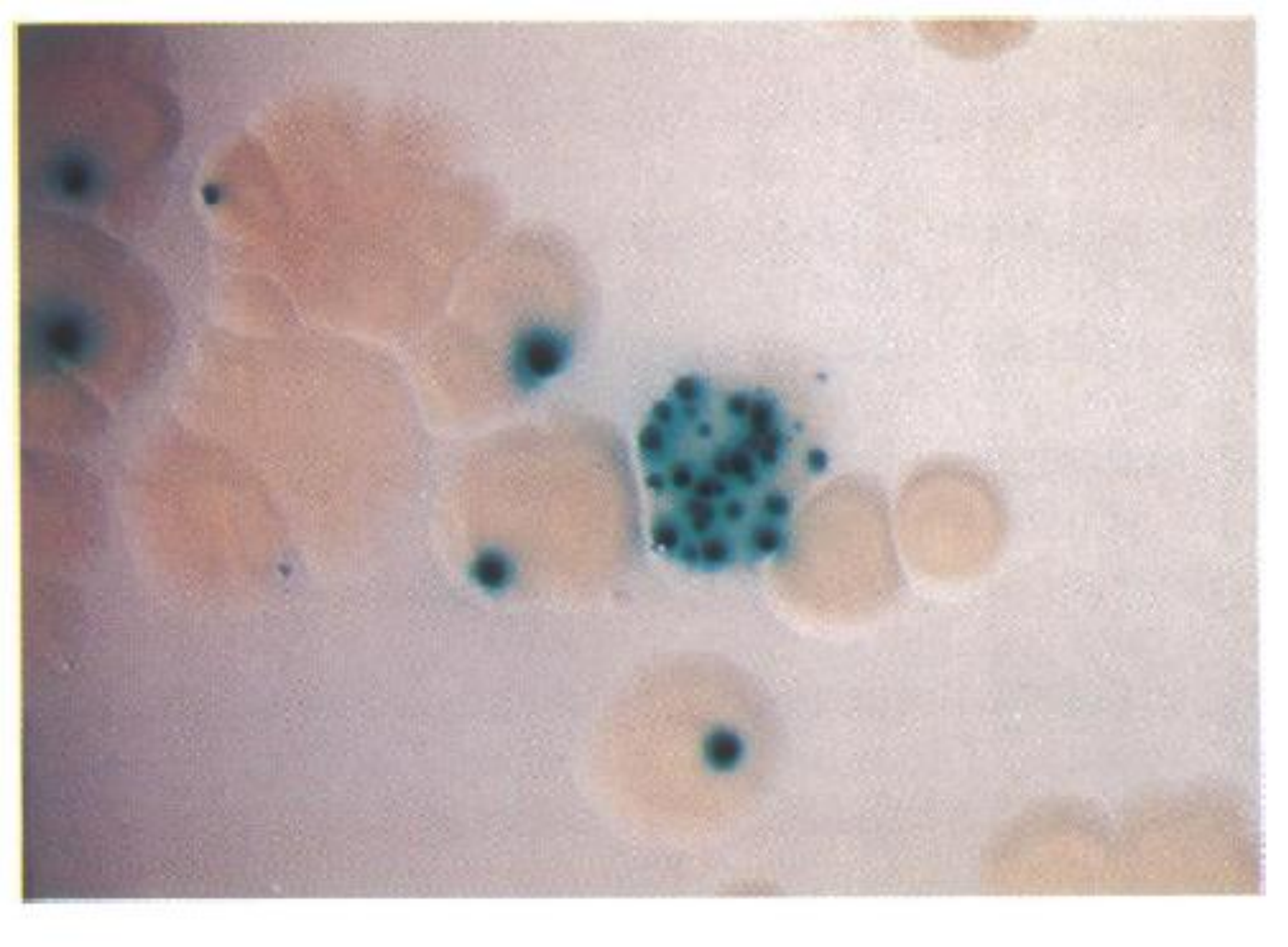
Ala — GCG — Lac$^-$

↓

Glu — GAG — Lac$^+$

GC → TA

Lac$^-$ → Lac$^+$



Identification of *MutY* and *MutM* in *E.coli*

The *mutY* gene: a mutator locus in *Escherichia coli* that generates G.C---T.A transversions.

Nghiem Y. Cabrera M, Cupples CG, Miller JH

Department of Biology, University of California, Los Angeles 90024.

We have used a strain with an altered *lacZ* gene, which reverts to wild type via only certain transversions, to detect transversion-specific mutators in *Escherichia coli*. Detection relied on a papillation technique that uses a combination of beta-galactosides to reveal blue Lac+ papillae. One class of mutators is specific for the G.C--T.A

***PNAS* 1988**

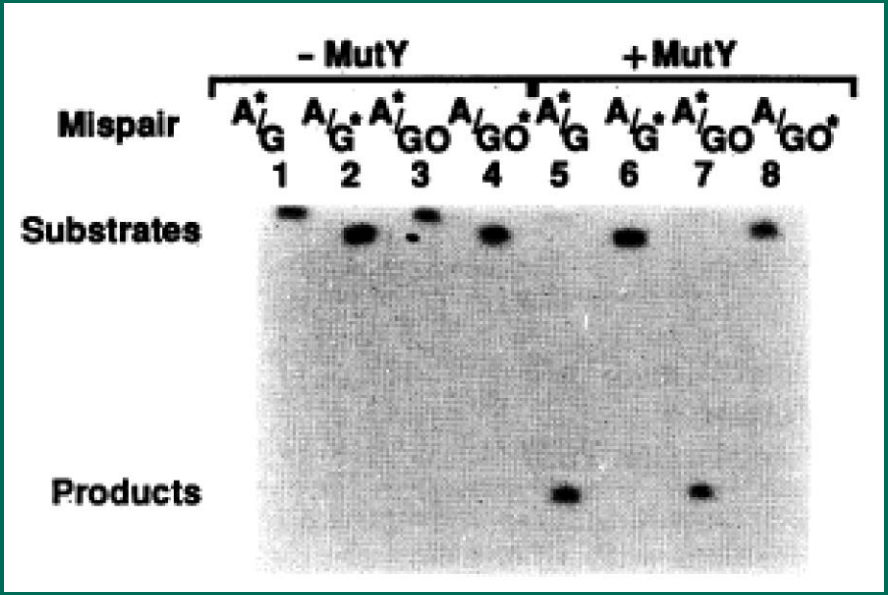
Cabrera M, Nghiem Y, Miller JH.

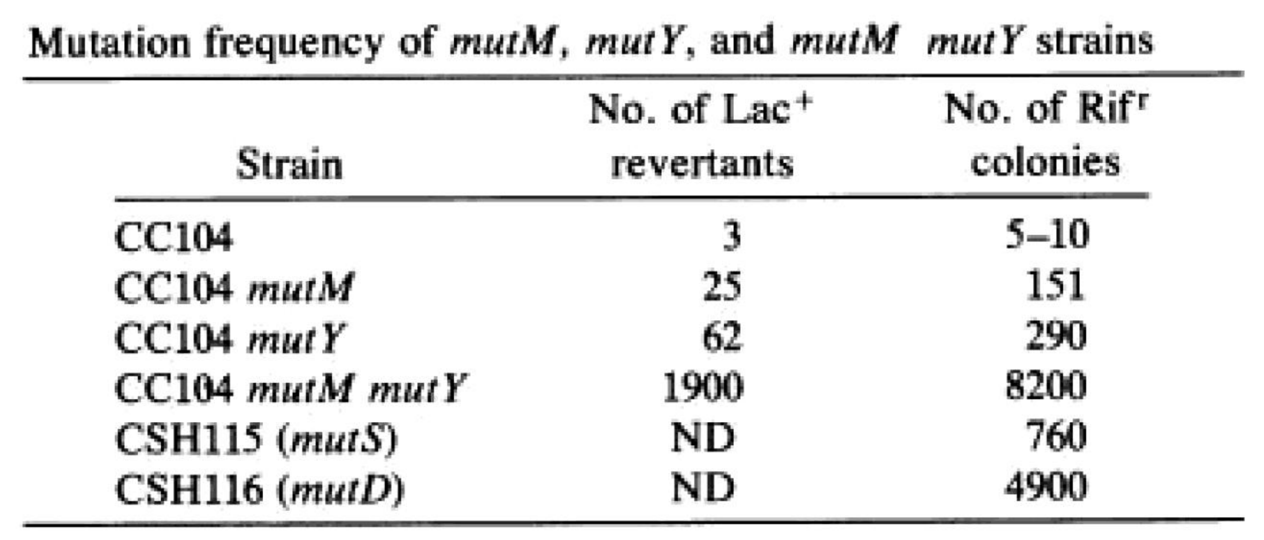
Department of Biology, University of California, Los Angeles 90024.

We used strains carrying specific *lacZ* alleles to identify a new mutator locus in *Escherichia coli*
which generates only G.C--T.A transversions among base substitutions. The locus, *mutM*, mapped near
the *cysE* locus, which is at 81 min on the genetic map.

***J Bacteriol* 1988**

MutY prevents G to T mutations in *E.coli*



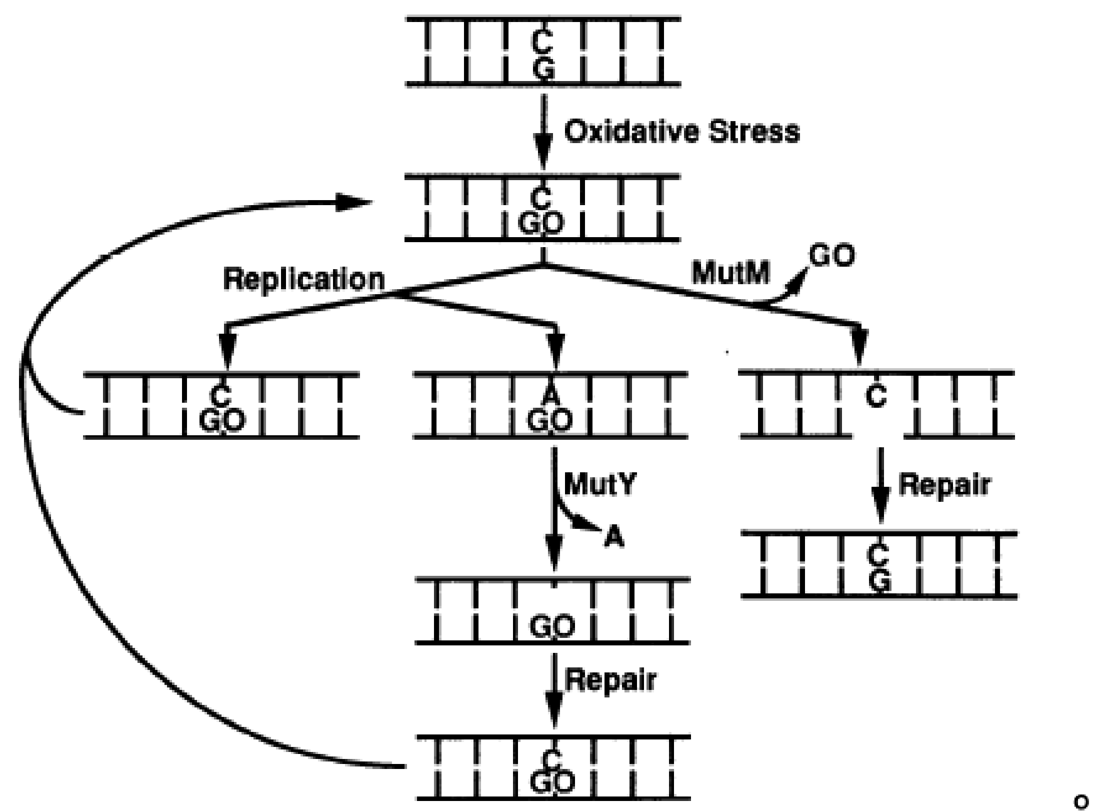


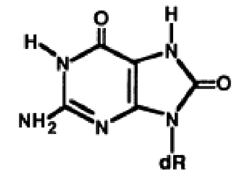
Mutation frequency of *mutM*, *mutY*, and *mutM mutY* strains

Strain	No. of Lac⁺ revertants	No. of Rif^r colonies
CC104	3	5–10
CC104 *mutM*	25	151
CC104 *mutY*	62	290
CC104 *mutM mutY*	1900	8200
CSH115 (*mutS*)	ND	760
CSH116 (*mutD*)	ND	4900

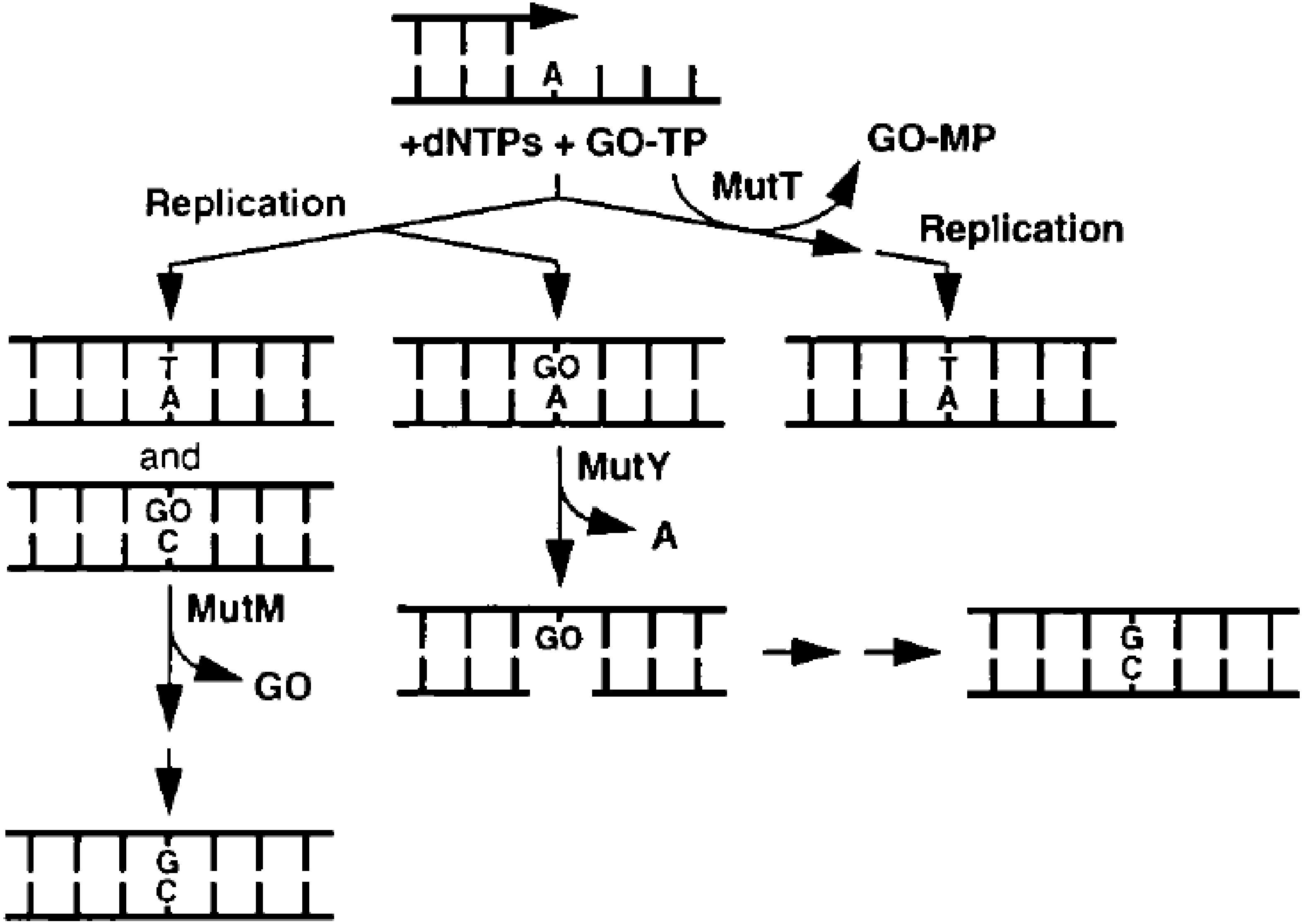
Michaels and Miller, *PNAS* 1992

Oxidative DNA damage repair





7,8-dihydro-8-oxoguanine (8-oxoG or GO)

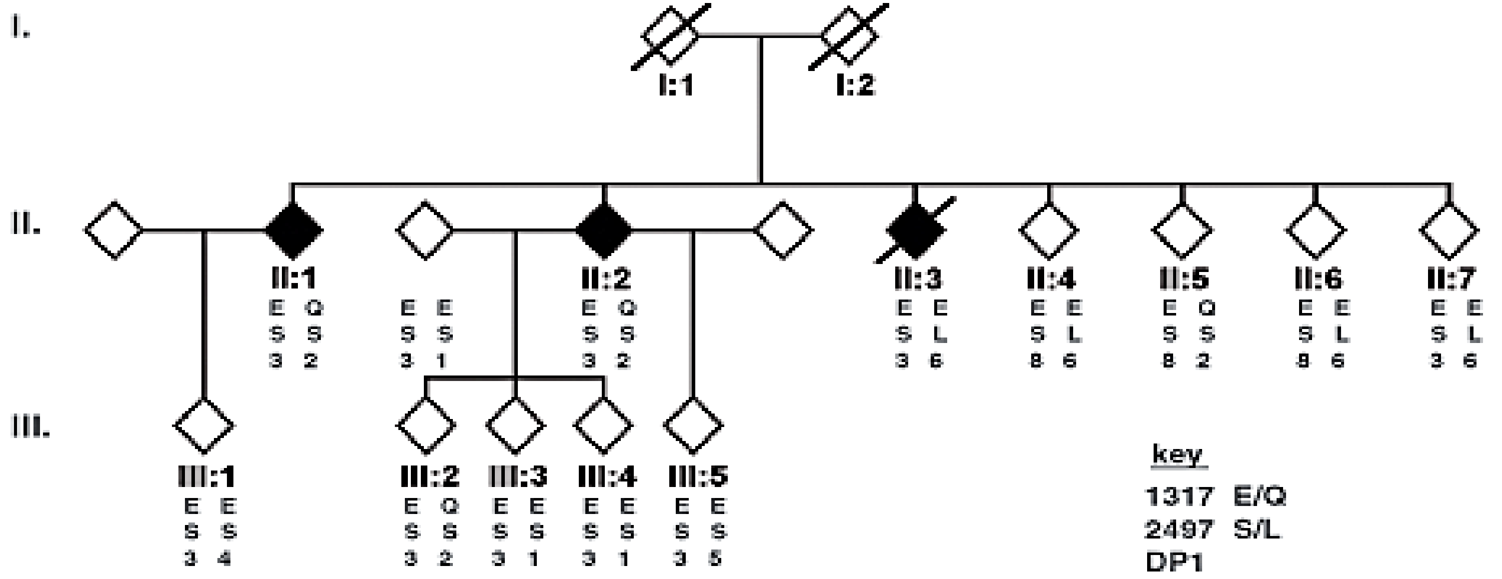

A
+dNTPs + GO-TP
GO-MP
MutT
Replication
Replication
T
A
GO
A
T
A
and
GO
C
MutY
A
MutM
GO
GO
G
C
G
C

Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors.

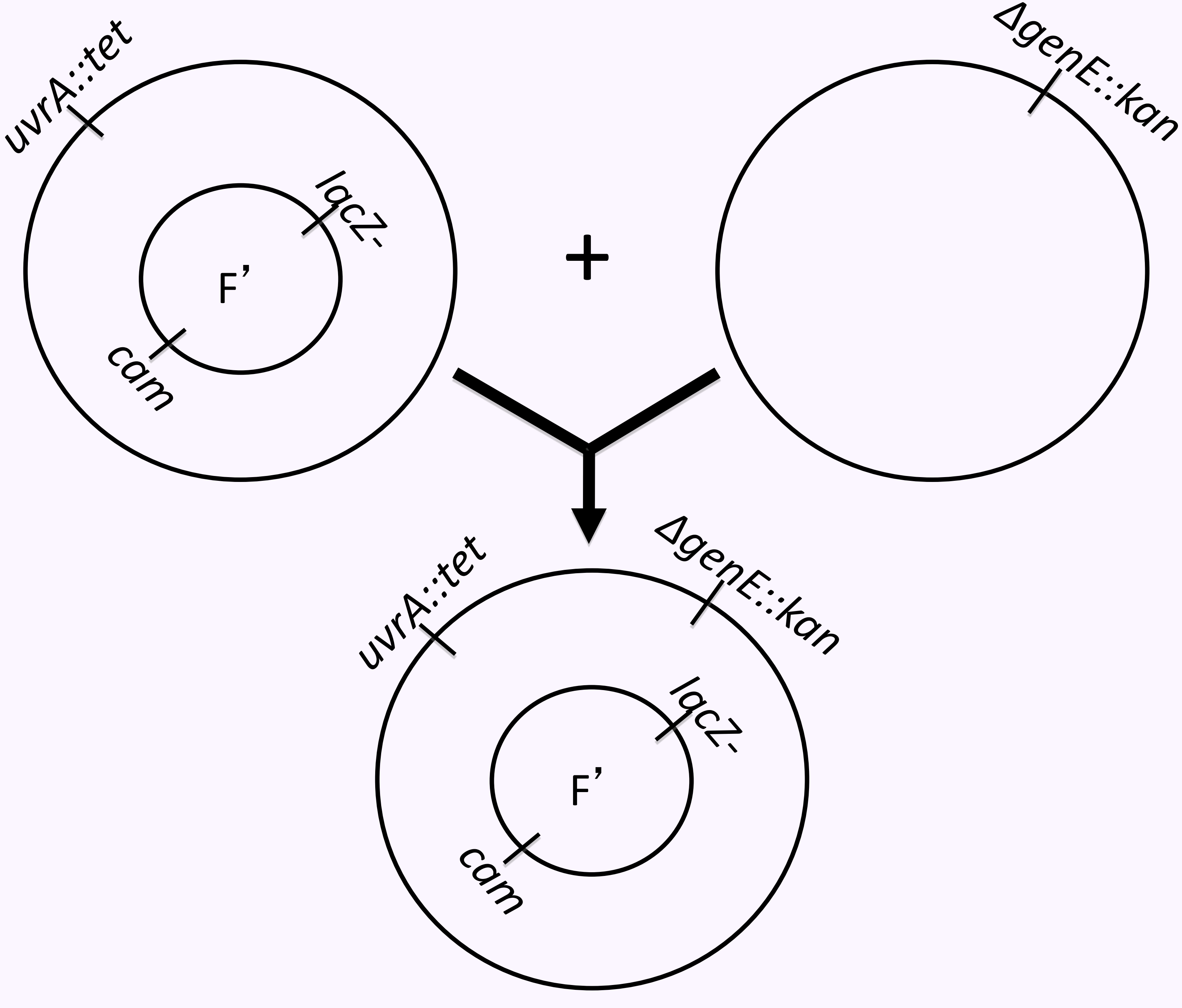
Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, Hodges AK, Davies DR, David SS, Sampson JR, Cheadle JP.

Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, UK.

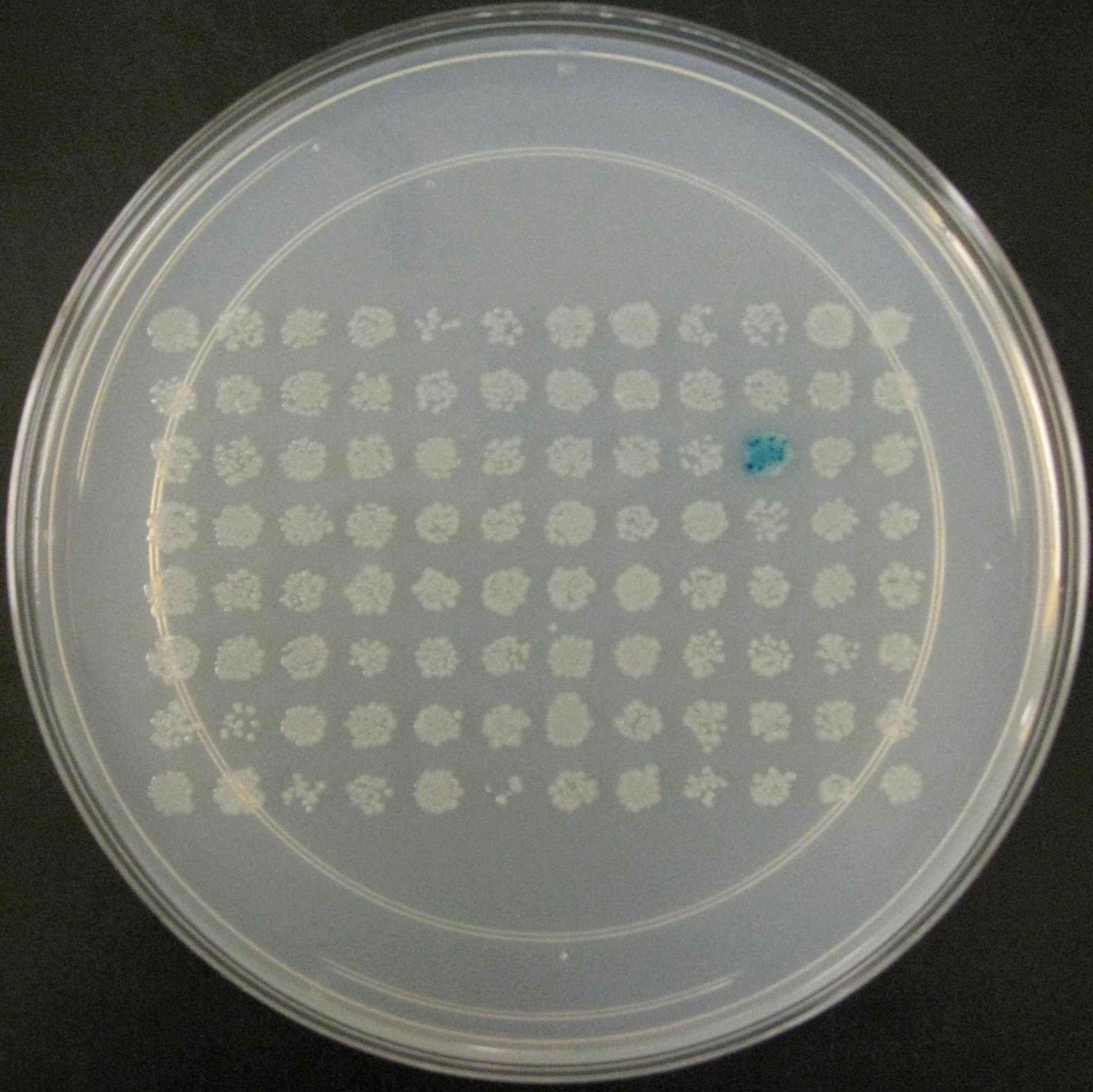
Inherited defects of base excision repair have not been associated with any human genetic disorder, although mutations of the genes mutM and mutY, which function in Escherichia coli base excision repair, lead to increased transversions of G:C to T:A. We have studied family N, which is affected with multiple colorectal adenomas



Nature Genetics 2002



uvrA::tet
lacZ-
F’
cam
+
ΔgenE::kan
uvrA::tet
ΔgenE::kan
lacZ-
F’
cam



Studies of mutagenesis and repair
in
Bacillus anthracis

B. anthracis genes (absent in *E. coli*) that are possibly involved in DNA replication, recombination, and repair

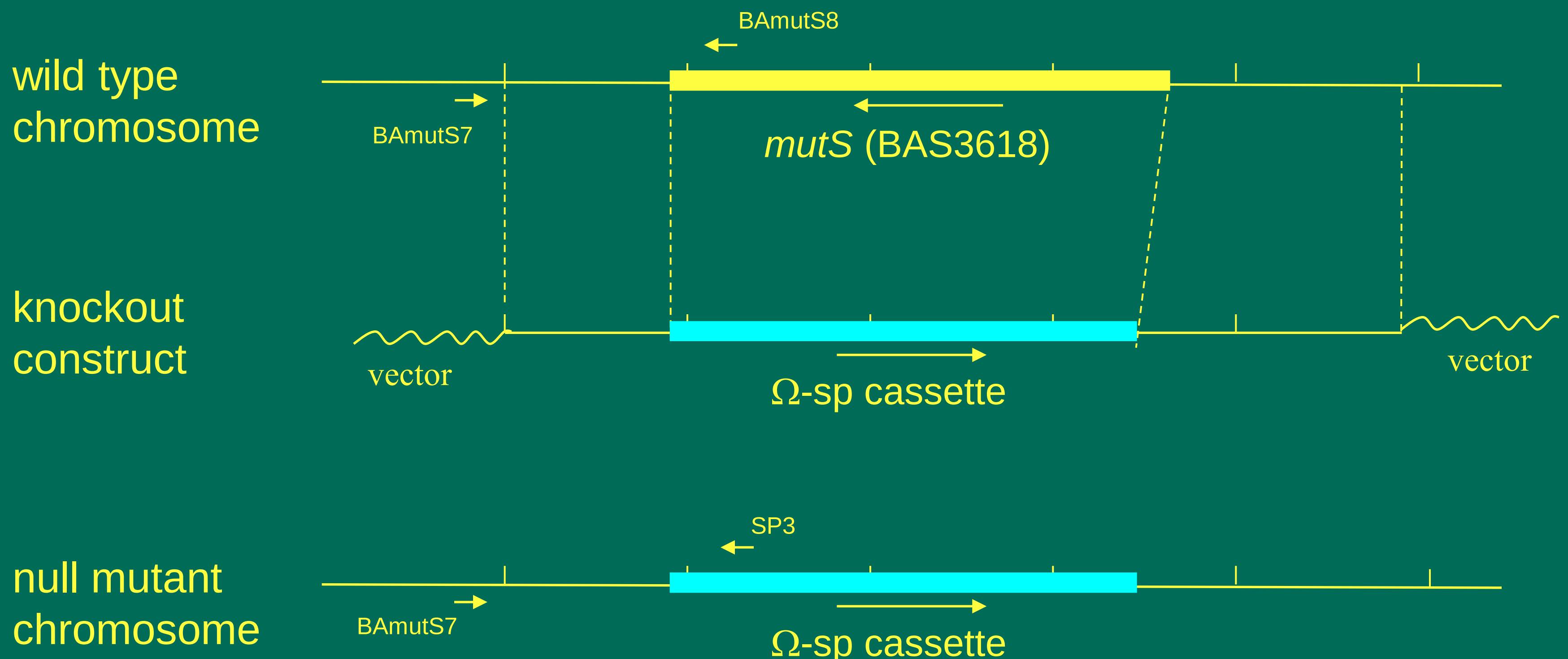
BAS number	COG	Predicted function
BAS0234	COG4294	UV-endonuclease, putative
BAS5196	COG4294	UV-endonuclease, putative
BAS1439	COG1573	uracil-DNA glycosylase family protein (BAS5250, 2nd UDG)
BAS4536	COG0827	hypothetical protein (putative adenine-specific DNA methylase)
BAS0852	COG1041	hypothetical protein (predicted DNA modification methylase)
BAS3332	COG2176	exonuclease family protein (putative dnaQ homolog)
BAS3404	COG2176	DNA polymerase, epsilon subunit, putative
BAS3669	COG2176	DNA polymerase, alpha subunit, Gram-positive type
BAS4780	COG2176	sporulation inhibitor KapD
BAS4451	COG1039	ribonuclease HIII
BAS2504	COG1372	intein homing endonuclease-related protein
BAS4109	COG1533	spore photoproduct lyase
BAS0039	COG1658	primase-related protein
BAS4858	COG1658	Toprim domain protein

BAS number	COG	Predicted function
BAS1831	COG1793	lipoprotein, putative (putative DNA ligase)
BAS4448	COG1796	PHP domain protein (polymerase X)
BAS0826	COG2094	methylpurine-DNA glycosylase family protein
BAS1291	COG2887	hypothetical protein (very little homology to RecB)
BAS2046	COG3547	transposase, IS110 family, OrfA
BAS2047	COG3547	transposase, IS110 family, OrfB
BAS4475	COG3611	DNA replication protein DnaB
BAS3807	COG3747	hypothetical protein (putative terminase)
BAS1060	COG3857	ATP-dependent nuclease, subunit B
BAS0424	COG3935	prophage LambaBa04, DnaD replication protein, putative
BAS0879	COG3935	DnaD domain protein
BAS1455	COG3935	DNA replication protein DnaD
BAS3088	COG3935	DnaD domain protein
BAS3828	COG3935	prophage LambaBa02, DNA replication protein
BAS5042	COG4095	comF operon protein 1
BAS2732	COG4912	hypothetical protein (predicted DNA alkylation repair enzyme)
BAS3091	COG4912	hypothetical protein (predicted DNA alkylation repair enzyme)
BAS4783	COG4912	hypothetical protein (predicted DNA alkylation repair enzyme)

Unique detoxification enzymes in *B. anthracis*

5	catalases
3	Fe-Mn superoxide dismutase
1	cytoplasmic Cu-Zn superoxide dismutase
1	bromoperoxidase
1	thiolperoxidase
8	thioredoxin proteins

B. anthracis knockout strategy



B. anthracis knockout mutants constructed

mutS (DNA mismatch repair protein)

mutY (A/G-specific adenine glycosylase)

mutM (formamidopyrimidine-DNA glycosylase)

mutM, *mutY*

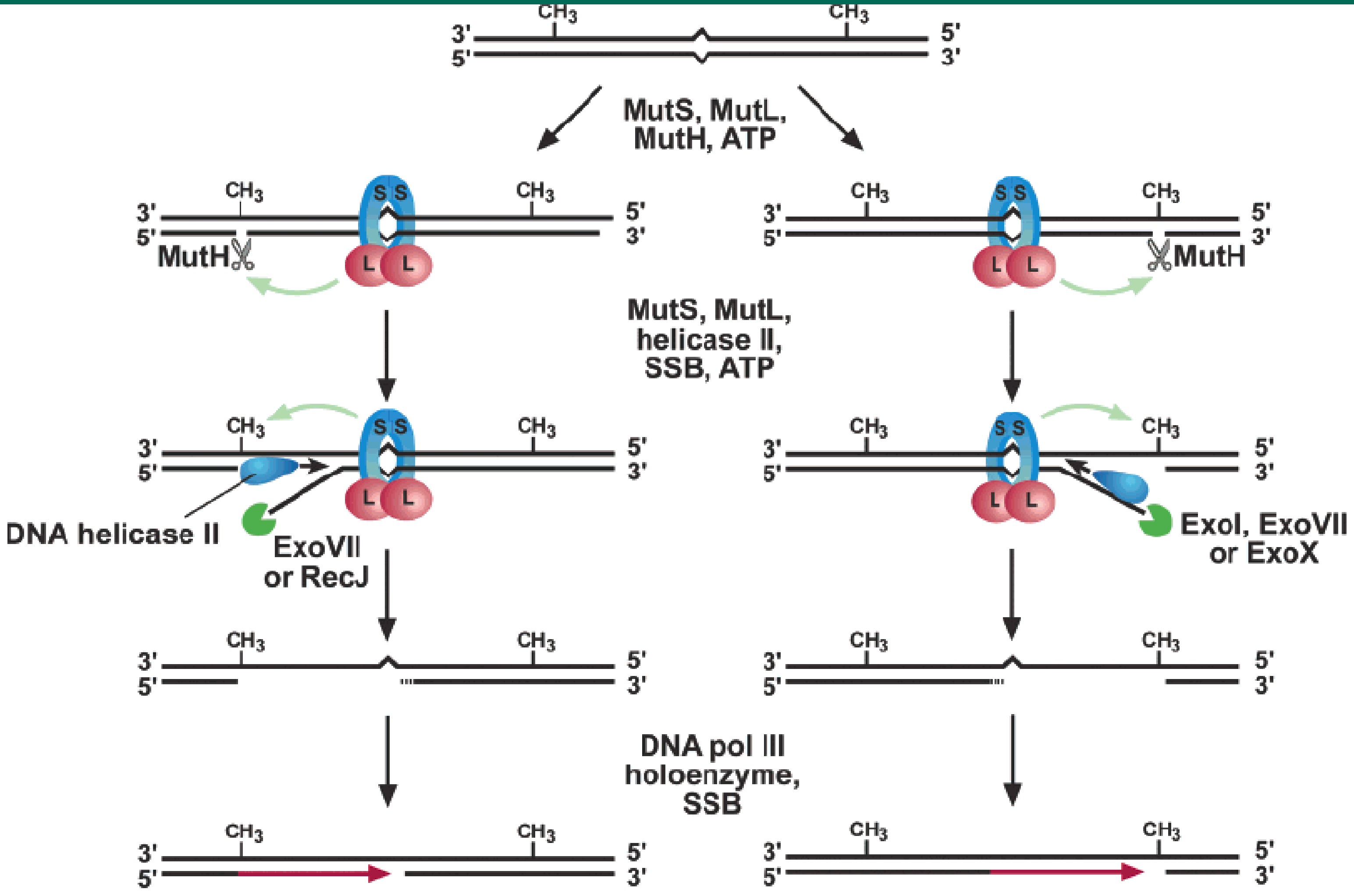
ndk (nucleoside diphosphate kinase)

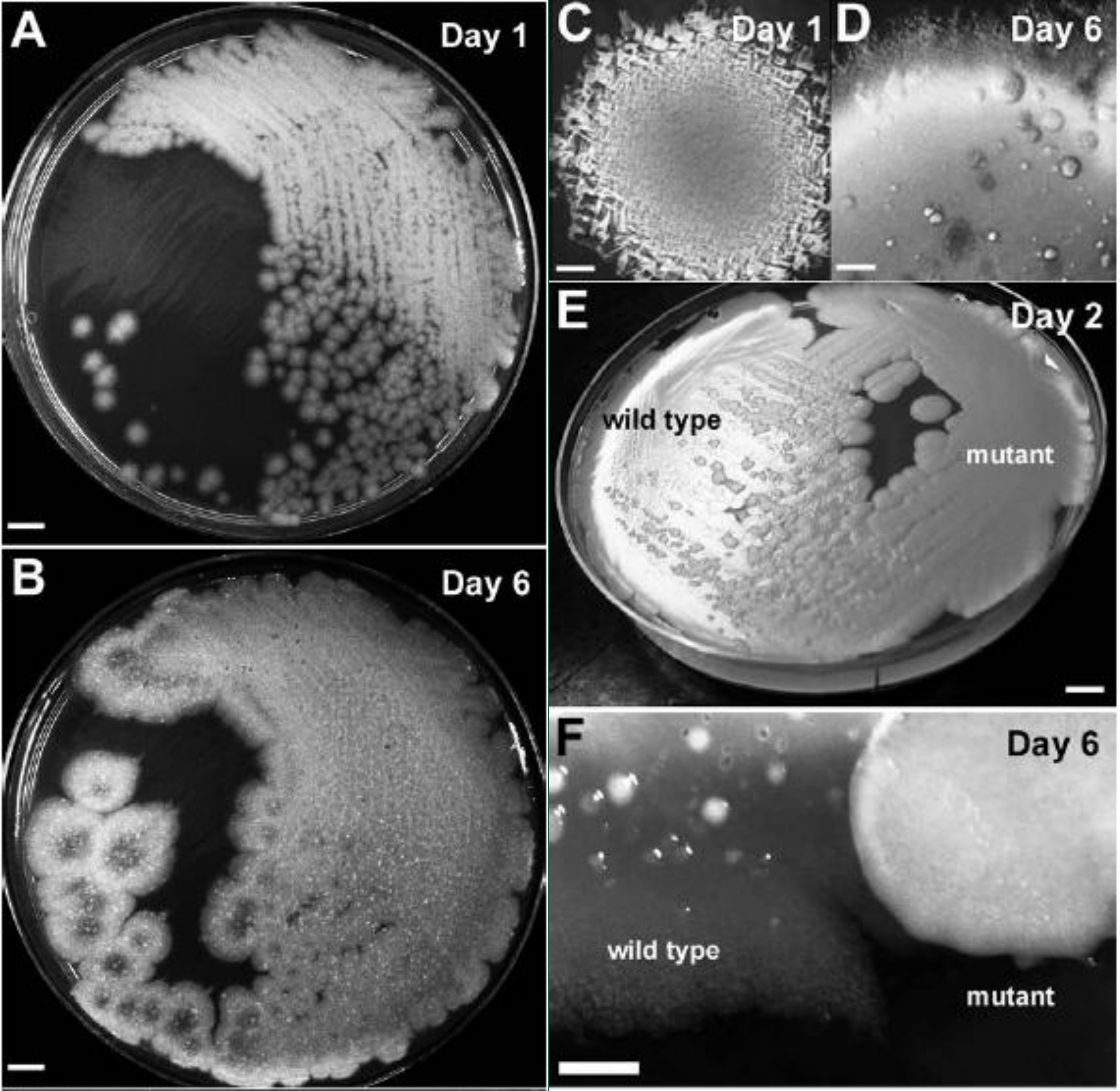
ndk, *mutS*

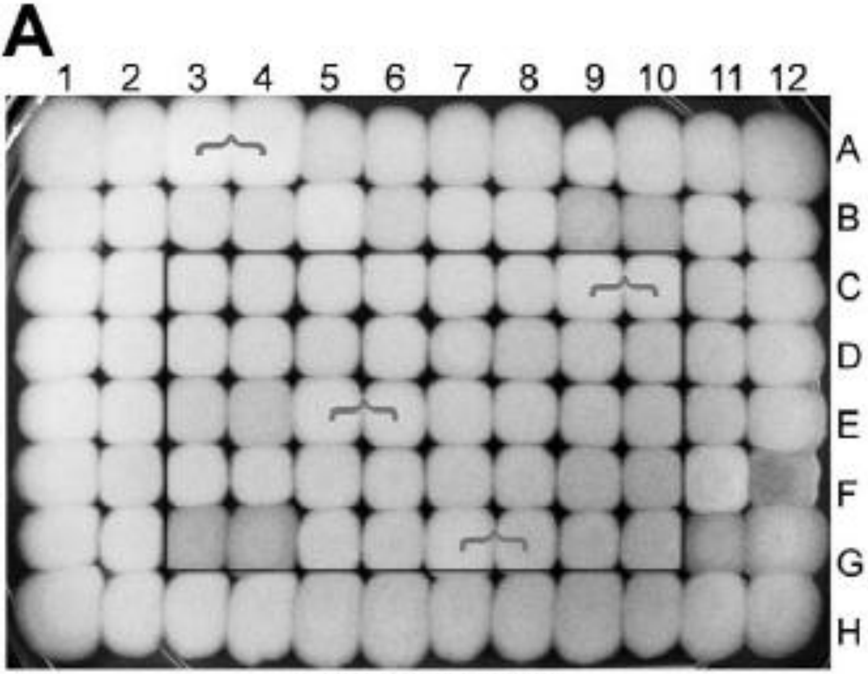
bromoperoxidase

B. Anthracis rpoB mutation frequencies (*f*) and rates (μ) x 10^9

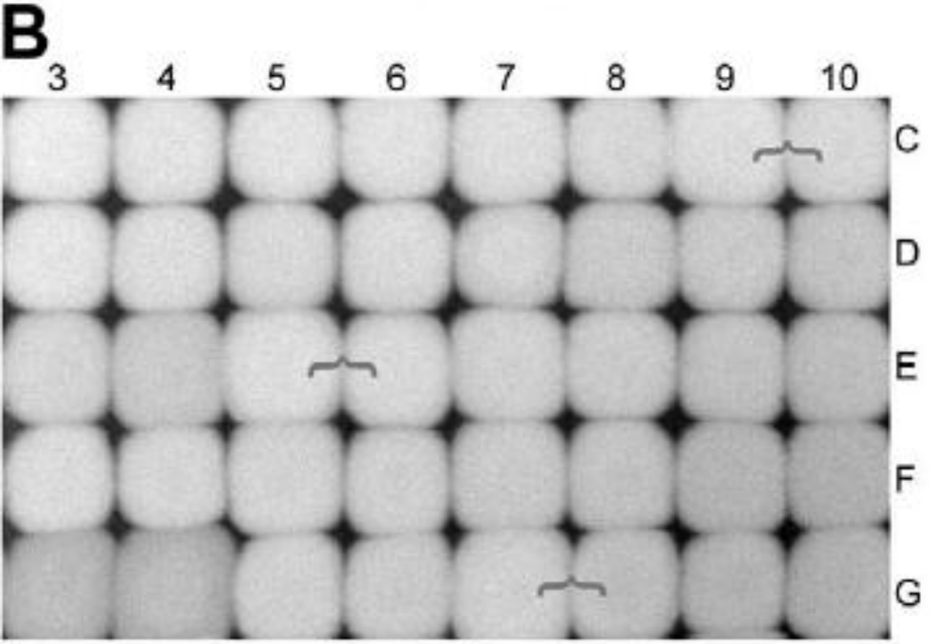
	f	μ
Wild type	2.8 (1.7-4.7)	1.6 (1.2-2.3)
mutY	33 (31-56)	9.4 (9.0-14)
mutY, *M*	2800 (2500-3300)	430 (390-500)
mutS	500 (480-550)	87 (84-94)


CH3
CH3
3'
5'
5'
3'
MutS, MutL,
MutH, ATP
CH3
CH3
S S
3'
5'
5'
3'
MutH
L L
CH3
CH3
S S
3'
5'
5'
3'
MutH
L L
MutS, MutL,
helicase II,
SSB, ATP
CH3
CH3
S S
3'
5'
5'
3'
L L
DNA helicase II
ExoVII
or RecJ
CH3
CH3
S S
3'
5'
5'
3'
L L
ExoI, ExoVII
or ExoX
CH3
CH3
3'
5'
5'
3'
CH3
CH3
3'
5'
5'
3'
DNA pol III
holoenzyme,
SSB
CH3
CH3
3'
5'
5'
3'
CH3
CH3
3'
5'
5'
3'

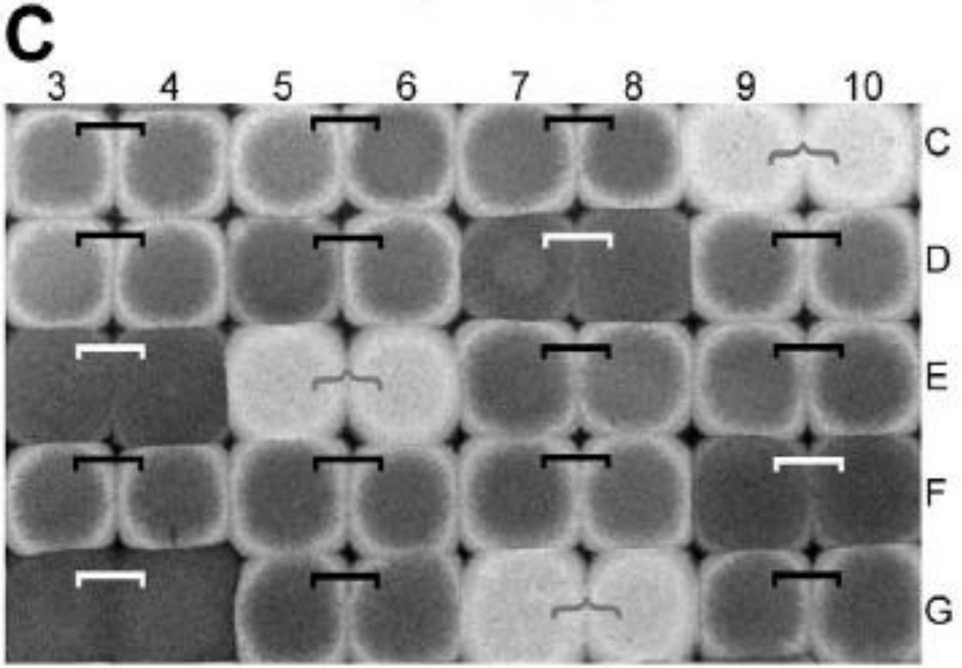

A
Day 1
B
Day 6
C
Day 1
D
Day 6
E
Day 2
wild type
mutant
F
Day 6
wild type
mutant



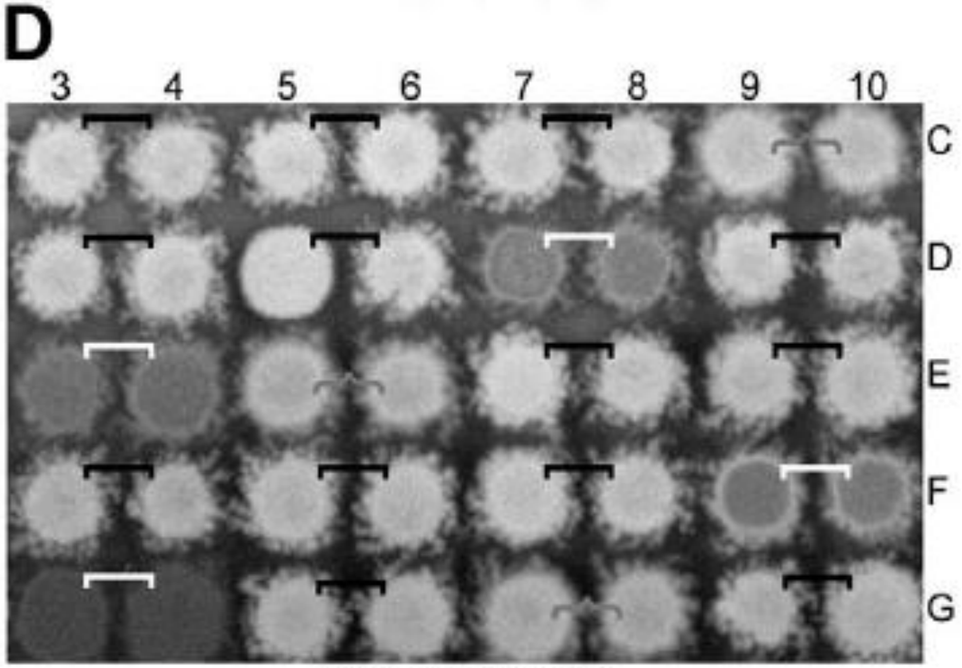
LB agar (Day 2)



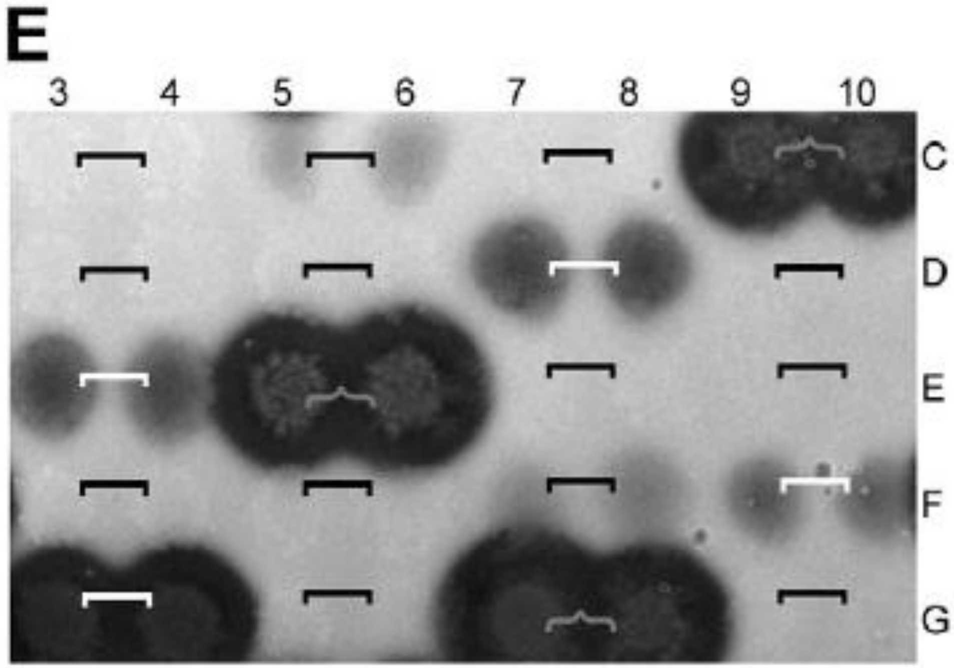
LB agar (Day 2)



LB agar (Day 6)



SM agar (Day 6)



milk agar (37°C, O/N)

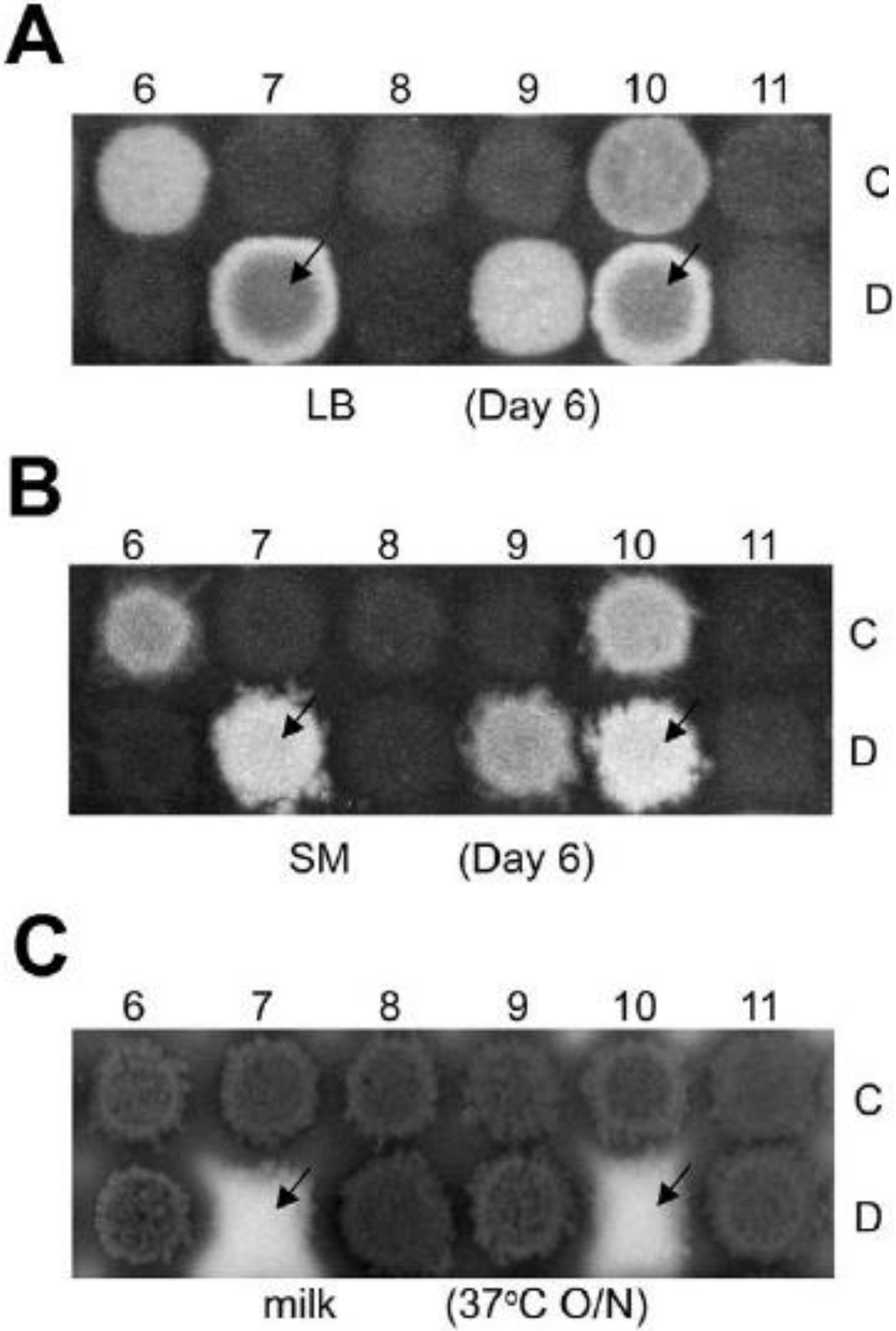
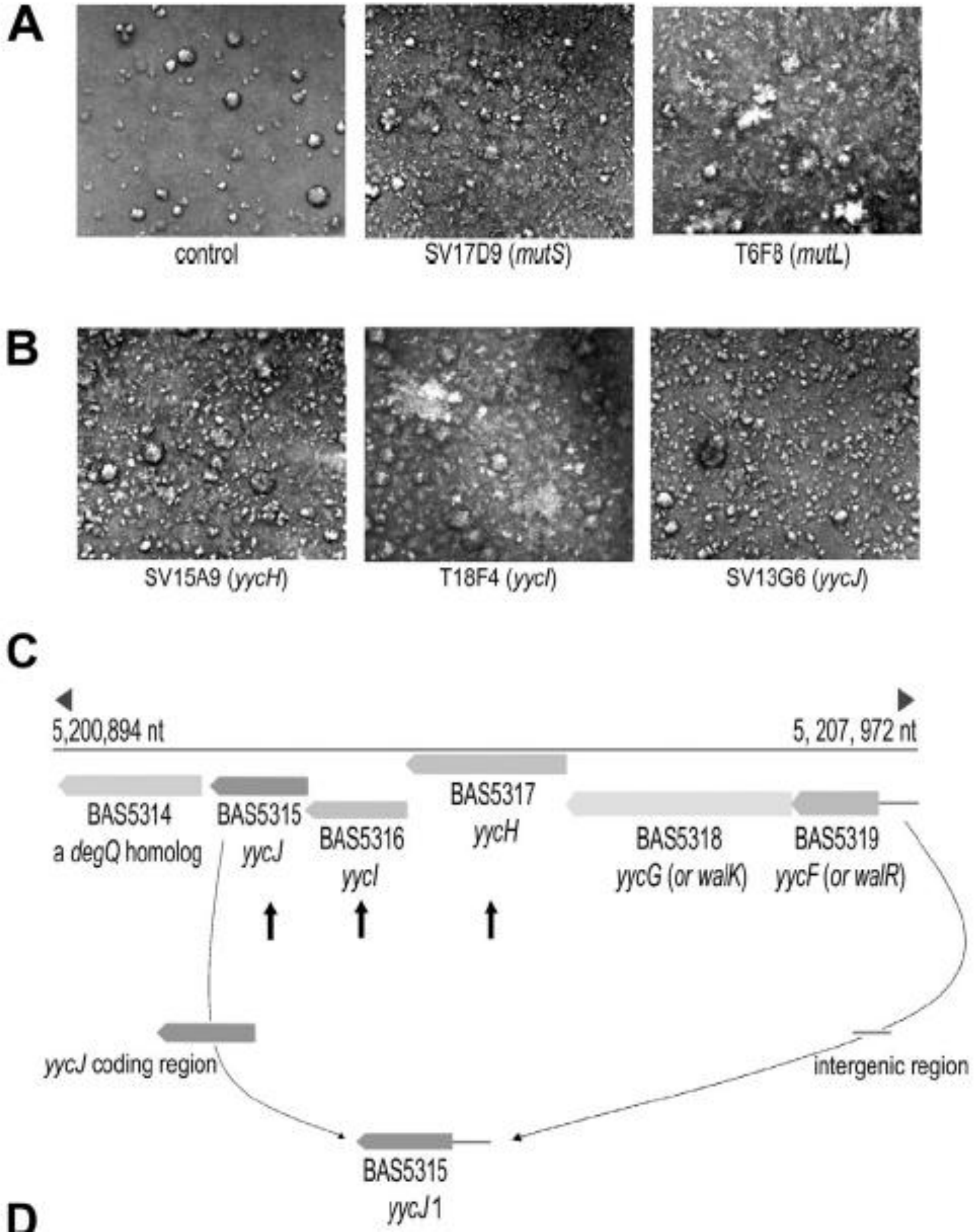


Fig. 3. A–C. Photographs showing two transposon-insertion mutants T17D7 and T17D10 (marked with black arrows), which display similar phenotypes as the group A papillation mutants. D–E. photographs of cells harbouring pUTE29 or pUTE29*nprR* on LB and milk plates.

A. On the LB plate.

B. On the sporulation (SM) plate.

C. On the milk plate.


A
control
SV17D9 (*mutS*)
T6F8 (*mutL*)
B
SV15A9 (*yycH*)
T18F4 (*yycI*)
SV13G6 (*yycJ*)
C
5,200,894 nt
5, 207, 972 nt
BAS5314
a *degQ* homolog
BAS5315
yycJ
BAS5316
yycI
BAS5317
yycH
BAS5318
yycG (*or walK*)
BAS5319
yycF (*or walR*)
yycJ coding region
intergenic region
BAS5315
*yycJ*1
D

Mutations in *nprR*

Type of change	Occurrence
Base substitutions	17
Small insertions and deletions	21
Tandem duplications	3
Large deletions	17
Total	58

80 bp tandem duplication

wild type

AGAG**GGTTAT** TGTGC..............CAA**GGTTAT** CATGAAACAGGCCTATATTA

80 bp

nprR mutant

AGAG**GGTTAT** TGTGC .... CAA**GGTTAT** TGTGC .... CAA**GGTTAT** CATGA

80 bp 80 bp

204 bp large deletion

wild type

TTTGC**TGATAAGG** ATGTACT .... ATTAAT**TGATAAGG** GAATTGATGCAGCAAA

204 bp

nprR mutant

TTTGC**TGATAAGG** GAATTGATGCAGCAAAACAAGAAGAGAGGTTTAATGCAAAATTA

Table 5. Tandem duplications detected in the *recJ* mutants.

No.	BAS0566	Occurrence[a]	
1	333–339, 7 bp	17	ATTTTG**AAATTAT**
2	921–963, 43 bp	12	TAGAT**TTAAATTA**
3	237–244, 8 bp	4	GCTGGA**T**G**AATG**
4	588–644, 57 bp	4	ATTAT**AATATAGC**
5	1011–1060, 50 bp	4	GGGAA**TT**GA**TGC**
6	222–237, 16 bp	3	GGATG**TGAA**G**GG**
7	401–420, 20 bp	3	CAC**TTGAAGAAG**
8	336–345, 10 bp	2	TTGAA**ATTATAAA**
9	338–347, 10 bp	2	GAA**ATTATAAATT**
10	384–426, 43 bp	2	GAA**AAGG**GA**TAT**
11	425–435, 11 bp	2	TGAAG**AA**G**GTAT**
12	463–500, 38 bp	2	GTTAT**TATA**T**ATG**
13	616–630, 15 bp	2	TTTAGAT**AT**T**CAT**
14	968–999, 32 bp	2	TTAAATT**AGAAG**
15	87–220, 134 bp	1	ATT**ATGT**CAG**GG**
16	133–140, 8 bp	1	AATG**GAA**AG**ATC**
17	213–223, 11 bp	1	TGTA**GAAG**AG**GA**
18	225–304, 80 bp	1	TGTG**AA**G**G**G**AA**
19	230–237, 8 bp	1	AGGGGAAGC**TGG**
20	326–385, 60 bp	1	GTTTT**GGAT**T**TTG**
21	418–504, 87 bp	1	AG**ATAGAT**T**GAAG**
22	422–508, 87 bp	1	AG**ATAGAT**T**GAAG**AA**GG**
23	509–554, 46 bp	1	AT**GGAA**GG**AGG**
24	599–670, 72 bp	1	CGC**TTG**T**ATATA**
25	736–781, 46 bp	1	AAAG**GTC**A**G**T**AT**
26	809–821, 13 bp	1	GATG**TACTTT**T**AG**
27	821–900, 80 bp	1	GCTATT**A**C**TT**T**AA**
28	1038–1060, 23 bp	1	GAGG**TTTAATGC**
29	1136–1172, 37 bp	1	CGTT**GTATAAAT**
Total		76	

a. Tandem duplications detected in *recJ* and *recJ'* mutants.

b. Tandem duplication sequences are underlined. Short homologous sequences are in

List of identified *Bacillus anthracis* mutator genes and their functions

No.	ORF	Product
Genes previously identified to be mutagenic when disrupted:		
1	*mutS*	DNA mismatch repair protein MutS
2	*mutL*	DNA mismatch repair protein MutL
3	*mutY*	A/G-specific adenine glycosylase
4	*polI-mutM*	DNA polymerase I formamidopyrimidine-DNA glycosylase
Additional genes identified in this study:		
5	*yycJ*	metallo-beta-lactamase family protein
6	*BAS4289*	helicase, putative
7	*recJ*	single-stranded-DNA-specific exonuclease

Distribution of mutations leading to Rif^r in *B. anthracis*

B. anthracis site (bp)*	Amino acid change	Base-pair change	Spontaneous		*mutS*	*mutY*	*mutY* *mutM*	5AZ	MNNG
1361	Q454R	AT→GC	15	24*	19	0	0	0	0
1400	H467R	AT→GC	17	23*	20	0	0	0	2
1399	H467Y	GC→AT	20	23*	1	0	0	0	31
1349	S450F	GC→AT	0	0	0	0	0	0	0
1415	S472F	GC→AT	1	4*	0	0	0	0	8
1415	S472Y	GC→TA	1	1	0	10	0	0	0
1360	Q454K	GC→TA	1	2*	0	27	30	0	0
1361	Q454L	AT→TA	0	0	0	0	0	0	0
1375	T459S	AT→TA	0	0	0	0	0	0	0
1402	K468Q	AT→CG	0	0	0	0	0	0	0
1400	H467P	AT→CG	1	1	0	0	0	0	0
1349	S450C	GC→CG	0	0	0	0	0	0	0
1399	H467D	GC→CG	0	0	0	0	0	17	0
1558	S453C	GC→CG	0	0	0	0	0	0	0
Total 14			**56**	**78***	**40**	**37**	**30**	**17**	**41**

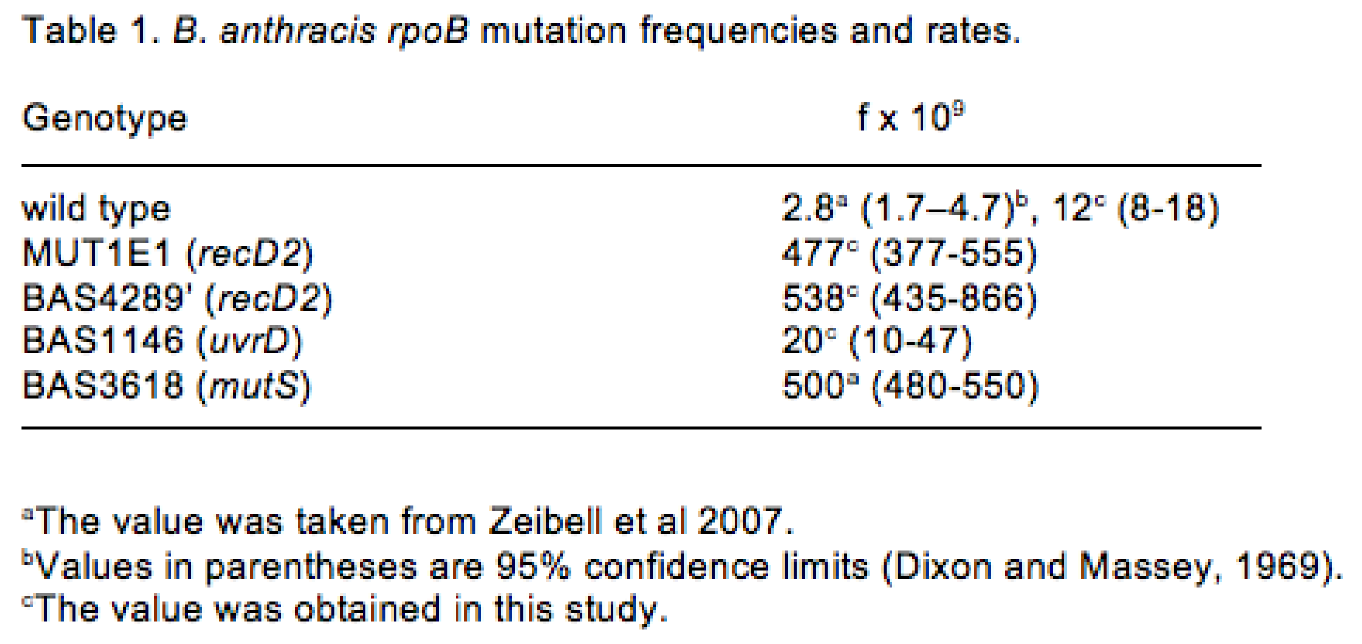


Table 1. *B. anthracis rpoB* mutation frequencies and rates.

Genotype	f x 10^9
wild type	2.8[a] (1.7–4.7)[b], 12[c] (8-18)
MUT1E1 (*recD2*)	477[c] (377-555)
BAS4289' (*recD2*)	538[c] (435-866)
BAS1146 (*uvrD*)	20[c] (10-47)
BAS3618 (*mutS*)	500[a] (480-550)

[a] The value was taken from Zeibell et al 2007.

[b] Values in parentheses are 95% confidence limits (Dixon and Massey, 1969).

[c] The value was obtained in this study.

Acknowledgement

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Madeline Yung
Cameron Sikavi

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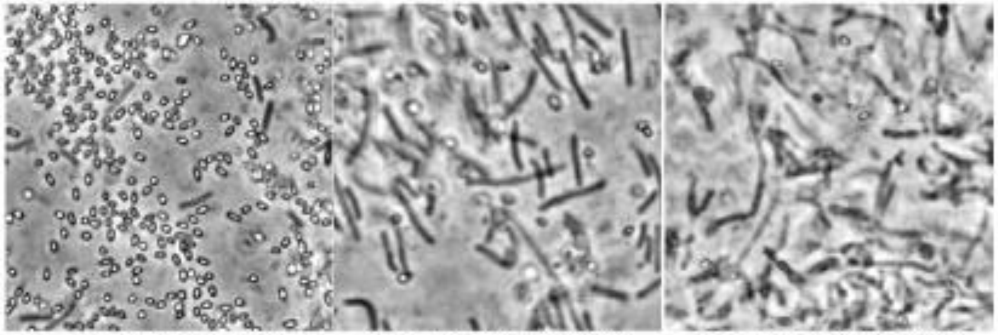
Katherine Kim
Bindu Patel
Vivian Shi
Andrea Chan
Hannah Hong
Jennifer Lee
Aileen Chang

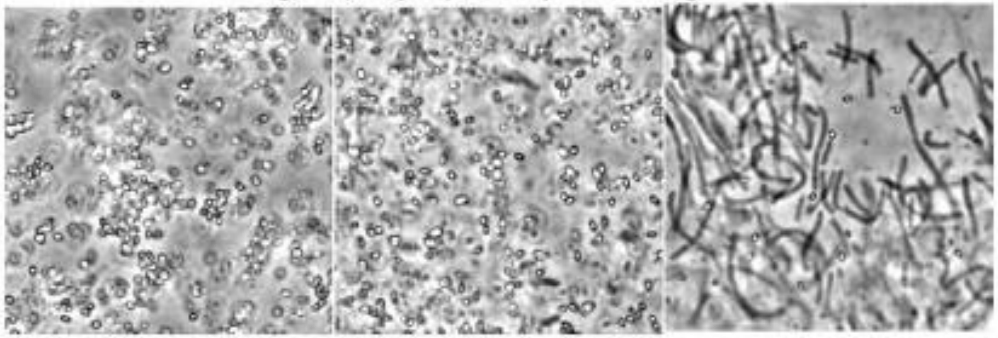
F

LB agar (Day 6), cell patch center



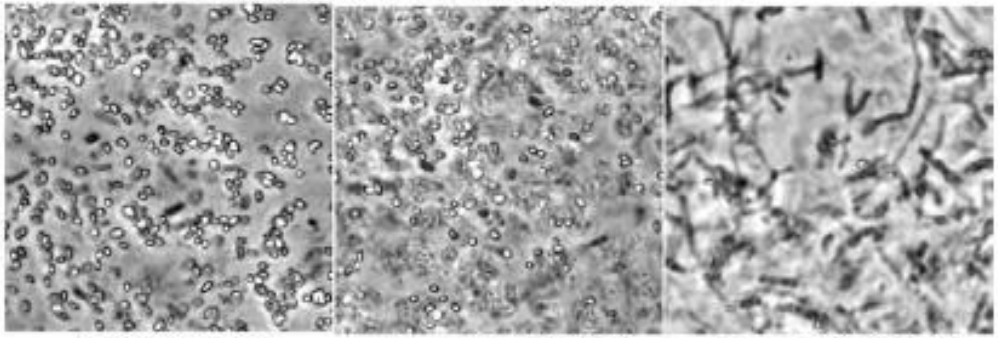
WT (T13E5) group A (T13C3) group B (T13G3)

LB agar (Day 6), cell patch edge



WT (T13E5) group A (T13C3) group B (T13G3)

SM agar (Day 6), cell patch center

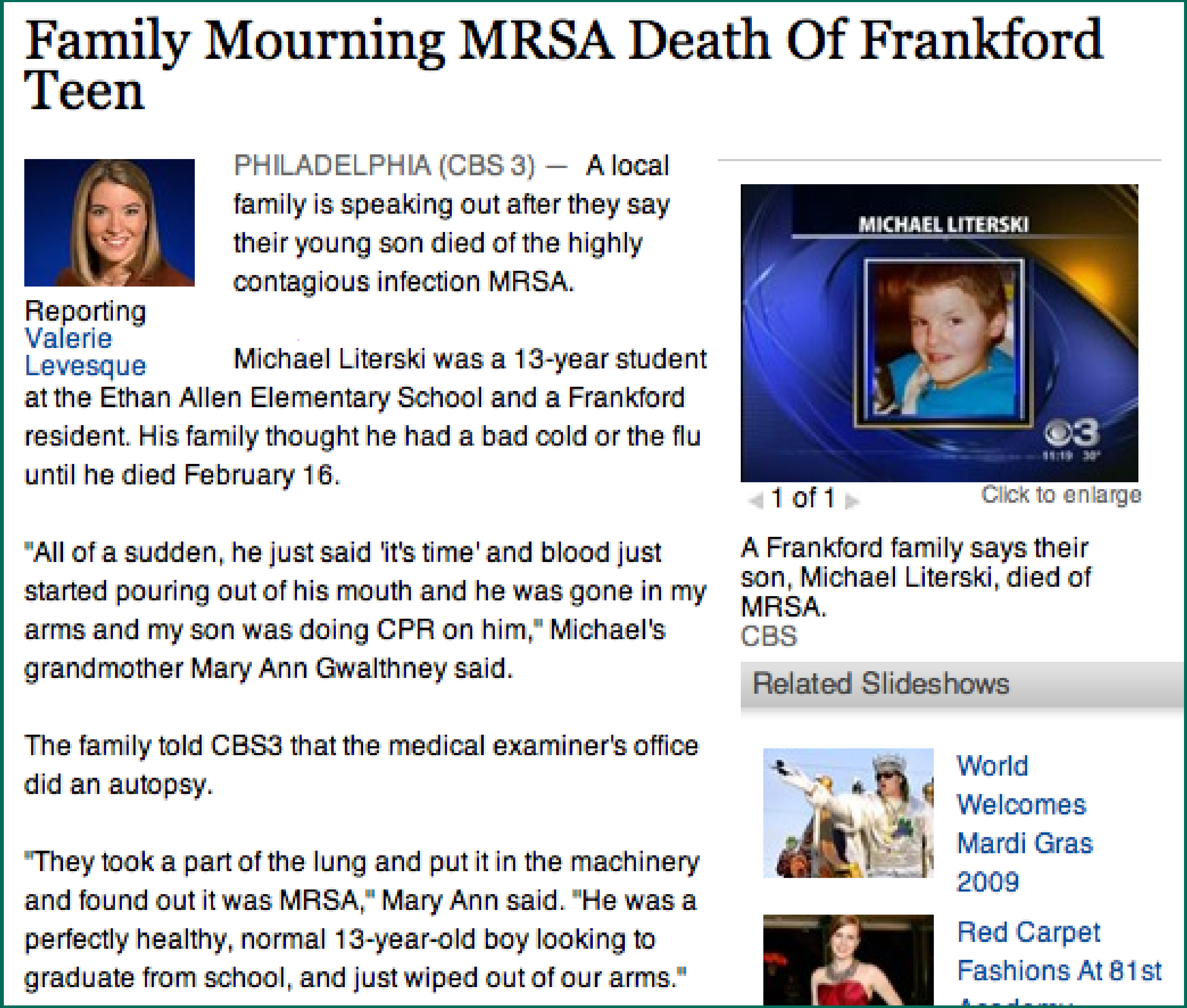


WT (T13E5) group A (T13C3) group B (T13G3)

Search for New Mutational Pathways

Brazilian amputee model dead at 20

- **STORY HIGHLIGHTS**
- **NEW:** Brazilian amputee model Mariana Bridi da Costa died early Saturday
- Da Costa's hands, feet were amputated after she contracted septicemia
- Da Costa placed sixth in the Miss Bikini International competition in China



Family Mourning MRSA Death Of Frankford Teen

Reporting
Valerie
Levesque

PHILADELPHIA (CBS 3) — A local family is speaking out after they say their young son died of the highly contagious infection MRSA.

Michael Literski was a 13-year student at the Ethan Allen Elementary School and a Frankford resident. His family thought he had a bad cold or the flu until he died February 16.

"All of a sudden, he just said 'it's time' and blood just started pouring out of his mouth and he was gone in my arms and my son was doing CPR on him," Michael's grandmother Mary Ann Gwalthney said.

The family told CBS3 that the medical examiner's office did an autopsy.

"They took a part of the lung and put it in the machinery and found out it was MRSA," Mary Ann said. "He was a perfectly healthy, normal 13-year-old boy looking to graduate from school, and just wiped out of our arms."

A Frankford family says their son, Michael Literski, died of MRSA.
CBS

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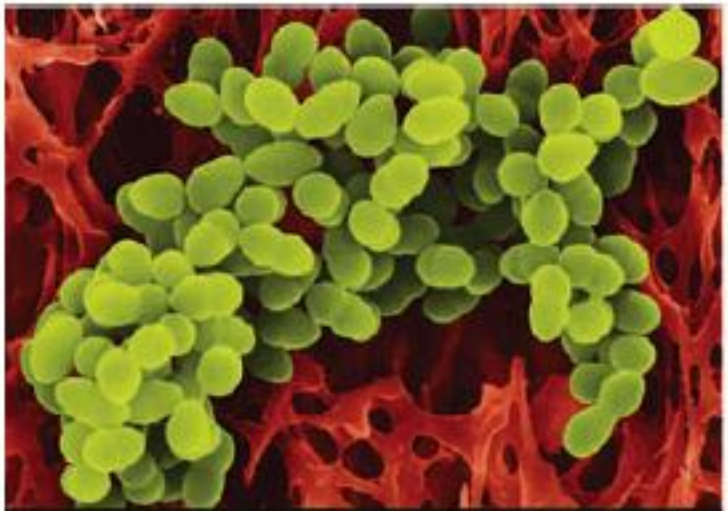
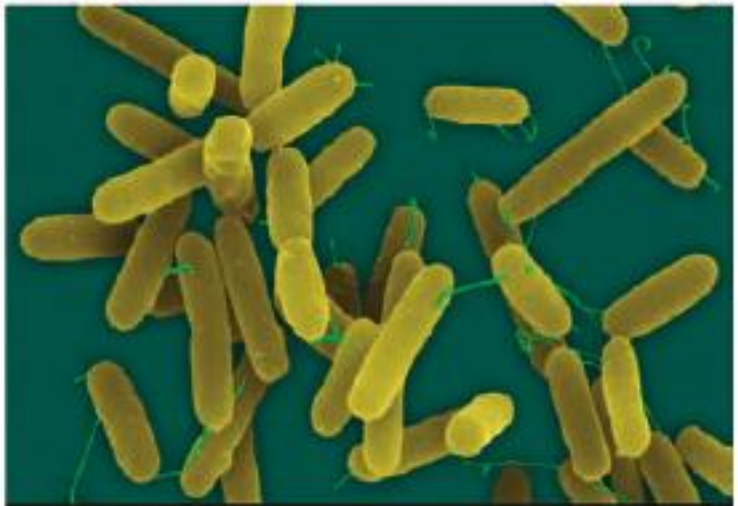


Staphylococcus aureus

Mycobacterium tuberculosis

Acinetobacter baumannii



Pseudomonas aeruginosa

Problem

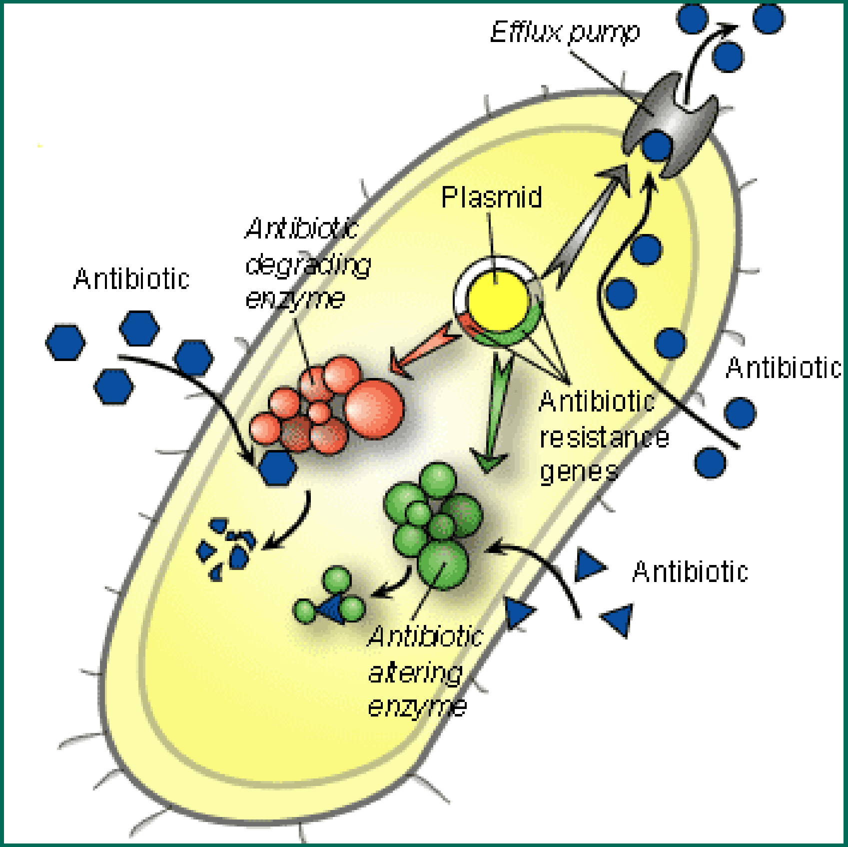
- We are losing the war on antibiotics
- Need to find new antibiotics/treatments
- Need to change behavior

Resistance Mechanisms

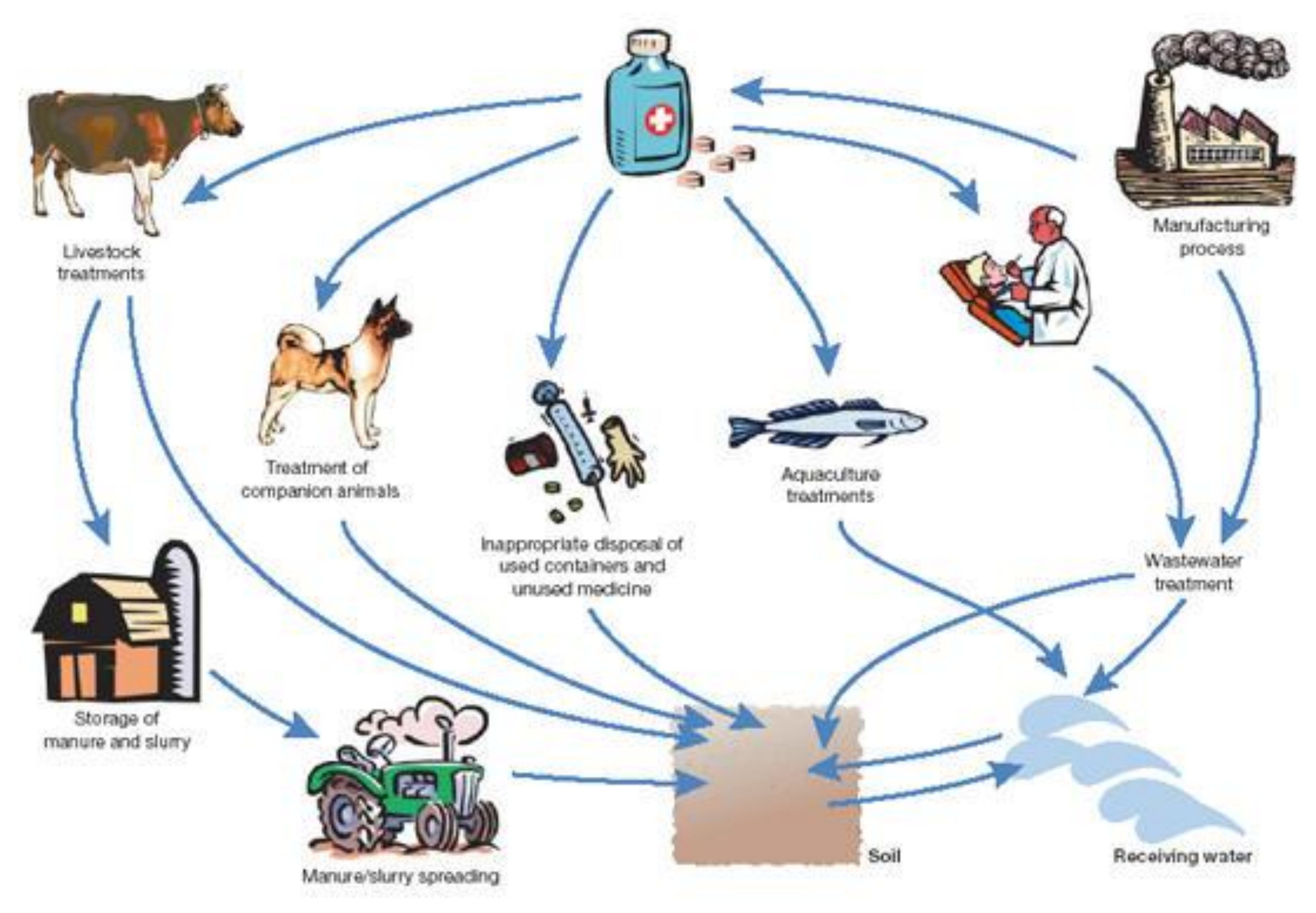
Altered receptors for the drug

Decreased entry into the cell

Destruction or inactivation of the drug

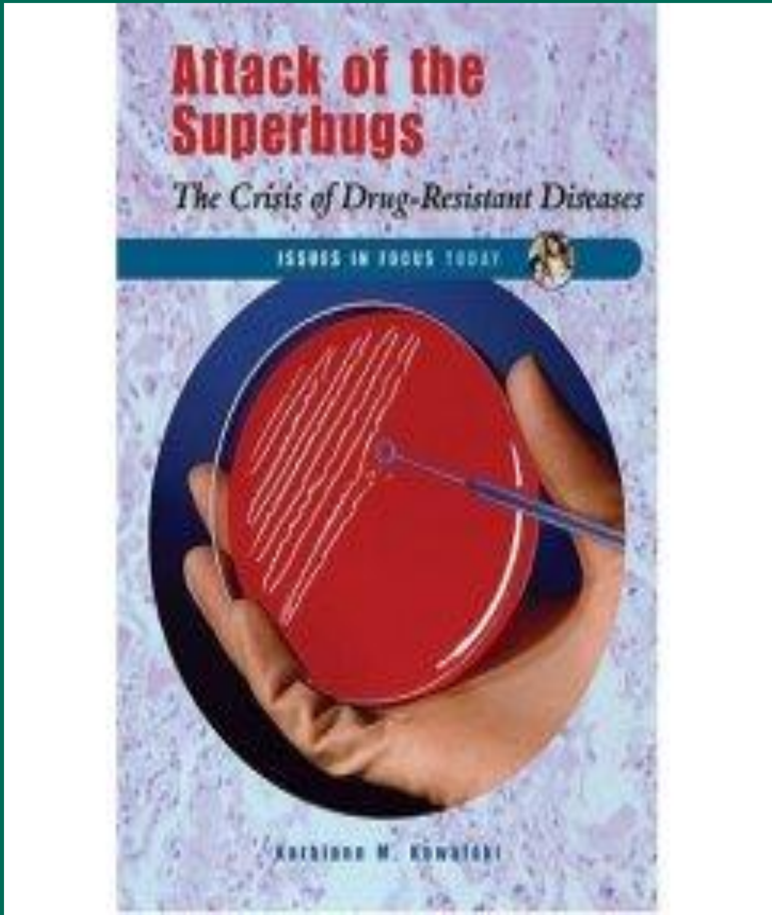


Route of Pharmaceuticals Entering Our Environment



Some Statistics

- more than 70 percent of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat them.
- Many infectious diseases are increasingly difficult to treat.
- Over 2 million fall prey to microbes once they get in the hospital, in this country alone. Some 90,000 die. About 70 percent of those are infected by drug-resistant bacteria. Costs for treatment of these infections approach $5 billion a year. Overall, the yearly toll exacted by drug-resistant infections in the United States is estimated to exceed $30 billion.



In the USA, more people die from MRSA than from AIDS

- In 2005
  - 20,000 people died from MRSA
  - 17,000 people died from AIDS

Statistics

- Farm animals in the United States receive 24.6 million pounds of antibiotics a year, which may be fueling the rise of drug-resistant bacteria, according to the Union of Concerned Scientists (UCS). UCS noted that about 70 percent of all antibiotics made in the United States are used to fatten up livestock.

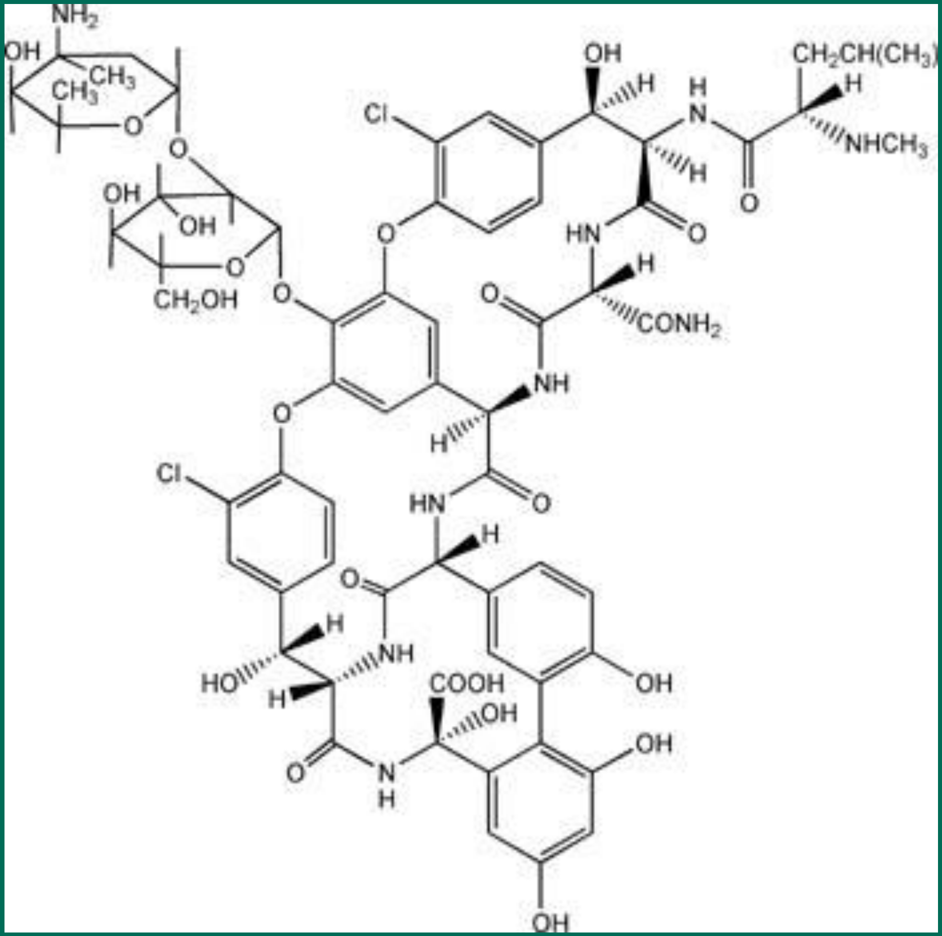
- Source: "Hogging It: Estimates of Antimicrobial Abuse in Livestock," by Margaret Mellon, Charles Benbrook, and Karen Lutz Benbrook, Union of Concerned Scientists, January 2001 (report available at www.ucsusa.org).

Approaches

- Find new antibiotics from natural sources
- Make chemical derivatives of existing antibiotics
- Find new targets in cell
- Find new combinations of existing drugs
- Find co-drug targets and co-drugs (potentiators)
- Exploit genomics – total knockout collections, metabolomic simulations

A successful example of a drug plus co-drug combination is the use of β-lactamase inhibitors together with β-lactams, such as penicillin

Vancomycin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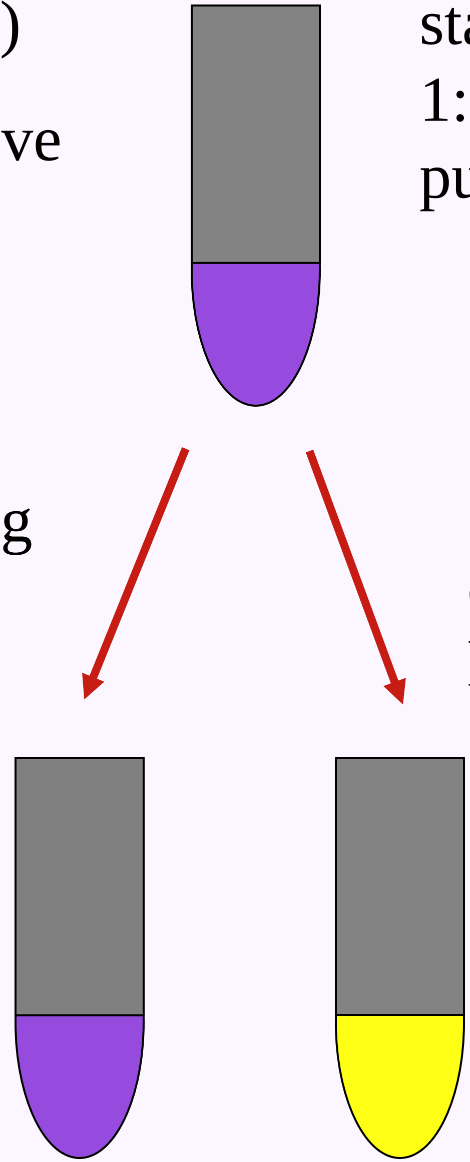
Strain A=WT (yellow)

Strain B=Drug sensitive mutant (purple)



starting mixture
1:20 Strain A: Strain B
purple

growth without drug

growth in presence of drug: strain B inhibited by low concentration of drug