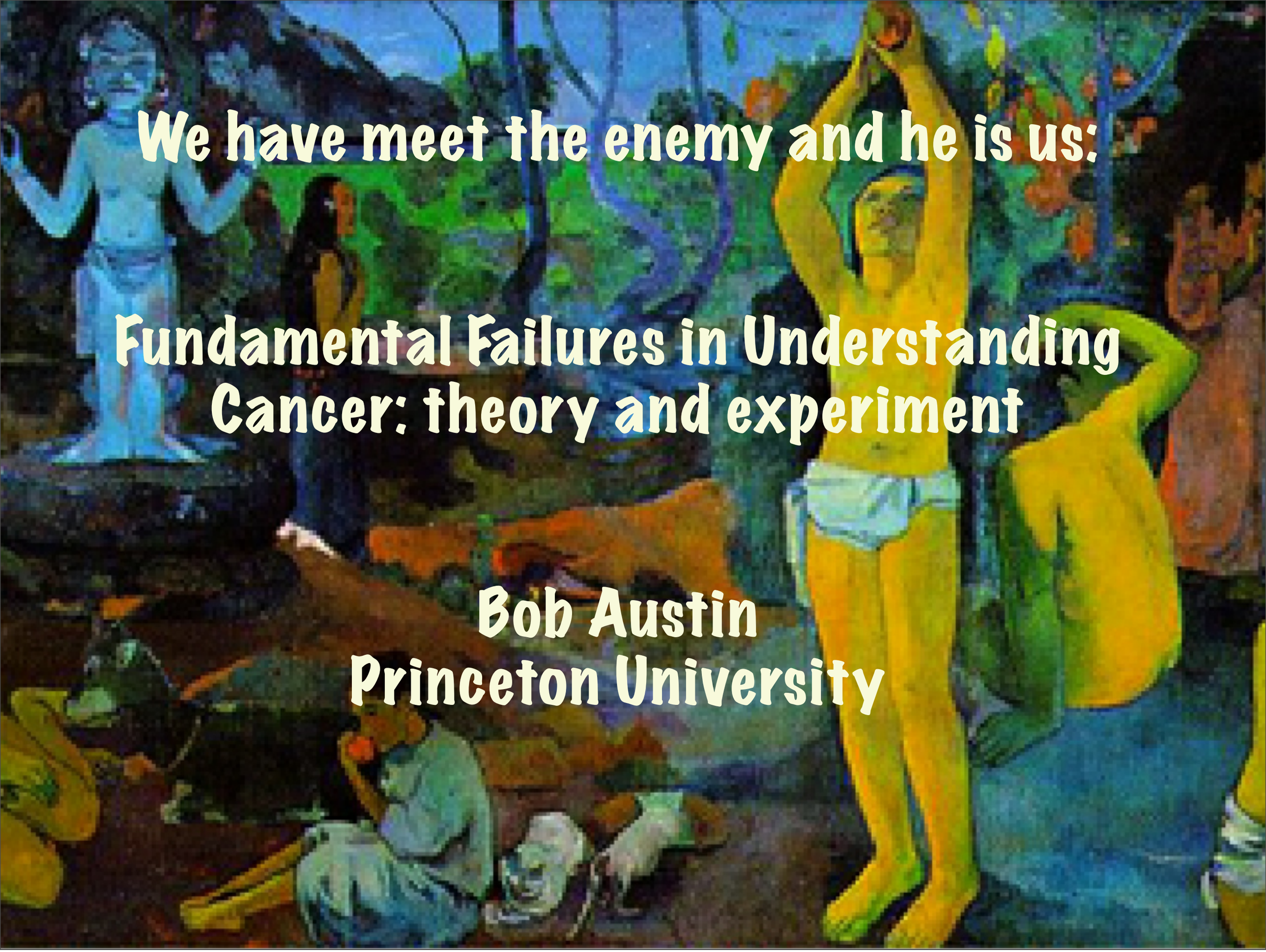
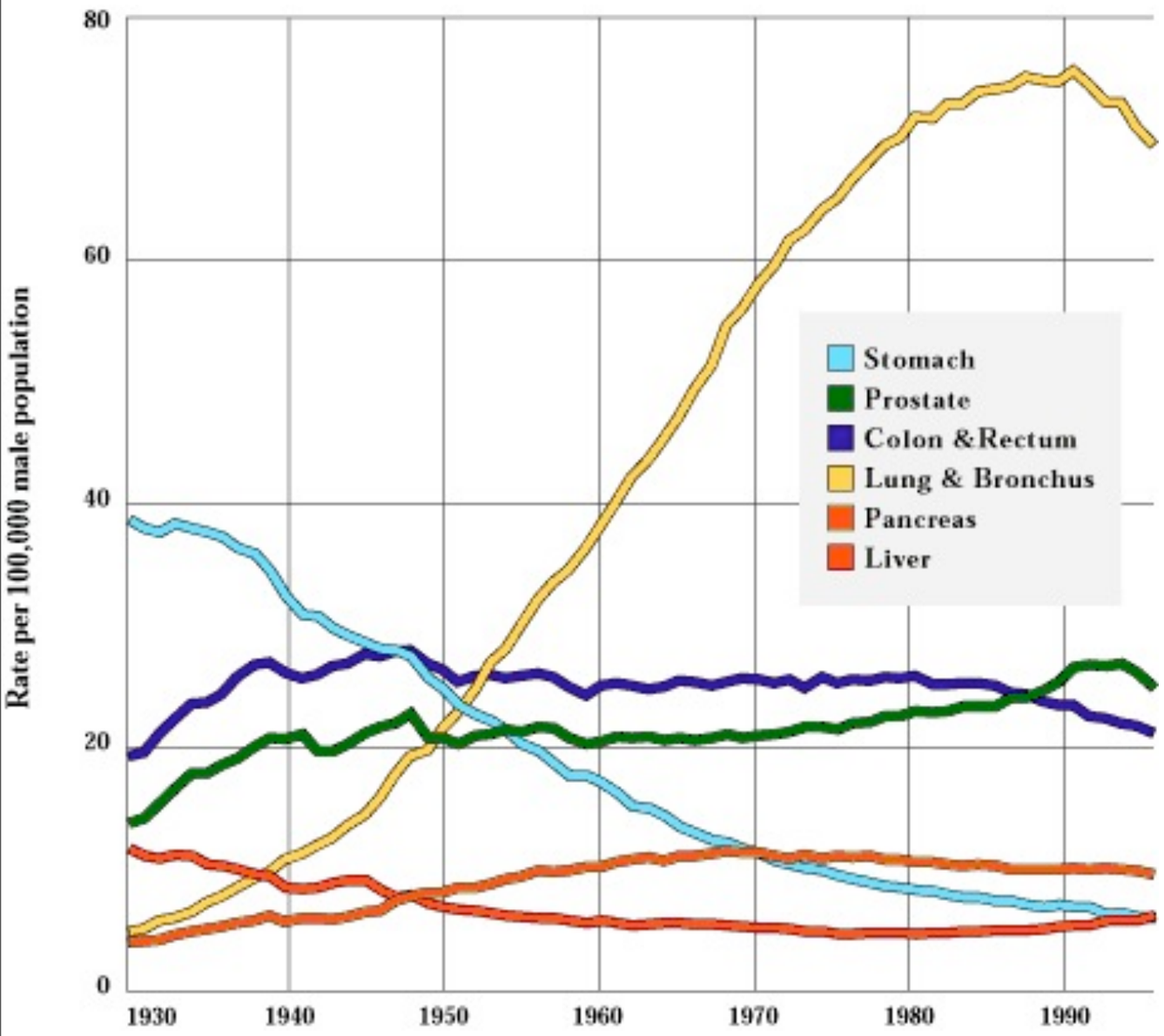
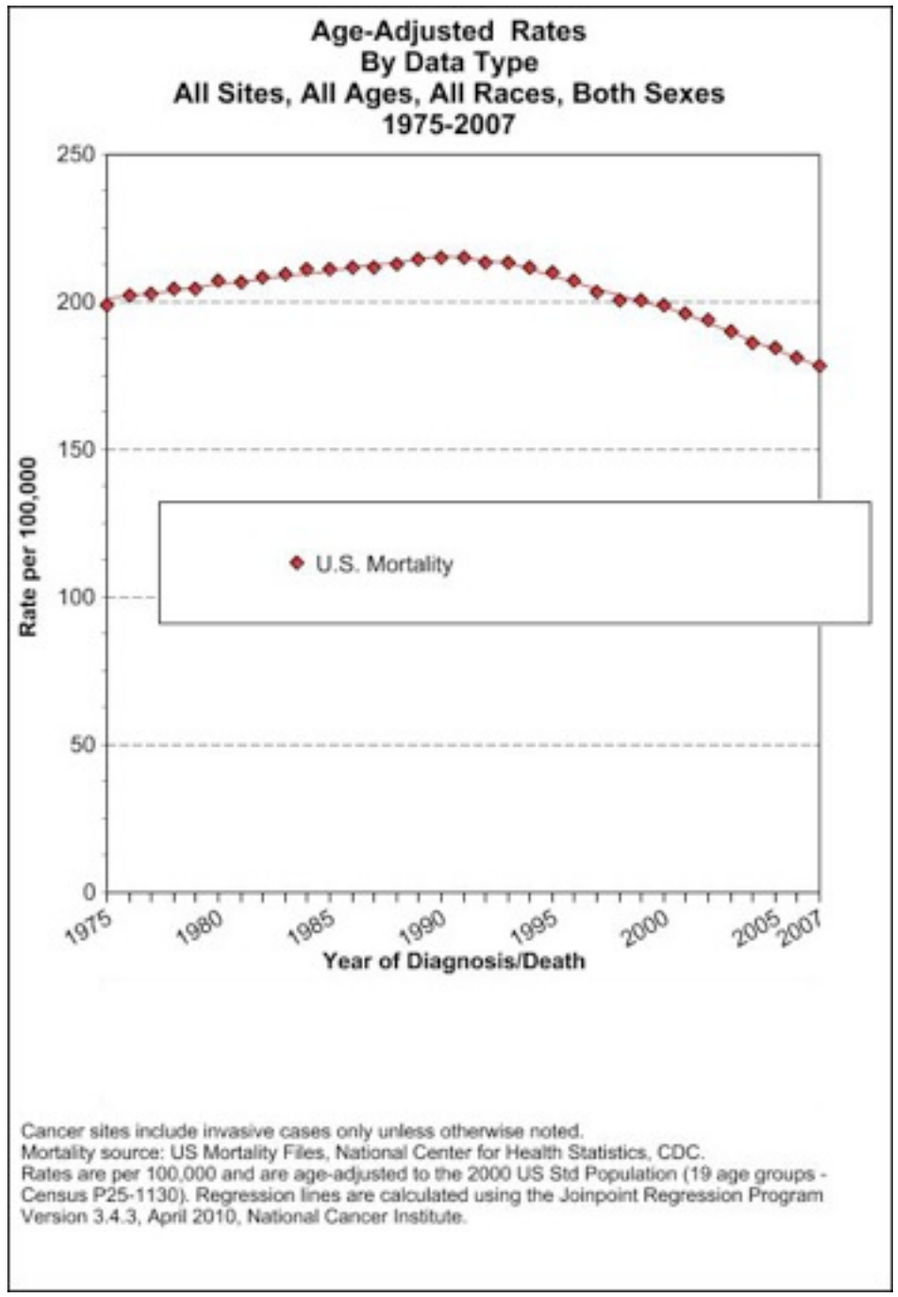

We have meet the enemy and he is us:
Fundamental Failures in Understanding Cancer: theory and experiment
Bob Austin
Princeton University

We seem to be not winning the "wars" against Cancer.





Except for a few remarkable exceptions, cancer mortality rates have been basically flat for 40 years. Nearly all the drops are due to prevention.

Economic Impact of Cancer

The National Institutes of Health (NIH) estimated the 2007 overall annual costs of cancer were as follows:

Total cost: $226.8 billion

Direct medical costs (total of all health expenditures): $103.8 billion

Indirect mortality costs (cost of lost productivity due to premature death): $123.0 billion

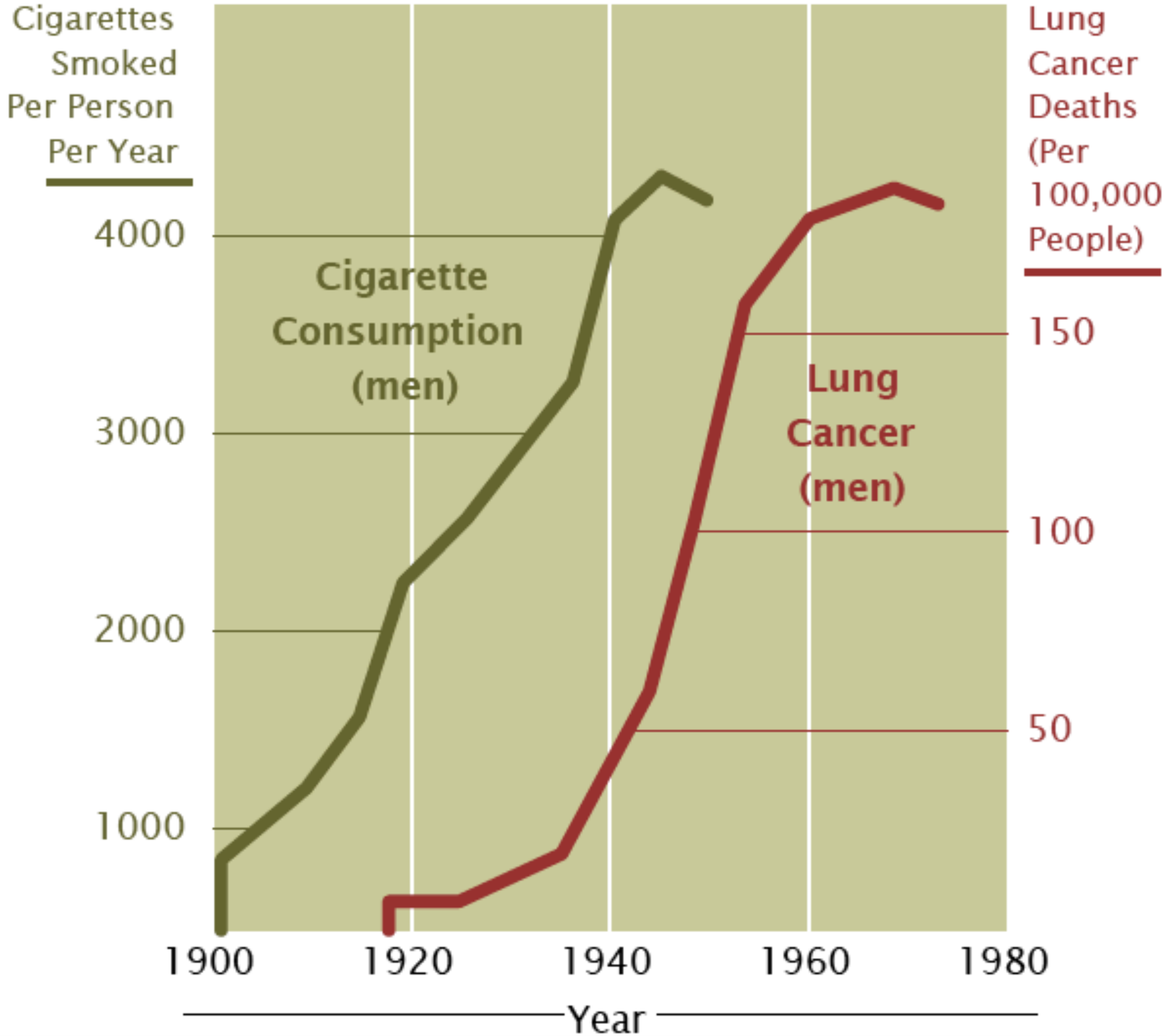
I. How to 1/2 the cancer rate and make 100 billion dollars/year with no physicists or biologists at all. Tomorrow.

Part (1/2?) of this chronic cancer condition is due to totally self-inflicted injuries:

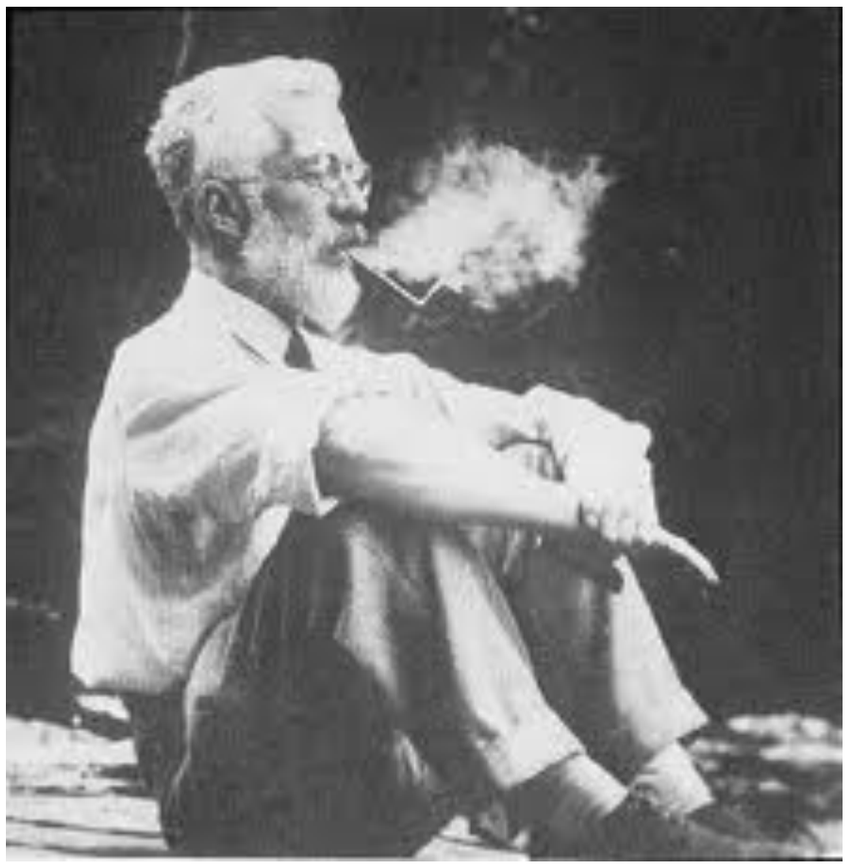
1) Obesity

NCI: "A projection of the future health and economic burden of obesity in 2030 estimated that continuation of existing trends in obesity will lead to about 500,000 additional cases of cancer in the United States by 2030.
This analysis also found that if every adult reduced their BMI by 1 percent, which would be equivalent to a weight loss of roughly 1 kg (or 2.2 lbs) for an adult of average weight, this would prevent the increase in the number of cancer cases and actually result in the avoidance of about 100,000 new cases of cancer."

2. Smoking. Duh. Unless you are R. Fisher.



Ronald A. Fisher: The Fundamental Equation of Evolution (1940)



Fisher was opposed to the conclusions of Richard Doll and A.B. Hill that smoking caused lung cancer.

"It has been suggested that the fact that Fisher was employed as consultant by the tobacco firms in this controversy casts doubt on the value of his arguments.

This is to misjudge the man. He was not above accepting financial reward for his labours, but the reason for his interest was undoubtedly his dislike and mistrust of puritanical tendencies of all kinds; and perhaps also the personal solace he had always found in tobacco."

SAN FRANCISCO | Thu Jun 7, 2012 1:00am IST

(Reuters) - California voters narrowly rejected a ballot measure that would have added a $1 tax to a pack of cigarettes in the state's primary election.

R.J. Reynolds and its affiliates spent $14.1 million on advertising, while Philip Morris USA and its affiliates spent $31 million.

By comparison, supporters of Proposition 29 spent $18 million, including contributions highlighted by acclaimed cyclist and cancer survivor Lance Armstrong and Michael Bloomberg, mayor of New York City.

But don't blame Big Tobacco, they are simply doing what any large and profitable organization does to protect their paychecks. You can count on it.

The California cancer research establishment did exactly the same thing: they were going to put the $1billion dollar windfall into "more research", i.e. increase their paychecks.

"More research" isn't working: stopping smoking works. Put the money into something useful, like deficit reduction.

On Jul 10, 2012, at 7:49 PM, Robert Austin wrote:
When are you going stop smoking, it's stupid.

stupid is goed!

u sound like a grandpa dude.

Juan E. Keymer (纪皇)

Assistant Professor
Department of BionanoScience
Faculty of Applied Science
Delft University of Technology
The Netherlands
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KeymerLab.TUDelft.nl

_____

3. "Something Else" Now the madness begins.

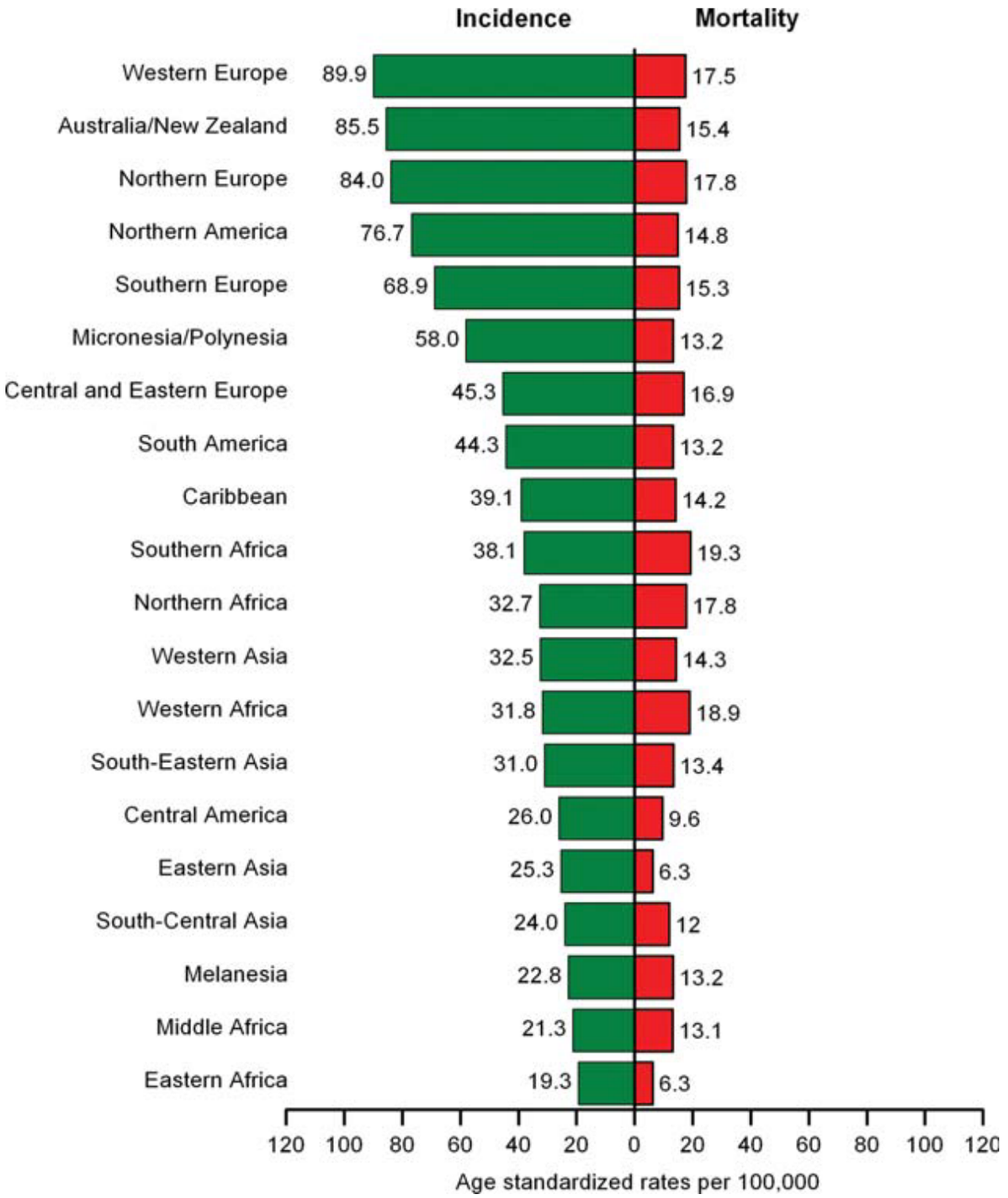


FIGURE 4. Age-Standardized Breast Cancer Incidence and Mortality Rates by World Area. Source: GLOBOCAN 2008.

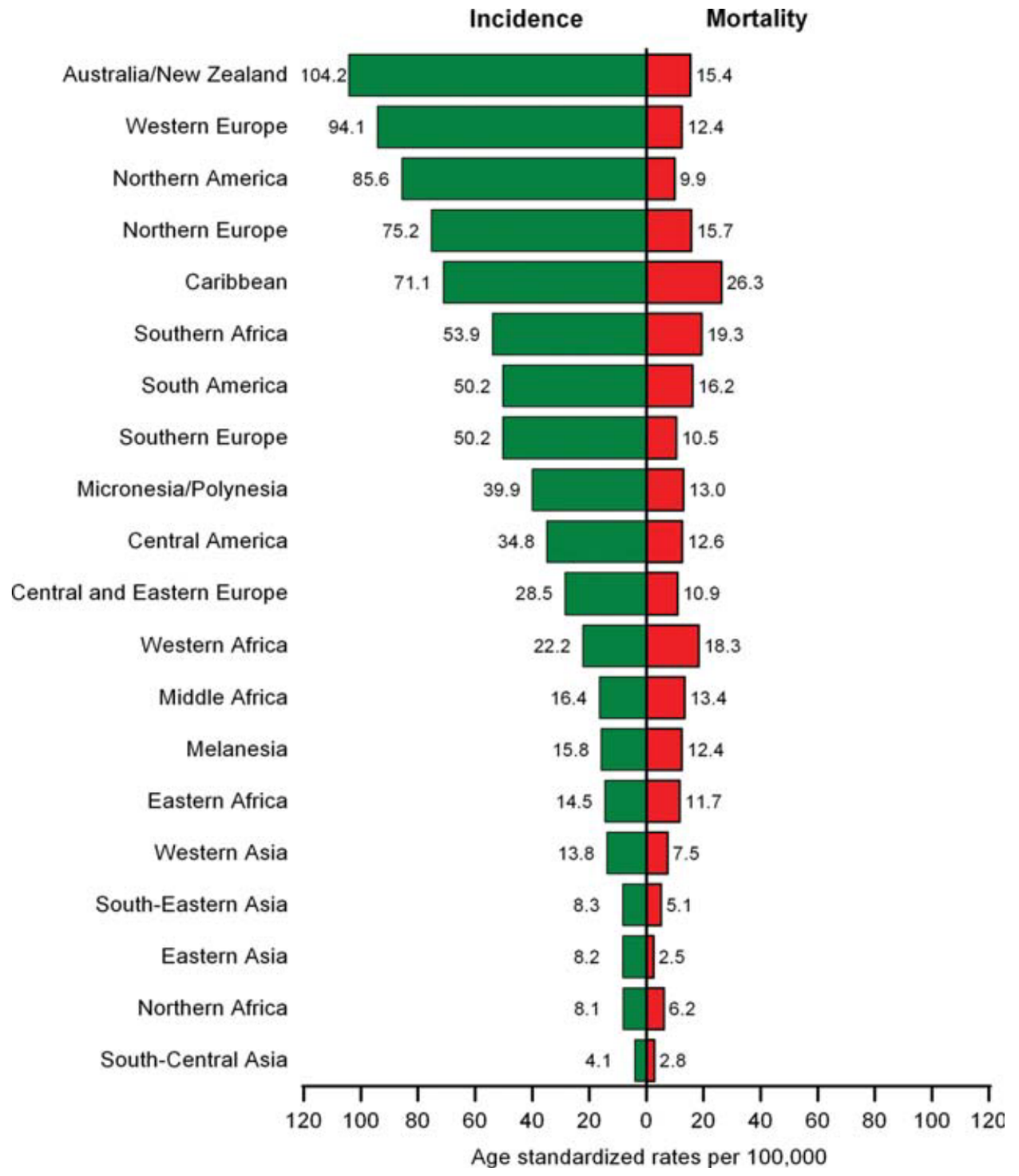


FIGURE 7. Age-Standardized Prostate Cancer Incidence and Mortality Rates by World Area. Source: GLOBOCAN 2008.

So, clearly we could lower cancer rates by at least 50% starting tomorrow, but it isn't going to happen, and never will.

A deeper question than human stupidity and greed: WHY does cancer even happen?

When you cut yourself, you don't bleed to death, your body fixes the wound.

Why doesn't the body fix the "wound that never heals?"

II. The three drivers of cancer progression due to chemotherapy.

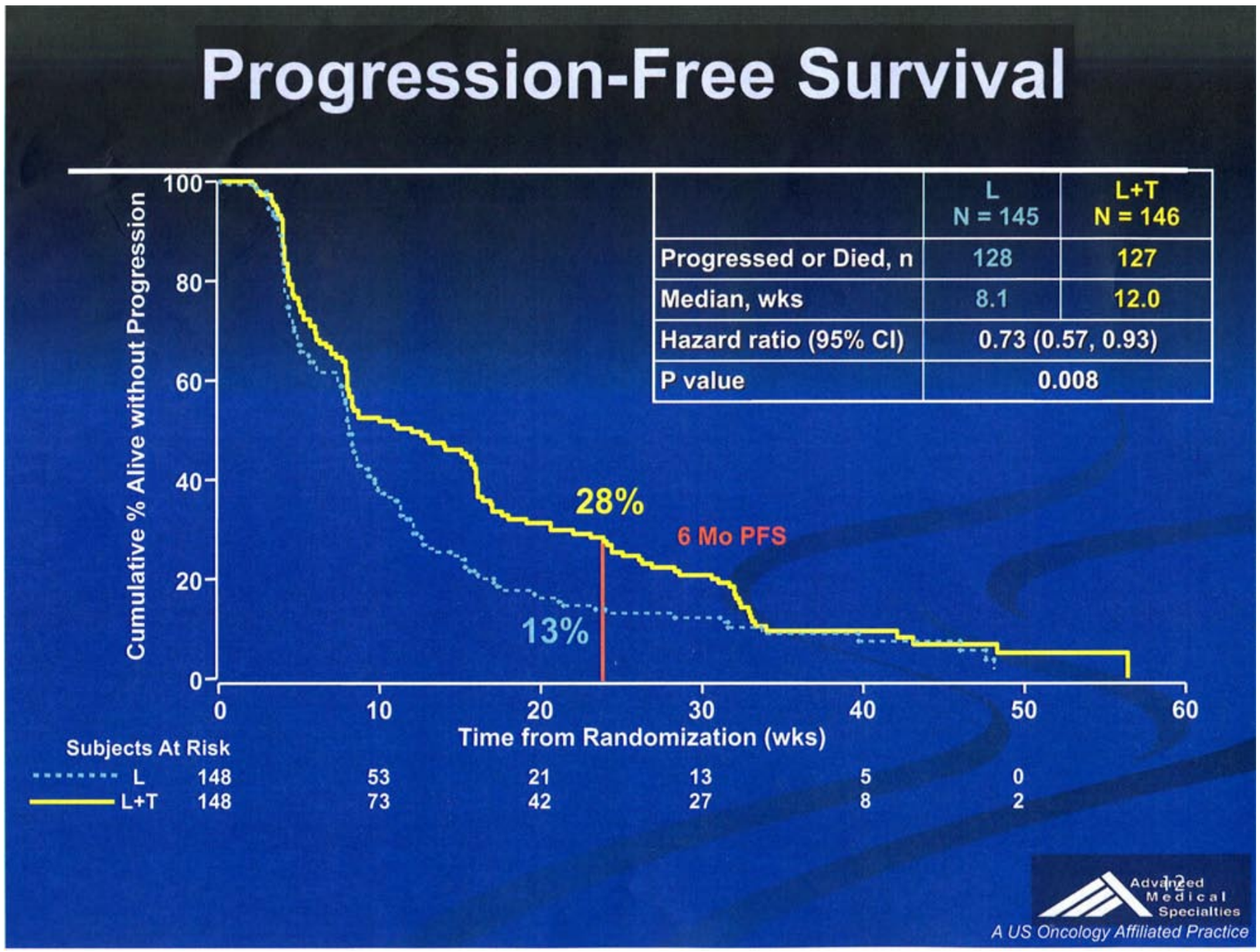
1. Genomic heterogeneity (intrinsic).

2. Mutation rate enhancement under stress (chemo).

2. Breaking the population down into a metapopulation of weakly connected small populations (surgery).

The result is a foregone conclusion: Progression, and I think metastasis.

Progression



(Kaplan-Meier curve)

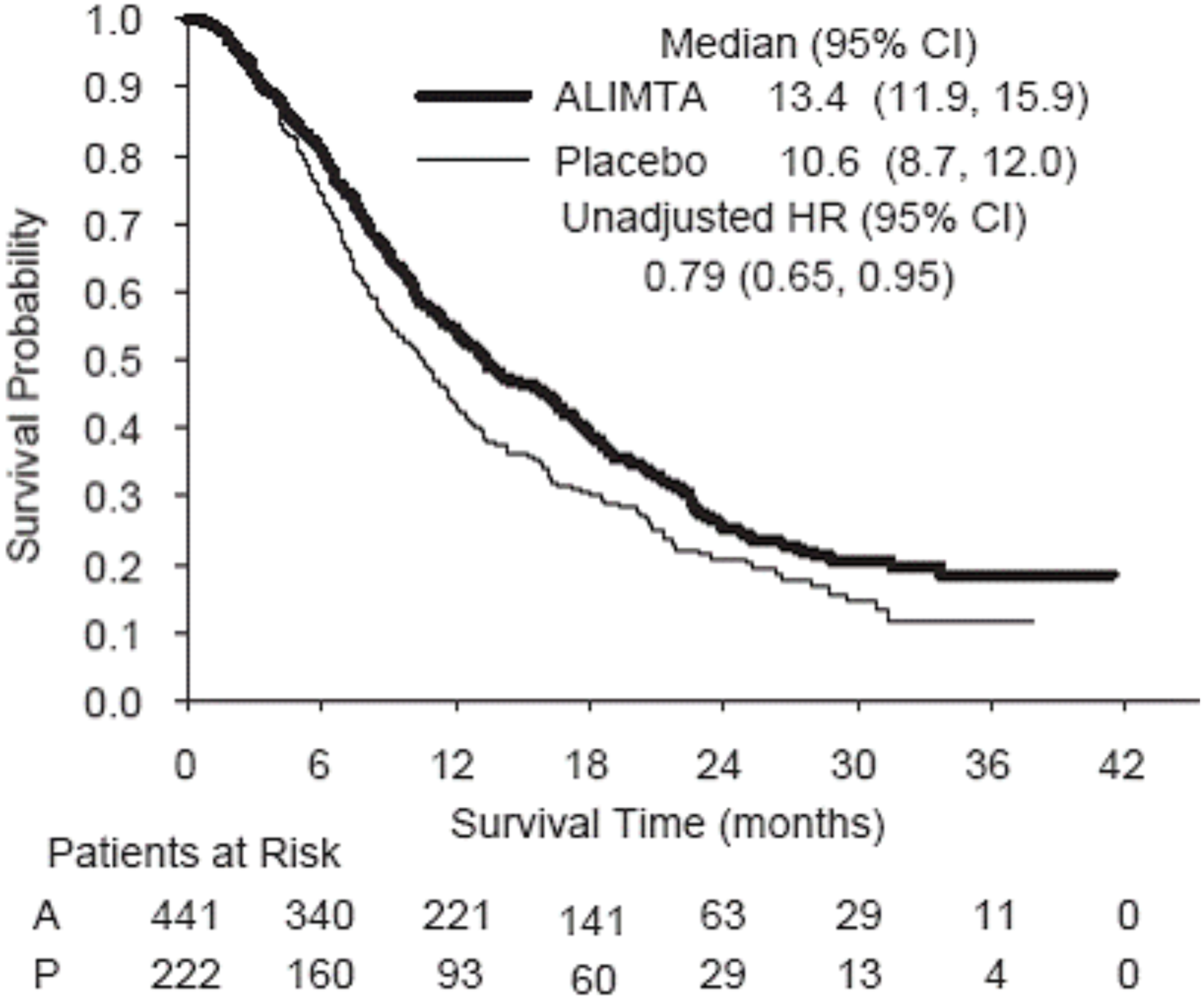
This is about what any oncologist deals with: RAPID EVOLUTION!

Interior of New Frick Chemistry Building at Princeton.

$350 million building primarily paid for from Prof. Ted Taylor's chemotherapy drug Alimata. It is a billion dollar a year seller.

Princeton sells the alumni on what a miracle it is, and how great an example of research.

NOT!



Until we understand the evolution of drug resistance, I wouldn't put much stock in the evening news when they talk (every week) about the latest cancer breakthrough. But I would buy Big Pharma stock.

My narrow view of neoclassical (Fisher) evolution modeling:

1) Successful mutations are random: $\Delta N = suN$

2) Mutation rates (u) are low: rate (u) of about $1/10^9$ mutations/basepair/generation.

$$u << 1$$

3) Most mutations are deleterious (reduce fitness). Selection coefficient very small:

$$s << 1$$

4) Evolution best studied in large numbers in big buckets, because of the low mutation rates and small selection coefficients. And it is really slow.

I think that is fundamentally wrong.

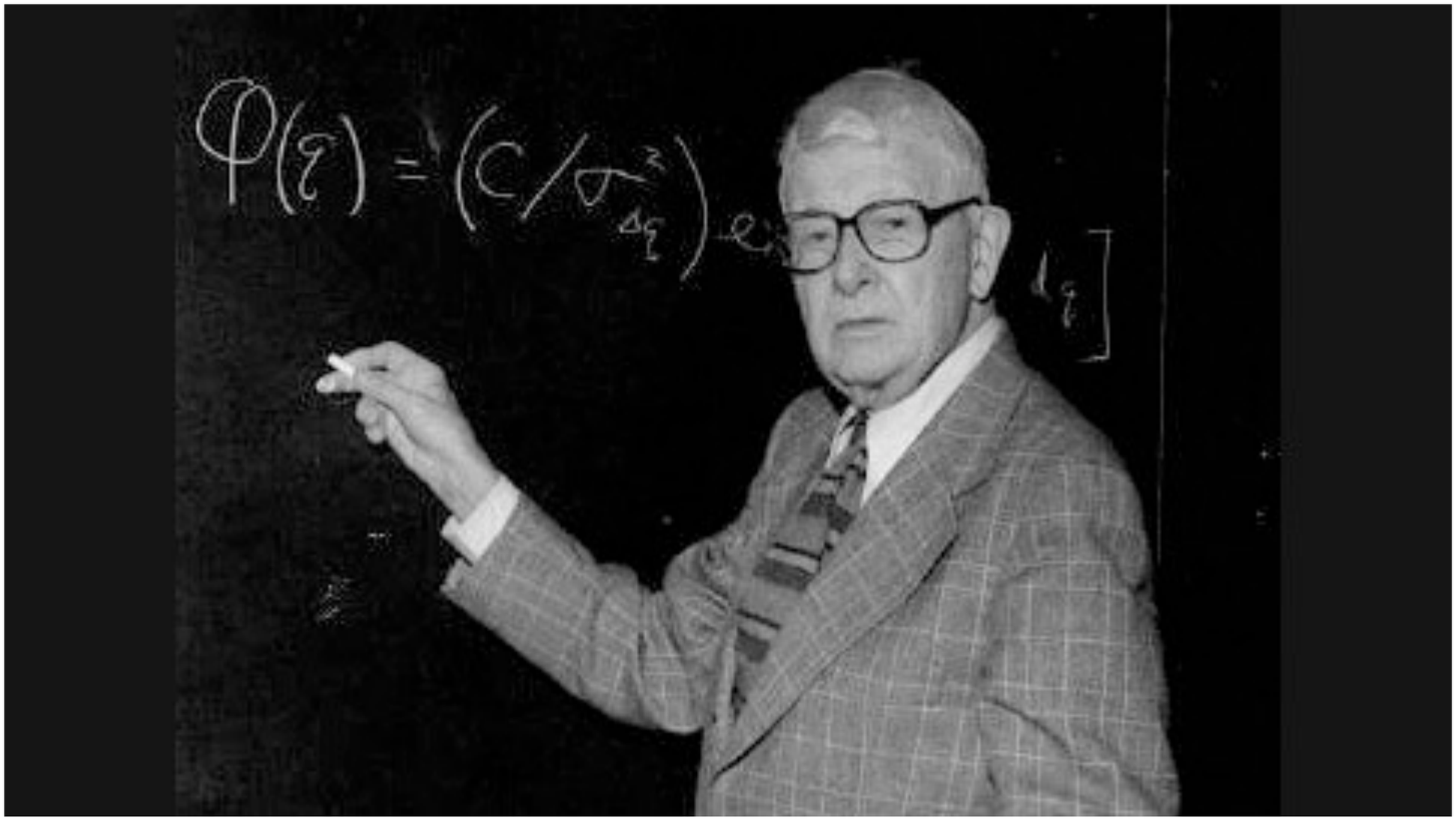
Newton: The fundamental equation of classical physics

$$\vec{F} = m\frac{d\vec{v}}{dt}$$

Fisher's fundamental equation of evolution:

$$\frac{d < F >}{dt} = \sigma^2 - \mu\Delta_\mu$$

Fisher's colleague, Sewall Wright, with whom he feuded famously (and was wrong), had a deeper way to view the dynamics of evolution.



Fisher's big mistake, which he never accepted (of course), was Wright's realization that not only is the VARIANCE in the genome necessary for evolution (if everybody is the same there is no evolution), but also the NUMBER of individuals N is important.

If you have a slight fitness advantage but have to compete with a large number of inferior individuals, you can't compete and you will go extinct.

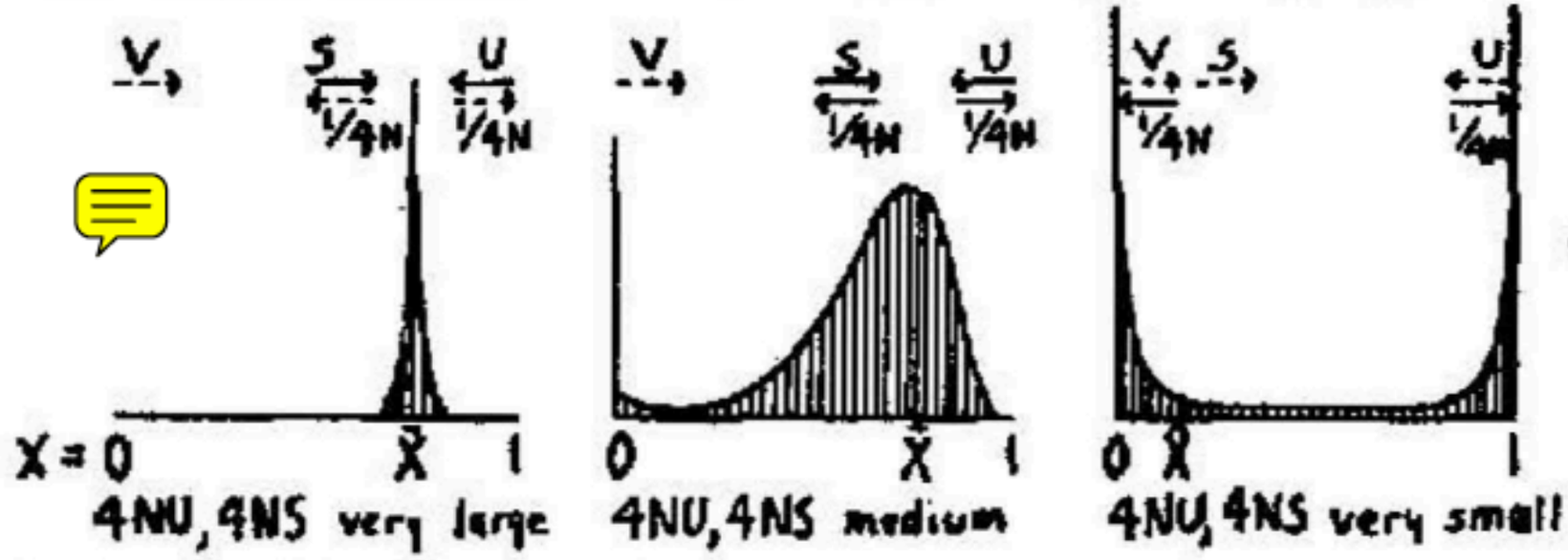
Boris: If you are awake, go back to sleep.

DISTRIBUTION OF GENE FREQUENCIES

SYMBOLS

A. Whole Species

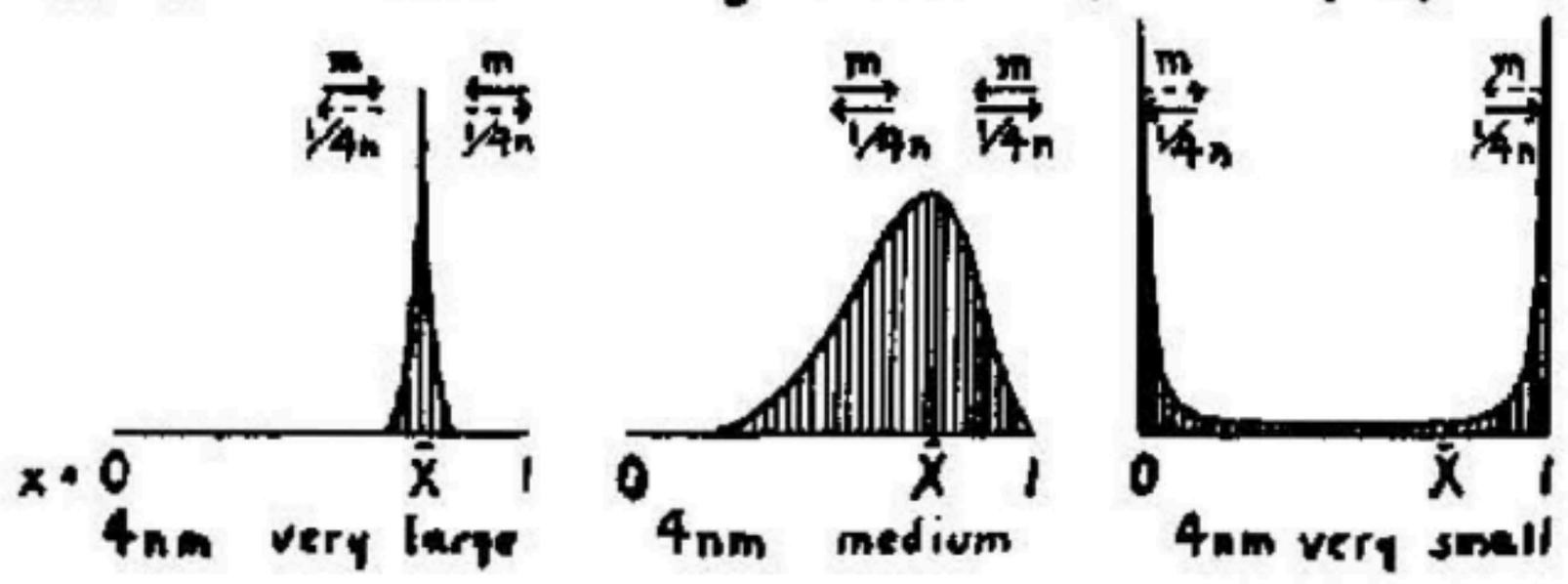
$$Y = Ce^{4NSX} X^{4NV-1} (1-X)^{4NU-1}$$



X gene frequency
Y probability
C coefficient
N population number
S selection coefficient
V,U mutation rates to and from gene, respectively, per generation

B. Local Race

$$y = Ce^{4nsx} x^{4nm\bar{X}-1} (1-x)^{4nm(1-\bar{X})-1}$$



x gene frequency
y probability
C coefficient
n population number
s selection coefficient
m population exchange with rest of species

The Origins of Antibiotic Resistance

Qiucen Zhang

A Dissertation

Presented to the Faculty

of Princeton University

in Candidacy for the Degree

of Doctor of Philosophy

To get an analytical expression of fixation time as a function of population size and fitness advantage, we need to solve the Kolmogorov Backward Equation [33]:

$$\frac{\partial \psi(q,t|p,0)}{\partial t} = V(p)\frac{\partial}{\partial p}\psi(q,t|p,0) + \frac{1}{2}D(p)\frac{\partial^2}{\partial p^2}\psi(q,t|p,0) \tag{2.2}$$

where $\psi(q,t|p,0)$ is the conditional probability to have frequency q in time t if the frequency is p at time 0. $V(p) = sp(1-p)$ and $D(p) = p(1-p)/N$. Considering the equilibrium state that $\partial\psi/\partial t = 0$, it's easy to solve eq.2.2 to get the probability of fixation $u(p)_{eq} = \lim_{t\to\infty} \psi(1,t;p,0)$:

[33] B. Drossel. Biological evolution and statistical physics. Advances in Physics, 50(2):209{295, 2001.

$$u(p)_{eq} = \frac{\int_0^p e^{-2\int_0^q D(x)/V(x)dx} dq}{\int_0^1 e^{-2\int_0^q D(x)/V(x)dx} dq} = \frac{1 - e^{-2N(0)sp}}{1 - e^{-2N(0)s}} \tag{2.3}$$

If the initial frequency is $1/N$, $u(p)_{eq}$ is:

$$u(p)_{eq} = \frac{1 - e^{-2s}}{1 - e^{-2Ns}} \tag{2.4}$$

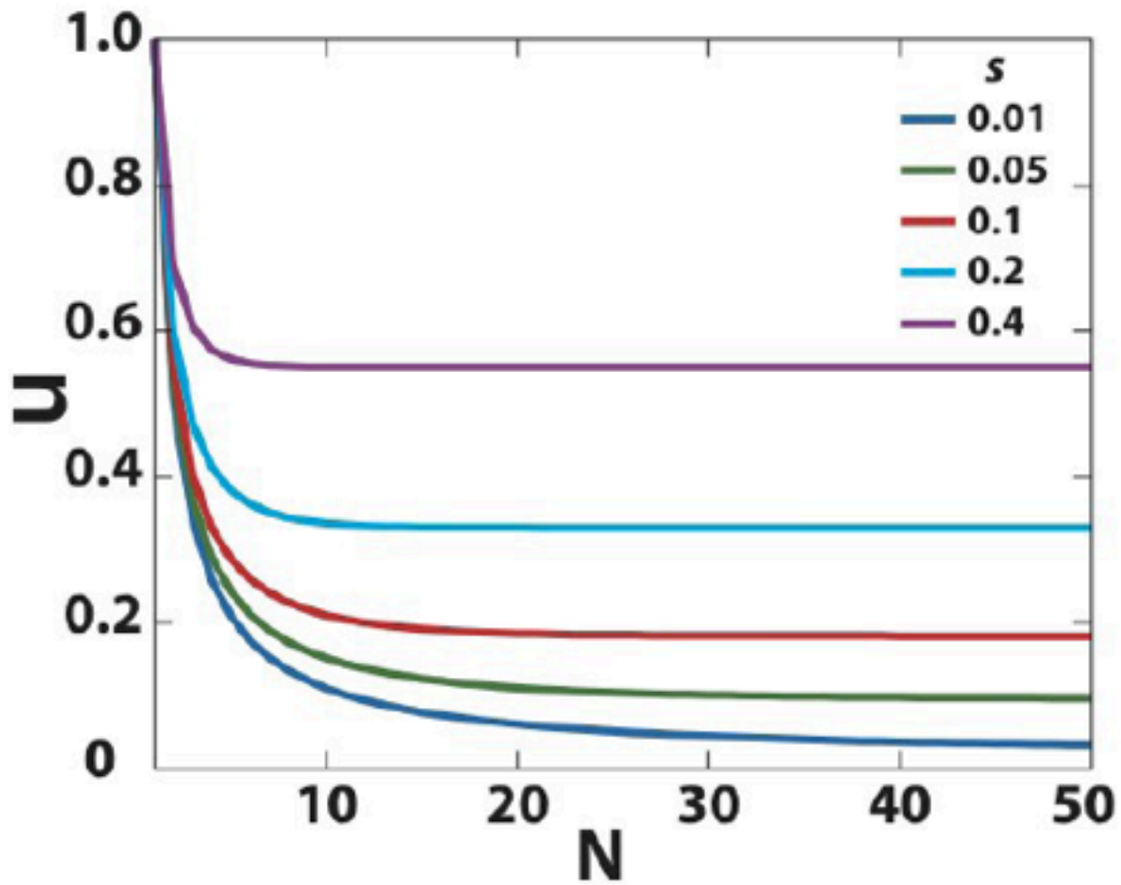


Figure 2.2: Probability of fixation at equilibrium as a function of population size N at different fitness advantage value s (how many extra children per cell per generation), assuming the initial frequency of the allele is $1/N$.

Does this mean that small N simply widens the heterogeneity?

Yes of course, and that is an important point, this drives evolution forwards.

But I think that small N also gives increasing weight to the less fitness increase mutations, which I expect would be the more frequent ones.

$$\frac{\partial P(s,N)}{\partial N} \sim \frac{e^{-s}}{(1-e^{-sN})^2} \sim \frac{e^{-s}}{sN} \sim \frac{1}{sN}$$

The TIME to fix also scales with N

The mean fixation time for a given initial frequency p would be [3]:

$$\bar{t}(p) = \frac{\int_0^\infty t(\partial u(p,t)/\partial t)dt}{\int_0^\infty (\partial u(p,t)/\partial t)dt}$$

For weak selection s, this is reduced to:

$$\bar{t}(p = 1/N) \simeq \frac{lnN}{s}$$

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$$\bar{t}(p) = \frac{\int_0^\infty t(\partial u(p,t)/\partial t)dt}{\int_0^\infty (\partial u(p,t)/\partial t)dt}$$

For weak selection s, this is reduced to:

$$\bar{t}(p = 1/N) \simeq \frac{lnN}{s}$$

Boris! I worship you!

Here is an example of numbers:

E. coli has about 4.5×10^6 basepairs in its genome. "It has been sequenced and annotated": annotated means we know the genes.

Suppose we wanted to evolve in E. coli resistance an antibiotic which blocks a gene needed for replication.

Suppose a specific single-nucleotide polymorphism (SNP) (i.e., A to T) is sufficient to block the antibiotic from binding (extremely unlikely, in fact wrong).

If the normal error rate u is 10^{-9} bp generation, only 1 bacterium in 1000 has a mutation anywhere in its genome with each generation.

A single bacterium reproducing under high stress so that the population does not change will need 10^9 generations to escape. Hopeless.

Or, of course if you had 10^9 individuals in each generation, even without growth, one "Einstein" would have the magic mutation and take off in exponential growth. Or would s/he?

This "Einstein" has to compete with 1 billion morons for food and space.

1) The probability of fixation decreases with increasing population size N for fixed s:

$$p_f \sim \frac{2s}{1 - \exp(-4Ns)}$$

2) The time to fix scales as 2N (big).

3) The time to lose scales as ln(N) (small).

On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient

Rutger Hermsen[1], J. Barrett Deris, and Terence Hwa[1]

Center for Theoretical Biological Physics and Department of Physics, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0374

Edited by* Nigel Goldenfeld, University of Illinois at Urbana-Champaign, Urbana, IL, and approved May 11, 2012 (received for review October 27, 2011)

The rapid emergence of bacterial strains resistant to multiple antibiotics is posing a growing public health risk. The mechanisms underlying the rapid evolution of drug resistance are, however, poorly understood. The heterogeneity of the environments in which bacteria encounter antibiotic drugs could play an important role. E.g., in the highly compartmentalized human body, drug levels can vary substantially between different organs and tissues. It has been proposed that this could facilitate the selection of resistant mutants, and recent experiments support this. To study the role of spatial heterogeneity in the evolution of drug resistance, we present a quantitative model describing an environment subdivided into relatively isolated compartments with various antibiotic concentrations, in which bacteria evolve under the stochastic processes of proliferation, migration, mutation and death. Analytical and numerical results demonstrate that concentration gradients can foster a mode of adaptation that is impossible in uniform environments. It allows resistant mutants to evade competition and circumvent the slow process of fixation by invading compartments with higher drug concentrations, where less resistant strains cannot subsist. The speed of this process increases sharply with the sensitivity of the growth rate to the antibiotic concentration, which we argue to be generic. Comparable adaptation rates in uniform environments would require a high selection coefficient ($s > 0.1$) for each forward mutation. Similar processes can occur if the heterogeneity is more complex than just a linear gradient. The model may also be applicable to other adaptive processes involving environmental heterogeneity and range expansion.

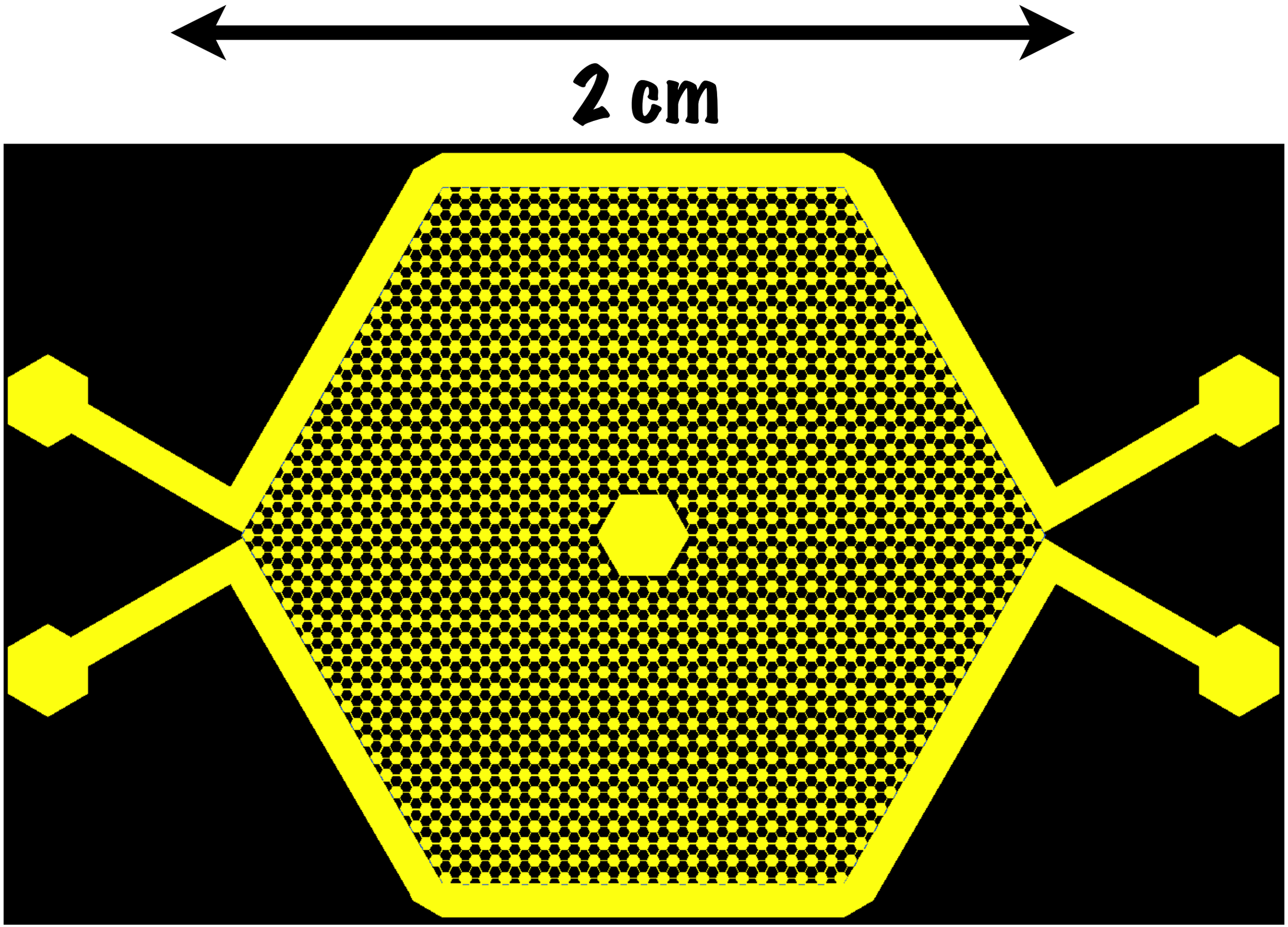
first passage processes | stochastic modeling | evolutionary ecology

who have a spectrum of immune responses. Antibiotic resistance therefore naturally evolves in heterogeneous environments.

In itself, the idea that environmental heterogeneity could promote the evolution of drug resistance is not new. Over a decade ago, it was proposed that heterogeneity could assist the evolution of drug resistance of HIV (9). Models suggested that, in homogeneous environments, the drug concentration has to be in a narrow range near the minimal inhibitory concentration (MIC) of the virus (called the *selective window*) for an effective selection of resistance: if the concentration is too high, both the wild type and feasible mutants are inhibited, whereas if it is too low, the wild type may out-compete the mutant (9). However, if the environment consists of two compartments, in one of which the drug does not penetrate well (a *sanctuary* or *reservoir*), the selective window is greatly enlarged. Samples from postmortem tissues of AIDS patients indeed suggest that compartmentalization in the central nervous system plays a role in the evolution of drug-resistant HIV strains (10).

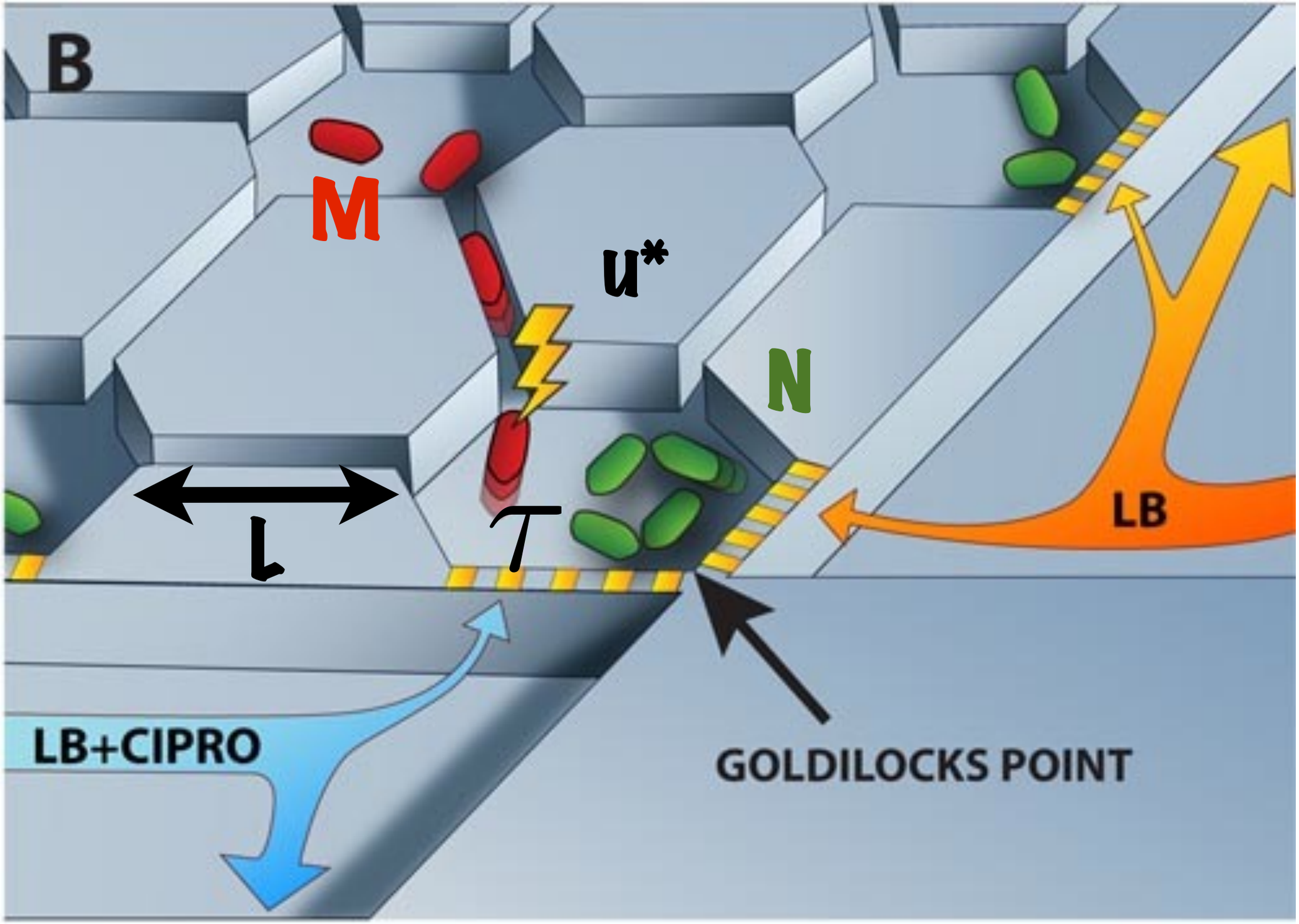
A similar effect could favor the evolution of antibiotic resistance in bacteria (8, 11, 12). Often, several mutations are required for a bacterium to obtain a medically relevant resistance level (13). In a homogeneous drug concentration, a single bacterium has to rapidly acquire these mutations to survive the treatment. If more than 2 specific mutations are required, this is unlikely (see *SI Text*). Heterogeneous environments, however, could provide sanctuaries, allowing these mutations to be selected one by one. Such ideas have led to the concept of "resistance-selective environments" as environments that favor the evolution of antibiotic resistance (11, 12).

Basic experiment: accelerate evolution without large flasks and years of time, using high stress gradients, mutagenic stress, wek coupling between small N populations.

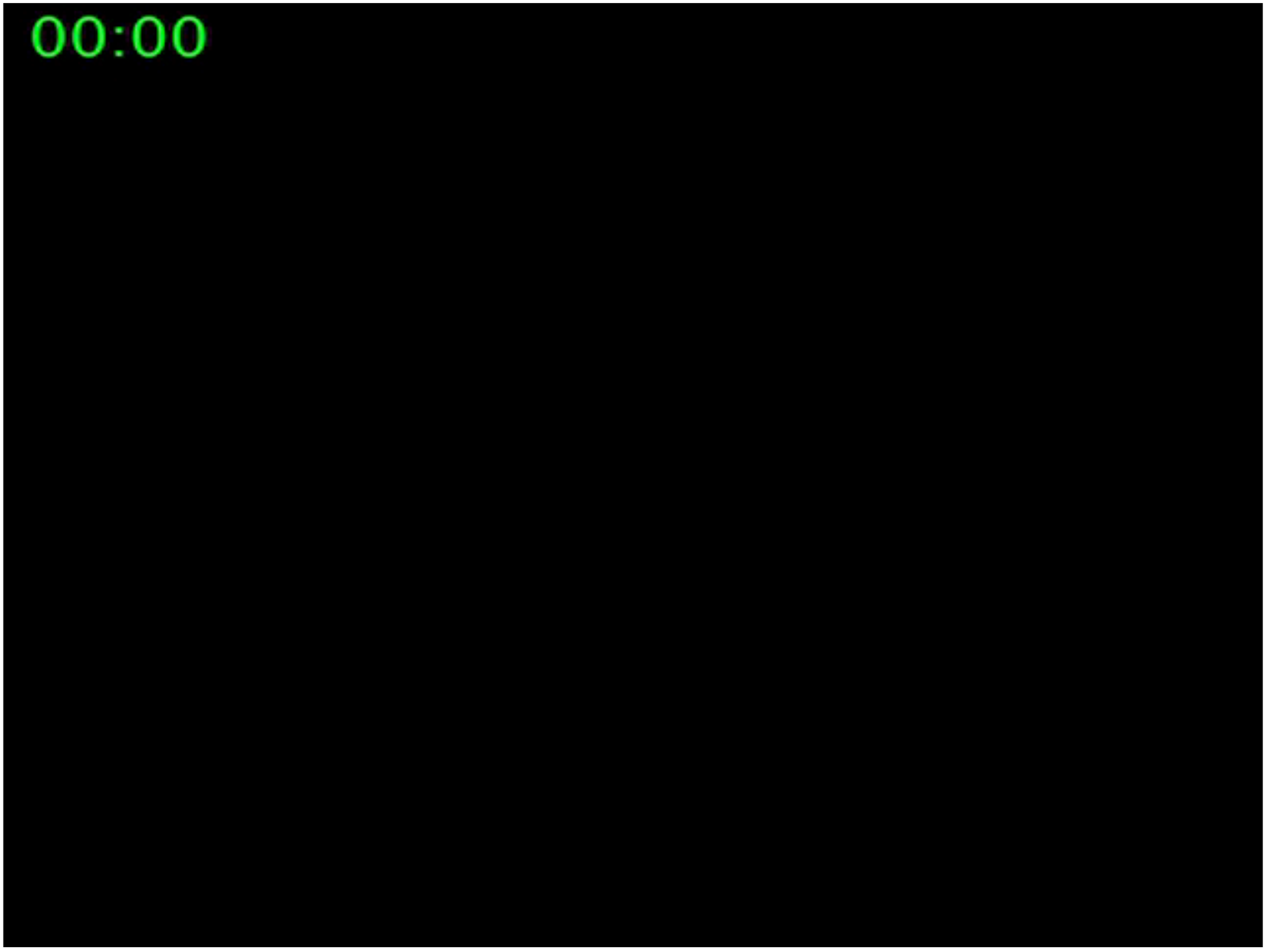


My attempt to realize Wright's Fitness Landscape

Goldilocks Points: Being at the Right Time at the Right Place

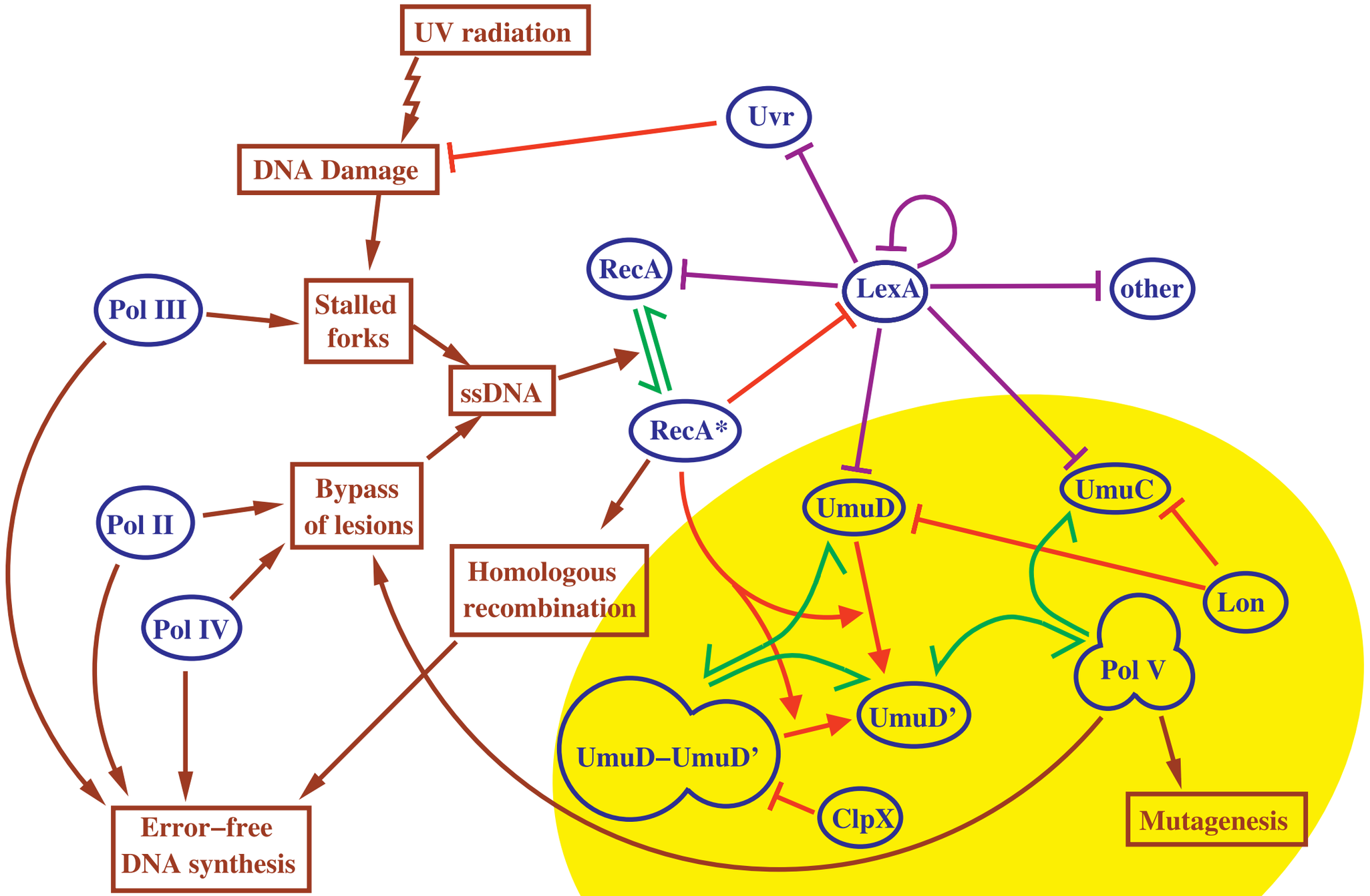


Stressor: mutagenic Cipro (x10^{4}) over generic rate.



x200 normal dose around bottom perimeter

Mutagenesis in Escherichia coli SOS Response: A Quantitative Model



Krishna et al. PloS Comp Bio 3(3) 2007

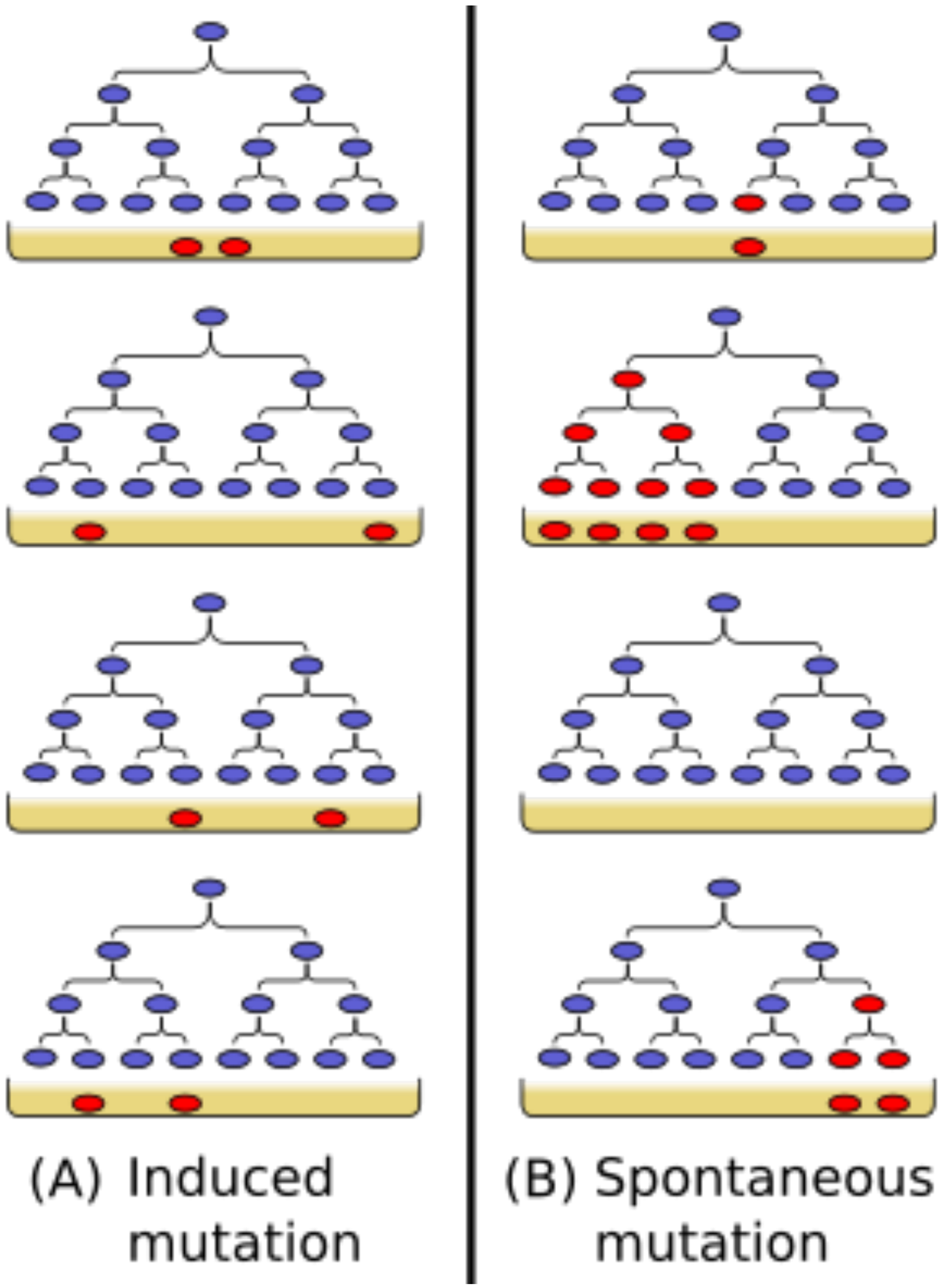
1. We can get rapid (10 hours) emergence of resistance to very high levels of Cipro (x20 MIC).

2. You need the Death Galaxy topology: simple "test tubes" don't do it.

3. Combination of spatial stress gradients AND organismal motility necessary.

4. It is de novo, not pre-existing mutations. I just can't kill the Delbruck beast in the Hotel California, but I keep trying.

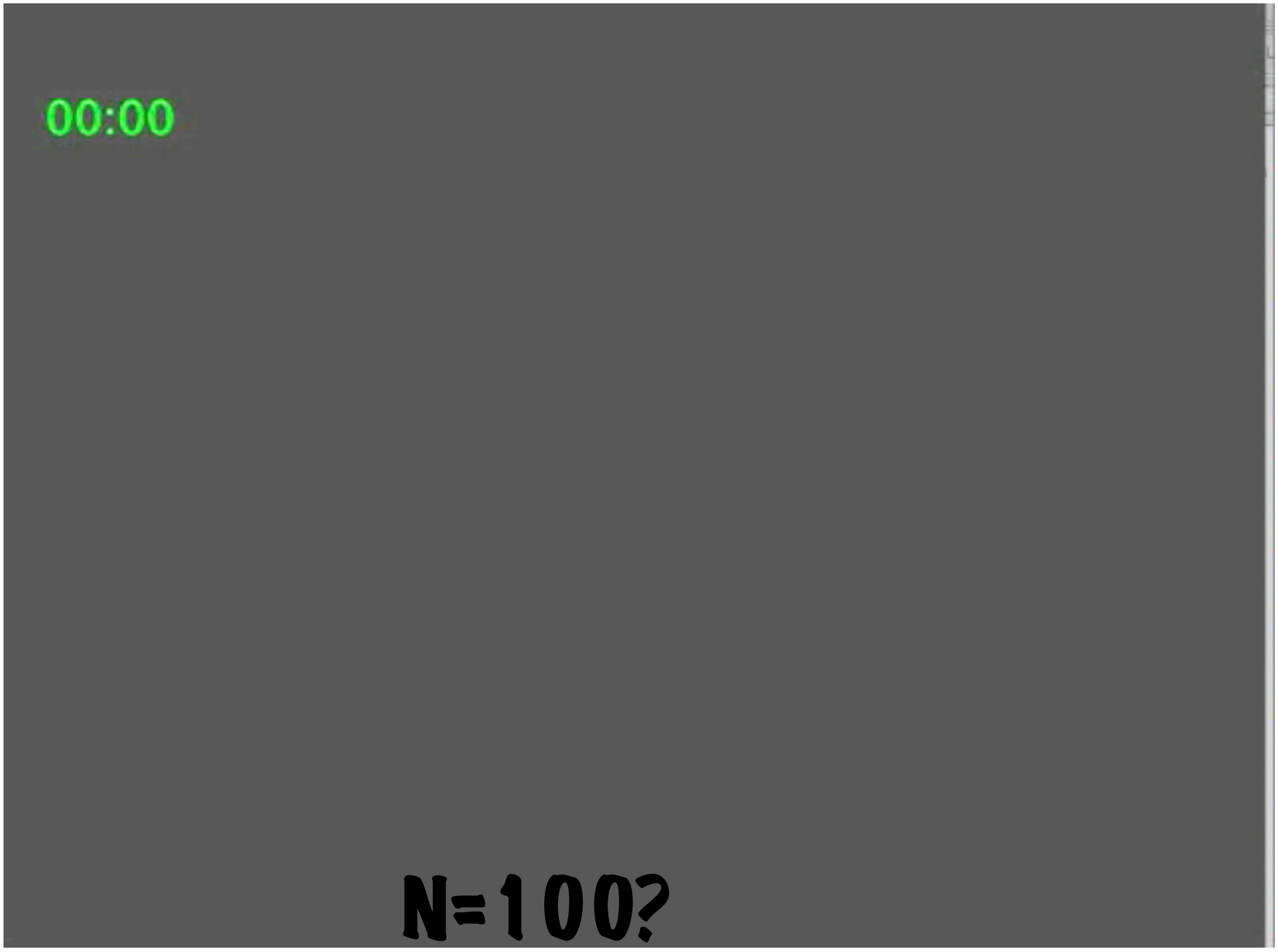
Max Delbruck: A very misleading experiment



What about population size N? If it is pre-existing low inoculation will show no resistance.

N=100?

What about population size N? If it is pre-existing low inoculation will show no resistance.



These information waves come from a modified form of Fisher-Kolmogorov Eq.:

$$\frac{\partial m}{\partial t} = \mu^*(x,y)(n + \nabla n \cdot \vec{L}) + r_m(x,y)m(1 - \frac{m+n}{K_h}) + \frac{L^2}{2\tau}\nabla^2 m$$

$$\frac{\partial n}{\partial t} = r_n(x,y)n(1 - \frac{m+n}{K_h}) + \frac{L^2}{2\tau}\nabla^2 n$$

The Fisher-Kolmogorov equation has soliton-like wave solutions with a minimum speed c:

$$c > 2\sqrt{DR}$$

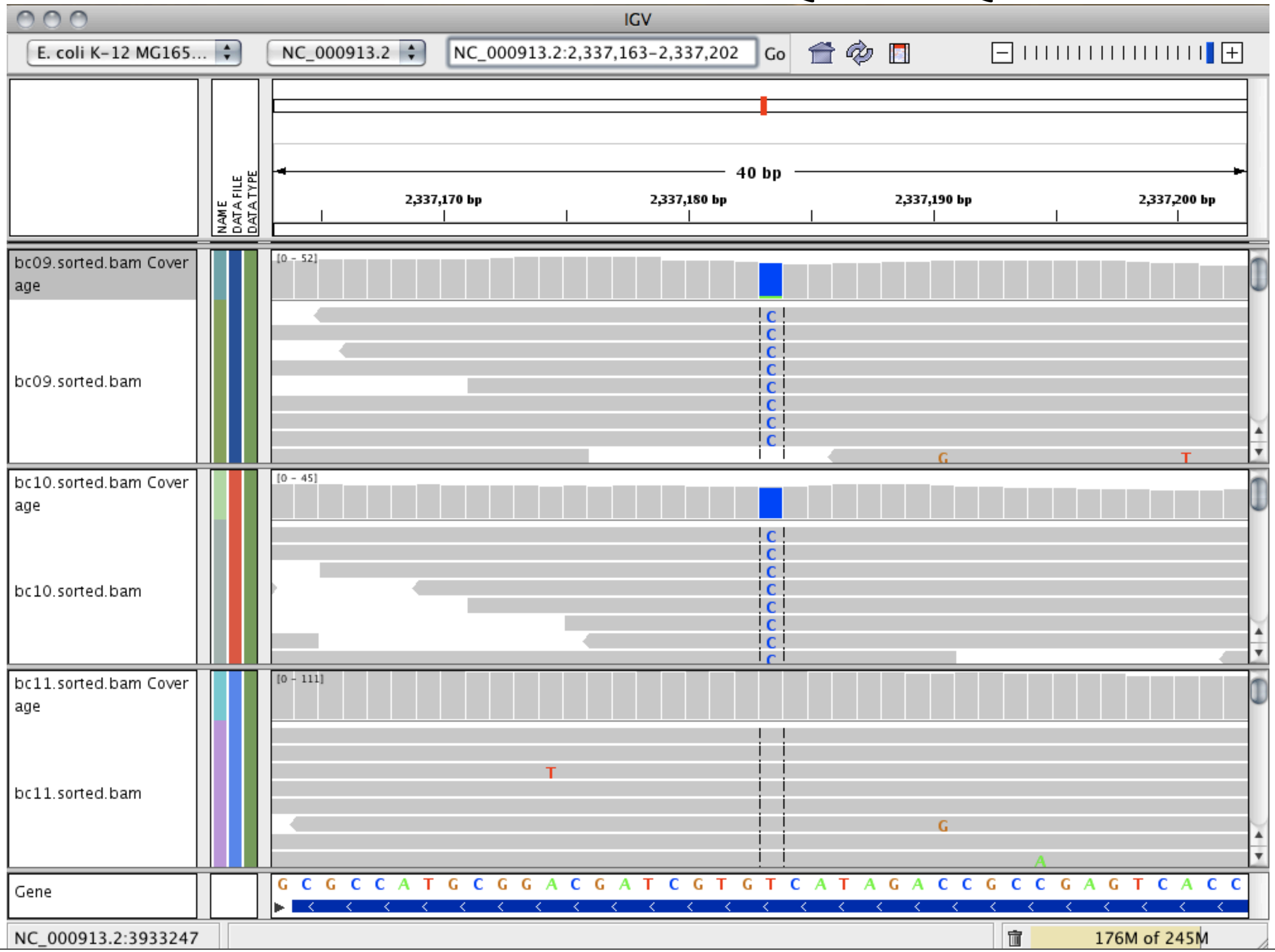
In spite of the complex nature of the process, the net result of this information wave is a rather surprisingly simple end result that like Special Relativity is quite elegant when all the calculations (which are straightforward but tricky) are done: the bacteria find 4 SNPs that solve the antibiotic problem compactly.

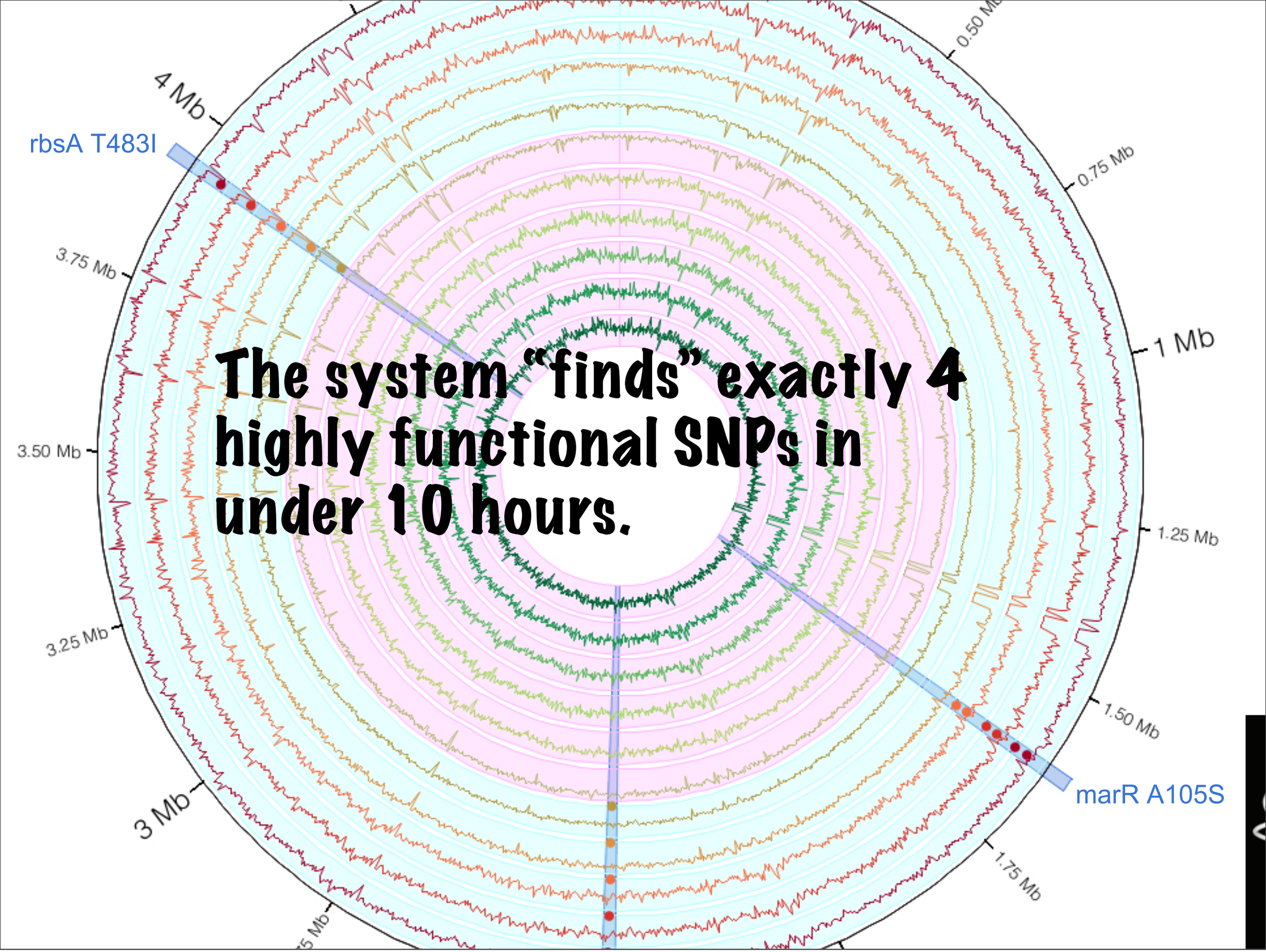
III. Deeper mysteries as we whistle in the dark.

Deep whole genome sequencing work (UCSC, Sequencing Core of the Princeton Physical Sciences Oncology Center) has revealed that the bacteria come up with a very clever and quick solution to the antibiotic problem in the Death Galaxy, very spookily so.

John Kim (UCSC) and Qiucen Zhang (PU)!

Nothing under x50 deep accepted.

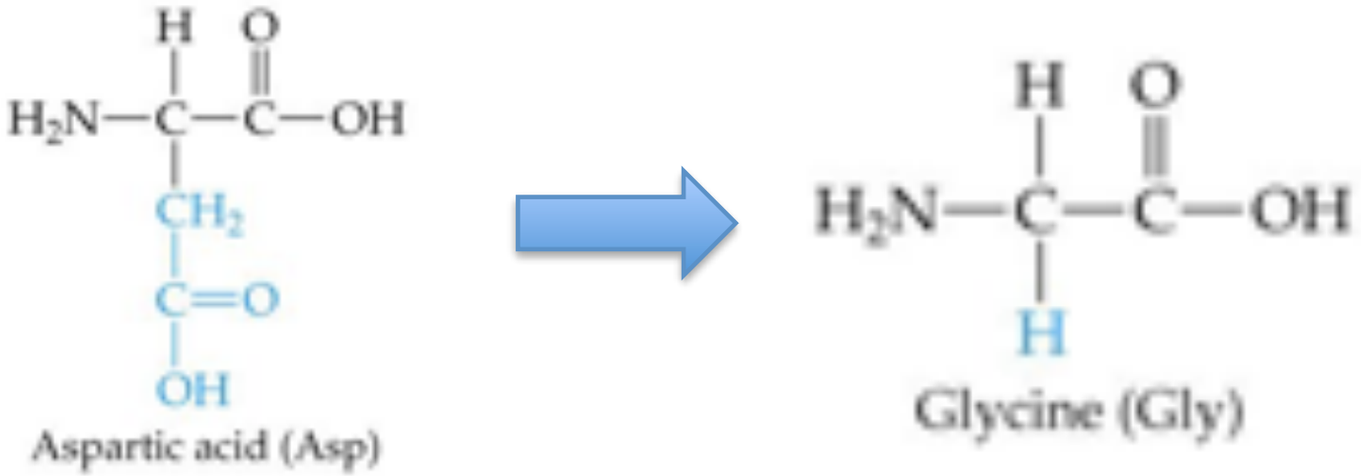




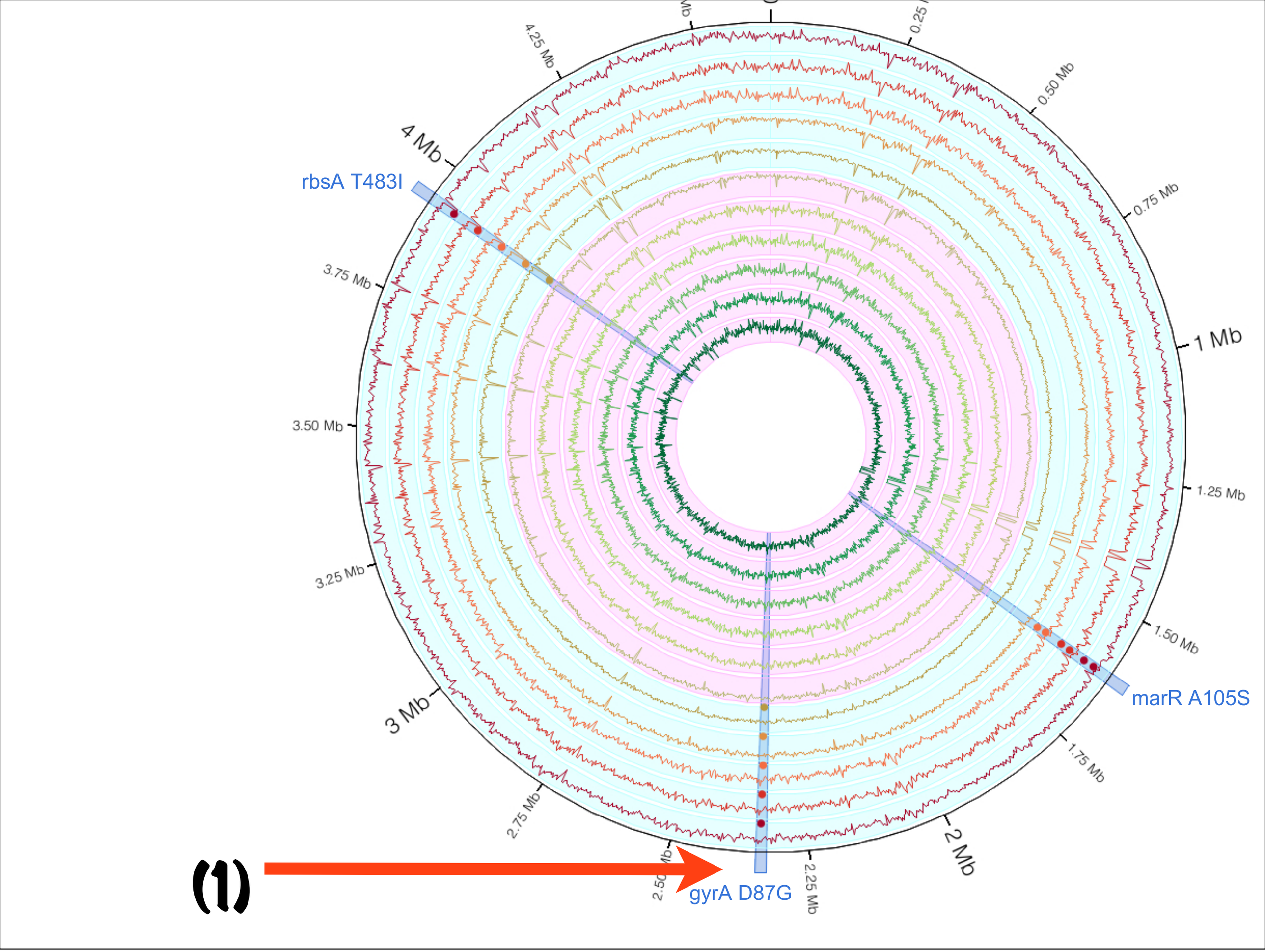
The system "finds" exactly 4 highly functional SNPs in under 10 hours.

1) Expected this: mutation in gyraseA where Cipro acts. SNP at locus 2,337,183. All samples.

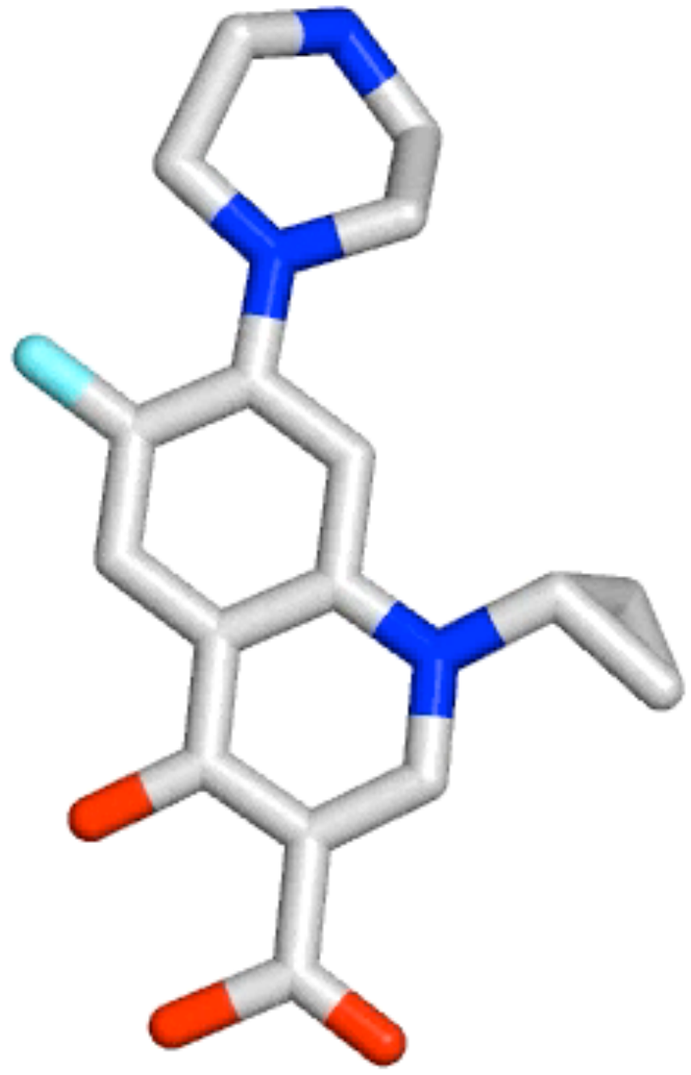
Missense Mutation in gyrA



- Mutations in gyrA have been shown to impart cipro-resistance to e. coli
- Previous studies show a D87N also imparts resistance
- Mutations that impart resistance most often occur in active binding site


rbsA T483I
marR A105S
gyrA D87G
(1)
4 Mb
3.75 Mb
3.50 Mb
3.25 Mb
3 Mb
2.75 Mb
2 Mb
1.75 Mb
1.50 Mb
1.25 Mb
1 Mb
0.75 Mb
0.50 Mb
0.25 Mb
4.25 Mb
2.25 Mb

How did the cell find "the" solution so fast?



2. We found another SNP in an unexpected place: pumps that remove toxins. Should have expected it. One SNP does not a phenotype make.

Mutation in gyrA alone is not enough to impart resistance

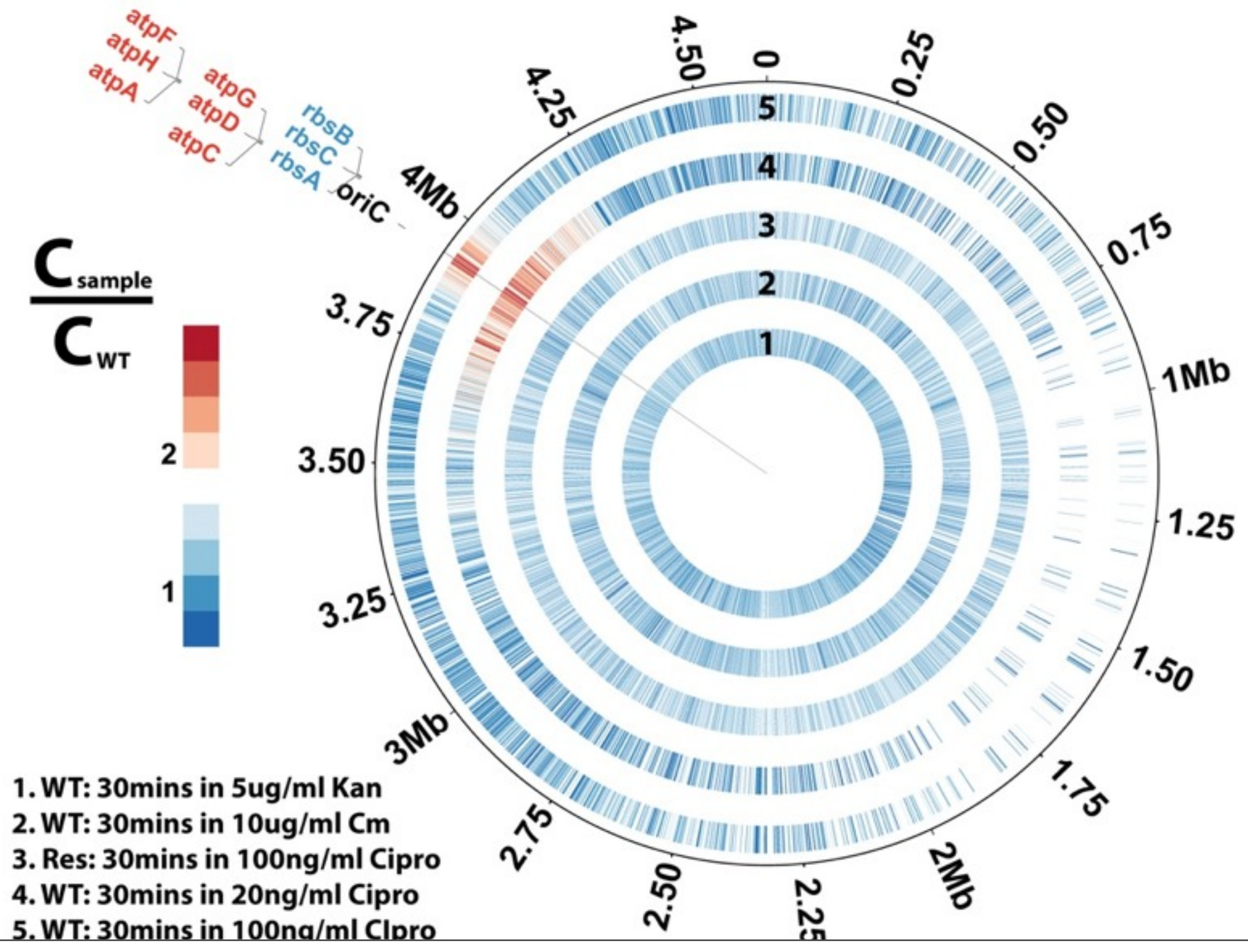
Table 1. Correlation between fluoroquinolone (ciprofloxacin/levofloxacin) susceptibility of 59 *Pseudomonas aeruginosa* clinical isolates and mutations in *gyrA* and *mexR* genes.

Fluoroquinolone susceptibility (no. of isolates)	Mutations in *gyrA* only	Mutations in *mexR* only	Mutations in both *gyrA* and *mexR*	No mutations in *gyrA* or *mexR*
Resistant (12)	4	0	4	4
Intermediate (6)	1	1	1	3
Susceptible (41)	1	12	1	27

- Correlation between resistance and a point mutation in gyrA is not 100%
- Resistant strains may not have a mutation in gyrA at all

Gorgani, N. (2009). Detection of point mutations associated with antibiotic resistance in Pseudomonas aeruginosa. International journal of antimicrobial agents, 34(5), 414-.

A missense A to T in base 3,933,247 in a region coding for the rbsA gene which is a component of the ribose ABC transporter complex and been previously reported to export other antibiotics (Erythromycin, Tylosin, and Macrolide).



Also found 2 SNPs (1,617,461: A to C and 1,617,460:C to G) in the marR operon.

The mar regulon identified in Escherichia coli (mar-Eco) plays a key role in the expression of a multidrug resistance phenotype, and specific mutations located in marR have been identified in resistant strains. The regulatory function associated with the marA locus simultaneously induces a decrease in antibiotic uptake by altering the porin content of the outer membrane and an increase of antibiotic ejection by activating efflux mechanisms. This response supports an efficient resistance to a range of commonly used antibiotics.

There is a problem. Where are the passenger mutations? We see 4 clear SNPs in functional places and nothing else.

If this came from random mutations, there should be lots of neutrals. There aren't any.

But directed mutations in the area of gyrA, although heretic and no doubt evil, don't seem to be there either. We see single spikes in the mutation landscape, and that is "troubling"

Personally, I find this pretty shocking:

Not only are we finding rapid emergence of antibiotic resistance in bacteria scaling down to very small numbers of bacteria, but also we see rapid and innovative finding of ways to bypass the antibiotics.

These mutations occur rapidly and in highly specific places that are highly functional.

I think the system knows what it is doing.

IV. Does it work for cancer cells: does breaking a population into a metapopulation of small numbers and putting a high stress gradient on them accelerate the evolution of resistance?

This is the core of our present "paradigm" of chemotherapy, and I would claim it is doomed to fail for the reasons I just stated in the bacterial work.

When will we accept that failure? Never.
Science advances one funeral at a time: Planck.

Trans-Network

Evolution of drug resistance of multiple myeloma in microfluidic "Death Alcatraz"

Amy Wu[1], Qiucen Zhang[2], Guillaume Lambert[3],
Zayar Khin[4], Ariosto Silva[4], Robert A. Gatenby[4],
James Perrot[5], Nader Pourmand[5],
Robert H. Austin[2], James C. Sturm[1]

[1]Electrical Engineering, Princeton University;
[2]Physics, Princeton University;
[3]Biology, New York University
[4]Moffitt Cancer Institute
[5]Bioengineering, UCSC

Look! →

Funded by Award Number U54CA143803
from the National Cancer Institute.

Note: NONE of this without NCI/PS-OC!

Multiple myeloma (from Greek myelo-; bone marrow),

Cancer of plasma cells, a type of white blood cell normally responsible for producing antibodies.

Abnormal plasma cells accumulate in the bone marrow.

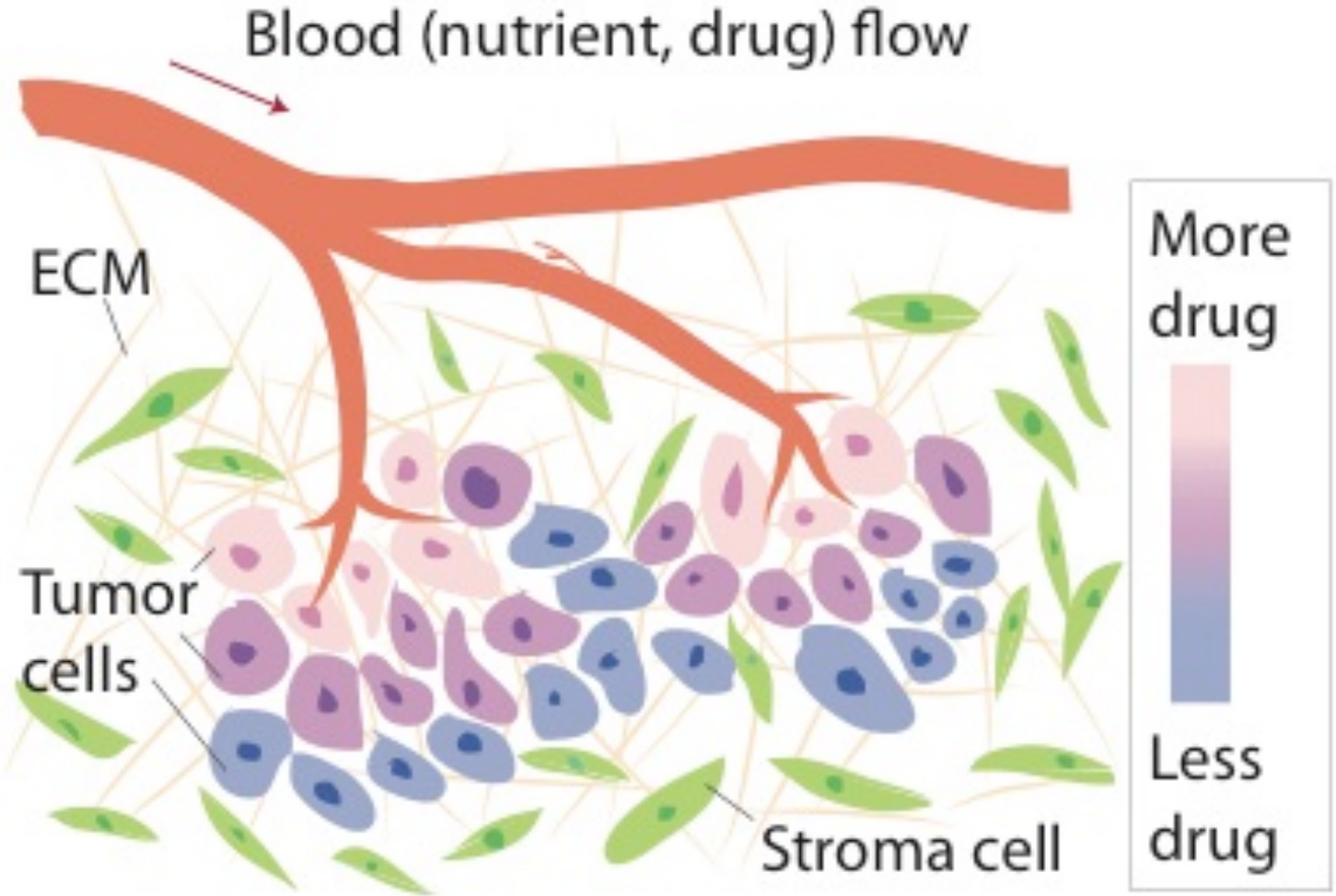
Most cases of myeloma also feature the production of a paraprotein—an abnormal antibody which can cause kidney problems.

Bone lesions often encountered (hence the name).

Myeloma is generally thought to be treatable but incurable, i.e. it always progresses.

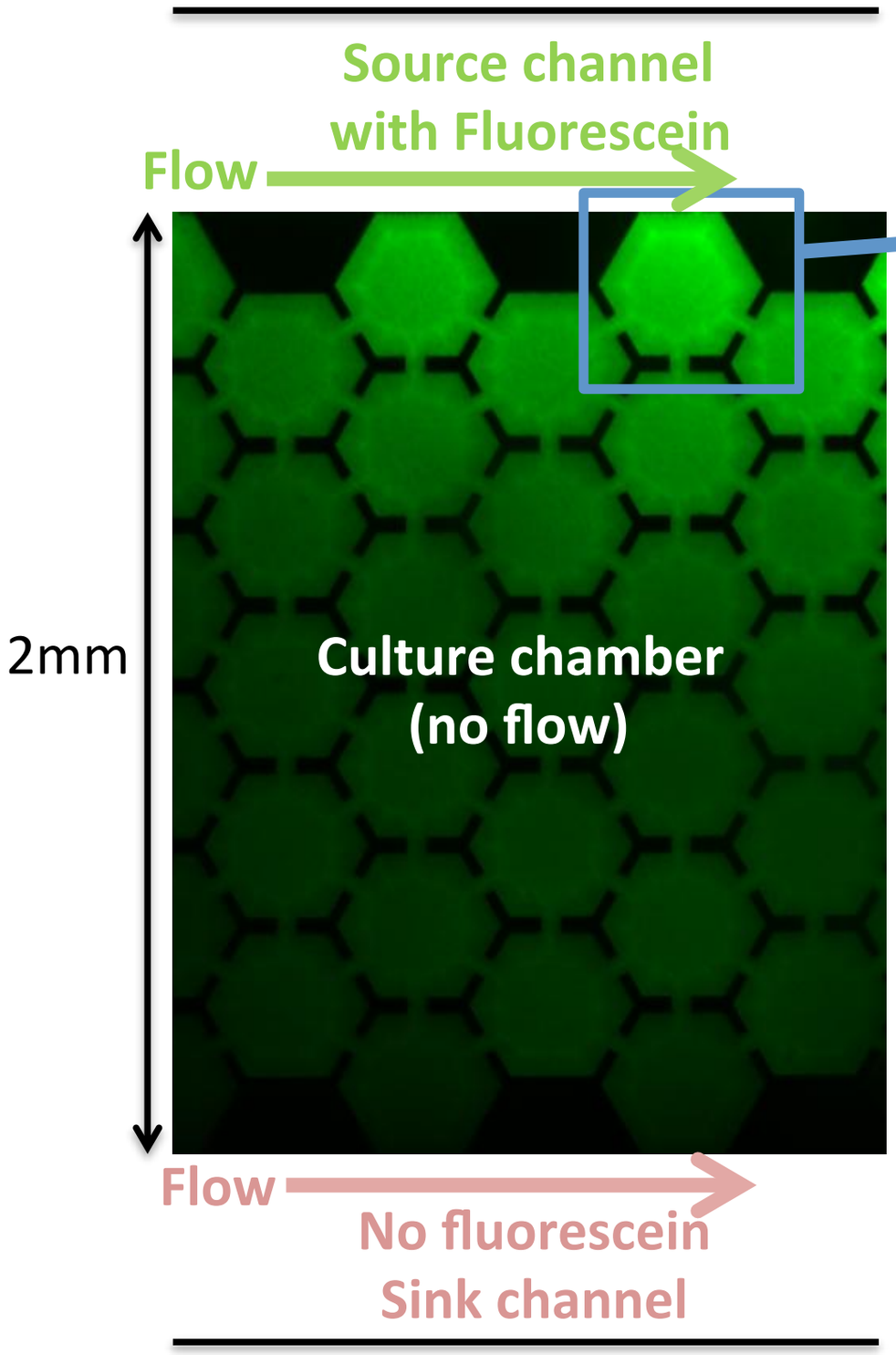
Cancer drug resistance and microenvironment

- Goal: study emergence of drug resistance in multiple myeloma (bone marrow cancer)

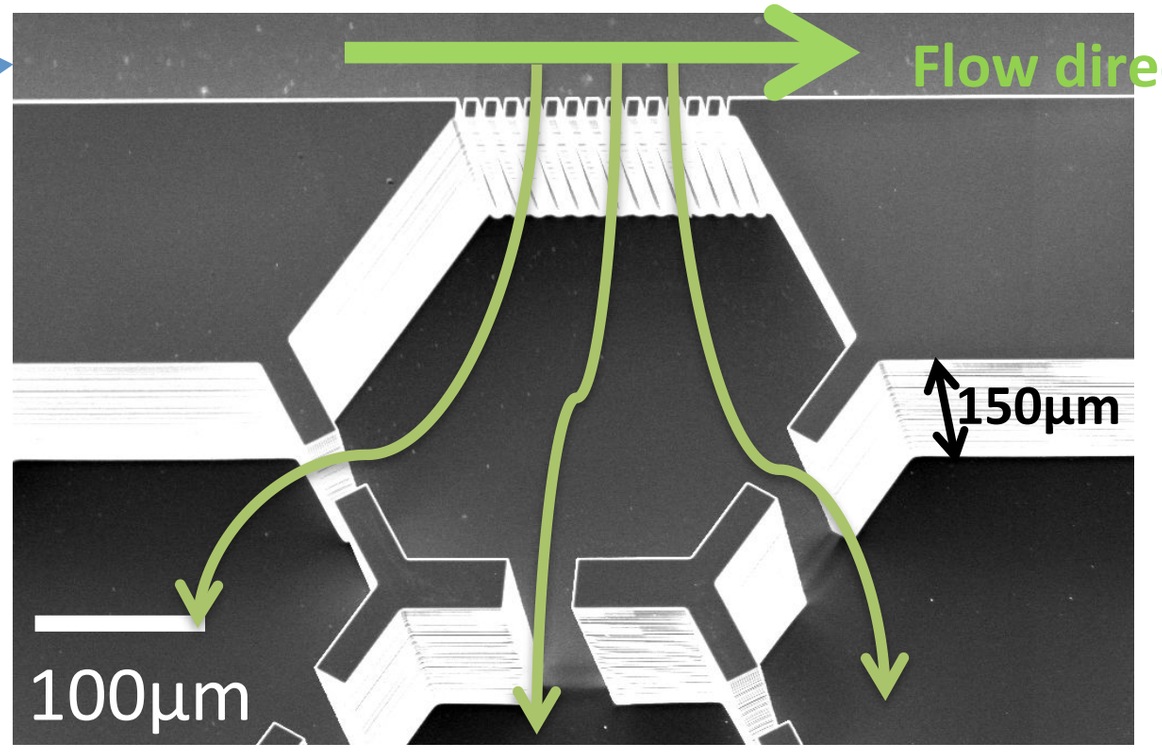


- Approaches
  1) Microfluidic design to construct drug gradients
  2) Microhabitats (increase fixation of mutation)
  3) MM and stroma interaction

Ref: Zhang et al, Science 2011



Microposts allow diffusion of biomolecules



- Recreate tumor microenvironment:
  - Stable drug gradient
  - Microhabitats allow cells to migrate, small population in each habitats is easy to fix mutation with growth advatage

Alcatraz: death row: engineers uncomfortable with death galaxy lack of control. Typical.

Project flow

Co-culture on-chip

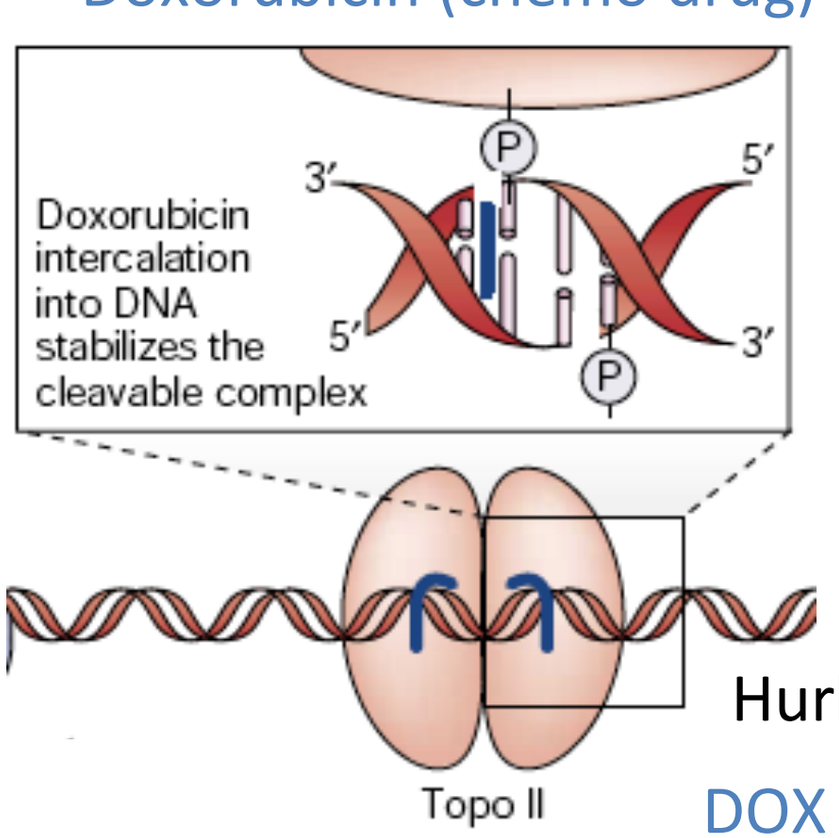
↓

Co-culture + steep DOX gradient (0-2000nM/2mm)

Bone marrow stroma (HS-5/GFP) + multiple myeloma (8226-S/RFP) cell lines

- 33% matrigel in culture chamber
- Under 5% CO_2, 37°C
- Observed growth of both cells over 200 hours.

- Doxorubicin (chemo drug)



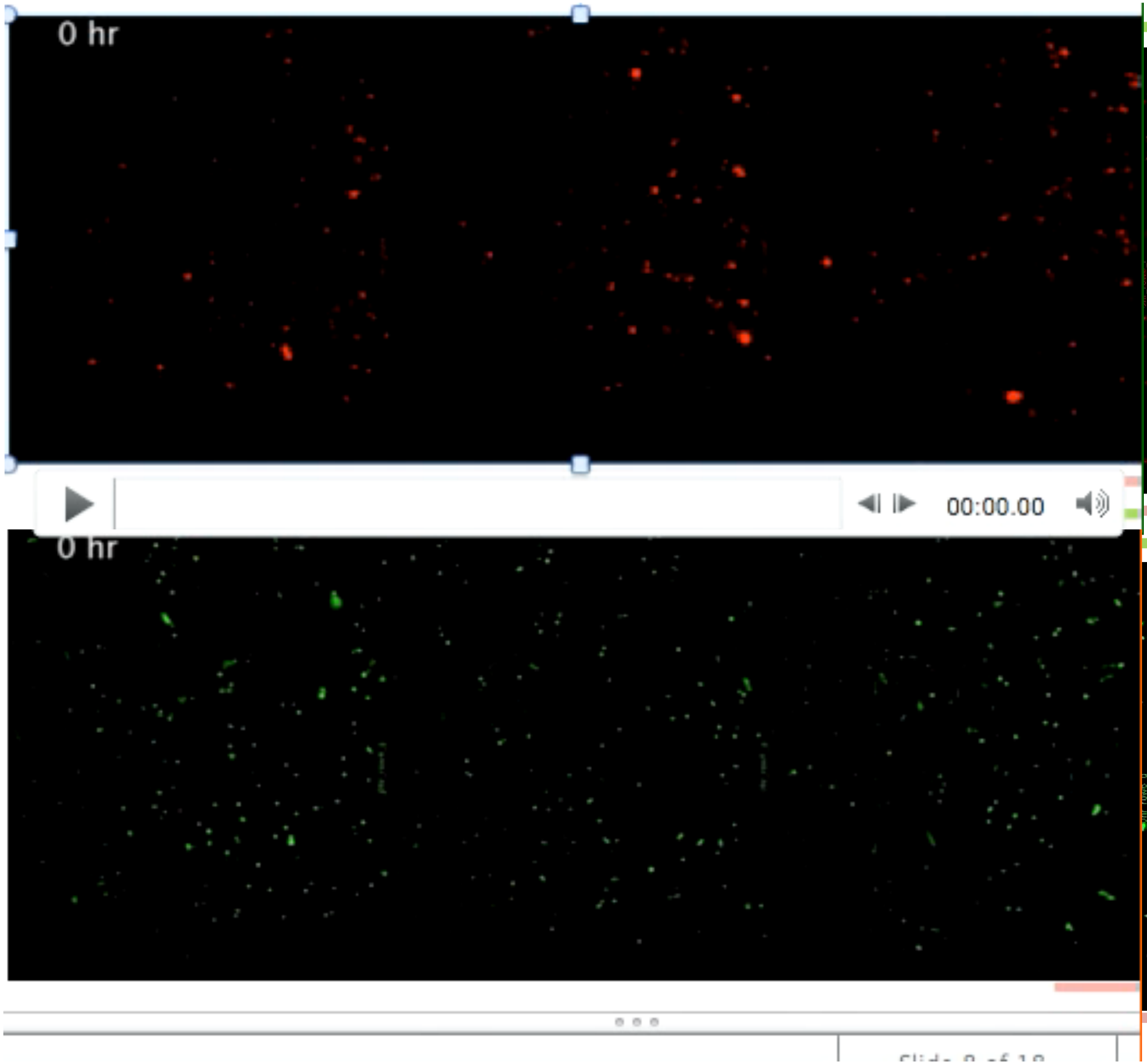
Hurley, Nat Rev Cancer 2002

DOX vs. Top II for cancer
≈ Cipro vs. Gyrase for E. Coli.

Medium DOX gradient (0-200nM/2mm) (movie)

Myeloma

Stroma



DOX 200nM

2mm

DOX 0nM

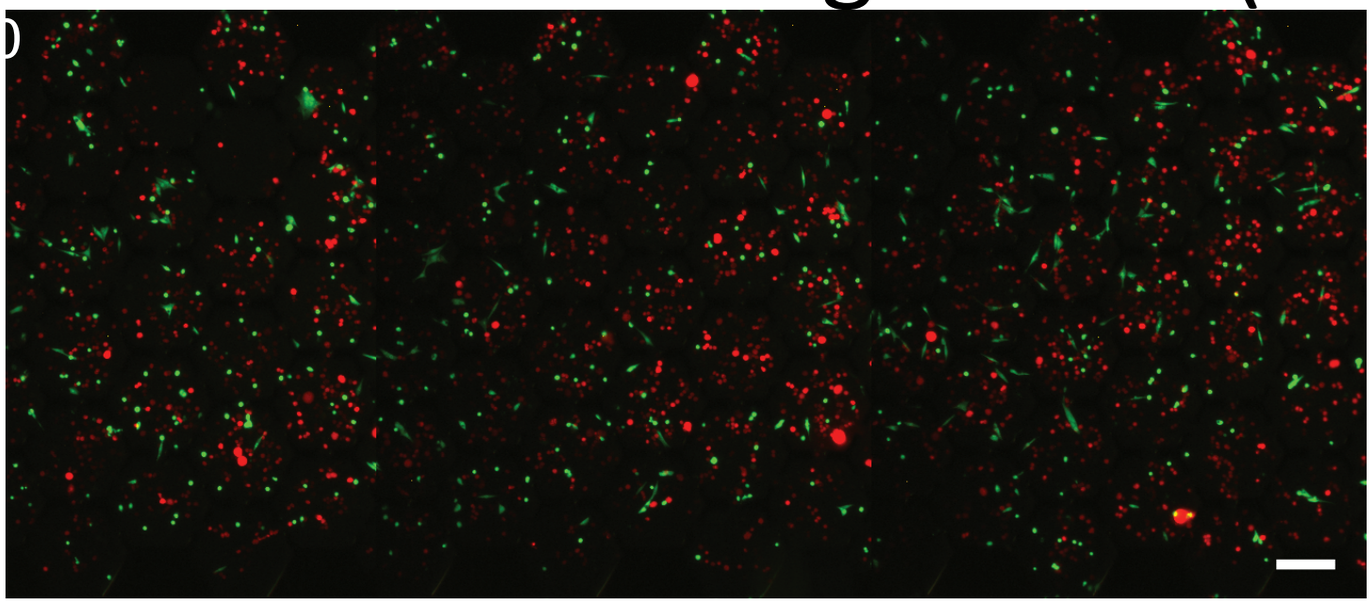
DOX 200nM

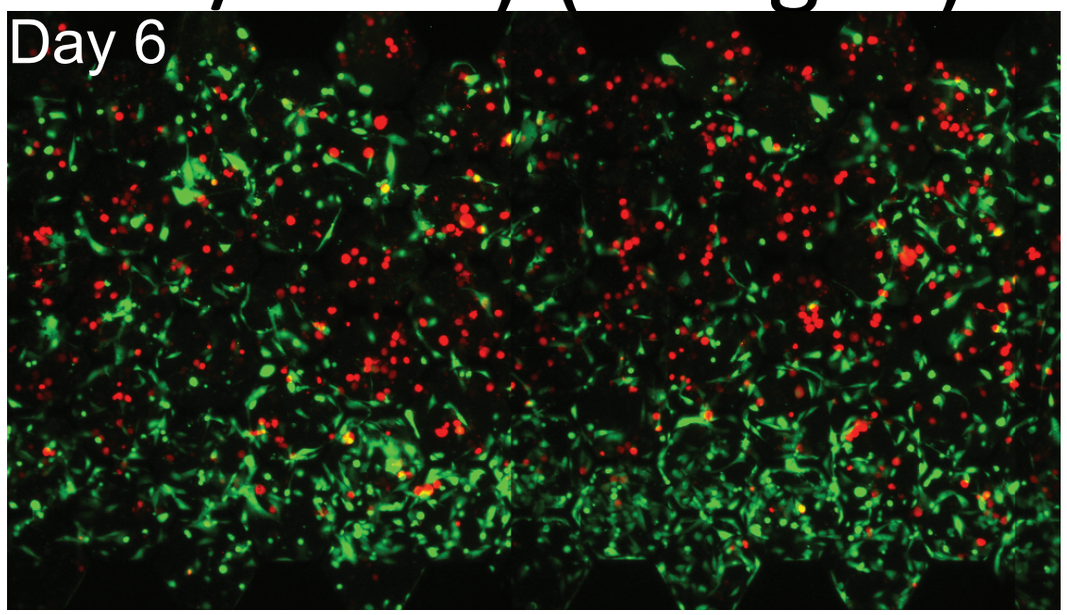
Growth!

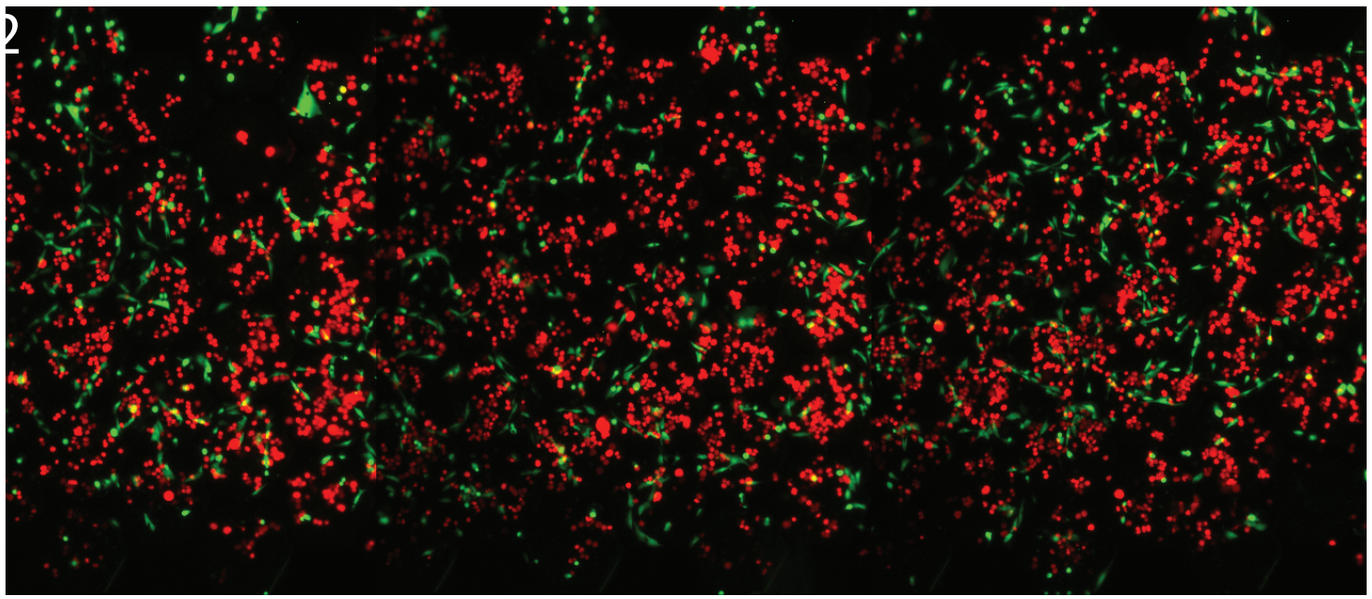
Stroma prosperous boundary

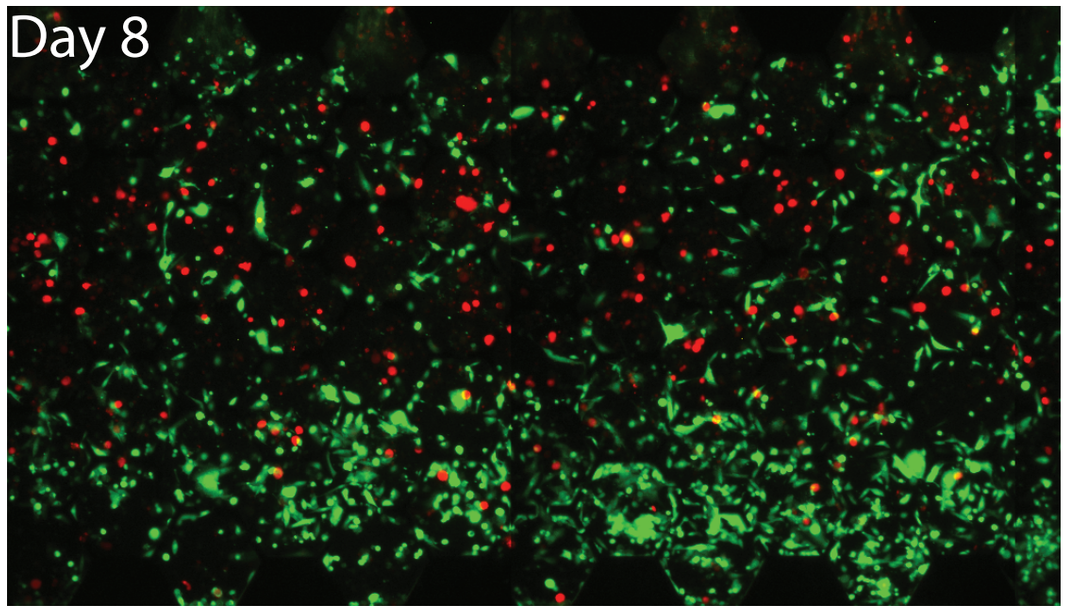
DOX 0nM

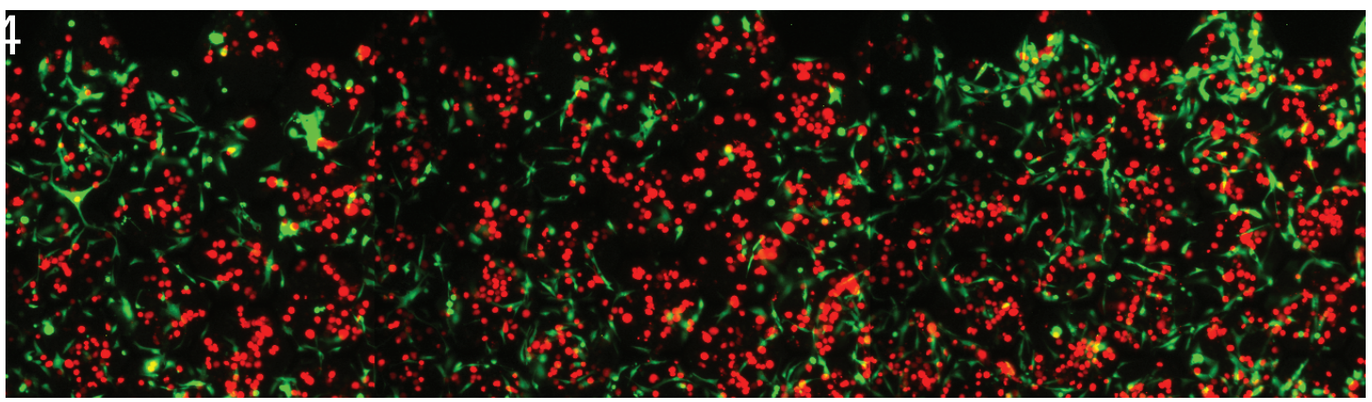
Medium DOX gradient (0-200nM/2mm) (merged)







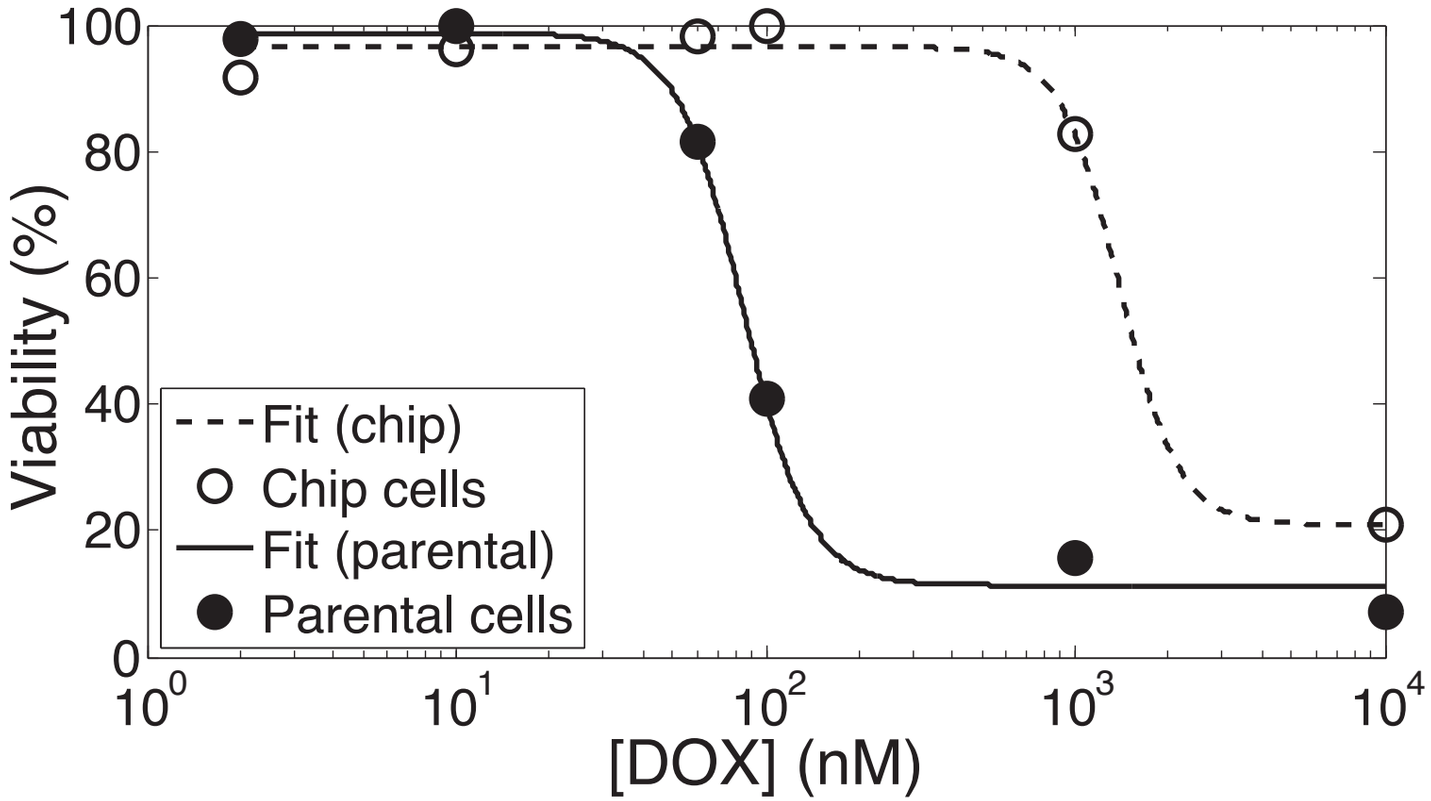




top: 0, bottom: 200nM
White line: 200um

Parental cells vs. cells from the chip

XTT Toxicity Assay



$$\text{Degree of cross-resistance (48 hour)} = \frac{IC_{50}(chip)}{IC_{50}(sensitive)} = \frac{1390}{85} = 16.3$$

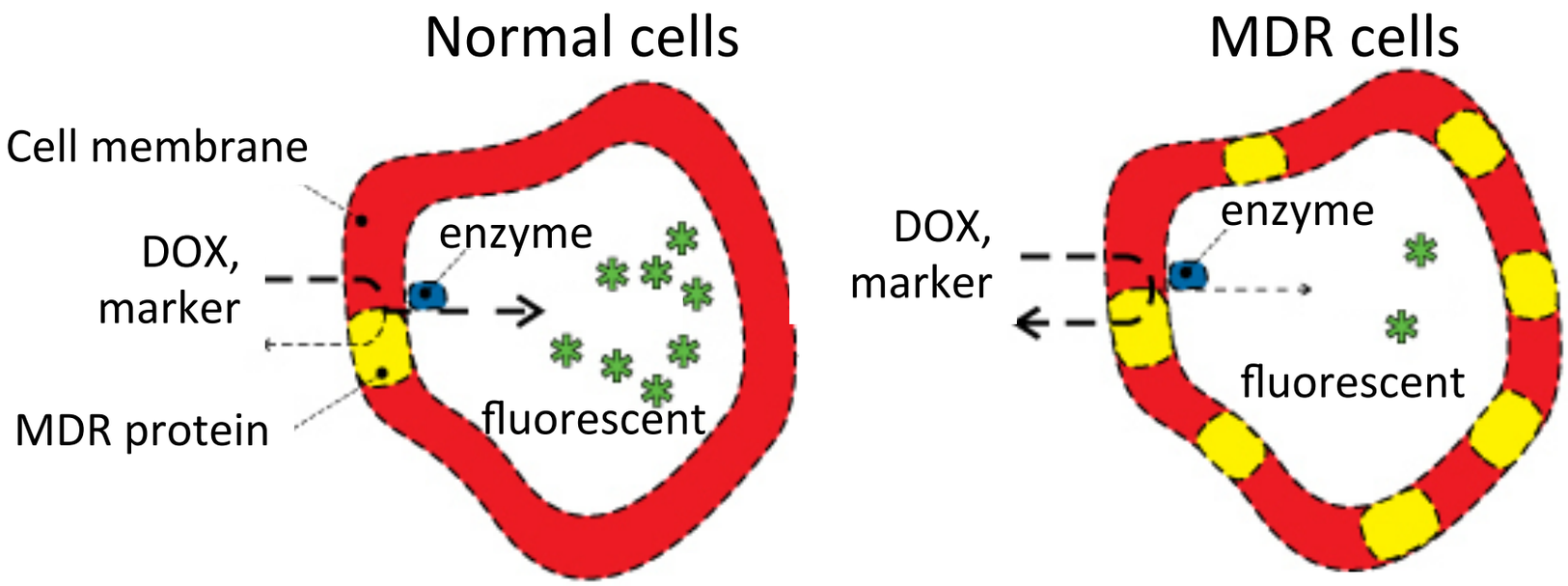
Note: degree of cross-resistance= 17.0 for resistance cells using the traditional protocol

Ref: Dalton et al, Cancer Research 1986(attached)

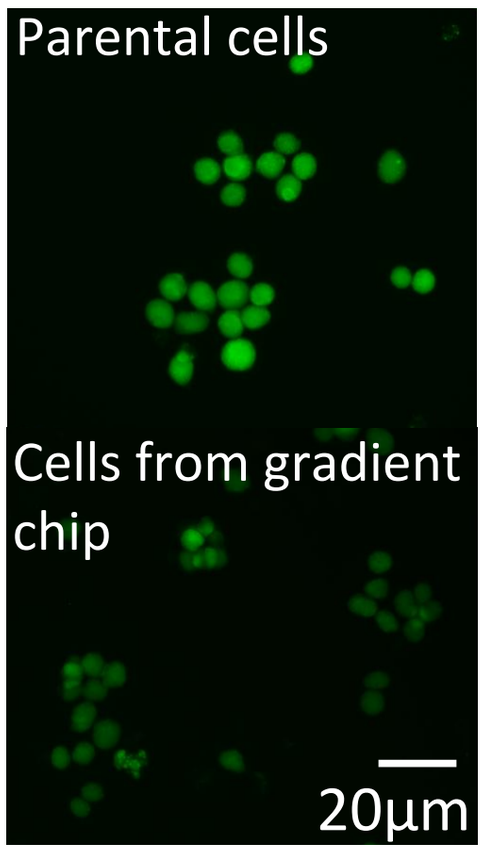
Parental cells vs. cells from the chip

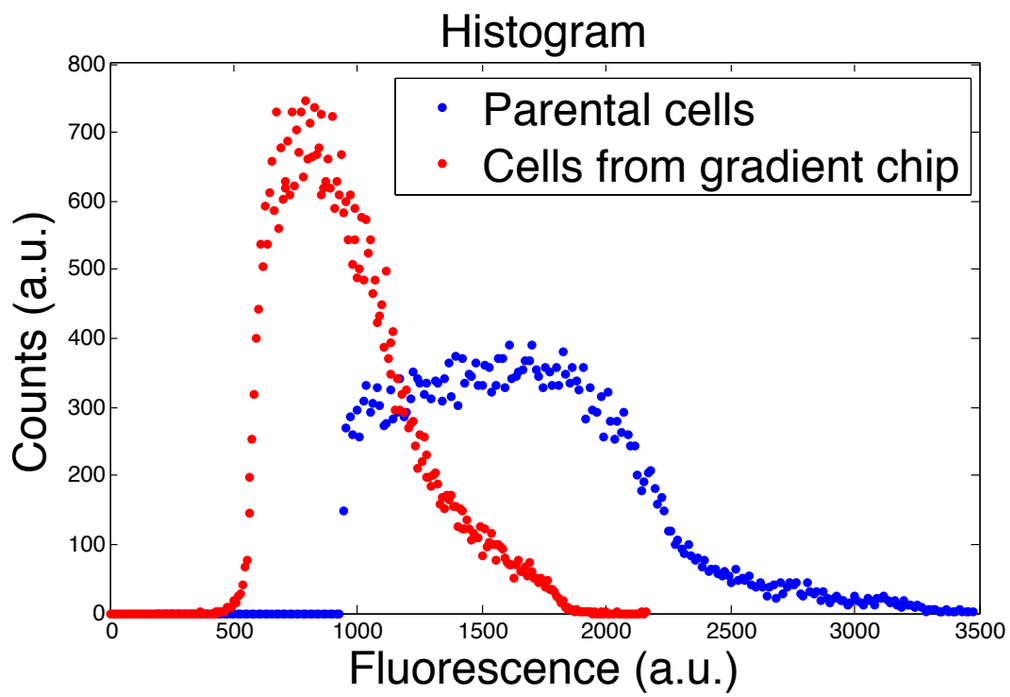
Multiple Drug Resistance (MDR)

- MDR transporter (metabolic drug pumps overexpressed by MDR cells)



- Resistant cells are dimmer than normal cells (MDR pumps out the fluorescent markers)





- Cells from gradient chip are dimmer than parental cells =>more MDR pumps =>more resistant!

(confirmed by western blot)

What about the genetic changes?
How much evolution occurred in
2 weeks?

Is the solution elegant and compact, or
a mess?

This is hot off my RAID servers and 1 solid week of sequence alignment at Princeton by Qiucen Zhang and Amy Wu, beating on 1 Tb of data from Nader Pourmand, Jimmy Perrott and John Kim, who are Gods in my opinion, and the Sequencing Core of the Princeton Center for Physical Oncology.

You are very badly mistaken if you believe in the $1000 genome. This was sequencing of all the exons (mRNA) in 2 cell lines: WT MM cancer cells and chip evolved resistant. 6 months of work.

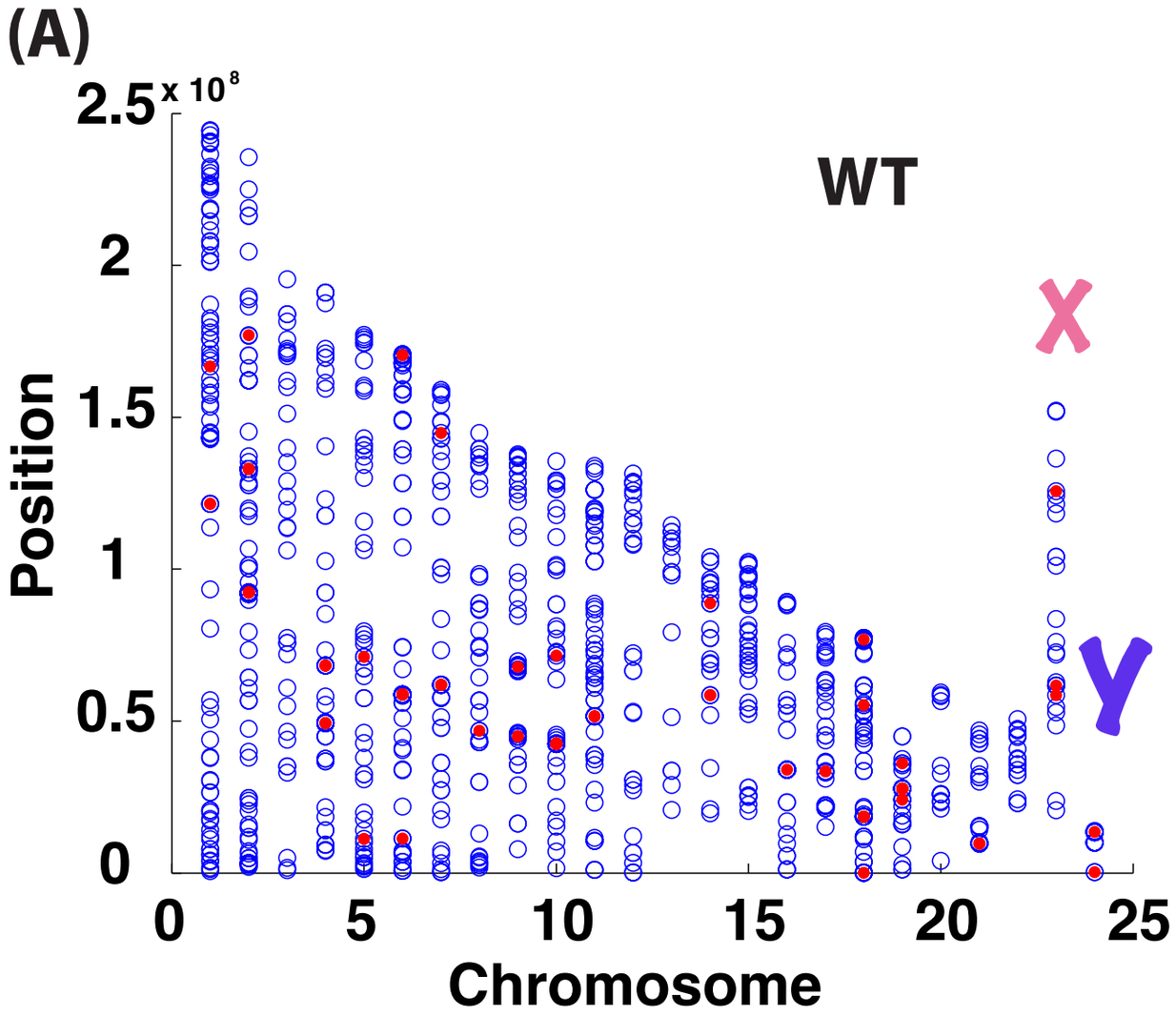
RNA-seq of MM

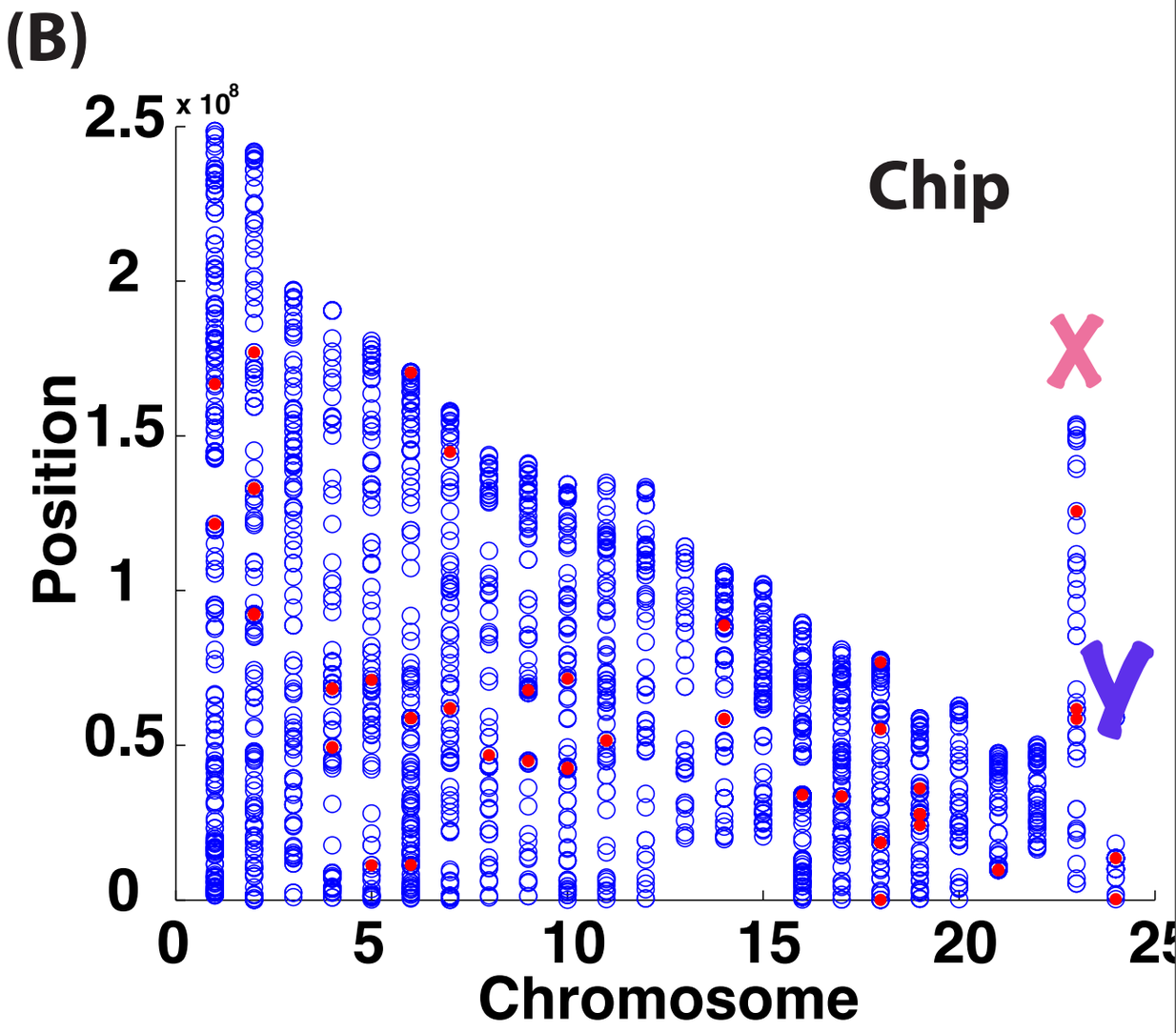
Note: 400,000,000 short reads, 50 bp per read, Illumina, mapped to H. sapien genome guide 37

A note of vicious reality: There were around 2400 SNPs for chip and 1200 for WT compared to the baseline Human Genome 37. The overlap is only 100 "common" SNPs.

For the remaining 2300 SNPs of chip, some locations don't have mapping coverage in WT. i.e. Some ABC pumps have SNPs in chip but no mapping in WT at same location, so cannot compare. After we filter out the incomparable SNPs, there are only 255 left.

Most don't map to known genes.





The red dots are "SNPs" common to WT and chip-evolved cells and so automatically culled out.

All the rest do not overlap, but coverage varies so many can be sequencing "noise". We demanded x20 coverage for both WT region and chip genome call.

So Darryl: I don't buy that evolution proceeds slowly in a tumor. If it did we could "cure" cancer, actually it is a losing game of whack-a-mole. Ask Steve Jobs in heaven.

I think that is some damn biology textbook talking which checked into the Hotel California/ Delbruck and just couldn't leave the place.

Boris, it is much worse than you think:

Email this morning from Qiucen Zhang (who is a genius) (but you don't want to mess with him)

Hi Bob,

1. Previously, we used a new version of "Samtools -mpileup | vcftools" to call SNPs based on some "embedded statistical test". At the end, I found it is bullshit and gave a lot of false calls including the SNPs in Amy's email.

2. The SNPs in my email are called by "Qiucen_snp_finder". My script used the following two criteria stated clearly:

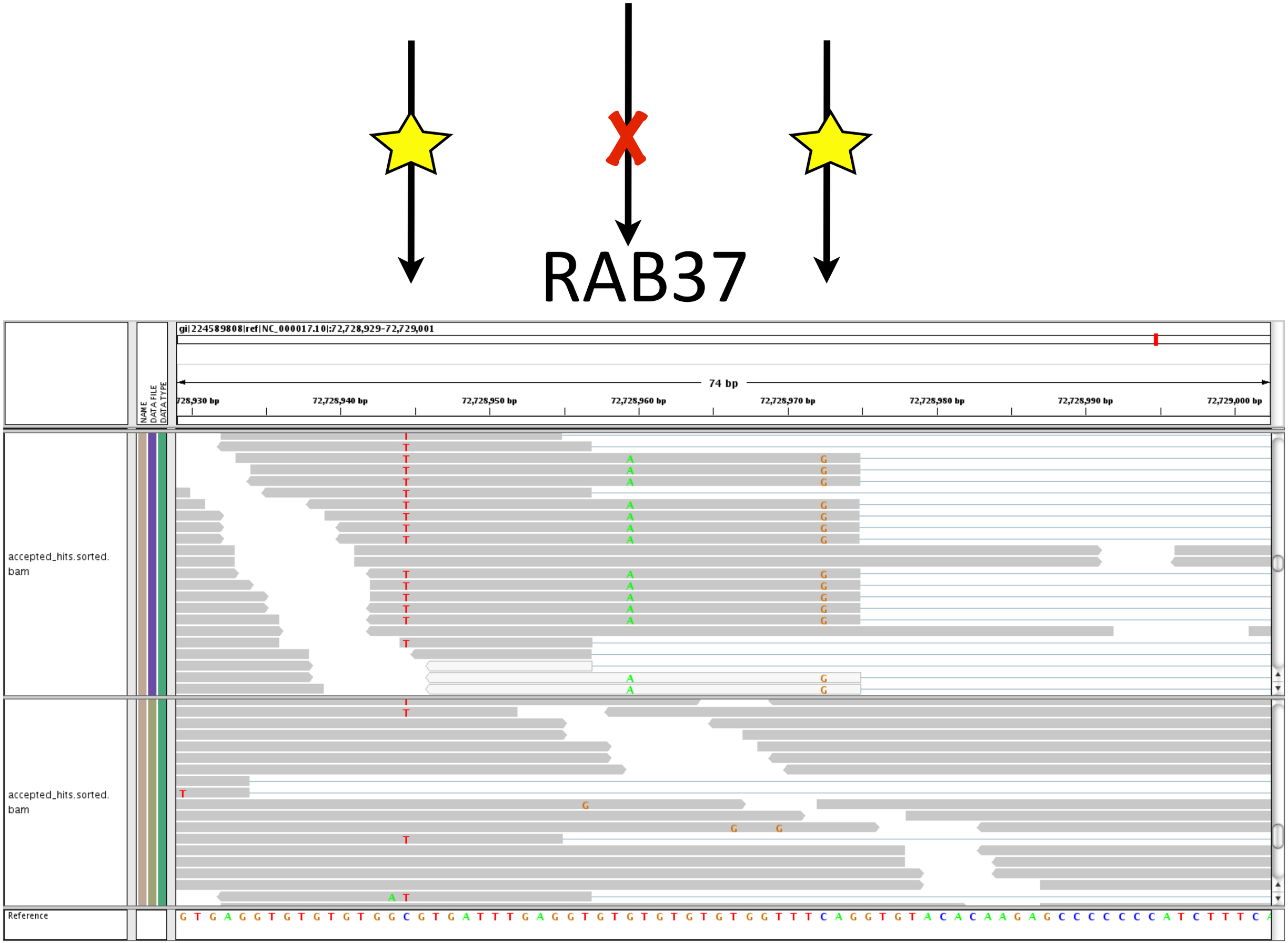
So the answer is: 5 SNPs we "believe" in. Could be (lots) more. P-scores are useless IMHO.

SNPs for chip cells

Chromosome	Position(bp)	Ref	SNP	Gene	Coverage
17	72,728,959	G	A	RAB27	74
17	72,728,972	C	G	RAB37	65
2	145,230,917	G	A	ZEB2	59
15	82,795,744	A	C	AGSK1	64
6	157,731,734	G	C	TMEM242	70

Note: these are the positions for Chip that are able to compare with WT (same location, WT also has coverage >20)
The following images show the SNPs location in IGV. Top row is Chip, bottom is WT

Note: Qiucen thinks this is complete bullshit.


RAB37
gi|224589808|ref|NC_000017.10|:72,728,929-72,729,001
74 bp
72,728,930 bp
72,728,940 bp
72,728,950 bp
72,728,960 bp
72,728,970 bp
72,728,980 bp
72,728,990 bp
72,729,000 bp
NAME
DATA FILE
DATA TYPE
accepted_hits.sorted.bam
accepted_hits.sorted.bam
Reference

Are these mutations interesting? Yes.

1+2) RAB27, 37 are part of the RAS (rat sarcoma) superfamily of small GTPases is broadly subdivided into five groups: Ras, Rho, Rab, Ran, and Arf.

Rab family proteins are important in regulating signal transduction and cellular processes such as differentiation, proliferation, vesicle transport, nuclear assembly, and cytoskeleton formation. However, some Rab proteins have been reported to be necessary for the adhesion and migration of cancer cells.

Although Ras and Rho family members have been strongly implicated in cancer progression, knowledge of Rabs action in this regard is limited. Some reports have also linked Rab GTPases with cancer cell migration and invasiveness

3) ZEB2 promotes the metastasis of gastric cancer and modulates epithelial mesenchymal transition of gastric cancer cells.

Over-expression of ZEB2 at the invasion front of colorectal cancer is an independent prognostic marker and regulates tumor invasion in vitro.

ZEB2 upregulates integrin α5 expression through cooperation with Sp1 to induce invasion during epithelial-mesenchymal transition of human cancer cells.

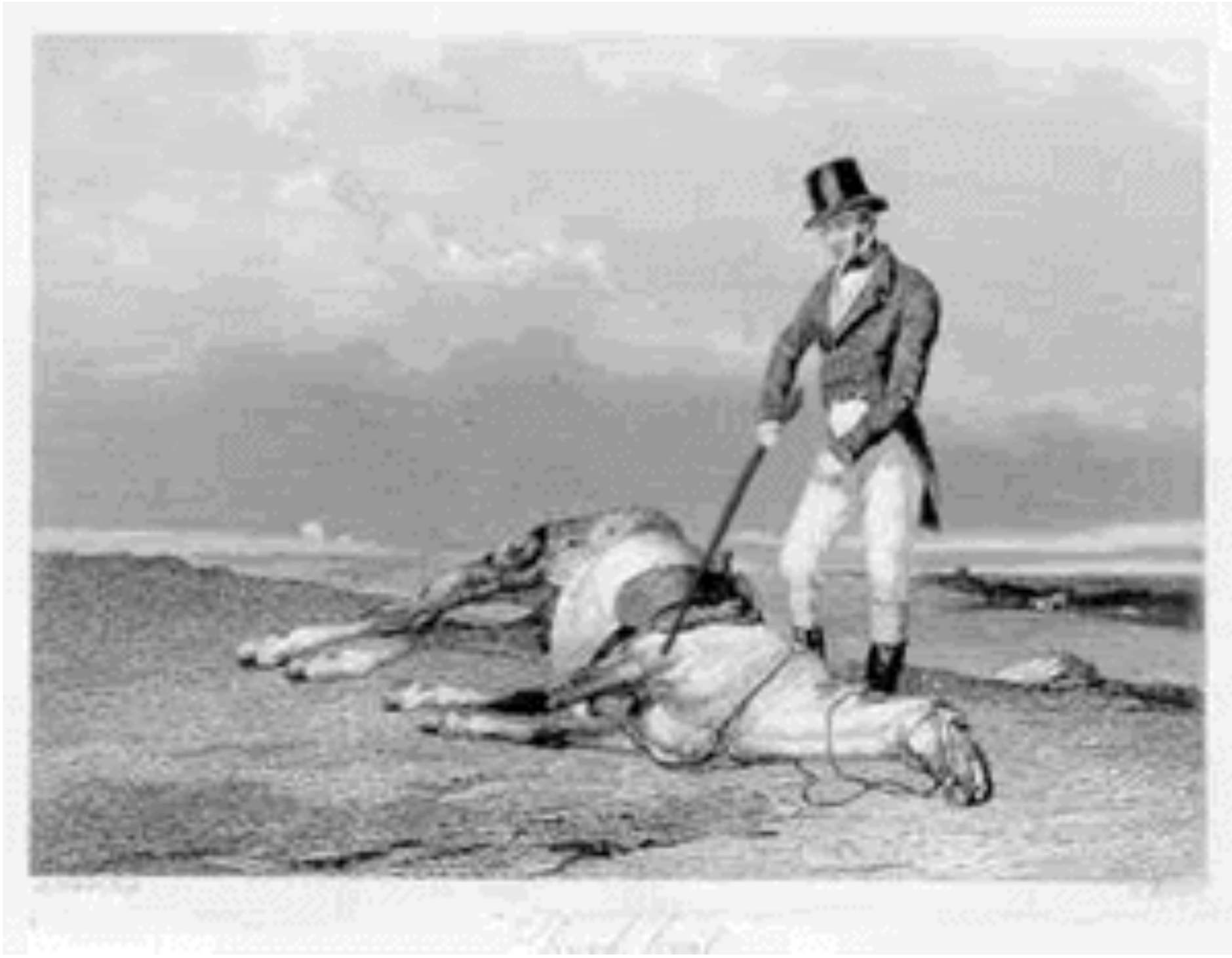
That is, it is a precursor to metastasis.

4) AGSK1: a novel carcinoma associated antigen. Metabolized and expressed in highly invasive cancer cells.

5) TMEM242. Only strange one. This protein is known to be involved in the MAP Kinase pathway, but I draw a blank with function.

Of the 5, 4 are clearly connected with invasiveness and metastasis. So my theory is we evolved a metastatic cancer in 2 weeks.

V. The Beatings Will Continue until the Patient Improves.



"Only 5 Percent of Cancer Research Funds Are Spent On Metastases, Yet It Kills 90 Percent of All Cancer Patients

ScienceDaily (June 1, 2010) — On average, about five percent of total cancer research funding is spent on investigating metastases (the spread of cancer cells around the body) in Europe, yet metastatic disease is the direct or indirect cause of 90 percent of all cancer deaths, according to an editorial in the European Journal of Cancer (EJC)."

Even Europeans can't get it right, and they drive small cars, take trains and have health care!

Metastases, rather than primary tumors, are responsible for most cancer deaths.

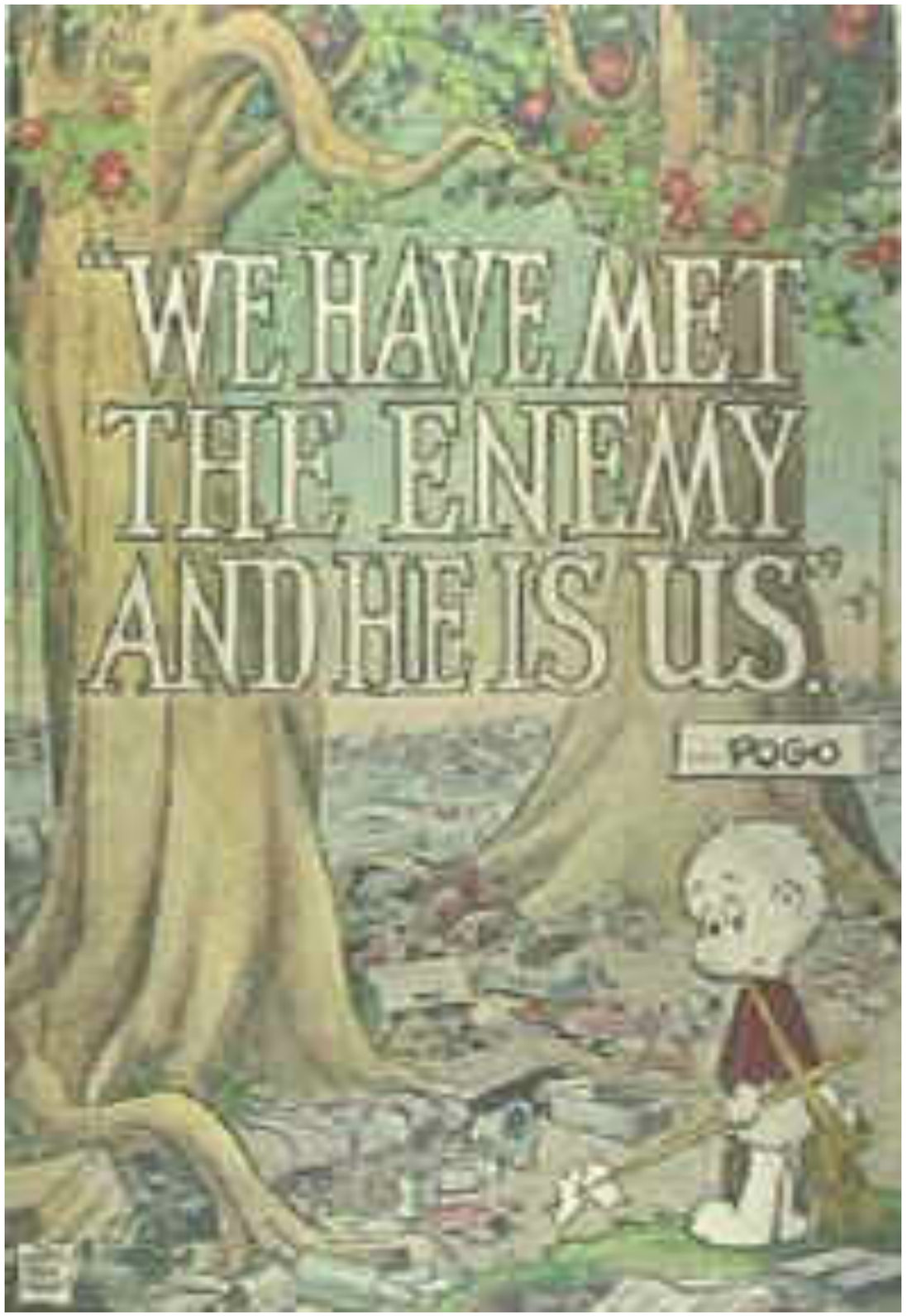
To prevent these deaths, improved ways to treat metastatic disease are needed. Blood flow and other mechanical factors influence the delivery of cancer cells to specific organs, whereas molecular interactions between the cancer cells and the new organ influence the probability that the cells will grow there.

Inhibition of the growth of metastases in secondary sites offers a promising approach for cancer therapy.

Perhaps the Princeton PS-OC is doing the right thing, learning how to drive "normal" cancer cells through the metastatic transition through stress gradients created by chemotherapy: the final end move in cancer's game to kill the host, which we have been aiding via our "paradigm".

But just as we won't fund the obvious ways to prevent cancer, at no cost, we won't support research on what what really kills, metastasis. That makes too much sense.

"We are all just prisoners here, of our own device"



Thanks!