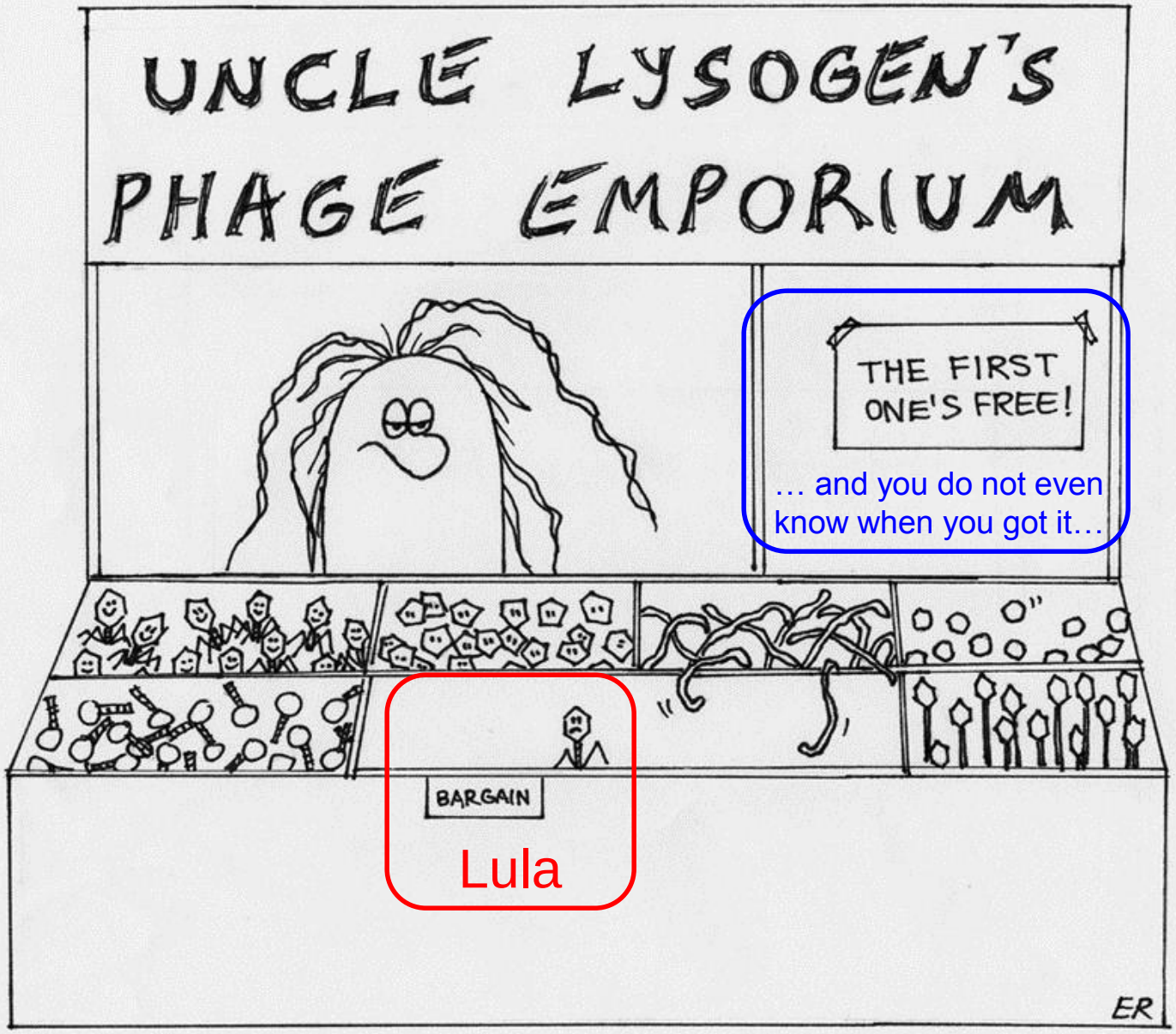
Рудольф Иосифович Салганик

Лаборатория как Среда Обитания:
Уроки Лулы

Andrei Kuzminov
Department of Microbiology
University of Illinois, Urbana-Champaign

Thanks to NIH and
ACS for funding us!



Cartoons by Ella Rotman

План доклада

— Как мы обнаружили, что мы поражены Лулой

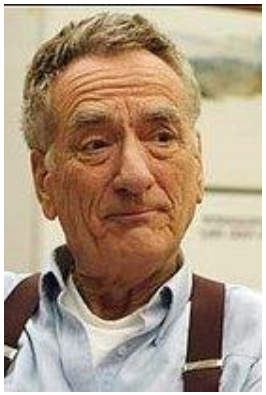
— Как мы вернули контроль над лабой

— Теория взаимного загрязнения экспериментального материала

— Почему Лула такая заразная?

— Человеческие помощники Лулы

— Если подозреваешь, что заражён Лулой — что делать?



Frank Stahl

(Очень) короткая история моей работы в США

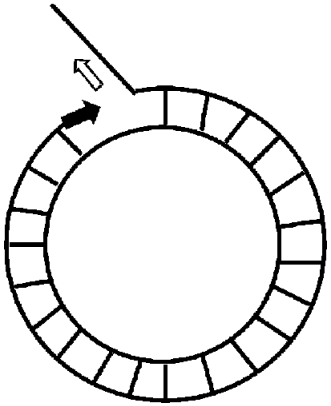
1990—2000
Орегон:
постдок

2000— н.в.
Иллиной:
ассистент,
доцент,
профессор

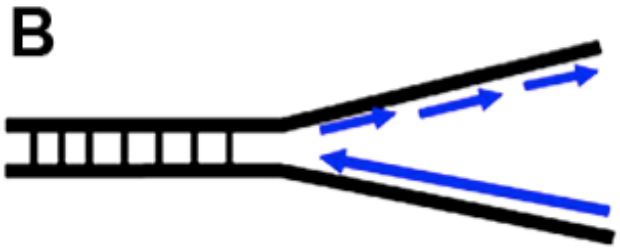
How we discovered that Lula was among us

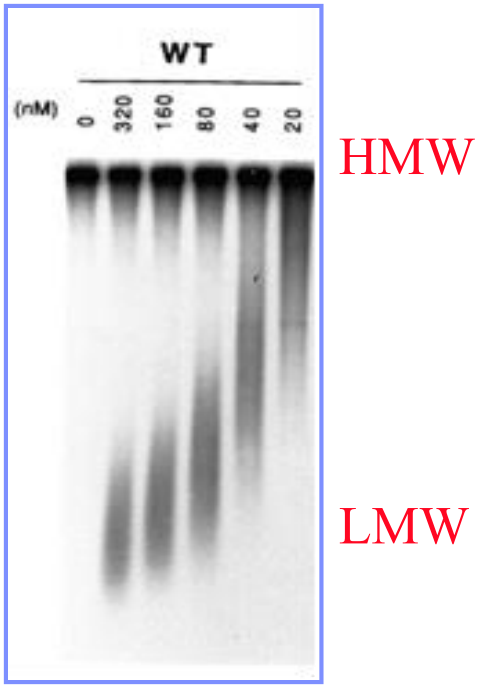
Luciana was studying DNA replication intermediates

Intermediates in DNA replication in vitro



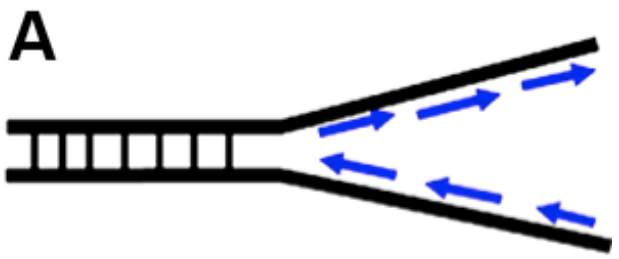
DNA replication is semi-discontinuous...



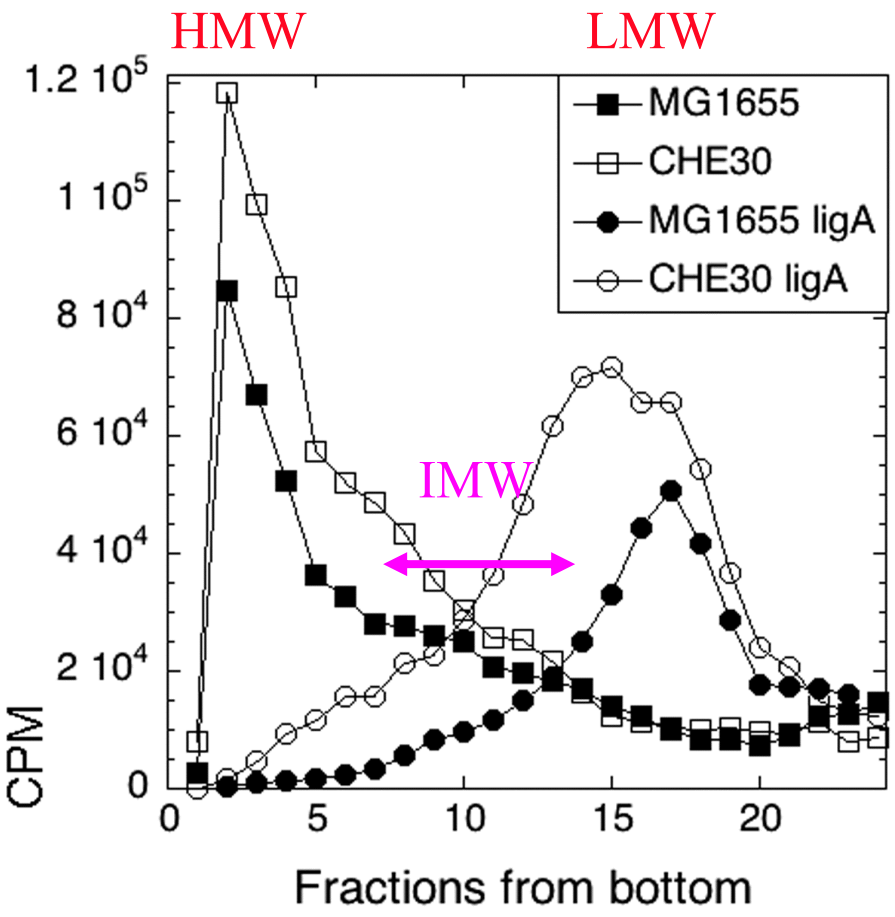


Marians et al.

Intermediates in DNA replication in vivo

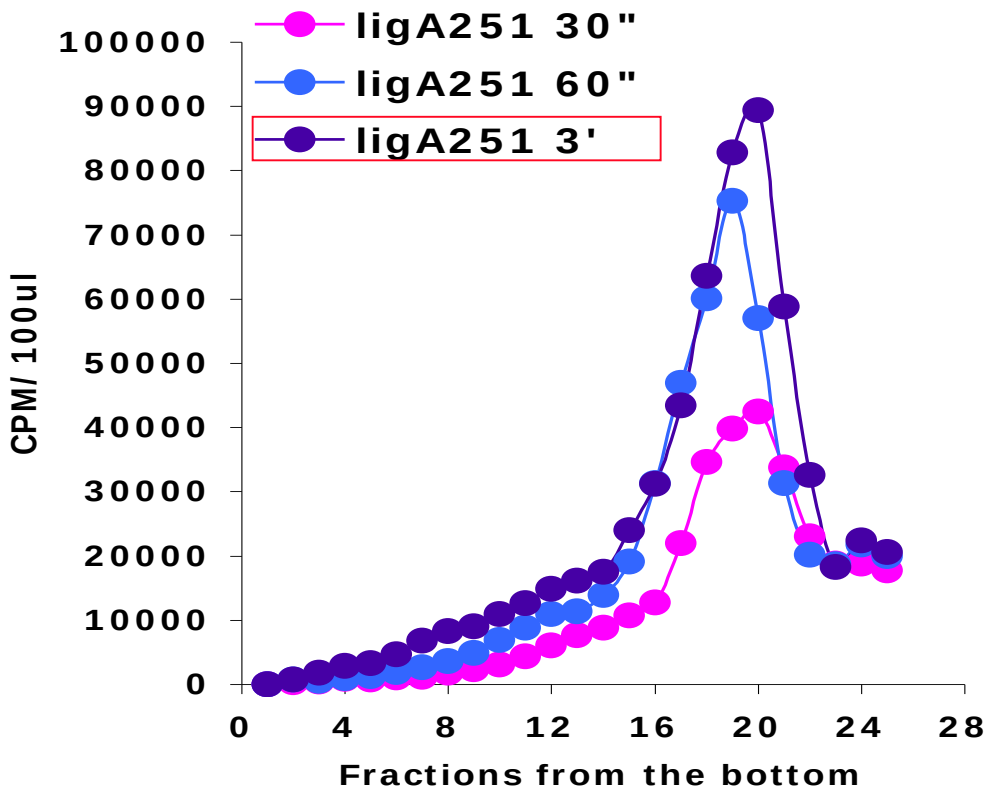


DNA replication is fully discontinuous...

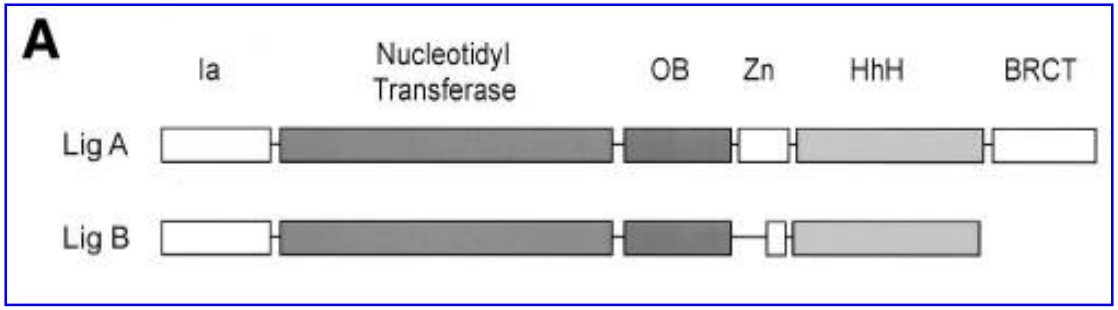


Okazaki et al., Amado and Kuzminov

Increasing preincubation time at 42° C



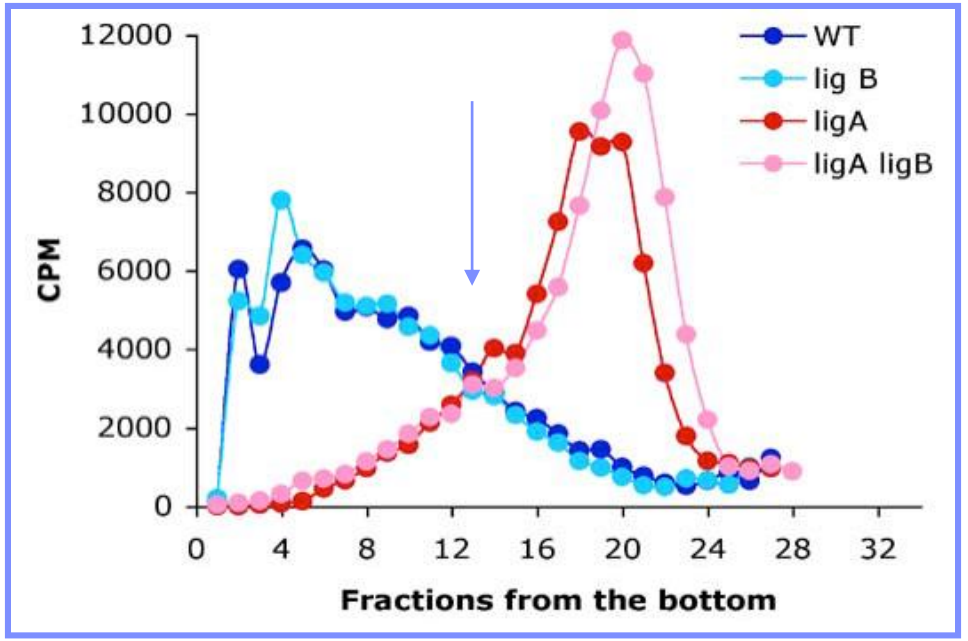
The second DNA ligase in *Escherichia coli*



-Lacks BRCT domain and two of the four Zn binding cysteines (essential for nick joining)

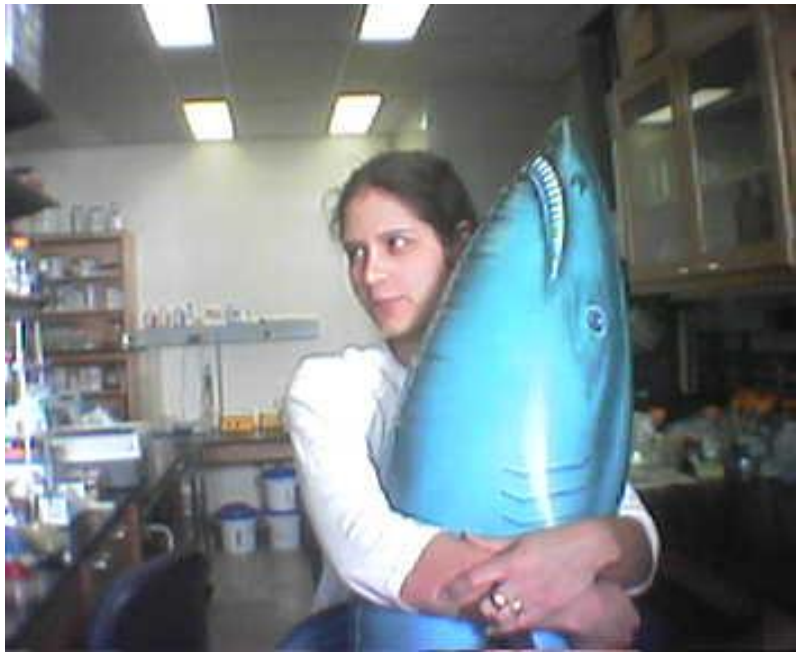
-Not much in vitro activity

Verl Sriskanda and Stewart Shuman, A second NAD+-dependent DNA ligase (LigB) in Escherichia coli Nucleic Acids Res. 2001 December 15; 29(24): 4930–4934.

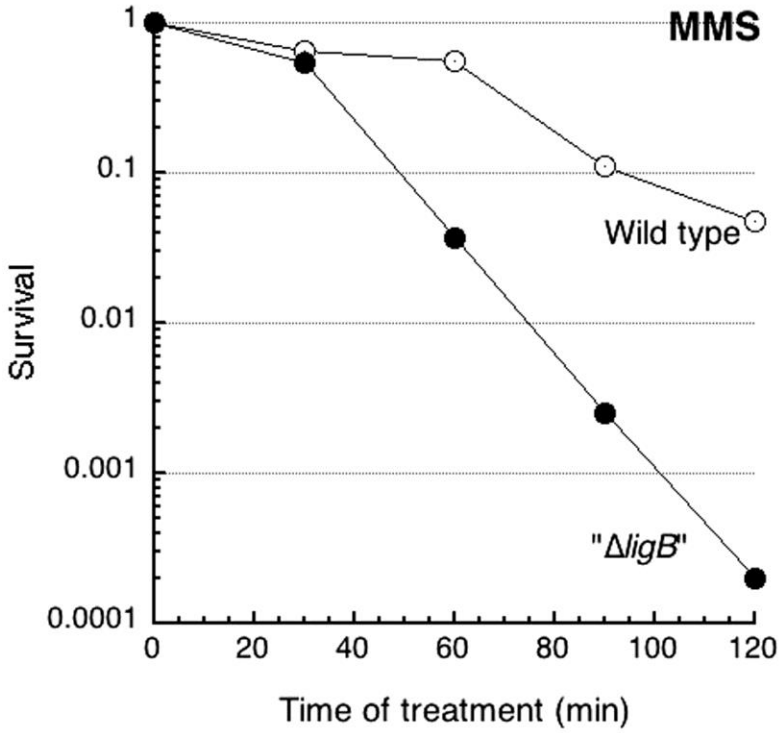


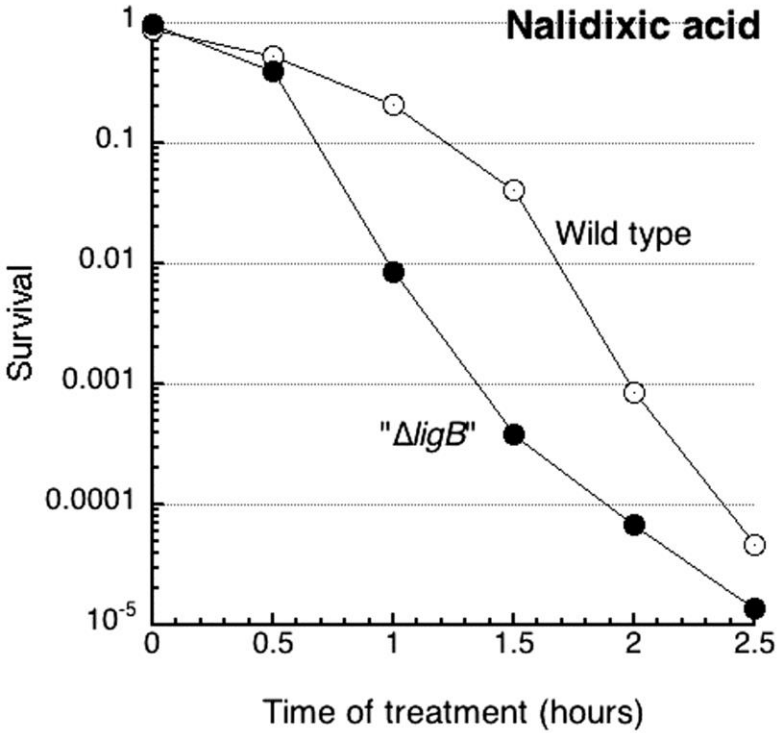
Thus, IMW replication intermediates are not due to LigB activity.

“Чтобы ещё такое сделать с *ΔligB* мутантом?”



Sensitivity of *ligB* mutants to DNA damage



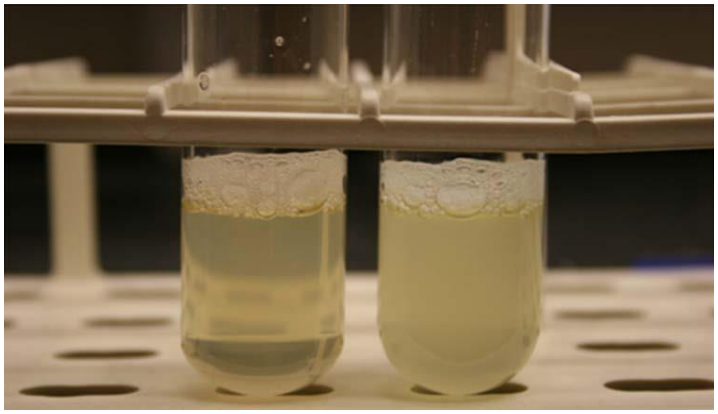


Окапи репарации ДНК!

Когда тебе кажется, что жизнь наконец тебе улыбнулась…

На самом деле она злорадно ухмыляется…

Лизис культур: след “Перуанского Фага”



— Rare, so was ignored for some time.

— Then I started teasing Luciana with this “Peruvian phage”, because nobody else in the lab was experiencing this.

— However, then partial lysis was noticed — infections were not *that* rare.

Nazca lines (Peru) — “Colibri”

— Eventually, Luciana noticed that only the WT cultures were lysing, as if the Δ*ligB* mutant was resistant to the phage.

— Naturally, the Δ*ligB* mutant was first suspected and then confirmed to carry the prophage, while the WT cells were sensitive to its lytic infection.

“Перуанский Фаг” превращается в “Орегонского фага”

— Luciana suspected that her DNA sensitivity phenotypes were due to the prophage, rather than the *ligB* defect. I thought it unlikely, knowing λ.

— She separated the prophage from Δ*ligB* and indeed found that the lysogens were DNA damage sensitive, while “clean” Δ*ligB* mutants were not. P1 transduction would move the phage, too, but not at 42° C.

— I thought that the “Peruvian” prophage was cool, but Luciana was disgusted and did not want any piece of this contamination.

— However, since she noticed that prophage moves from strain to strain by P1 transduction, she analyzed her strain constructions and traced the source of her contamination back to one of **my** strains that I constructed in Oregon and brought with me.

— Thus, the “Peruvian phage” turned into an “Oregonian phage”.

Заражение проектов у других людей

— Лусиана проверила все свои штаммы на заражение и нашала ещё несколько заражённых.

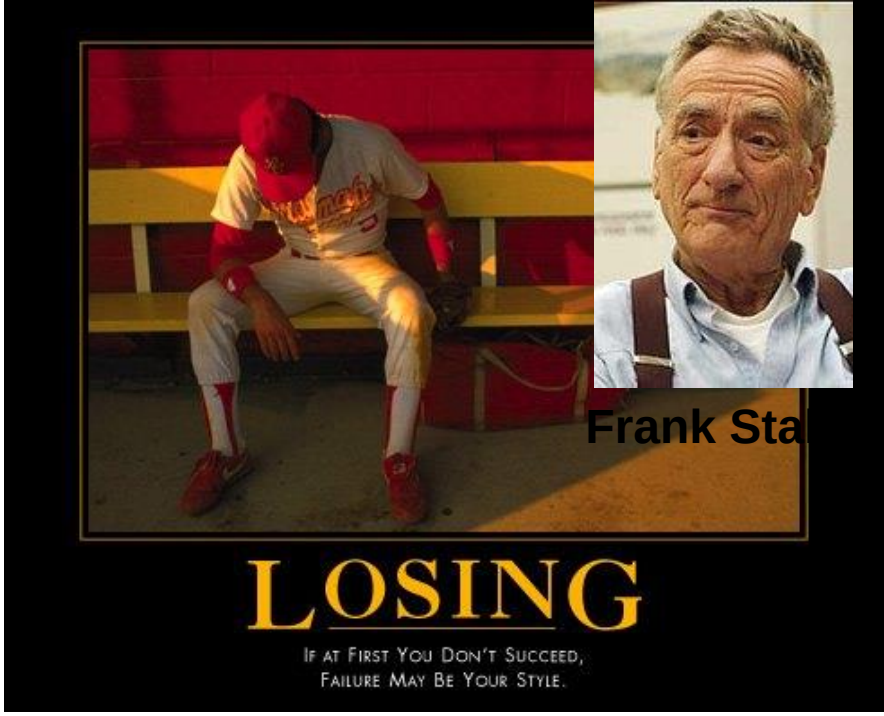
— Тут уж вся лаба бросилась проверяться на заражение “Орегонским Профагом”. Всё оказалось довольно печально…

— Некоторые проекты были заражены почти полностью, в то время как другие проекты (у тех же людей) не пострадали. Среднелабораторный уровень заражённости был порядка 10%.

— В основном профаг передвигался P1- трансдукцией, но изредка попадались и случаи прямого заражения, вероятно через совместное накачивание.

— Этоот инцидент с фагом меня сильно смущал, так как я вышел из “Фаговой лабы” и должен был знать “такие вещи”. Но у Франка я работал только с “лямбдой” и ничего подобного никогда не встречал.

Грустный конец невесёлой истории?



Frank Sta

— When I asked Frank whether he new there was a non-Lambda prophage contamination of at least one strain in his collection, and it was capable of spreading by P1 transduction, he told me he was NOT interested to hear the details.

— I felt this was the lowest part of my career:

— I did not know what to do besides the damage control measures like buying filter tips and reconstructing contaminated strains;

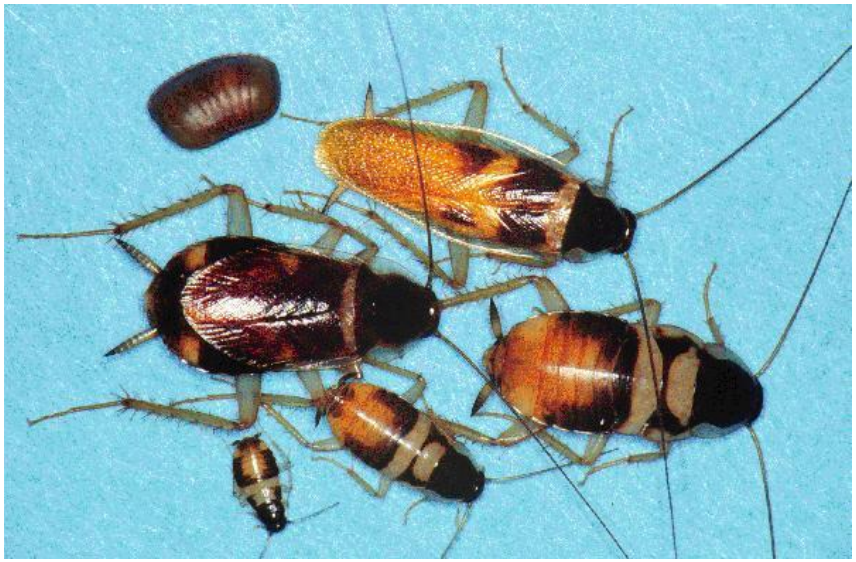
— Students openly ridiculed me, because, in their opinion, I was supposed to recognize prophage contaminations early on;

— My own advisor made it clear it was some kind of a taboo.

— On the bright side, I was naturally interested in the “Oregonian phage”, but who would volunteer working with “contamination”?

Как мы вернули утерянный контроль над лабораторией

Психические аспекты, мешающие борьбе с загрязнением



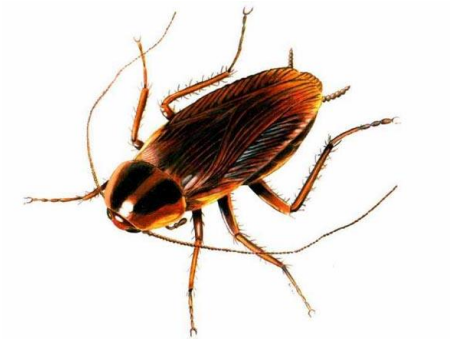
— Психологическое отвращение.

— Био-загрязнение = заражение.

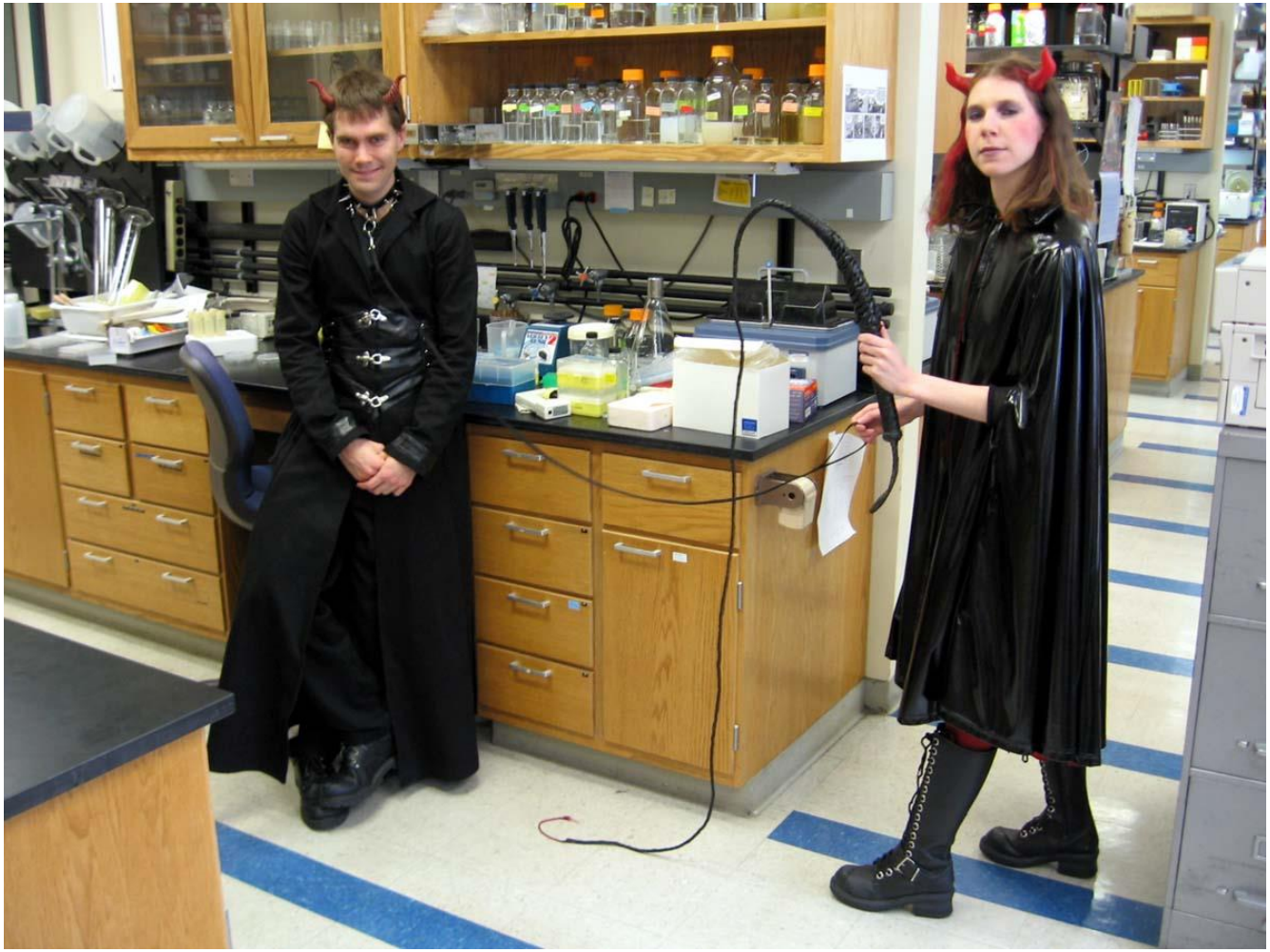
— Кто балуется с загрязнением, пачкается сам...

Ella was studying the chromosomal abnormalities in Δ*seqA* mutants

Certain parts of her project were heavily contaminated by the “Oregonian prophage”, although, luckily, no conclusions were influenced by it.

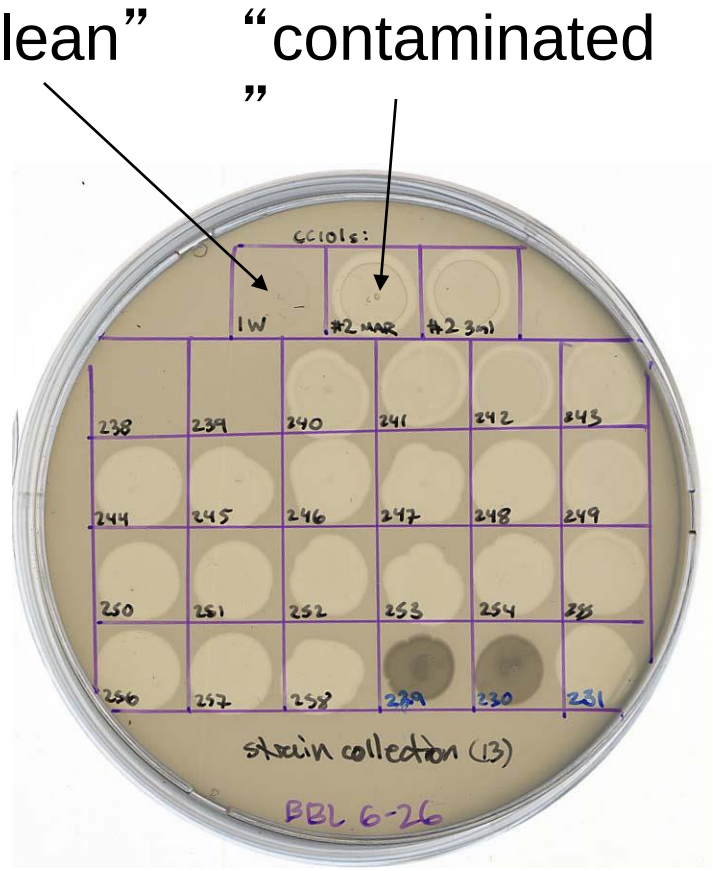


Ella and cockroaches

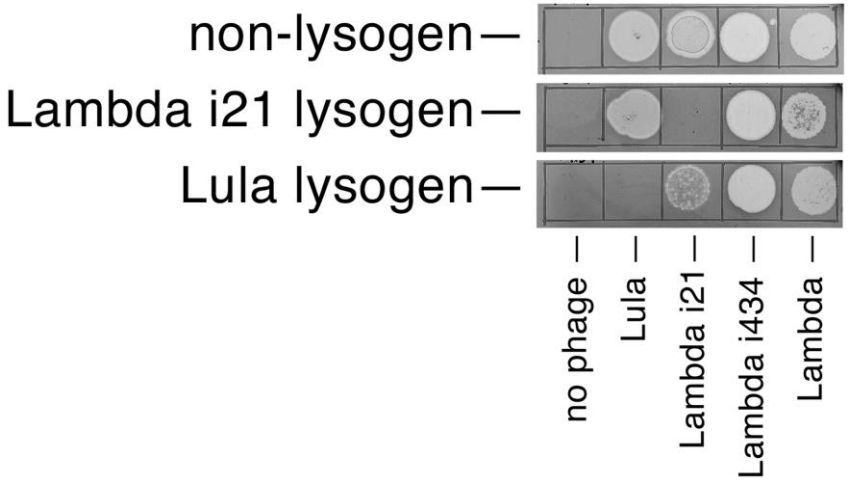


Елла быстро установила размах загрязнения

— She streamlined the protocol for detection of contamination and tested a lot of strains:

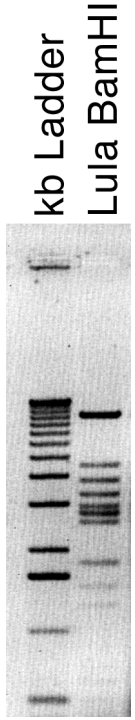


Элла сделала “заразу” своим питомцем



— Like me, she thought that phages were cool, especially if they were unknown.This phage was definitely not lambda by its immunity.

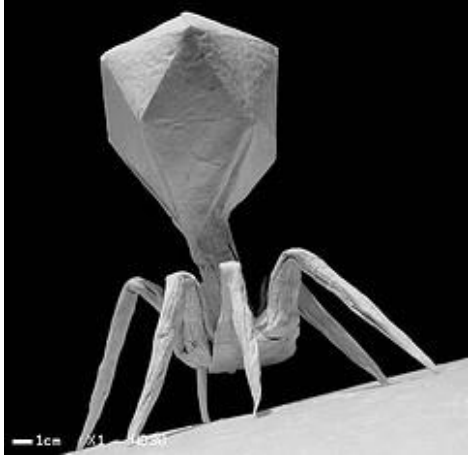
— Ella purified DNA of the phage and found that its restriction pattern was (expectedly) different from Lambda.



— Sequencing from the ends of the cloned fragments revealed that the phage had no strong homology to anything published at that time, but some weak homology to some lambdoid phages.

— So, the contamination was a temperate lambdoid phage.

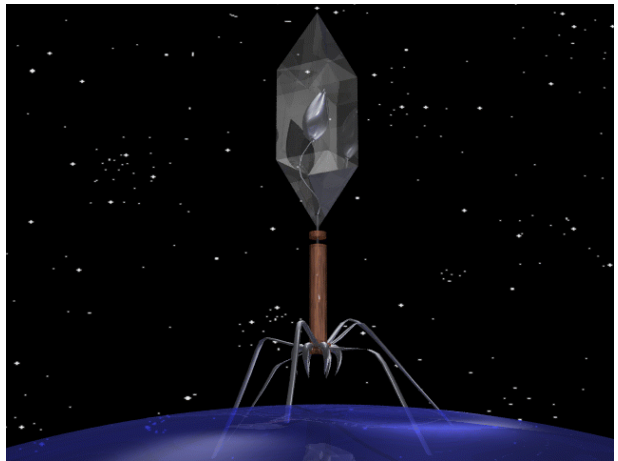
Как назвать Заразу?



— No names were coming from students, so half-jokingly I started calling the phage "Lula", which combines the names of both Luciana and Ella.

— Neither student liked the name, and it took time to be accepted. “A perfect name for exotic dancer”.

— *Lula* means "squid" in Portugese, and I found comparisons of phages with squids especially appropriate.



А тут ещё…

— I was not sure about where the Lula characterization was going or whether the story is publishable at all.

— In the meantime, Ella was diligently checking all the incoming strains for prophage contamination.

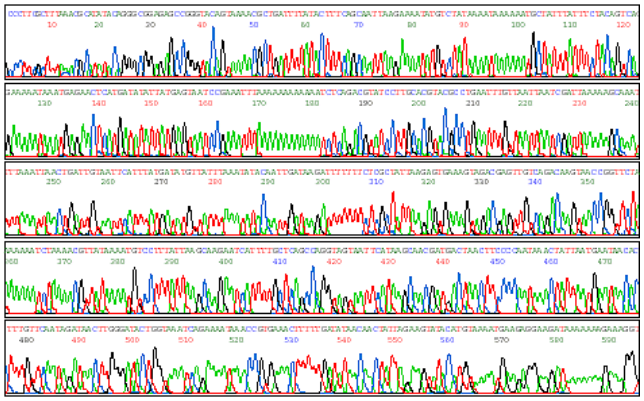
— She found a few, coming both from individual labs, as well as from the *E. coli* Genetic Stock Center.

— We felt better about it, — we were not the only ones contaminated after all!

— However, the surprising finding was that all patterns of genomic digestion of contaminant phages from various sources matched the one of Lula!

— So Lula was infecting *E. coli* collections across the country!

Нуклеотидная последовательность Лулы



— At this point we decided to sequence Lula.

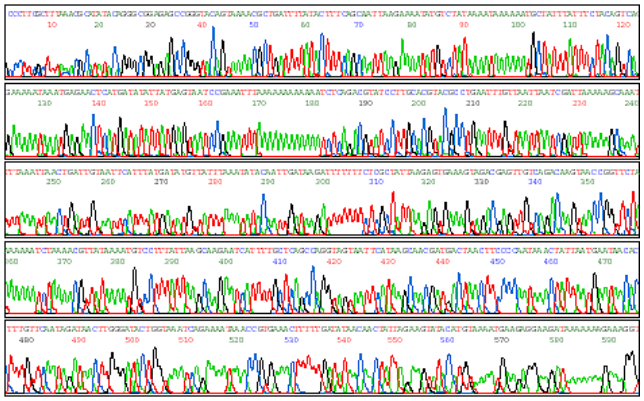
— Lula turned out to be a close relative of phi80 — a long-forgotten lambdoid phage from the 60s and 70s.

— phi80 was found in Japan and at one point was almost as popular as Lambda.

— Lots of Lambda-phi80 hybrids were generated.

— At that time, phi80 sequence was "undetermined” (only a few short fragments were in the database) but we started asking around.

Lula = phi80



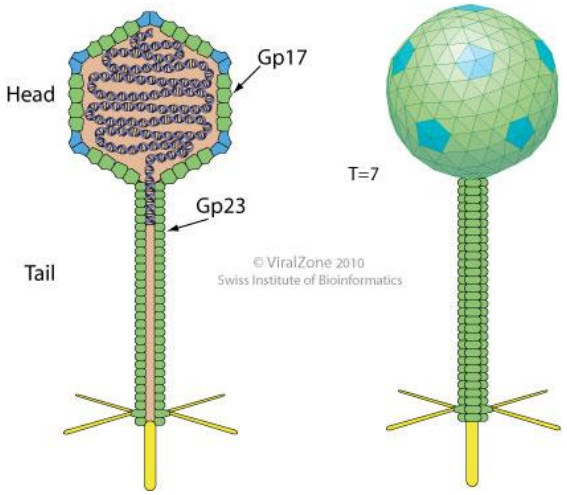
— Eventually, Sherwood Casjens (Utah) said that Guy Plunkett III should have it.

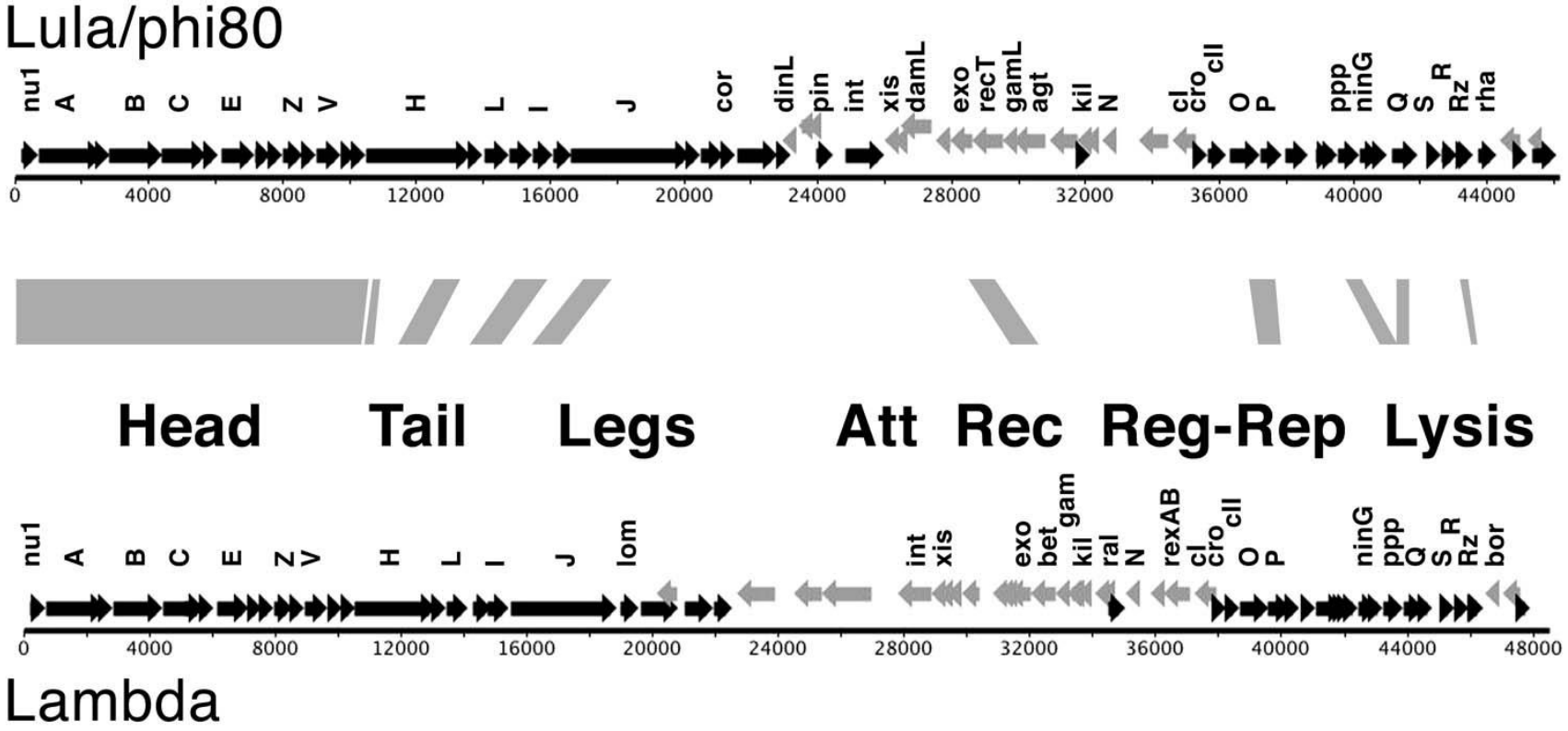
— Guy used to be with Blattner, but Fred already closed his lab at Wisconsin. Luckily for us, Guy was still around, and even still had access to his old sequencing gels!

— It turned out that they have sequenced phi80 many times over, because they used it to test their new sequencing protocols. But never published the sequence.

— Anyway, Lula and phi80 turned out to be identical, — down to a single bp!

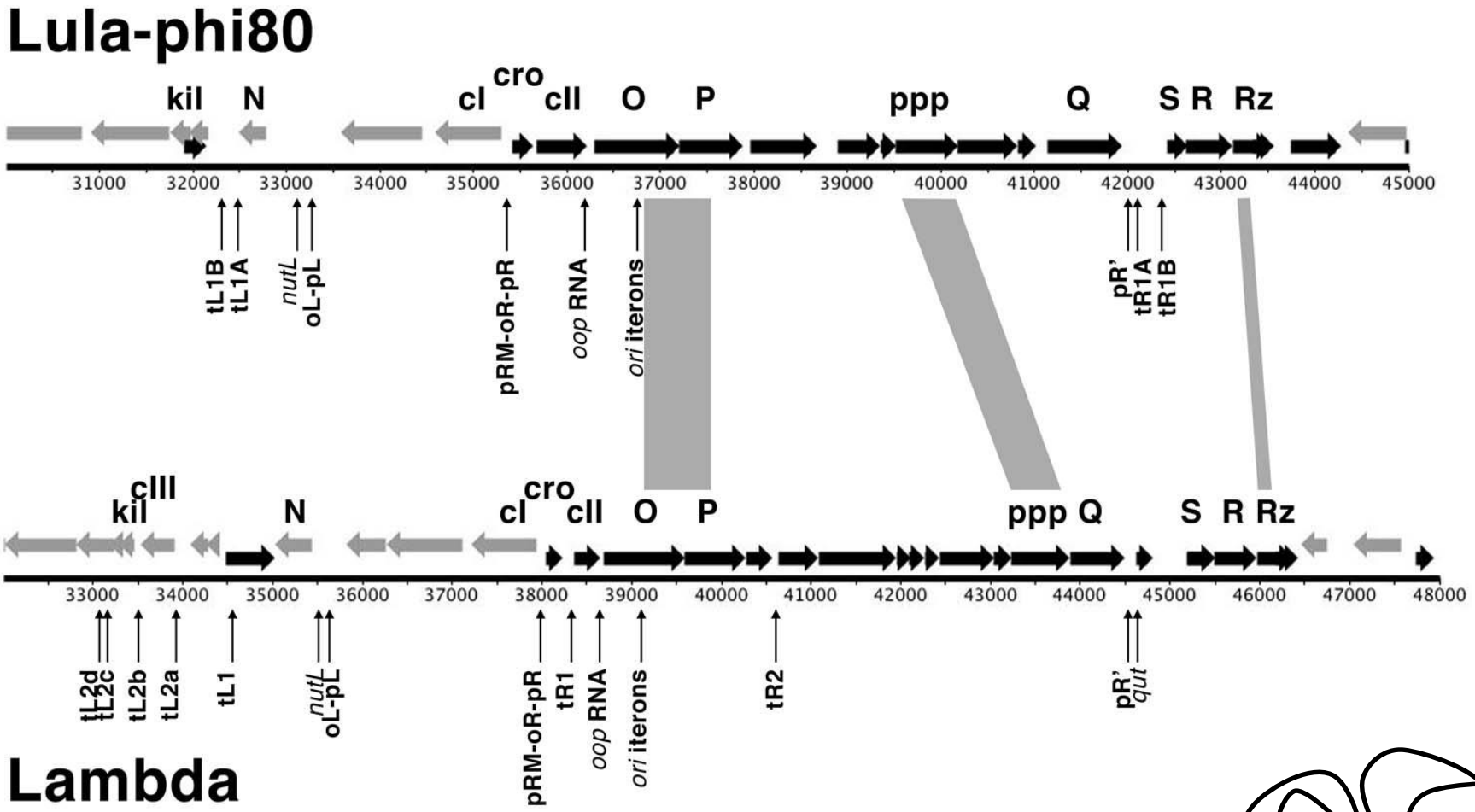
Lula/phi80 genome overall layout



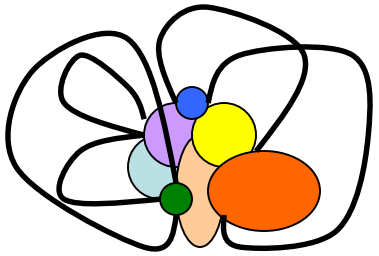


The overall genome structure was surprisingly Lambda-like, although real homology — 70% to lambda, 80% to N15, was restricted to the head/tail region.

Lula/phi80 control region

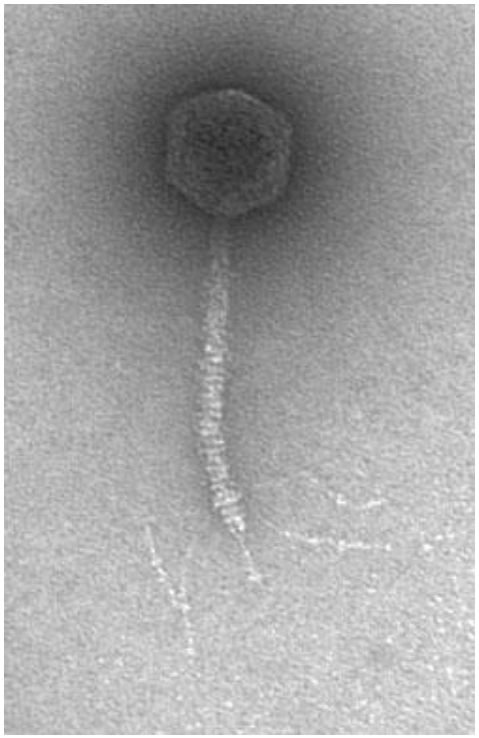


The reason for this remarkable layout conservation?

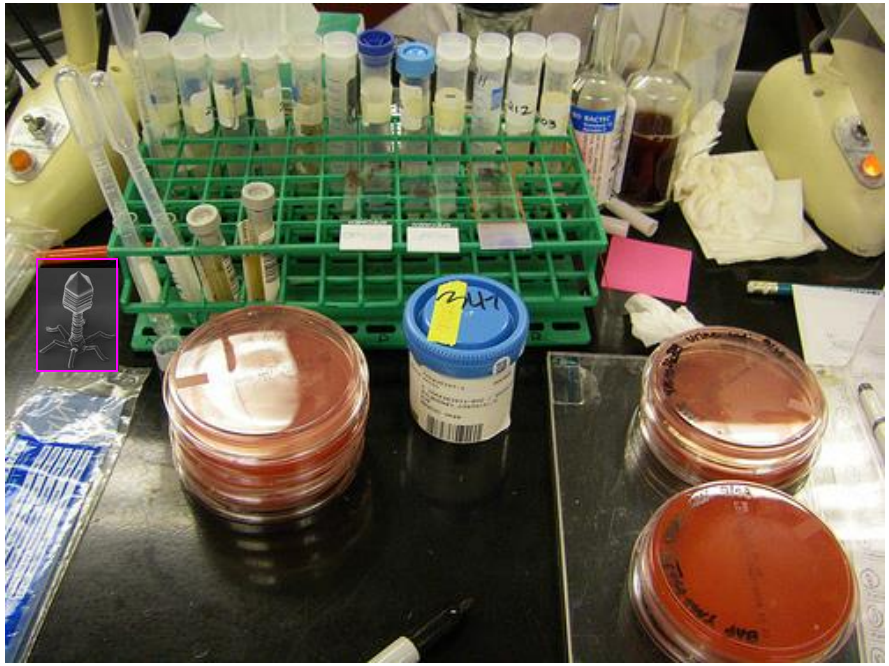


— the specificity determinant was HK022/HK97/T1/N15 mixture, with a significant human homology (two genes are “human”)!

— Thus, Lula contaminated not only the *E. coli* collections across the US, — it actually penetrated the human genome databases!



Лула прижилась в лабах



— We may see messy lab benches here, but for Lula it is a livable habitat.

— But then, how does it do it? Is Lula the only organism that can defy our controls over the laboratory environment?

The theory of cross-contamination

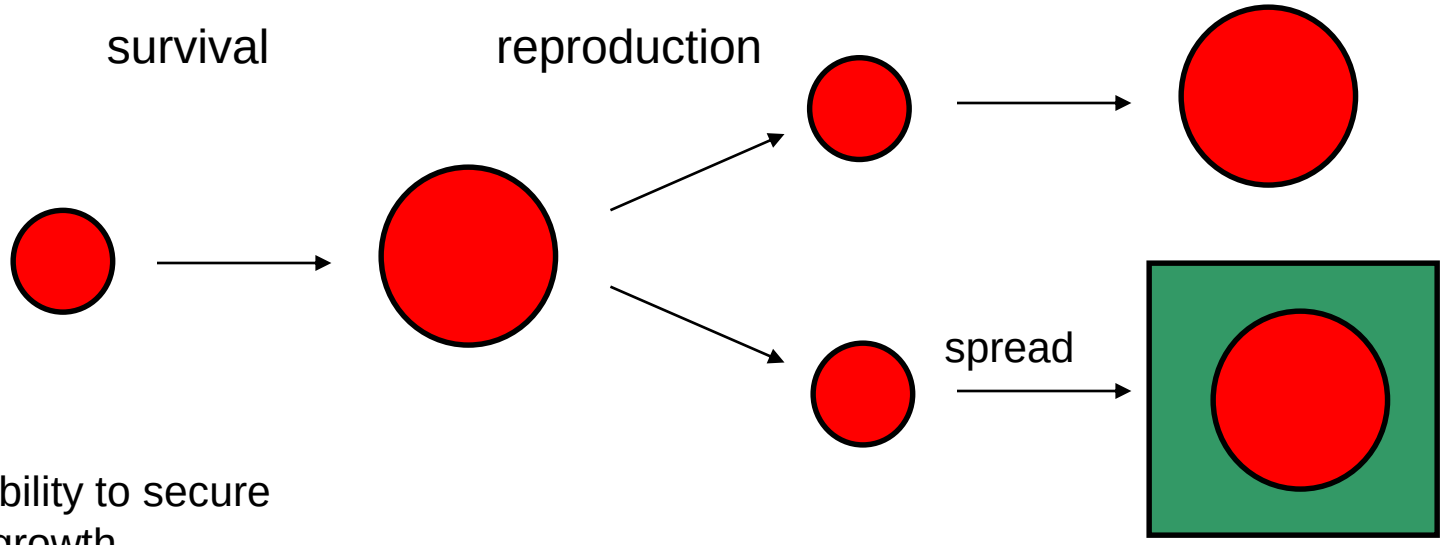
Организмы приспособлены к своей среде обитания

Выживание: способность добывать ресурсы для роста

Размножение: выведение потомства

Распространение: завоевание новых ниш

Адаптация = выживание + размножение + распространение



Survival: the ability to secure resources for growth.

Reproduction: the ability to leave progeny.

Spread: the ability to settle in new niches

Лаборатория как среда обитания?

— Лабораторные организмы не котролируют свой рост, размножения и паспространение — поэтому лаборатория для них не является средой обитания.

— Экспериментатор сам контролирует адаптацию экспериментальных организмов.

Лаборатория так устроена чтобы как раз НЕ быть средой обитания

... precisely by denying a chance to adapt to it!

How to prevent Life from turning laboratory into an environment?

Protocols

— growth limited in time (temporal)

— growth limited in space (growth vessels)

Homogeneity (restriction to a particular strain of experimental organism by…)

— seeding with individual organisms, colonies, etc.

— checking phenotypes

— monitoring growth characteristics

Barriers to cross-contamination

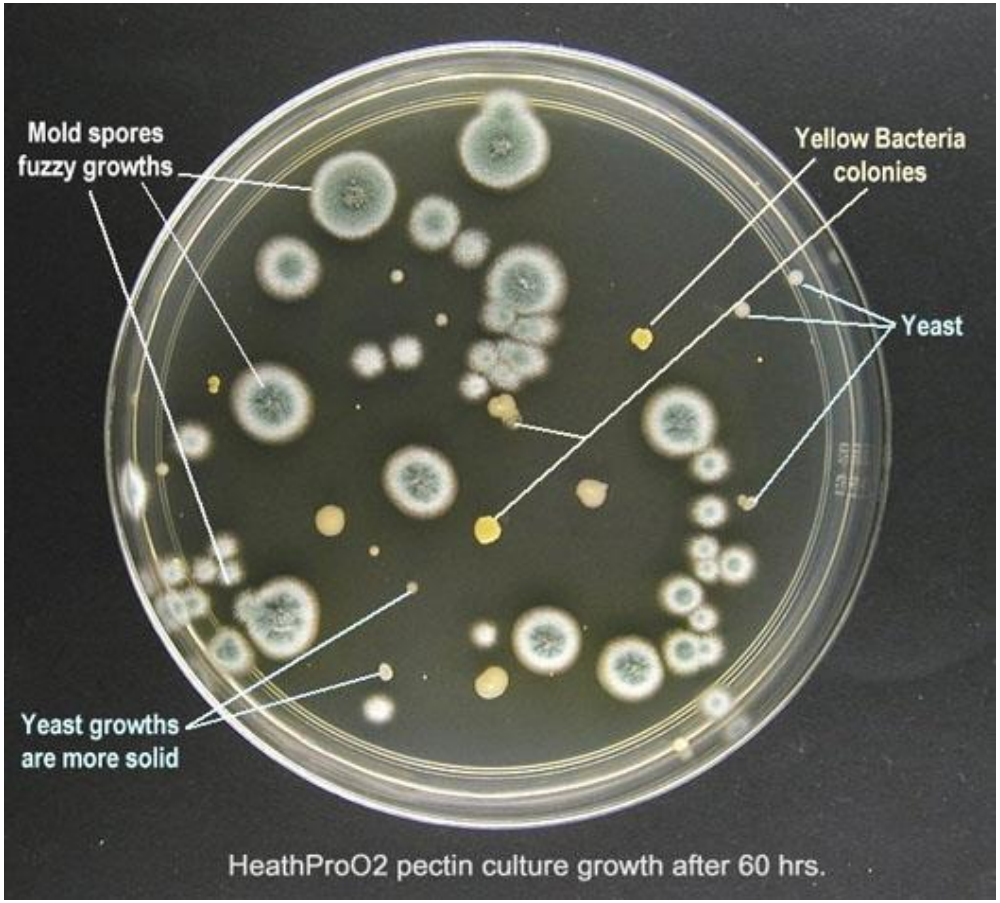
— separate and closed growth vessels

— single-use manipulation tools

— sterilization

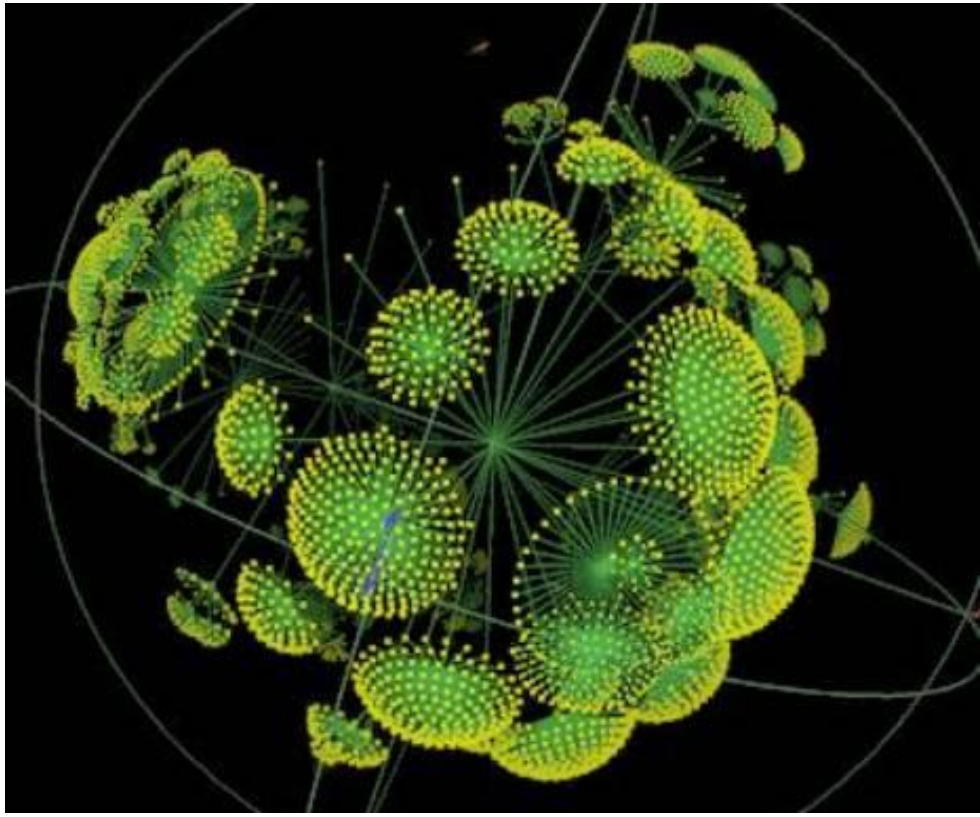
— frequent verification

Example: preventing co-habitation



А можно ли вообще превратить лабораторию в среду обитания?

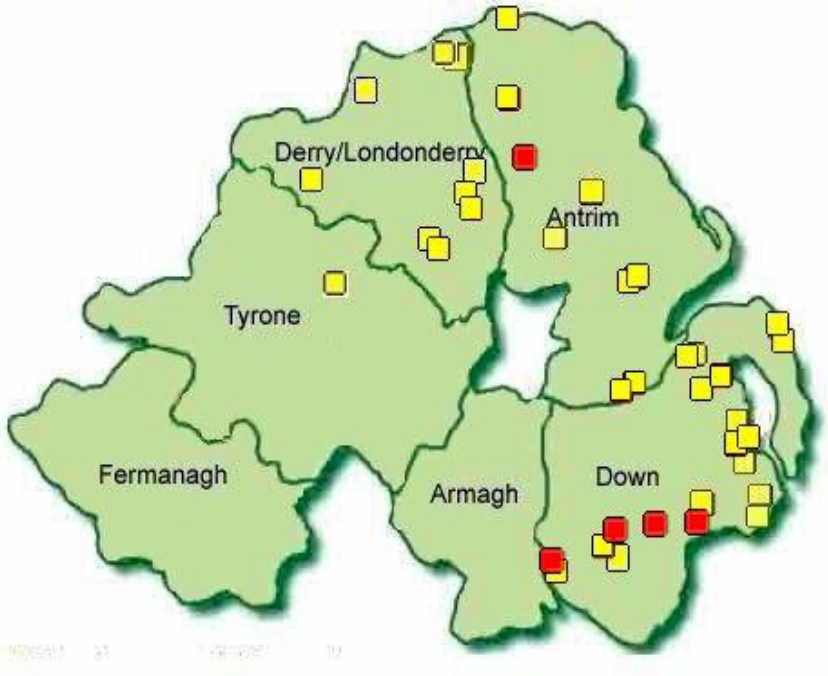
— Cross-contamination that is not recognized as as such may allow unauthorized growth, multiplication and spread for a significant period of time.



Example #1: cross-contamination, which is not immediately apparent

— Cross-contamination of clinical samples with the positive-control strains of pathogenic bacteria is a well-known, if under-appreciated, challenge for testing laboratories.

In ecology terms, this is "spread to new niches"



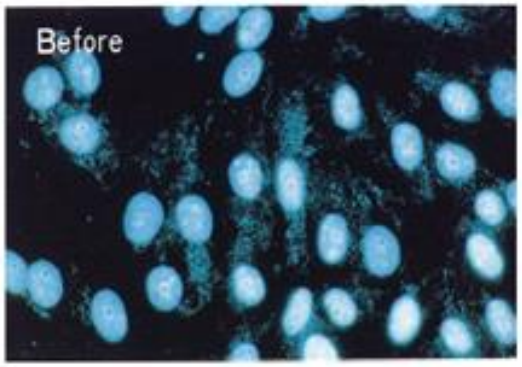
"false outbreaks"

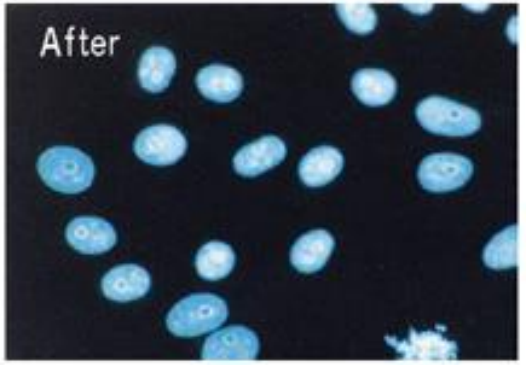
Lab spread tricks learned

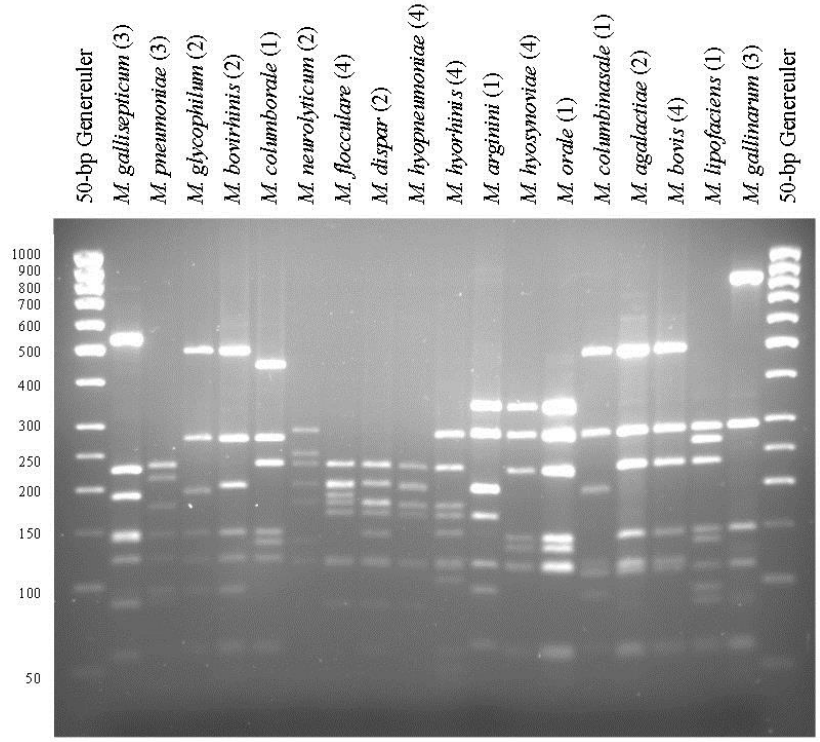
1. Experimental material mimicry.

2. A careless tech or grad student is your best friend.

Example #2: mycoplasma contamination of cell cultures







— Conservatively, at least 30% of all cell cultures are contaminated.

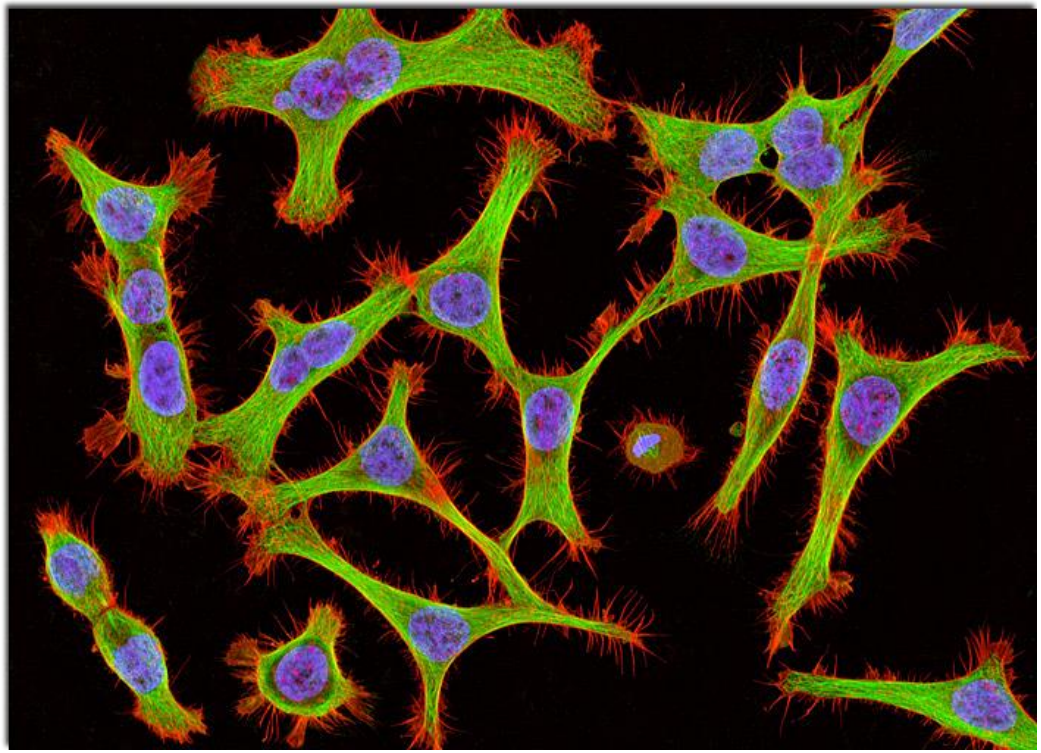
— In some countries, this number goes up to 90%.

Lab spread tricks learned

— Commensals go unnoticed

— Lab protocol free-riding

Example #3, HeLa cells



— Cross-contamination in cell culture is quite common, because of mimicry.

— However, one particular contaminant stands out.

— At least 10% and may be up to 20% of all cell lines out there are either contaminated with HeLa cells or *are* HeLa cells.

— Although the problem was discovered in 1967, it is only worse today…

— Thus, HeLa cells multiply and spread in the lab, using it as environment.

Lab spread tricks learned

— Covert productivity (based on mimicry).

— Spreading through aerosol.

Example #4, easily the most disturbing one

— We thought that Lula cross-contamination may still be unique because it is:

— a contamination of rapidly-growing bacteria, which are hard to cross-contaminate with something other than related bacteria (because of fast growth)

— a cross-contamination with a phage (a virus).

— Then we learned that cross-contamination with uncharacterized viruses in cell cultures is pervasive, but tends to be ignored until something really bad happens.

Nothing is new under the Sun…

So, how do our barriers to cross-contamination stack up against the tricks of spread?

Barriers to cross-contamination

— separate and closed growth vessels

— single-use manipulation tools

— sterilization

isolate

— frequent verification of phenotypes and growth characteristics

— going back to collection

check & discard

Spread tricks

— experimental material mimicry in conjunction with faster replication

OR

— Experimental material commensalism (again, with faster replication)

— spreading through aerosol

— lab protocol hitchhiking

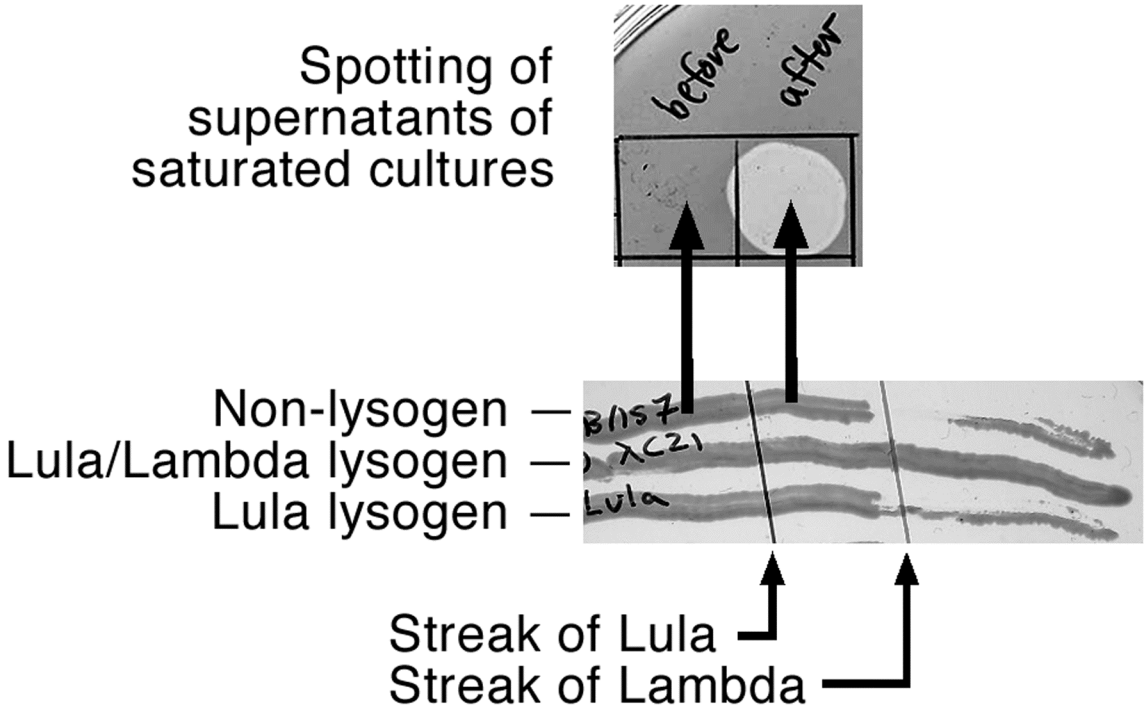
— sneaking in during new strain construction

Characterization of Lula

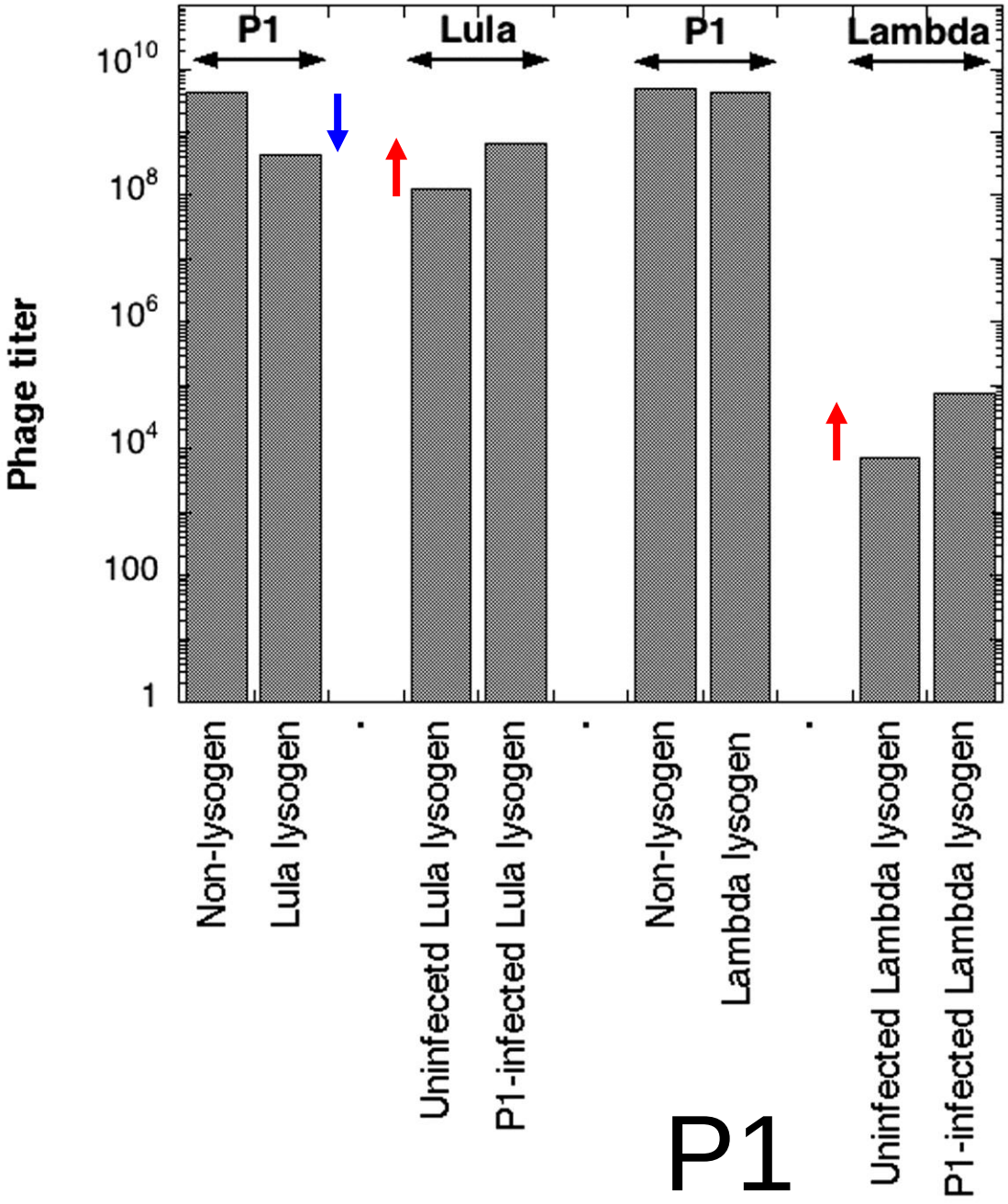
We decided to learn tricks of Lula's spread

— By characterizing several aspects of Lula's infection, having Lambda as a negative control.

Lysogeny test

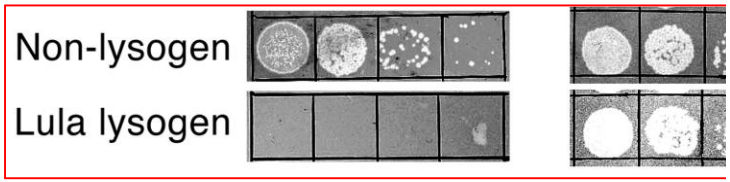


Interaction with lytic phages

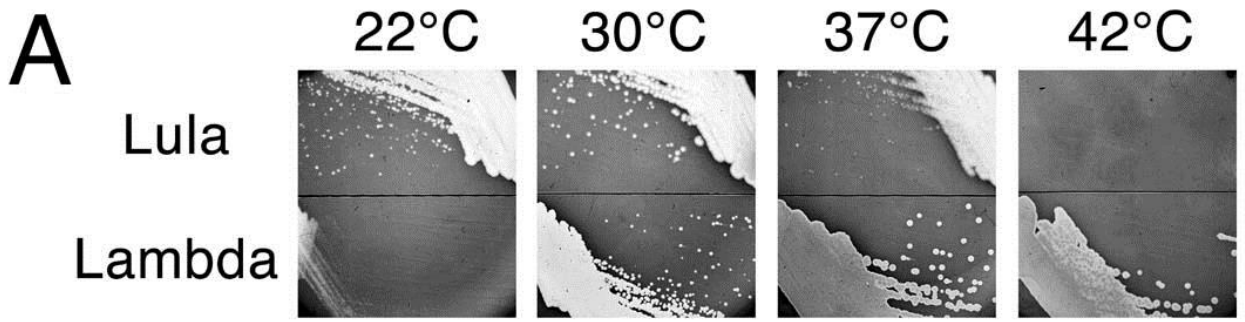


P1

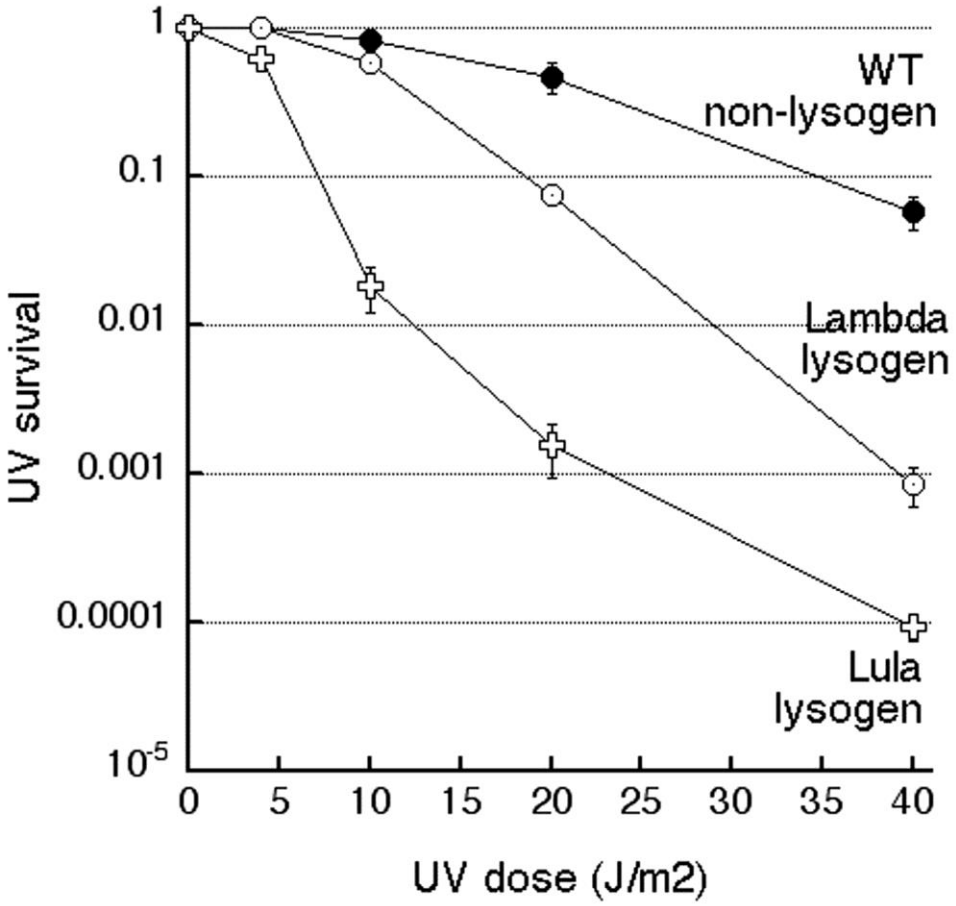
T4



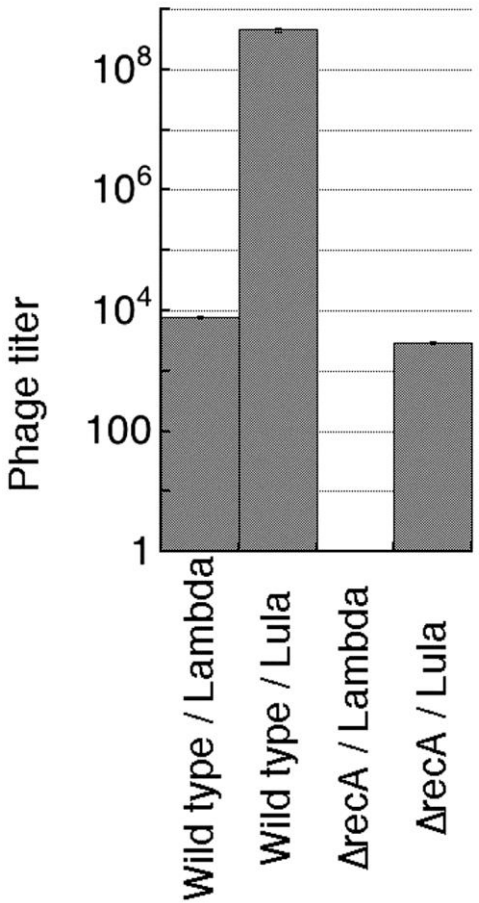
The temperature gradient

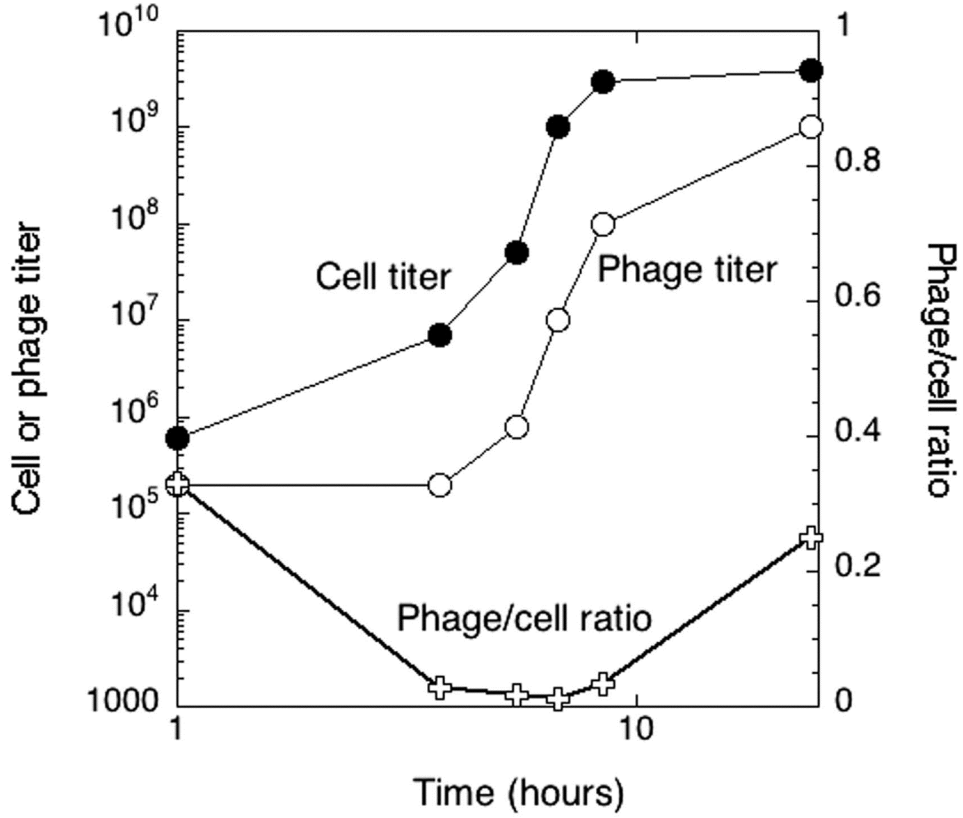


UV killing


1
0.1
0.01
0.001
0.0001
10^{-5}
UV survival
0
5
10
15
20
25
30
35
40
UV dose (J/m2)
WT
non-lysogen
Lambda
lysogen
Lula
lysogen

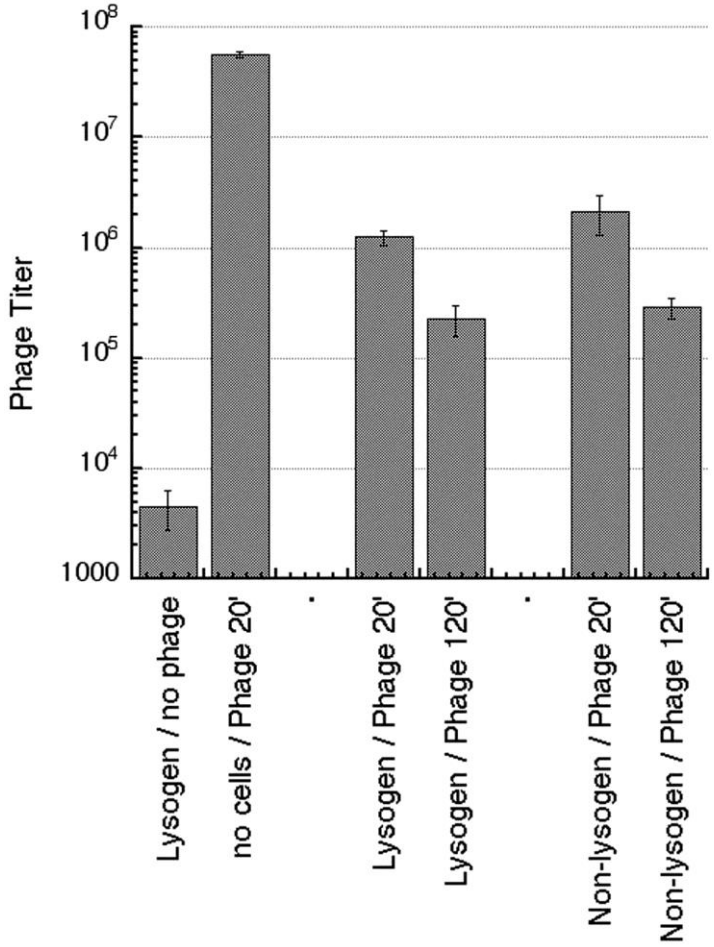
Culture titer



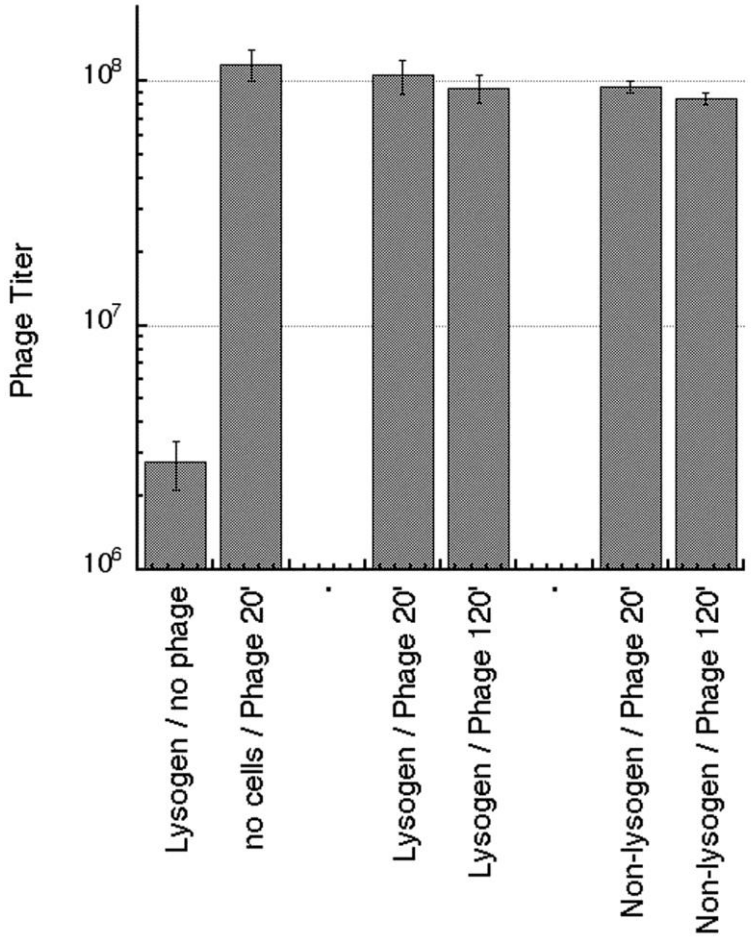


Stability in saturated cultures

Lambda

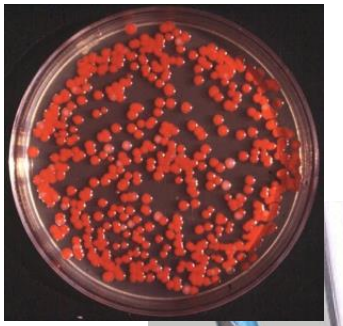


Lula

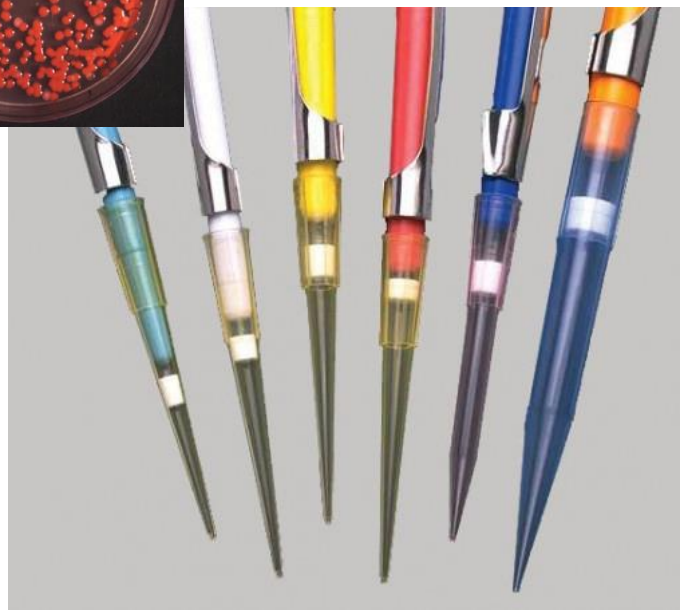


Creatures that spread in the lab should be resistant to either earosolation or desiccation

— Many laboratory procedures, including all types of handling of liquid cultures, generates aerosols.



— Try to find references for these "common facts".

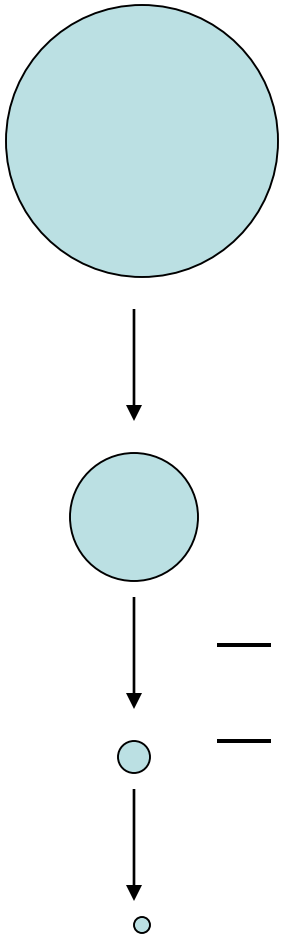


— Back in 1940s it was documented that shaking cultures, opening microtubes and pipetting are all aerosol generators.

— The worst culprits, in fact, are ...

Why orbital (rotary) shakers, rather than reciprocal ones?

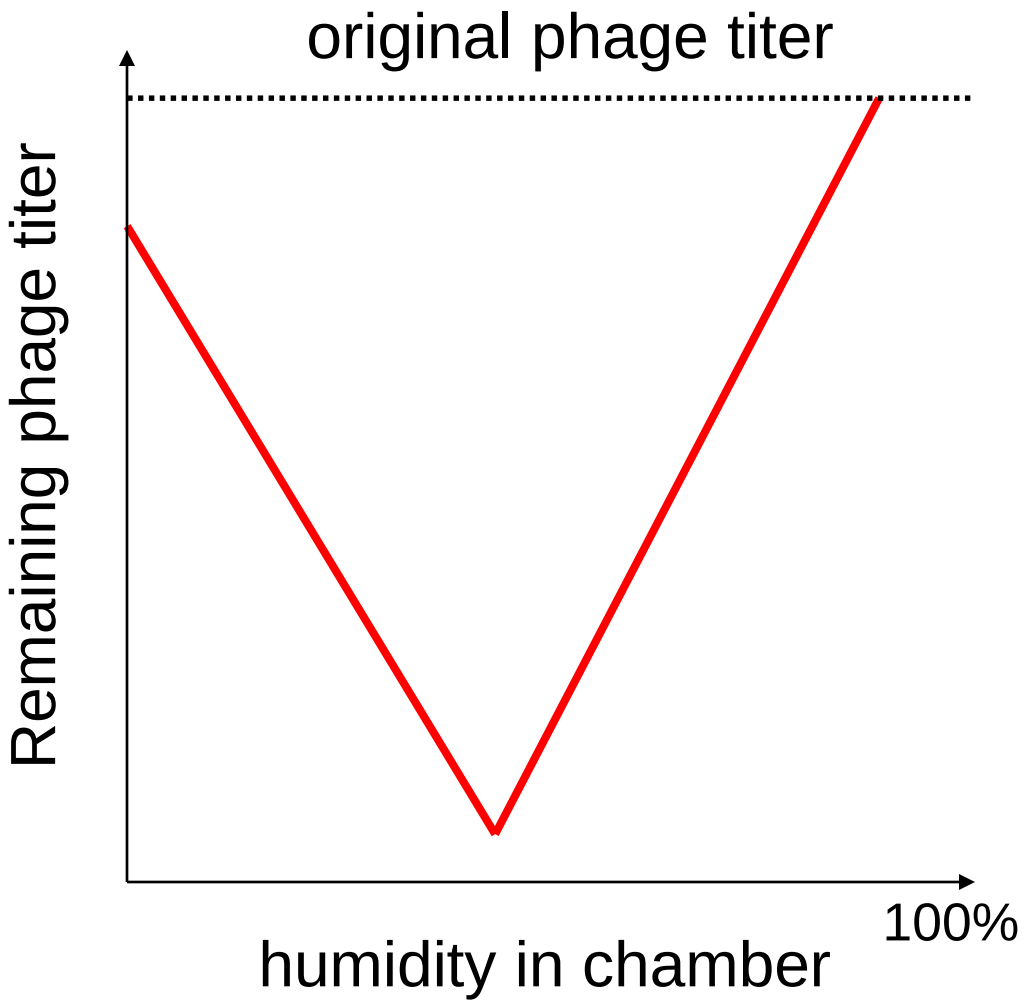
The challenge of small droplets



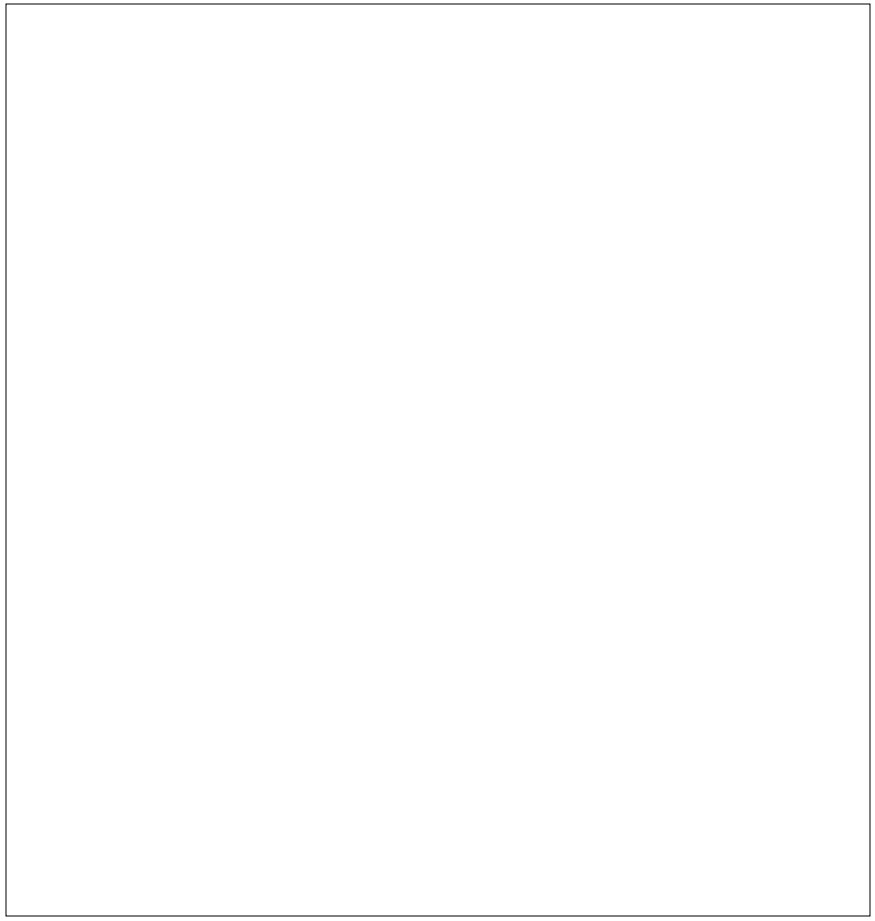
— Surface tension;

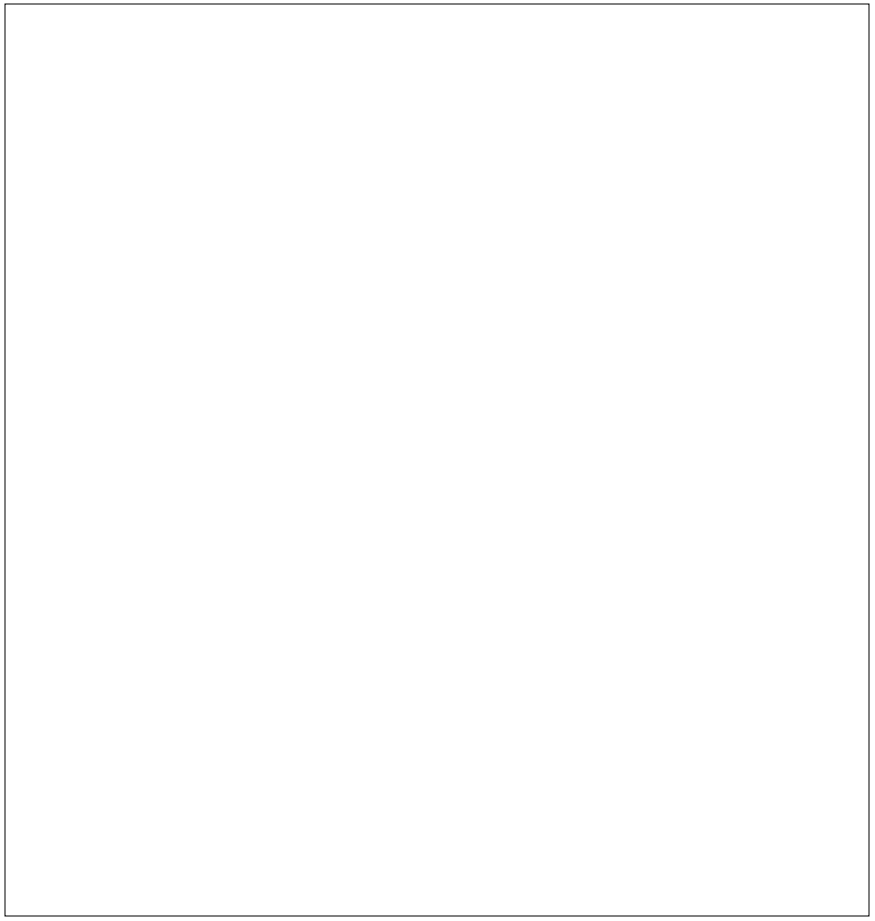
— desiccation

Survival of phages in aerosols



Resistance to aerosolation





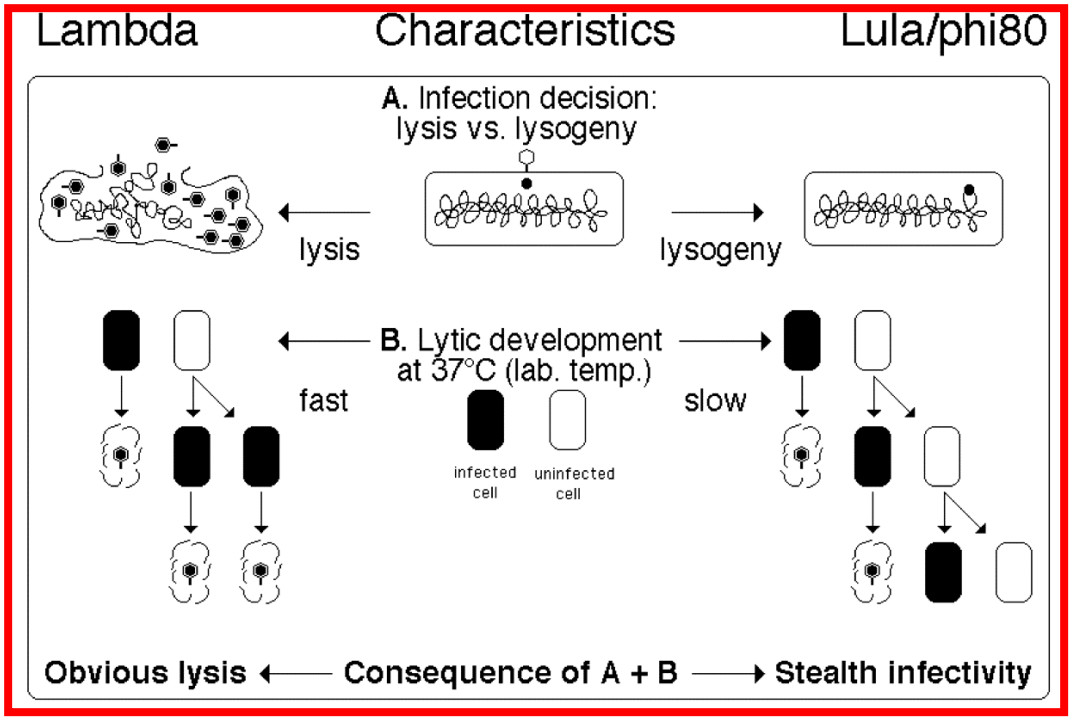
Summary of our findings-1

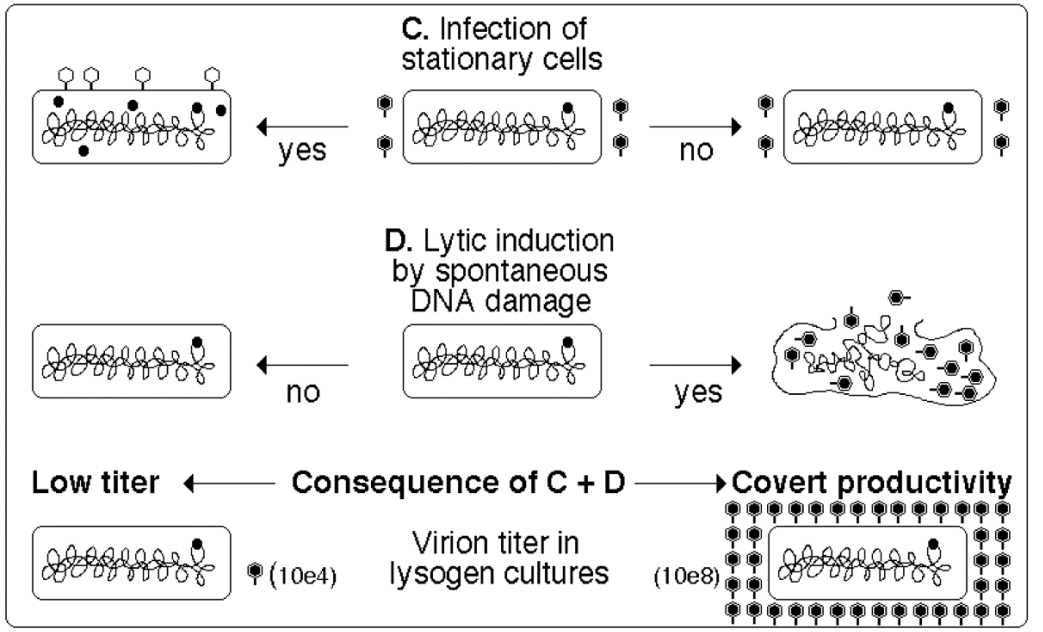
A priori, generic qualifications for cryptic horizontal spread in the laboratory environment should include:

1) stability against aerosolation/desiccation, as aerosols are likely to be the major horizontal spread mechanism in the laboratory;

2) either experimental material commensality or mimicry, to hide the non-sanctioned growth;

3) stealthy infectivity — efficient infection of diverse non-contaminated materials with a minimal subsequent evidence of contamination.





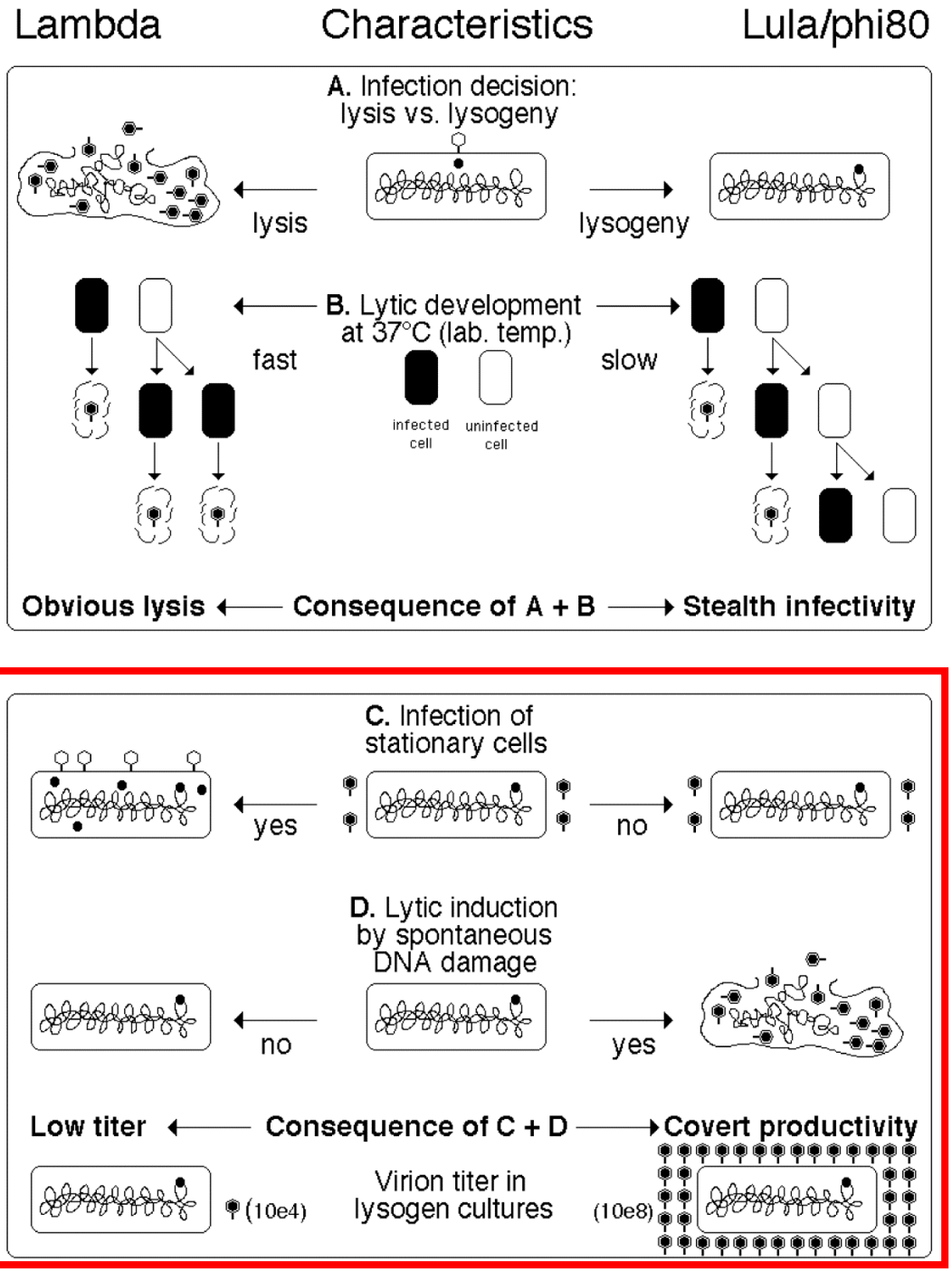
Summary of our findings-2

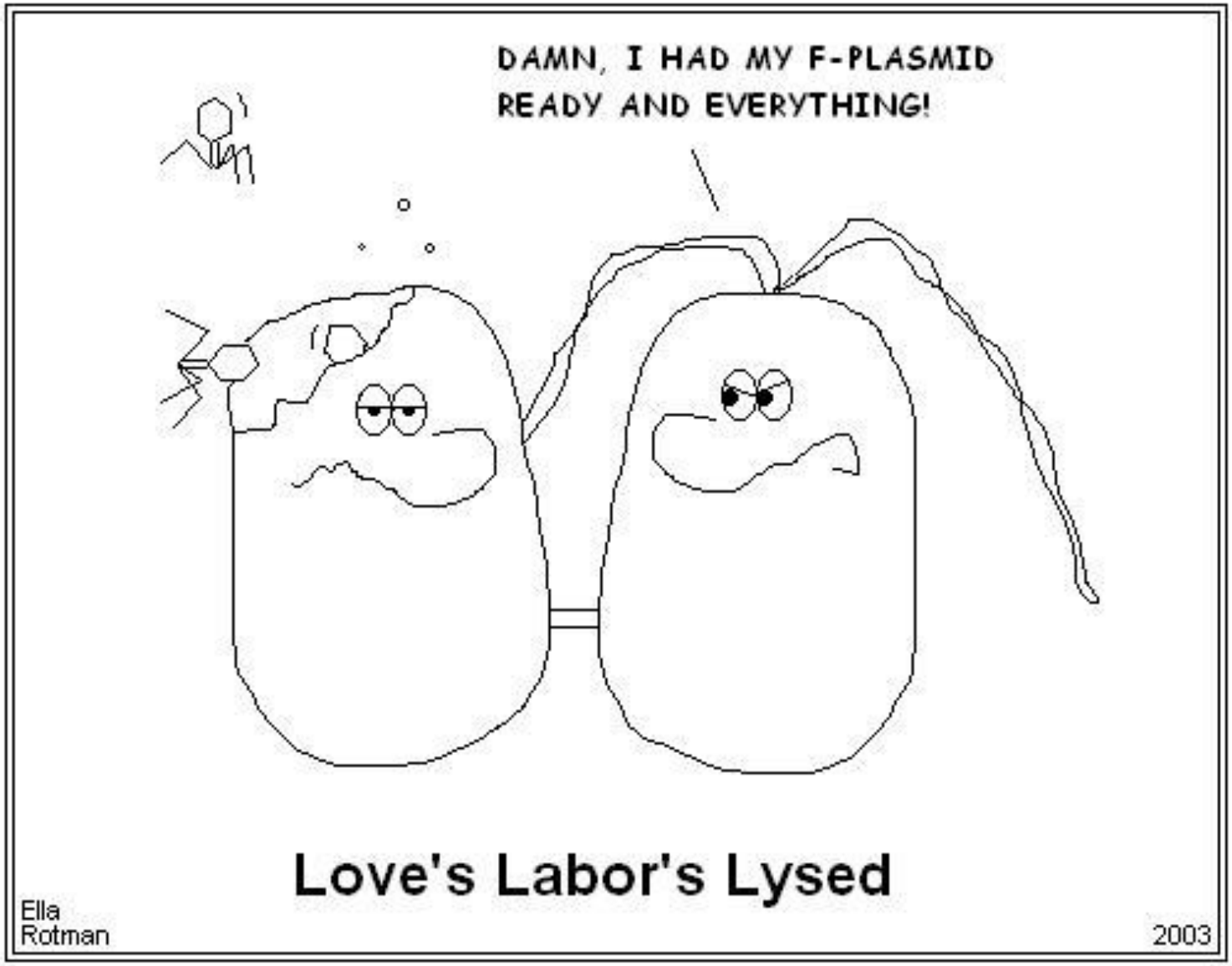
Additional qualifications for survival via horizontal spread in the laboratory, which we, *a posteriori*, can identify as:

4) covert productivity — continuous production of the agent by the contaminated research material to the highest possible level which is still inconspicuous, achieved via crude synchronization of replication of the agent with the one of the research material;

5) stability against the distinct challenges of the laboratory environment (like survival in saturated cultures);

6) "protocol hitchhiking" — facilitated spread of the agent via common laboratory practices and protocols.




DAMN, I HAD MY F-PLASMID
READY AND EVERYTHING!
Love's Labor's Lysed
Ella
Rotman
2003

The Human Accomplices of Lula

The social factors

1. The tragedy of the commons (carelessness (blissful ignorance), contamination aversion (pride)).

2. Blaming the victim (unknowingly).

3. T1 scare

4. Unwillingness to accept responsibility (waiting for the scapegoat)

5. Lack of vigilance. The Cassandra syndrome.

1. The tragedy of the commons

Shared facilities — the primary battleground of the spread.

Curators of the shared facilities are the ones that are in a position to observe, react to and document the contamination.

Multiple users do not share common practices, sensitivities to contamination.

— Carelessness (blissful ignorance)

— Contamination aversion (pride)

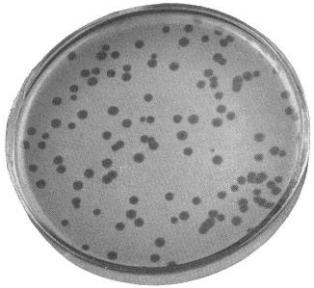
2. Blaming the victim

— "Contaminated" users have lysogens, — therefore their strains do not lyse.

— "Clean" users have non-lysogens, — therefore their strains tend to lyse in contaminated central facilities.

— Those whose strains lyse are presumed to have "dirty cultures" and are blamed for their problem by exactly the culprits that bring in the contaminated strains (which do not lyse — thus, giving their owners "immunity" from suspicion).

3. T1 scare



— Lula lysogens are resistant to infection with a lytic bacteriophage T1, with whom phi80 shares the receptor, FhuA (TonA).

— Thus, it is likely that many *ton* (T1-resistant) mutants isolated early on, when T1 was perceived as a problem, were in fact Lula lysogens. The T1-scare helped spread Lula contamination early on.

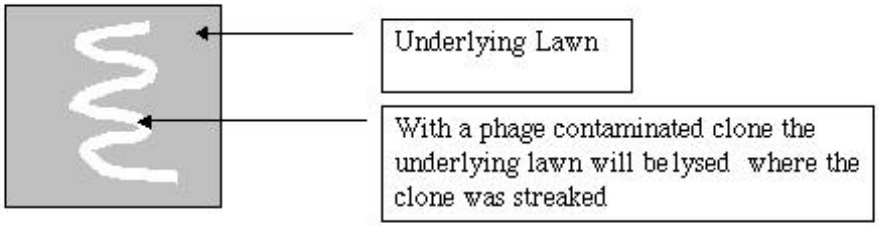
— Ironically, it is also likely that some of the Lula-caused infections were misidentified as T1 infections, exaggerating the scare.

— The current evidence for this is ongoing infection of BAC libraries with "T1-like" phages that definitely behave like temperate (non-lytic) phages.

http://bacpac.chori.org/phage_testing_protocol.htm

http://www.empiregenomics.com/resources/faq/131-t1-phage-information

http://www.lifesciences.sourcebioscience.com/clone-products/image-/source-bioscience-lifesciences-gene-sets/phage-contamination/phage-testing-assay.aspx



4. Unwillingness to accept responsibility: Contribution Games clarify the situation

— … A cousin of the Prisoner's Dilemma (PD), called the Contribution Games (CG) described by Rasmusen (2007), shows why people are often unwilling to accept responsibility and do something about it.
— In the CG, two parties have a choice of accepting responsibility or avoiding it. The nature of the game reflects the idea that taking responsibility is a public good. Once someone takes a responsibility, everyone benefits from it.
— According to CG, one party is willing to make the contribution or accept the responsibility, but (s)he prefers that someone else does it. Thus, mutually avoiding a responsibility is mildly destructive, while accepting responsibility together has a small net payout. However, having the other party accept responsibility *when the first party does not* is clearly the best option — it avoids tainting one's reputation. Hypothetical payouts for this CG game:

	B accepts	**B avoids**
A accepts	3 / 3	1 / 5
A avoids	5 / 1	-1 / -1

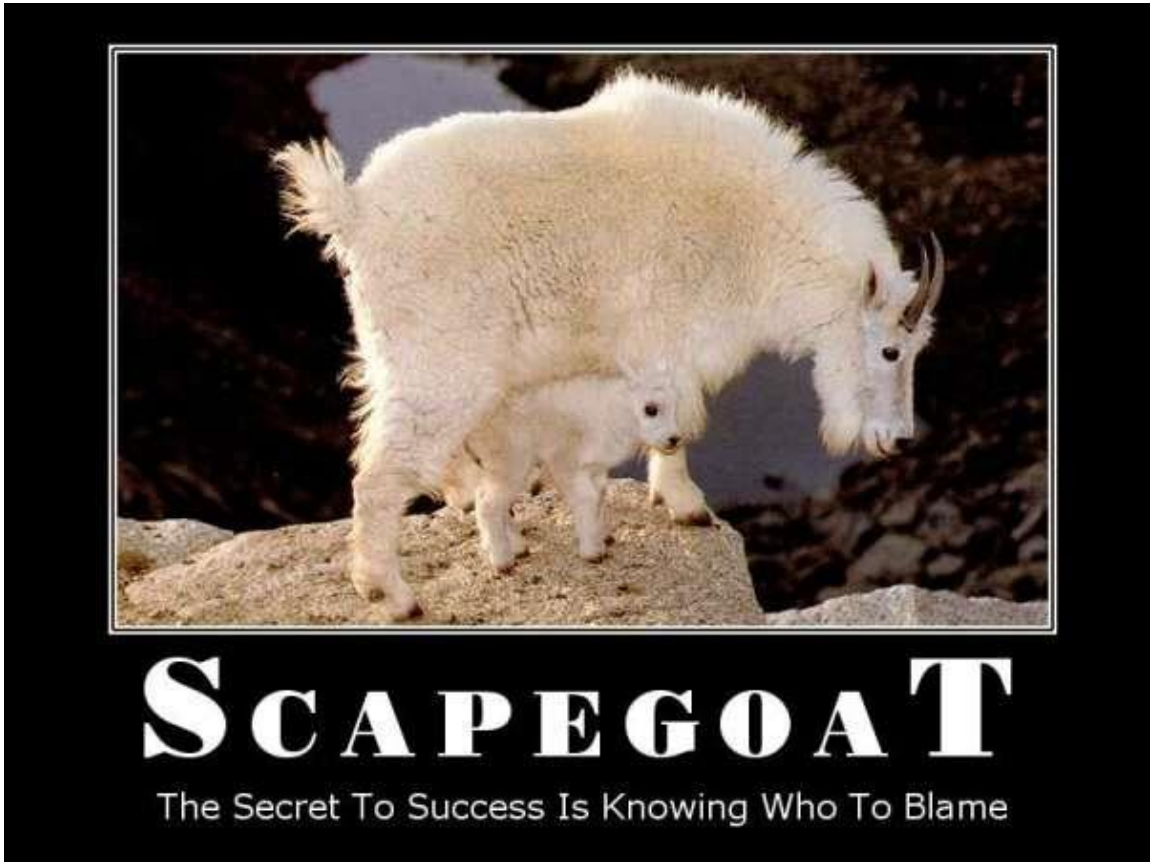
Reputation = 2

4. Avoiding responsibility and waiting for the scapegoat

— Remarkably, if there are N parties, and each party uses the same probability of avoiding the responsibility, the probability that any one party avoids the responsibility increases asymptotically toward 1 as N increases.

— In other words, the more people involved with the problem, the lower is the likelihood that any one person or party will accept responsibility and hence do something about it. (Taboo)

— Rasmusen makes clear the lesson:
"A situation like this requires something to make one of the pure-strategy equilibria a focal point. The problem is divided responsibility. **One person must be made responsible** …"



4. And why were *we* chosen to be responsible?

— We study DNA repair, and Lula makes cells DNA damage sensitive;

— We grow our cells at 28° C, the optimal temperature for Lula lytic development;

— Our main shakers are reciprocal ones;

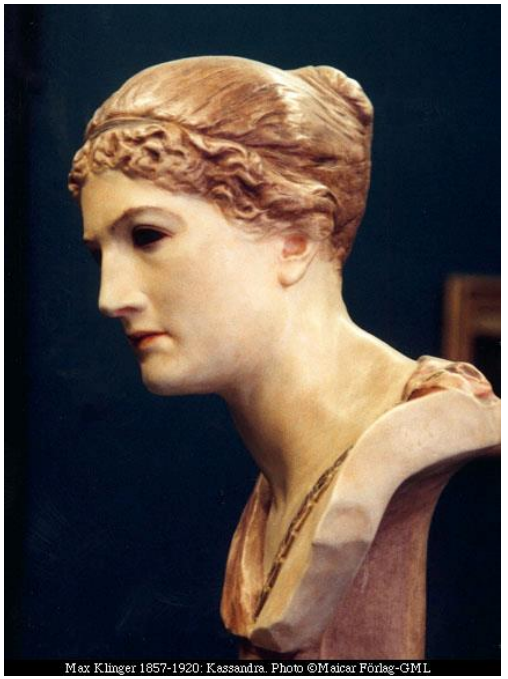
— We know how to work with phages;

— Lower aversion to "contamination"?

5. Lack of Vigilance

— Just because you do not want to be paranoid about it, does not mean that Lula is not around..

5. The Cassandra syndrome



WIKI: The "Cassandra syndrome" is a term applied in situations in which valid warnings or concerns are dismissed or disbelieved.

Helpful Hints

What can be done to minimize cross-contamination and spread of Lula/phi80

— When handling Lula cultures, use **aerosol barriers**:

— filtered tips for pipets.

— Cotton plugs for the flasks (on flasks or tubes with metal or plastic closures, seal tops with Parafilm).

— Avoiding reciprocal shakers will reduce aerosols (even though aeration will suffer).

— To inhibit Lula lytic infection, use **42° C** incubation as much as practical. This works both during culture incubation and plate incubation (for example, after P1 transduction).

— To kill Lula virions, grow *E. coli* and other enterics in the presence of **0.1% SDS**. If growth in the presence of SDS is impossible, at least add SDS to the spent culture medium and wash all the culture vessels with strong detergents.

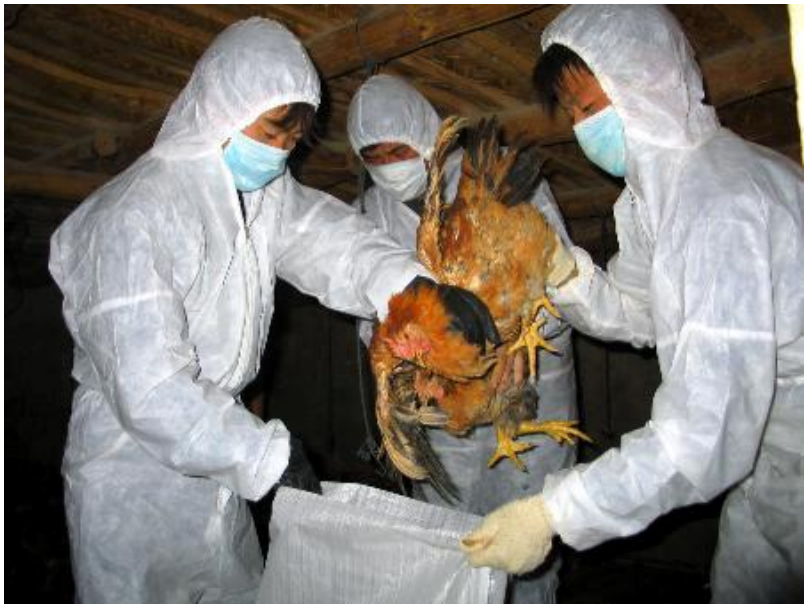
What can be done to stop cross-contamination and spread of Lula completely

— Check all your existing strains and P1 lysates for Lula contamination.

— Dispose of the identified lysogens and rebuild the lost strains (if needed).

— Check any new strain that is to be deposited into the collection, whether imported or built in the lab, for Lula contamination (takes several hours).

— Stop using shared facilities.




Lula.
£4.99

Helacyton gartleri

Stan Gartler

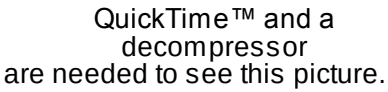
Due to their ability to replicate indefinitely, and their non-human number of chromosomes (HeLa cells have a modal chromosome number of 82, with four copies of chromosome 12 and three copies of chromosomes 6, 8, and 17.), HeLa was described by Leigh Van Valen (Department of Ecology and Evolution at the University of Chicago) as an example of the contemporary creation of a new species, *Helacyton gartleri*, named after Stanley M. Gartler, who Van Valen credits with discovering "the remarkable success of this species."

His argument for speciation depends on three points:

* The chromosomal incompatibility of HeLa cells with humans.
* The ecological niche of HeLa cells.
* Their ability to persist and expand well beyond the desires of human cultivators.

It should be noted that this definition has not been followed by others in the scientific community, nor, indeed, has it been widely noted.

As well as proposing a new species for HeLa cells, Van Valen proposes in the same paper the new family Helacytidae and the genus Helacyton.


QuickTime™ and a
decompressor
are needed to see this picture.