

# Biodiversité du microbiome digestif et bactériophages

Laurent DEARBIEUX

POUR LA RECHERCHE, POUR LA SANTÉ,  
POUR DEMAIN



RICAI 2018

38ème Réunion Interdisciplinaire  
de Chimiothérapie Anti-Infectieuse

Je n'ai pas de lien d'intérêt potentiel à déclarer

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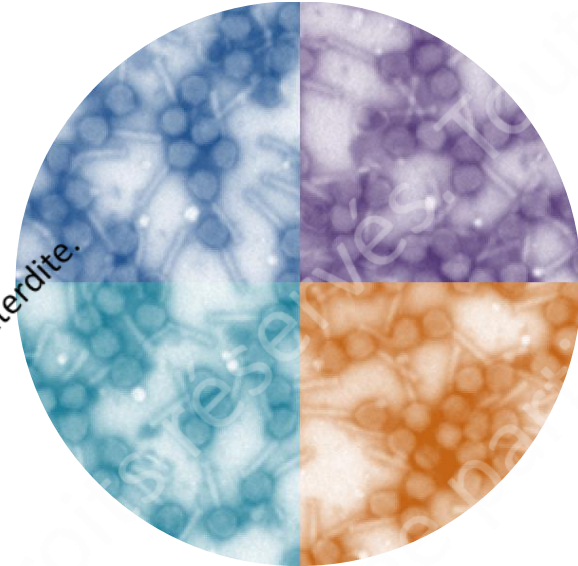
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# What's in OUR gut ?



**10<sup>10+</sup>**  
**Cells**

Bacteria, archaea, fungi...



**10<sup>10+</sup>**  
**Viruses**

Bacteriophages, enteric viruses

# How to describe and analyze our microbiota ?

## DNA sequencing



## Bacteria

16S rRNA or  
Deep sequencing

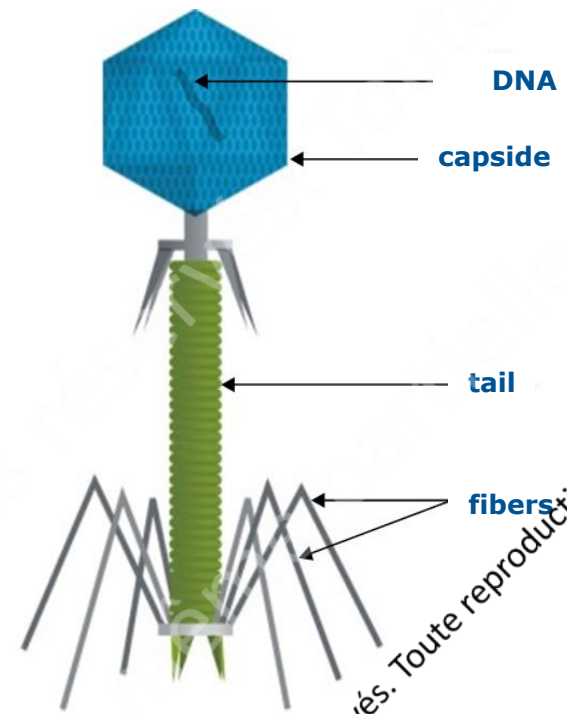
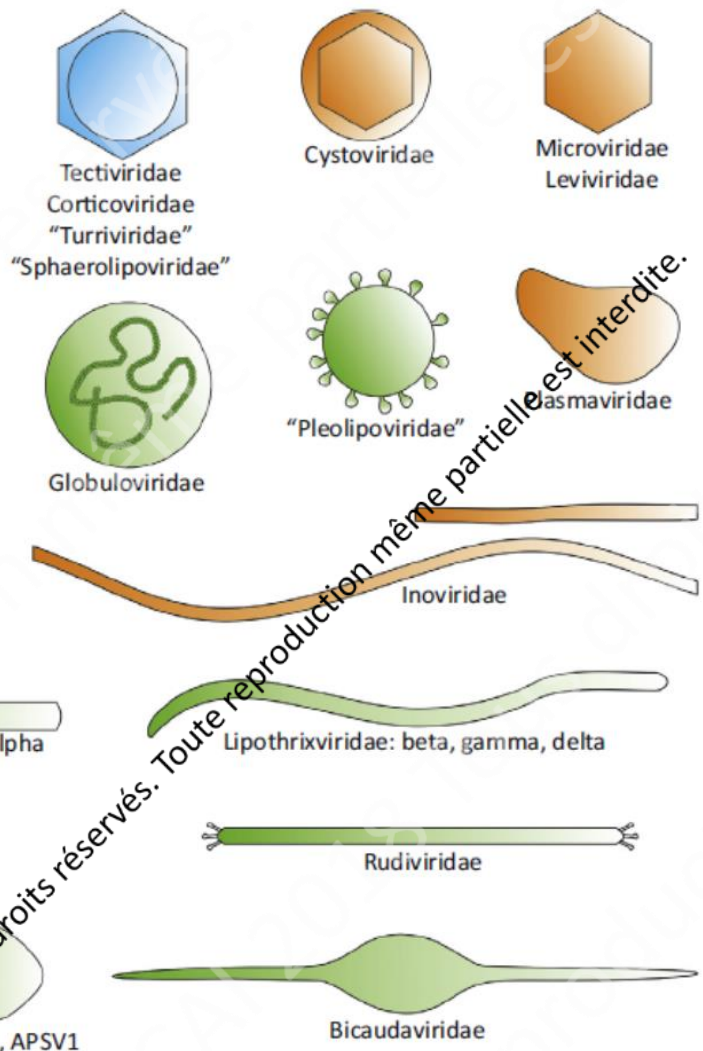
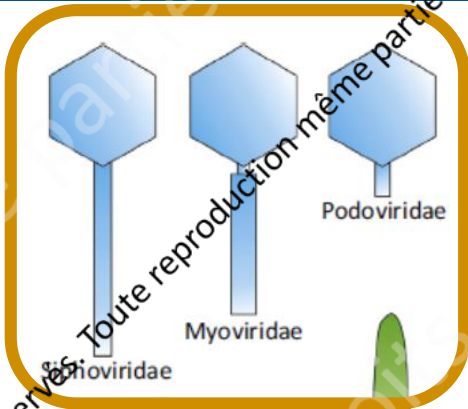
Microbiome  
(bacteriome)

## Bacteriophages

No 16S rRNA, **no marker**  
Deep sequencing only

Virome  
(phageome)

# Bacteriophages belong to viruses infecting microbes



## The T4 phage model

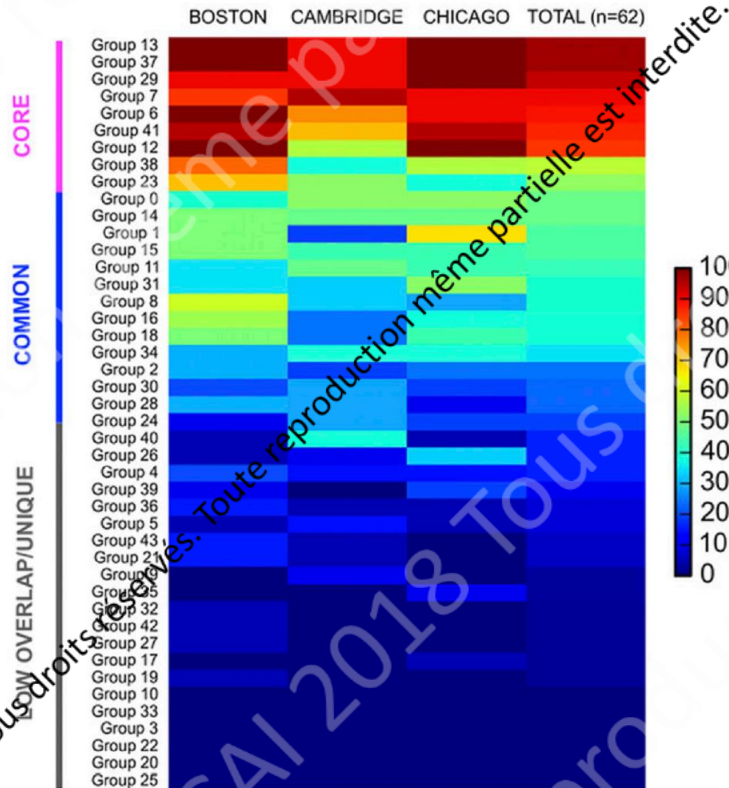
96% of bacteriophages reported belong to *Caudovirales* family of viruses

# Which bacteriophages are there ?



## Healthy human gut phageome (HGP)

Pilar Manrique et al. PNAS 2016, August 29



9 phage groups (incl. Crass)  
(incl. 9 complete genomes)

13 phage groups  
(incl. 23 complete genomes)

22 phage groups  
(incl. 39 complete genomes)

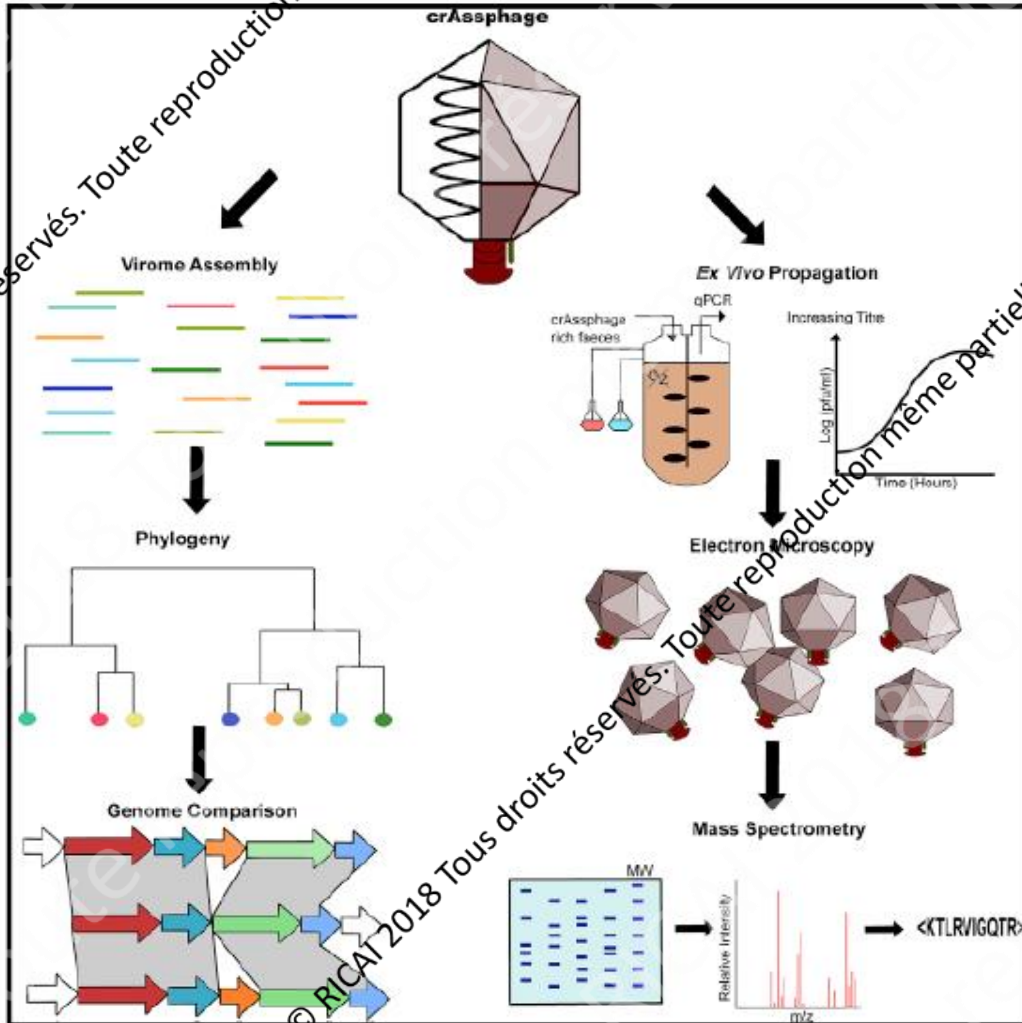
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# 4 years to isolate the bacterium infected by crAssphage !

## Cell Host & Microbe

### Biology and Taxonomy of crAss-like Bacteriophages, the Most Abundant Virus in the Human Gut

Guérin et al., *Volume 24, Issue 5*,  
14 November 2018 Pages 653-664



## In Brief

CrAssphage is the most abundant human gut-associated virus. Guerin et al. identify 249 crAss-like phage genomes and classify them into four subfamilies and ten candidate genera that differ among human populations. These *in silico* predictions are combined with *ex vivo* propagations, electron microscopy imaging, and mass spectrometry detection.

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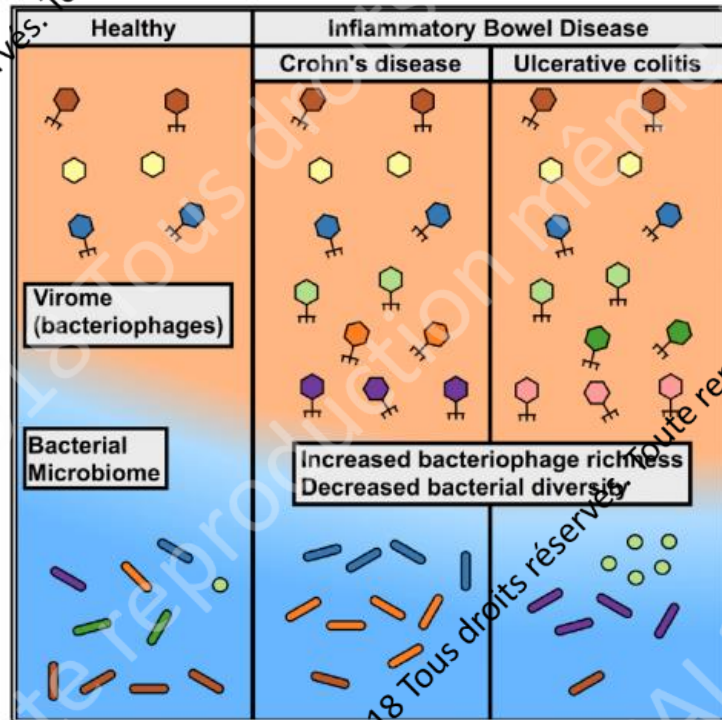
# Why do we care about bacteriophages ?

Cell

Article

## Disease-Specific Alterations in the Enteric Virome in Inflammatory Bowel Disease

Graphical Abstract



Authors

Jason M. Norman, Scott A. Handley, ..., Miles Parkes, Herbert W. Virgin

**In Brief:**

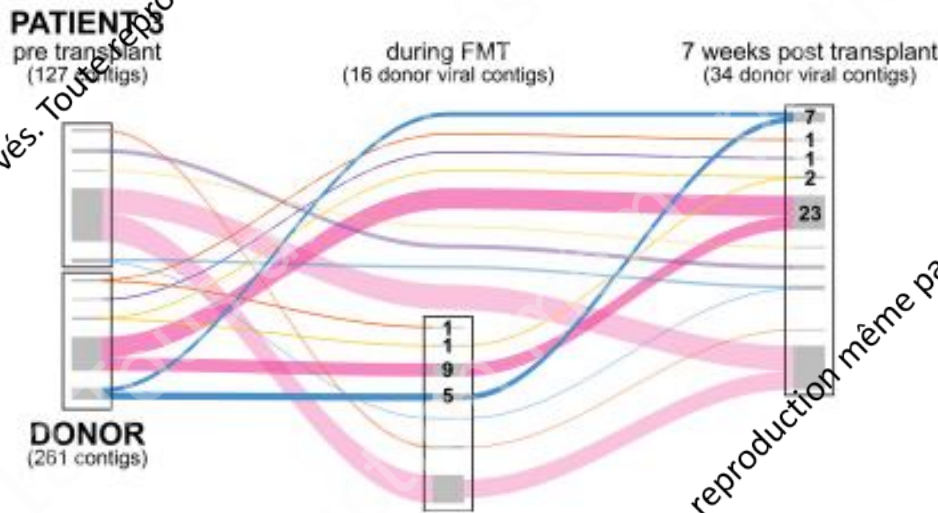
The enteric virome is abnormal in multiple cohorts of inflammatory bowel disease patients, exhibiting disease-specific features that **are not explained** by changes in bacterial diversity and richness.

**Other examples of viral dysbiosis include: AIDS, Diabetes, Autism...**



# How far can bacteriophages influence our microbiota ?

Transfer of Viral Communities between Human Individuals during Fecal Microbiota Transplantation  
*Chehoud et al., 2016 mBio 7(2):e00322-16*

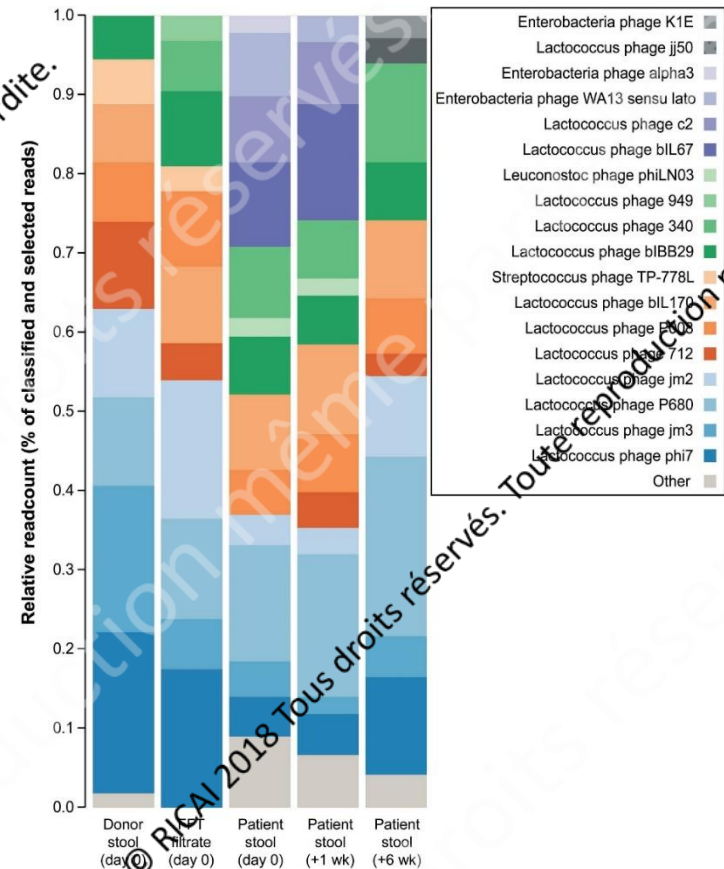


Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation

*Draper et al., 2018 Microbiome (2018) 6:220*

## Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With *Clostridium difficile* Infection.

Ott et al. *Gastroenterology*, 2017



# How rapidly bacteriophages colonize the GIT ?

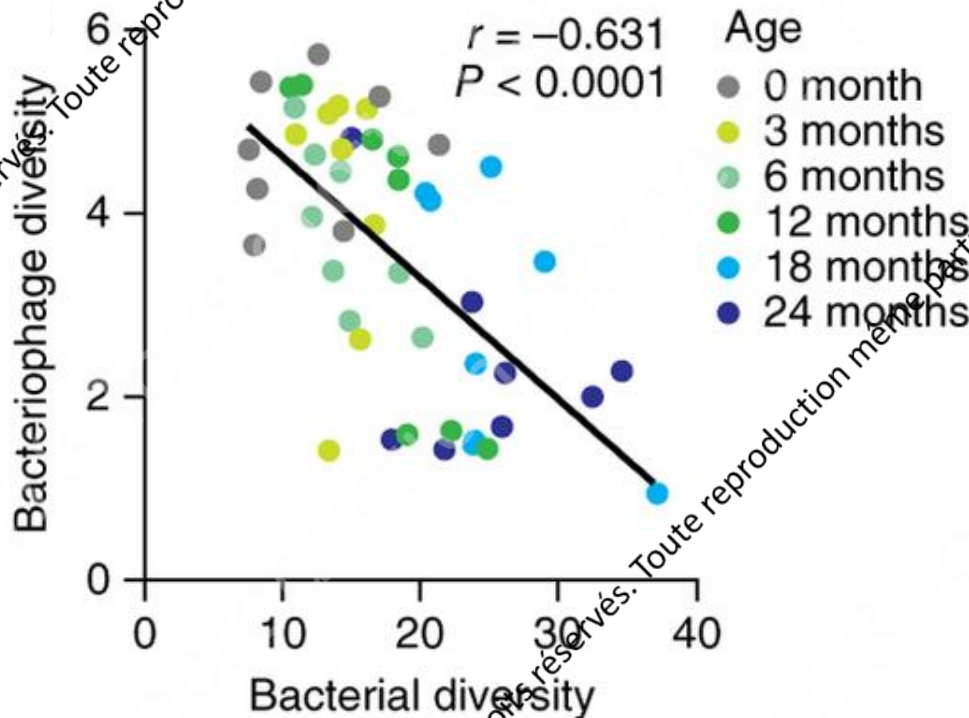
Early life dynamics of the human gut virome and bacterial microbiome in infants  
 Lim et al. *Nature Medicine*, 2015 Oct;21(10):1228-34



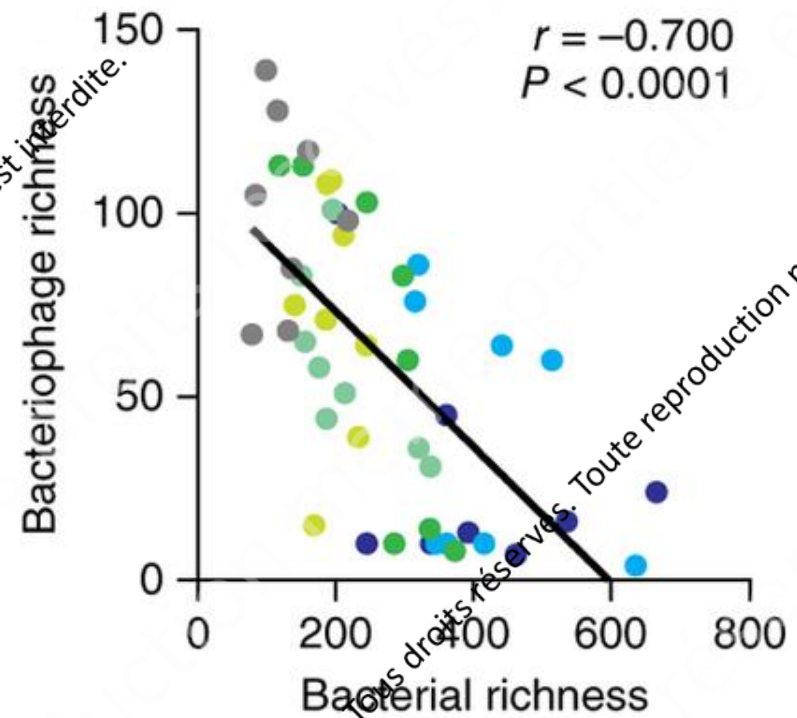
# Variations of bacteriophages within 24 months of life

**Inverted correlation** richness/diversity of bacteria and bacteriophages

## Diversity



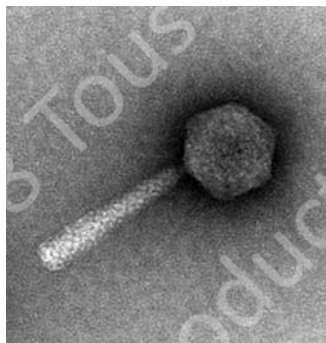
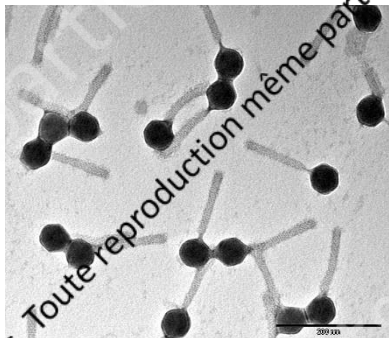
## Richness



**Why and how** bacteriophages are diverse and abundant at birth ?

Association of bacteria and bacteriophages by sequences: not yet !

# Identification of drivers bacteria: bacteriophages



**Virulent bacteriophages / *E. coli* / Mice gut**

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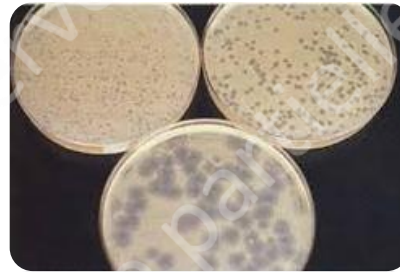
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# Virulent bacteriophages isolation

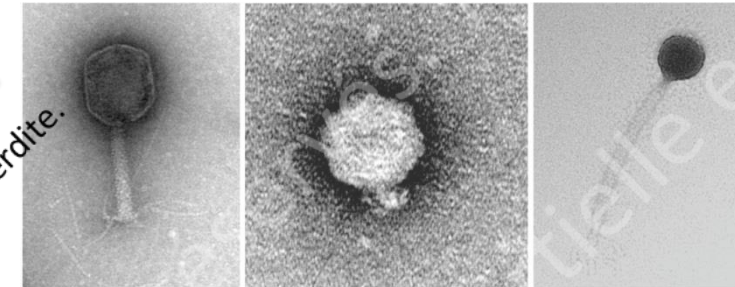
## isolation



## purification



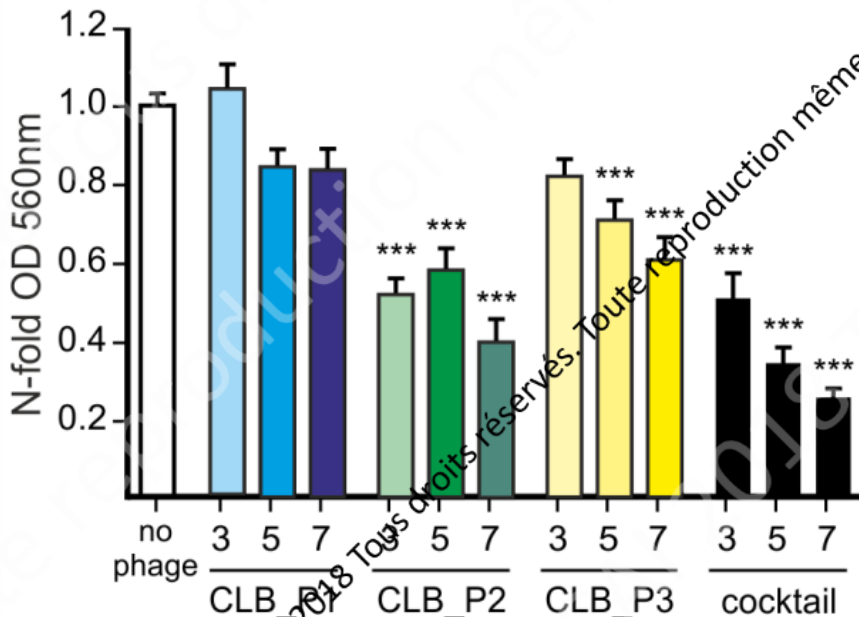
## characterization



myoviridae

podoviridae

siphoviridae

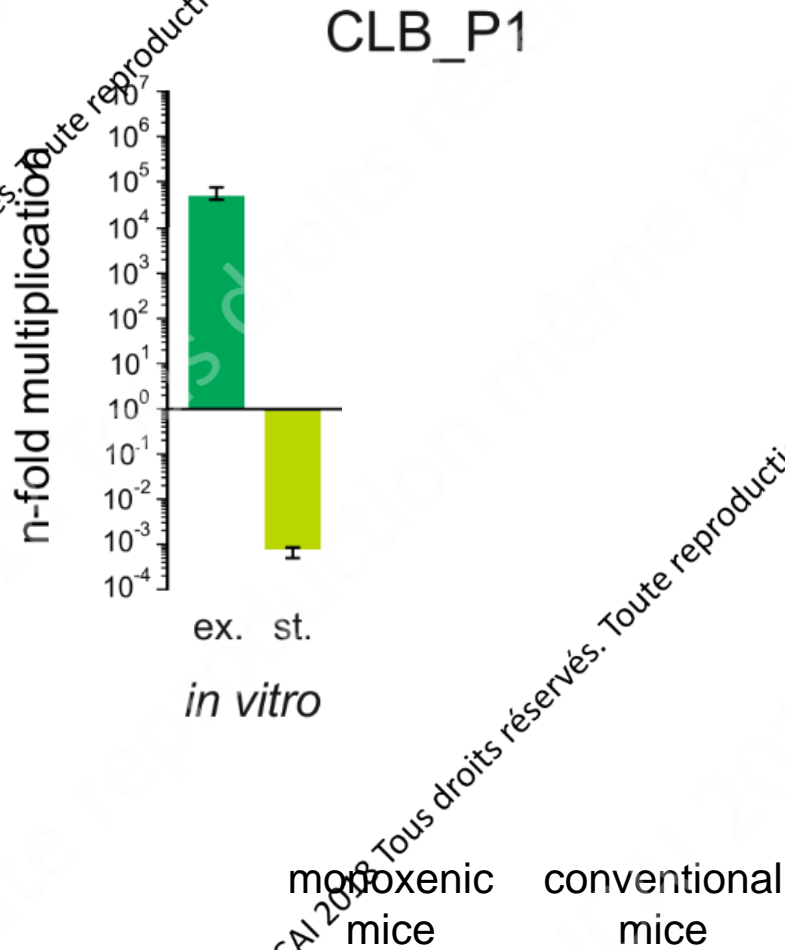


## formulation



Synergistic effect of the cocktail on biofilms

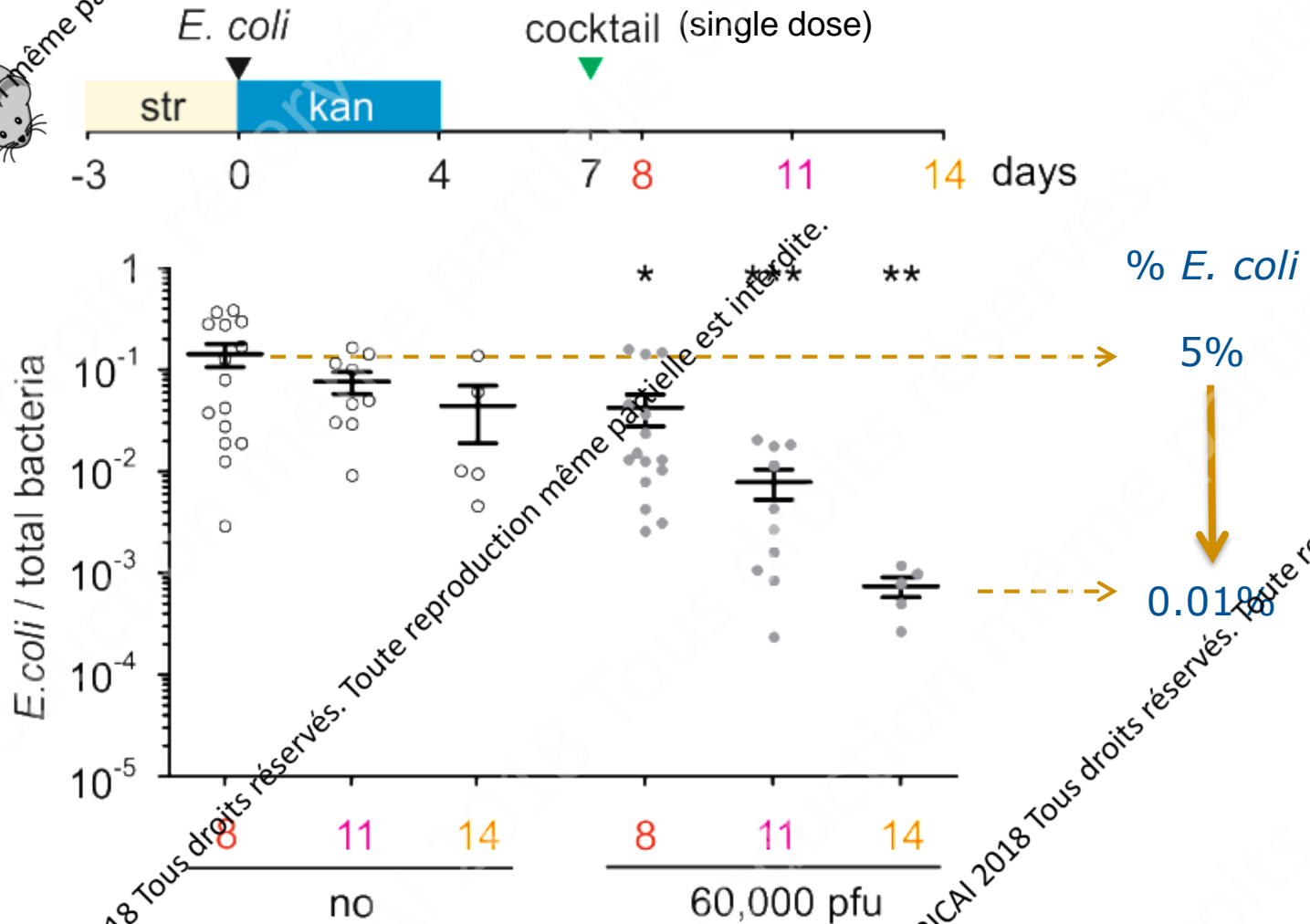
# Biogeography of bacteriophages replication



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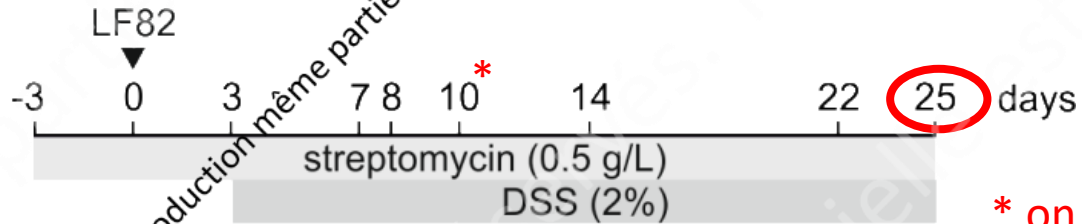
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# Gradual reduction of *UPEC* colonization



without impact on microbiota composition (16S)

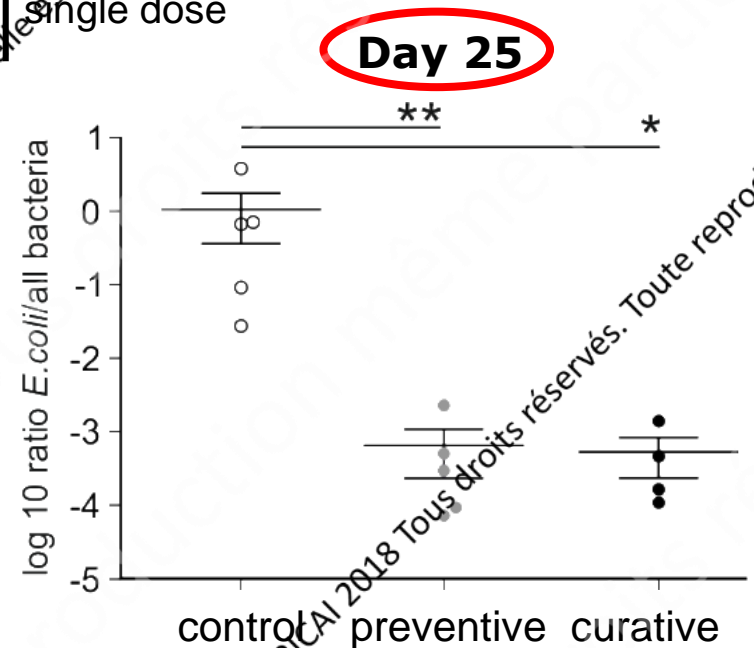
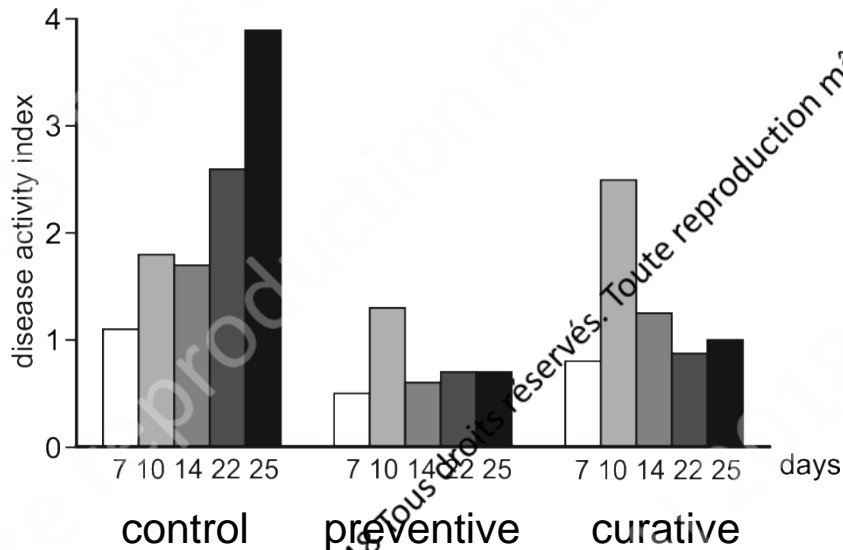
# Targeting Adherent Invasive *E. coli* (AIEC)



Conventional mice

\* on day 10 colitis symptoms observed

- ▲ PBS (control group, n=5)
  - ▲ cocktail (preventive group, n=5)
  - ▲ cocktail (curative group, n=5)
- Single dose



Sustainable reduction of AIEC in both ileum and colon

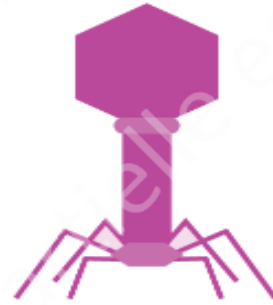


# Long term interactions in the gut



**Pathogenic *E. coli* LF82**

Adherent Invasive *E. coli* (AIEC)



**Phage LF82 P10**

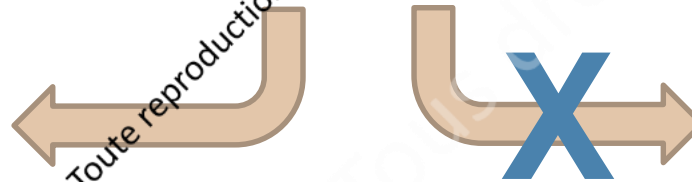
Myoviridae Felix-O1 like



**Commensal *E. coli* MG1655**

K12 strain

**HOST**

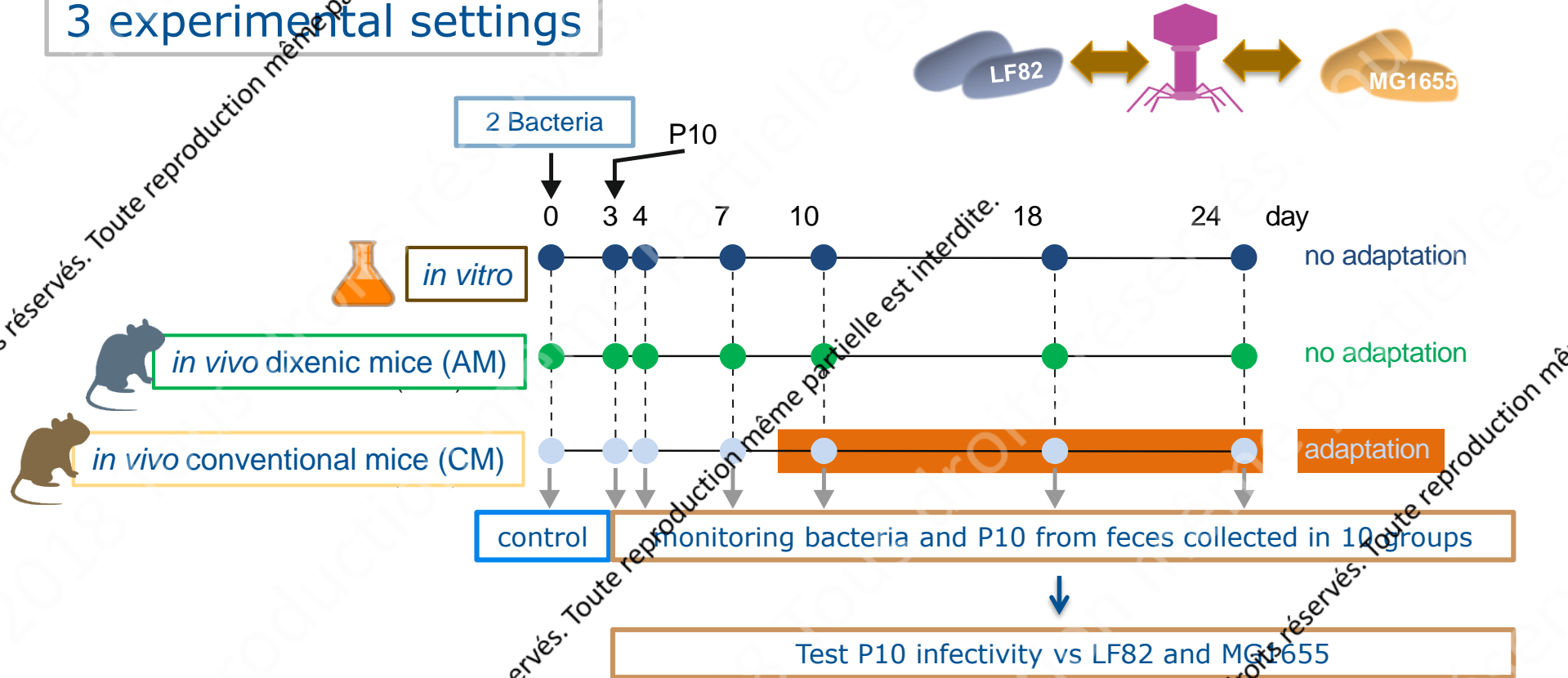


**No infection**  
**No adsorption**

**Can P10 evolve to acquire the ability to infect a new host?**

# A model of coevolution

## 3 experimental settings



**Host jump observed only in conventional mice**

# Bacteriophage populations genomics

Whole evolving bacteriophage populations



*In vitro*



Axenic



Conventional  
(adapted - and non adapted)



Phages isolation

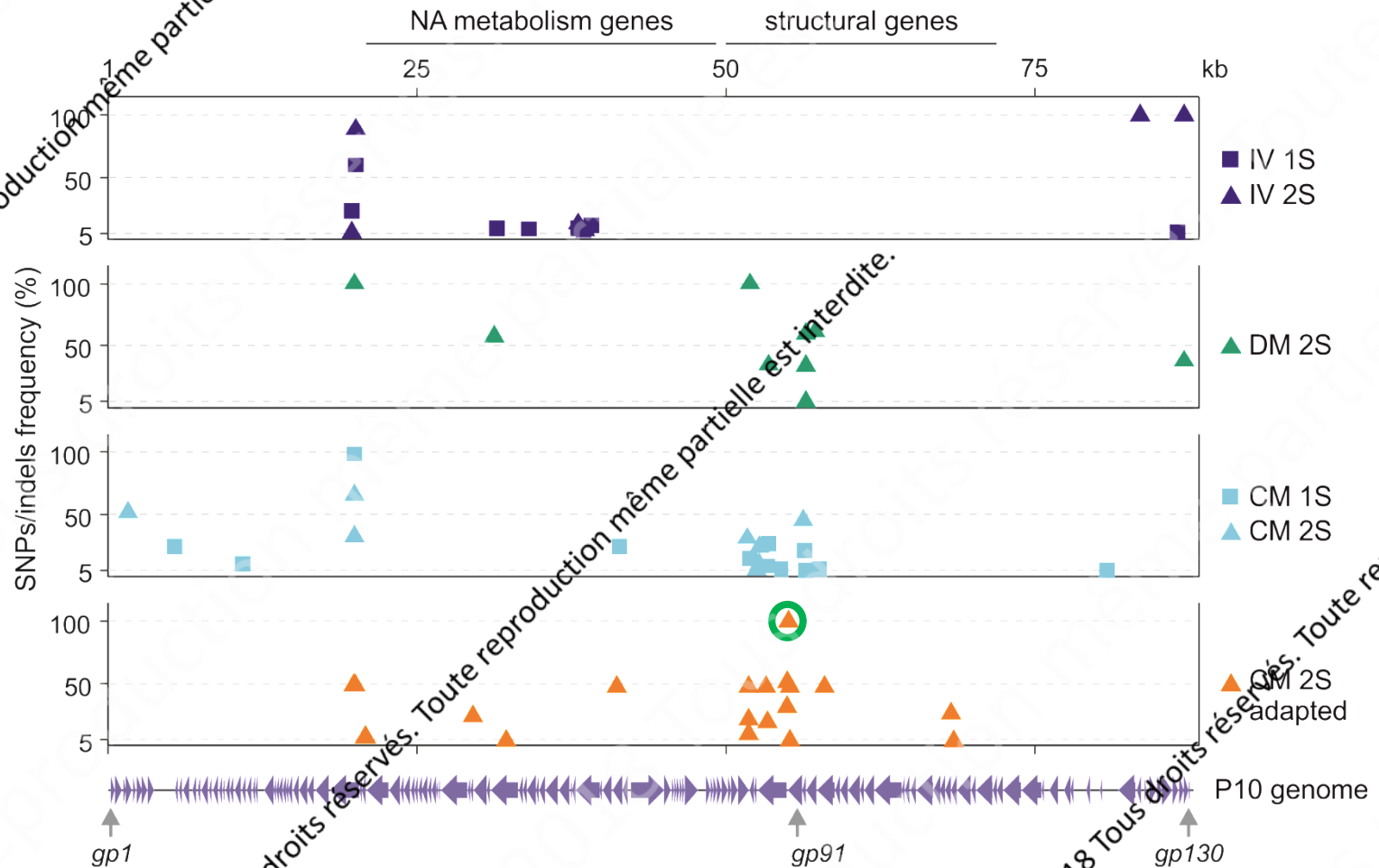


DNA sequencing



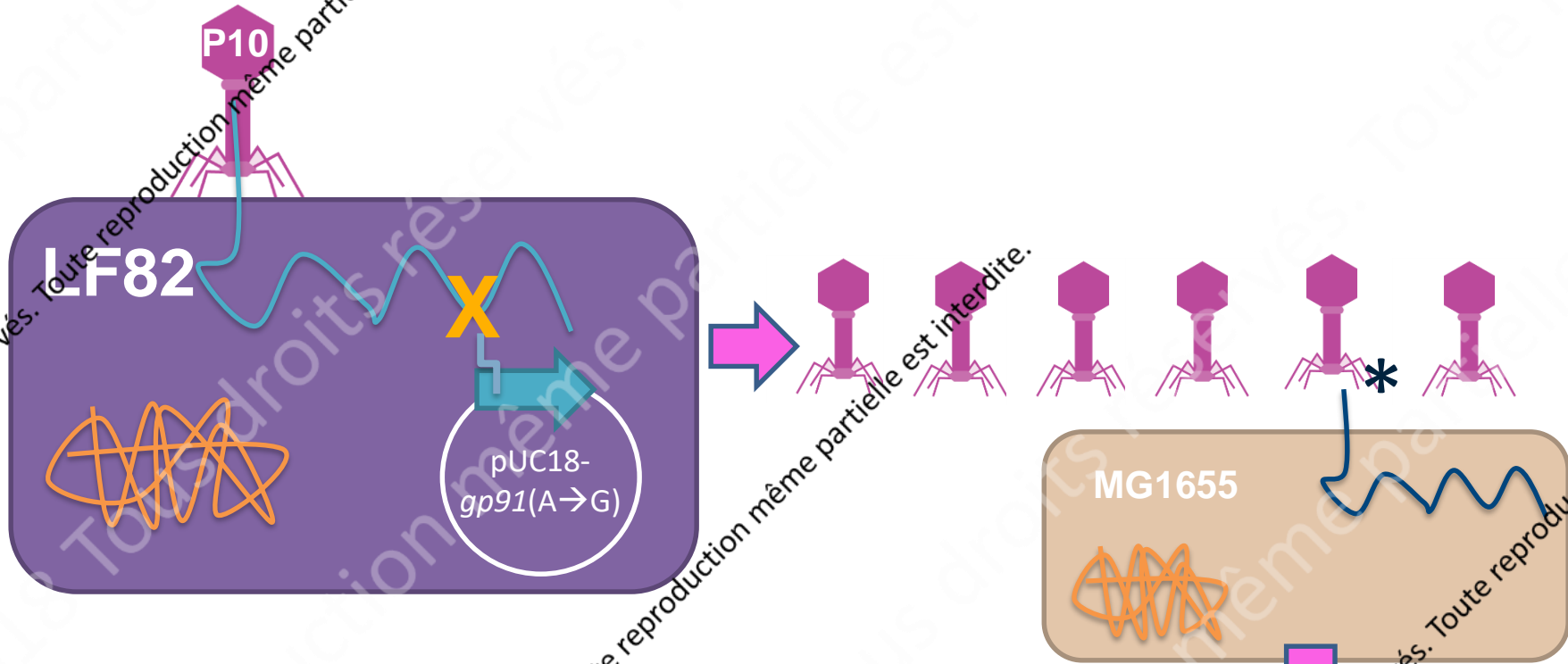
Variant calling  
versus the  
ancestral P10

# A unique SNP specific of conventional mice



**Y284H mutation in GP91 is unique in conventional mice**

# Is a unique SNP sufficient ?



**All recombinant phages carry mutation Y284H**

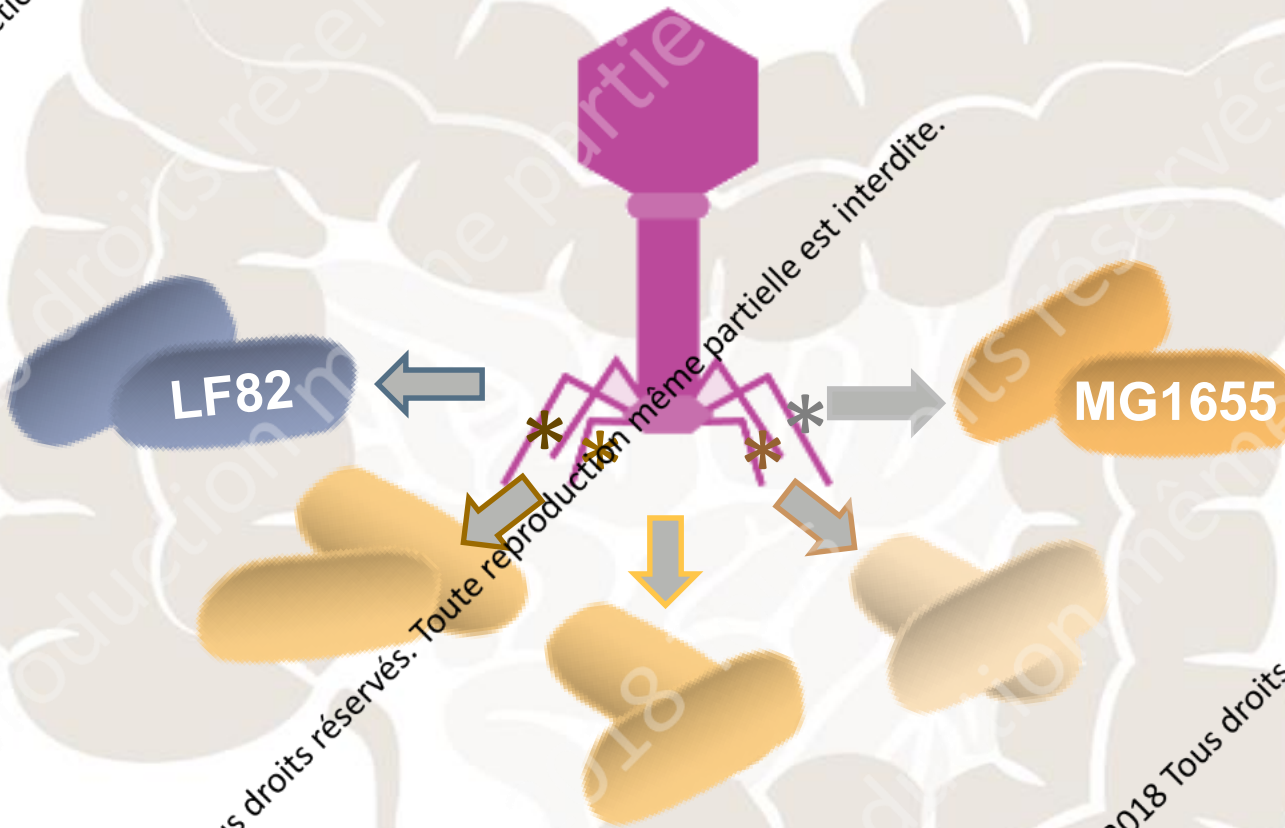
Recombinant 1	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 2	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 3	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 4	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 5	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 6	CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Ancestral	CCGATAGGCCACCGCCTGCTTCATATGTCAATCTGTGGTTATGAGCACC
	*****

**MG1655**

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# Why a single SNP is not selected *in vitro*?

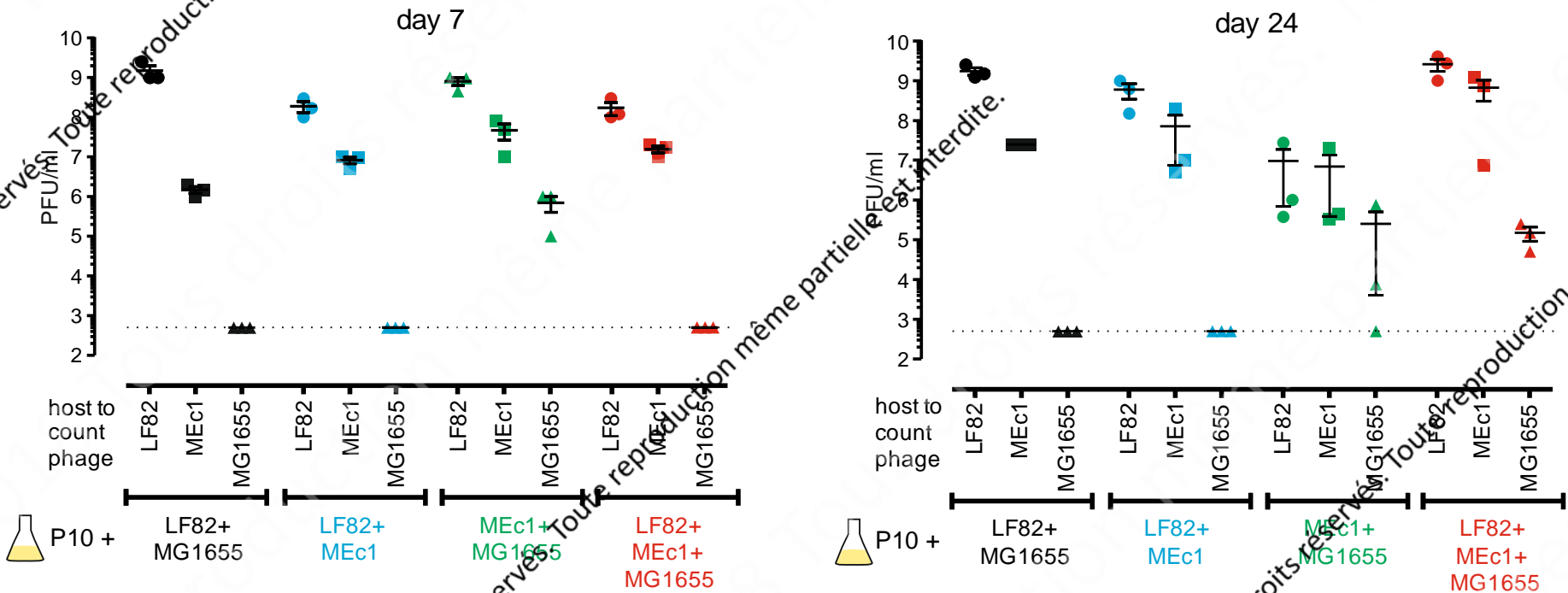
## HOW ?



Does it requires an intermediate step?

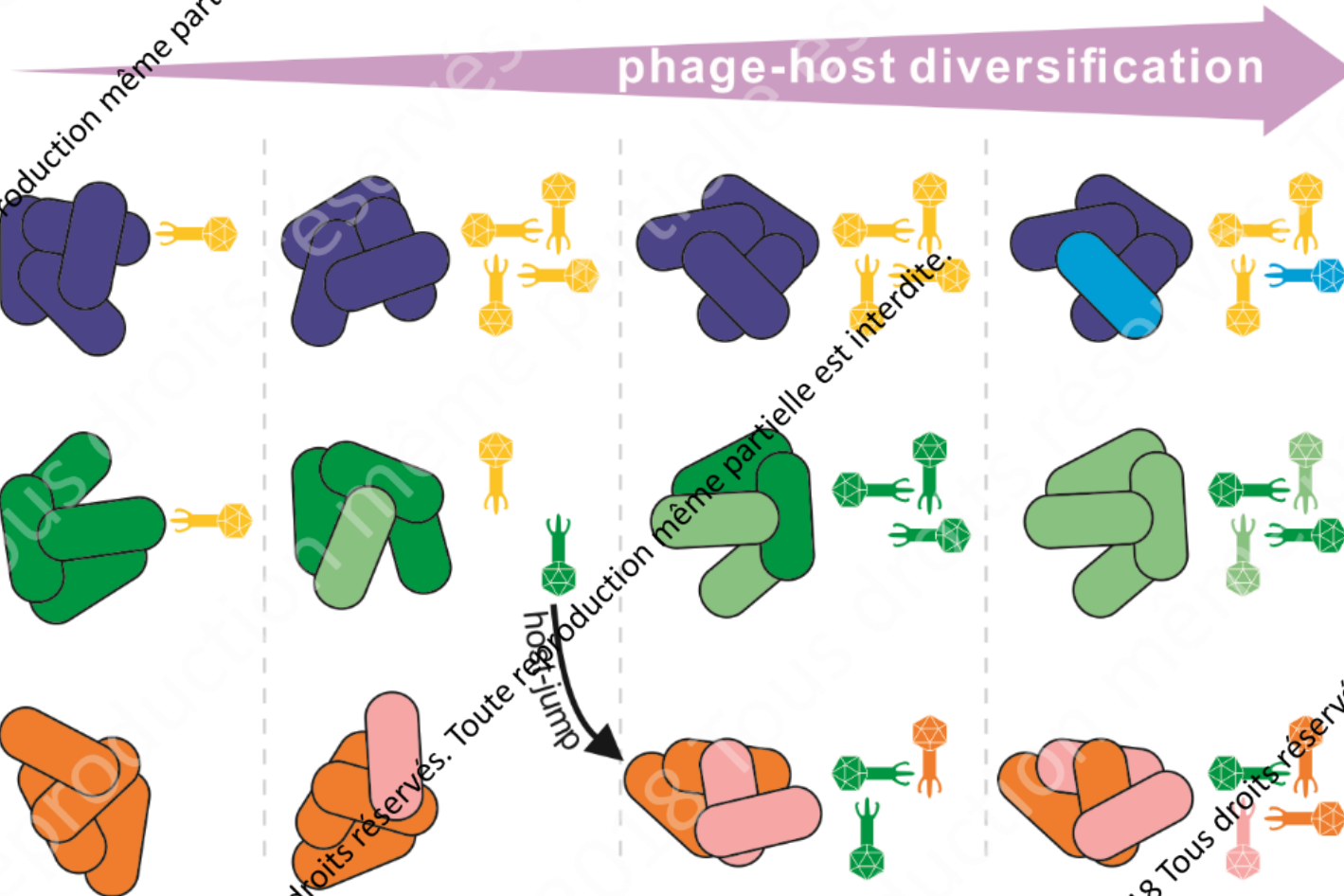
# A single intermediate host is sufficient

We isolated a murine *E. coli* strain (MEc1) from control groups



We can recapitulate host jump *in vitro* when adding only strain MEc1

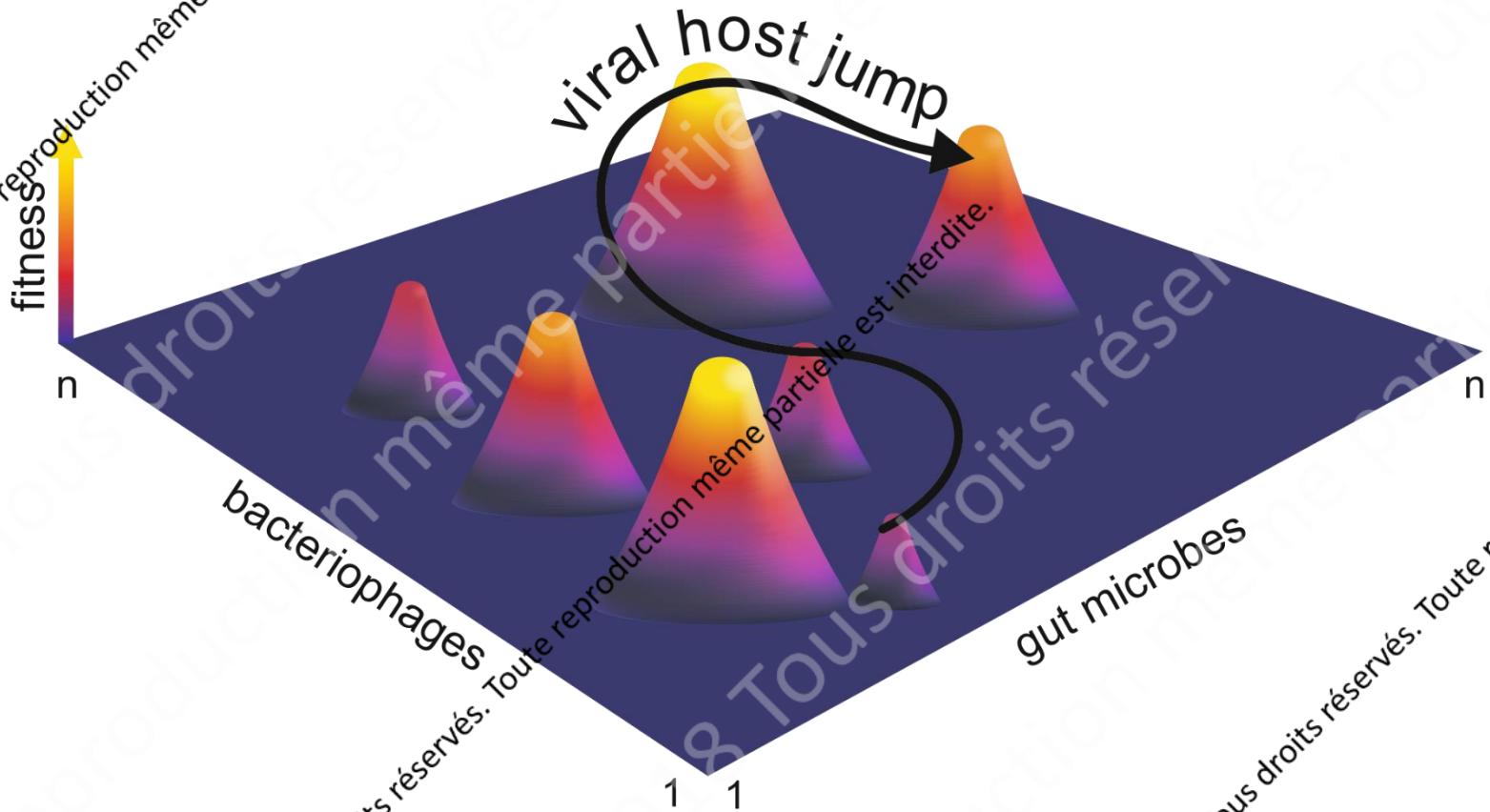
# Microbiota is driving bacteriophage evolution



A diverse population of bacteria will offer multiple opportunities for bacteriophages to differentially propagate within the gut by host jumps.



# Bacteriophage/Bacteria interactions and evolution



A dynamic fitness landscape required for viral persistence ?

# Interactions Bacteriophages Bacteria in Animals



Mathieu De JODE (PhD)

Baptiste GABORIEAU (MD, M2)

Marta LOURENÇO (PhD)

Raphaëlle DELATTRE (MD-PhD)

Quentin LAMY-BESNIER (PhD)

Dwayne ROACH (Post doc)

Rob Lavigne (K.U. Leuven)

Angus Buckling (Univ. Exeter)

James Di Santo (I. Pasteur)

Spencer Shorte (I. Pasteur)

Jean-Damien Ricard (H. L. Mourier)

Erick Denamur (H. Bichat)

Stephen Lory (Harvard Medical School)

Guy Schoehn (IBS Grenoble)

Joshua Weitz (Georgia Tech.)



[www.p-h-a-g-e.org](http://www.p-h-a-g-e.org)



[www.phages.fr](http://www.phages.fr)



[www.isvm.org](http://www.isvm.org)

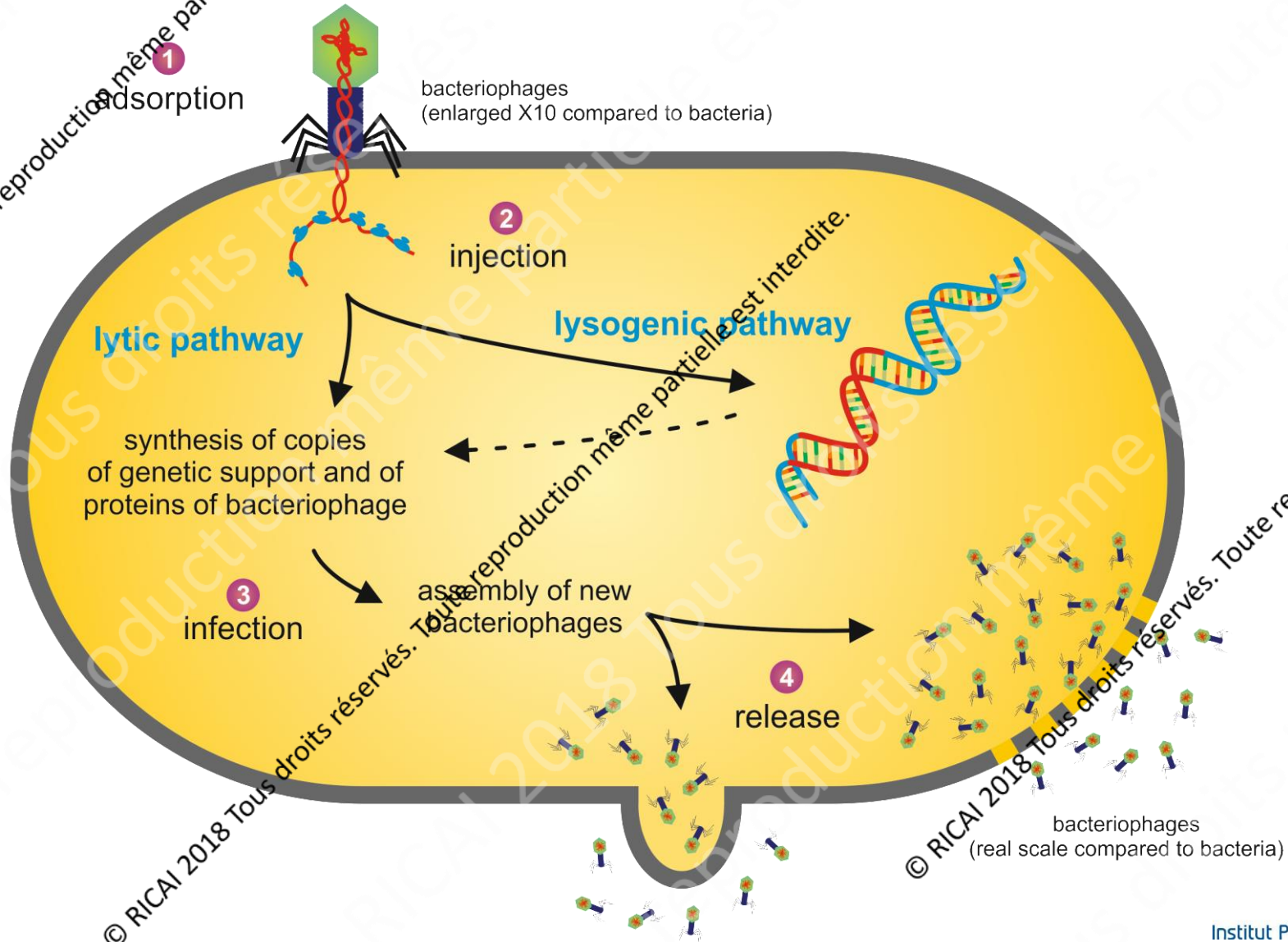
Fondation reconnue  
d'utilité publique  
habilitée à recevoir  
dons et legs

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75724 Paris Cedex 15

[www.pasteur.fr](http://www.pasteur.fr)

<https://research.pasteur.fr/en/team/group-laurent-debarbieux/>

# Bacteriophages: virulents vs. temperate



# Bacteriophages are specific (host range is limited)

PAK strain

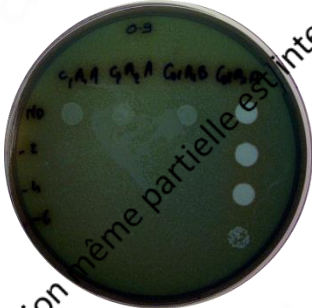
O:6



O:4



O:9



O:10

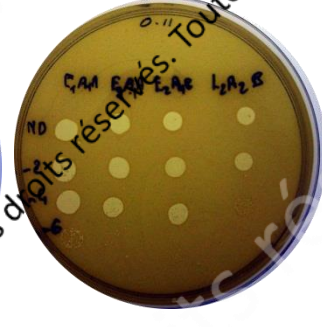


O:11

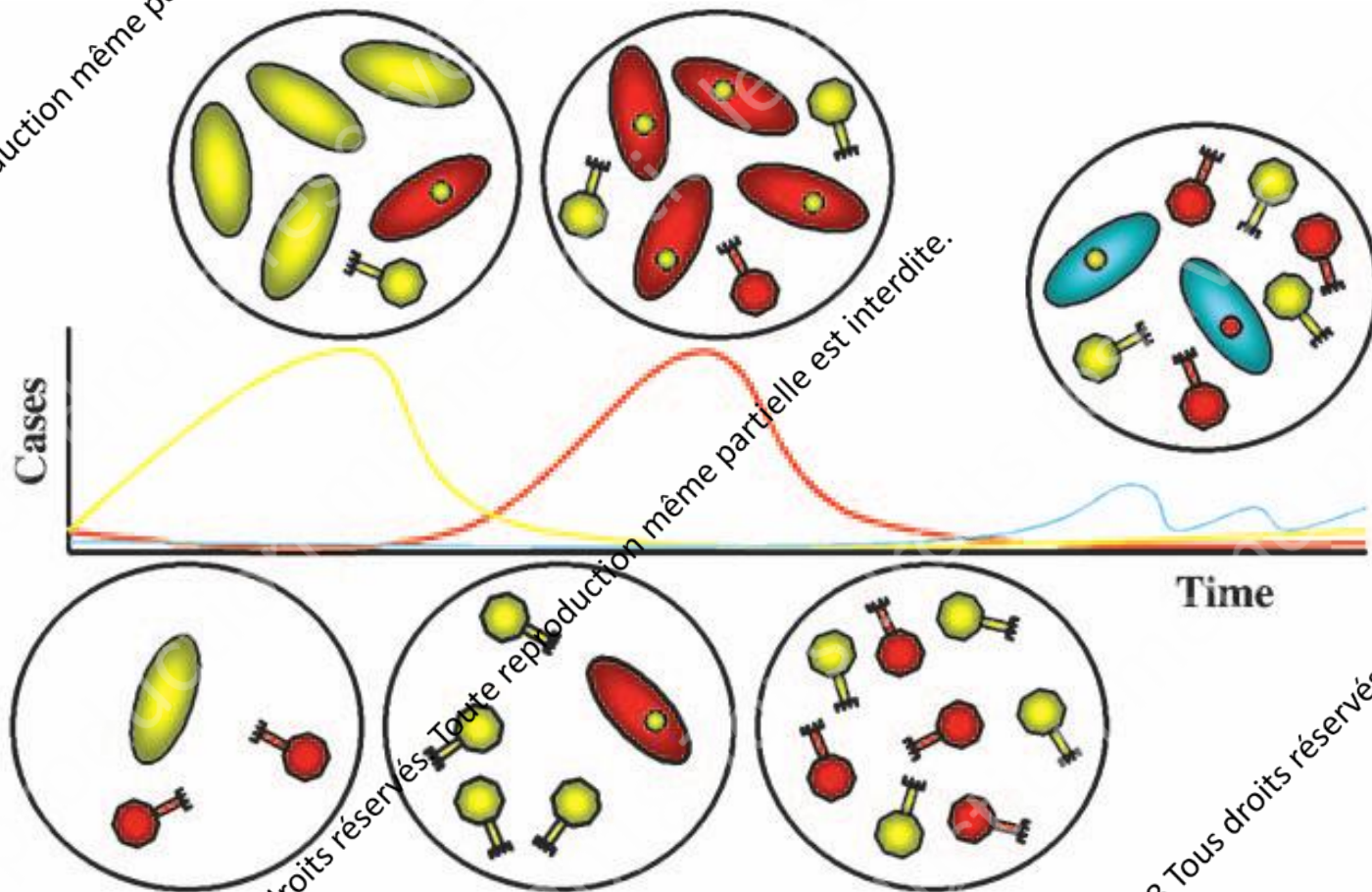


clinical isolates (serotypes)

ND  
-2  
-4  
-6



# Coevolution bacteriophages/bacteria



Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages  
Faruque et al., 2005, PNAS, vol 102, p1702

# Examples of “approved” compassionate treatments

In France, 2 patients treated in 2016/2017, not yet published

In Belgium:

**Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicemia in a patient with acute kidney injury—a case report.**

Jennes S, Merabishvili M, Soentjens P, Pang KW, Rose T, Keersebilck E, Soete O, François PM, Todorescu S, Verween G, Verbeken G, De Vos D, Pirnay JP.

*Crit Care*. **2017 Jun** 4;21(1):129. doi: 10.1186/s13054-017-1409-y. No abstract available.

PMID: 28583189

In the USA:

**Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection.**

Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM, Taplitz R, Smith DM, Kerr K, Kumaraswamy M, Nizet V, Lin L, McCauley MD, Strathdee SA, Benson CA, Pope RK, Lencux BM, Picel AC, Mateczun AJ, Cilwa KE, Regeimbal JM, Estrella LA, Wolfe DM, Henry MS, Quinones J, Salka S, Bishop-Lilly KA, Young R, Hamilton T.

*Antimicrob Agents Chemother*. **2017 Sep** 22;61(10). pii: e00954-17. doi: 10.1128/AAC.00954-17.

Print 2017 Oct. PMID:28807909

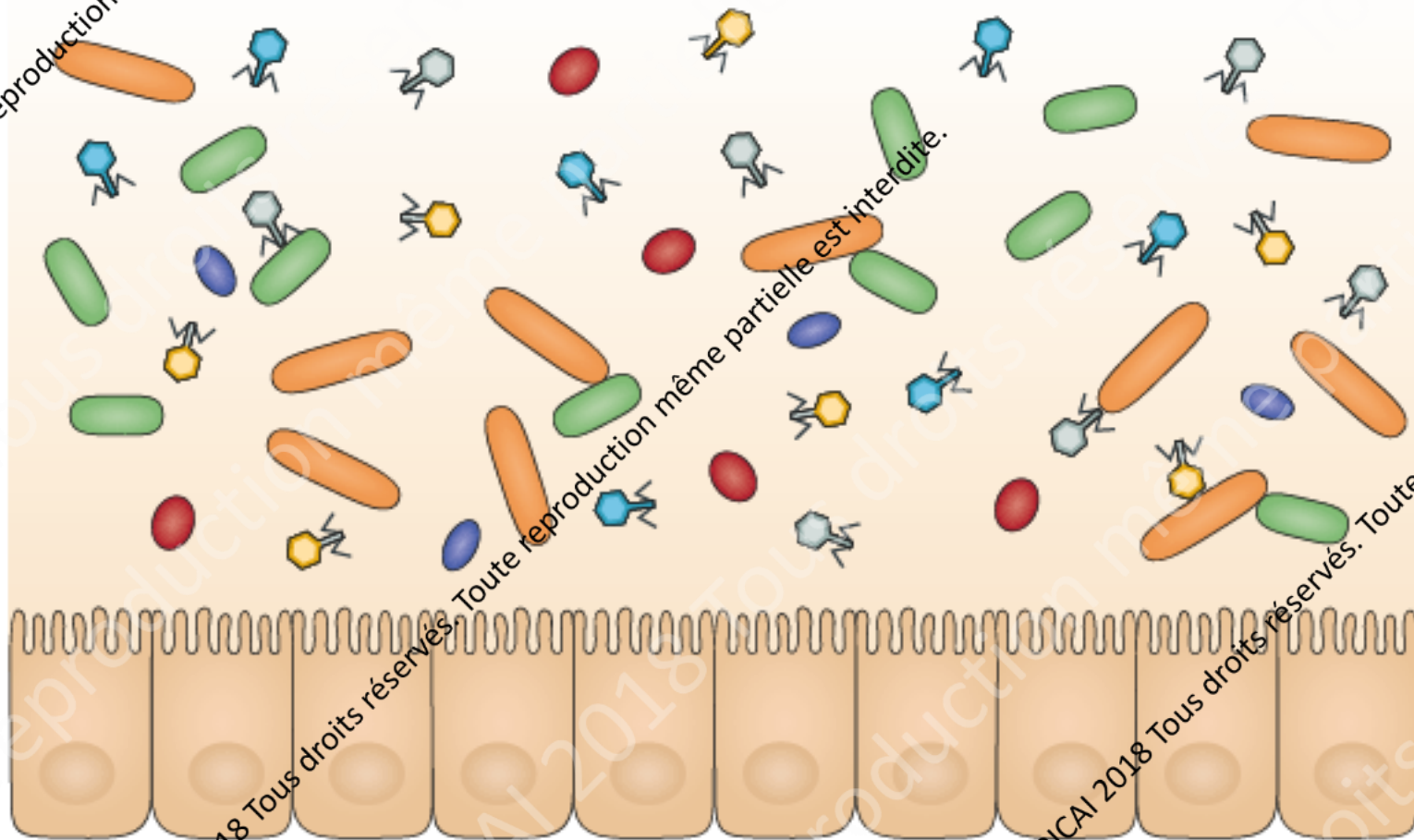
**Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa***

Chan BK, Turner PE, Kim S, Mojibian HR, Eleftheriades JA, Narayan D

*Evol Med Public Health*. **2018 Mar** 8;2018(1):60-66.

We will not **ESKAPE** Phage Therapy !!!

# Association bacteria/bacteriophages ?



Sequence analysis are not yet powerful enough !