

*Infections à mycoplasmes chez les animaux d'élevage-
facteurs de virulence et nouveaux vaccins*

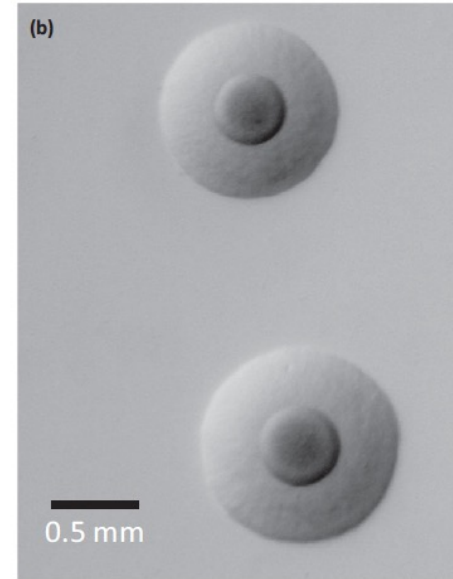
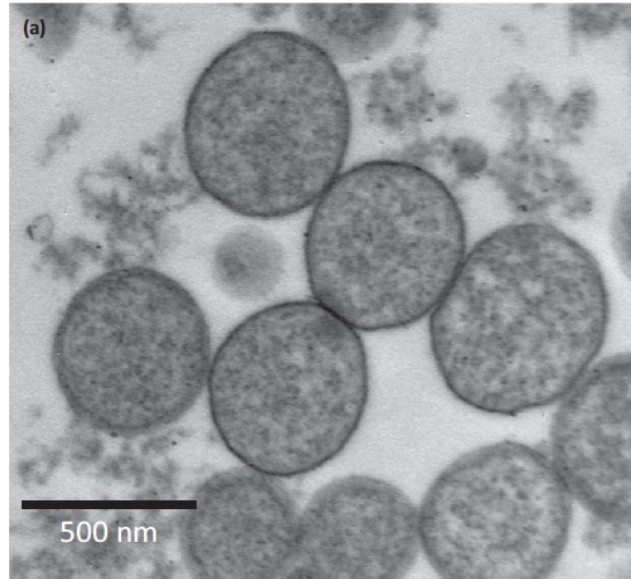
Alain Blanchard, PR

UMR INRAE 1332 Biologie du fruit et pathologie

alain.blanchard@u-bordeaux.fr



Mycoplasmas (*Mollicutes*)



- No cell wall
- Genome ranging from ~ 600-2200 kbp
- Low GC content (24-30%)
- UGA as tryptophan
- Limited metabolic pathways
- **Self-replicating** in axenic media

Briefly, what do we know about mycoplasmas?

- Colonize a large variety of hosts
- Mycoplasmas, among the most evolved organisms
- Mycoplasmas and the concept of a minimal cell
- Build a minimal cell/chassis starting with a mycoplasma cell
 - Rationale
 - What's the use?
 - Progress made in the INRA laboratory
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 - Immunoglobulin-binding proteins + Ig protease (MIB-MIP)

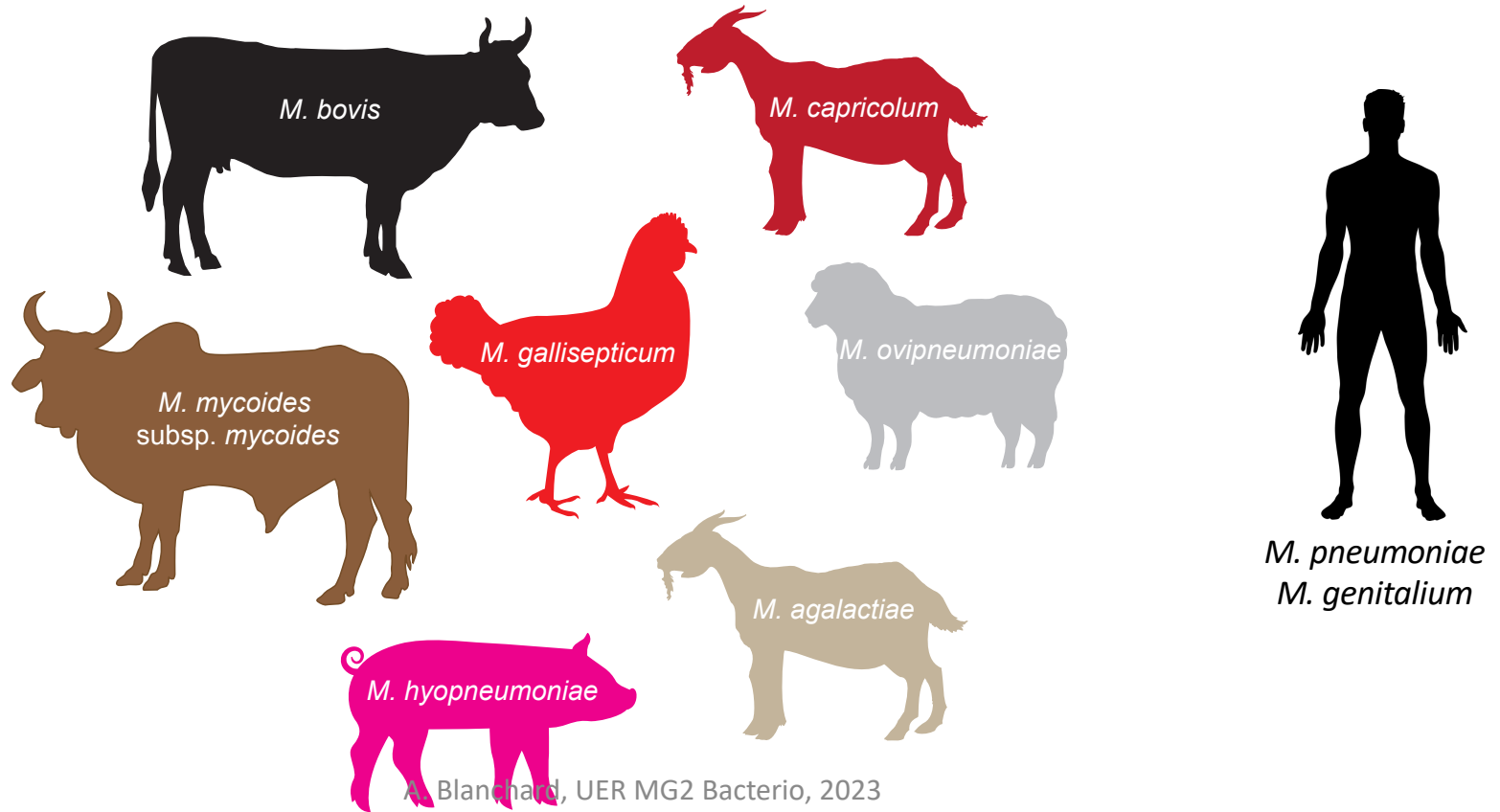
Over 100 *Mycoplasma* or *Ureaplasma* species are successful pathogens of a wide range of animals and human



- High morbidity, low mortality
- Chronic infection
- Predilection for the mucosal surface of the respiratory and reproductive tract as well as for the joints
- For several: no satisfactory control (preventive or therapeutic)

Citti and Blanchard. Trends Microbiol. 2013 Apr;21(4):196-203.

Several mycoplasma species have a strong economical impact



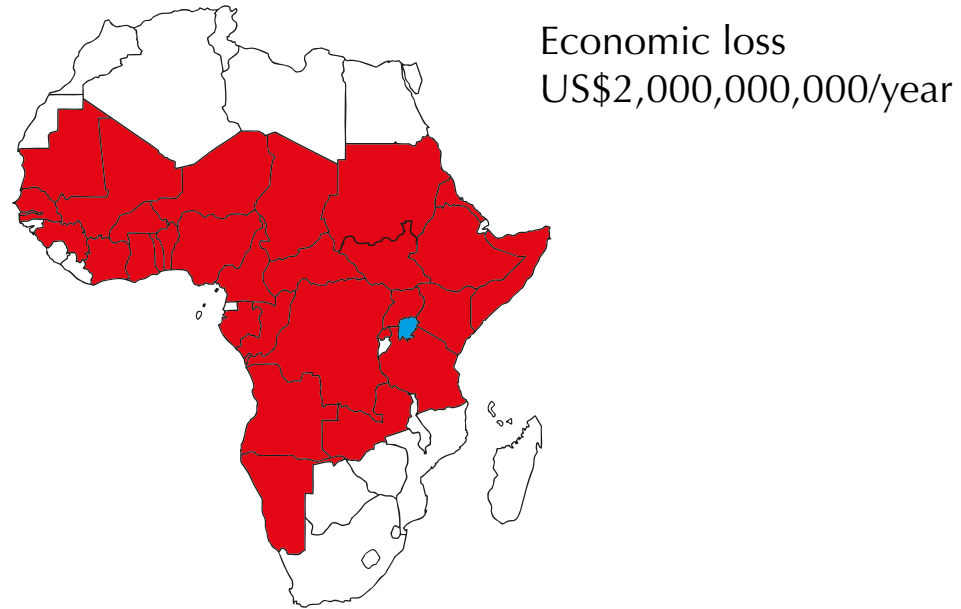
What is Contagious Bovine Pleuropneumonia, CBPP?

- *Mycoplasma mycoides*
subsp. *mycoides* (Mmm)
- acute, subacute or chronic respiratory disease
- Affects bovidae
- Causes financial losses
 - Reduced Productivity
 - Mortality
 - Trade restrictions

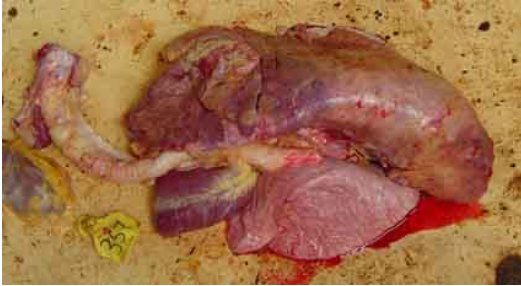


↗
Cow with CBPP

Geographic Distribution of CBPP (2010-2013)



CBPP - Pathology



CBPP - Control Strategies

- Live vaccine
 - Limited Efficacy, side effects and limited duration of immunity
- Quarantine
 - Control of animal movement
 - Test and Slaughter including compensation
- Antibiotics
 - Not recommended, but practiced

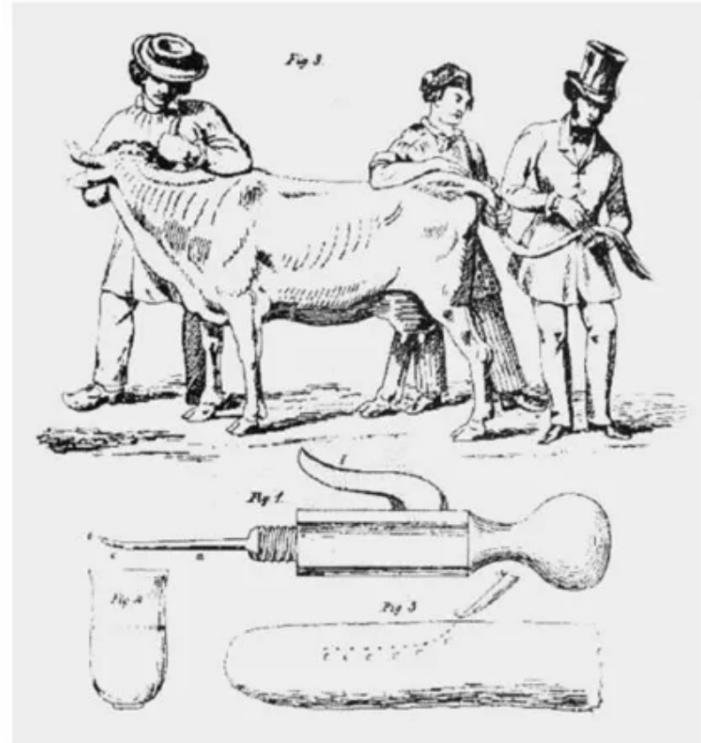
CBPP vaccination



**Willem's
Reaction**

Jores, J., Baldwin, C., Blanchard, A. et al. Contagious Bovine and Caprine Pleuropneumonia: a research community's recommendations for the development of better vaccines. *npj Vaccines* 5, 66 (2020).
<https://doi.org/10.1038/s41541-020-00214-2>

Willems and the PPCB vaccination



<https://veteriankey.com/a-brief-history-of-veterinary-vaccines/>

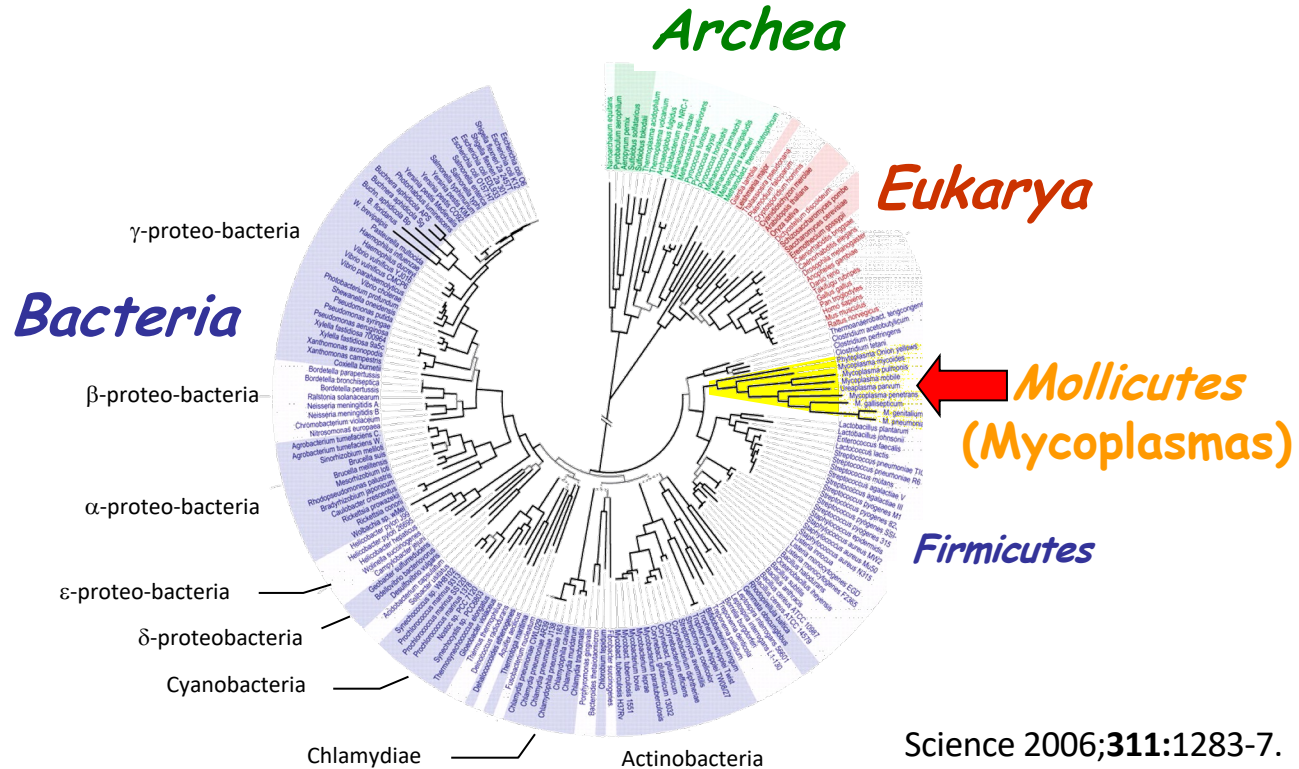
The correct way to vaccinate cattle against contagious bovine pleuropneumonia. Source: (From C.A. Spinage: "Cattle plague: a history," Kluwer; "The tail method of inoculation for pleuropneumonia", and the reference is Keulen, 1854. With permission.).

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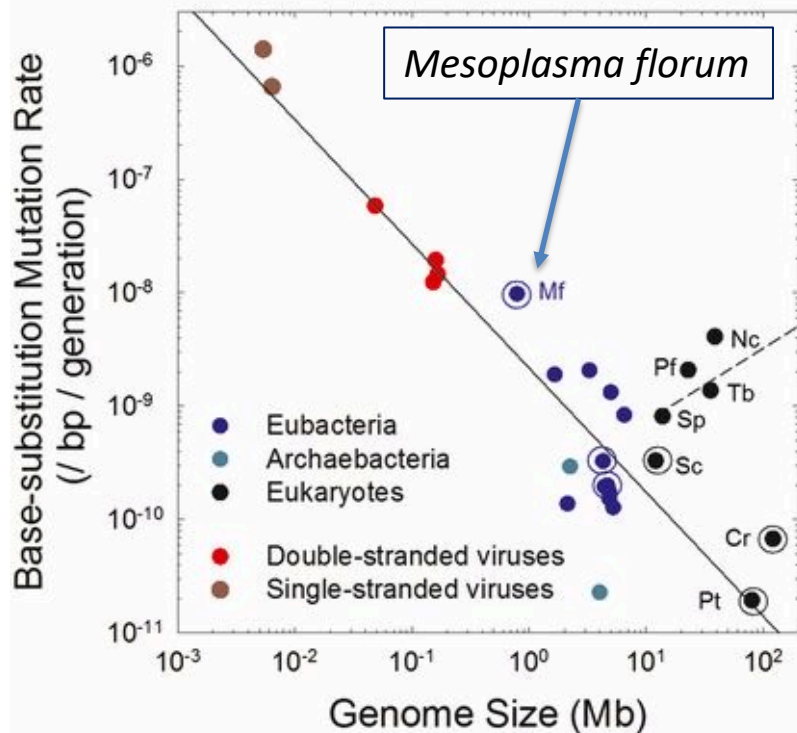
Mycoplasmas as some of the most evolved bacteria

Mollicutes (Mycoplasmas) derived from firmicutes during evolution



Science 2006;311:1283-7.

Mycoplasmas as fast-evolving bacteria, a reality



OPEN ACCESS Freely available online

PLOS GENETICS

Ultrafast Evolution and Loss of CRISPRs Following a Host Shift in a Novel Wildlife Pathogen, *Mycoplasma gallisepticum*

Nigel F. Delaney¹, Susan Balenger², Camille Bonneaud^{1,2a}, Christopher J. Marx¹, Geoffrey E. Hill², Naola Ferguson-Noel³, Peter Tsai⁴, Allen Rodrigo^{4,2b}, Scott V. Edwards^{1*}

« nucleotide substitution rate of $0.8-1.2 \times 10^{-5}$ per site per year »

« ...the sequencing of 30 *M. florum* lines after 2,000 cell divisions yields the highest known rate of base-substitutional mutation for any unicellular organism, $9.78 (0.71) \times 10^{-9}$ per site per cell division ».

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What is a minimal cell?

- « Minimal cell » is a concept
- Minimal cells comprise only essential genes and biomolecular machinery for basic life¹
- Essential genes are absolutely required for the survival of an organism and are therefore considered the foundation of life²
- Most of the work in this field has been performed using bacteria as a cellular chassis

¹Current Opinion Biotechnol 2010;**21**:697-703.

²Trends Cell Biol 2011;**21**:562-568.

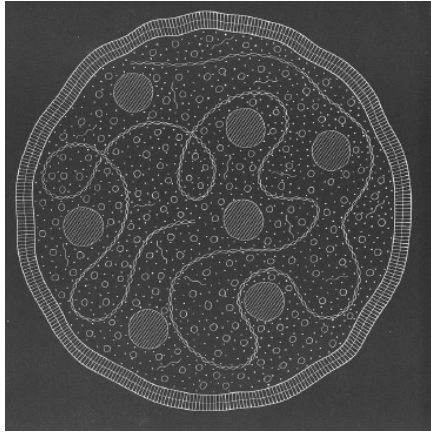
Mycoplasmas, the « smallest living cells »

The Smallest Living Cells

1962

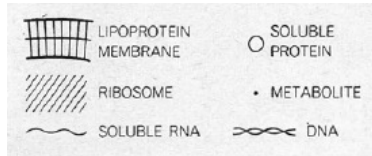
A microbe known as the pleuropneumonia-like organism gives rise to free-living cells smaller than some viruses. They suggest the question: What are the smallest dimensions compatible with life?

by Harold J. Morowitz and Mark E. Tourtellotte



From the “Scientific American” 1962; **206**:117-26

- NASA supports attempts to assemble a living cell from its separated components.
- It did not work but it gave a considerable push to the field of membrane reconstitution.



Shmuel Razin

Mycoplasma genomes among the first to be sequenced



Claire Fraser



Craig Venter



ARTICLES

The Minimal Gene Complement of *Mycoplasma genitalium*

Claire M. Fraser,* Jeannine D. Gocayne, Owen White, Mark D. Adams, Rebecca A. Clayton, Robert D. Fleischmann, Carol J. Bult, Anthony R. Kerlavage, Granger Sutton, Jenny M. Kelley, Janice L. Fleischman, Janice F. Weidman, Keith V. Small, Mina Sandusky, Joyce Fuhrmann, David Nguyen, Teresa R. Utterback, Deborah M. Saudick, Cheryl A. Phillips, Joseph M. Merrick, Jean-Francois Tomb, Brian A. Dougherty, Kenneth F. Bollt, Ping-Chuan Hu, Thomas S. Lucier, Scott N. Peterson, Hamilton O. Smith, Clyde A. Hutchison III, J. Craig Venter

Mycoplasma genitalium has the smallest genome (580 Kbp, ~500 genes) for a self-replicating organism

Mycoplasmas are members of the class Mollicutes, a large group of bacteria that lack a cell wall and have a characteristically low G + C content (1). These diverse organisms are parasites in a wide range of hosts including humans, animals, insects, plants, and cells grown in tissue culture (1). Aside from their role as potential pathogens, *Mycoplasmas* are of interest because of their reduced genome size and content relative to other prokaryotes.

Mycoplasma genitalium is thought to contain the smallest genome for a self-replicating organism (580 kb) and represents an important system for exploring a minimal functional gene set (2). *Mycoplasma genitalium* was originally isolated from urethral specimens of patients with non-gonococcal urethritis (3) and has since been shown to exist in parasitic association with ciliated epithelial cells of primate genital and respiratory tracts (4).

The strategy and methodology for whole-genome random ("shotgun") sequencing and assembly was similar to that previously described for *Haemophilus influenzae* (5, 6). To facilitate ordering of contigs, each template was sequenced from both ends. A total of

9,946 sequencing reactions were performed by five individuals using an average of eight AB 373 DNA sequencers per day for a total of 8 weeks. Assembly of 8472 high-quality *M. genitalium* sequence fragments along with 299 random genomic sequences from Peterson et al. (7) was performed with the TIGR ASSEMBLER (8). The assembly process generated 39 contigs (size range, 636 to 73,351 base pairs [bp]) that contained a total of 3,806,480 bp of primary DNA sequence data. Contigs were ordered by ASM_ALIGN, a program that links contigs on the basis of information derived from forward and reverse sequencing reactions from the same clone.

ASM_ALIGN analysis revealed that all 39 gups were spanned by an existing template from the small-insert genome DNA library (that is, there were no physical gaps in the sequence assembly). The order of the contigs was confirmed by comparing the order of the random genomic sequences from Peterson et al. (7) that were incorporated into the assembly with their known position on the physical map of the *M. genitalium* chromosome (9). Because of the high stringency of the TIGR ASSEMBLER, the 39 contigs were searched against each other with GRAST (a modified FASTA [10]) to detect overlaps (<30 bp) that would have been missed during the initial assembly process. Eleven overlaps were detected with this approach, which reduced the total number of gups from 39 to 28.

Templates spanning each of the sequence gaps were identified, and oligonucleotide primers were designed from the se-

quencing data to resolve ambiguities.

Ninety-nine percent of the *M. genitalium* genome was sequenced with better than single-sequence coverage, and the mean sequence redundancy was 6.5-fold. Although it is extremely difficult to assess sequence accuracy, we estimate our error rate to be less than 1 base in 10,000 on the basis of frequency of shifts in open reading frames (ORFs), overall quality of raw data, and fold coverage. The *M. genitalium* sequence (version 1.0) has been deposited in the Genome Sequence Database (GSDb) with the accession number L43967 (11).

Genome Analysis

The *M. genitalium* genome is a circular chromosome of 580,070 bp. The overall G + C content is 32% (A, 34%; C, 16%; G, 16%; and T, 34%). The G + C content across the genome varies between 27 and 37% (using a window of 5000 bp), with the regions of lowest G + C content flanking the presumed origin of replication for this organism (see below). As in *H. influenzae* (3), the ribosomal RNA (rRNA) operon (44%) and the transfer RNA (tRNA) genes (52%) in *M. genitalium* contain a higher G + C content than the rest of the genome, which may reflect the necessity of retaining essential G + C base pairing for secondary structure in rRNAs and tRNAs (12).

The genome of *M. genitalium* contains 74 Eco RI fragments, as predicted by both cosmid mapping data (9) and sequence analysis. The order and sizes of the Eco RI fragments determined by both methods are

4,573
on of

C. M. Fraser, J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, J. L. Fleischman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Saudick, C. A. Phillips, and J. C. Venter are at the Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA. J. M. Merrick is in the Department of Microbiology, State University of New York at Buffalo, Buffalo, NY 14244, USA. J. H. Hutchison III is at the University of Medicine and Dentistry, Newark, NJ 07102, USA. K. F. Bollt, P. C. Hu, T. S. Lucier, S. N. Peterson, and C. A. Hutchison III are at the University of North Carolina at Chapel Hill, Chapel Hill, NC 27598, USA.

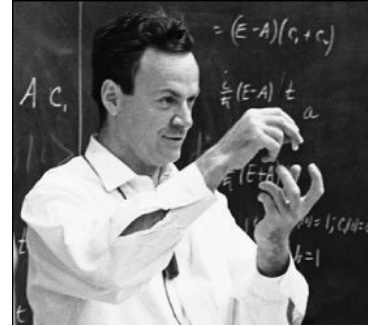
*To whom correspondence should be

Science 1995; 270:397-403.

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Build life to understand it?



« *What I cannot create I do not understand* »

Richard Feynman, 1988. Nobel Prize 1965.

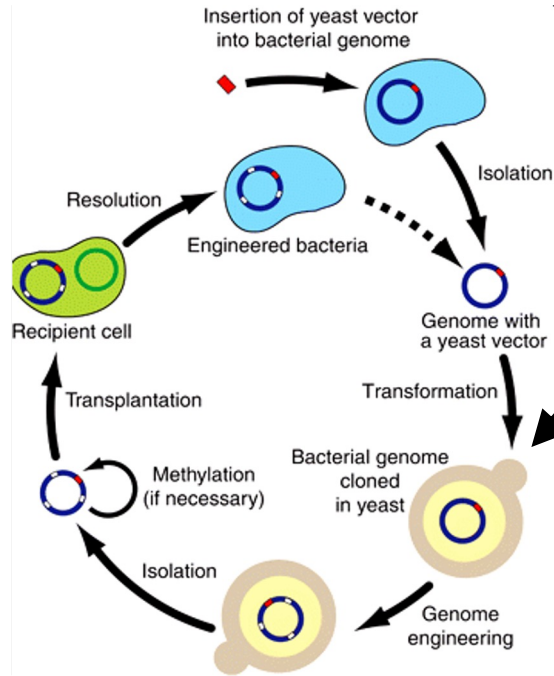
➔ moto of Synthetic Biology

Build life to understand it

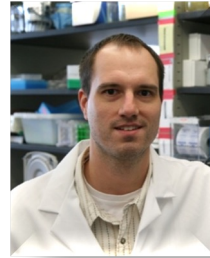
Biologists and engineers should work together: synthetic biology reveals how organisms develop and function, argue **Michael Elowitz** and **Wendell A. Lim**.

A. Blanchard, UER MG2 Bacterio, 2023

Synthetic Biology: mycoplasma genome engineering



Science 2009; **325**:1693-6.



Daniel Gibson



Carole Lartigue



Craig Venter

Synthetic genome

J. Craig Venter
INSTITUTE

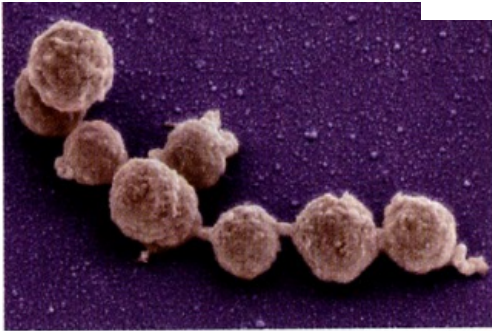


Hamilton Smith, Clyde Hutchinson



John Glass

THE 50 BEST INVENTIONS OF THE YEAR

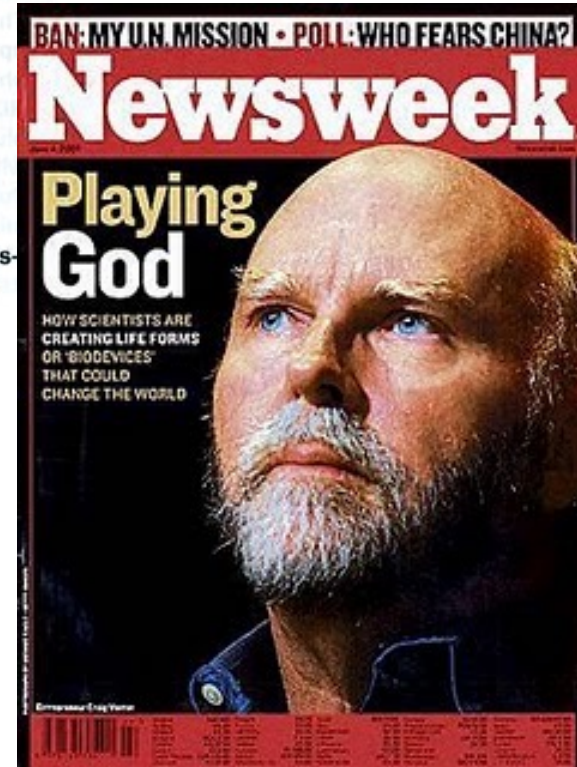


Time (NO. 3)

First Synthetic Cell

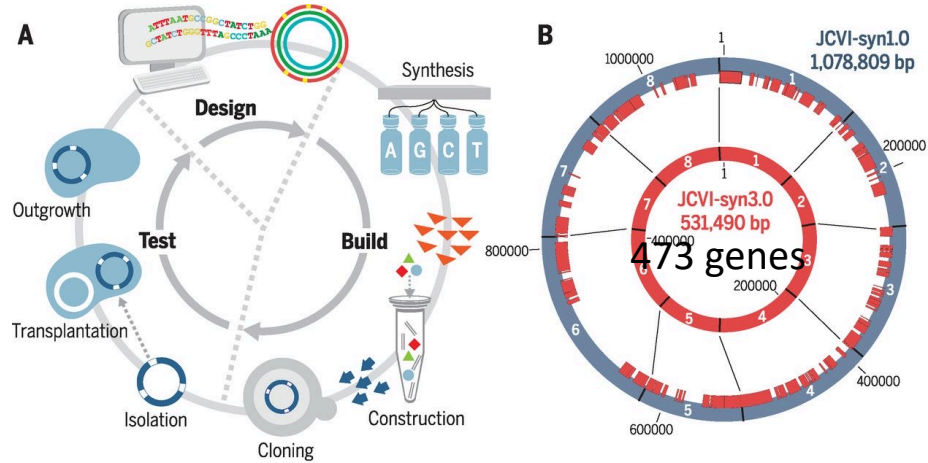
Creating life in the lab? It wasn't such a stretch for J. Craig Venter, who successfully co-mapped the human

The Economist's

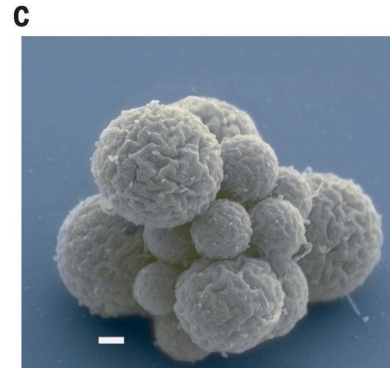


The synthesis of a new minimal cell

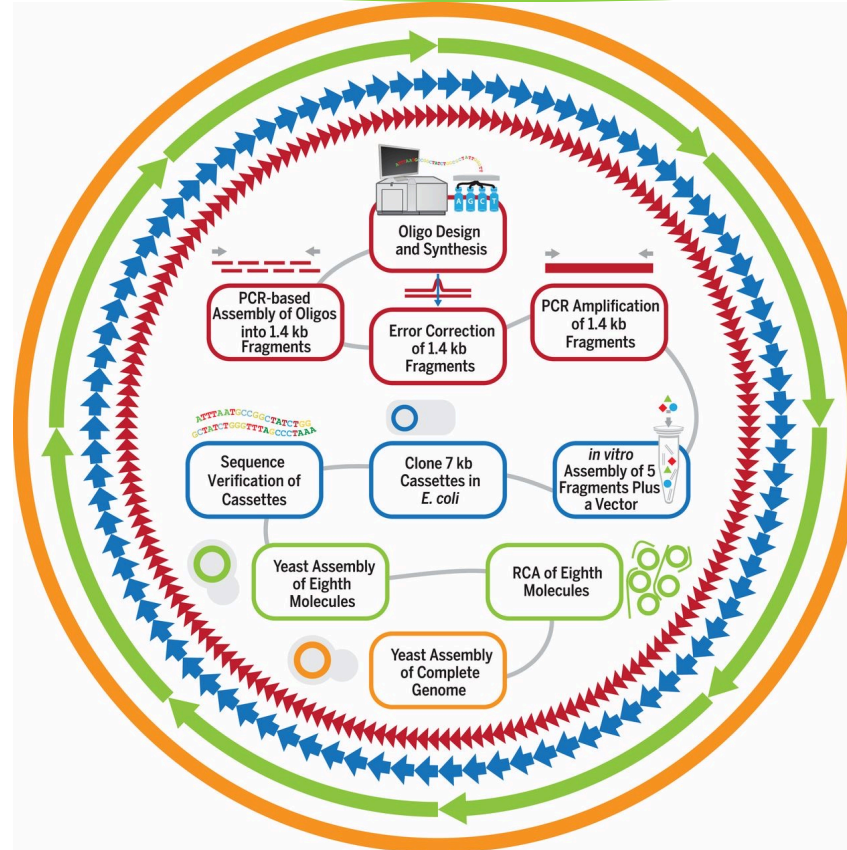
Four design-build-test cycles produced JCVI-syn3.0.



Hutchison et al. Science. 2016
25;351(6280):aad6253.

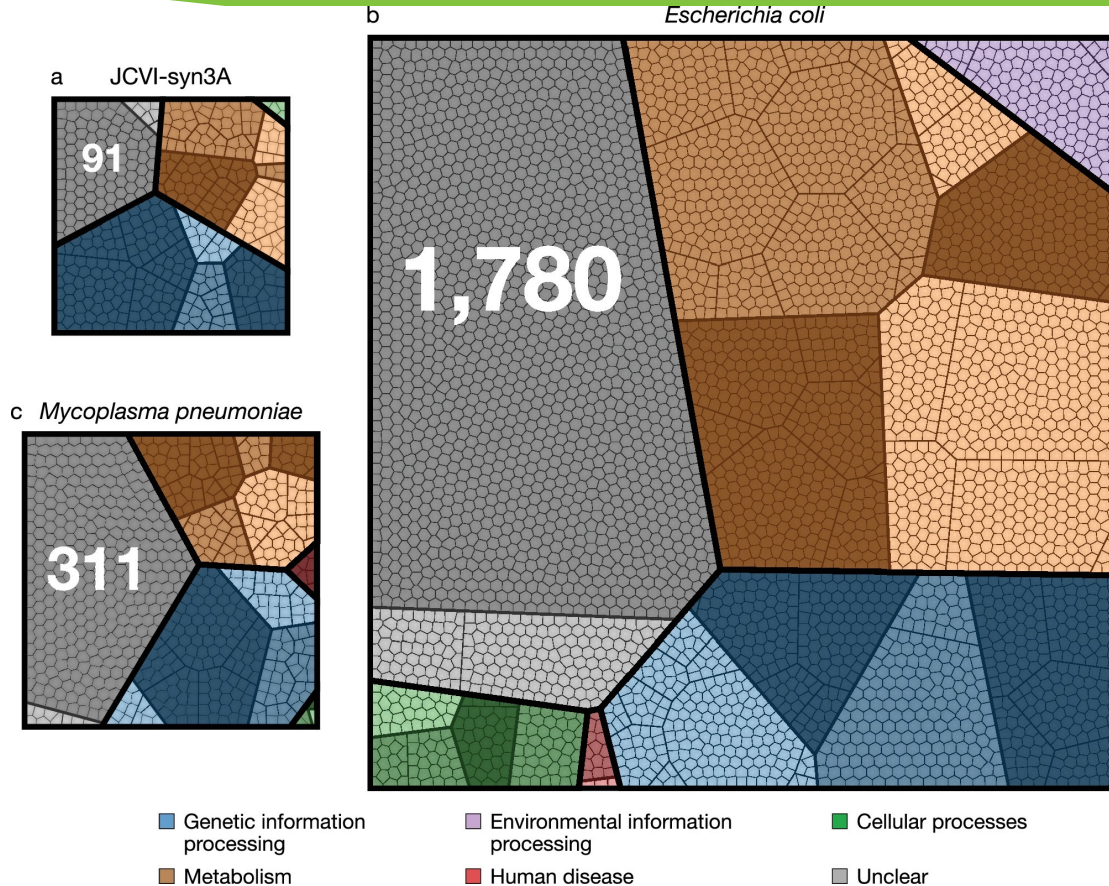


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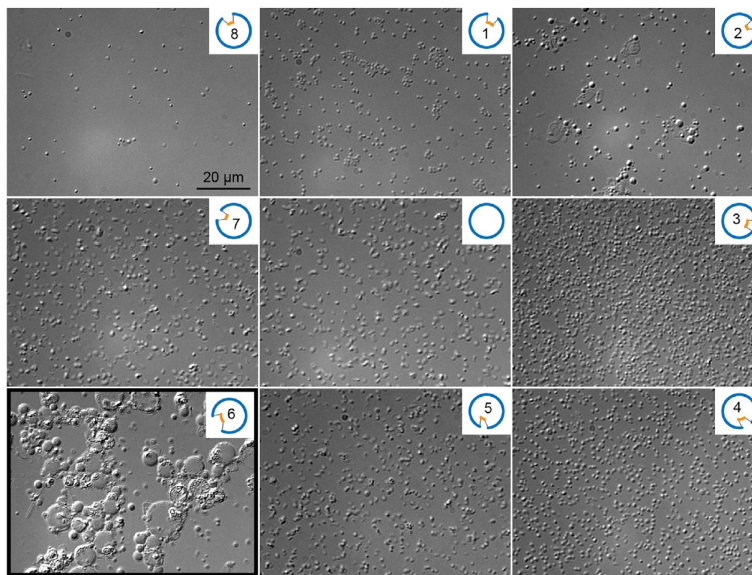
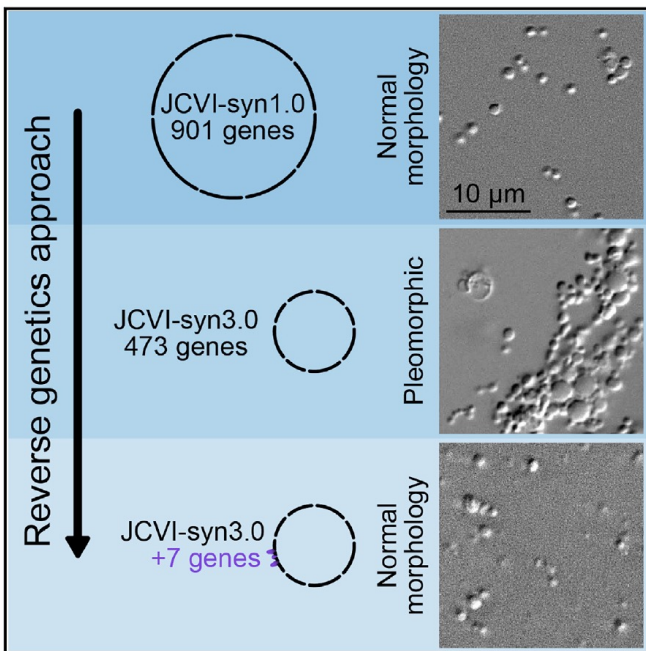


Hutchison et al. Science. 2016
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The synthesis of a new minimal cell



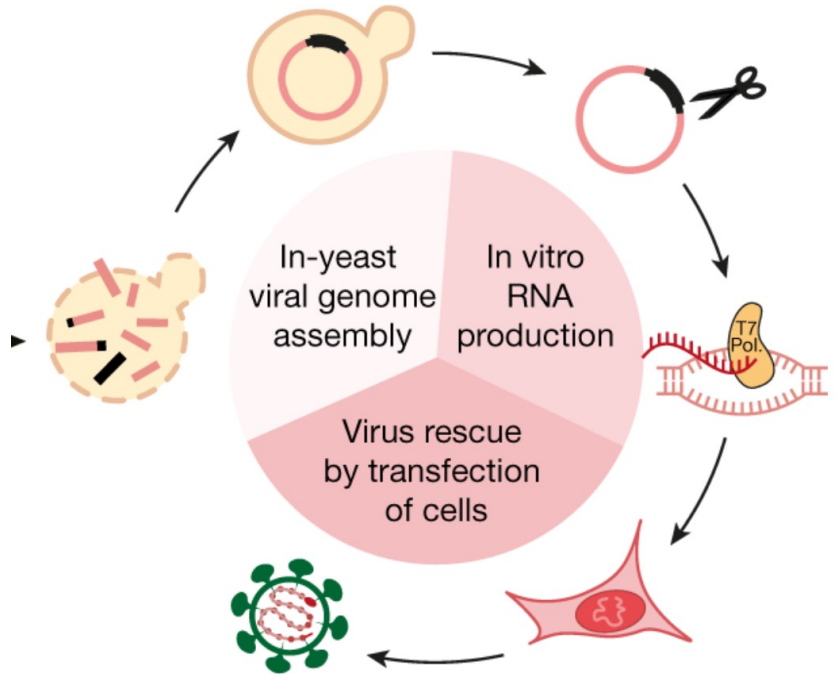
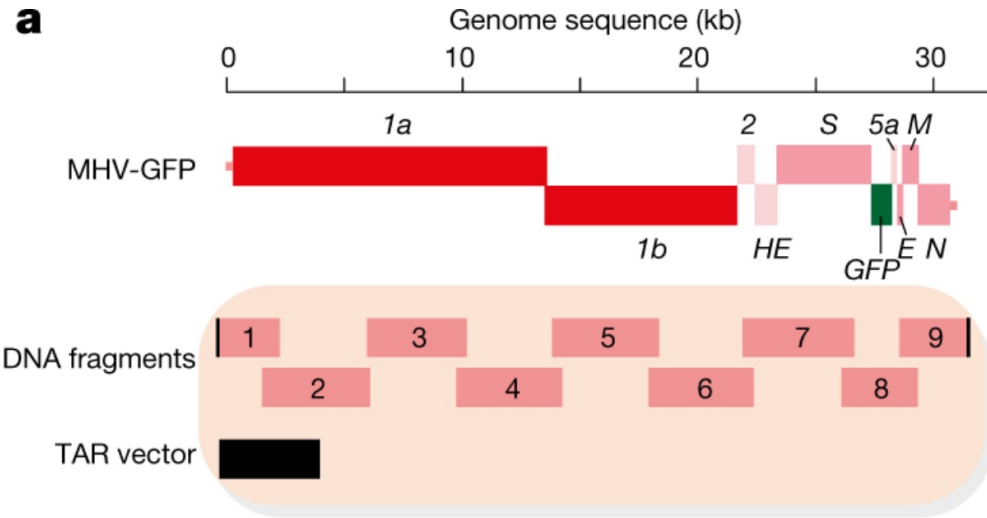
The synthesis of a new minimal cell: fixing cell division problems



Serial deletions from Syn1.0 genome identified a genome region (6) that encodes factors regulating cell shape/division

Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform

[Tran Thi Nhu Thao](#), [Fabien Labrousseau](#), [...] [Volker Thiel](#) [✉](#)



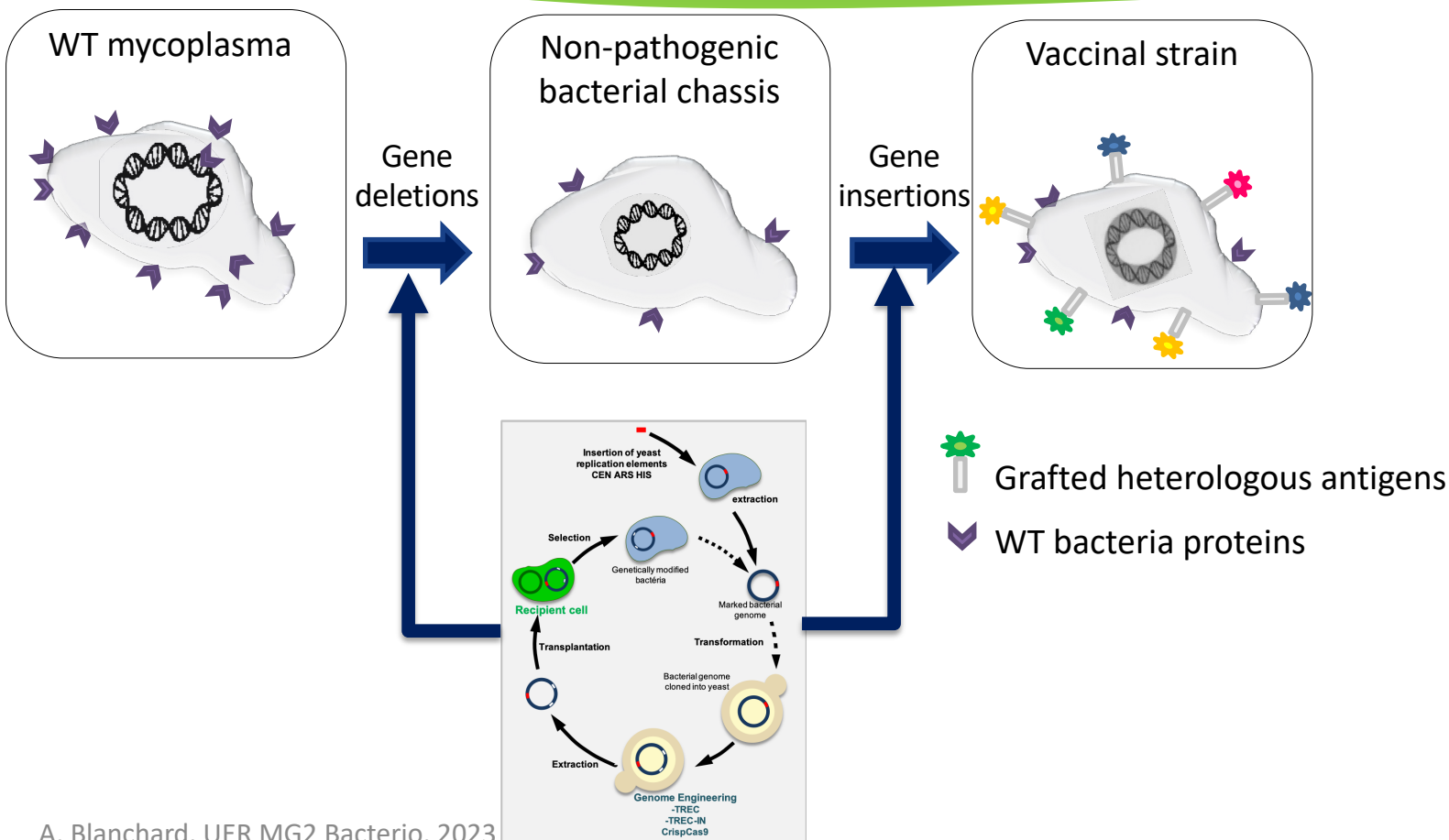
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What's the use?

- Building minimal cells → foundation of life
- Functional genomics
- Building chassis
 - Vaccines
 - Delivery systems
- Others

From a mycoplasma chassis to a candidate vaccine



From a mycoplasma chassis to a delivery system

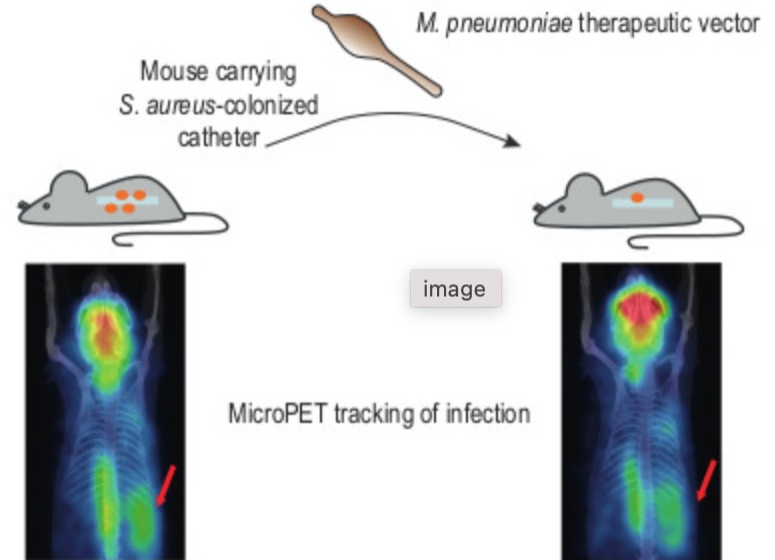
Article



molecular
systems
biology

Engineering a genome-reduced bacterium to eliminate *Staphylococcus aureus* biofilms *in vivo*

Victoria Garrido^{1,2,†} , Carlos Piñero-Lambecka^{1,3,†} , Irene Rodríguez-Arce^{1,2} , Bernhard Paetzold^{1,4} , Tony Ferrar¹ , Marc Weber¹ , Eva García-Ramallo^{1,3} , Carolina Gallo¹ , María Collantes^{5,6} , Iván Peñuelas^{5,6} , Luis Serrano^{1,7,8,*} , María-Jesús Grillo^{2,**} & María Lluch-Senar^{1,3,9,***}

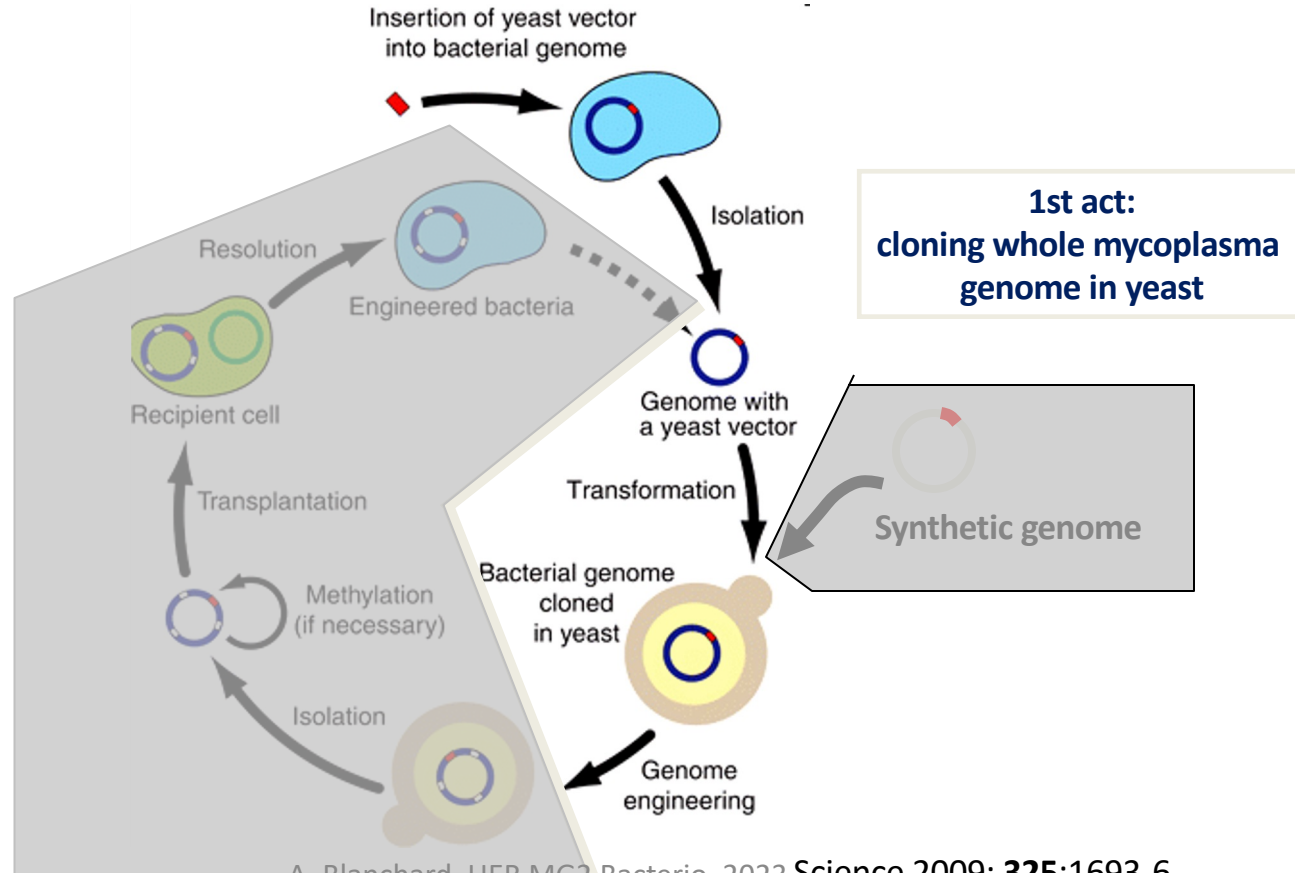


Mol Syst Biol (2021)17:e10145 <https://doi.org/10.15252/msb.202010145>

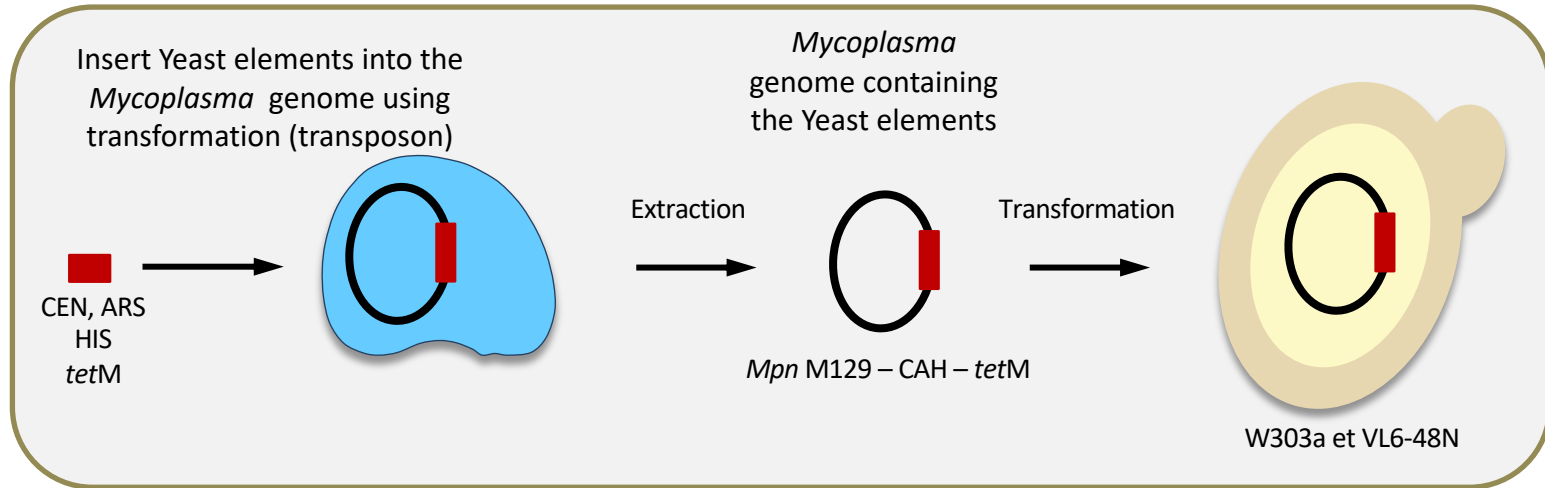
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Mycoplasma genome engineering, a Synthetic Biology trilogy



Transformation of a mycoplasma genome in yeast

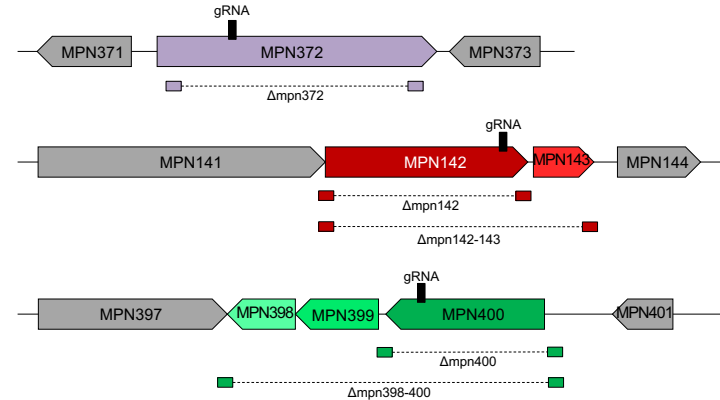
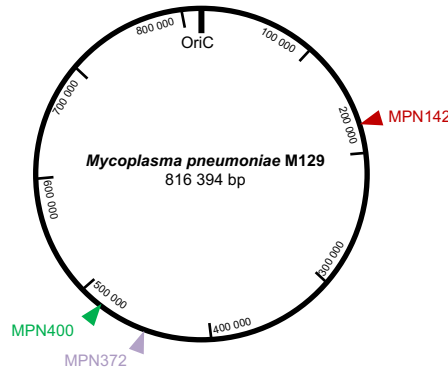
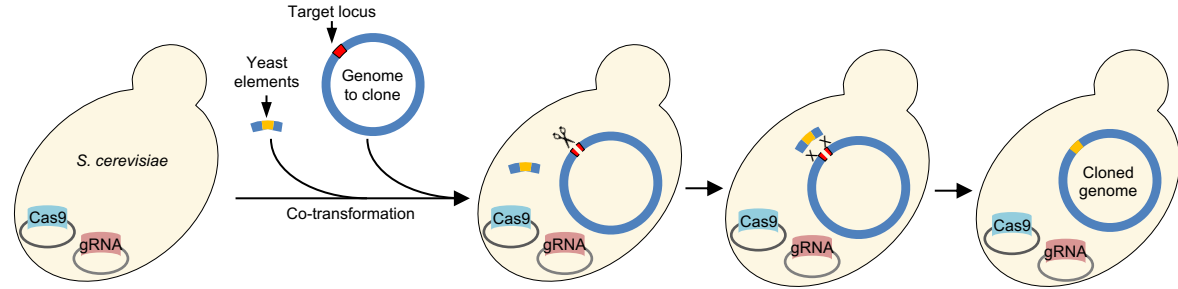


Lartigue *et al*, 2009 ; Benders *et al*, 2010 ; Labroussaa *et al*, 2016

Problems with this approach:

- random insertion of the yeast elements in the *Mycoplasma* genomes
- It takes a few weeks

CRasPy-cloning applied to the *M. pneumoniae* genome cloning in yeast



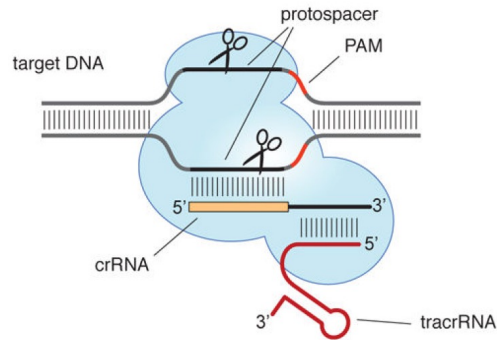
"This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634942."

E. Ruiz et al, ACS Synth Biol. 2019 Oct 30. doi:
10.1021/acssynbio.9b00224.

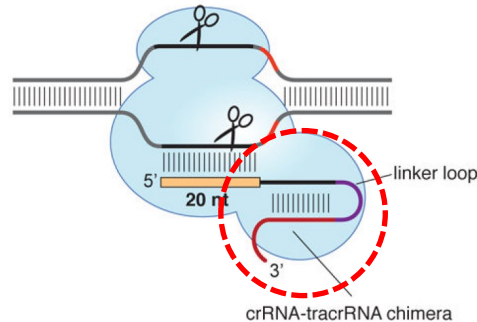
A. Blanchard, UER MG2 Bacterio, 2023

Game-changing finding in 2012

Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by single chimeric RNA



Martin Jinek, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, **Jennifer A. Doudna, and Emmanuelle Charpentier**. *Science*. 2012 Aug 17; 337(6096): 816–821.
Cited more than 17.000 times

Emmanuelle Charpentier

Jennifer A. Doudna

The 2020 Chemistry Laureates

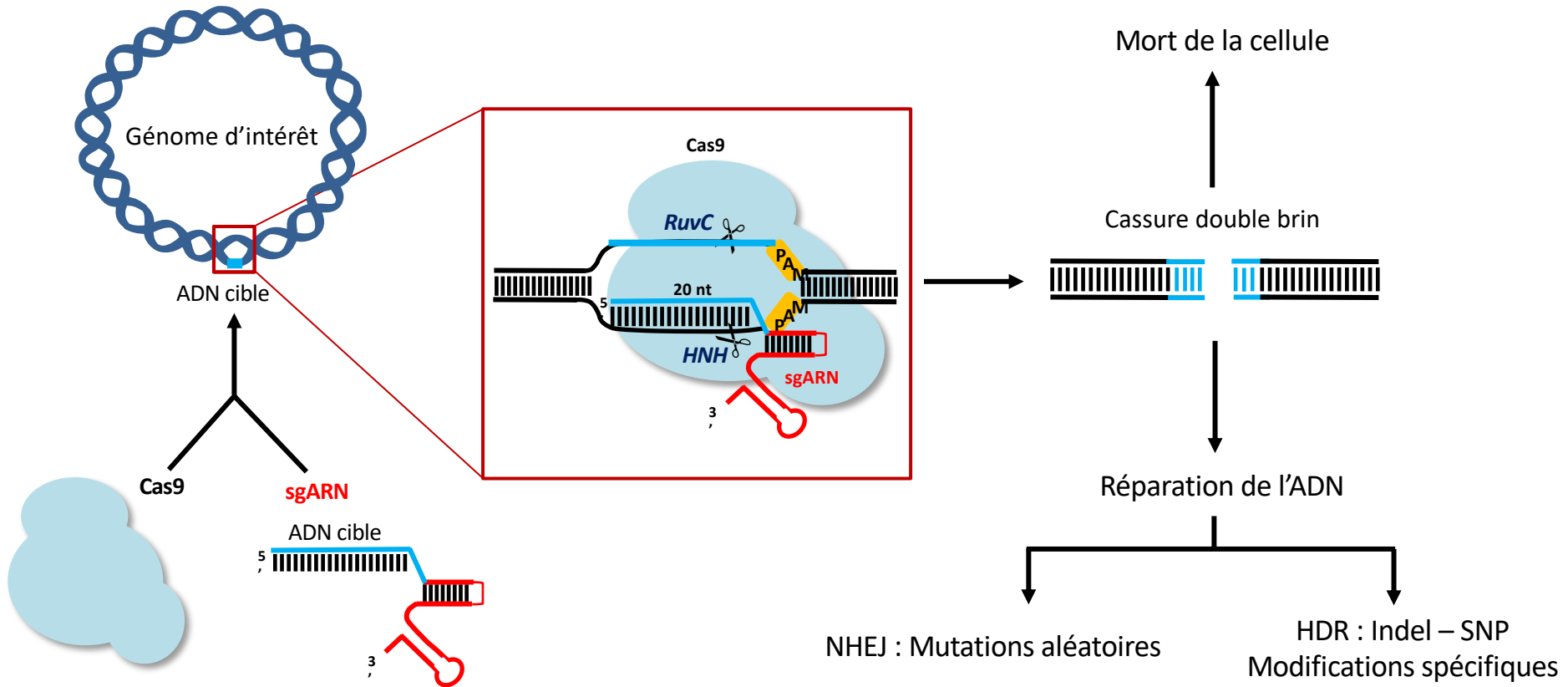
The 2020 Nobel Prize in Chemistry is awarded to [Emmanuelle Charpentier](#) and [Jennifer A. Doudna](#) “for the development of a method for genome editing”.

Since Charpentier and Doudna discovered the CRISPR/Cas9 genetic scissors in 2012 their use has exploded. The genetic scissors have taken the life sciences into a new epoch and, in many ways, are bringing the greatest benefit to humankind.

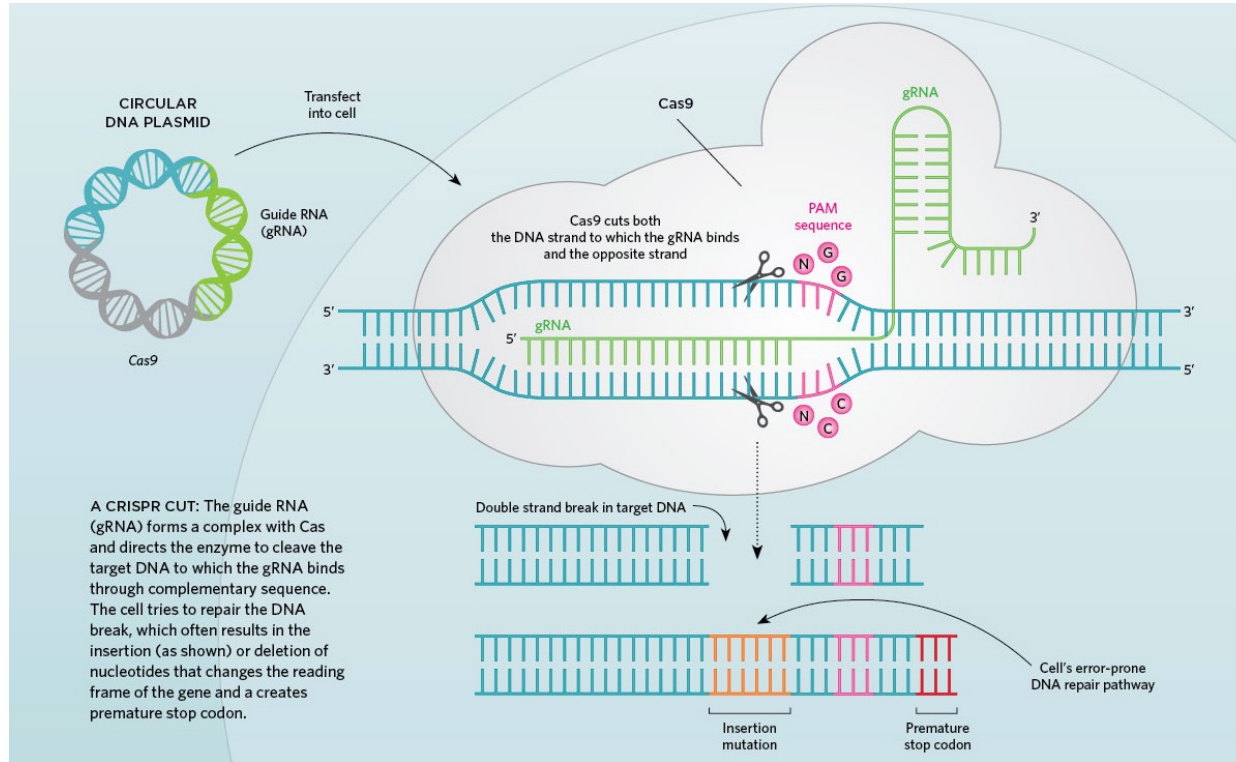


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CRISPR/Cas9 l'outil d'ingénierie

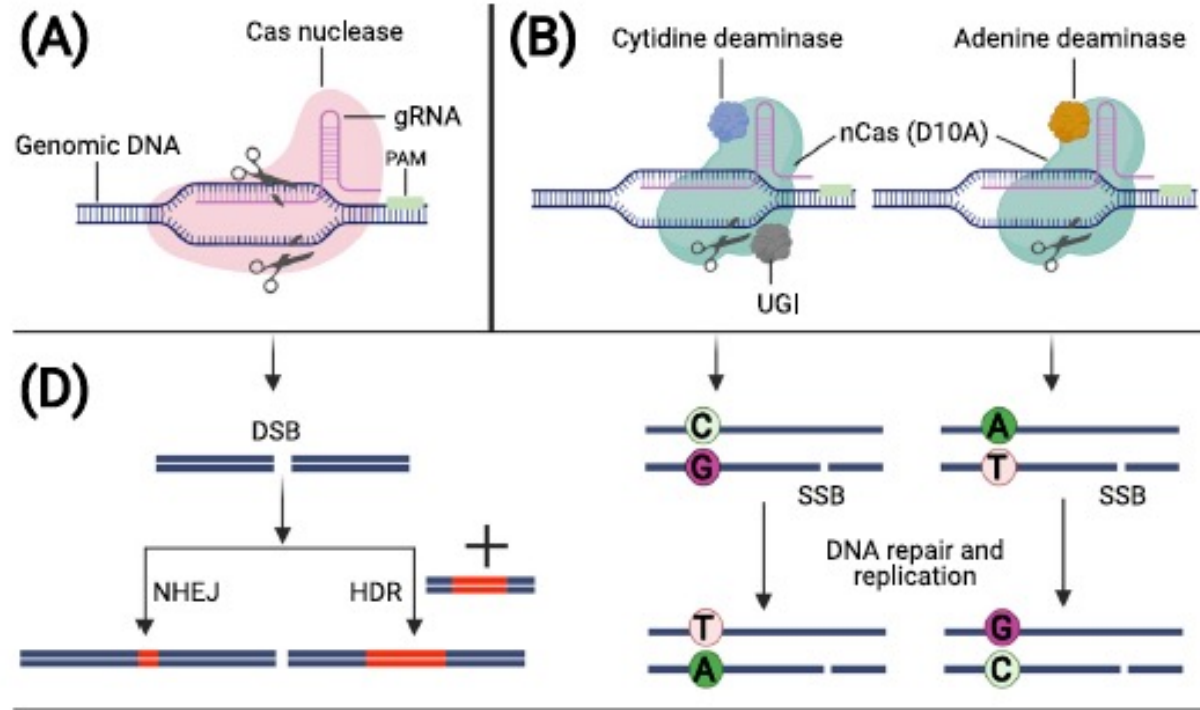


Utilisation de CRISPR-Cas9, une révolution dans l'ingénierie génomique



Tiré de http://www.the-scientist.com/images/March2014/CRSPR_full.jpg

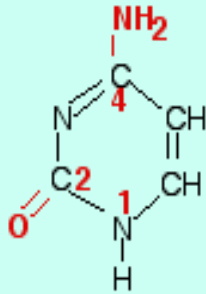
From CRISPR-Cas9 to CRISPR-Cas-base editing



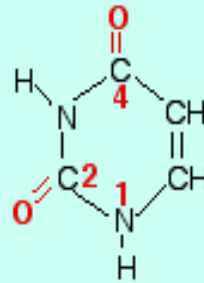
Trends in Plant Science 2021, Vol. 26, No. 11, pp1133-1152. <https://doi.org/10.1016/j.tplants.2021.06.015>



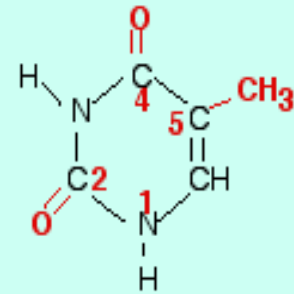
Pyrimidine



Cytosine (C)

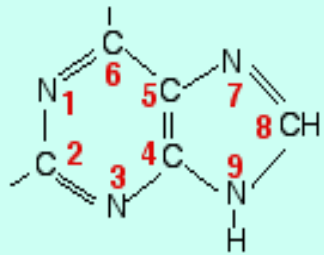


Uracile (U)

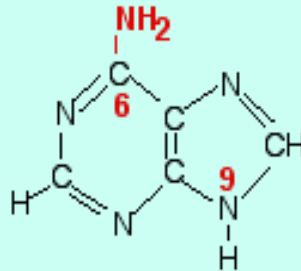


Thymine (T)

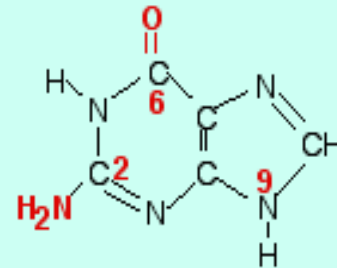
e



Purine



Adénine (A)

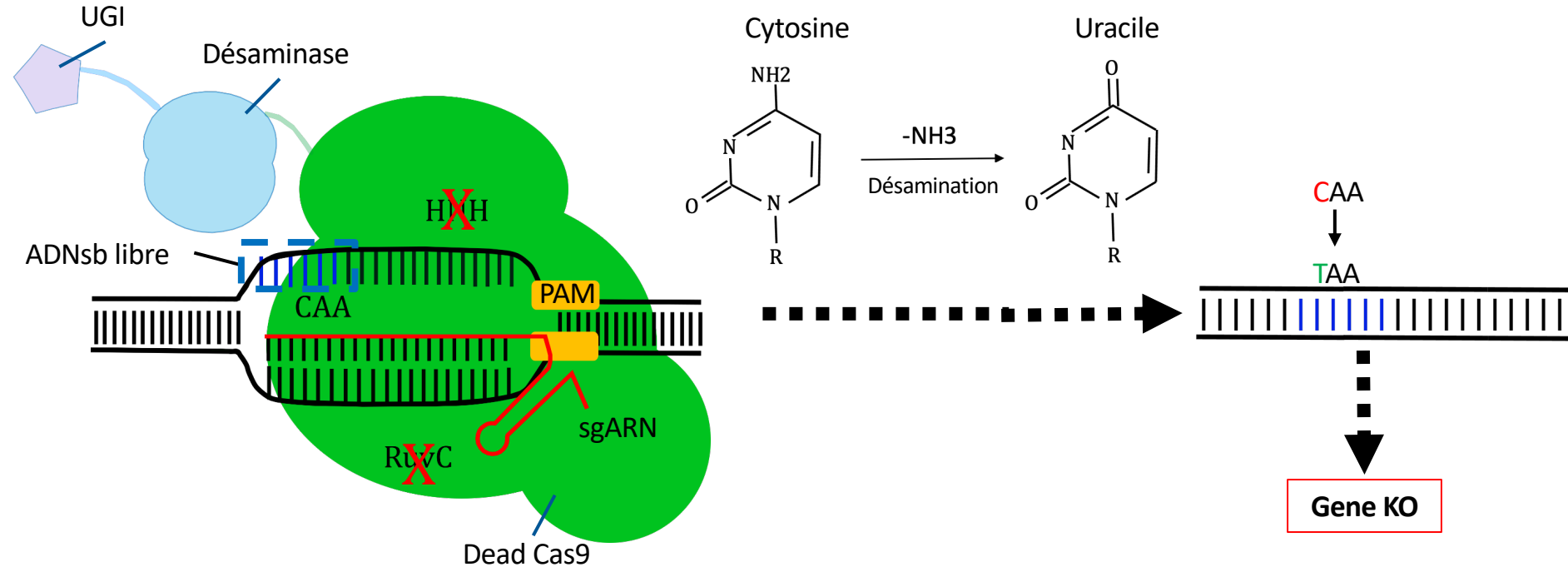


Guanine (G)

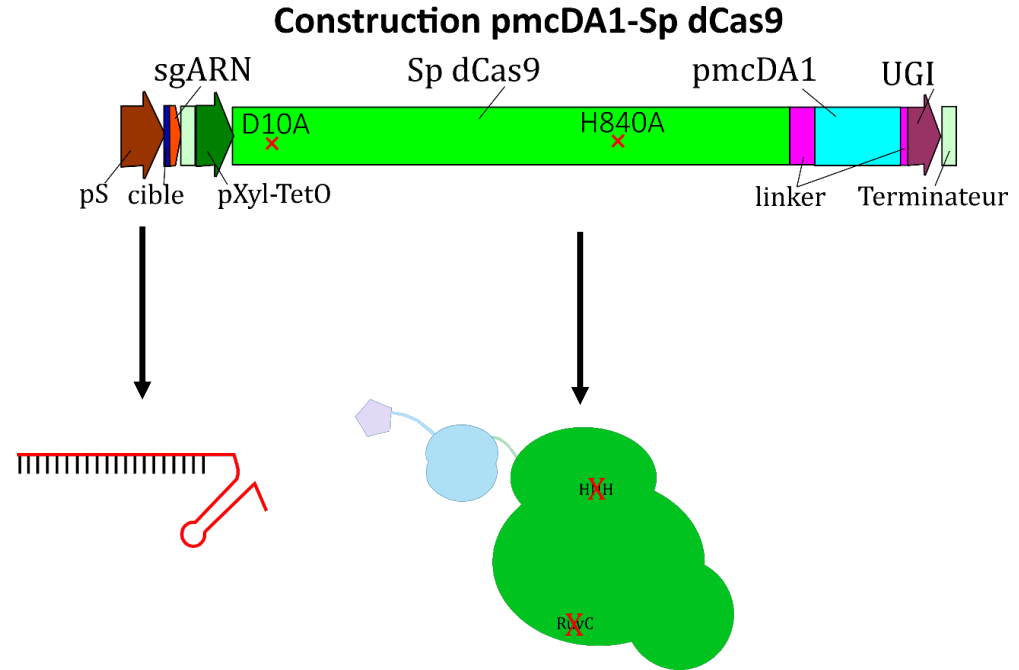
Noter que les pyrimidines présentent de fortes similitudes, ainsi:

- l'uracile est une cytosine désaminée
- l'uracile est une thymine déméthylée

- Principe des systèmes d'édition de bases

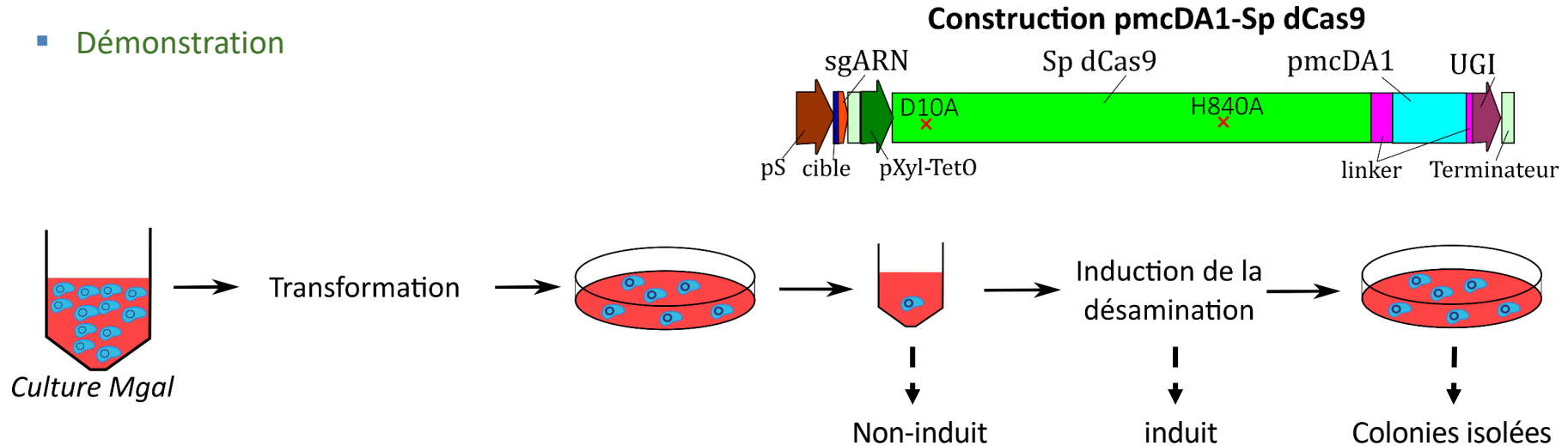


■ Démonstration



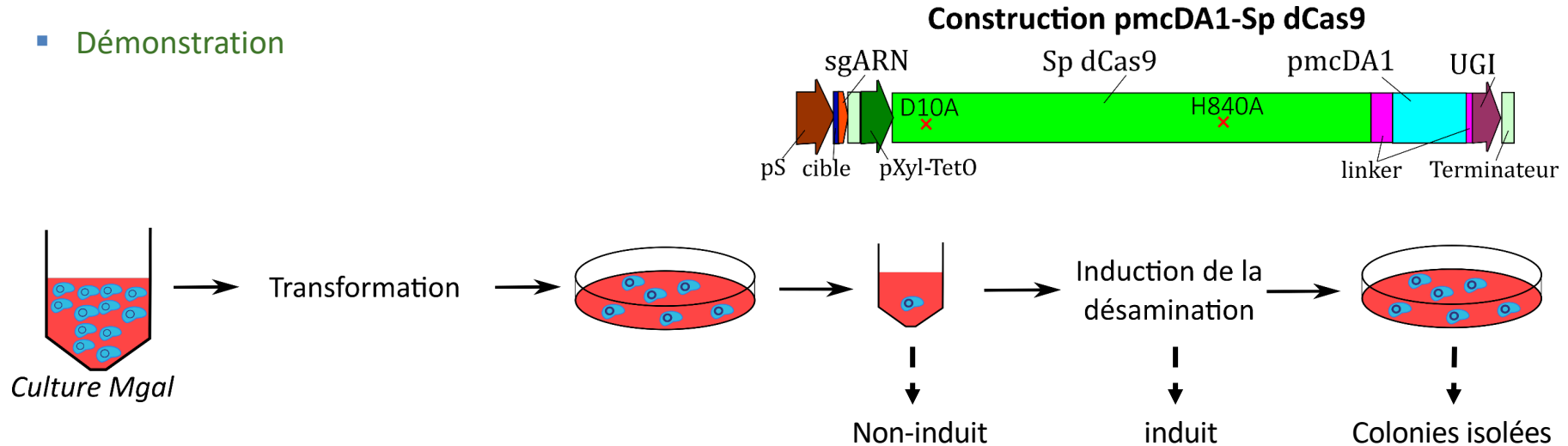
Ipoutcha et al Appl Environ Microbiol. 2022 Sep 13;88(17):e0099622. doi: 10.1128/aem.00996-22

■ Démonstration



Ipoutcha et al Appl Environ Microbiol. 2022 Sep 13;88(17):e0099622. doi: 10.1128/aem.00996-22

■ Démonstration

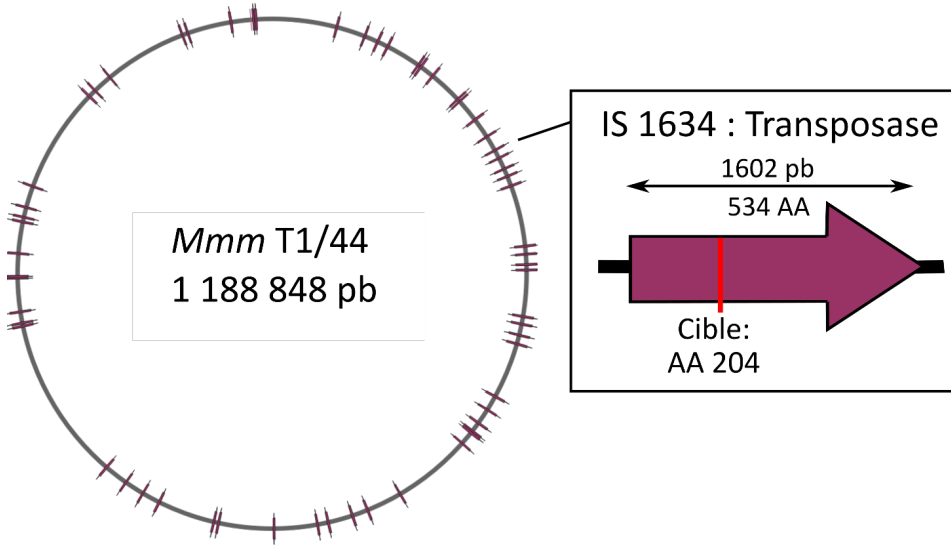


Cible *ksgA* : T G A C **C** A A A A T A T T A T T A A T A **T G G**



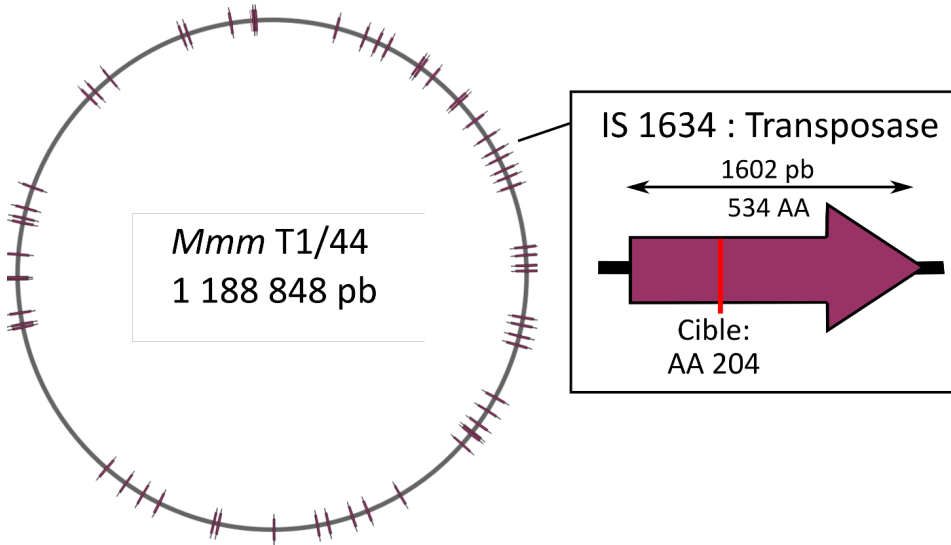
Cytosine cible pouvant générer un codon stop

- Repousser les limites du système

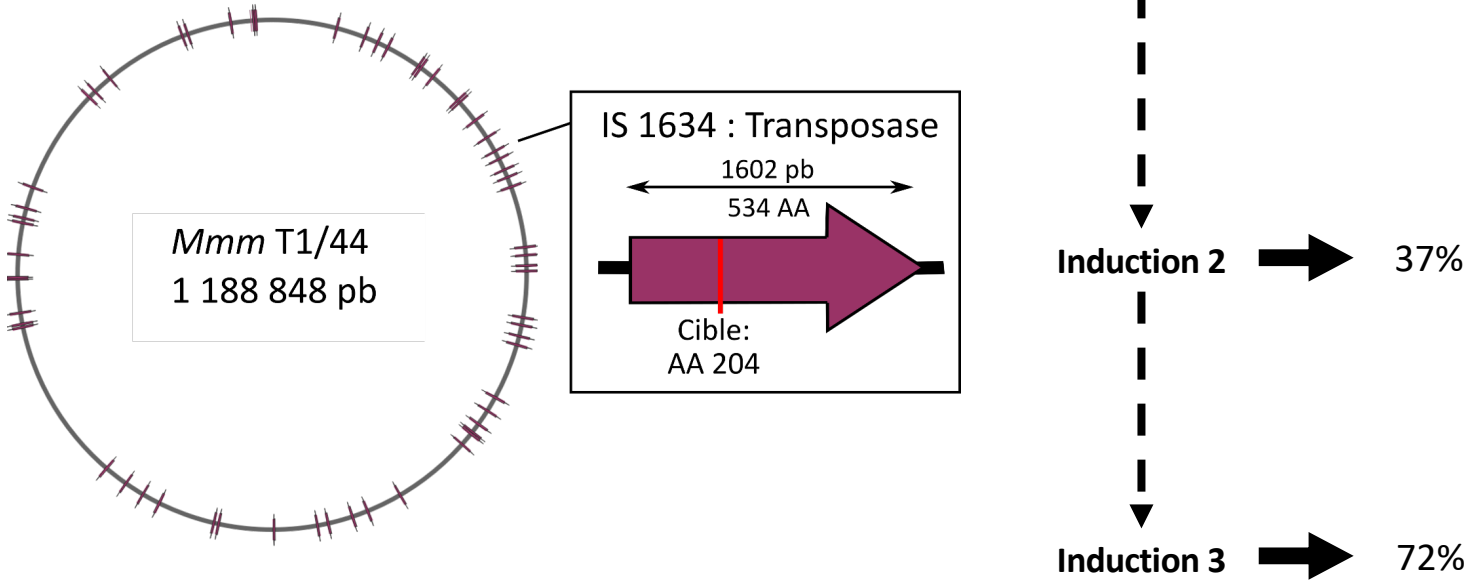


- Repousser les limites du système

Induction 1 → 5%



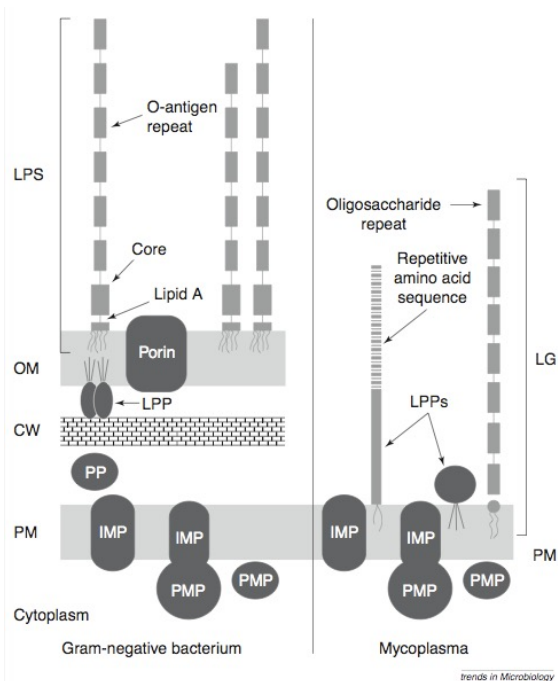
- Repousser les limites du système



Briefly, what do we know about mycoplasmas?

- Colonize a large variety of hosts
- Mycoplasmas, among the most evolved organisms
- Mycoplasmas and the concept of a minimal cell
- Build a minimal cell/chassis starting with a mycoplasma cell
 - Rationale
 - What's the use?
 - Technical progress made in the INRA laboratory
- **Pathogenicity and virulence factors**
 - Mostly extracellular but for some are able to survive intracellularly
 - No typical virulence factors (no typical Type III or IV secretion system, few or no toxin, very few regulators or signal sensing systems such as 2-component systems)
 - Adhesins are essential for virulence
 - Interference of their metabolism with that of their host or toxicity from their metabolism (H_2O_2 production)
 - Variable surface antigens (evasion from the immune system)
 - Immunoglobulin-binding proteins + Ig protease (MIB-MIP)

Mycoplasma lipoproteins, variable surface antigens



Veterinary Microbiology 153 (2011) 44–50



Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Review

The central role of lipoproteins in the pathogenesis of mycoplasmoses

G.F. Browning*, M.S. Marenda, A.H. Noormohammadi, P.F. Markham

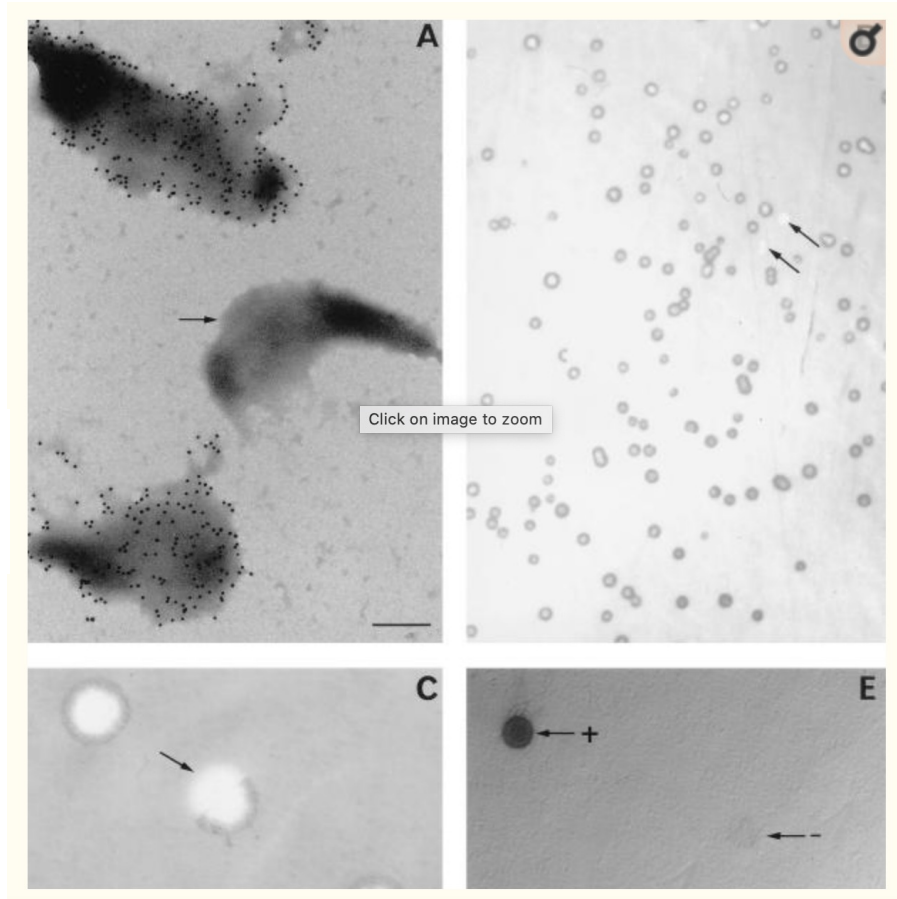
Asia-Pacific Centre for Animal Health, Faculty of Veterinary Science, The University of Melbourne, Parkville, Victoria 3010, Australia

Chambaud et al., 1999, Trends Micro

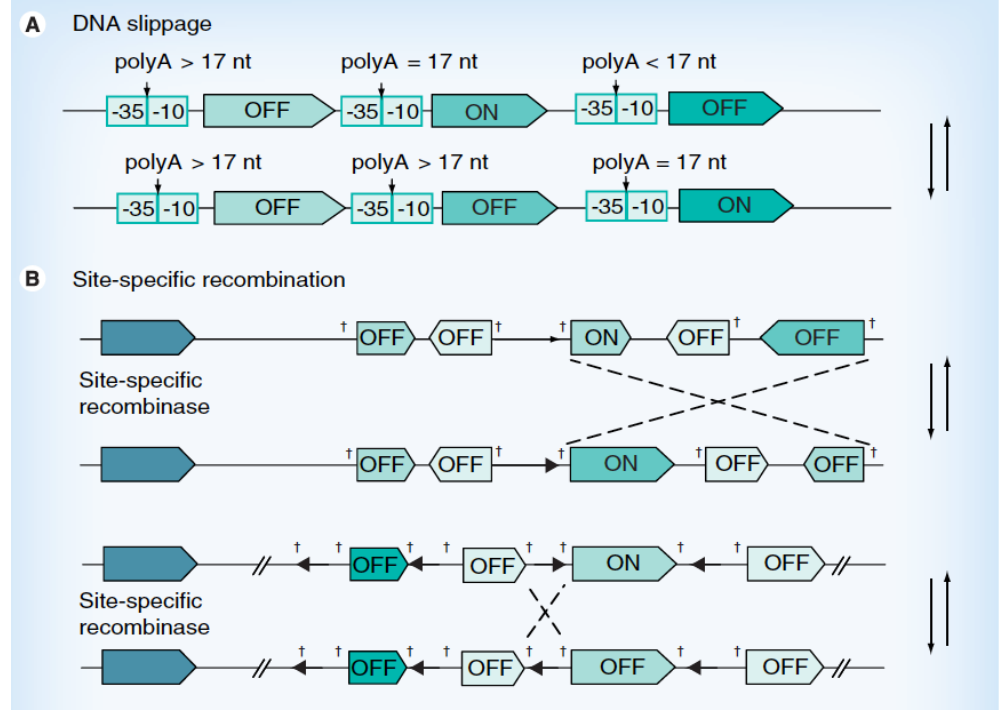
A. Blanchard, UER MG2 Bacterio, 2023

Variation antigénique de la lipoprotéine P35 chez *Mycoplasma penetrans*

Infect Immun. 1999 Apr;67(4):1569-78.
Phase variations of the *Mycoplasma penetrans* main surface lipoprotein
increase antigenic diversity
O Neyrolles et al



Mécanismes moléculaires correspondant à la variation antigénique chez les mycoplasmes



Citti et al

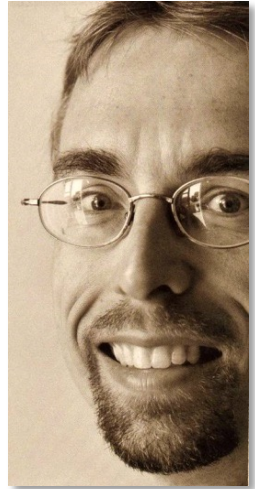
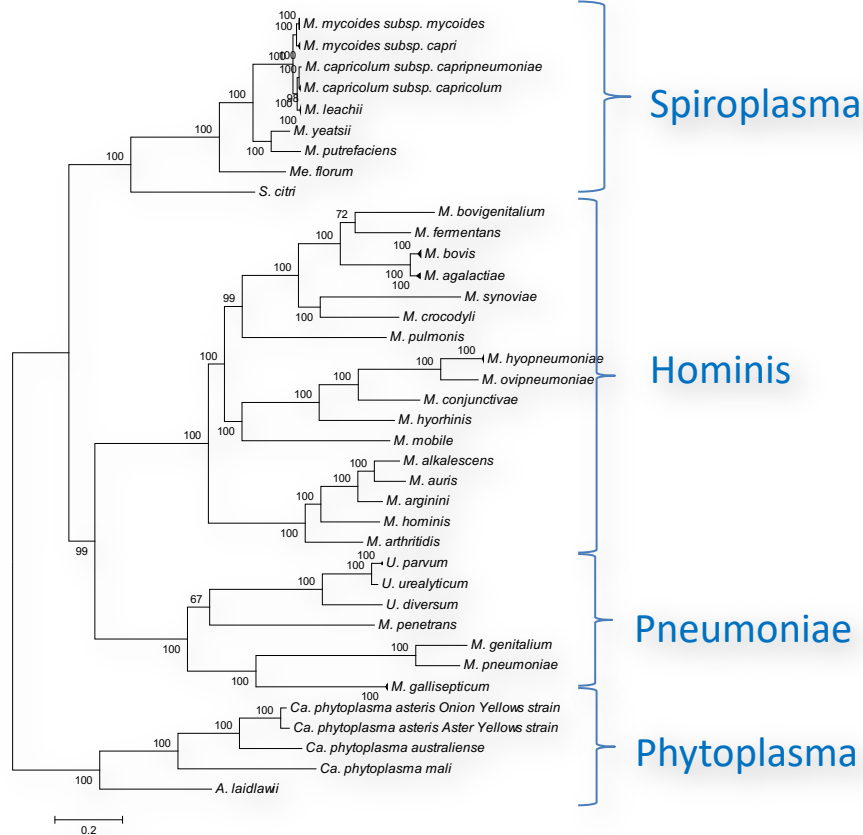
Future Microbiol. (2010) 5(7), 1073–1085

A. Blanchard, UER MG2 Bacterio, 2023

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Virulence/host factors are often exchanged by HGT among bacteria



P. Sirand-Pugnet

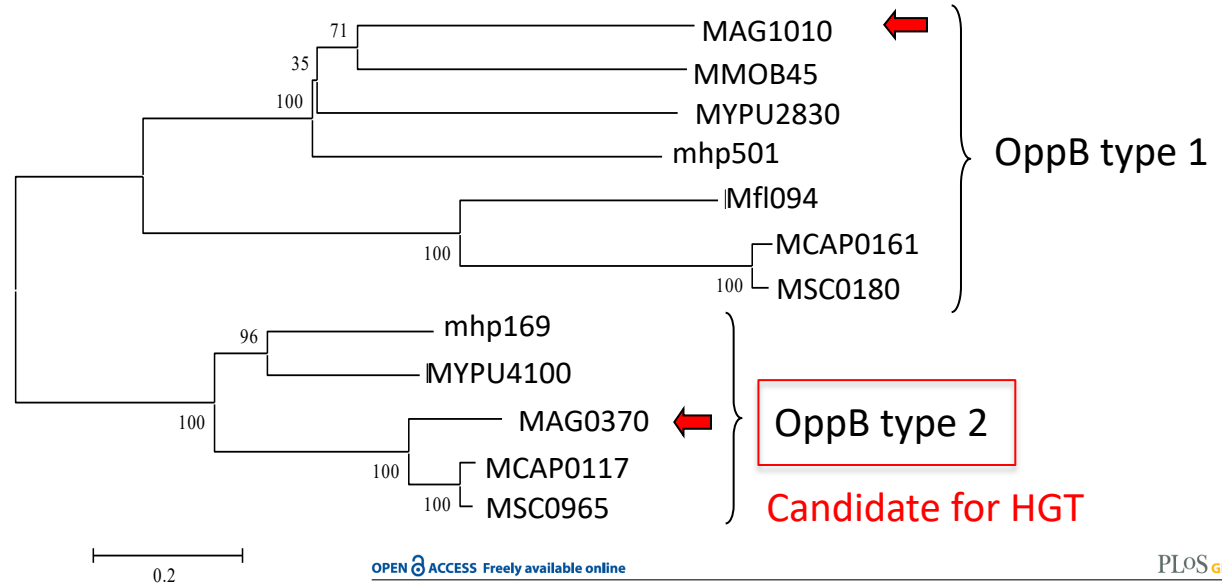
How to identify putative horizontal gene transfer (HGT) events?

Redoing this phylogenetic tree for each ortholog in mycoplasmas and looking for incongruence in the tree compared to the « true » phylogenetic tree

Virulence factors are often exchanged by HGT among bacteria

METHOD

- Build sets of orthologs by using clustering methods*
- For each set of orthologs build a phylogenetic tree
- Look for incongruence as compared to the « true phylogenetic tree »

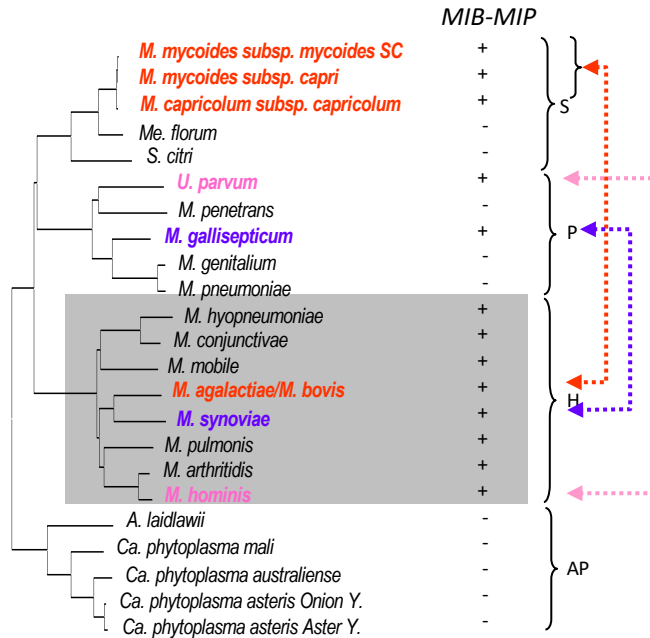


*: BMC Bioinformatics. 2011;12:457. doi: 10.1186/1471-2105-12-457.

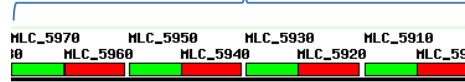
Being Pathogenic, Plastic, and Sexual while Living with a Nearly Minimal Bacterial Genome

Pascal Sirand-Pugnet^{1,2}, Carole Lartigue^{1,2,a}, Marc Marends^{3,a,b}, Daniel Jacob⁴, Aurélien Barré⁴, Valérie Barbe⁵, Chantal Schenowitz⁵, Sophie Mangenot⁵, Arnaud Couloux⁵, Beatrice Segurens⁵, Antoine de Daruvar⁴, Alain Blanchard^{1,2}, Christine Citti^{3*}

Horizontal transfer of a locus including repeated genes

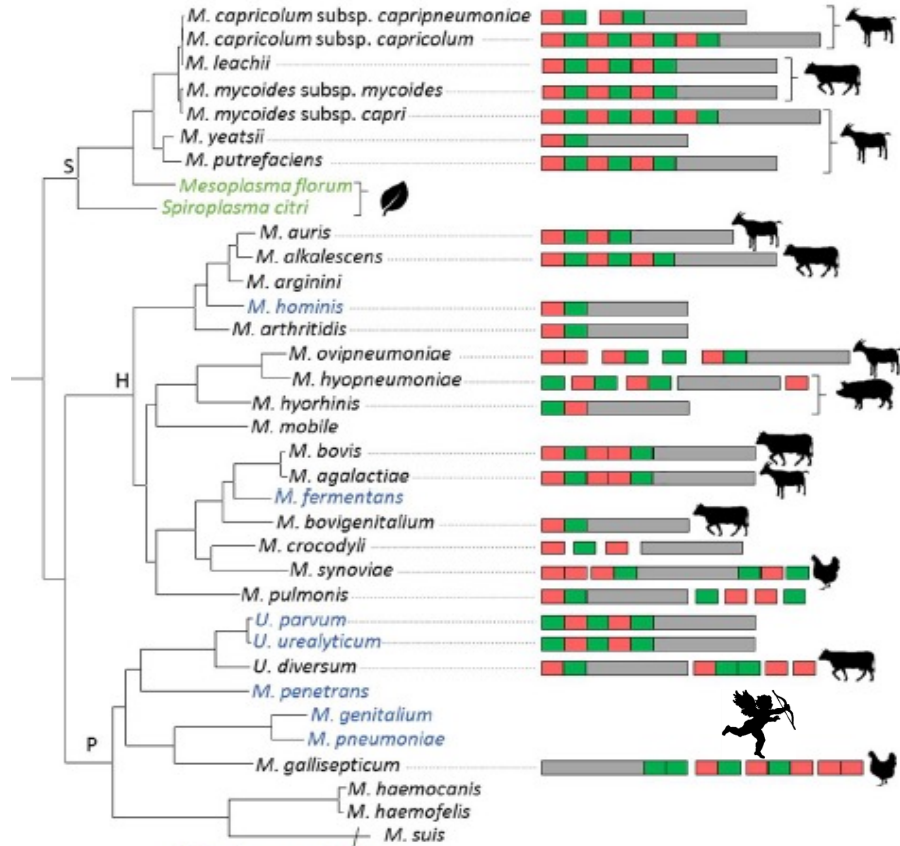


1 to 4 MIB-MIP



- Typical organisation: 1 to 4 copies of MIB-MIP
- Probable origin: Hominis ancestor
- HGT predicted between species from the same host (ruminant, bird, human)

Looking for HGT in mycoplasmas



The genes found in the operon are **specific to *Mollicutes***, widespread in animal pathogenic species, but absent from all plant pathogenic and most human pathogenic species.



Y. Arfi

Arfi Y et al. 2016. Proc Natl Acad Sci U S A. **10**;113(19):5406-11.

In search of a functional annotation in *M. mycoides* subsp. *capri*

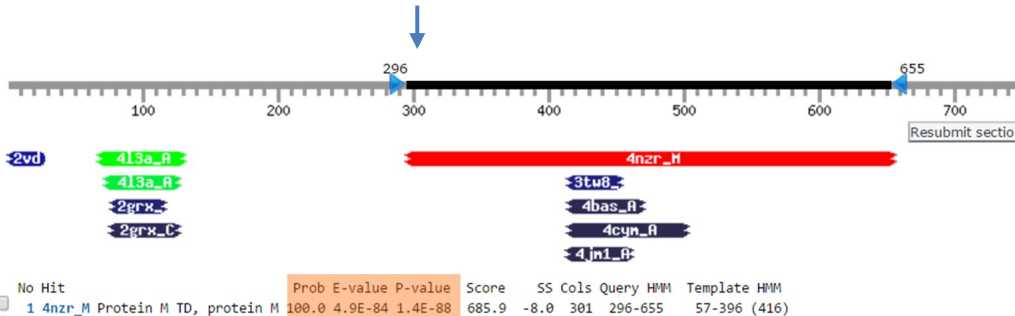


"0583" - 750 aa; 84 kDa

BlastN/BlastP → **no informative hit**

Looking for distant homologs using HHPred:

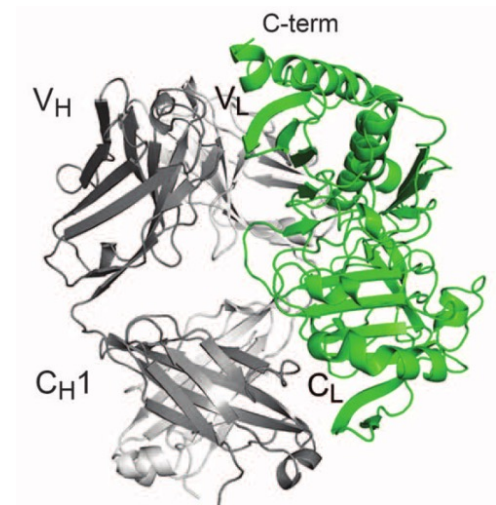
AA sequence → **Predicted structure** → Search PDB



One domain of 0583 is structurally similar to a characterized protein

➤ **Hypothesis: "0583" is an immunoglobulin-binding protein**

4nzm is a domain of an Ig-binding protein found in *M. genitalium*: Protein M



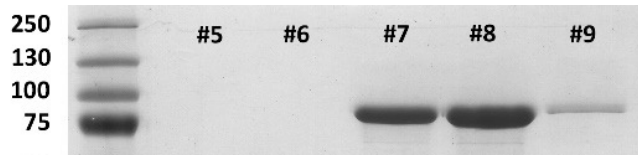
Grover *et al.* Science **343**, 656–61 (2014).

In search of a functional annotation in *M. mycoides* subsp. *capri*

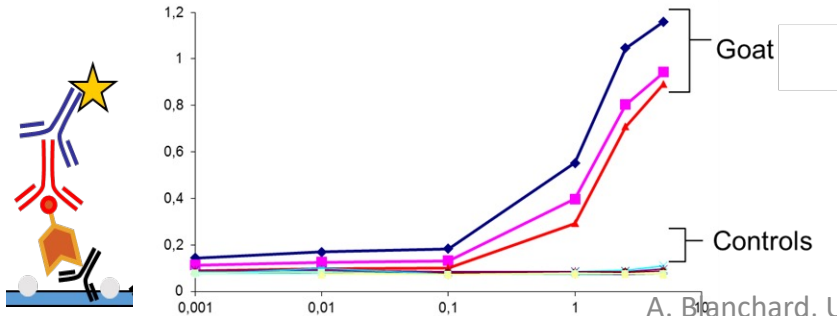


Biochemical approach

Recombinant protein – His tag in Nter –
Expressed in *E. coli*. Ni-NTA + SEC purification

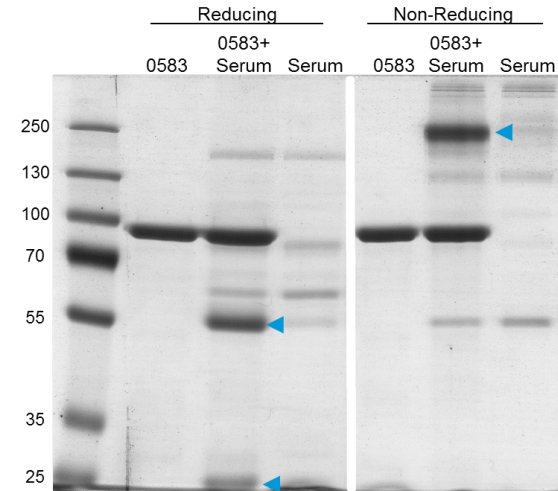


ELISA assay: goat serum is coated on the plate, and probed with 0583.
After washing, the presence of the His-tag is checked.



A. Blanchard, UER MG2 Bacterio, 2023

Pull-down assay: 0583 is coated on Ni-NTA agarose.
Goat serum is flowed over the resin. After washing,
bound proteins are eluted.



"<" LC MS/MS
→ Goat IgG

➤ 0583 is an immunoglobulin-binding protein (MIB)

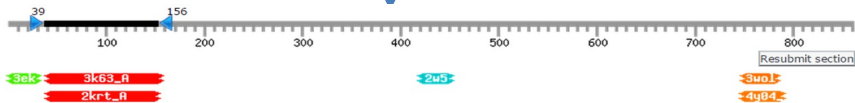
In search of a functional annotation in *M. mycoides* subsp. *capri*



"0582" - 847 aa; 97 kDa

BlastN/BlastP → no informative hit

Looking for distant homologs using HHPred:



No	Hit	Prob E-value	P-value	Score	SS	Cols	Query HMM	Template HMM	
1	3k63_A Conserved hypothetical	99.5	1.8E-14	5.1E-19	133.1	9.1	110	39-156	2-118 (126)
2	2krt_A Conserved hypothetical	99.3	8.8E-12	2.5E-16	114.8	10.1	107	39-153	7-120 (121)
3	3wo1_A DAP BII, dipeptidyl ami	90.8	0.16	4.5E-06	61.1	4.0	37	748-784	622-658 (698)
4	4y04_A Peptidase S46; space, w	90.1	0.27	7.7E-06	59.4	5.0	44	747-790	643-697 (726)
5	2w5e_A Putative serine protease	85.2	0.74	2.1E-05	44.7	4.0	36	756-792	124-159 (163)
6	2ggv_B NS3, non-structural pro	82.5	0.77	2.2E-05	46.7	2.8	22	753-774	128-149 (185)
7	3mmg_A Nuclear inclusion prote	82.2	1.7	4.6E-05	46.1	5.3	49	744-792	136-185 (241)
8	3e90_B NS3 protease; trypsin-1	81.8	0.86	2.4E-05	46.8	2.9	22	753-774	133-154 (198)
9	2p7f_B Serine protease NS3; fl	81.6	0.67	1.9E-05	46.8	2.0	22	753-774	114-135 (172)

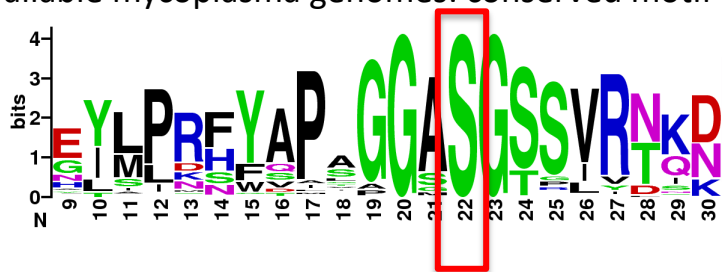
Multiple low-confidence hits pointing towards Serine proteases.

Catalytic serine of the hits in the homology region

```
Q ss_pred          cccccCCCCCccEeCCCCCEEEEEEccc
Q Fri_Jun_05_09: 716 PRFYAPAGGASGSVRTKDNKLLAVYHASN
Q Consensus       716 ~~~~~~p-GGASGSSVRnqnneLVGIyfas~
                   +.+-.||-|+|+.+|+|-|.+.
T Consensus      623 lstdITGGNSGSPV~n~~geligl~fdgn
T 3wo1_A         623 LSDLDTTGGNSGSPVLDAHGKLVGLAFDNGI
T ss_dssp        ~~~~~CCCTTCCTCEEECTTCCEEEEEEEEC
T ss_pred        EeeCCCCCCCCCCCCCCCCCEEEEEecCc
```

Catalytic Serine

Sequence alignment of 0582 homologs found in all available mycoplasma genomes: conserved motif



➤ Hypothesis: 0582 encodes a **serine protease**

In search of a functional annotation in *M. mycoides* subsp. *capri*

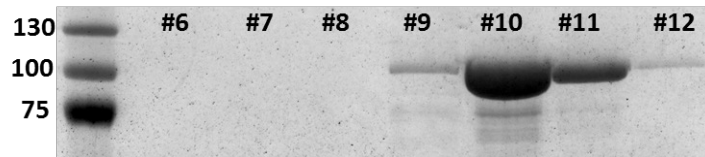


Biochemical approach

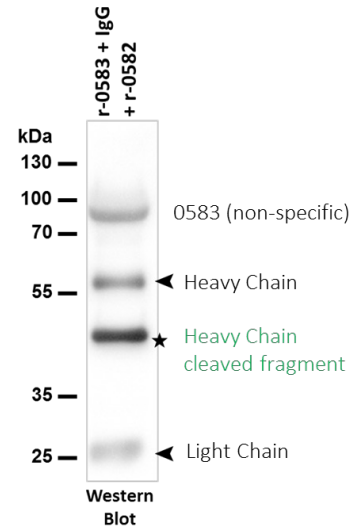
Recombinant protein – His tag in Nter .

Expressed in *E. coli*. Ni-NTA + SEC purification

→ **Non-toxic – not a broad spectrum protease**

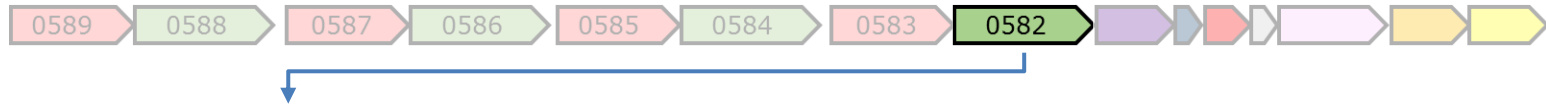


Cleavage assay: 0582 is mixed with purified 0583 and IgG



- 0582 is an immunoglobulin protease (MIP) that cleaves the Ig heavy chain, producing a 44 kDa fragment

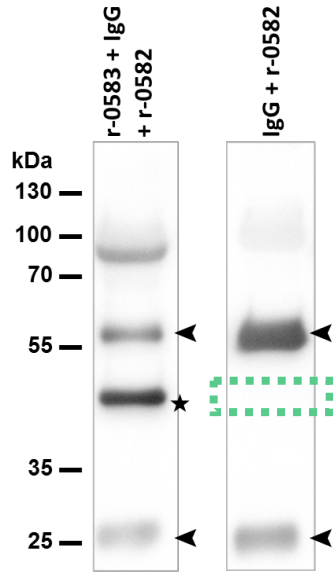
MIP is only active once the complex MIB-Ig has been formed



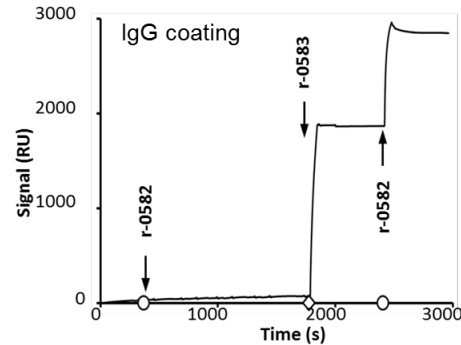
0582 (MIP) has atypical characteristics

0582 is inactive by itself, and is recruited by 0583 (MIB)

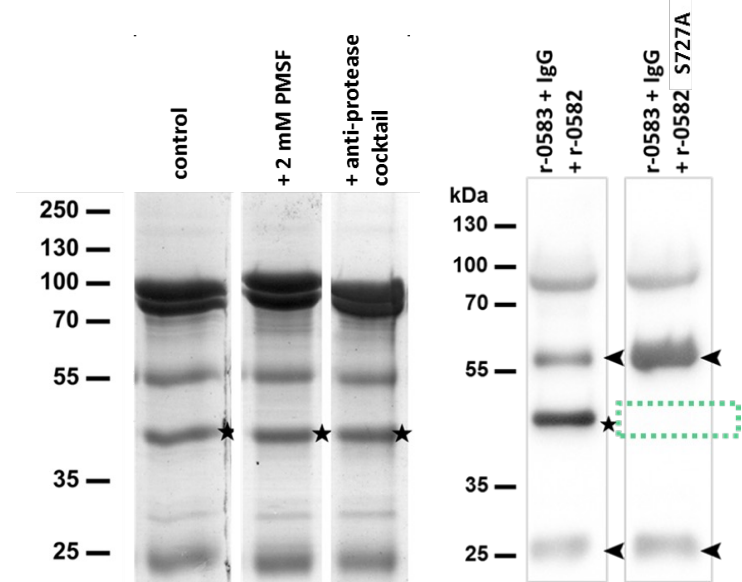
0582 is not sensitive to serine protease inhibitors, but is inactive if the conserved serine is mutated.



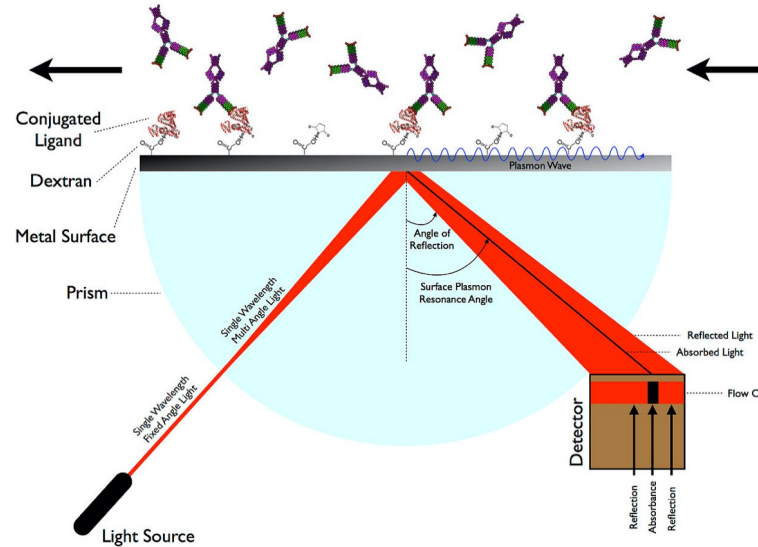
WB – Ac anti-Ig



Surface Plasmon Resonance



Résonance des plasmons de surface



"Résonance plasmon de surface." *Wikipédia, l'encyclopédie libre*. 10 mai 2020, 16:37 UTC. 10 mai 2020, 16:37

<http://fr.wikipedia.org/w/index.php?title=R%C3%A9sonance_plasmon_de_surface&oldid=170698150>.

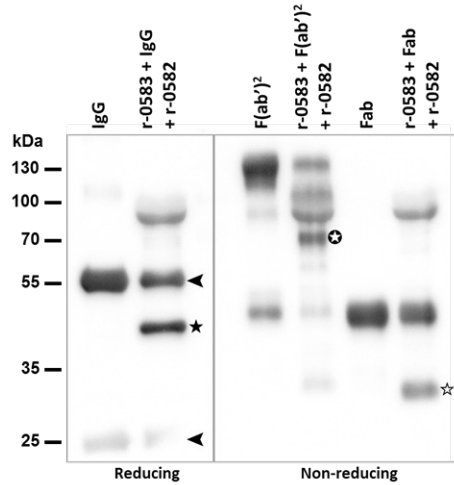
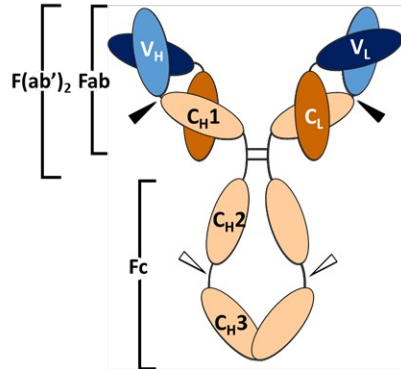
<https://www.youtube.com/watch?v=sM-VI3alvAI>

MIB-MIP is an Ig-cleaving atypical system



0582 cleaves antibodies at an atypical site

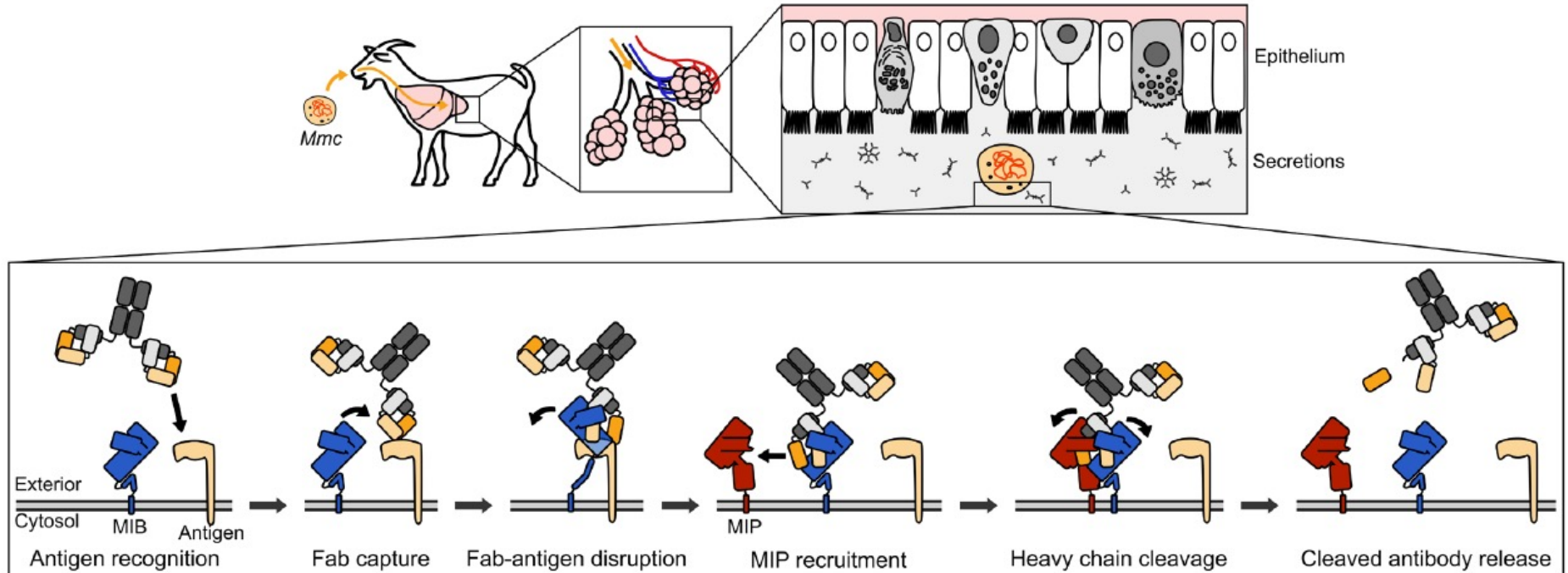
Cleavage assay: 0582 is able to cleave whole Ig, $F(ab')_2$ and Fab fragment, in presence of 0583.



Amino-sequencing: the cleavage site amino-terminal region has the sequence **VSSAST**, which is located at the linker between the V_H and C_H3 domains.

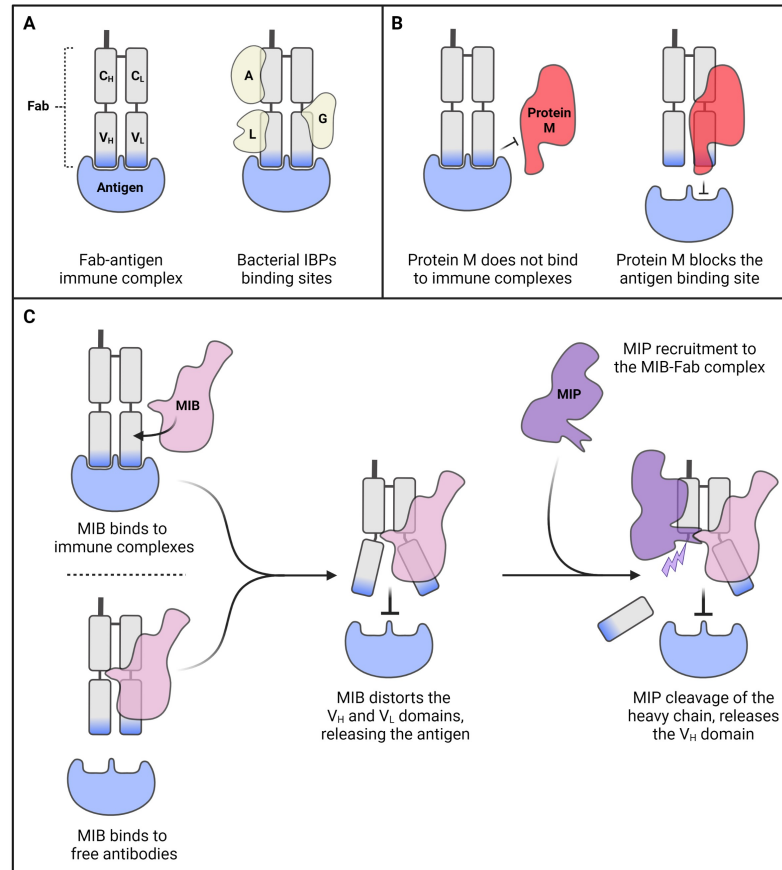
MNPLWTLFLVLSAPRGVLSQVRLQESGPSLVKPSQTLSTCTVSGFSLTYNAVSWVRQA
PGKALEWLGGIDGTVGLNPALKVRLTITRDTSKSQVSLALNNALTDDSAVYYCAKGDDY
PNYIDYWGPGLLVSV**VSSAST**TPPKVYPLTSCCGDTSSSIIVTLGCLVSSYMPEPVTVTWNS
GALTSGVHTFPAVLQSSGLYSLSSMVTVPASTSGAQTFCICNVAHPASSTKVDKRVEP

MIB-MIP working model



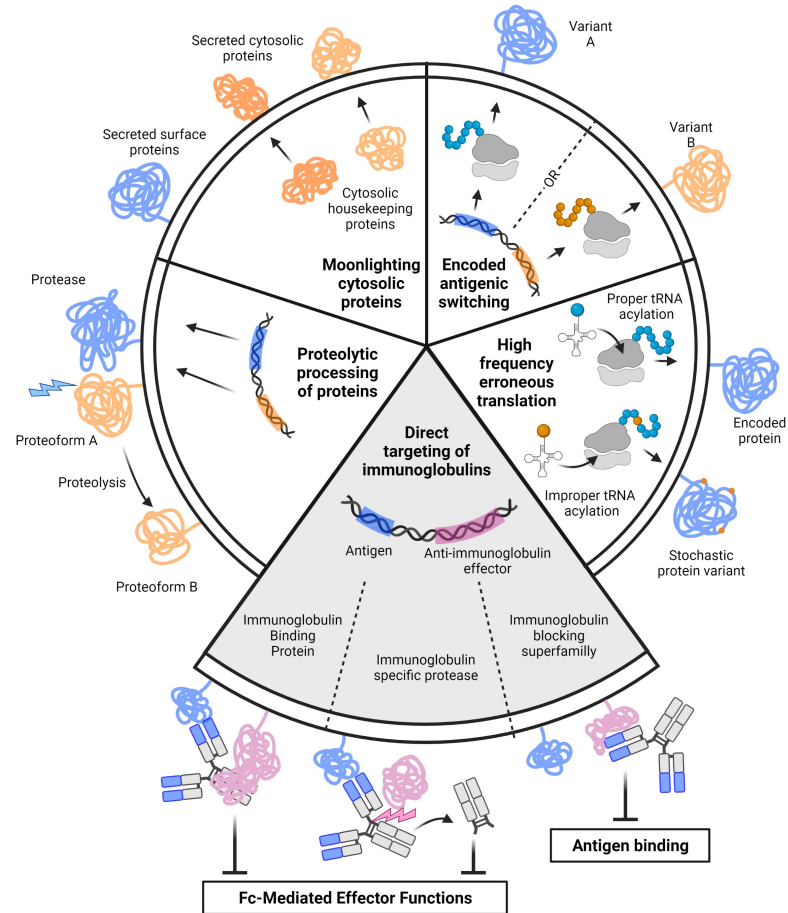
The mycoplasma surface proteins MIB and MIP promote the dissociation of the antibody-antigen interaction, *Sci Adv.* 2021 Mar 5;7(10):eabf2403. DOI: (10.1126/sciadv.abf2403)

Proposed model of Ig capture and cleavage by MIB-MIP



Arfi Y et al. 2021. mBio, 12;
DOI: 10.1128/mBio.01974-21.

Un palette de mécanismes pour échapper à la réponse immunitaire



Arfi Y et al. 2021. mBio, 12;
DOI: 10.1128/mBio.01974-21.

Thank you for your attention



Cecilia



Anne



Flavio



Paul



Joseph



Jared



Elise



+ Suchismita Chandran (JCVI)

A. Blanchard, UER MG2 Bacterio, 2023



ILRI

INTERNATIONAL
LIVESTOCK RESEARCH
INSTITUTE

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UNIVERSITÄT
BERN

J. Craig Venter[®]
INSTITUTE

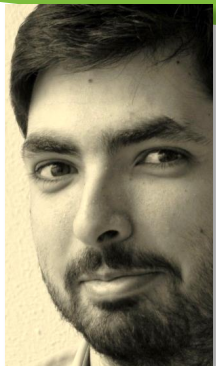


"This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634942."

université
de BORDEAUX



L. Béven



Y. Arfi



C. Lartigue-Prat



A. Blanchard



G. Gourgues



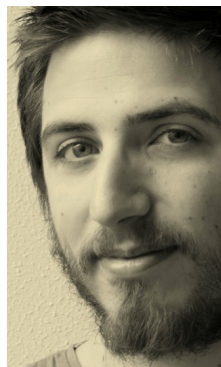
P. Sirand-Pugnet



Dubrana



V. Talenton



I. Tsarnopoulos



V. Y. Valverde
Timana

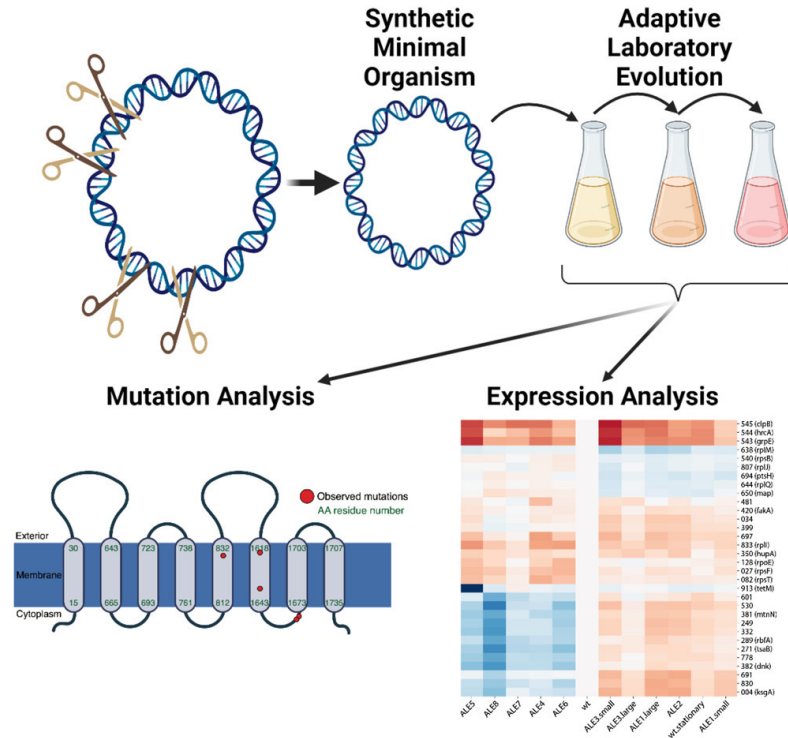


L. Garcia

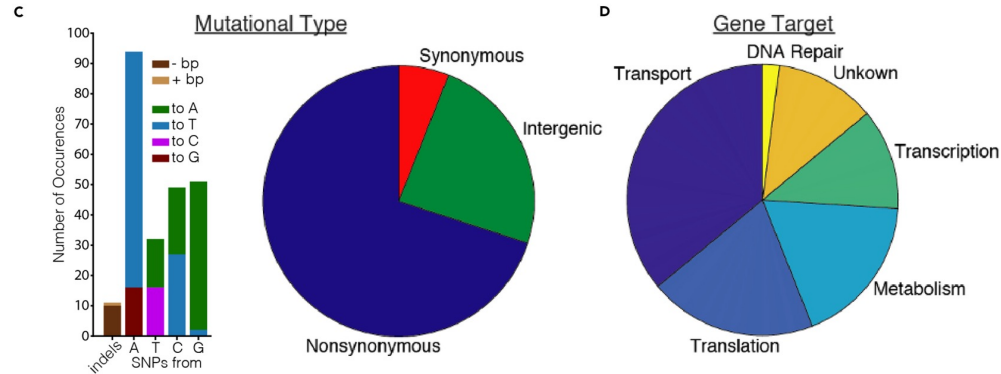
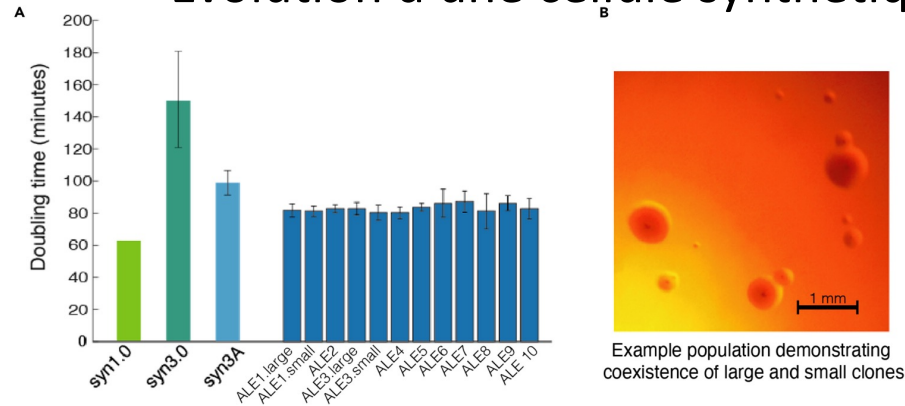


E. Ruiz

Evolution d'une cellule synthétique



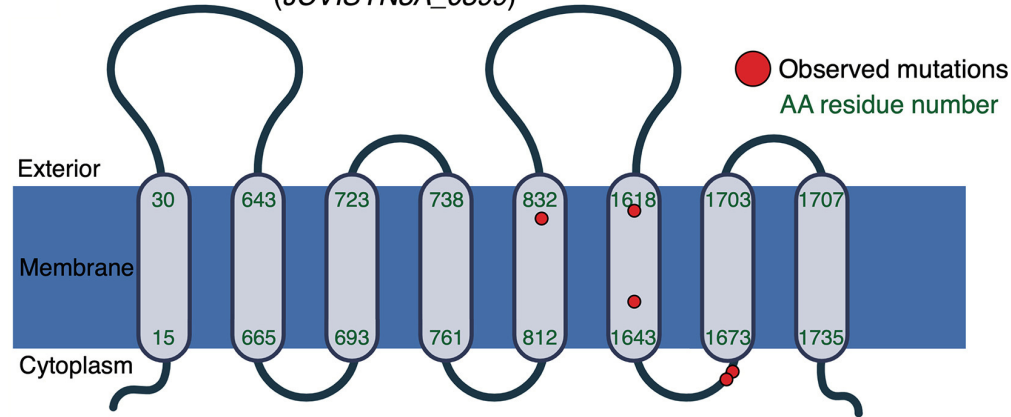
Evolution d'une cellule synthétique



Evolution d'une cellule synthétique

A

Uncharacterized ABC Transporter
(*JCVISYN3A_0399*)



B

13x TA repeat in unevolved syn3A

...ACATATTGCATTTATATATATATATATATATATATATATGAATGTATG...

TA multiplicities observed after evolution:

9x (one strain)

10x (one strain)

11x (three strains)

61 bp upstream of
uncharacterized
lipoprotein
(*JCVISYN3A_0481*)