

Synthesis, Engineering and Transplantation of Bacterial Genomes

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Synthetic biology approaches

→ **Genome synthesis and assembly**

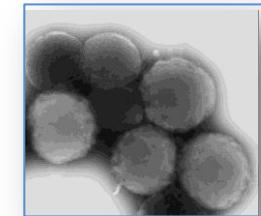
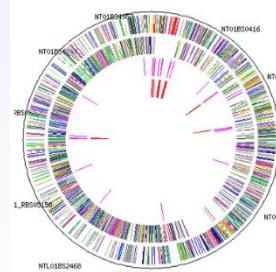
→ **Genome transplantation**

→ **Genome engineering**

What has been done?

What are the current challenges?

The initial concept



Design

Synthesis Assembly Engineering

Transplantation

J. Craig Venter
INSTITUTE

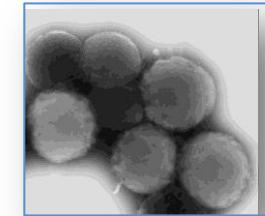
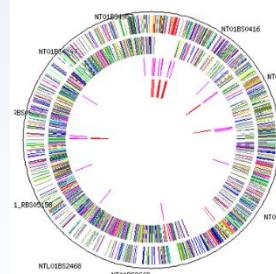
J. Craig Venter

**Daniel Gibson
Clyde Hutchison
Hamilton Smith**

Carole Lartigue

John Glass

The initial concept



Design

Synthesis Assembly Engineering

Transplantation



Let's start by copying nature !

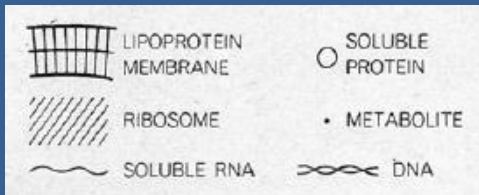
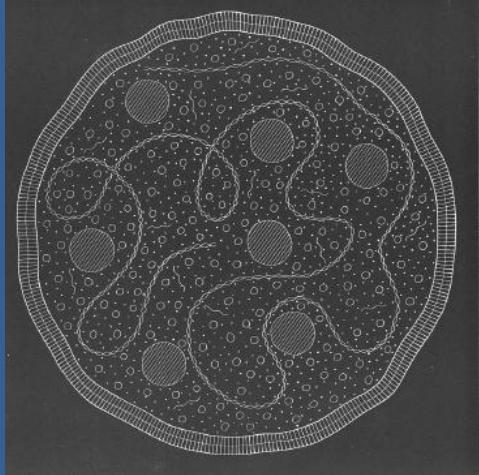
Mollicutes, minimal bacteria

The Smallest Living Cells

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



Unsuccessful attempts to assemble a living cell from its separated components (=proto-synthetic biology?).
This program was supported by NASA.

Mollicutes, minimal bacteria

Mycoplasma genomes among the first to be sequenced

■ ARTICLES ■

Science 1995; 270:397-403.

The Minimal Gene Complement of *Mycoplasma genitalium*

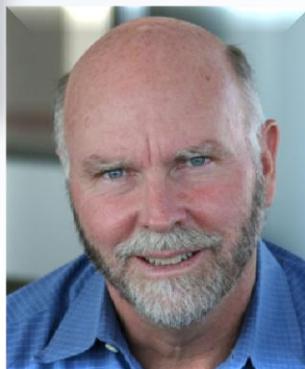
Claire M. Fraser,* Jeannine D. Gocayne, Owen White, Mark D. Adams, Rebecca A. Clayton, Robert D. Fleischmann, Carol J. Bult, Anthony R. Kerlavage, Granger Sutton, Jenny M. Kelley, Janice L. Fritchman, Janice F. Weidman, Keith V. Small, Mina Sandusky, Joyce Fuhrmann, David Nguyen, Teresa R. Utterback,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

The complete nucleotide sequence (580,070 base pairs) of the *Mycoplasma genitalium* genome, the smallest known genome of any free-living organism, has been determined by [REDACTED] cient for closure. All electropherograms were visually inspected with TIGR EDITOR.

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



Claire Fraser



Craig Venter



ratory 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

C. M. Fraser, J. D. Gocayne, O. White, M. D. Adams, R. K. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, J. L. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, C. M. Saudek, C. A. Phillips, and J. C. Venter are at the Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA. K. F. Merrick is in the Department of Molecular Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10464, USA. B. A. Dougherty, H. O. Smith are at the Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. K. F. Bott, P. C. Hu, T. S. Lucier, S. N. Peterson, and C. A. Hutchison III are at the University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA.

*To whom correspondence should be addressed.

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 resolved against each other with GRASTA [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 gaps from 39 to 28.

Templates spanning each of the sequence gaps were identified, and oligonucleotide primers were designed from the sequences at the end of each contig. All gaps were less than 300 bp; thus, a primer walk from both ends of each template was sufficient

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* (5), 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 in agreement, with one apparent discrepancy between coordinates 62,708 and 94,573 in the sequence. However, reevaluation of

Mollicutes, minimal bacteria

Gene inactivation by transposon mutagenesis

Global Transposon Mutagenesis and a Minimal Mycoplasma Genome

Clyde A. Hutchison III,^{1,2*} Scott N. Peterson,^{1*†} Steven R. Gill,¹
Robin T. Cline,¹ Owen White,¹ Claire M. Fraser,¹
Hamilton O. Smith,^{1‡} J. Craig Venter^{1‡§}

Science. 1999; **286**:2165-9.



Essential genes of a minimal bacterium

John I. Glass, Nacyra Assad-Garcia, Nina Alperovich, Shibu Yooseph, Matthew R. Lewis, Mahir Maruf,
Clyde A. Hutchison III, Hamilton O. Smith*, and J. Craig Venter

Proc Natl Acad Sci U S A. 2006; **103**:425-30. 1

100 genes out of 482 can be INDIVIDUALLY disrupted *in vitro*



Minimal genome = 382 genes ???

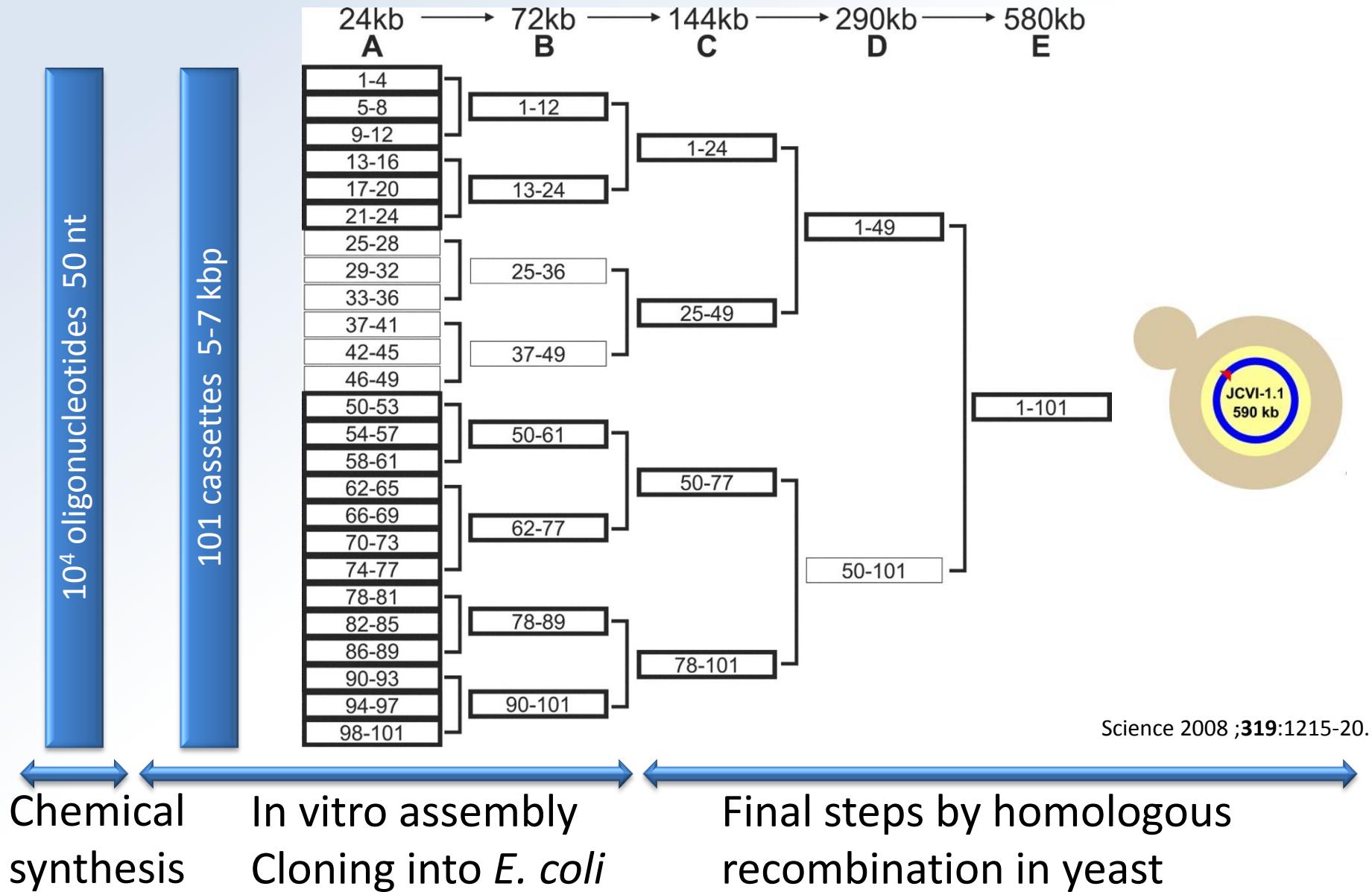
From the computer to the living cell

→ Genome synthesis and assembly

→ Genome transplantation

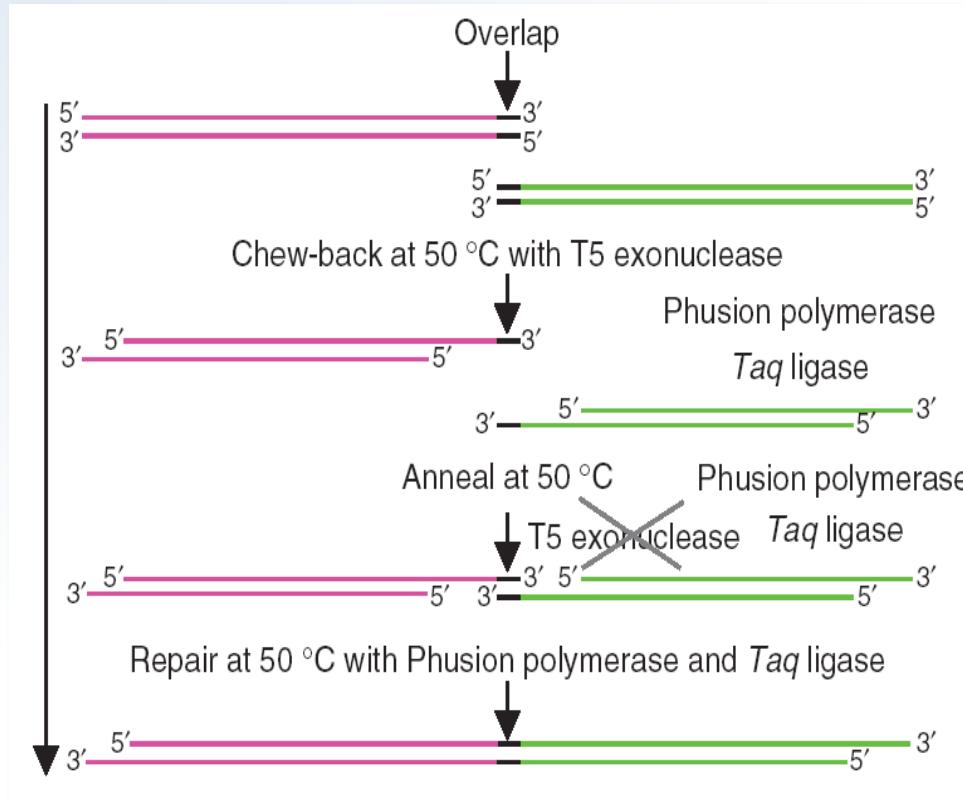
→ Genome engineering

M. genitalium genome synthesis and assembly



Genome assembly: chew-back anneal or Gibson's method

in vitro recombination + E. coli cloning



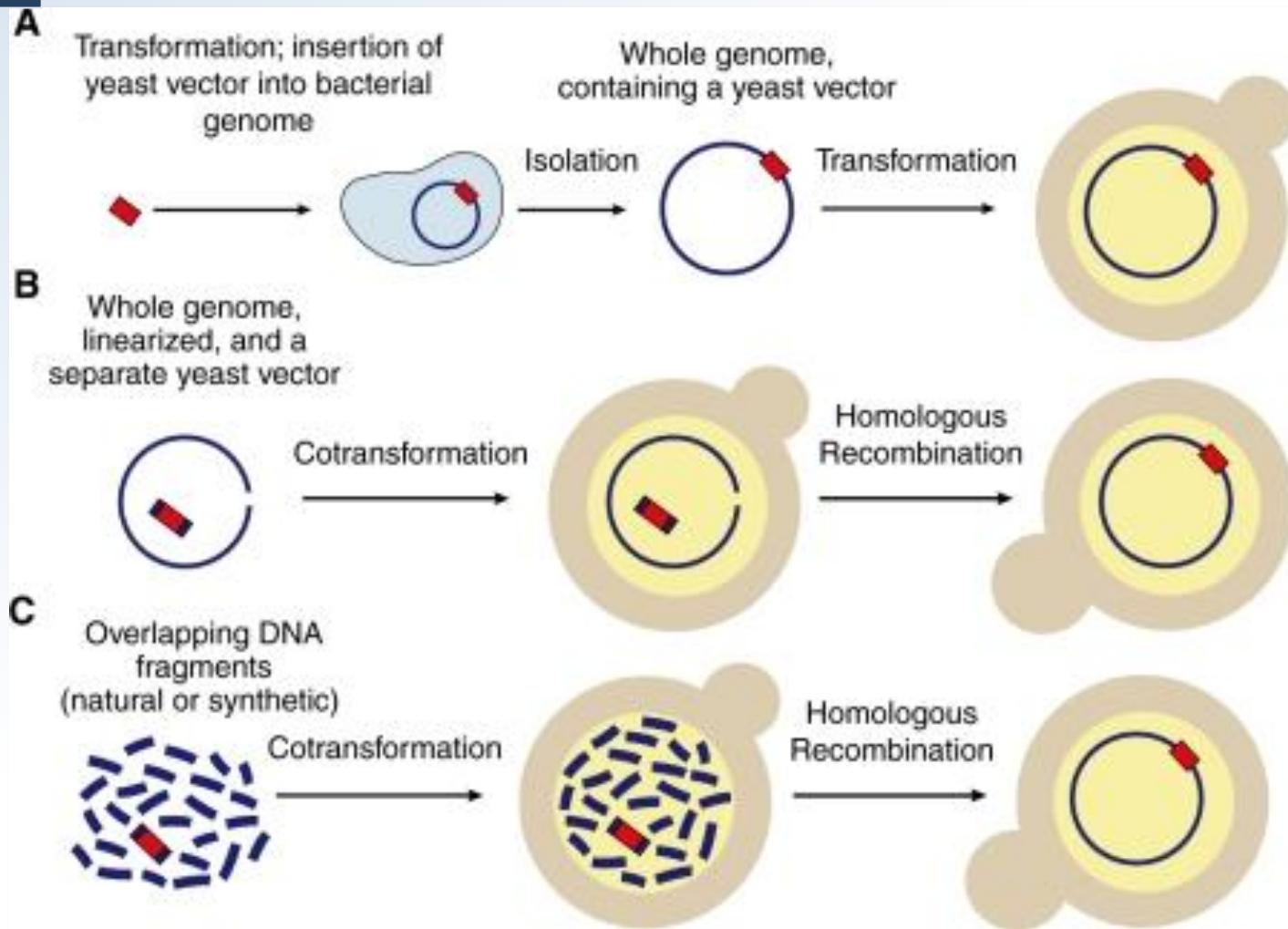
From a few kbp to ~300 kbp

Genome assembly: chew-back anneal



Genome cloning and assembly in yeast

3 methods:



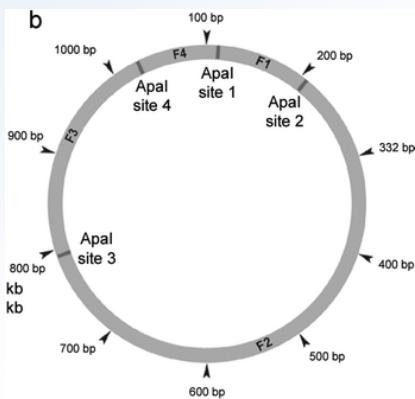
What are the limits?

- Size?
- G+C content?
- Genetic code?

Genomes cloned in yeast

MOLLIICUTES

	Genome size (Mbp)	% G+C	Genetic code	Yeast cloning
<i>Mycoplasma genitalium</i>	0.58	32	Non standard	✓
<i>Mycoplasma pneumoniae</i>	0.81	41	Non standard	✓
<i>Mycoplasma mycoides</i> subsp. <i>capri</i>	1.1	24	Non standard	✓
<i>Mycoplasma capricolum</i>	1.1	25	Non standard	✓
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i>	1.2	24	Non standard	✓
<i>Acholeplasma laidlawii</i>	1.5	32	Universal	✓



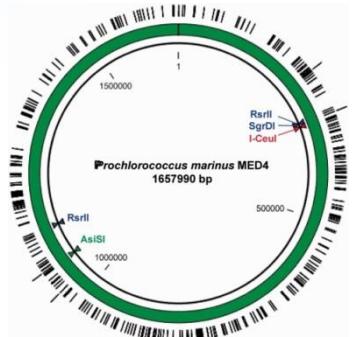
Acholeplasma laidlawii
1.5 Mpb



Successfull cloning required the inactivation of a gene coding a surface anchored endonuclease

Genomes cloned in yeast

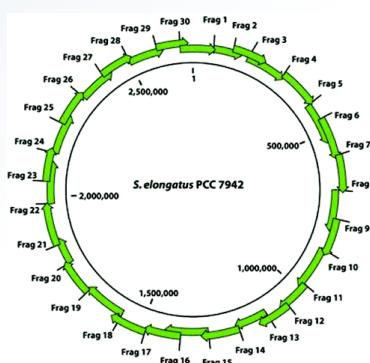
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MOLLIICUTES	<i>Mycoplasma genitalium</i>	0.58	32	Non standard
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	<i>Acholeplasma laidlawii</i>	1.5	32	Universal
CYANOBACTERIES	<i>Prochlorococcus marinus</i>	1.6	31	Universal



Prochlorococcus marinus
1.6 Mpb

Genomes cloned in yeast

	Genome size (Mbp)	% G+C	Genetic code	Yeast cloning
MOLLIICLES	<i>Mycoplasma genitalium</i>	0.58	32	Non standard
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	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i>	1.2	24	Non standard
	<i>Acholeplasma laidlawii</i>	1.5	32	Universal
CYANOBACTERIES	<i>Prochlorococcus marinus</i>	1.6	31	Universal
	<i>Synechococcus elongatus</i>	2.7	55	Universal



Synechococcus elongatus
2.7 Mpb

- . Cloning of ~150kb fragments
- . Addition of autonomous replicating sequences (ARS) allowed the cloning of 454 kbp fragments

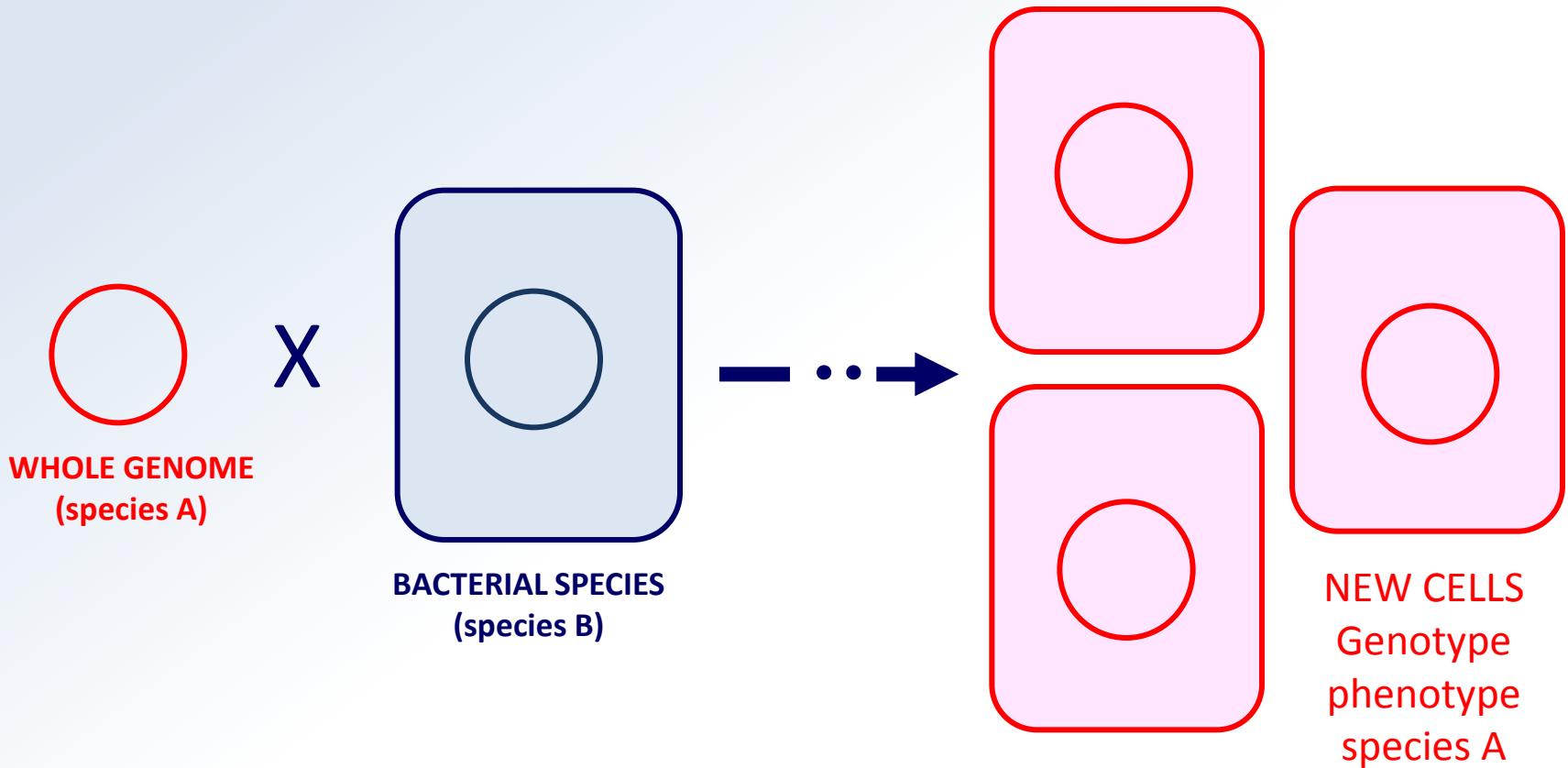
From the computer to the living cell

→ Genome synthesis and assembly

→ Genome transplantation

→ Genome engineering

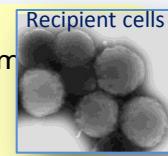
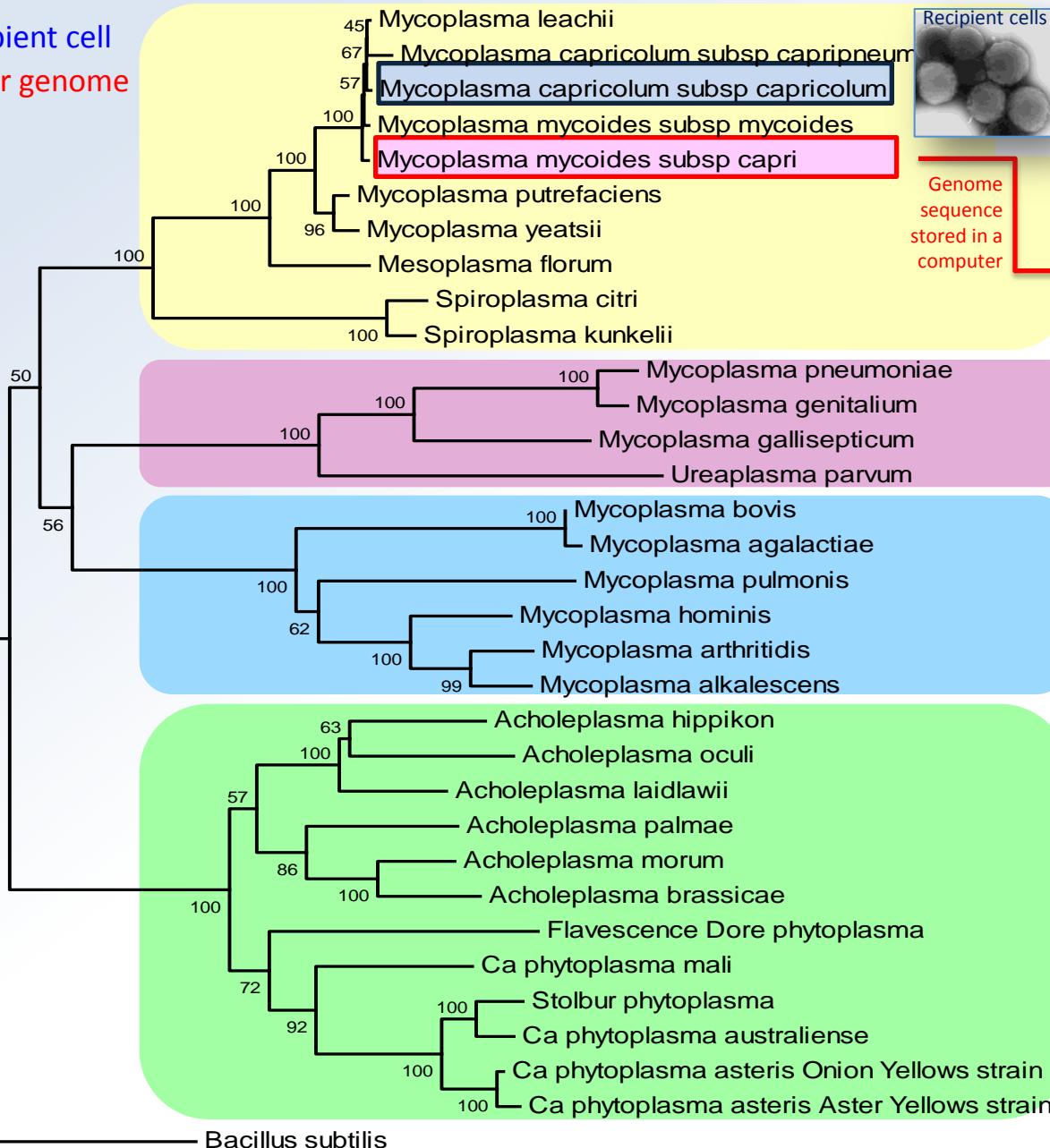
Genome transplantation



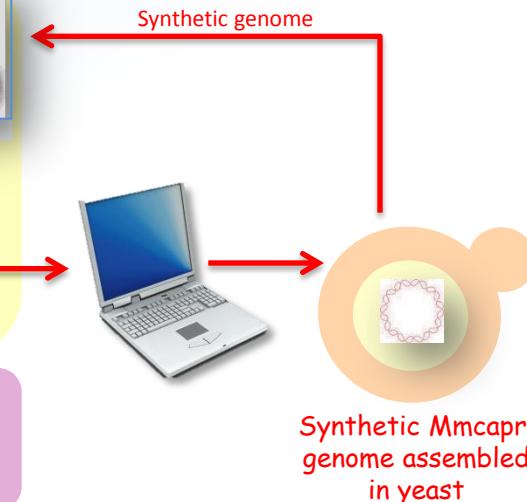
Installation of either **synthetic** or **natural** genome into a recipient cytoplasm such that the donor genome becomes the new operating system of the cell

Transplantation of SYNTHETIC genome from YEAST to mycoplasma

Recipient cell
Donor genome



Genome sequence stored in a computer



Synthetic Mmcapri genome assembled in yeast

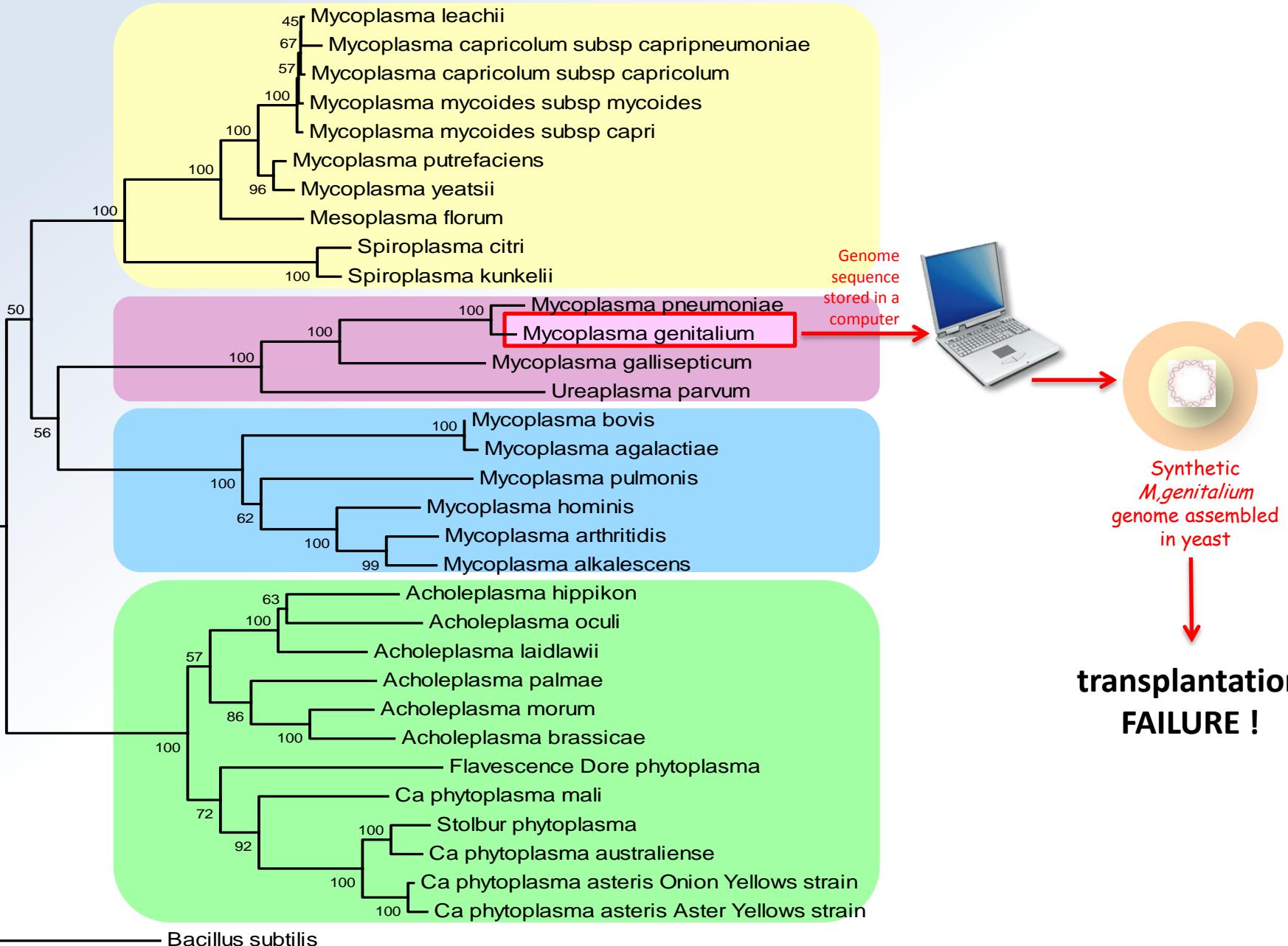
Successful transplantation



C. LARTIGUE

Science. 2007; 317:632-8.
Science. 2009; 325:1693-6
Science. 2010 329:52-6.

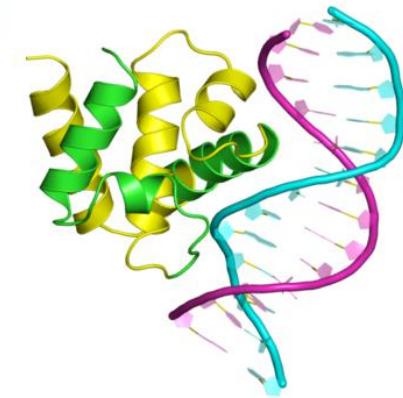
Transplantation of SYNTHETIC genome from YEAST to mycoplasma



Genome transplantation

What are the limits?

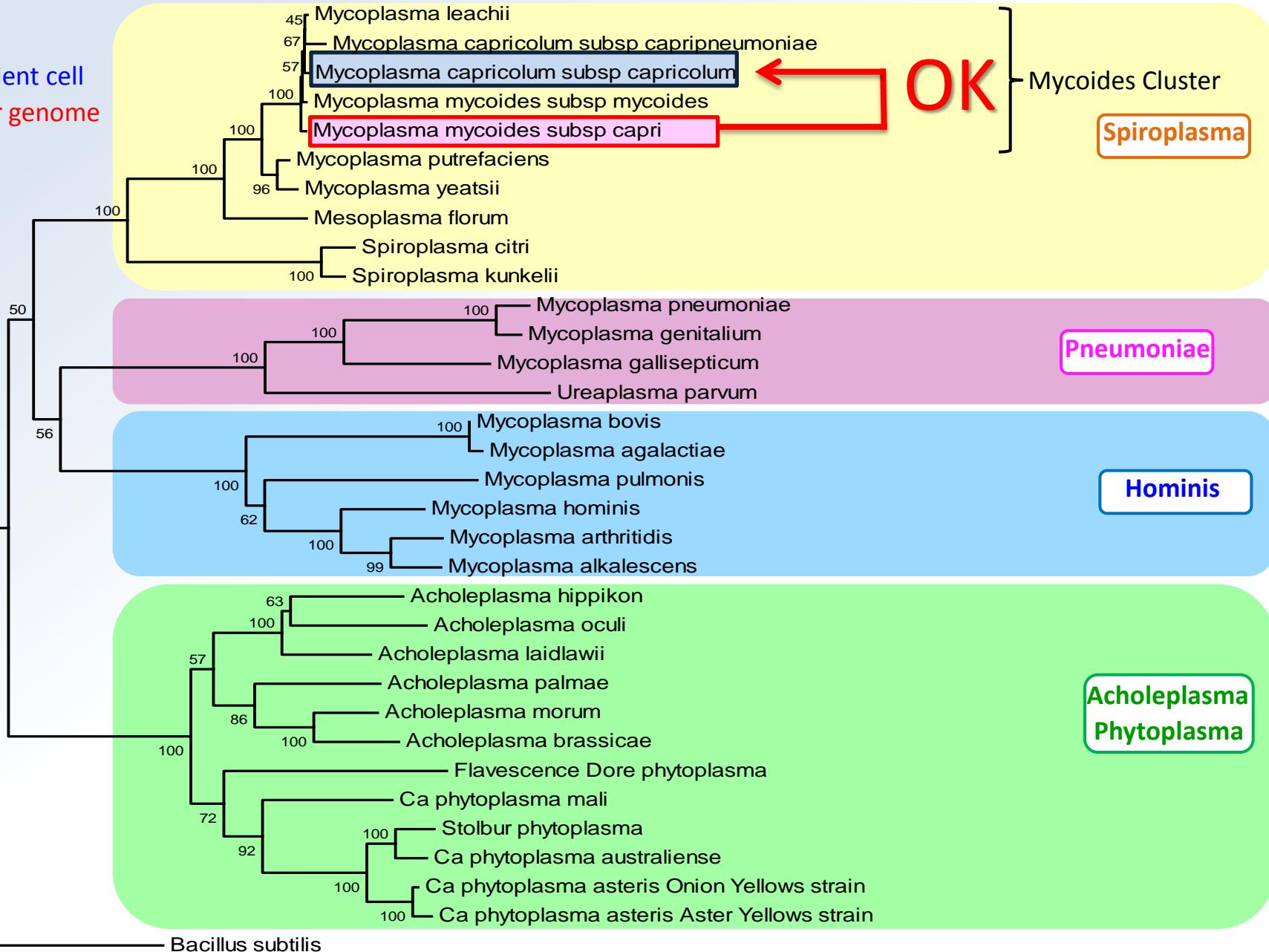
- “Do not forget the chassis!”¹
 - The transplanted genome must be tolerated in cell (restriction-modification systems)
 - The molecular machinery of the recipient cell must be compatible enough to initiate the replication of the transplanted genome (check with heterologous *oriC* plasmids)
 - The molecular machinery of the recipient cell must be able to express the genes encoded by the transplanted genome
➔ need for new recipient cells
- Portability



¹A. Danchin. FEBS Lett. 2012 Jan

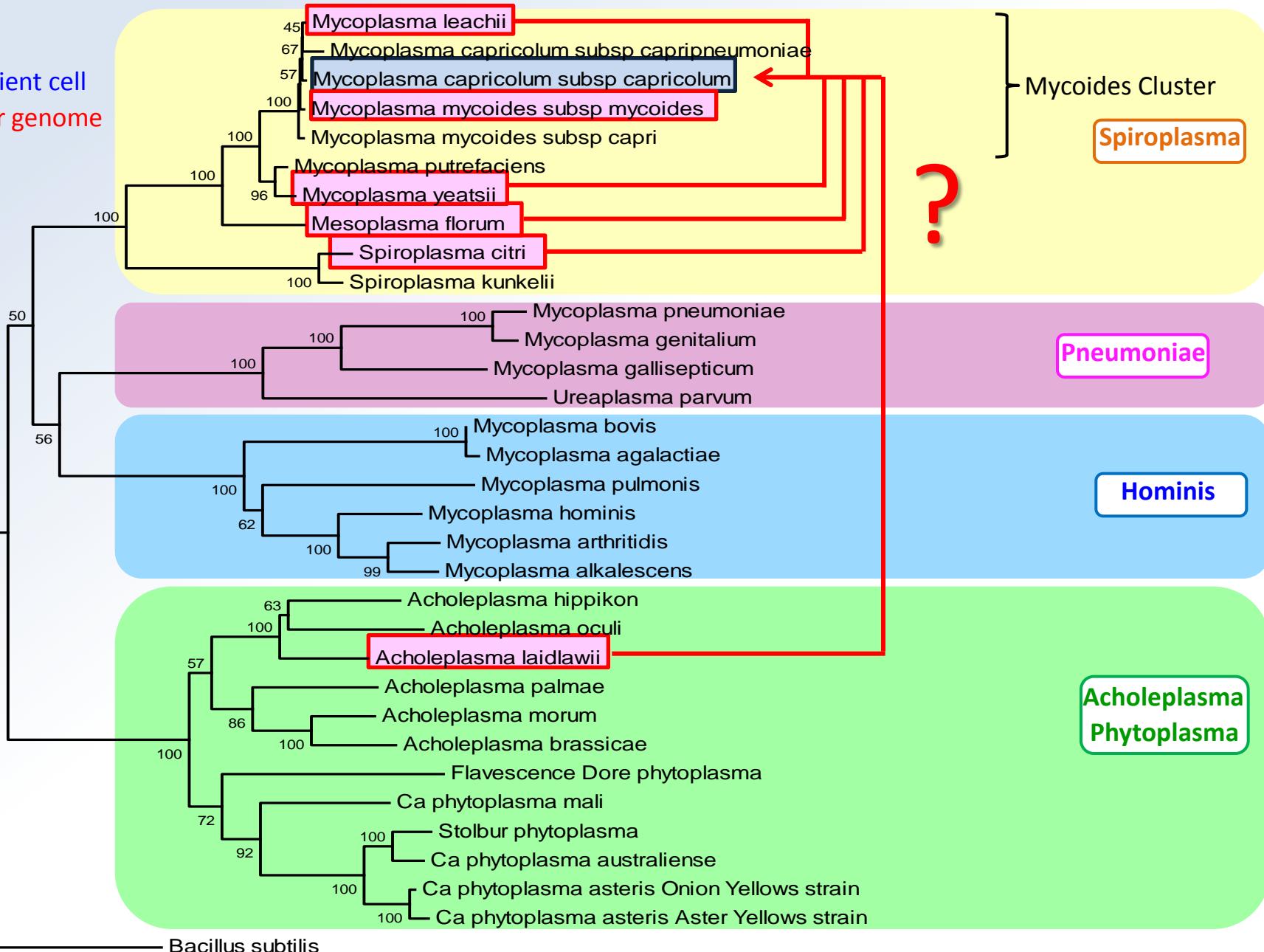
Extending genome transplantation

Recipient cell
Donor genome



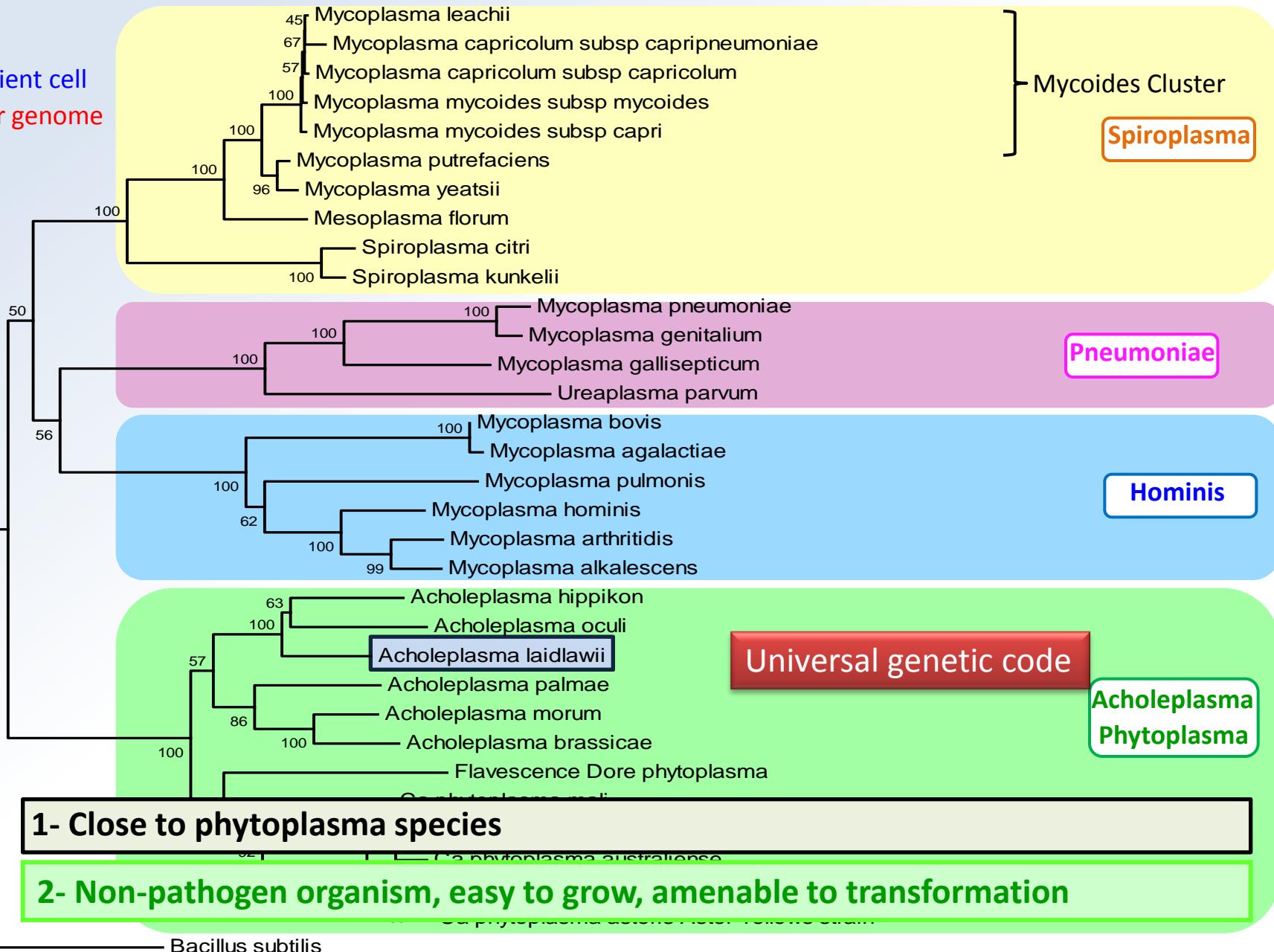
Extending genome transplantation

■ Recipient cell
■ Donor genome

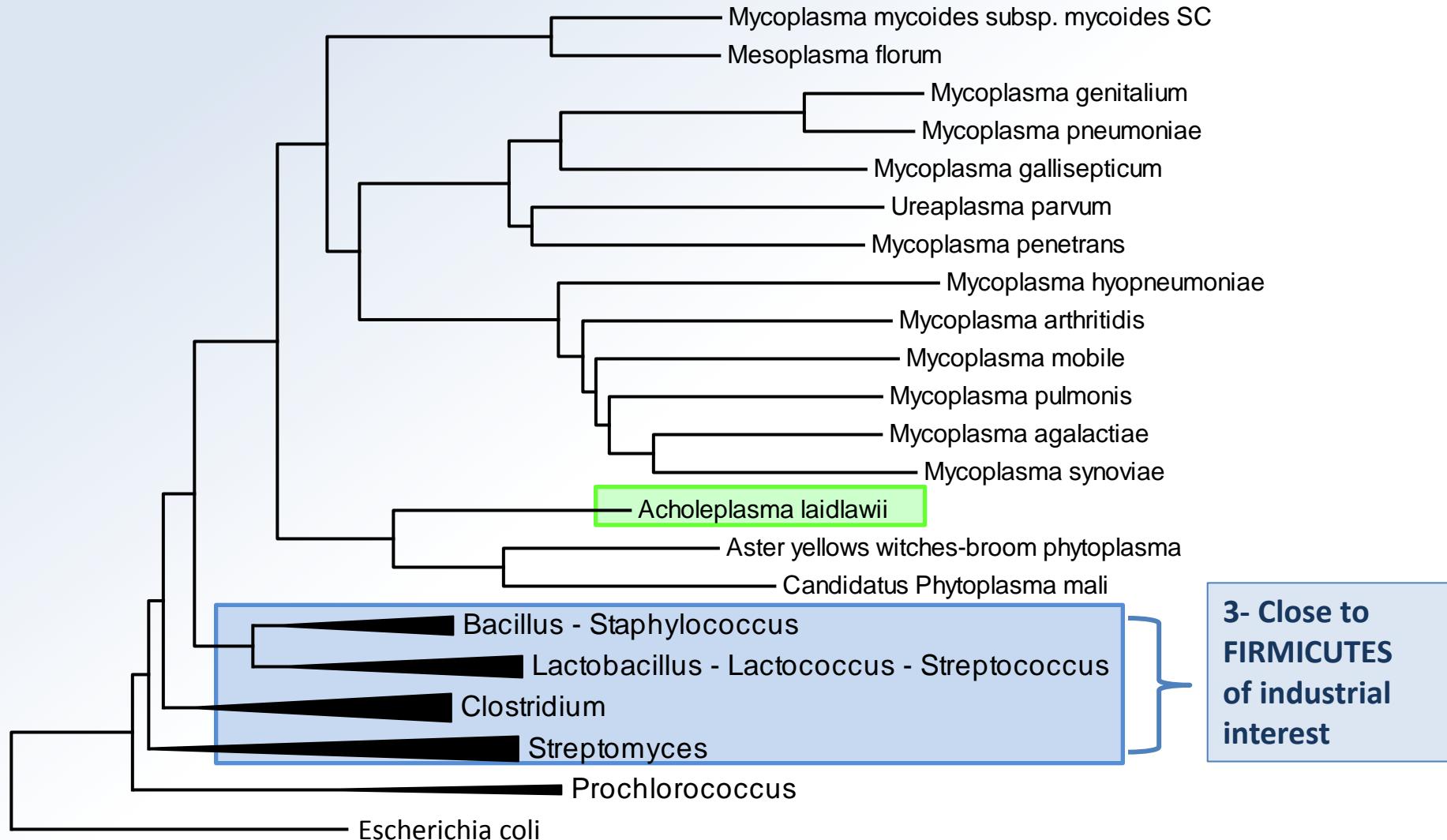


Search for other recipient cells

 Recipient cell
 Donor genome



More benefits from *Acholeplasma* genus



From the computer to the living cell

- Genome synthesis and assembly
- Genome transplantation
- Genome engineering

Genome engineering, some current projects

- Genome down-sizing (JCVI)
- Genome re-organisation (JCVI)
- Engineering of uncultivated bacteria (INRA)
- Development of a mycoplasma vaccine
(JCVI-INRA-ILRI)

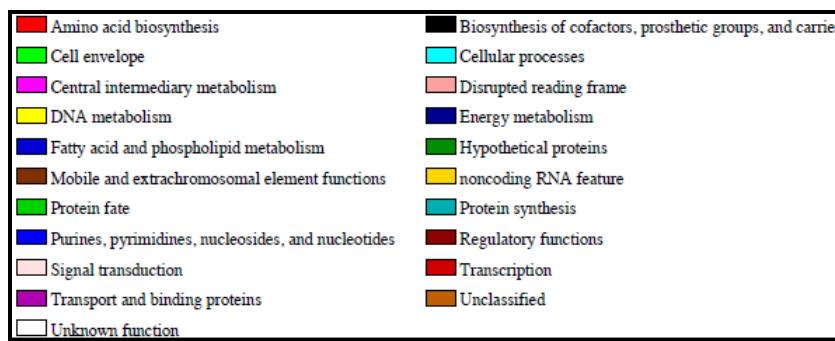
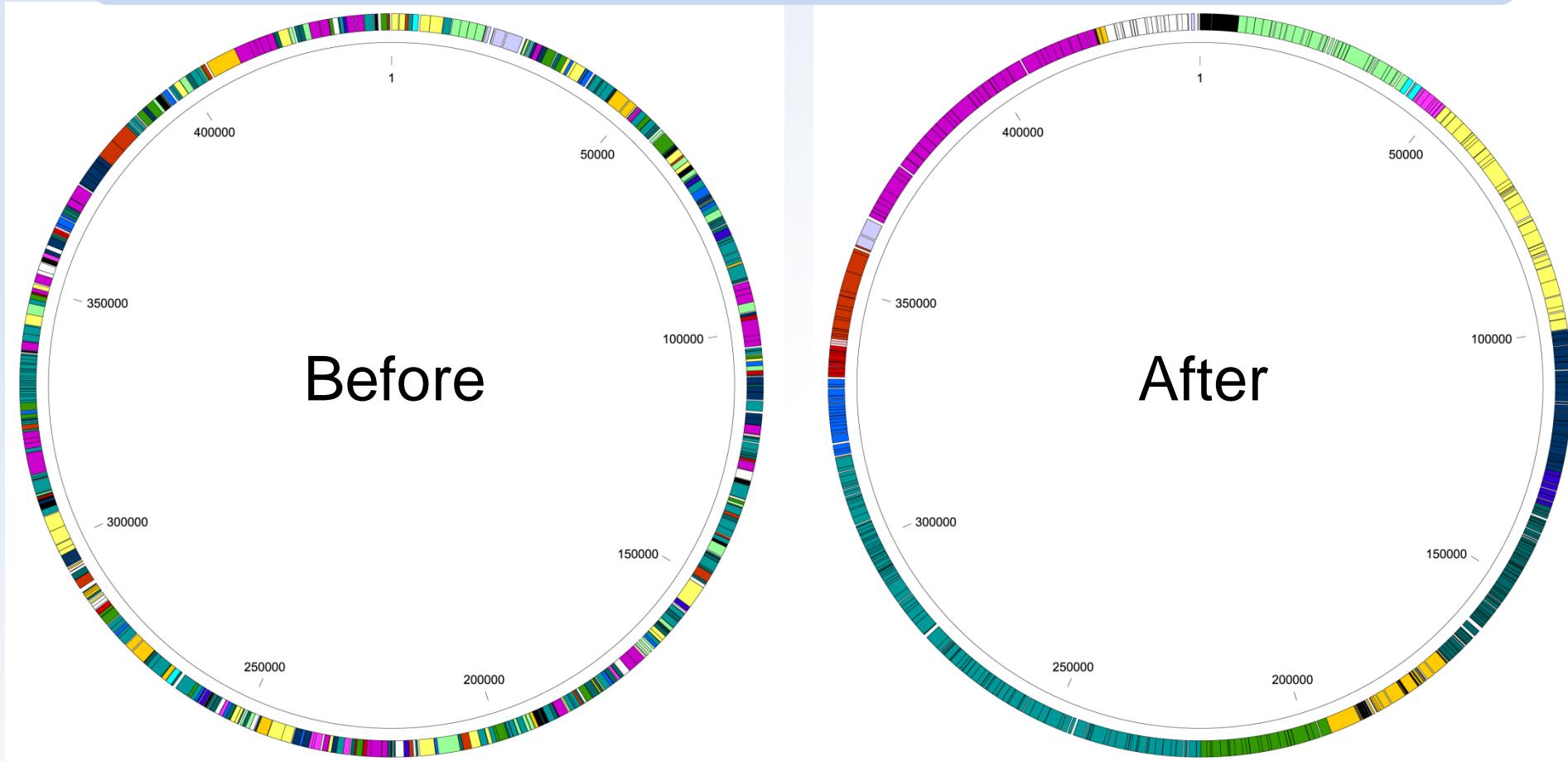
Genome down-sizing

Minimizing the genome of *Mycoplasma mycoides* JCVI-syn1.0 using two different approaches:

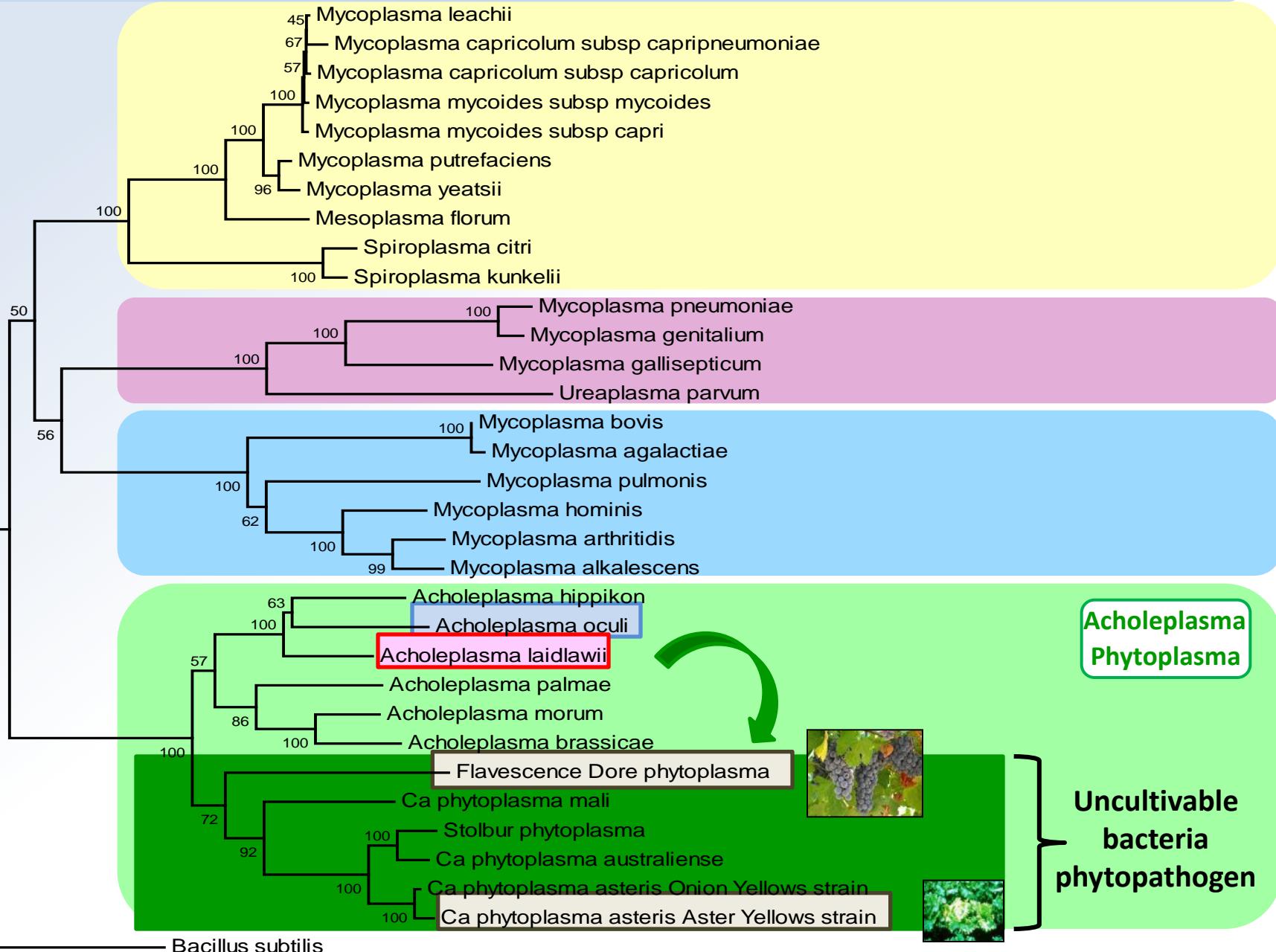
Top down approach involving **iterative deletion** of non-essential large gene clusters (phase I) and followed by deletion of small clusters and individual non-essential genes (phase II).

Bottom up approach involving **design of a minimal genome** based on the totality of our information on viable single gene transposon knockouts and viable single or multiple cluster deletions. The designed minimal genome will then be **chemically synthesized**.

Genome re-organisation (defragmentation)

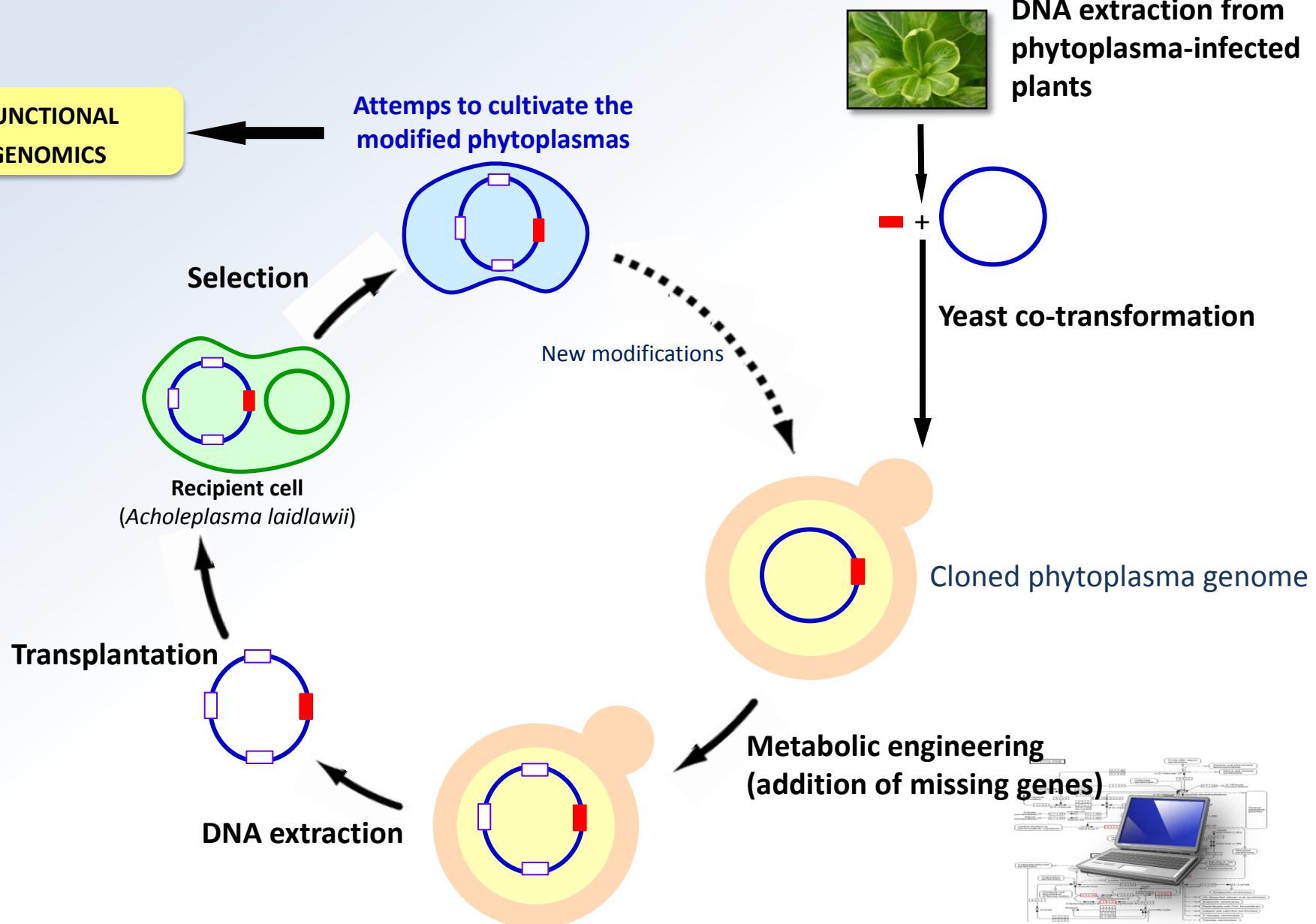


Genome engineering of uncultivated bacteria



Genome engineering of uncultivated bacteria

FUNCTIONAL
GENOMICS



Development of a mycoplasma vaccine

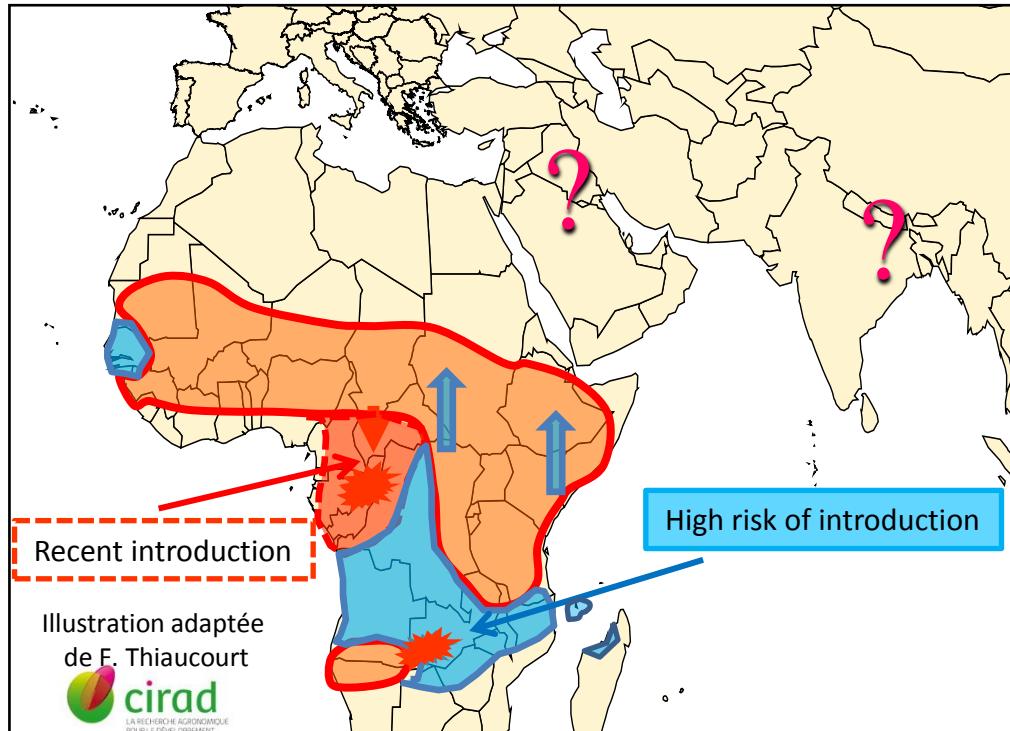
→ **MYCOPLASMA MYCOIDES SUBSP. MYCOIDES CAUSAL AGENT OF CONTAGIOUS BOVINE PLEUROPNEUMONIAE**

➤ Current situation

- Endemic disease
- Continue to spread in sub-saharan Africa
 - ❖ Recent introductions (Gabon, Congo)
- Situation in Asia not well known
- Important economic losses
- Threat for Europe

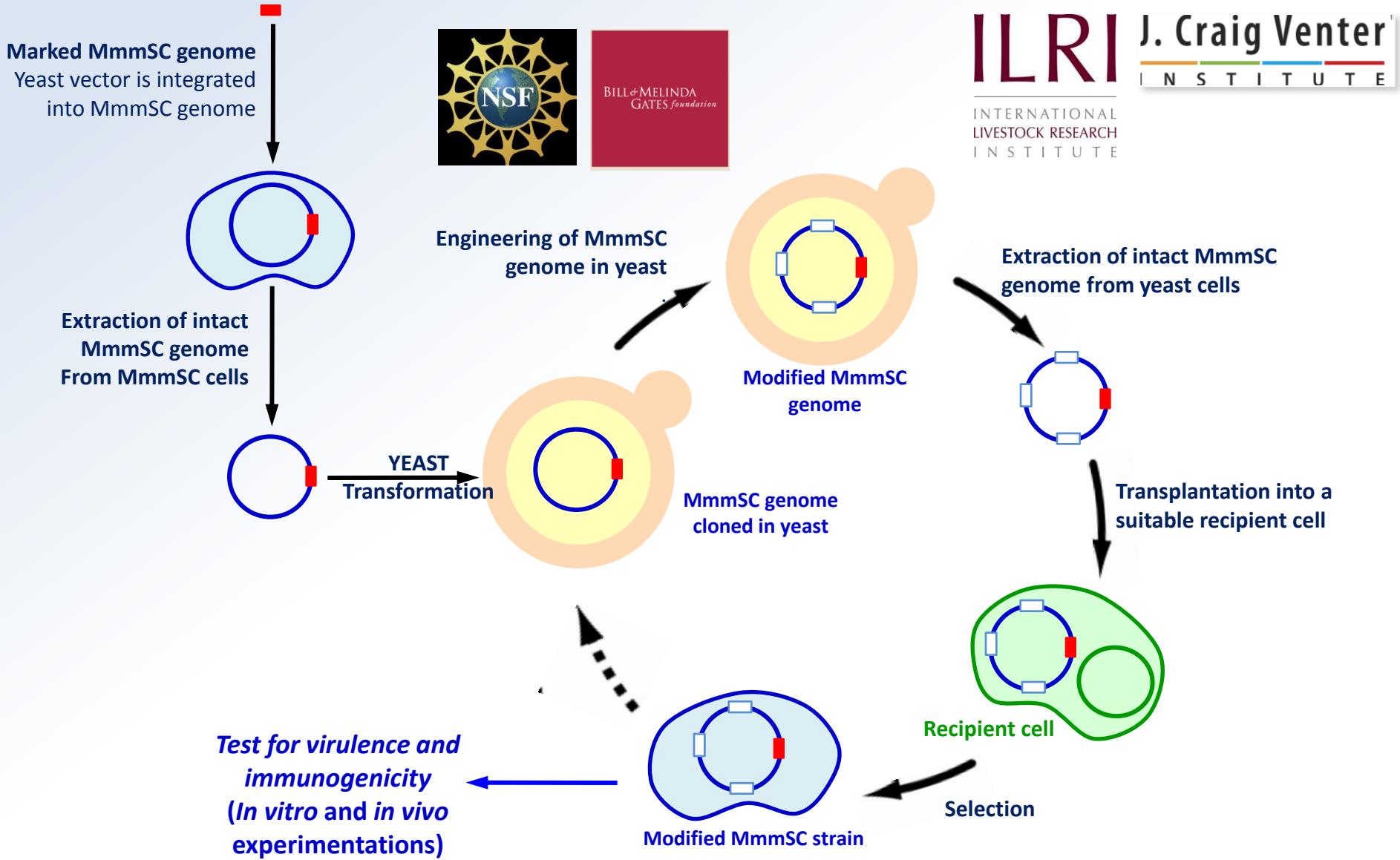


➡ **Urgent need for an improved vaccine**



Development of a mycoplasma vaccine

CBPP vaccine development



Conclusions

- The three technologies, Genome synthesis and assembly, Genome transplantation and Genome engineering have huge potential in both academic and applied research
- Some limits are identified:
 - Genome synthesis and assembly
 - cost
 - Genome cloning in yeast:
 - size and G+C% of cloned genomes
 - Genome transplantation:
 - complementarity between genome and cellular chassis
 - portability of the method
 - Genome engineering
 - constraints of genome architecture

Thank you for your attention



Goethe's 'Faust' depicting Mephistopheles creating a homunculus, Bibliothèque des Arts Décoratifs, Paris, France. 1854.



A. Blanchard



P. Sirand-Pugnet



A. Lebaudy



C. Lartigue



C. Charenton



G. Gourgues