Synthesis, Engineering and Transplantation of Bacterial Genomes

Carole Lartigue
*(remplacée par Alain Blanchard)*

UMR 1332
INRA, Villenave d’Ornon, France
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

Daniel Gibson
Clyde Hutchison
Hamilton Smith

Carole Lartigue
John Glass
The initial concept

- Design
- Synthesis
- Assembly
- Engineering
- Transplantation

Let’s start by copying nature!
Unsuccessful attempts to assemble a living cell from its separated components (=proto-synthetic biology?). This program was supported by NASA.

From the “Scientific American” 1962; 206:117-26
Mollicutes, minimal bacteria

Mycoplasma genomes among the first to be sequenced

Science 1995; 270:397-403.

The Minimal Gene Complement of Mycoplasma genitalium


The complete nucleotide sequence (560,070 base pairs) of the Mycoplasma genitalium genome, the smallest known genome of any eubacterial species, has been determined by the TIGR Sequencing Center. The genome, which is 1,044,053 nucleotides in length, encodes 438 genes for protein-coding sequences, 32 genes for RNA molecules, and 9 genes for transfer RNA (tRNA) molecules. The overall GC content of the genome is 36%, which is similar to that of other mollicutes. The genome contains a high proportion of GC-rich regions, which may be important for the survival of this organism in its host, the human body. The genome also contains a high proportion of GC-rich regions, which may be important for the survival of this organism in its host, the human body. The genome also contains a high proportion of GC-rich regions, which may be important for the survival of this organism in its host, the human body. The genome also contains a high proportion of GC-rich regions, which may be important for the survival of this organism in its host, the human body. The genome also contains a high proportion of GC-rich regions, which may be important for the survival of this organism in its host, the human body.

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

Craig Venter

Claire Fraser
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 Venter1†

Essential genes of a minimal bacterium

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

Chemical synthesis

In vitro assembly

Cloning into *E. coli*

Final steps by homologous recombination in yeast

10^4 oligonucleotides 50 nt

101 cassettes 5-7 kbp

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

in vitro recombination

Gibson Assembly™ Master Mix
Instruction Manual

NEB #E2611S/L
10/50 reactions

From a few kbp to ~300 kbp

Repair at 5' and 3'
Genome cloning and assembly in yeast

3 methods:

A. Transformation; insertion of yeast vector into bacterial genome

B. Whole genome, linearized, and a separate yeast vector

C. Overlapping DNA fragments (natural or synthetic)
Genome cloning in yeast

What are the limits?

- Size?
- G+C content?
- Genetic code?
# Genomes cloned in yeast

<table>
<thead>
<tr>
<th>MOLICUTES</th>
<th>Genome size (Mbp)</th>
<th>% G+C</th>
<th>Genetic code</th>
<th>Yeast cloning</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma genitalium</em></td>
<td>0.58</td>
<td>32</td>
<td>Non standard</td>
<td>✓</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>0.81</td>
<td>41</td>
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</tr>
<tr>
<td><em>Mycoplasma mycoides subsp. capri</em></td>
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<td>24</td>
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<tr>
<td><em>Mycoplasma mycoides subsp. mycoides</em></td>
<td>1.2</td>
<td>24</td>
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<tr>
<td><em>Acholeplasma laidlawii</em></td>
<td>1.5</td>
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Successfull cloning required the inactivation of a gene coding a surface anchored endonuclease.
## Genomes cloned in yeast

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**Prochlorococcus marinus**
1.6 Mbp
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</tr>
<tr>
<td>Synechococcus elongatus</td>
<td>2.7</td>
<td>55</td>
<td>Universal</td>
<td>×</td>
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- 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
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

Mycoplasma leachii  
Mycoplasma capricolum subsp capripneumoniae  
Mycoplasma capricolum subsp capricolum  
Mycoplasma mycoides subsp mycoides  
Mycoplasma mycoides subsp capri  
Mycoplasma putrefaciens  
Mycoplasma yeatsii  
Mesoplasma florum  
Spiroplasma citri  
Spiroplasma kunkelli

Mycoplasma pneumoniae  
Mycoplasma genitalium  
Mycoplasma gallisepticum  
Ureaplasma parvum

Mycoplasma bovis  
Mycoplasma agalactiae  
Mycoplasma pulmonis  
Mycoplasma hominis  
Mycoplasma arthritidis  
Mycoplasma alkalescens

Acholeplasma hippikon  
Acholeplasma oculi  
Acholeplasma laidlawii  
Acholeplasma palmae  
Acholeplasma morum  
Acholeplasma brassicae  
Flavescence Dore phytoplasma  
Ca phytoplasma mali  
Stolbur phytoplasma  
Ca phytoplasma australiens  
Ca phytoplasma asteris Onion Yellows strain  
Ca phytoplasma asteris Aster Yellows strain

Bacillus subtilis

C. LARTIGUE

Successful transplantation

Transplantation of SYNTHETIC genome from YEAST to mycoplasma

- Mycoplasma leachii
- Mycoplasma capricolum subsp capripneumoniae
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- Stolbur phytoplasma
- Ca phytoplasma australiensae
- Ca phytoplasma asteris Onion Yellows strain
- Ca phytoplasma asteris Aster Yellows strain
- Bacillus subtilis

Genome sequence stored in a computer

Synthetic *M. genitalium* genome assembled in yeast

transplantation FAILURE!
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

**Phylogenetic Distance on Transplantation**

- Mycoplasma leachii
- Mycoplasma capricolum subsp capripneumoniae
- Mycoplasma capricolum subsp capricolum
- Mycoplasma mycoides subsp mycoides
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- Mycoplasma putrefaciens
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**Recipient cell**

**Donor genome**

**Mycoplasma**
- Mycoplasma bovis
- Mycoplasma agalactiae
- Mycoplasma pulmonis
- Mycoplasma hominis
- Mycoplasma arthritidis
- Mycoplasma alkalescens

**Acholeplasma**
- Acholeplasma hippikon
- Acholeplasma oculi
- Acholeplasma laidlawii
- Acholeplasma palmae
- Acholeplasma morum
- Acholeplasma brassicae

**Spiroplasma**
- Spiroplasma citri
- Spiroplasma kunkeli

**Mycoides Cluster**
- Mycoplasma leachii
- Mycoplasma capricolum subsp capripneumoniae
- Mycoplasma capricolum subsp capricolum
- Mycoplasma mycoides subsp mycoides
- Mycoplasma mycoides subsp capri
- Mycoplasma putrefaciens
- Mycoplasma yeatsii
- Mesoplasma flororum
- Spiroplasma citri
- Spiroplasma kunkeli

**Pneumoniae**
- Mycoplasma pneumoniae
- Mycoplasma genitalium
- Mycoplasma gallisepticum
- Ureaplasma parvum

**Hominis**
- Mycoplasma bovis
- Mycoplasma agalactiae
- Mycoplasma pulmonis
- Mycoplasma hominis
- Mycoplasma arthritidis
- Mycoplasma alkalescens

**Achloplasma**
- Acholeplasma hippikon
- Acholeplasma oculi
- Acholeplasma laidlawii
- Acholeplasma palmae
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- Acholeplasma brassicae

**Phytoplasma**
- Flavescence Dore phytoplasma
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**Bacillus subtilis**
Extending genome transplantation

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Bacillus subtilis

Recipient cell
Donor genome

Mycoides Cluster
Spiroplasma
Pneumoniae
Hominis
Phytoplasma
Influence of phylogenetic distance on transplantation

Mycoides Cluster

- Spiroplasma

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Pneumoniae

- Mycoplasma pneumoniae
- Mycoplasma genitalium
- Mycoplasma gallisepticum
- Ureaplasma parvum

Hominis

- Mycoplasma bovis
- Mycoplasma agalactiae
- Mycoplasma pulmonis
- Mycoplasma hominis
- Mycoplasma arthritidis
- Mycoplasma alkalescens

Acholeplasma

- Acholeplasma laidlawii
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Universal genetic code

1- Close to phytoplasma species

2- Non-pathogen organism, easy to grow, amenable to transformation
More benefits from *Acholeplasma* genus

- Mycoplasma mycoides subsp. mycoides SC
- Mesoplasma florum
- Mycoplasma genitalium
- Mycoplasma pneumoniae
- Mycoplasma gallisepticum
- Ureaplasma parvum
- Mycoplasma penetrans
- Mycoplasma hyopneumoniae
- Mycoplasma arthritidis
- Mycoplasma mobile
- Mycoplasma pulmonis
- Mycoplasma agalactiae
- Mycoplasma synoviae
- Acholeplasma laidlawii
- Aster yellows witches-broom phytoplasma
- Candidatus Phytoplasma mali

3- Close to FIRMICUTES of industrial interest

- Bacillus - Staphylococcus
- Lactobacillus - Lactococcus - Streptococcus
- Clostridium
- Streptomyces
- Prochlorococcus
- Escherichia coli
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)
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)

Before

After
Influence of phylogenetic distance on transplantation
Genome engineering of uncultivated bacteria

Uncultivable bacteria phytopathogen
FUNCTIONAL GENOMICS

Attemps to cultivate the modified phytoplasmas

Selection

Recipient cell
\((Acholeplasma\ laidlawii)\)

Transplantation

DNA extraction

New modifications

Yeast co-transformation

Cloned phytoplasma genome

DNA extraction from phytoplasma-infected plants

Metabolic engineering (addition of missing genes)
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

Marked MmmSC genome
Yeast vector is integrated into MmmSC genome

Extraction of intact MmmSC genome from MmmSC cells

Engineering of MmmSC genome in yeast

MmmSC genome cloned in yeast

Modified MmmSC genome

Extraction of intact MmmSC genome from yeast cells

Transplantation into a suitable recipient cell

Modified MmmSC strain

Selection

Recipient cell

Test for virulence and immunogenicity (In vitro and in vivo experimentations)
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
Goethe's 'Faust' depicting Mephistopheles creating a homunculus, Bibliotheque des Arts Décoratifs, Paris, France. 1854.