Genome Transplantation in Bacteria: Changing One Species to Another

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Hypothesis: Genome Transplantation

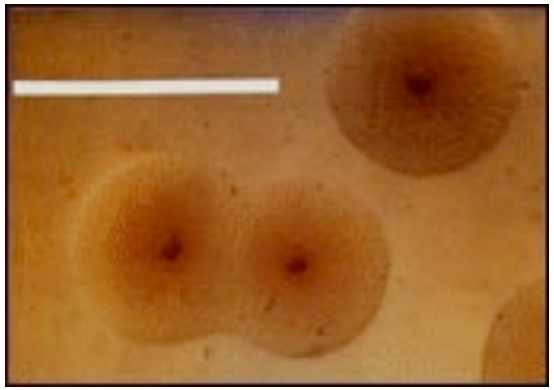
- Transfer whole intact genomes from one bacterial species to another, changing the recipient bacteria to the donor species
  - entirely replace the recipient genome with the donor genome
  - No recombination between original and new DNA
  - Results in cells with same genotype and phenotype as donor cell

Synthetic Genomics

- Synthetic genetic modification of the genetic code of organisms to produce a desired behavior
- Potential to construct useful microorganisms
- Goal of Venter lab: chemically synthesize genomes to define the minimal genome necessary for life
  - "bottom up" approach
- Competing technology with evolutionary methods (e.g. MAGE)

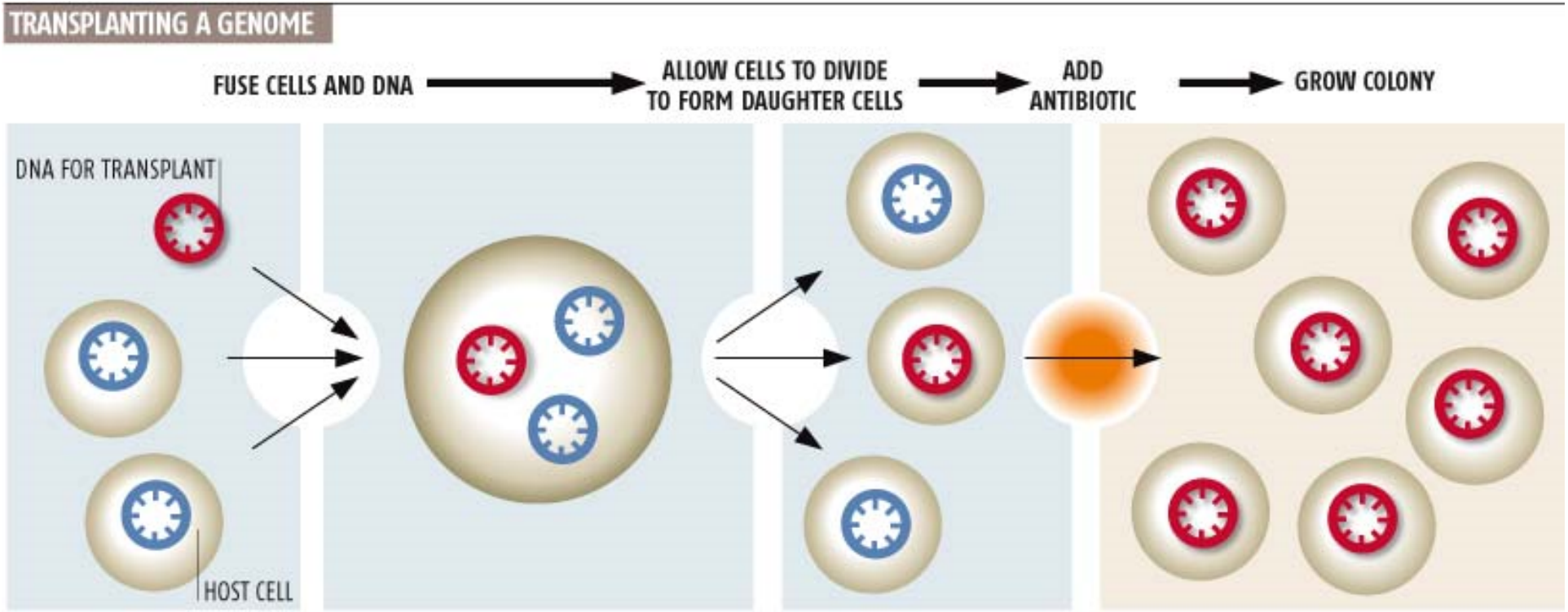
Mycoplasmas as experimental organisms

- Small genomes
- Altered genetic code (UGA → Tryptophan)
- No cell wall
- M. myocides LC plasmids are viable in M. capricolum



- Key assumption: these physical properties will facilitate genome transplantation

Overview of Genome Transplantation

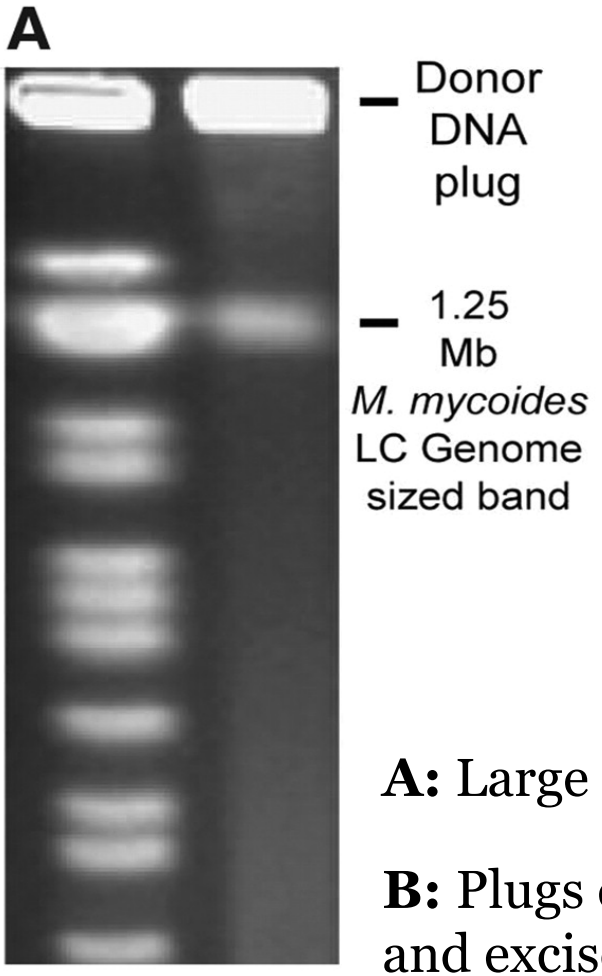


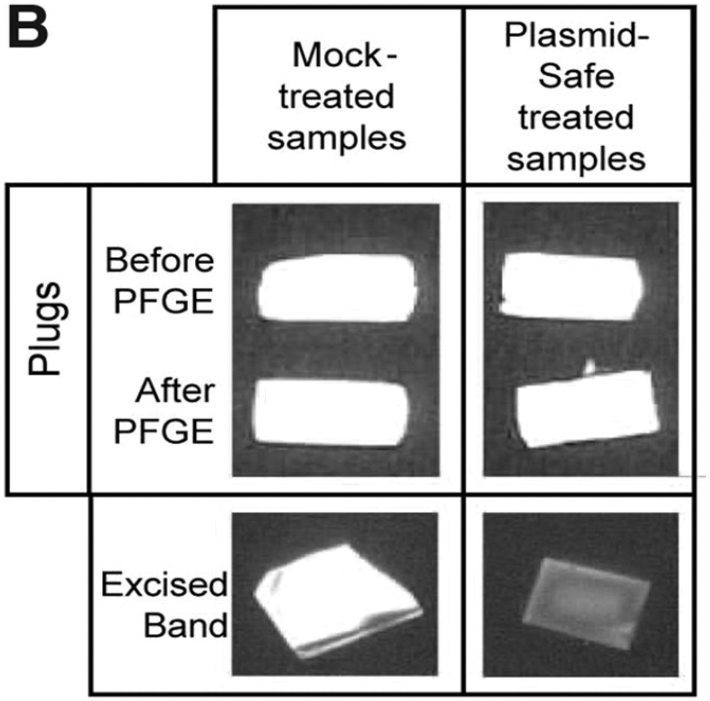
Selection: Tetracycline resistance
Screening: β-galactosidase (lacZ) genes
Main challenge: isolating intact genome and avoiding recombination

Gentle Isolation of Intact Donor Genome

- *M. mycoides* grown containing tetracycline resistance and lacZ genes
- Cells suspended in agarose blocks and distributed into plug molds
- Cells were lysed and proteins digested with proteinase K
- Intact genome isolated through pulsed-field gel electrophoresis (PFGE)

Isolated Donor Genome is Circular

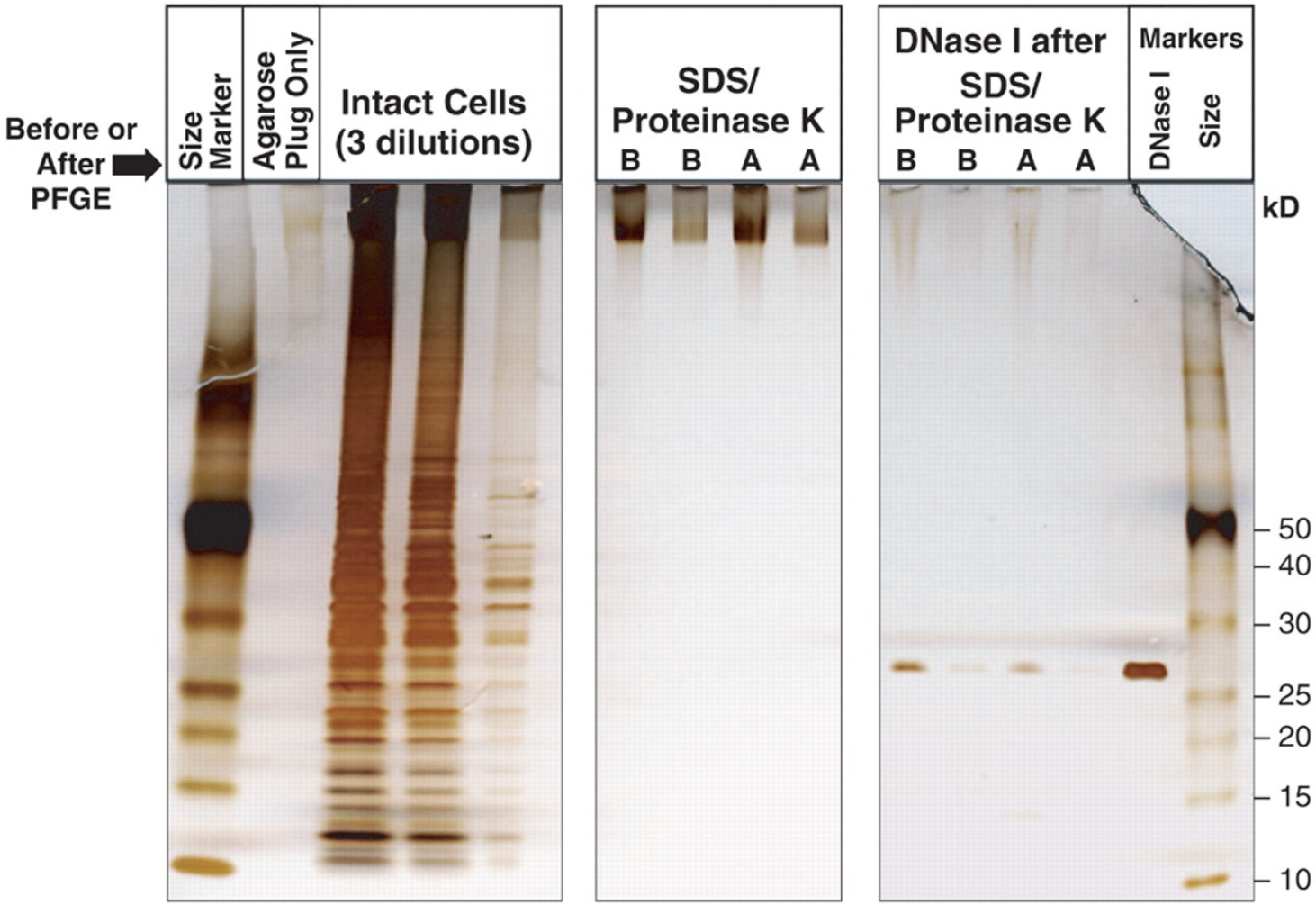




A: Large amount of circular DNA remain in the plug

B: Plugs contain circular double stranded DNA, and excised band is linear

Isolated DNA is naked



Naked DNA is desired as a proof of concept for inserting synthetic genomes

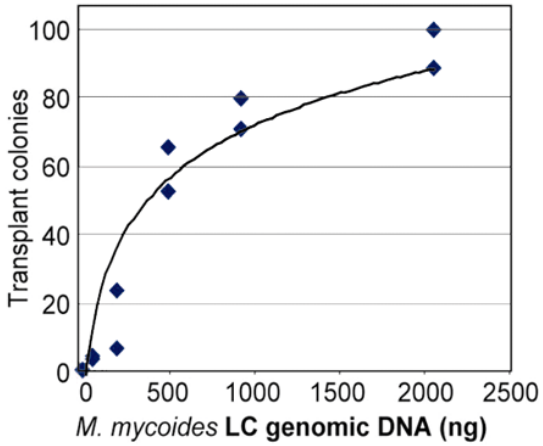
Genome Transplantation into *M. capricolum*

- Prepared M. mycoides genome was transplanted into nucleotide-starved M. capricolum cells, at low efficiencies
- Blue colonies (*M. mycoides)* formed after 3 days
- Smaller blue and white colonies (*M. capricolum)* formed after 10 days
  - Result of recombination?

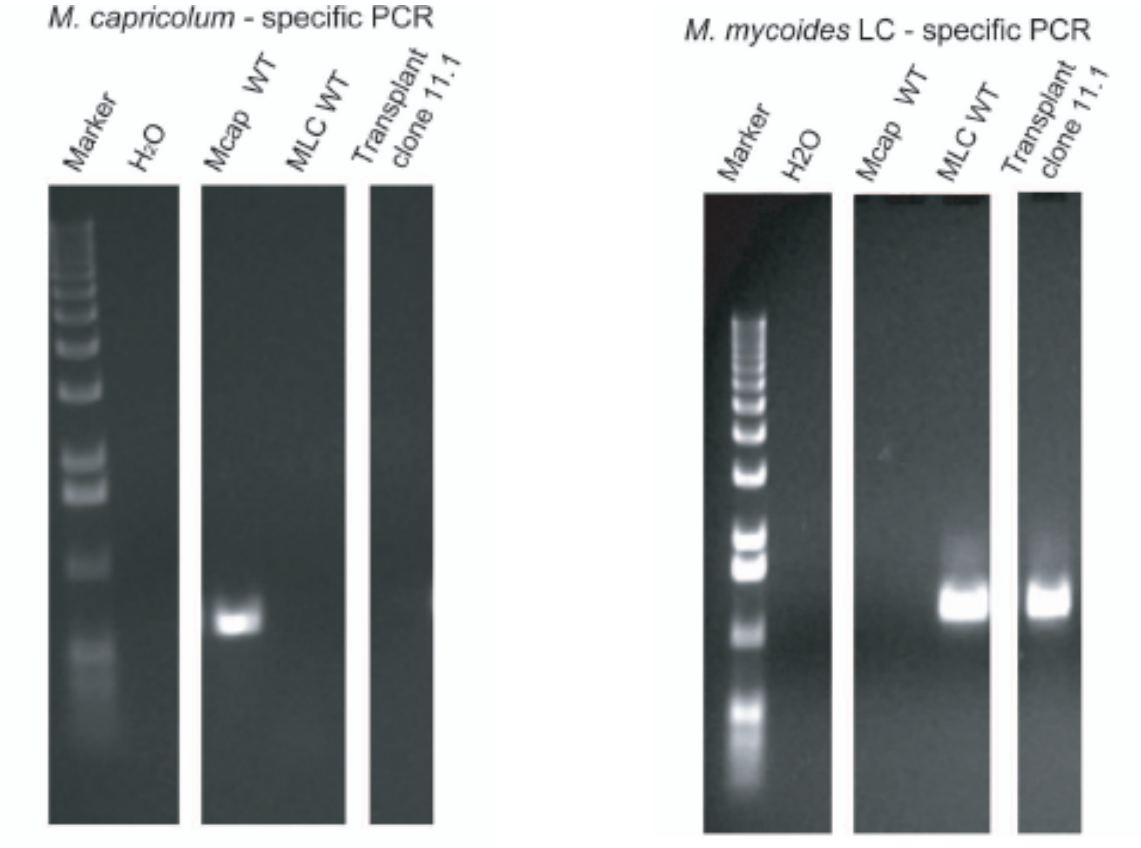
Genome Transplantation into *M. capricolum*

Maximum efficiency: 1 in 150,000 cells were transplanted

Experiment date	Number of colonies: Negative controls: No donor DNA	Number of colonies: Negative controls: No recipient cells	Number of colonies: *M. mycoides* LC transplants	Total *M. capricolum* recipient cells
3/28/06	0	0	1	4×10^9
4/13/06	2*	0	~65	8×10^8
4/19/06†	0	0	1	1×10^8
5/25/06	0	0	1	6×10^8
6/07/06	0	0	16	5×10^8
6/08/06	0	0	17	2×10^8
6/28/06	0	0	8	7×10^8
7/06/06	0	0	3	6×10^9
9/07/06	0	0	2	3×10^{10}
11/17/06‡	0	0	~100	2×10^8
11/24/06‡	0	0	~100	5×10^8
12/13/06	0	0	20	4×10^8
1/04/07	0	0	17	5×10^7
1/18/07	0	0	20	2×10^7
3/01/07	0	0	24	6×10^7
3/20/07‡	0	0	134	5×10^7
3/21/07‡	0	0	81	3×10^7
3/29/07‡	0	0	132	2×10^7



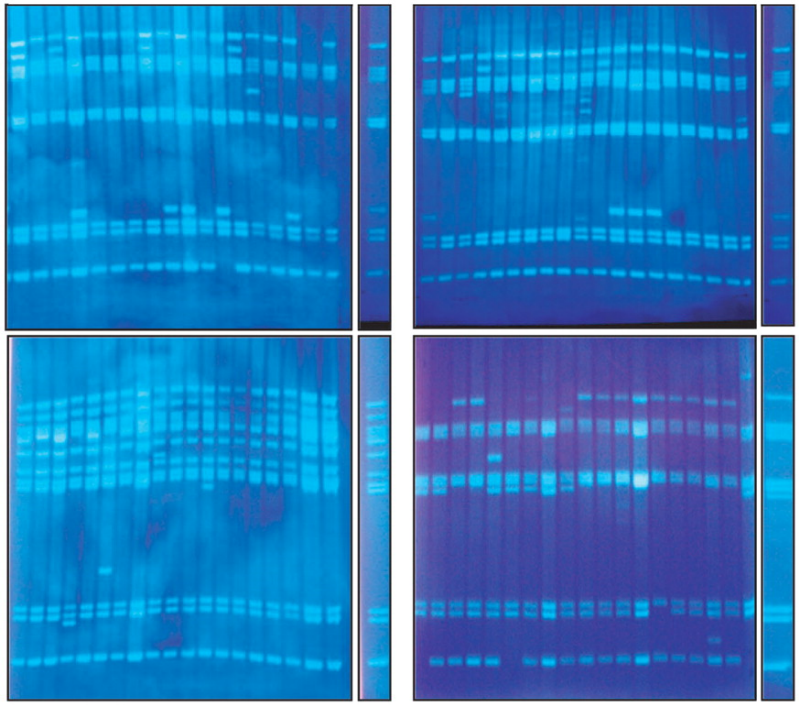
Transplants display *M. mycoides* Genotype



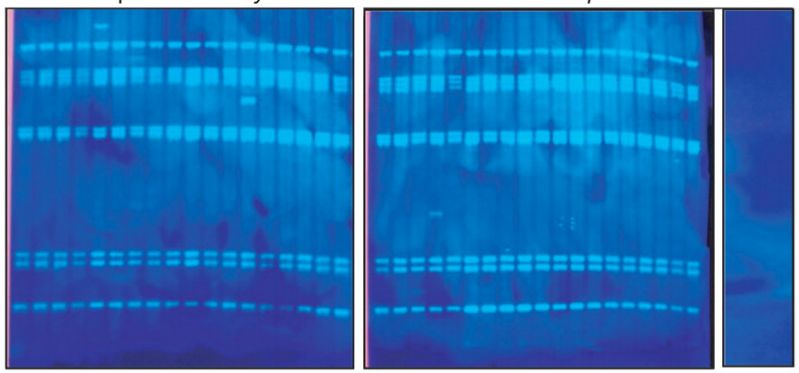
- *M. Mycoides* specific IS1296 PCR worked for wild type *M. Mycoides* and the transplant
- Still possible for a recombination of IS1296, lacZ, and tetM to destroy the arginine deiminase gene

Transplants display *M. mycoides* Genotype

A Transplants and donor genome profiles



B Untransplanted *M. mycoides* LC clones and wt *M. capricolum*

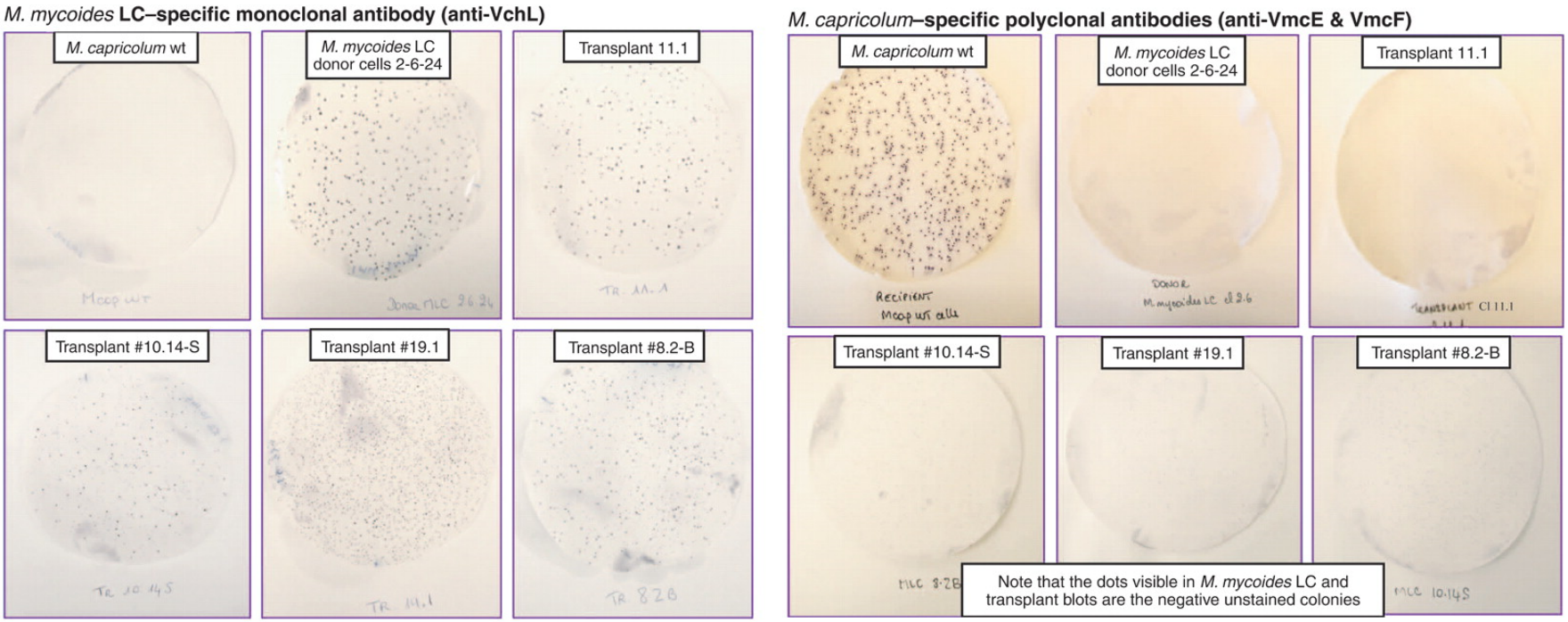


- 92% (34/37) of WT *M. mycoides* clones and 59% (44/75) of the transplants were identical to the donor

- Variations can be explained by IS element transposition during the transitional period

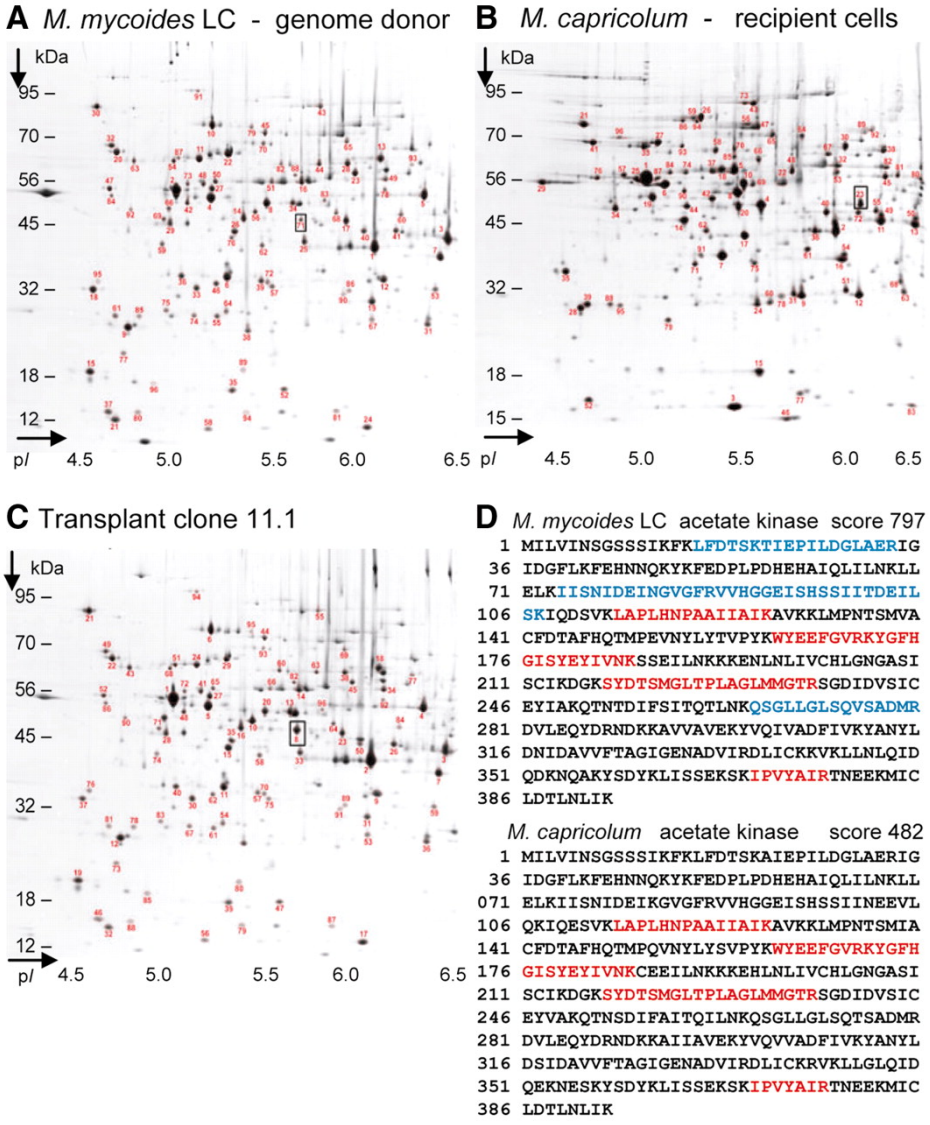
- Subsequently, sample sequencing of random sequence reads from transplants all matched the *M. mycoides* sequence

Transplants display *M. mycoides* Phenotype



- *M. mycoides* specific antibodies bind transplant blots with same intensity as wild-type
- *M. capricolum* specific antibodies do not bind to transplant blots

Transplants display *M. mycoides* Phenotype



- Two-dimensional electrophoresis with mass spectrometry

- Transplants and *M. mycoides* show identical protein spot patterns

- *M. capricolum* spot patterns varies by more than 50%

Unknowns

- Mechanism of transplant
- Can this method be broadly applied to other bacterial species
- Is the similarity of the donor and recipient organisms a limitation?
- Can this technique be used to successfully transplant a synthetic genome?

Future Work

- Transplantation of a synthetic genome using this technology
- Use of synthetic genomes to define a minimal genome sufficient for cellular life
- Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. Gibson DG, et al. Science 2008
- Ethical implications of synthesizing a minimal genome

Formation of Protocell-like Vesicles in a Thermal Diffusion Column

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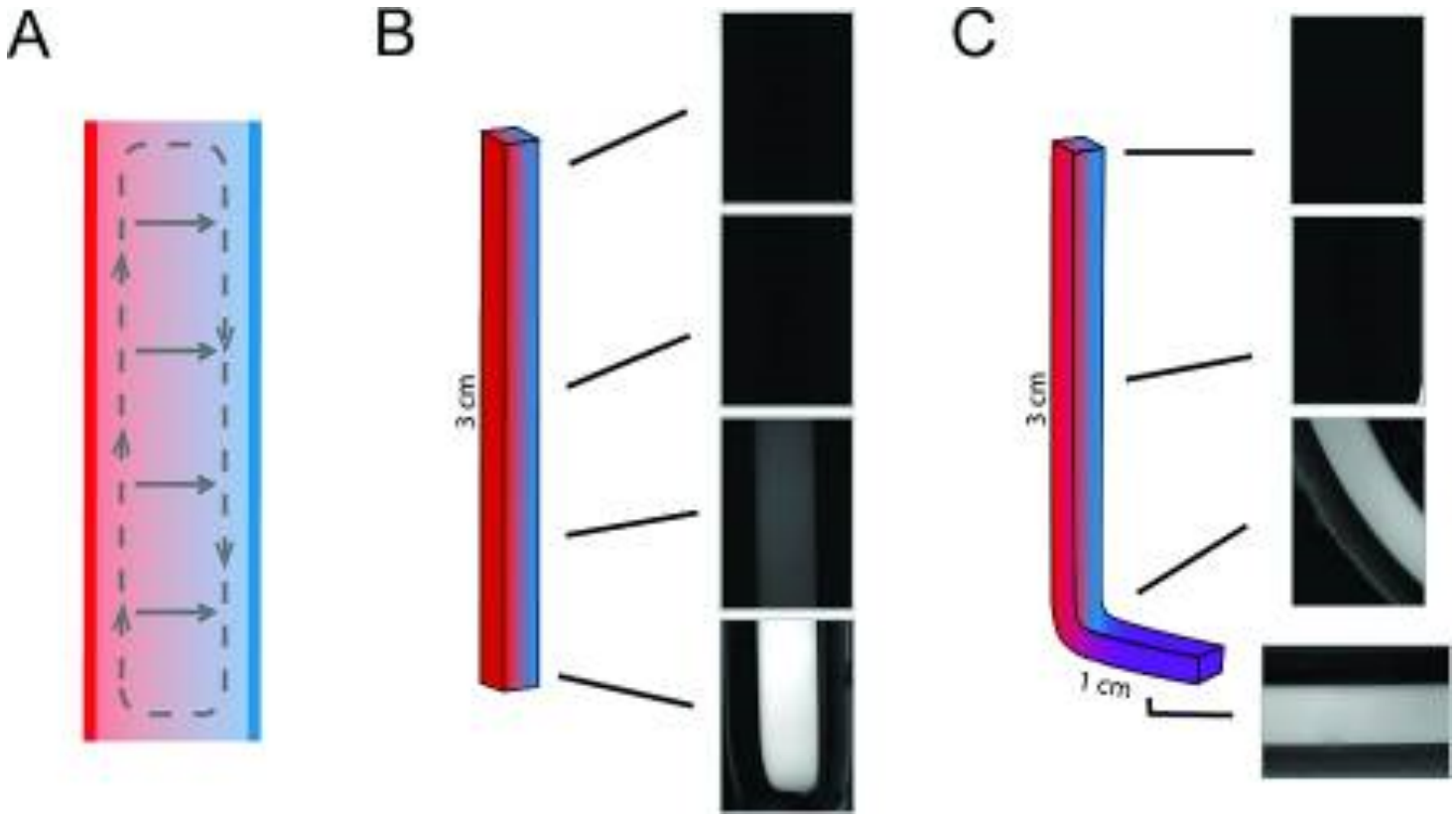
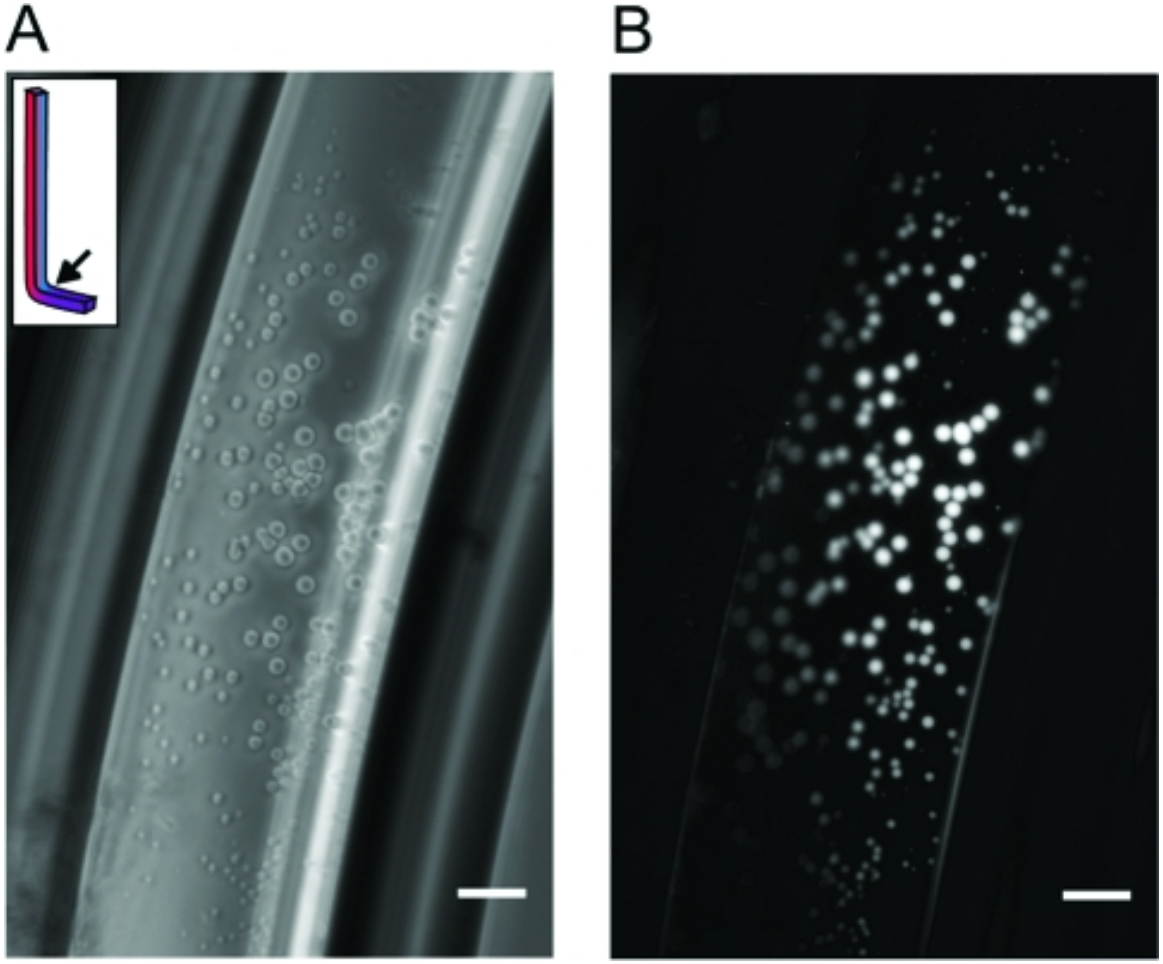
Thermal diffusion columns concentrate small molecules



Locally concentrated oleate forms vesicles



“bottom up” approach