Lartigue, C.; Glass, J.I.; Alperovich, N.; Pieper, R; Parmar, P.P.; Hutchison III C.A.; Smith, H.O.; Venter, J.C.

Genome Transplantation in Bacteria: Changing One Species to Another

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Objectives of Study

- Genome Transplantation
  - To completely replace the whole genome of a bacterial cell with one from another species
  - Resulting cells have genotype and phenotype of input genome (recipient genome completely replaced)

- Requirement for establishment of field of Synthetic Genomics
  - Useful for research into functions of individual genes
  - Minimal genetic components for life –cell lines with minimal extraneous metabolic pathways – more efficient cells
  - Facilitate construction of new microorganisms specific for energy production, environmental stewardship, and medicine.
  - Chemically synthesized chromosomes transplanted into cellular milieu and activated into viable living cells to carry out genetic engineering in a more extensive and systematic way

Previous Work

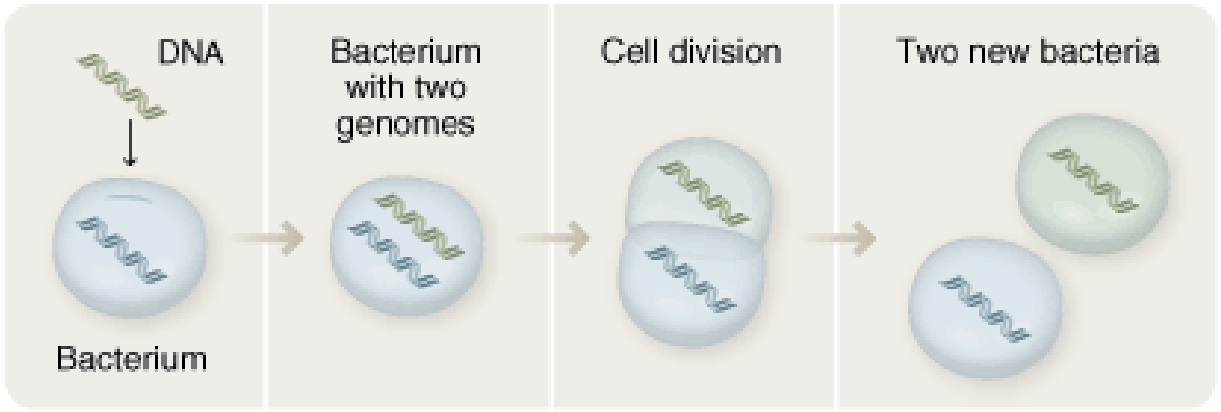
- **1944** - Ability of bacteria to take up naked DNA and integrate it to form genetic recombinants
- **1992** – Artificial chromosomes up to 300 kbp transplanted
- **2001** – 30% replacement of DNA
- **2005 & 2007** – authors reported incompatibility between host and recipient genomes
  - Almost entire genome *Synechocystis* PCC6803 into *B. subtilis*
  - *Haemophilus influenzae* into *E. coli*

- Transplantation of nuclei common in vertebrates

Use of *Mollicutes*

- Small Genomes (~1 million bp)
  - Easier to synthesize
  - Less likely to break during transplant
- Fast growing – faster testing iterations
- Lack of cell wall

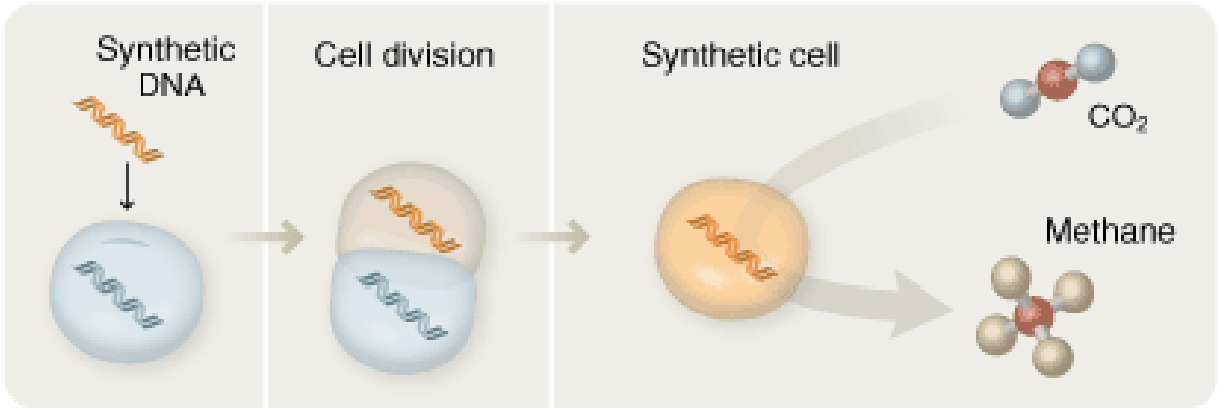
- Specific Cell Lines
  - Donor - *Mycoplasma mycoides* Large Colony strain GM12
  - Recipient - *Mycoplasma capricolum* strain California kid
  - 76.4% of 1083 kbp of *M. mycoides* could be mapped on 1010 kbp *M. capricolum*



A complete set of DNA from one species of bacteria is removed and inserted into another species of bacteria.

When the cell divides, the two genomes may go into different daughter cells.

One of the new cells is identical to cells of the donor species, and one is identical to cells of the host species.



If researchers were able to create a synthetic genome, the transplantation process might be able to create synthetic cells.

In theory, synthetic cells could be designed to have useful properties such as the ability to efficiently convert carbon dioxide into methane.

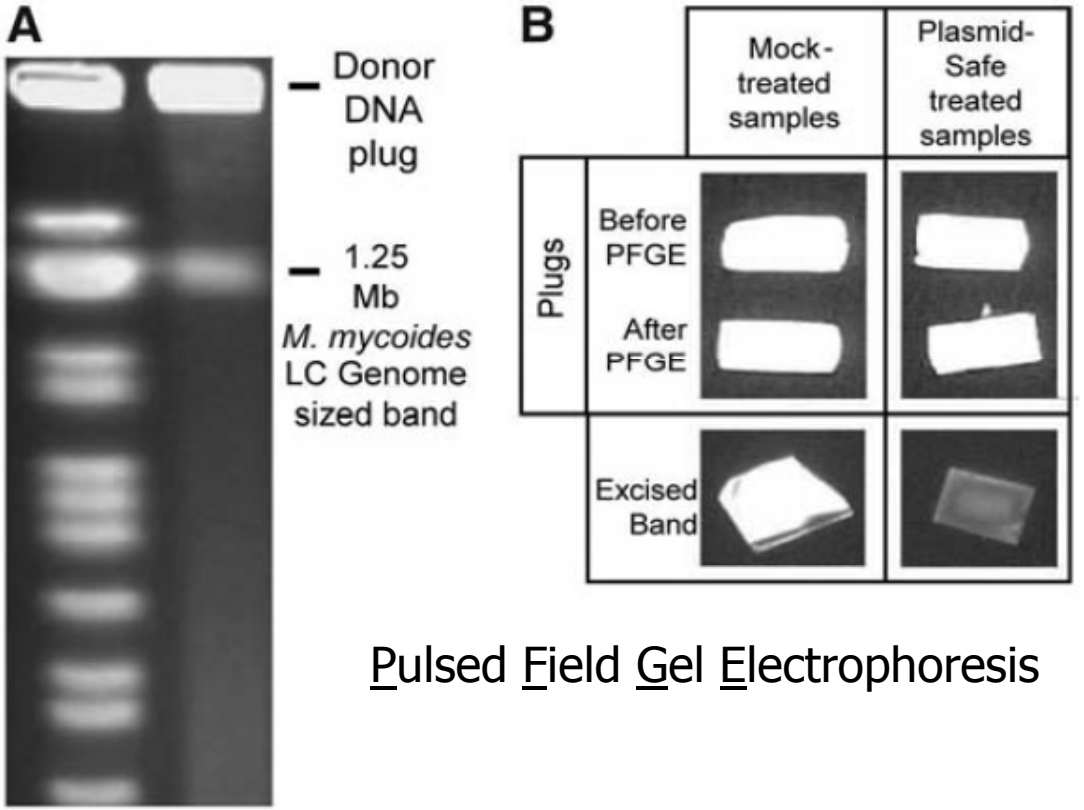
June 29, 2007 The New York Times

Donor DNA Prep

- Whole *M. mycoides* DNA isolation
  - Cells grown at 37°C in basic SP4 media (+AB)
  - Spun into pellet and resuspended in Tris/sucrose
  - Washing repeated and samples were warmed to 50°C and LMT agarose was added

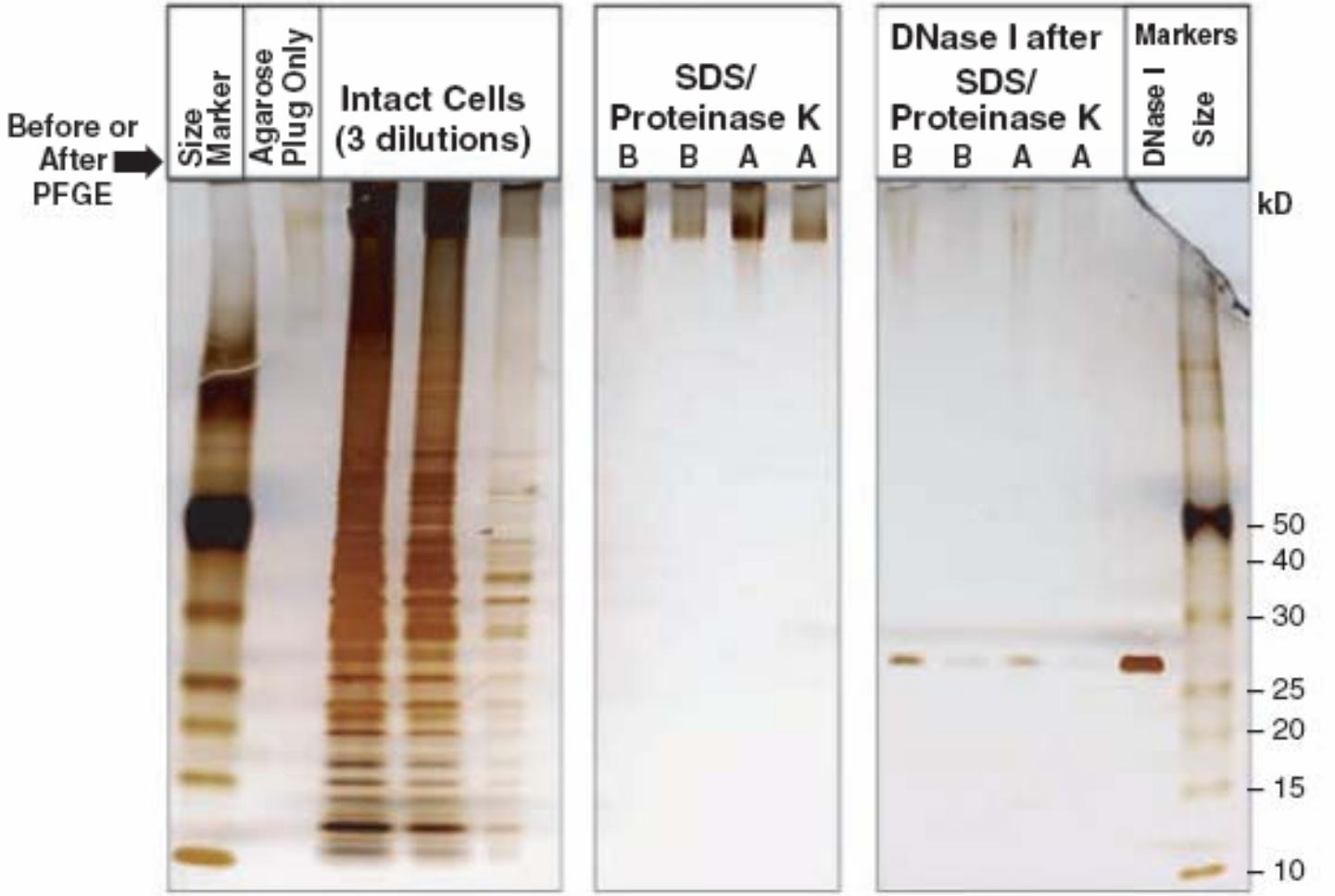
- Digested proteins, lipids, RNAs, and sheared DNA removed by electrophoresis and enzymes to form Naked DNA

Confirming intact DNA



Pulsed Field Gel Electrophoresis

Confirmation by SDS polyacrylamide gels



Recipient Cell Prep

- *M. capricolum* grown, centrifuged into pellet, reconstituted in simple media (SP4) and yeast transfer RNA, and stored on ice.
- Naked DNA liberated - melt at 65°C with β-agarase.
- DNA and *M. capricolum* incubated with "Fusion Buffer" (buffers, salts, PEG8000)
- Recipient cells sometimes encapsulated donor DNA
- Solutions plated and colonies picked and grown in broth with tetracycline to kill residual *M. capricolum*
- Neg. controls – no recipient cells or no donor DNA

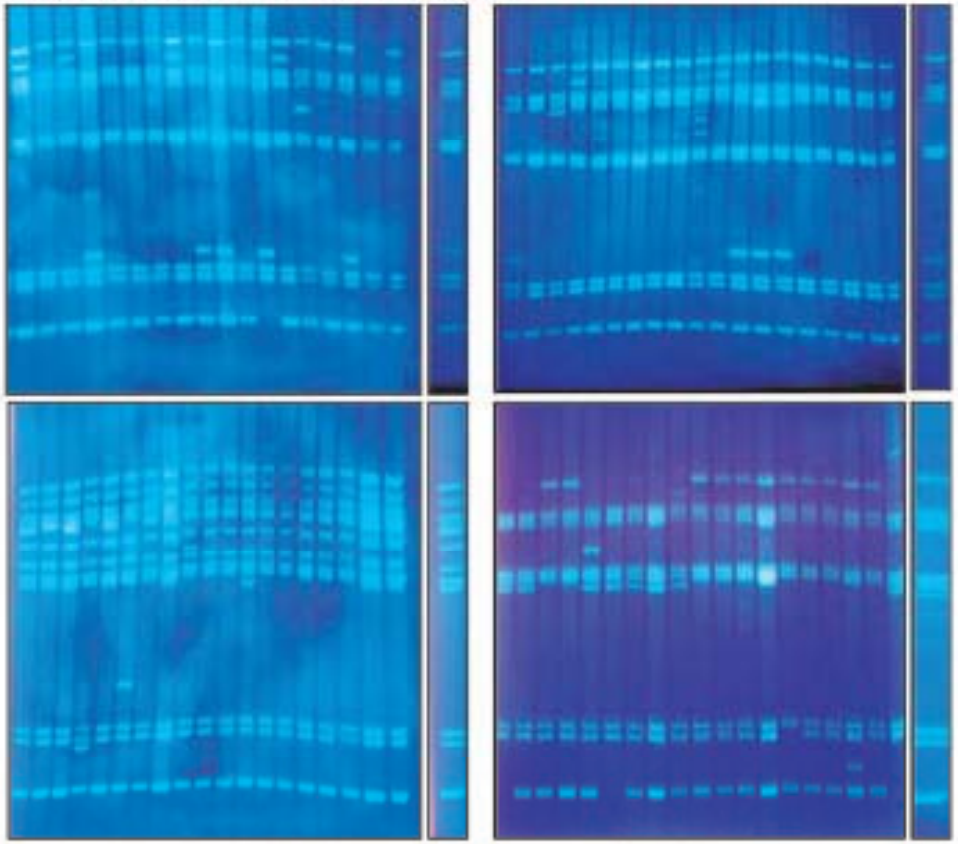
Experiment date	Number of colonies			Total *M. capricolum* recipient cells
	Negative controls		*M. mycoides* LC transplants	
	No donor DNA	No recipient cells		
3/28/06	0	0	1	4×10^9
4/13/06	2*	0	~65	8×10^8
4/19/06†	0	0	1	1×10^8
5/25/06	0	0	1	6×10^8
6/07/06	0	0	16	5×10^8
6/08/06	0	0	17	2×10^8
6/28/06	0	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

*We attribute these two colonies to laboratory error, and we never saw any colonies on the no-donor-DNA control plates in any later experiments. †After this experiment, we did six experiments not listed here that produced no transplant clones. ‡We attribute the higher genome transplantation efficiency in these experiments to the inclusion of streptomycin in the SP4 medium used to grow the *M. mycoides* LC donor genomes.

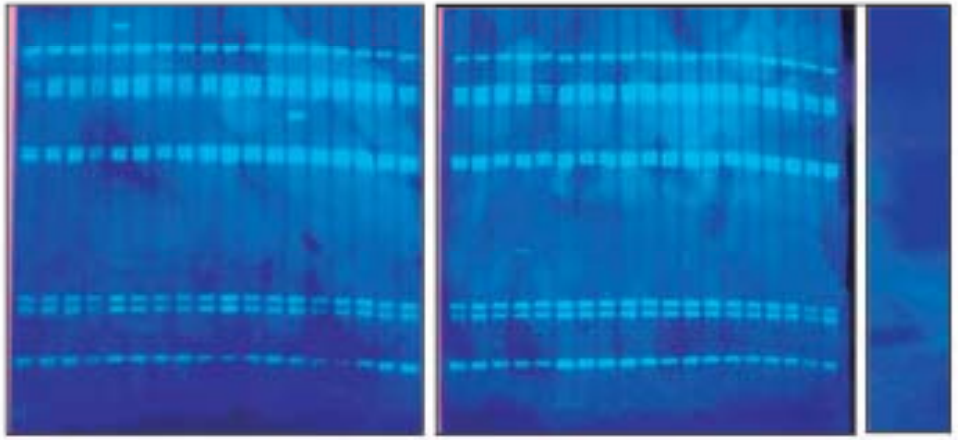
Analysis of Transplants

- Colonies are blue and tetracycline resistant but could represent recombinant genes
- Analysis of 1300 random sequence reads showed all matched donor sequence
- Genotype analysis
  - PCR – generated amplicon only with *M. capricolum* template DNA but not with *M. mycoides* wild-type or with transplanted clones
  - Southern Blot analysis of donor an recipient mycoplasmas

A Transplants and donor genome profiles



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

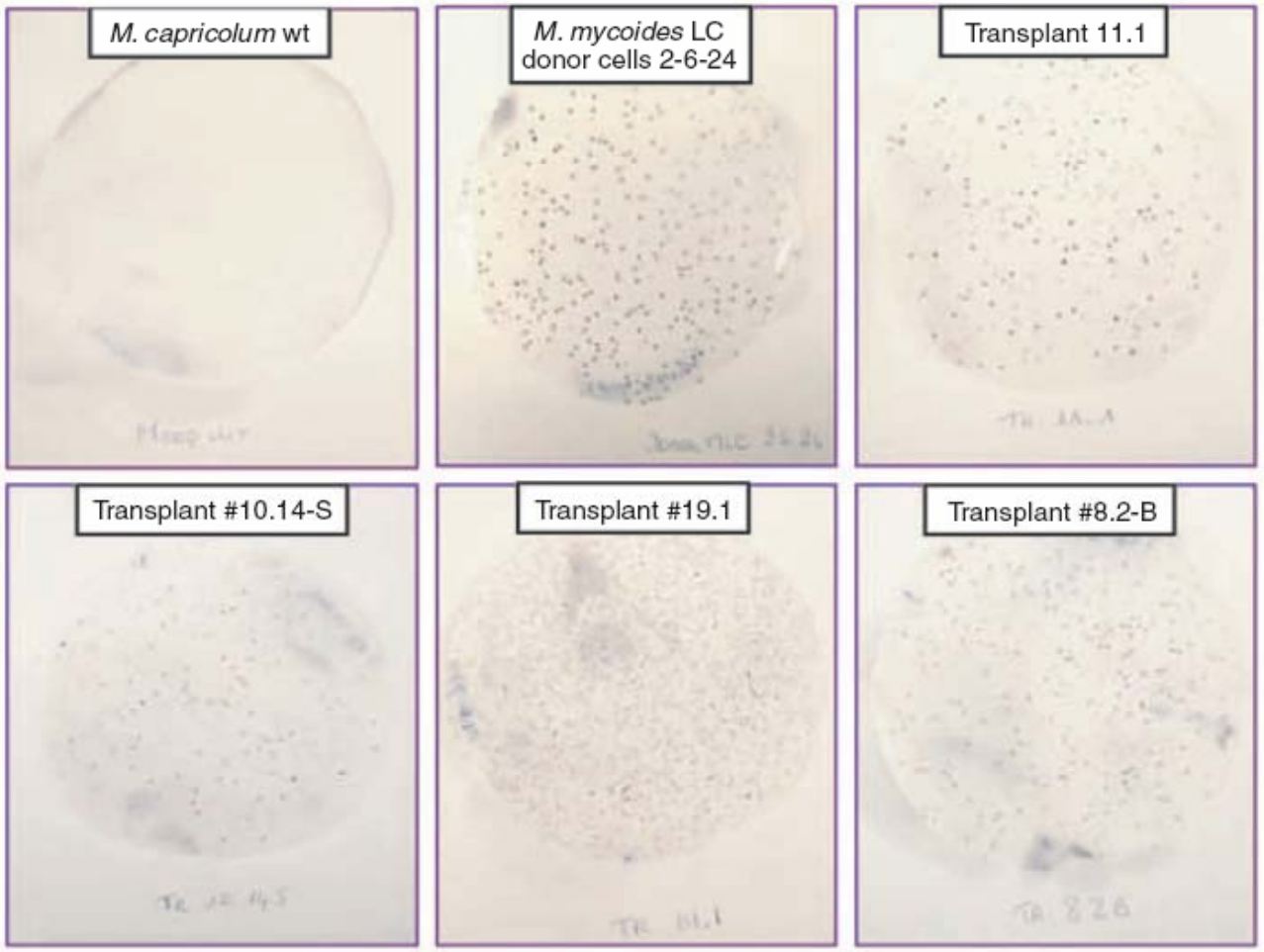


Phenotype analysis

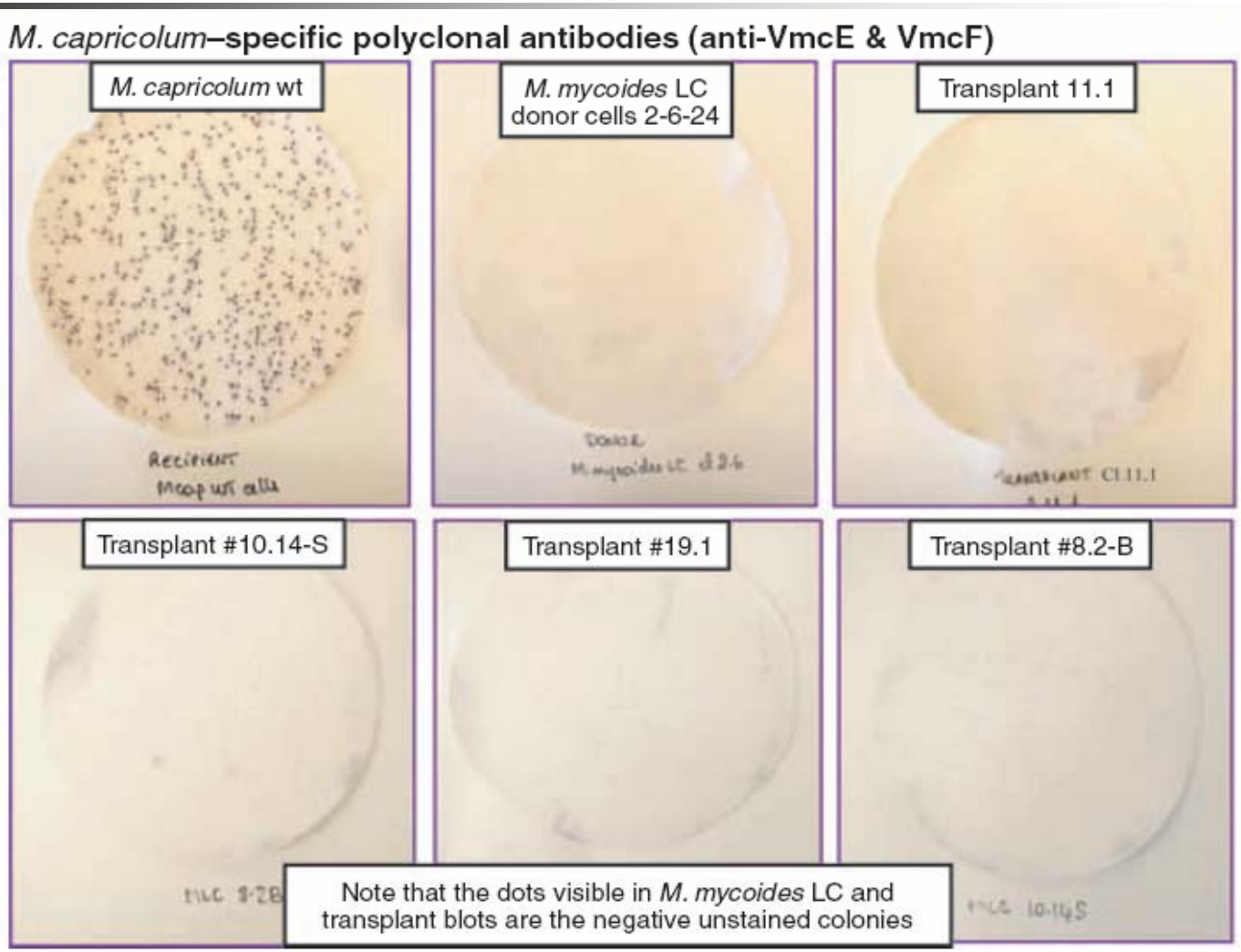
- Single-gene products
  - Colony-Western blots – probed for specific antibodies specific to surface antigens for each species
  - In both assays *M. mycoides* specific antibodies bound the transport blots with the same intensity as the original *M. mycoides* LC

Colony Hybridization

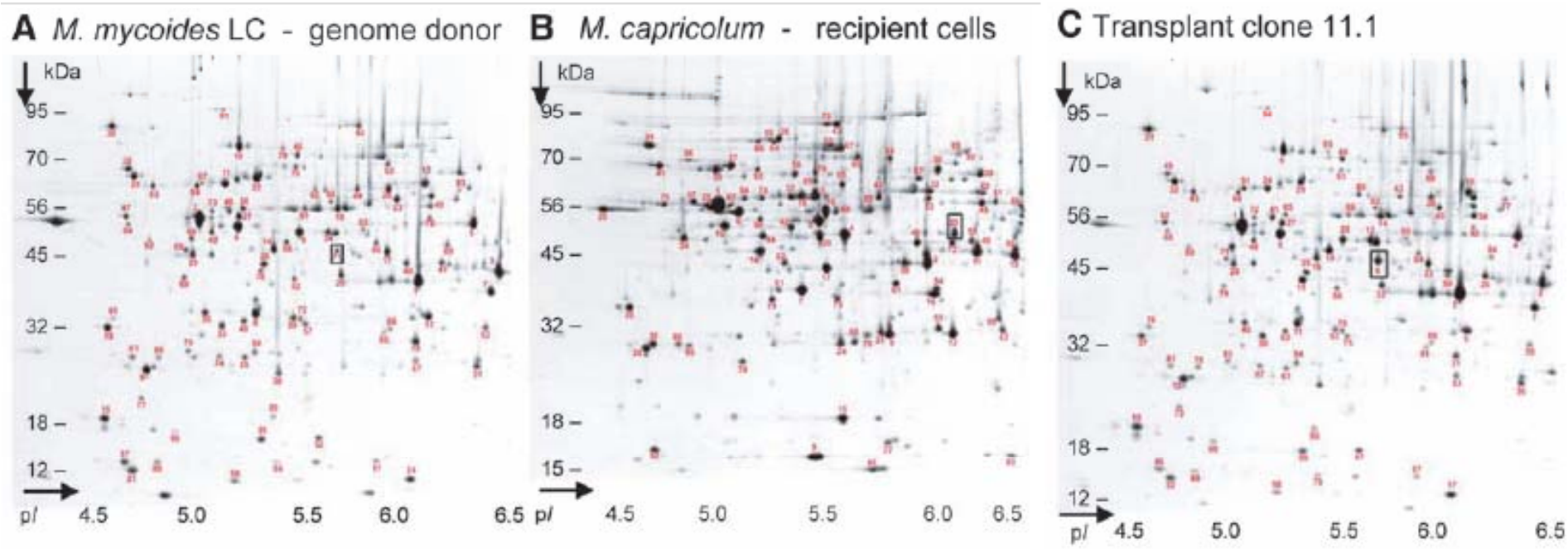
M. mycoides **LC–specific monoclonal antibody (anti-VchL)**



Colony Hybridization

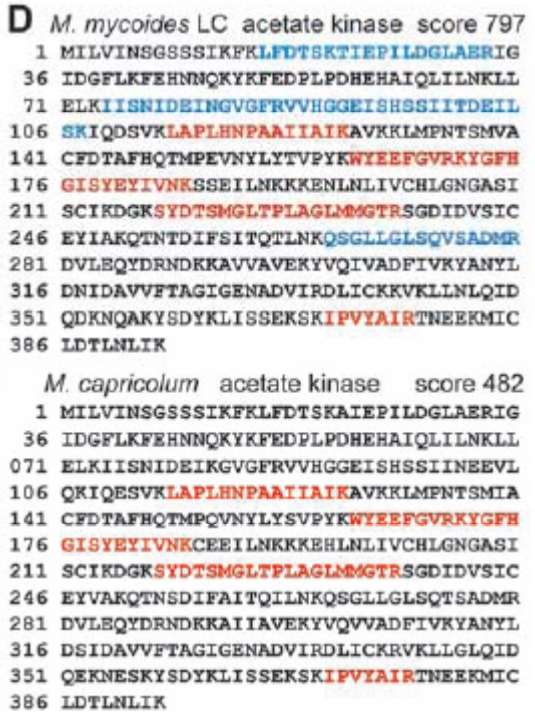


Proteomic Analysis – 2D Gels



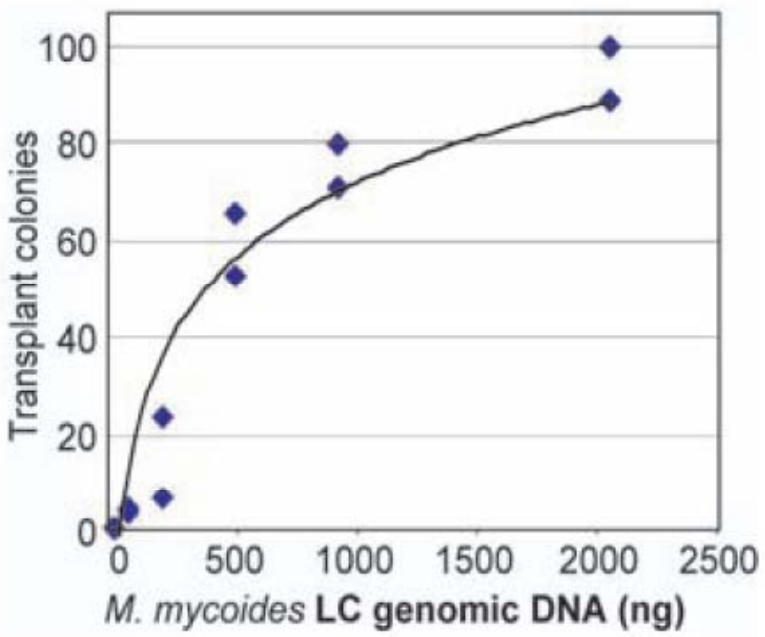
Peptides in Acetate Kinase

- Peptides matching only the species of donor
- No recombination after transplantation and before cell division



Optimizing Transplant η

- Varied
  - Recipient cells
  - Genomic DNA used
- Optimal when recipient cells at 2.5×10^7
- Optimal DNA is at higher concentrations, but plateaus.

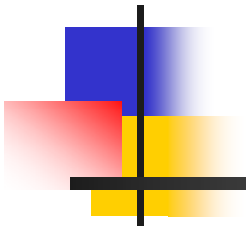


Summary

- Discovered a form of DNA transfer that permits recipient cells to be platforms for production of new species with use of modified or manmade genomes take control of living cells
  - Non-natural transplantation (no recombination)
  - The achievement marks a significant step toward ultimate goal of creating a synthetic organism to carry out genetic engineering in a more extensive and systematic way
- Genome completely transplanted as confirmed by multiple methods
- Simple in concept, difficult in execution – majority of research was proving the extent of transplantation

Study Limitations

- Not sure of mechanism
- Efficiency issues - most efficient expt. only 1 cell in ~150 K transplanted
- Only small DNA strands transferred
- Species were close relatives – other species are a question – not a universal formula
- George Church, a leading systems biologist at Harvard Medical School - the new report was "good science" but that it had been achieved in an organism, Mycoplasma, that is unsuitable for industrial uses.



Questions