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Genome Transplantation in Bacteria: Changing One Species to Another

Carole Lartigue, John I. Glass,* Nina Alperovich, Rembert Pieper, Prashanth P. Parmar, Clyde A. Hutchison III, Hamilton O. Smith, J. Craig Venter

As a step toward propagation of synthetic genomes, we completely replaced the genome of a bacterial cell with one from another species by transplanting a whole genome as naked DNA. Intact genomic DNA from *Mycoplasma mycoides* large colony (LC), virtually free of protein, was transplanted into *Mycoplasma capricolum* cells by polyethylene glycol–mediated transformation. Cells selected for tetracycline resistance, carried by the *M. mycoides* LC chromosome, contain the complete donor genome and are free of detectable recipient genomic sequences. These cells that result from genome transplantation are phenotypically identical to the *M. mycoides* LC donor strain as judged by several criteria.

It has been known ever since Oswald Avery's pioneering experiments with pneumococcal transformation more than six decades ago, that some bacteria can take up naked DNA (*1*). This DNA is generally degraded or recombined into the recipient chromosomes to form genetic recombinants. DNA molecules several hundred kilobase pairs (kb) in size can sometimes be taken up. In recent studies with competent *Bacillus subtilis* cells, Akamatsu and colleagues (*2*, *3*) demonstrated cotransformation of genetic markers spread over more than 30% of the 4.2-megabase pair (Mb) genome using nucleoid DNA isolated from gently lysed *B. subtilis* protoplasts. Artificial transformation methods that employ electroporation or chemically competent cells are now widely used to clone recombinant plasmids. Generally, the recombinant plasmids are only a few kilobase pairs in size, but bacterial artificial chromosomes (BACs) greater than 300 kb have been reported (*4*). Recombinant plasmids coexist with host-cell chromosomes and replicate independently. Two other natural genetic transfer mechanisms are known in bacteria. These are transduction and conjugation. Transduction occurs when viral particles pick up chromosomal DNA from donor bacteria and transfer it to recipient cells by infection. Conjugation involves an intricate mechanism in which donor and recipient cells come in contact and DNA is actively passed from the donor into the recipient. Neither of these mechanisms involves a naked DNA intermediate.

In this paper, we report a process with a different outcome, which we call "genome transplantation." In this process, a whole bacterial genome from one species is transformed into another bacterial species, which results in new cells that have the genotype and phenotype of the input genome. The important distinguishing feature of transplantation is that the recipient genome is entirely replaced by the donor genome. There is no recombination between the incoming and outgoing chromosomes. The result is a clean change of one bacterial species into another.

Work that is related to the process we describe in this paper has been carried out or proposed for various species. Itaya *et al*. transferred almost an entire *Synechocystis* PCC6803 genome into the chromosome of a recipient *B. subtilis* cell using the natural transformation mechanism. The resulting chimeric chromosome had the phenotype of the *B. subtilis* recipient cell. Most of the *Synechocystis* genes were silent (*5*). A schema for inserting an entire *Haemophilus influenzae* genome as overlapping BACs into an *Escherichia coli* recipient has also been proposed; however, those authors have pointed out difficulties arising from incompatibility between the two genomes (*6*). Transplantation of nuclei as intact organelles into enucleated eggs is a well-established procedure in vertebrates (*7*–*9*). Our choice of the term "genome transplantation" comes from the similarity to eukaryotic nuclear transplantation in which one genome is cleanly replaced by another.

Genome transplantation is a requirement for the establishment of the new field of synthetic genomics. It may facilitate construction of useful microorganisms with the potential to solve pressing societal problems in energy production, environmental stewardship, and medicine. Chemically synthesized chromosomes must eventually be transplanted into a cellular milieu where the encoded instructions can be expressed. We have long been interested in defining a minimal genome that is just sufficient for cellular life (*10*, *11*) and have advocated the approach of chemically synthesizing a genome as a means for testing hypotheses concerning the minimal set of genes. The societal and ethical implications of this work have been explored (*12*, *13*).

Fabricating a synthetic cell by this approach requires the introduction of the synthetic genome into a receptive cytoplasm. We chose mycoplasmas, members of the class *Mollicutes*, for building a synthetic cell. This choice was based on a number of characteristics specific to this bacterial taxon. The essential features of mycoplasmas are small genomes, use of UGA to encode tryptophan (rather than a stop codon), and the total lack of a cell wall. A small genome is easier to synthesize and less likely to break during handling. The altered genetic code facilitates cloning in *E. coli* because it curtails the expression of mycoplasma proteins. The absence of a cell wall makes the exterior surfaces of these

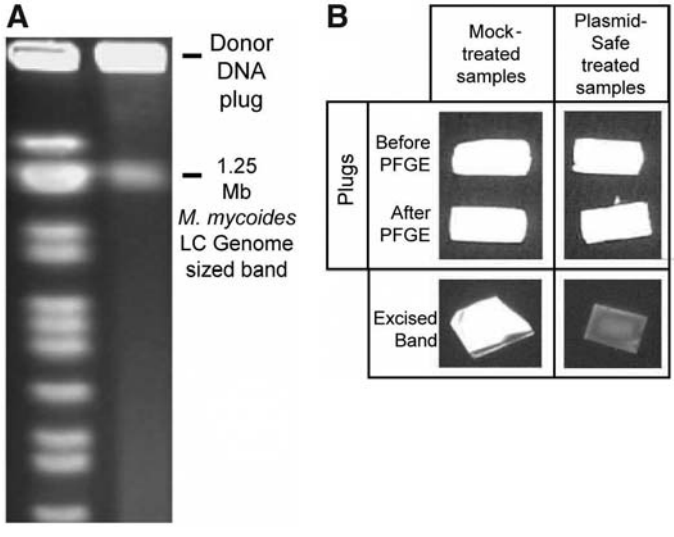


Fig. 1. Demonstration that the DNA in the blocks was intact and circular, whereas the DNA in the band that migrated into the gel was linear. (**A**) A pulsed-field gel loaded with a plug containing *M. mycoides* LC DNA. The 1× TAE buffer gel was separated by electrophoresis for 20 hours and then stained with SYBR gold. The marker lane contains Bio-Rad *Saccharomyces cerevisiae* genomic DNA size markers. Note the large amount of DNA remaining in the plug. (**B**) The plugs are shown either before PFGE or after PFGE, and the genome sized band produced after PFGE, and either with or without treatment with the Plasmid-Safe DNase. The nuclease enzyme digests linear DNA, but has no effect on circular duplex DNA. These data indicate the band of DNA that migrated into the gel was exonuclease-sensitive and, therefore, linear.

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bacteria similar to the plasma membranes of eukaryotic cells and may simplify our task of installing a genome into a recipient cell by allowing us to use established methods for insertion of large DNA molecules into eukaryotic cells.

We elected to develop our genome transplantation methods using two fast-growing mycoplasma species, *Mycoplasma mycoides* subspecies *mycoides*, Large Colony strain GM12, and *Mycoplasma capricolum* subspecies *capricolum*, strain California kid, as donor and recipient cells, respectively. They divide every 80 and 100 min, respectively. These organisms are both opportunistic pathogens of goats, but can be grown in the laboratory under Biosafety Level 2 conditions. In preparation for our experiments, it was necessary to sequence both genomes and compare them to determine the degree of relatedness. We found that 76.4% of the 1,083,241-bp draft sequence of the *M. mycoides* LC genome (*14*) could be mapped to the 1,010,023-bp *M. capricolum* genome (*15*), and this content matched on average at 91.5% nucleotide identity. The remaining ~24% of the *M. mycoides* LC genome contains a large number of insertion sequences not found in *M. capricolum*.

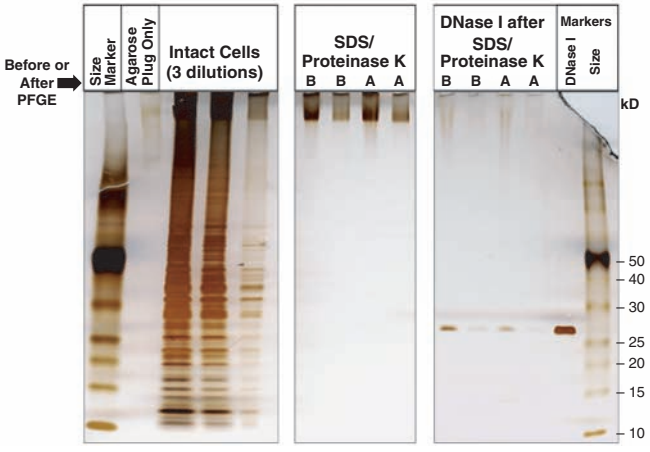


Fig. 2. SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of isolated *M. mycoides* LC DNA in agarose blocks shows that there were no detectable proteins associated with the DNA. The gels were silver-stained. (**Left**) The three lanes labeled "Intact cells" were three dilutions of *M. mycoides* LC cells that were boiled in SDS and loaded onto the gel. (**Middle**) Agarose blocks with the *M. mycoides* LC DNA that were boiled in SDS and loaded on the protein gel either before (B) or after (A) PFGE. (**Right**) To determine whether the material at the top of the gel was protein or DNA, we treated the blocks, before and after PFGE, with DNase I. One of the markers was DNase I.

Table 1. Results of a series of transplantation experiments.

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.

At the outset, we explored a number of methods for genome transplantation. The process had three key phases: isolation of intact donor genomes from *M. mycoides* LC, preparation of recipient *M. capricolum* cells, and installation of the isolated genome into the recipient cells. We chose our donor and recipient cells for genome transplantation on the basis of our observation that plasmids containing a *M. mycoides* LC origin of replication complex (ORC) can be established in *M. capricolum*, whereas plasmids with an *M. capricolum* ORC cannot be established in *M. mycoides* LC (*16*).

Donor Genomic DNA Preparation

Manipulation of whole chromosomes in solution exposes the DNA to shear forces that can cause breakage. Thus, it was important to minimize genome manipulation during the detergent and proteolytic enzyme treatments by suspending the cells in agarose blocks. Intact chromosomes were immobilized in the resulting cavern in the agarose that originally held the cell. Digested protein components, lipids, RNAs, and sheared genomic DNAs could then be removed by dialysis or electrophoresis from the immobilized intact genomic DNA.

Whole, intact genomic DNA isolation was performed using a CHEF Mammalian Genomic DNA Plug Kit from Bio-Rad. Briefly, we grew *M. mycoides* LC cells containing tetracycline-resistance (*tetM*) and β-galactosidase genes (*lacZ*) (*17*) at 37°C to moderate density in SP4 medium (*18*), supplemented with 10 μg/ml of tetracycline and, in some experiments, 10 μg/ml of streptomycin. Fifty to 100 ml of cultured cells was reduced to a pellet by centrifugation at 4575*g* for 15 min at 10°C. We resuspended cells in 20 ml of 10 mM Tris (pH 6.5) plus 0.5 M sucrose; spun as before; and resuspended again in 1 ml (~1 to 5 × 10^9 cells/ml). We incubated the cell suspension for 15 min at 50°C, then mixed it with an equal volume of 2% low-melting-point (LMP) agarose in 1× TAE buffer [40 mM Tris-acetate and 1 mM EDTA]. After 5 min at 50°C, the mixture of cells and LMP agarose (2 ml) was distributed in 100-μl aliquots into plug molds. The 20 plugs solidified at 4°C. Embedded mycoplasma cells were lysed and proteins were digested at 50°C for 24 hours by addition of 6 ml of proteinase K reaction buffer [100 mM EDTA (pH 8.0), 0.2% sodium deoxycholate, and 1% sodium lauryl sarcosine] with 240 μl of proteinase K (>600 U/ml). The 20 plugs were then washed four times at room temperature for 1 hour in 20 ml of 1× Tris-EDTA buffer [Tris-HCl (20 mM) and EDTA (50 mM), (pH 8.0)] with agitation and stored in 10 ml of Tris-EDTA buffer at 4°C.

We wanted to confirm that our gentle preparation of the genomic DNA yielded intact circular molecules. We subjected some agarose plugs to pulsed-field gel electrophoresis (PFGE) in a 1% LMP gel in TAE, with contour-clamped homogeneous electric field (*19*) (CHEF DR III, Bio-

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Rad). Pulse times were ramped from 60 to 120 s over 24 hours at 3.5 V/cm. After migration, plugs were removed from the wells and stored in 10 ml of Tris-EDTA buffer (as described above) at 4°C until used as source of intact genomic DNA for chromosome transplantation experiments. During PFGE, intact circular bacterial chromosomes become caught in the agarose and do not migrate, whereas full-length linearized DNA, as well as smaller DNA fragments, RNAs, proteins, and any other charged cellular molecules remaining after the detergent and enzyme digestion were removed from the plug by electrophoresis (*20*). A SYBR gold (Molecular Probes)–stained pulsed-field gel (Fig. 1A) showed a band of DNA that had the same electrophoretic mobility as a 1.125-Mb linear DNA size marker (about the same size as the *M. mycoides* LC genome), plus an intense band at the position of the wells, which suggested that a large amount of DNA was still in the plugs. Extensive digestion of the plug and the excised ~1.125-Mb band with Plasmid-Safe adenosine triphosphate (ATP)–dependent deoxyribonuclease (DNase) (Epicentre Biotechnologies) clearly degraded the excised ~1.125-Mb band (Fig. 1B). Plasmid-Safe ATP-dependent DNase digests linear double-stranded DNA to deoxynucleotides and, with lower efficiency, closed-circular and linear single-stranded DNA. The enzyme has no activity on nicked or closed-circular double-stranded DNA or supercoiled DNA. This is compatible with the presence of a large amount of circular genomic DNA in the plug. As we became more experienced with genome isolation, the amount of apparently linearized DNA in our preparations diminished.

We analyzed the plugs to confirm that the DNA encased in them was naked. Plugs loaded on SDS polyacrylamide gels after boiling in SDS showed no detectable protein by silver staining, which indicated that the majority of the DNA was naked (Fig. 2). In order to make sure that the DNA was completely deproteinated during the genome transplantation, agarose plugs treated with detergent and proteinase K were subjected to liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) on an ion-trap mass spectrometer (*21*). Five *M. mycoides* peptides, each for a different protein and from a separate plug, were identified (table S1). Because LC-MS/MS analysis is very sensitive and provides excellent sequence coverage, the peptide quantities are extremely small. Only one peptide per protein was detected, which makes it highly unlikely that any undigested proteins were present in these agarose plug samples. In addition, we detected no *M. mycoides* LC peptides in plugs not exposed to PFGE. There was also a background in the samples run on PFGE of many peptides not encoded by *M. mycoides* LC, such as keratin peptides. All of these peptides, including the five encoded by *M. mycoides* LC, could be contaminants introduced during the PFGE.

The final step in donor genome preparation entailed liberation of the DNA from agarose encasement. Before transplantation experiments, the agarose plugs containing *M. mycoides* LC genomic DNA (before or after PFGE) were washed 2 times 30 min in 1 ml of 0.1× Tris-EDTA buffer [Tris-HCl (2 mM) and EDTA (5 mM) (pH 8.0)] with gentle agitation. The buffer was completely removed, and the agarose plugs were melted at 65°C with 1/10th volume of 10× β-agarase buffer [10 mM bis Tris-HCl (pH 6.5) and 1 mM EDTA] for 10 min. The molten agarose was cooled for 10 min to 42°C and incubated overnight at the same temperature with 2.5 units of β-agarase I (New England Biolabs) per 100 μl of plug. We calculated each plug contained ~10 μg of DNA (~8×10^9 genomes).

Recipient Cell Preparation and Genome Transplantation Reaction Conditions

We prepared the *M. capricolum* recipient cells in a 6-ml culture of SOB medium (*22*) containing 17% fetal bovine serum and 0.5% glucose. Incubation was at 37°C until the medium pH was 6.2. Cells (5 to 50 × 10^7 cells/ml) were then spun in a centrifuge at 4575*g* for 15 min at 10°C. As pH decreased from 7.4 to 6.2, regular ovoid *M. capricolum* cells changed shapes dramatical-

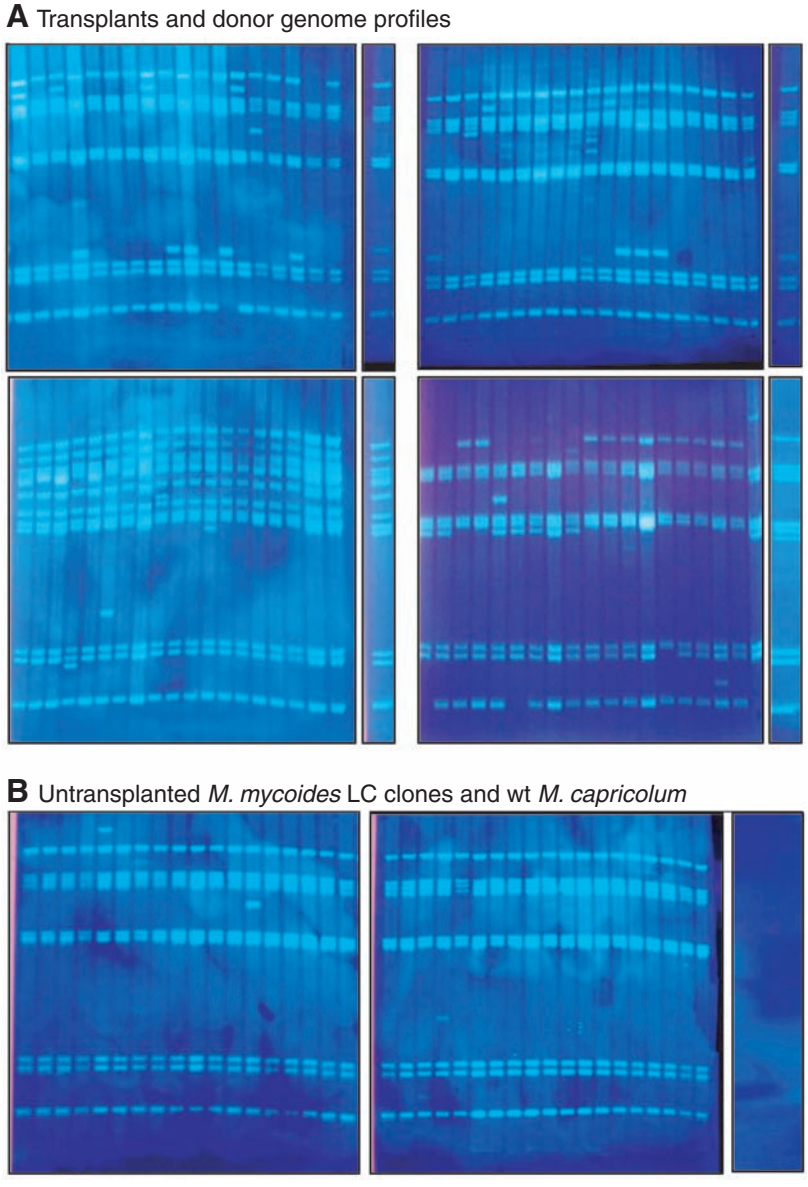


Fig. 3. Southern blots of (**A**) 75 transplants and (**B**) 37 different *M. mycoides* LC filter clones. The blots were probed with a PCR amplicon that hybridized to the IS1296 insertion sequences. Although different samples all had multiple copies of the IS1296, they had slightly different patterns on the blots, which indicated movement of the element. For the transplants (A), the donor cell genomes are shown in the single lanes. As a control (B), Southern blots of recipient cells (wild-type *M. capricolum*) are shown in the single lane. The IS196 probe from *M. mycoides* LC genomic DNA was amplified by PCR using primers IS1296P1F (AAGCGTTTAGAATAGAAGGGCTA) and IS1296P1R (CTGAATTGTACAGGAGACAATCC).

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ly. Cells became longer, thinner, and branched. In poor medium, inhibition of DNA replication due to nucleotide starvation is known to induce branching in *M. capricolum* cells (*23*, *24*). Cells were washed once [Tris 10 mM and NaCl 250 mM (pH 6.5)], resuspended with 200 µl of $CaCl_2$ (0.1 M), and held on ice for 30 min. During that period, 20 µl of β-agarase–treated plugs (~50 ng/µl) were delicately transferred into 400 µl of SP4 medium without serum [SP4 (–)], with wide-bore genomic pipette tips, and incubated 30 min at room temperature. For the genome transplantation, *M. capricolum* cells mixed with 10 µg of yeast transfer RNA (Invitrogen) were gently transferred into the 400 µl of SP4 (–) containing 20 µl of *M. mycoides* LC whole-genomic DNA. An equal volume of 2× fusion buffer [Tris 20 mM, NaCl 500 mM, $MgCl_2$ 20 mM, polyethylene glycol 8000 (PEG; USB Corporation no. 19959) 10%] was added, and the contents were mixed by rocking the tube gently for 1 min. After 50 min at 37°C, 10 ml of SP4 was added, and the cells were incubated for 3 hours at 37°C to allow recovery. Finally, cells were spun at 4575*g* for 15 min at 10°C, resuspended in 0.7 ml of SP4, and plated on SP4 agar plates containing 3 µg/ml tetracycline and 150 µg/ml X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside).

The plates were incubated at 37°C until large blue colonies, putatively *M. mycoides* LC, formed after ~3 days. Sometimes, after ~10 days smaller *M. capricolum* colonies, both blue and white, were visible. Thus, all of these colonies were tetracycline-resistant, as evidenced by their surviving the antibiotic selection, and only some expressed β-galactosidase. These colonies might be the result of recombination. We observed that these colonies appeared after almost twice as many days as it took for the transplants to become visible (*25*). Individual colonies were picked and grown in broth medium containing 5 µg/ml of tetracycline. During propagation, the tetracycline concentration was progressively increased to 10 µg/ml. When we first developed this technique, we subjected all plugs to PFGE. Later, we found this step was unnecessary. We observed no significant difference in transplantation yield as a result of PFGE of the plugs.

Every experiment included two negative controls. To ensure that the *M. mycoides* genomic DNA contained no viable cells, one control was processed exactly as described above except no *M. capricolum* recipient cells were used. Similarly, in another control, *M. capricolum* recipient cells were mock-transplanted without any donor DNA. The results of a series of experiments are shown in Table 1. No colonies were ever observed in controls lacking recipient cells; thus, the donor DNA was free of any viable contaminating *M. mycoides* LC cells. When donor DNA and recipient cells were both present, from 1 to >100 putative transplants were obtained in individual experiments. As we became more experienced with this technique, the yield of transplant colonies increased.

Analysis of Putative Transplants

The blue, tetracycline-resistant colonies resulting from *M. mycoides* LC genome transplantation were to be expected if the genome was successfully transplanted. However, colonies with that phenotype could also result from recombination of a fragment of *M. mycoides* LC genomic DNA containing the *tetM* and *lacZ* genes into the *M. capricolum* genome. To rule out recombination, we examined the phenotype and genotype of the transplanted clones.

Genotype analysis. We analyzed several transplant clones after synthesis with the polymerase chain reaction (PCR) using primers specific for each species to determine whether the putative transplants had *M. mycoides* LC sequences other than the selected *tetM* and *lacZ* marker genes. We used PCR primers specific for IS1296 insertion sequences, which are present in 11 copies in the sequenced *M. mycoides* LC genome, but are absent in the *M. capricolum* genome. Similarly, we used PCR primers specific for the *M. capricolum* arginine deiminase gene,

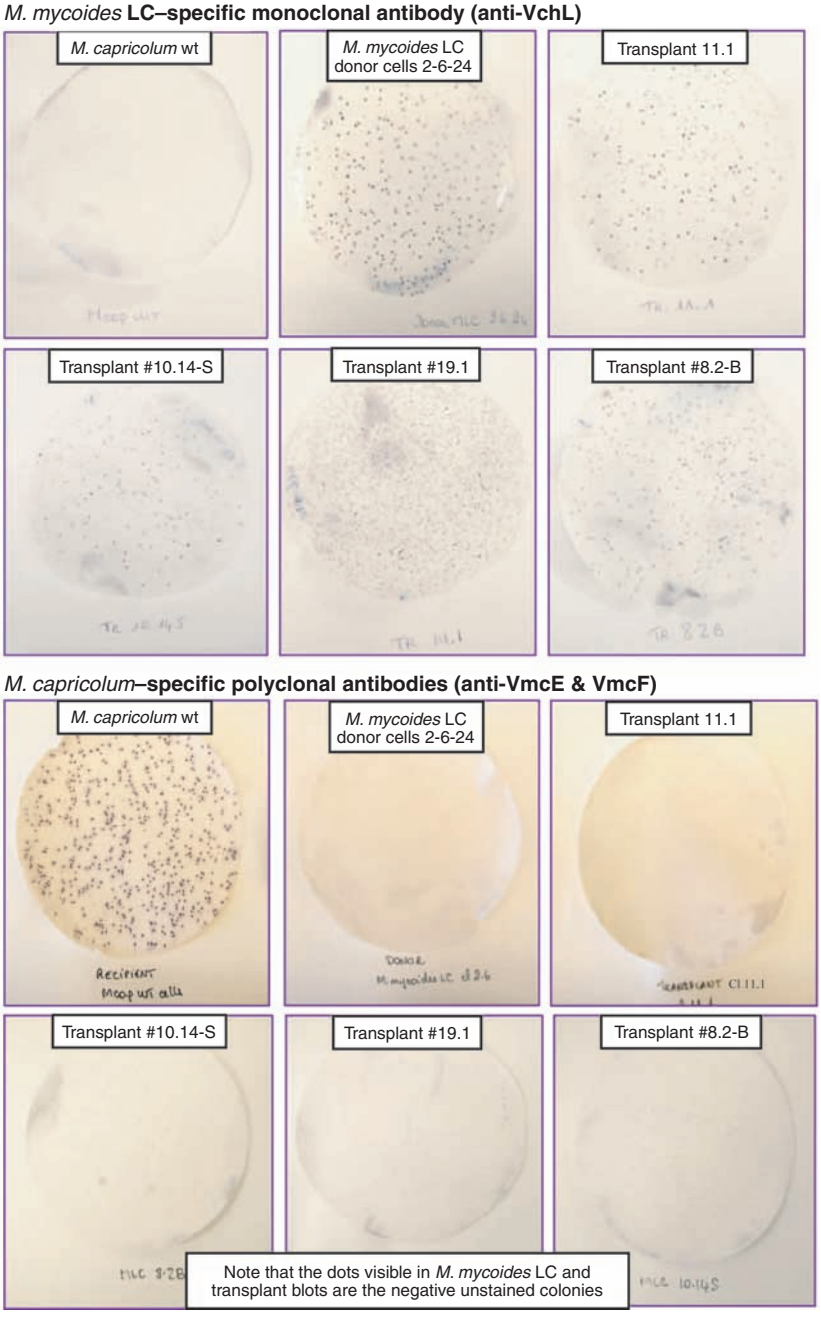


Fig. 4. Colony hybridization of the *M. mycoides* LC (genome donor), *M. capricolum* (recipient cell), and transplants from four different experiments that were probed with a polyclonal antibody specific for the *M. capricolum* VmcE and VmcF surface antigens or with monoclonal antibodies specific for the *M. mycoides* LC VchL surface antigen (*29*).

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which is not present in *M. mycoides* LC. The IS1296 PCR produced an amplicon only when the template was the *M. mycoides* wild-type strain or was one of the transplanted clones. Similarly, the *M. capricolum* arginine deiminase PCR generated an amplicon with the *M. capricolum* template DNA, but not with the *M. mycoides* LC wild-type DNA or DNAs from transplant clones. The PCR experiments left open the possibility that fragments of the *M. mycoides* LC genome containing an IS1296, the *tetM* gene, and the *lacZ* gene had recombined into the *M. capricolum* genome in such a way that they destroyed the arginine deiminase gene (fig. S1). A more convincing genotypic analysis that looked at the overall genome used Southern blot analysis of the donor and recipient mycoplasmas and a series of putative transplants. Genomic DNA from each of those species was digested with the restriction enzyme Hind III and run on a 1% agarose gel. Southern blots were prepared and probed with IS1296 sequences. As expected, no probe hybridized to the wild-type *M. capricolum* lane (Fig. 3A). We did this analysis on every transplant we obtained, as well as a series of *M. mycoides* LC clones (Fig. 3B). Analysis of Southern blots of 37 wild-type *M. mycoides* LC clones and 75 putative transplants showed that 34 (92%) and 44 (59%), respectively, were essentially identical to the *M. mycoides* LC donor DNA blot; the rest showed variations in the banding patterns. We assume that variation was the result of IS element transposition. We hypothesize that mobility of the IS1296 element may be somewhat suppressed in *M. mycoides* LC cells. However, there may be no suppression of transposon mobility immediately following introduction of the donor genome into the *M. capricolum* cytoplasm. This is evidence of a transitional period when the *M. mycoides* LC donor genomes reside in a cellular milieu whose *M capricolum* content is initially high, but diminishes with each cell division. Next, we did sample sequencing of whole-genome libraries generated from two transplant clones. Our analysis of more than 1300 random sequence reads from the genome of each clone (totaling ~1.09 million bases for each clone) showed that all reads matched *M. mycoides* LC sequence (*26*). We cannot rule out the possibility that small regions of the donor genomes recombined with identical regions of *M. capricolum* recipient cell genome; however, those regions would be very small. There are 20 identical regions of between 395 and 972 base pairs. The above results were all consistent with the hypothesis that we have successfully introduced *M. mycoides* LC genomes into *M. capricolum* followed by subsequent loss of the capricolum genome during antibiotic selection.

Phenotype analysis. We examined the phenotype of the transplanted clones in two ways. In one, we looked at single-gene products characteristic of each of these two mycoplasmas. Using colony-Western blots, we probed donor and recipient cell colonies and colonies from four different transplants with murine antibodies specific for the *M. capricolum* VmcE and VmcF surface antigens and with murine antibodies specific for the *M. mycoides* LC VchL surface antigen. In both assays, *M. mycoides* LC VchL–specific antibodies bound the transplant blots with the same intensity as it bound the *M. mycoides* LC blots (Fig. 4). Similarly, the antibodies specific for the *M. capricolum* VmcE and

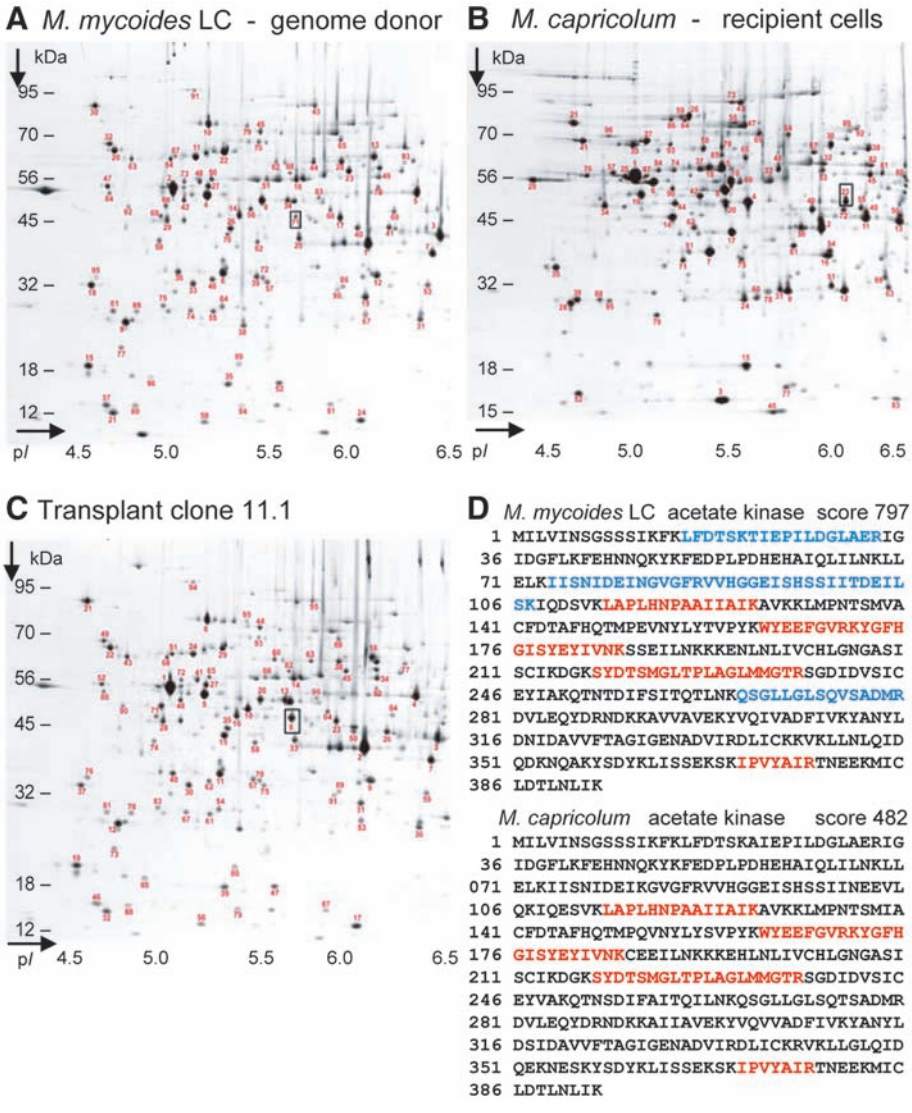


Fig. 5. Proteomic analysis. Two-dimensional gels were run using cell lysates from (**A**) *M. mycoides* LC, (**B**) *M. capricolum*, and (**C**) a transplant clone (11.1). Standard conditions were used for the separation of protein spots in the first dimension on immobilized pH gradient (IPG) strips (pH range 4 to 7) and in the second, SDS-PAGE, dimension (molecular mass 8 to 200 kD) (*30*). The gels were stained with Coomassie brilliant blue G-250, and 96 spots were excised from each of the gels. Spots 71 (A), 23 (B), and 8 (C) were identified as acetate kinase. (B) *M. capricolum* acetate kinase showed a clear alkaline pH shift. The sequence coverage map for trypsin-digested peptides obtained from MALDI-MS peptide mass fingerprint (PMF) data localizes peptide sequences of acetate kinase [spot 8 (C)] matching mass/charge ratio (*m/z*) values in the PMF. Peptide sequences in red were identical to the two *Mycoplasma* species; peptide sequences in blue were unique to *M. mycoides* LC.

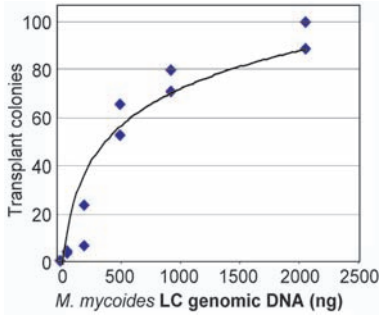


Fig. 6. Genome transplantation as a function of the amount of *M. mycoides* LC genomic DNA transplanted. Transplant colonies were observed on two different plates. We observed no colonies on either the no-recipient-cell control or the mock-transplanted control plates.

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VmcF did not bind the to the transplant blots. In the second, proteomic analysis, cell lysates of all three strains were examined by using differential display in two-dimensional electrophoresis (2-DE) gels, followed by identification of proteins spots with matrix-assisted laser desorption ionization (MALDI) mass spectrometry. The 2-DE spot patterns of the *M. mycoides* LC and the transplanted clone were identical within the limits of 2-DE; however, the *M. capricolum* 2-DE spot patterns were very different. More than 50% of the respective spots could not be matched among the gels (Fig. 5, A to C). More evidence was gained from MALDI-MS data that the transplant proteome was identical to the *M. mycoides* LC proteome and did not have any *M. capricolum* features. For nearly 90 identified spots of the transplant, confidence scores obtained with the Mascot algorithm were invariably equal or higher for *M. mycoides* LC than for *M. capricolum* proteins, despite high sequence homologies; although there were nine protein spots with confidence scores that indicated they were derived from *M. capricolum* genes, each case proved to be an artifact of either sequencing errors or gene boundary annotation errors (table S2). As an example, Fig. 5D visualizes peptides in acetate kinase matching only the sequence of the respective *M. mycoides* LC protein. Thus, the phenotypic assays affirmed that the transplants were likely *M. mycoides* LC and were not the result of a *M. capricolum–M. mycoides* LC mosaic produced by recombination between the donor and recipient cell genomes after the transplantation of the *M. mycoides* LC genome and before the two genomes segregate during cell division.

Optimization of Genome Transplantation Efficiency

To determine what factors govern genome transplantation efficiency, we varied the number of *M. capricolum* recipient cells and the amount of *M. mycoides* LC genomic DNA used in transplantation experiments. Transplant yield was optimal when 10^7 to 5×10^7 cells were used. At lower donor DNA concentrations, there was a linear relation between the amounts of genomic DNA transplanted and transplant yield. Yields began to plateau at higher donor DNA concentrations (Fig. 6).

Concluding Remarks

These data demonstrate the transplantation of whole genomes from one species to another such that the resulting progeny are the same species as the donor genome. However, they do not explain the mechanism of the transplant. This is not natural DNA transformation, where linear DNA enters the cytoplasm and recombines into the resident chromosome. Our genome transplantation does not entail recombination, and our donor molecule is circular. In addition, our recipient mycoplasma cells have not been shown to be competent for natural transformation, nor are any DNA uptake genes identified in the *M. capricolum* genome. We presume that organisms carrying both donor and recipient cell genomes occurred at least transiently at early times after transplantation. Only 1 recipient cell in ~150,000 was transplanted in our most efficient experiments. This low efficiency has so far prevented a demonstration of transient mosaicism. Although our donor and recipient are distinct species, they are phylogenetically close relatives. Genome transplantation works for the species we have chosen, but we do not know for what other species it will work.

Because mycoplasmas are similar to mammalian cells with respect to their lack of a cell wall, we experimented with a series of approaches that are effective for transferring large DNA molecules into eukaryotic cells. These included cation- and detergent-mediated transfection, electroporation, and compaction of the donor genomes using various cationic agents. None of those approaches proved effective for whole-genome transplantation (see SOM). Our PEG-based method may be akin to PEG-driven cell fusion methods developed for eukaryotic cells. To test this hypothesis, two parental strains of *M. capricolum*, one carrying a *tetM* marker in the chromosome and the other one with the chloramphenicol-resistance marker (CAT) in a stable ORC plasmid, were both prepared as "recipient" cells, mixed, and incubated in the presence of the fusion buffer as described above for transplantation experiments. We plated cells on SP4 agar containing both tetracycline (3 μg/ml) and chloramphenicol (50 μg/ml). In the presence of 5% PEG, we obtained progeny resistant to both antibiotics. No colonies grew in the absence of 5% PEG. The number of colonies increased ~30 times when we pretreated cells with $CaCl_2$. Sequencing analysis of 30 clones showed that all had both the *tetM* and CAT markers in the cells at the expected chromosomal and plasmid locations. Thus, we concluded that with our PEG-based method, *M. capricolum* cells fuse. Those results agree with membrane studies by Rottem and colleagues demonstrating that fusion of *M. capricolum* cells is maximal in 5% PEG (*27*). Gene transfer into *Mycoplasma pulmonis* was also mediated by PEG at concentrations likely to fuse cells, albeit only small DNA segments are transferred (*28*). We can imagine that, in some instances, the cells may fuse around the naked *M. mycoides* LC genomes. Those genomes, now encapsulated in *M. capricolum* cytoplasm, express the *tetM* protein, which allows the large fused cells to grow and divide once plated on the SP4 agar containing tetracycline. Cells lacking the *M. mycoides* genome do not grow. Eventually, now, in the absence of PEG and through a process of cell division and chromosome segregation, normal, albeit tetracycline-resistant, β-galactosidase–producing *M. mycoides* cells produce large blue colonies on the plate. This basic approach of PEG-mediated genome transplantation may allow other species to be transplanted with naked genomes containing antibiotic-resistance genes.

Some bacterial cells have multiple large chromosomes. This suggests the existence of natural mechanisms for chromosome transfer between species. However, we have no evidence that genome transplantation as described here occurs in nature. We observed that in the absence of treatment with detergent and proteinase K, nucleoids from *M. mycoides* LC cells would not produce transplants. Given the improbability of the natural occurrence of free-floating bacterial genomes that are both deproteinized and intact, genome transplantation could be a phenomenon unique to the laboratory. Still, we have discovered a form of bacterial DNA transfer that permits recipient cells to be platforms for the production of new species with the use of modified natural genomes or manmade genomes generated by the methods being developed by synthetic biologists.

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25. To minimize the risk of contaminating our transplant cultures with *M. mycoides* LC cells from our donor genome preparation process, we used three different

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hoods for our cell culture work: one for *M. mycoides* LC donor cell preparation, one for *M. capricolum*, and one for working with transplant clones.
26. There was no sequence that was unique to *M. capricolum*. Of the 24 reads that did not match the *M. mycoides* LC or *M. capricolum* genome sequences, most were either very short reads (<200 bases) or the result of chimeric clones, which is to be expected owing to the active transposons in *M. mycoides* LC and also as part of library construction. The data for the two transplant clones that were sequenced are posted at the National Center for Biotechnology Information, NIH, NCBI Trace File Archives (accession numbers 1807995910 through 1807998555).
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REPORTS

Quantum Hall Effect in a Gate-Controlled *p-n* Junction of Graphene

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The unique band structure of graphene allows reconfigurable electric-field control of carrier type and density, making graphene an ideal candidate for bipolar nanoelectronics. We report the realization of a single-layer graphene *p-n* junction in which carrier type and density in two adjacent regions are locally controlled by electrostatic gating. Transport measurements in the quantum Hall regime reveal new plateaus of two-terminal conductance across the junction at 1 and 3/2 times the quantum of conductance, e^2/h, consistent with recent theory. Beyond enabling investigations in condensed-matter physics, the demonstrated local-gating technique sets the foundation for a future graphene-based bipolar technology.

Graphene, a single-layer hexagonal lattice of carbon atoms, has recently emerged as a fascinating system for fundamental studies in condensed-matter physics (*1*), as well as a candidate for novel sensors (*2*, *3*) and postsilicon electronics (*4*–*10*). The unusual band structure of single-layer graphene makes it a zero-gap semiconductor with a linear (photon-like) energy-momentum relation near the points where valence and conduction bands meet. Carrier type—electron-like or holelike—and density can be controlled by using the electric-field effect (*10*), obviating conventional semiconductor doping, for instance via ion implantation. This feature, doping via local gates, would allow graphene-based bipolar technology devices comprising junctions between holelike and electron-like regions, or *p-n* junctions, to be reconfigurable using only gate voltages to distinguish *p* (hole-like) and *n* (electron-like) regions within a single sheet. Although global control of carrier type and density in graphene using a single back gate has been investigated by several groups (*11*–*13*), local control (*8*, *9*) of single-layer graphene has remained an important technological milestone. In addition, *p-n* junctions are of great interest for low-dimensional condensed-matter physics. For instance, recent theory predicts that a local step in potential would allow solid-state realizations of relativistic (Klein) tunneling (*14*, *15*) and a surprising scattering effect known as Veselago lensing (*16*), comparable to scattering of electromagnetic waves in negative-index materials (*17*).

We report the realization of local top gating in a single-layer graphene device that, combined with global back gating, allows individual control of carrier type and density in adjacent regions of a single atomic layer. Transport measurements at zero perpendicular magnetic field B and in the quantum Hall (QH) regime demonstrate that the functionalized aluminum oxide (Al_2O_3) separating the graphene from the top gate does not significantly dope the layer nor affect its low-frequency transport properties. We studied the QH signature of the graphene *p-n* junction and found new conductance plateaus at 1 and $3/2\,e^2/h$, consistent with recent theory addressing equilibration of edge states at the *p-n* interface (*18*).

Graphene sheets were prepared via mechanical exfoliation using a method (*19*) similar to that used in (*10*). Graphite flakes were deposited on 300 nm of SiO_2 on a degenerately doped Si substrate. Inspection with an optical microscope allowed potential single-layer regions of graphene to be identified by a characteristic coloration that arises from thin-film interference (Fig. 1A). These micrometer-scale regions were contacted with thermally evaporated Ti/Au (5/40 nm) that was patterned using electron-beam lithography. Next, a ~30-nm layer of oxide was deposited atop the entire substrate. As illustrated (Fig. 1B), the oxide consisted of two parts, a nonconvalent functionalization layer (NCFL) and Al_2O_3. This deposition technique (*19*) was based on a recipe successfully applied to carbon nanotubes (*20*). The NCFL serves two purposes. One is to create a noninteracting layer between the graphene and the Al_2O_3, and the other is to obtain a layer that is catalytically suitable for the formation of Al_2O_3 by atomic layer deposition (ALD). The NCFL was synthesized by 50 pulsed cycles of NO_2 and trimethylaluminum (TMA) at room temperature inside an ALD reactor. Next, five cycles of H_2O-TMA were applied at room temperature to prevent desorption of the NCFL. Lastly, Al_2O_3 was grown at 225°C with 300 H_2O-TMA ALD cycles. To complete the device, a second step of electron-beam lithography defined a local top gate (5/40 nm Ti/Au) covering a region of the device that includes one of the metallic contacts.

A completed device, similar in design to that shown in the optical image in Fig. 1A, was cooled in a ^{3}He3 refrigerator and characterized at temperatures T of 250 mK and 4.2 K. Differential resistance, $R = dV/dI$, where I is the current and V the source-drain voltage, was measured by standard lock-in techniques with a current bias

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