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SYNTHETIC BIOLOGY

Avoiding the chop

Over the past few years researchers have made substantial progress in the quest to create synthetic microorganisms, for which a necessary step is the transplantation of a synthetic genome into a bacterial cell. However, until now, this has proved to be an elusive goal. Therefore, the recent transplantation and modification of a bacterial genome in yeast and the transfer of this genome to a different bacterial strain to produce viable cells is an important advance for the synthetic biology field.

J. Craig Venter and colleagues previously demonstrated that the genome of one bacterium could be replaced with that of another and reasoned that the same would be true for a synthetic genome. However, when they chemically synthesized a bacterial genome, assembled it in a yeast system and tried to transplant the synthetic genome into a different recipient strain, they were unable to recover any transformed cells.

The authors took a new route to work out what the roadblock was. First, they transformed bacteria (*Mycoplasma mycoides*) with a vector containing sequences that would allow the *M. mycoides* genome to propagate in yeast as an artificial chromosome. They then transferred this *M. mycoides* genome to yeast and found that it could be stably maintained. By inserting a cassette with yeast selection markers into the bacterial genome, they deleted a non-essential restriction endonuclease from the *M. mycoides* genome — demonstrating that yeast tools can be used to modify bacterial genomes outside their native cellular environment.

However, when the authors tried to transplant *M. mycoides* genomes from yeast to recipient *Mycoplasma capricolum* cells, once again they failed to recover any transplanted cells. They guessed that the unmethylated transplanted *M. mycoides* genomes were being digested by restriction enzymes in the recipient strain — an important bacterial defence against foreign DNA. To overcome this defence, the authors tried two strategies: first, they knocked out the only restriction enzyme in *M. capricolum* cells; and second, they protected the donor *M. mycoides* DNA by *in vitro* methylation using *M. capricolum* or *M. mycoides* cellular extracts or purified methylases. Both of these strategies worked, and implementation of either allowed *M. mycoides* genomes that had been modified in yeast to be successfully transplanted into *M. capricolum* cells.

As there are few selection markers that can be used in mycoplasma systems and these bacteria have low recombination activity, the genetic manipulation of *M. mycoides* is limited. However, the maintenance of bacterial genomes in yeast described in this study will allow the manipulation of bacterial genomes through the vast array of genetic tools that can be implemented in yeast. This opens up the possibilities of creating complex genome modifications, such as multiple deletions, insertions and rearrangements, and producing novel bacterial genomes with altered traits. The next challenge will be to apply this strategy to bacterial species other than mycoplasmas, as many microorganisms of clinical or industrial importance are difficult to genetically manipulate.

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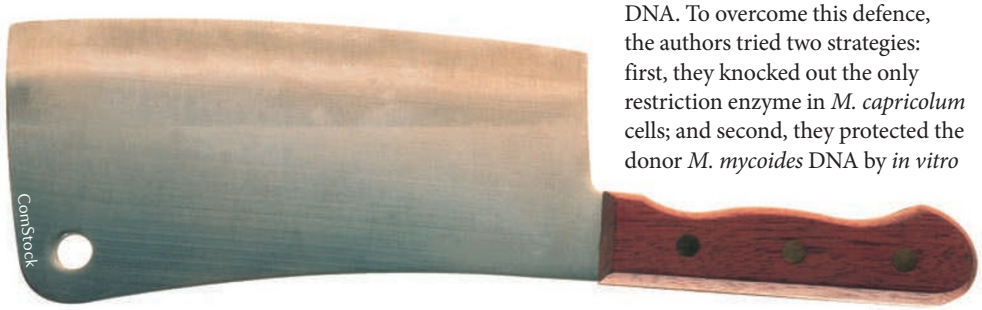
> “…the maintenance of bacterial genomes in yeast described in this study will allow the manipulation of bacterial genomes…”



ORIGINAL RESEARCH PAPER Lartigue, C. *et al.* Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* 20 Aug 2009 (doi:10.1126/science.1173759)
FURTHER READING Benner, S. A. & Sismour, A. M. Synthetic biology. *Nature Rev. Genet.* **6**, 533–543 (2005)