Sharpening the CRISPR-Cas scissors

CRISPR-Cas lets scientists make precise changes to DNA. The combination of protein and RNA looks for the specified piece of DNA and makes a cut. Different DNA can then be inserted in that position to repair the cut. PhD candidate Thomas Swartjes came up with an elegant method for improving the snipper proteins (nucleases).

CRISPR-Cas is a bacterial defence system. But what works in one place in the DNA does not necessarily do so in another place. Some applications require customized scissors. Swartjes thought up a way to use evolution to let nature further develop the existing nucleases (the scissors). He uses bacterial sex as the instrument in that evolution.

Bacteria don't actually have sex, but geneticists compare the process of conjugation to sex. 'Conjugation is when bacteria stick to one another and transfer genetic material,' explains Swartjes. 'The bacterium forms protrusions on the exterior that function like a kind of lasso. They capture another bacterium and draw it in, which is followed by the transfer of a plasmid.'

The idea is to use evolution and conjugation to develop snipper proteins that get a bit more effective than their predecessor every time

The plasmid, a loose piece of circular DNA, can then spread through a population of bacteria via conjugation (and replication). Swartjes is fascinated by this process. The title of his thesis is *Horizontal Dancing* for Bacteria. 'Horizontal dancing is a euphemism for sex, and a parallel for the horizontal transfer of genes between neighbouring bacteria. This is in contrast to the vertical transfer of genes through division.

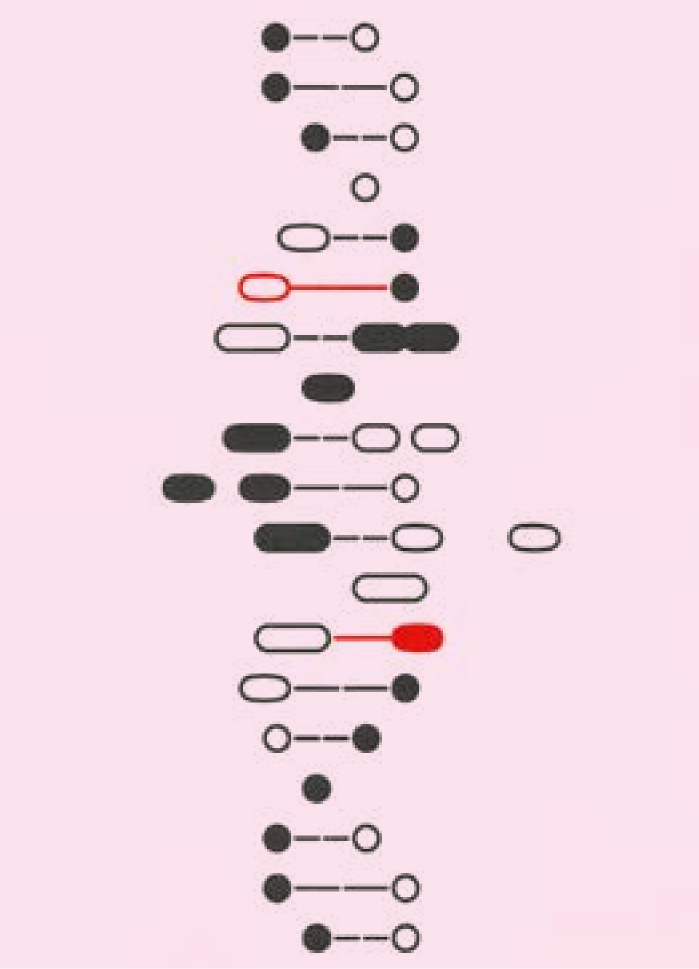
Inhibition

Swartjes' idea is to use evolution and conjugation to develop nucleases that get a bit more effective than their predecessor every time. He uses bacterium cells (*E.coli*) with two plasmids: one with the gene for the snipper protein Cas-9 and one with genes that inhibit conjugation. 'The challenge for the Cas-9 protein is to mutate so that it can snip the inhibitor plasmid. If it manages that, the inhibition preventing conjugation is removed and the Cas-9 gene is transferred.'

The slightly improved Cas-9 then spreads through the bacterial culture through successive conjugations (sex). In each new cell, a new challenge awaits with intact inhibitor plasmid that has to be overcome. Evolution helps make the scissors increasingly more effective, more efficient and faster. And not much lab work is needed either. The idea is that nature does the work.

But there is still some way to go. The principle of conjugation and a plasmid spreading through a population works, says Swartjes. 'But I want a plasmid that only transfers the gene for Cas-9, not all the other genes needed for that process. I tried to place those genes on the bacterium chromosome. But then it suddenly stops working.'

That is the point at which the PhD thesis ends. But not the research. 'As long as it looks promising, we will see if someone can continue with the research,' says Swartjes. 'It is a nice, elegant system, but if there are too many obstacles, we'll stop working on it. But we haven't reached that point yet.' RK



Cover image of the PhD thesis *Horizontal Dancing for Bacteria* by Thomas Swartjes.