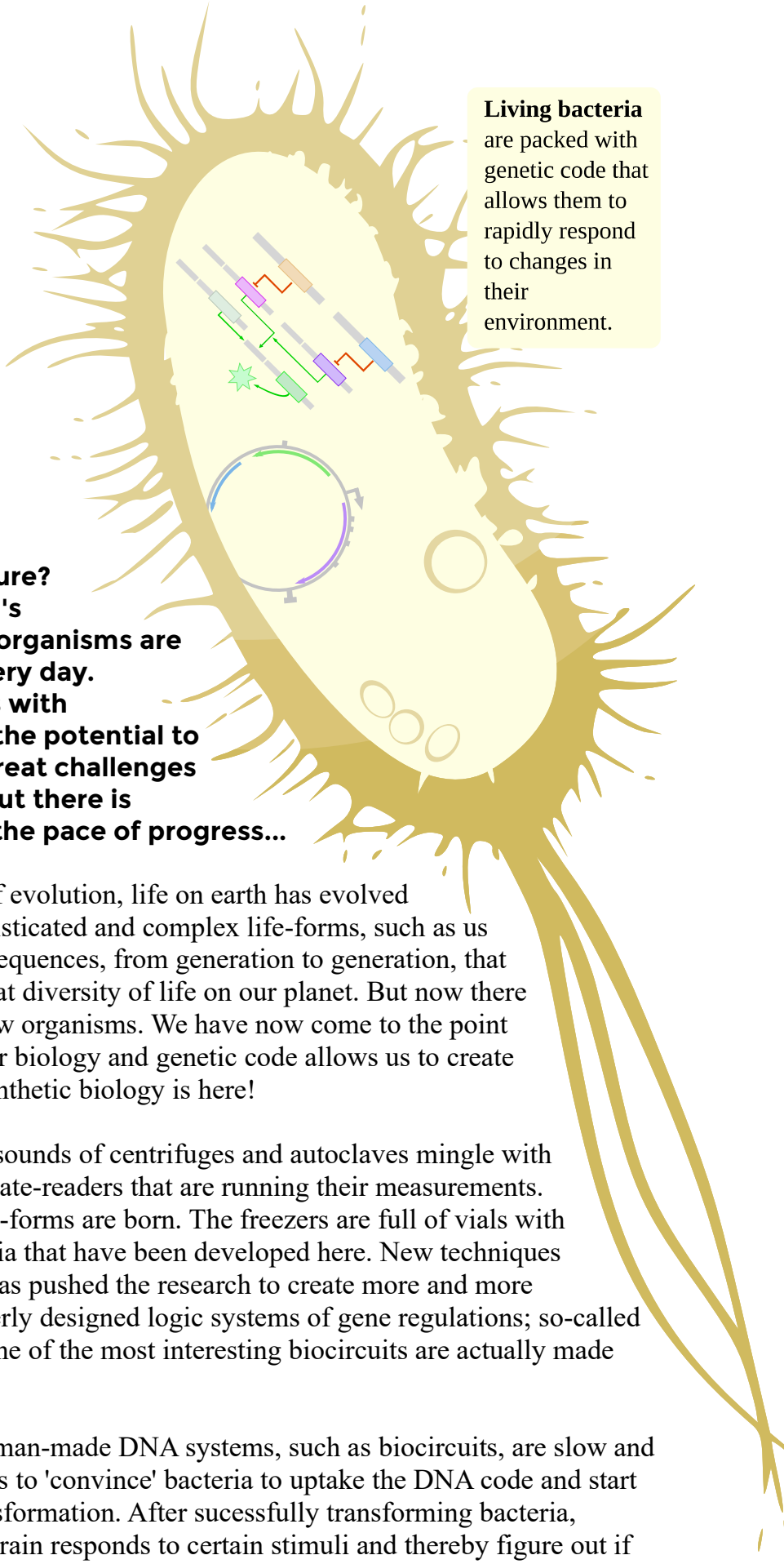
How to create life in a test-tube



Living bacteria are packed with genetic code that allows them to rapidly respond to changes in their environment.

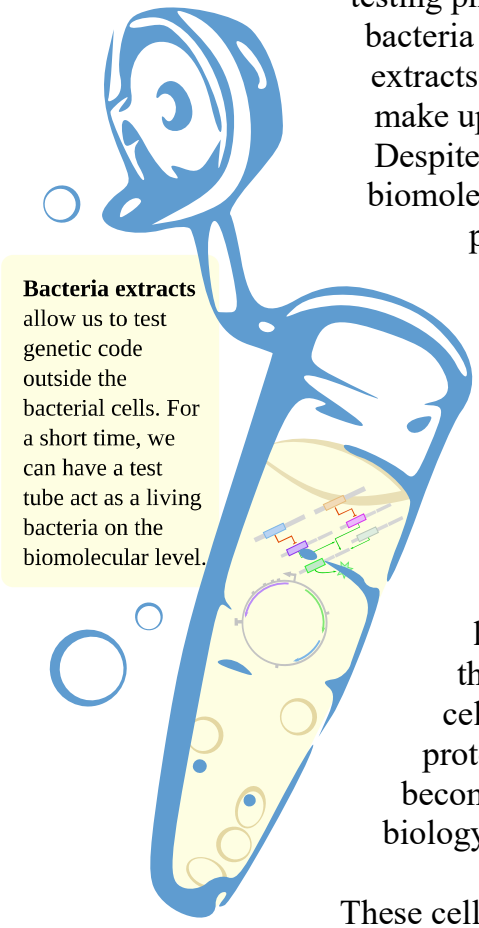
What will life look like in the future? With new technology, man-kind's attempts to genetically modify organisms are excelling further and further every day. Soon we could create life-forms with amazing new capabilities, with the potential to solve (or create?) some of the great challenges that humanity is facing today. But there is something that still holds back the pace of progress...

Ever since the first stumbling steps of evolution, life on earth has evolved from simple single cells to very sophisticated and complex life-forms, such as us humans. It is the tiny shifts in DNA sequences, from generation to generation, that has driven evolution to create the great diversity of life on our planet. But now there is a new driving force for creating new organisms. We have now come to the point where our understanding of molecular biology and genetic code allows us to create and change the very fabric of life. Synthetic biology is here!

In the biocircuits lab on Caltech, the sounds of centrifuges and autoclaves mingle with the occasional buzz coming from a plate-readers that are running their measurements. This is one of the labs where new life-forms are born. The freezers are full of vials with the many genetically modified bacteria that have been developed here. New techniques and lower costs of processing DNA has pushed the research to create more and more complex cells, that now contain cleverly designed logic systems of gene regulations; so-called 'biocircuits'. However, in this lab, some of the most interesting biocircuits are actually made outside the living cells.

The conventional way of testing our man-made DNA systems, such as biocircuits, are slow and very costly. The main strategy used, is to 'convince' bacteria to uptake the DNA code and start using it, a process called genetic transformation. After sucessfully transforming bacteria, scientists can test how the bacterial strain responds to certain stimuli and thereby figure out if

their DNA works as expected. The long time and tedious work needed to test just a simple biocircuit with this approach is really limiting the research progress.



Bacteria extracts allow us to test genetic code outside the bacterial cells. For a short time, we can have a test tube act as a living bacteria on the biomolecular level.

Luckly, there is a way to by-pass the use of living cells in the initial testing phase of a designed biocircuit. This can be done by using bacteria extracts: so-called 'cell-free expression systems'. These extracts are simply a mix of proteins and enzymes which together make up the cell machinery needed to produce proteins from DNA. Despite that there are no living cells in the extracts, the biomolecules remain intact for some time and can do their work, providing 'life' to the test tube for a few hours. Once a biocircuit has been designed, the testing can be done very rapidly by simply mixing the DNA strands with the extract. The rest is just biochemistry. After an hour or so, the output from the reaction can be measured.

In this thesis work, several biocircuits have been built and tested with cell-free extracts. One of the highlights of this prototyping has been the assembly of logic AND-gates, some of which have previously only been tested in living bacteria. The results showed that the biocircuits have the same general function in bacteria as when tested outside cells, supporing the idea of using cell-free extracts for protoyping biocircuit modules. Perhaps this design strategy will become widely adopted in the future, to speed up synthetic biology research?

These cell free extracts could potentially accelerate the development of genetically modified organisms and bio-inspired sensors and assays. However, there is yet a long way to prove the usability of this new design process, if the research community is going to adopt it on a large scale. Meanwhile, more tests will have to be done, with new and more advanced biocircuits to validate the use cell-free extracts for rapid prototyping of biocircuits.

Text and Illustration: Anton Frisk