Körber Stiftung

Körber European Science Prize 1995

Genetic Probes in Environmental Research

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Genetic probes are short sections of genetic material with which scientists can specifically prove the presence of certain genes. They are particularly suitable for identifying microorganisms which were previously impossible or difficult to detect.

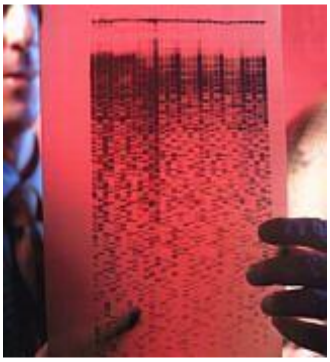
Karl-Heinz Schleifer and Rudolf Amann. In the Körber project they identify and classify microbes and develop a collection of genetic probes.
(Photo: Friedrun Reinhold)

Conceived and distributed in all living organisms by nature three and a half billion years ago, the hereditary molecule, DNA, is a perfect code. It encrypts all structures and functions of the living being. Millions to billions of four different symbols called bases are lined up in each cell, seemingly at random – whether bacteria or blood corpuscles. In abbreviated form, the cipher symbols of this chemical language are A, C, G and T. Their words, i.e. the actual information units, are the genes. Just as the words in our language each consist of different sequences of letters, genes are also made up of a certain sequences of bases – sometimes just a few hundred, but occasionally also several million. In accordance with these base sequences of the genes, the cells produce their proteins. These contribute to the make-up of each cell on the one hand, while directing and dominating their countless metabolic reactions on the other. DNA is a double-stranded molecule, with both strands complementing one other and only two of the four bases forming pairs, namely A and T, and C and G. If the sequence of one strand is, for example, G-T-C-A, that of the other strand must then be C-A-G-T. The complementary strands of DNA can be very easily separated in the laboratory, for example by heating them in boiling water. If the temperature is then reduced, the complementary strands join up again. Many modern molecular analysis methods are based on this phenomenon – including those that work with genetic probes.

Bo Barker Jørgensen and Nils Revsbach.
(Photo: Friedrun Reinhold)

It is genetic probes like these that are the focus of the research project which is to be supported by the Körber Foundation starting in 1995. Genetic probes are short, single-strand DNA pieces comprising just a few thousand bases. Researchers can isolate them from an organism or produce them themselves using state-of-the-art equipment. The base sequence of each genetic probe is usually known to the scientists. Using the genetic probes, for example, certain genes can be identified. These tests work because the genetic probe concerned can possibly pair itself with the unknown, just previously heated and hence single-stranded DNA. The molecules are attracted more strongly to each other, the better a base sequence fits that of the unknown

DNA. For example, a probe with the components A-A-A-A-A attaches itself perfectly to a molecule with the letters T-T-T-T-T, less well to DNA with the base sequence T-T-G-T-C, and not at all to DNA with the sequence G-C-A-A-C. Just how strong the molecular liaison ultimately turns out to be is relatively simple to determine for scientists: Prior to each test, they mark their genetic probe – with either radioactive bases or bases coupled to a dye. The more the pair glows or lights up colorfully, the better the probe and the unknown molecule have congregated.



The nucleic acids deciphered following gel electrophoretic separation form the basis of all life. They are also the target structures for the genetic probes.
(Photo: Friedrun Reinhold)

Genetic probe studies are of particular importance in the world of microorganisms because researchers can use them to identify bacteria which to date were impossible or very difficult to detect. The researchers 'knock up' their genetic probes using the DNA of already known species or DNS which they have isolated directly from the environment. So they know their biochemical tracker dogs extremely well. Then they check whether and how well the probes bond with certain genes of another, unknown bacterium. The microbiologists focus their analyses above all on the rRNA molecules and their genes. There are three reasons for this. Firstly, rRNA molecules occur very frequently in each individual cell, which makes them very easy to detect. Secondly, researchers are able to manufacture probes of varying specificity in this area. And thirdly, it is the rRNA molecules which form the basis for the classification of microbes based on their genetic affinity. In this way, based on the rRNA molecules, bacteria of higher organisms such as animals can be separated, and different types of bacteria can also be differentiated from one another.

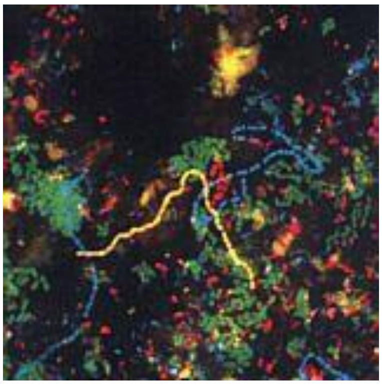
Scientists at the Technische Universität München have designed genetic probes which are complementary to rRNA molecules of a wide range of bacteria groups. In this way, they are able to determine certain microorganisms in a mixed culture rapidly and reliably. If the researchers observe a very strong molecular attraction to rRNA molecules of a particular bacterium, then it is closely related to the nucleus from which the genetic probe concerned originates. If the probe and target RNA form a weak bond, the organisms are only very remotely related. In the meantime, the microbiologists have refined their methods to the point where they permit comparisons in the natural habitats of the organisms – for example directly in the soil, water, or air.

The bacterial life that exists in sewage in purification plants is referred to by biologists as a classic black box. Nothing precise is known about it. Shedding light on this dark area, primarily using genetic probe technology, as well as other high-tech methods, is one of two focal points of the internationally renowned researchers whom the Körber Foundation is honoring with its European Science Prize 1995. The foundations of microbial genetic probe analysis were laid by scientists from the Technische Universität München. Karl-Heinz Schleifer, holder of the Chair for Microbiology there for more than 20 years, has concerned himself with the identification and classification of microbes since the very beginning of his scientific career. The interest of his co-worker Rudolf Amann in genetic probes and their application in microbial ecology and evolution was awakened during a period of research in the

USA. Since then, he and Schleifer have built up one of the world's largest collections of molecular trackers – a collection which both of them want to extend further within the project. Using the probes, they will search specifically for bacteria directly in the sewage – without previously growing them – and initially ascertain the physical distribution of the organisms. It is then possible to draw conclusions on what it is that particular microbes accomplish – or not, as the case may be.

Jiri Wanner
(Photo: Friedrun Reinhold)

Here is an example: Microbiologists have known for more than 20 years that bacteria known as Acinetobacter occur in purification plants. As a result of the conventional detection methods, engineers have since assumed that it is mainly these microbes that remove phosphate. They have therefore always optimized their systems with a view to making things as comfortable as possible for Acinetobacter – in order for it to flourish. Unfortunately, the experts were backing the wrong horse: New studies with genetic probes have revealed that other phosphate-exterminating bacteria populate sewage far more prolifically; it is these that the engineers should really have been taking care of. In the Körber project the scientists hope to uncover similar errors. They will be helped not only by the genetic probes, but also by confocal laser scanning microscopy. This method makes it possible, for example, to isolate a sludge floc from the sewage and to observe the distribution of bacteria in the floc. In combination with genetic probe analysis this enables the researchers to draw conclusions as to which microbes are active in the floc and where.



Evidence of different bacteria in activated sludge by means of fluorescence marked nucleic acid probes. (Photo: TU Munich)

The team in Munich receives further support from Bo Barker Jørgensen and Niels Peter Revsbech. The two scientists are key figures in the development and application of electrochemical and optical micro-probes. These probes measure which chemical reactions take place in the environment of the bacteria and which molecules are involved. Bo Barker Jørgensen has been Director of the Max Planck Institute for Marine Microbiology in Bremen since 1992. He began his career at the University of Aarhus where one of his students, namely Niels Peter Revsbech, introduced the electrochemical probes. Revsbech still works in the Department of Ecology and Genetics at the University of Aarhus. The knowledge gained by means of the electrochemical probes and redox dyes will round off the overall picture of microbe traffic in sewage.

All the results will feed the work of Jiri Wanner. Towards the end of the project, the engineer from the Prague Institute for Chemical Technology plans to build a completely new kind of purification plant – at first on a laboratory scale – which is optimized for bacterial life. Wanner is regarded as an expert on water technologies and is already involved in several international research projects.

Ulf Göbel from the Institute for Microbiology at the Berlin Charité and Erik C. Böttger from the Institute for Medical Microbiology at the Hannover Medical School are working on the project's second focal area:

To discover previously non-traceable bacterial pathogens using the 'gene detective' and to introduce the method to diagnostic practice. Göbel is one of the pioneers when it comes to the development and application of genetic probes in medicine. He was a scholarship holder at the German Research Foundation. The same applies to his colleague Böttger. The two physicians will be tapping the rich resources of the Munich genetic probe gallery, as well as newly developing their own molecular tracker dogs. The researchers expect that they will then be able to swiftly detect infections which were previously difficult to trace, and to do so directly in the tissue itself. In particular, this would be of eminent importance to people whose immune system is weakened and is therefore highly susceptible to bacterial infections. Göbel and Böttger are thinking, for example, of AIDS, tumor or transplant patients, for whom the course of bacterial infections is crucially linked to the timely identification of the pathogens. Only a rapid and reliable diagnosis can smooth the way for a vital treatment with antibiotics.

In addition, the physicians want to tread further new ground within the project itself. Genetic probe analysis offers the opportunity to throw light on the confusing diversity of polymicrobial infections. These include periodontitis (inflammation of the gums), in which it is assumed that several hundred different microorganisms are involved. Furthermore, the researchers want to examine other, almost unexplored bacterial symbiotic communities – in the intestine, for example. The 'members' of the intestinal flora largely evade the physicians, yet they have a considerable influence on the functions of the intestine. If the individual microbes had been recorded first and their dynamic unraveled, it would then be much easier to develop ways of detecting disorders in these complex ecosystems and influencing them by means of therapies.

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(Photo: Friedrun Reinhold)

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