GENSENSOR-NANOPARTS

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Project co-ordinator	Universitaet Bremen, Centre for Applied Gensensorik (CAG)		
Partners:	CAG, Exiqon, Generi Biotech, Miltenyi and PRI		
Name of lead contractor	CAG-Management		
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1.0	12.09.2007	Creation and final Version	Prof. Dietmar Blohm (based on documents from all partners)

The following table shows the distribution status of the document's versions. Usually every version will be available on the GenSensor-Nanoparts website, so this will not be mentioned specially.

Version	Date	Distributed to	Distributed from
1.0	07.11.2007	Dr. Faure and all work package leaders	Prof. Dietmar Blohm

Content

1 Project execution

After a self-financing extension by 6 months the GenSensor-Nanoparts project was successfully finished after 42 months total time end of July 2007.

Scientifically the essential objectives have been reached but the results achieved are not suitable yet for immediate technical solutions. Further research is necessary in the fields of bioinformatics, RIfS measurement instrumentation and DNA analytics.

1.1 Project objectives

The project aims to contribute to the further development of the DNA microarray field, which is scientifically and commercially an important part of the genomic revolution going on in bioscience since about 15 years. The main objective of the GenSensor-Nanoparts project is to fill the gap in DNA analytics still existing between extracting nucleic acids from the biological material and analysing it by using microarrays. It is of general importance for biological research, clinical diagnostics and industrial applications irrespective whether the microarrays are used for gene expression analysis or for identifying and genotyping organisms.

Project goal is the reliable detection of nucleic acid molecules specifically in such cases when they are low abundant and hard to detect like a needle in the haystack. To achieve this goal the projects was trying to develop a sequence selective extraction method by which minor abundant molecules of nucleic acids can be enriched and analysed even in the presence of an extreme excess of non-target sequences.

The basic idea of the project is to use the synthetic nucleic acid LNA (locked nucleic acid) which is known to exhibit extremely strong sequence dependent binding to biological DNA and RNA. LNA containing oligonucleotides bound to extremely small magnetic nano-particles are expected to bind in the extraction broth even very rare DNA and RNA molecules and

make them isolatable by magnetic means. After transferring to the microarrays of the complexes formed during extraction consisting of the nano-beads and the target DNA which is sequence specifically bound to nano-beads according to the sequences of the LNA covering them, the nano-beads are expected to be detectable much more sensitive then the pure DNA or RNA molecules, since the nano-beads generate much stronger signals then single molecules.

The most important advantage of this system compared with most of the presently used microarray platforms is that there is no need to label the nucleic acid before they can be analysed by microarrays. A label free measurement system for microarrays has been established as “Gensensor” using the Reflectrometric interference spectroscopy (RIfS) as signal detection method. So, the innovative key element is the double-functional role the nano-beads play as tool for sequence selective nucleic acid extraction and as enhancer of the RIfS signal on the microarray.

The objective of the GenSenso-Nanopart project is the realization of this concept and to proof it by identifying finally a few selected microorganisms in milk.

Although there are many successful commercial products available for extracting nucleic acids from biological materials, this concept is new and is expected to bring about not only improved DNA-analytical equipment but also a new class of reagents, the sequence specific LNA-containing nano-beads.

As a further scientific outcome of the project, an universally applicable method for enriching low abundant DNA and RNA molecules during the extraction procedure is expected to be developed.

These scientific and technical results described were expected to be reached by working out the following project steps:

Bioinformatics groups define sequences specific for 5 model microorganisms and design *in silico* target DNA and capture oligonucleotides for microarrays and LNA-containing Nano - Beads.

A SME biotech company prepares the DNA stretches from the organisms and verifies the sequences experimentally which had previously been selected and optimized *in silico* by the research groups.

Another SME biotech company synthesises the special LNA-containing oligo-nucleotides.

The fourth step is the synthesis of the magnetic nano-beads and coupling the LNA-containing oligonucleotides to them.

Step five is the adaptation of the RIfS detection system to be able to use the nano-beads as signal enhancing elements.

Establishment of a highly sensitive DNA assay to monitor the selective extraction process and working out the extraction protocol in detail is the next step.

Finally the functionality of the complete system will be tested by using it for the identification from milk of those microorganisms the sequences of which had been selected at the beginning of the project.

A further objective of the project was to help bringing the two products mentioned into the market.

And finally it was a goal of this project to promote the co-operation between companies and research institutions in the different countries of the EU and to generate a personal network in this field. Realising the last aspects and creating an effective and pleasant working atmosphere was mainly the task of the project management.

1.2 List of participants

Project Participants

Partici-pant No.	Participant's Name	Participant's Acronym	Country	Participant's function and role in the project
1	University of Bremen, Centre of Applied Gensensorik (CAG)	CAG, consisting of three groups: - CAG-Biotec, Bremen, - CAG-Bioinformatics, Bremerhaven - CAG-RIfS, Tübingen	Germany	Project co-ordinator and leader of 5 WP's (part of bioinformatics, RIfS as microarray read-out, development of assay, extraction protocol, project management, dissemination and exploitation)
2	Exiqon A/S	Exiqon	Denmark	SME, Leader of 1 WP (synthesis of LNA-oligonucleotides)
3	Generi Biotech s.r.o.	Generi Biotech	Czech Republic	SME, Leader of 1 WP (experimen-tal testing of target sequences and preparing nucleic acid samples)
4	Miltenyi GmbH	Miltenyi	Germany	Industry, Leader of 1 WP (deve-lopment and production of LNA-covered nano-beads)
5	Plant Research International B.V.	PRI	The Nether-lands	Private, non commercial company, Leader of 1 WP (bioinformatics to select gene sequences *in silico*)

The project consortium has mostly been established by personal contacts at previous research co-operations. All partners met once before the project started to discuss a reasonable and complementary consortium structure. All partners started from the beginning on and worked until its end although some of the work packages ended already after 12 months.

The relation between the number of participants and the total cost structure of the consortium (3 companies, 2 research institutions) shows that the main intension of the project was to

create knowledge which can quickly implemented as market products preferably by the partner companies. The fact that 2 out of the 3 companies involved in the GenSensor-Nanoparts project are SMEs points to the commitment of the project to draw special attention to SMEs to strengthen their competitiveness and support their engagement in the European market.

For more detail about the GenSensor-Nanoparts project and the consortium see the web site www.gensensornanoparts.uni-bremen.de

1.3 Description of the work performed and end results

The consortium was kept small to work as efficiently and goal-oriented as possible. The five project partners are closely located in central Europe (see Fig. 1) enabling an intensive communication not only by media but in person.

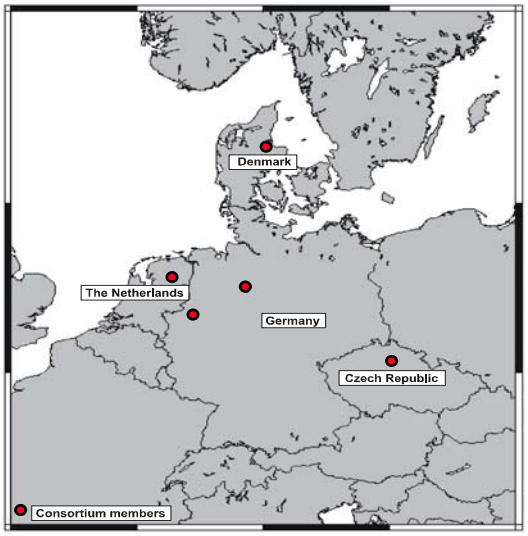

Fig. 1 Location of the project partners

The work performed can be summarized by describing the end results, which consist of the following achievements:

Unique DNA sequences highly specific for each of the following five microorganisms were defined *in silico* at the whole genome level: *Salmonella enterica ssp. enterica serovar Typhimurium LT2, Salmonella enterica* ssp. *enterica serovar Typhi*, *Listeria monocytogenes* Li 2, *Staphylococcus aureus* ssp. Mu50 and *Staphylococcus epidermidis*.

PCR primers, DNA-captures for microarrays, LNA-captures for nano-beads and target DNA sequences were positively selected experimentally and best LNA-DNA pairs were used as model system for selective extraction experiments.

Different types of LNA-coupled magnetic nano-beads were developed and used to “fish” target DNA of the model organism mentioned above.

Different extraction media like buffer, milk, cell lysate and mixtures containing chaotrophic agents to free the nucleic acids from other components of the biological material to be extracted are demonstrated to allow the sequence selective extraction.

Experimental difficulties in using the PCR as extraction assay in the presence of an excess of nano-beads lead to the conception of a new padlock-based DNA-analytical method and to its experimental realisation (not yet finished).

These achievements correspond to the essentials of the objectives of the GenSensor-Nanoparts project, which is therefore considered to be fulfilled successfully.

As additional conclusion it can be stated, that the results obtained did not satisfy all hopes as expected at the project start, because

The reasons of certain differences between hybridization properties of capture-target pairs predicted *in silico* and estimated experimentally could not be clarified because of lack of resources during the project, but this problem was recognized as a very important scientific problem for this field.

Quantification of the efficiency of selective extraction was not yet possible since the PCR is not reliable in the presence of the special nano-beads, a problem which could be solved only partially.

The RIfS signal enhancing effect of the nano-beads expected to increase the sensitivity of the microarray read-out has been worked out nicely but the signal to noise ratio is still very high.

At the Dresdener Sensorsymposium in December 2007 the following contribution will be presented (in German since the symposium will be held in German):

„GenSensor Nanoparts – nano-biotechnische Komponenten eines bioanalytischen Mikroarray-Systems“

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Abstract:

Ein Konsortium bestehend aus drei Firmen und vier akademischen Forschungseinrichtungen aus der Tschechischen Republik, Dänemark, Deutschland und den Niederlanden arbeiten gemeinsam an der Entwicklung einer Methode zur Optimierung von wesentlichen Arbeitsschritten in der Mikroarray-basierten Genanalyse. Im allgemeinen umfasst die Mikroarray-Methodik fünf Teile, 1. Extraktion der zu analysierenden Nukleinsäuren aus biologischen Proben, 2. Bereitstellung der relevanten Zielsequenzen durch Amplifikation, cDNA-Synthese und/oder Labelling 3. Hybridisierung der so gewonnenen Sequenzen auf Mikroarrays, 4. Erfassung der aus Hybridisierungsexperimenten erhaltenen Signale und 5. Datenanalyse. Während die Hybridisierung, Signalerfassung und Datenanalyse in einem erweiterten Mikroarray-basierten Gensensorsystem zusammengefasst werden können, werden innovative Lösungen für den Gewinn von quantitativen Ergebnissen aus dem System benötigt, welche den wirklichen Grad an verschiedenen, aus biologischen Proben zu bestimmenden Nukleinsäuren widerspiegelt. Zur Analyse von Proben, die eine große Anzahl an unterschiedlichen Sequenzen enthalten, muss die Komplexität der Nukleinsäuremischung reduziert werden. Diese Aufgabe wird durch die neue, zu entwickelnde nano-biotechnische Komponente gelöst, die nur eine bestimmte Gruppe an Sequenzen während der Extraktion erkennt, diese Nukleinsäuren ohne weitere Aufbereitung auf den Mikroarray-Chip zu überführen erlaubt und außerdem die Nachweisgrenze verbessert. Die nanostrukturierten Oberflächen dieser Komponenten, die Teil eines hochentwickelten online Hybridisierungs-Mikroarray („Gensensor“) sind, haben darüber hinaus das Ziel, die Robustheit und Zuverlässigkeit der DNA-Mikroarray Analyse zu verbessern, v.a. wenn geringste Mengen an Nukleinsäuren in einem Extrakt neben einem Überschuss an anderen Nukleinsäuren detektiert und quantifiziert werden sollen. Dies ist in der biomedizinischen Diagnostik, in der Qualitätskontrolle von Nahrungs- und Futtermittelprodukten, bei der Ermittlung der Genexpression von seltenen mRNA Spezies und in vielen anderen Gebieten der bio-analytischen Forschung und klinischen sowie industriellen Routine der Fall. Das Ziel ist die Entwicklung eines integrierten Systems für schnelle, markierungsfreie und kostengünstige Analysen von DNA oder RNA in hochkomplexen Proben.

In addition, at the public satellite workshop entitled “Nanotechnology for DNA-analytics” of the EuroNanoForum 2007 in Düsseldorf, 19.-21. June 2007, organised by the GenSensor-Nanoparts project management two detailed descriptions of the project were presented.

1.4 Description of methodologies and approaches employed

DNA microarray technology is an interdisciplinary field of research and a worldwide market segment in the size of about 500 million Euro. The market products are kits for DNA extraction, microarray chips, DNA synthesis service to produce microarray captures (mostly oligonucleotides), hybridization instruments and tools, laser scanner and diverse types of computer software. Since there are no technical standards yet established in this field most of these products are under continuous improvement and technical development.

The GenSensor-Nanoparts project is embedded in this process. Therefore the consortium includes not only specialists working in the different fields but industrial players active in this field as well as research institutions more interested in developing new basic methodologies and approaches.

The experimental work was mostly concerned with developing and producing of special nano-particles in connection with nucleic acid chemistry, molecular genetics, bioinformatics and biochemistry.

The approach started with the new concept of combining two new ideas, namely to realise the sequence selective nucleic acid extraction by using LNA-covered nano-beads and to use these nano-beads also for increasing the read-out of the hybridization signal on the microarray. Both new ideas could be realised so that the new concept could be demonstrated to work. For its technical realisation further investigations and developments are necessary.

1.5 Achievements of the project related to the state-of-the-art

1.5.1 Advances against the state-of-the-art

To our knowledge neither the new concept nor the two methods constituting it as described in section 1.3 had been published before or during the course of the GenSensor-Nanoparts project. There are market products know already for more than 20 years for isolating mRNA using their poly(A) stretches – an analogy to the sequence specific extraction, but individual sequences specific for certain microorganisms have never been used before as extraction method in connection with LNA and a label-free read-out of microarrays.

So, in comparison with the state-of-the-art this project was and is still highly innovative. The main achievement of the project is to demonstrate clearly that this innovative principle is working even if not all technical problems could be solved finally.

1.5.2 Impact on the industry sector

The results of the GenSensor-Nanoparts project have shown that a sequence selective extraction of nucleic acids from milk, cell lysates and other matrices is feasible. Therefore, this new method might be a useful tool for improving certain aspects of microarray

technology and can lead to the new industrial products for this field. Most conceivable are products related to the new type of nano-beads to which sequence specific LNA-containing DNA-captures are coupled. In addition, the application spectrum of LNA-based tools can be expanded and finally new RIfS-based equipment for label-free detection of hybridization signals on microarrays is envisioned. But all developments still need more research and development effort.

1.5.3 Impact on the research sector

The results obtained have shown that the extraction of nucleic acids of preselected sequences from crude biological material is possible. This process needs further characterisation and optimisation. In addition the project gave reason to develop a new padlock-based DNA-analytical method which probably will have a positive impact on the research sector of molecular genetics, genomics and microarray technology in general.

This outcome might encourage other research groups to develop and to use DNA microarrays for further applications in the academic as well as in the clinical and industrial sectors.

1.6 Dissemination and use

The GenSensor-Nanoparts project implemented a dissemination and exploitation strategy to make the academic and commercial aspects compatible and useful. The scientific project partners were mainly contributing to the scientific community by presenting results to the academic world at scientific conferences (see above), and the industrial partners approached their customers and business partners. These activities together with additional measures taken mostly in public media stimulated the awareness for the project and its results.

The strategy defines tasks for the market analysis and targets for the identification of economic benefits. The dissemination and use plan of the GenSensor-Nanoparts project has been carried out according to the activities described. In the part related to the dissemination plan, activities are listed and described, which give an update of the exploitable achievements and the putative perspectives.

The consortium is aware that the future socio-economic impact of the results of the GenSensor-Nanoparts project can be improved by a systematic evaluation of questions related to technology transfer, patenting, standardisation and market prospects which is still going on.