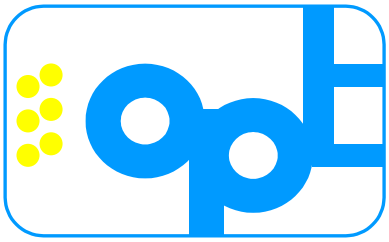


Contract no. 016727

OPTOLABCARD

Mass produced Optical Diagnostic Labcards based on Micro and Nano SU8 Layers

INSTRUMENT: STREP

PRIORITY: FP6-2004-IST-NMP-2

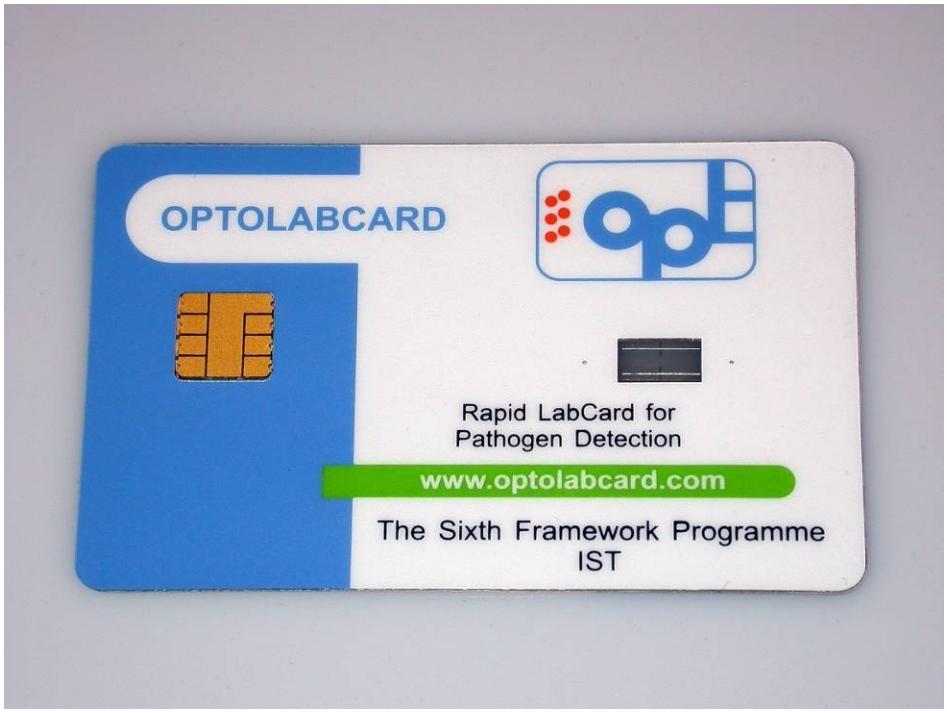
OPTOLABCARD Second year Executive Summary

Start date of project: 1st September 2005 Duration: 36 months

Lead partner for deliverable: IKERLAN

Revision: v1.0

Project co-funded by the European Commission within the 6th Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	✓
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including Commission Services)	
CO	Confidential, only for members of the consortium (including Commission Services)	



OPTOLABCARD Coordinator
Dr. Jesús M. Ruano-López
Microsystems area
IKERLAN
Pº J. M. Arizmendiarrieta, 2
20500 Arrasate-Mondragón (Gipuzkoa)
SPAIN
Tel.: +34 943 71 24 00 Fax: +34 943 79 69 44
E-mail: jmruano@ikerlan.es http: www.ikerlan.es
IK4 Research Alliance

INDEX

PROJECT STATUS: TECHNICAL PROGRESS AND ACHIEVEMENTS.....4

DIFFUSSION.....11

PROJECT STATUS: TECHNICAL PROGRESS AND ACHIEVEMENTS

Last year, we ended up with a wide range of SU8 processes and a first prototype chip where real sample concentration and amplification took place. As a summary, we could say that last year, we provided a first prototype of the chip. This second year we have prepared the components (external valves, heaters, concentration, amplification) to integrate a first functional prototype of the system by November.

Despite this card is an important breakthrough and achievement of the project. It is important to mention that this labcard is currently fabricated by an expensive method even for prototyping. The fabrication is based on a modified Printed Circuit Technology and assembly one by one. The card would be cheaper if standard smartcard fabrication technologies are applied.

Let´s summarise the achievements reached in this second year:

- A set of microfluidic components have been developed based on elastic membranes bonded to the SU8 and on embedded cantilevers in microchannels. The combination of these processes gives us a unique opportunity to fulfil the requirements of the PCR chips. The membranes valves will close the PCR chamber where 1 bar of pressure is generated (no leakage is allowed). The cantilevers' valves will work as flow control direction (some leakage is permitted) and the burst valve and the electrolysis pump will inject the PCR reagents into the chamber. Due to the patenting process carried out at this moment, we can not provide more information of the microfluidic control achievements. In any case a prototype valves have been fabricated as can be seen in Figure 1.

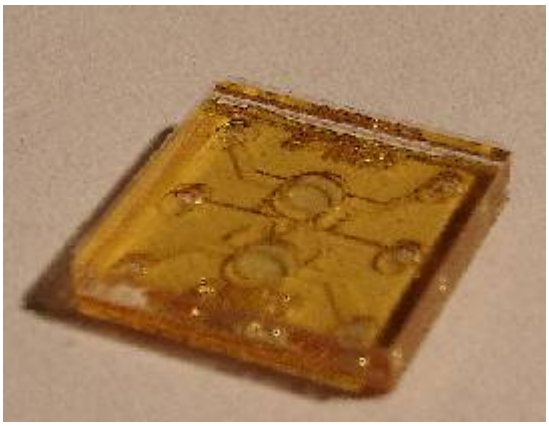
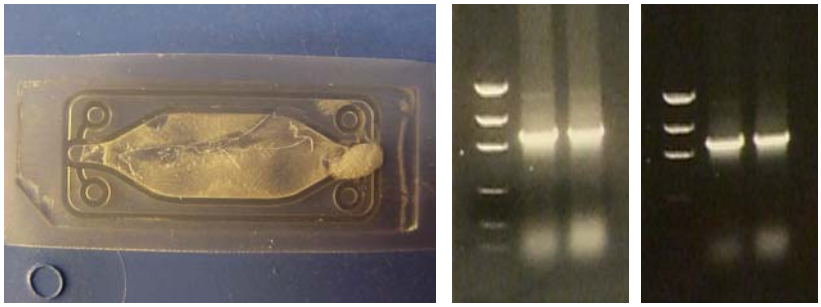


Figure 1: Picture of the valve prototype

- Regarding the stored reagents, it is worthwhile to mention the high level of achievement of this activity. We have freeze dried reagent on chip that then has been used to carry out a PCR. This is as far as we know, the first time that has been achieved. It was necessary the commitment of three partners: MIC, Ikerlan and VET (previously named DFVF) to achieve this result. Special care, dedication and effort were carried out by Dr. Bang from VET to achieve such a high-class results.

A) Freeze-dried and PCR on "Open" chip from MIC



B) Freeze-dried and PCR on "Close" chip from IKERLAN

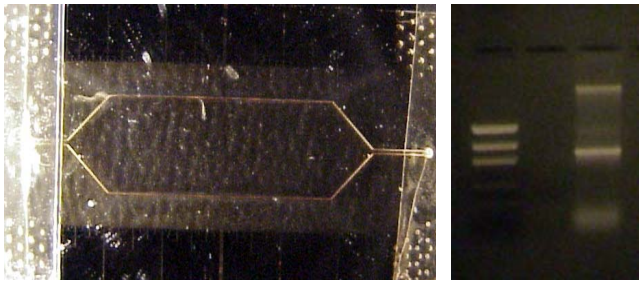
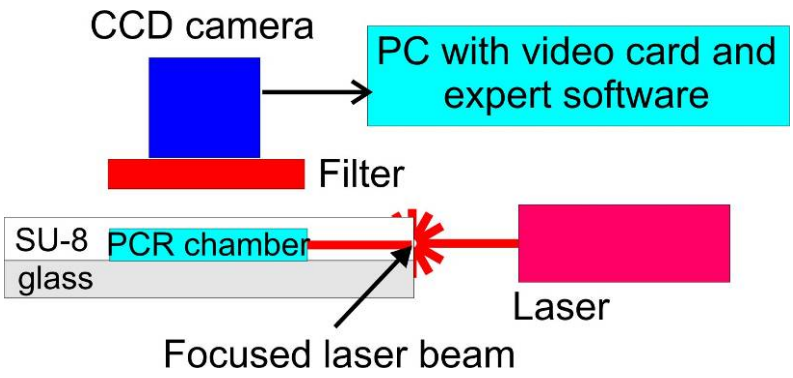


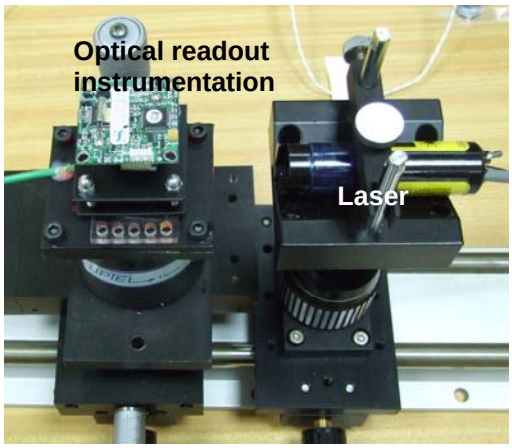
Figure 2. On chip Freeze-dried PCR mixture: in A) "open" chip and in B "close" chip and PCR results

- On top of that, Ikerlan and Gaiker have been carrying out a gelification on chip under the frame of a Spanish project in collaboration with Biotools S.L. A study was carried out to adapt their gelification process in a closed chip. Preliminary results show that gelification on chip is feasible. However, the sample has not been validated yet by PCR.
- A thorough validation protocol (including sample preparation to specific detection by qPCR) off chip at the laboratory level was carried out by Gaiker, VET and BIOEF. This has allowed to find the right PCR cocktail to ensure a PCR reaction despite of the inhibitory effect of the SU8 and the magnetic beads. Gaiker and BIOEF has optimized a protocol when clinical samples are used, able able to recover *Salmonella typhimurium* cells from inocula containing 10 colony forming of units per mililitre of test sample (stool sample). Gaiker has developed a very specific and highly sensitive *Salmonella* spp. Real-Time PCR assay OFF CHIP (we are able to quantify cell number of a diluted Salmonella isolate, with a detection limit of 1 logarithmic order of colony forming units) and efficiently reproducible.
- The optical readout instrumentation co-working with Real-Time PCR disposable chip has been successfully developed and tested. The detection limit of DNA is 3 – 5 ng/ml what, is sufficient to fulfil project requirements. The system is made of commercially accessible components, the key component is new informatics method of data collection and processing with a use of the specialized software co-working with the developed instrumentation for the real-time analysing of the fluorescence signal which has been developed by us. The Real-Time PCR amplification of the DNA of Campylobacter bacteria has been successfully carried out and characterized using this optical setup. Currently, the best solution is application of IKERLAN Gaiker chip, where high detection limits and easy laser light coupling have been achieved. (Figure 3).

a)



b)



c)

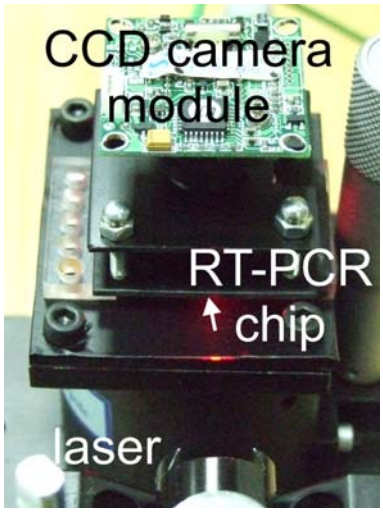


Figure 3. The measurement set-up with IKERLAN chip and side-wall edge coupling of laser light: a) scheme, b) view of the stand, c) detail of the stand – the CCD module positioned a few millimetres above the chip

- A thorough optical characterisation has been carried out demonstrating that we have enough sensitivity to carry out real time PCR. In that respect, as previously the salmon sperm DNA (2,5 ng/ml – 2,5 µg/ml) and TO-PRO 3 (0,5 µM) were used in tests. The detection limit was about 5 ng/ml (Figure 4 and Figure 5). This result shows that the direct coupling of the light did not cause decreasing of the detection limits (in comparison to result obtained with MIC DTU chip), the sensitivity of this configuration meets all of the specifications of the OPTOLABCARD project.

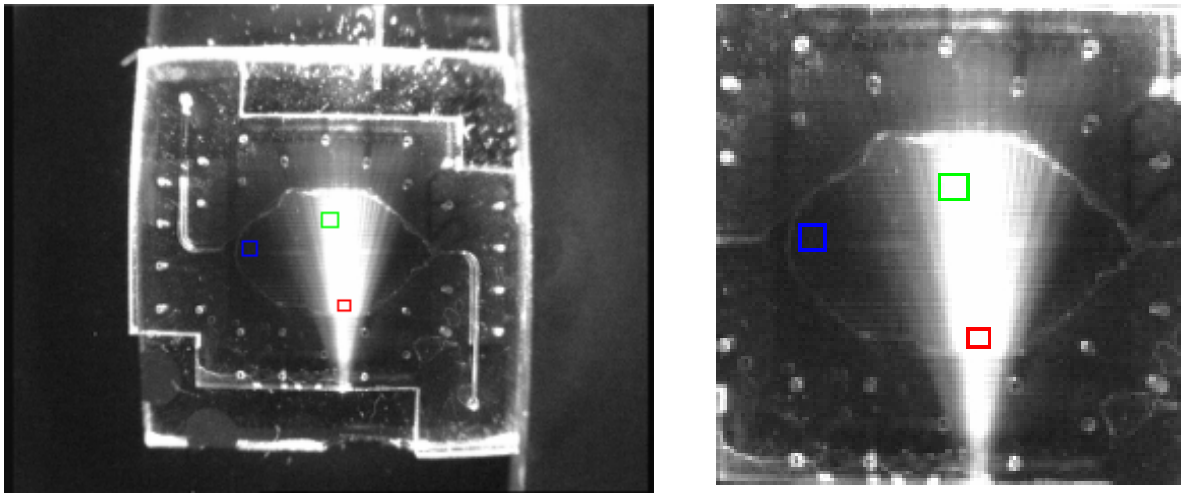


Figure 4. The chip at tests (left) and enlarged PCR chamber sector. Areas of analysis justified by the software marked with squares.

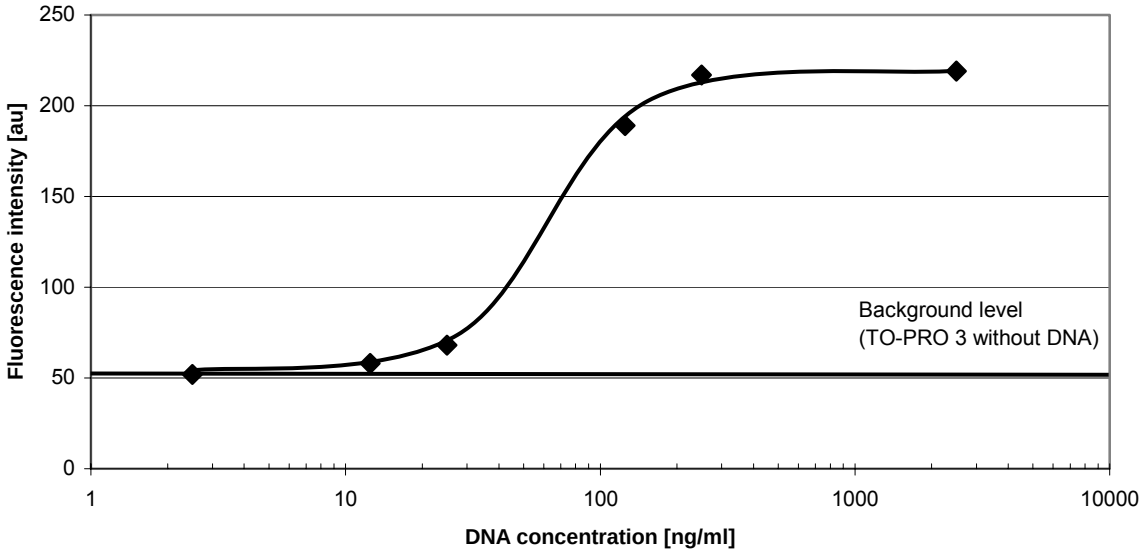


Figure 5. Fluorescence intensity as function of DNA concentration

- Several improvements were achieved in comparison with last year: (i) less PCR mixture (from 2.5 µl to 0.6 µl) was required; (iv) less time (25 minutes) was needed for the complete analysis and (v) a higher sensibility (CT=15 vs CT= 19) was obtained. See Figure 6.

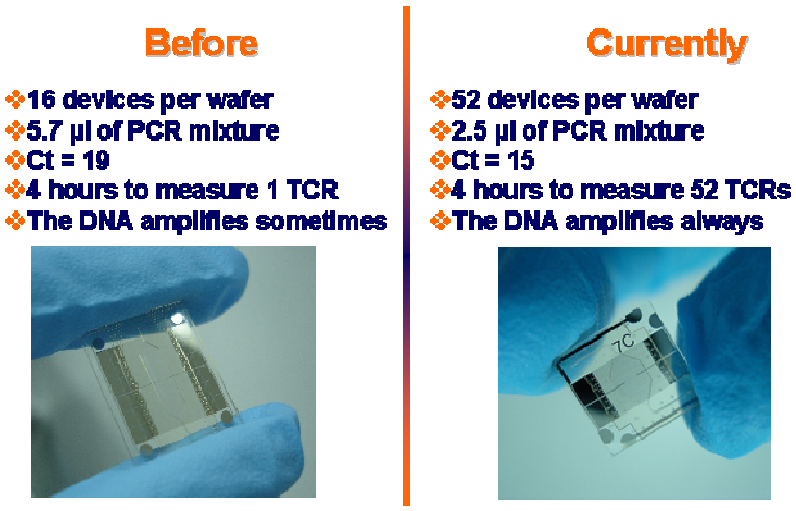


Figure 6: Summary of the improvements carried out regarding the one chamber approach

- The PCR chip size has been from 3.36 cm^2 to 1cm^2. (See Figure 7).

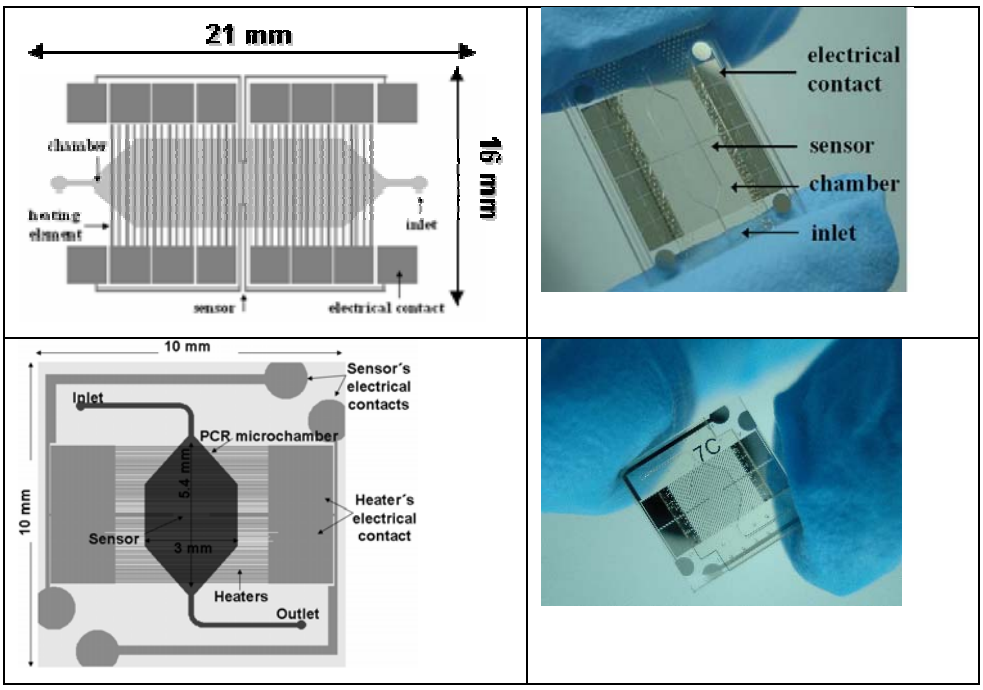


Figure 7. Comparison of the first year and second year chip Left) Schematic top view of the SU-8 chip. It consists of a rhombus shape microchamber, a resistive Ti/Pt sensor and two parallel heaters; Right) Picture of the chip

- This smaller chip has been also packaged following the same concept that the used in the previous design. Nevertheless in this case, the capsule can be open or close without the need of screws. It uses a latch or bolt.

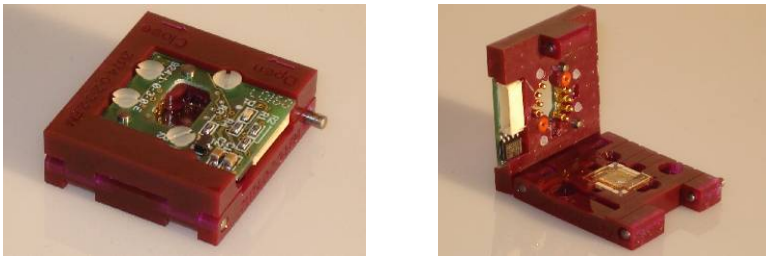
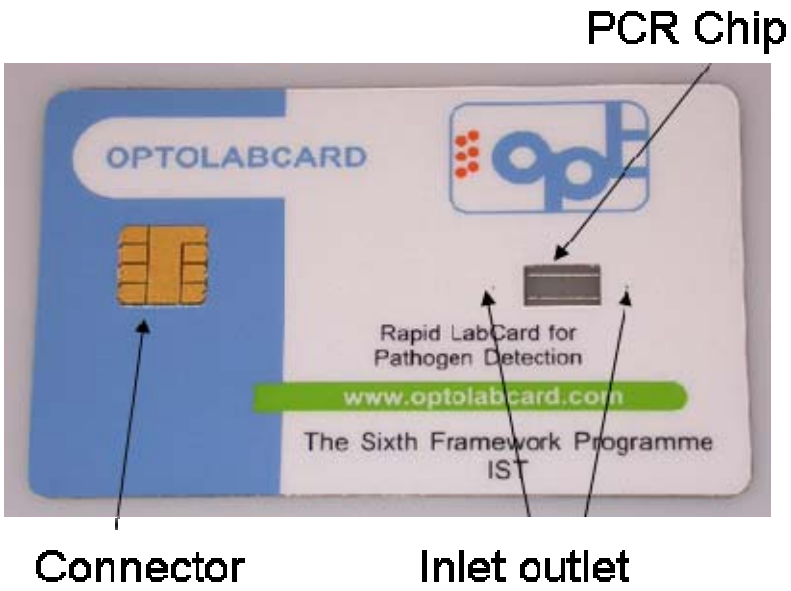


Figure 8. Photographs of the fabricated PCR microdevices: closed and open packaged device.

- This second year we have fabricated and package a SU8 chip in a Smart Card as can be seen in the figure:



- A business model with a strategy to place the above presented product and process potential into the targeted market has been designed. This strategy combine a portfolio able to assure the R&D investments and technology skills on microfluidics and process know-how needed to perform the shift from small lot “hanging fruit” opportunities (present opportunities) to the “pot of gold” opportunities, usually related with mass production skills (see exploitation plan deliverable).

- In order to get a high robustness during qPCR assays, an internal amplification control (IAC) is being included. In a PCR without an IAC, a negative response (no band or signal) can mean that there was no target sequence present in the reaction. But it could also mean that the reaction was inhibited due to malfunction of the thermal cycler, incorrect PCR mixture, poor polymerase activity, and, not least, the presence of inhibitory substances in the sample matrix. Conversely, in a PCR with an IAC, a control signal will always be produced when there is no target sequence present. When neither IAC signal nor target signal is produced, the PCR has failed. Thus, when a PCR-based method is used in routine analysis, an IAC, if the concentration is adjusted correctly, will indicate false-negative results. It is the false-negative results that turn a risk into a threat for the population, whereas a false-positive result merely leads to a clarification of the presumptive results by retesting the sample. GAIKER is working in the IAC design.

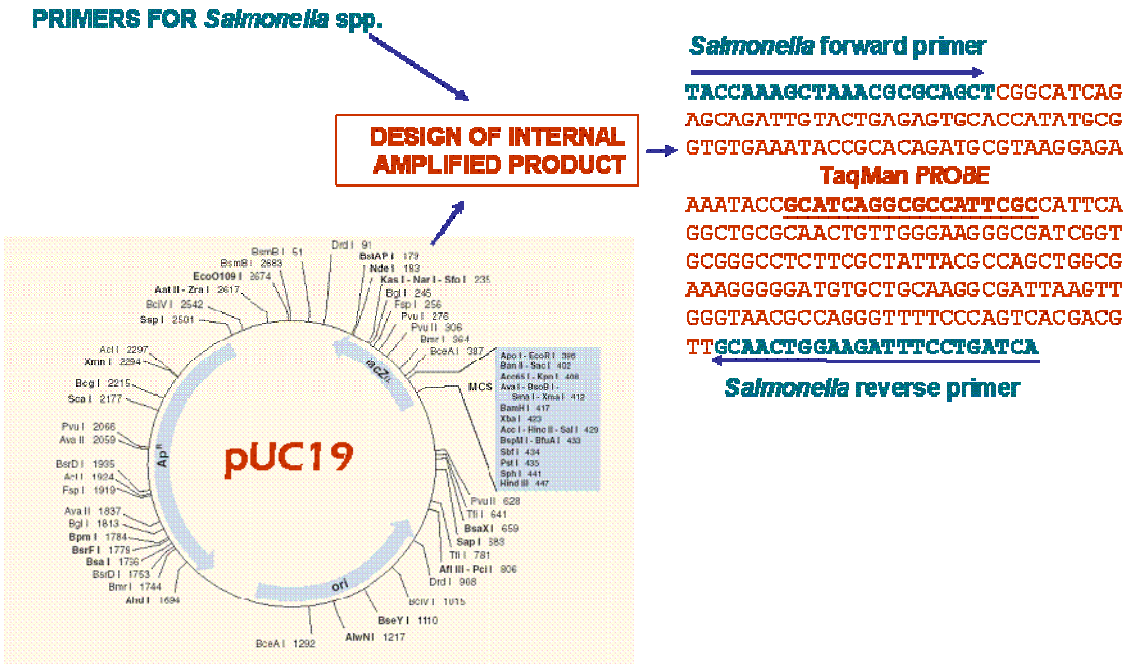


Figure 9. Genetic construction for IAC

DIFFUSSION

In the second year of the project, the consortium has participated and attended the following conferences:

1) Transducers/Eurosensors 2007 in Lyon, France, on 10-14, June, 2007
2) MNE 2007 in Copenhagen, Denmark, on 23-26 September, 2007
3) MicroTAS 2007, in Paris, France, on 7-11 October 2007

For publications, the consortium has been involved in preparation and join publication on the following papers:

A. Journal papers:

- Kristian Smistrup, Minqiang Bu, Anders Wolff, Henrik Bruus, Mikkel Fougt Hansen, Theoretical analysis of a new, efficient microfluidic magnetic bead separator based on magnetic structures on multiple length scales, Microfluidics and Nanofluidics, In press. DOI: 10.1007/s10404-007-0213-0
- Minqiang Bu, Troels Balmer Christensen, Kristian Smistrup, Anders Wolff, Mikkel Fougt Hansen, Characterization of a Microfluidic Magnetic Bead Separator for High-Throughput Applications, Sensors and Actuators A: Physics, in review
- Minqiang Bu, Kristian Smistrup, Anders Wolff, Mikkel Fougt Hansen, Parametric study on a microfluidic magnetic bead separator for high-throughput applications, Draft has been prepared and to be submitted to Journal of Micromechanics and Microengineering
- T.B. Christensen, C. M Pedersen, D.D. Bang, and A. Wolff (2007). Sample preparation by cell guiding using negative dielectrophoresis. Microelectronic Engineering 84: 1690-1693.
- Gudnason H., Matin Dufva, D.D. Bang and Anders Wolff (2007). Camparison of multiple DNA dyes for real-time PCR and investigation of the effect of dye concentration on amplification and DNA melting curve analysis (Accepted for publication in Nucleic Acid Research)
- Jesus M.Ruano-Lopez. Fabrication strategies to integrate 3D microfluidic networks with biosensors to manufacture Lab on a Chip devices. Measurement + Control, Vol.40, No. 4, pp. 111-115
- Maria Agirregabiria, Francisco Javier Blanco, Javier Berganzo, Asier Fullaondo, Ana María Zubiaga, Kepa Mayora, Jesús Miguel Ruano-López. Sodium dodecyl sulfate-capillary gel electrophoresis of proteins in microchannels made of SU-8 films. Electrophoresis, Vol.27, No. 18, pp. 3627-3634
- F J Blanco, M Agirregabiria, J Berganzo, K Mayora, J Elizalde, A Calle, C Dominguez and L M Lechuga, Microfluidic-optical integrated CMOS compatible devices for label-free biochemical sensing, Journal of Micromechanics and Microengineering, Vol.16, No.5, pp. 1006-1016

B. Conference proceedings:

- Minqiang Bu, Troels Balmer Christensen, Kristian Smistrup, Anders Wolff, Mikkel Fougt Hansen, Theoretical and experimental investigation on the capturing behaviour of a novel microfluidic magnetic bead separator for high-throughput applications, in Proceeding of Micro and Nano Engineering (MNE2007), Copenhagen, Denmark, September 23-26, 2007, accepted
- R. Walczak, J. A. Dziuban, J. Koszur, A. Wolff, D. D. Bang and M. Bu, CCD camera-based optical readout system for RT-PCR DNA analyzer: towards rapid and cheap detection of pathogens in food and clinical samples in nanograms per millilitre concentration of DNA, in: Proceedings of MicroTAS'07, Paris, France, October 11-14, 2007, accepted
- Monica Brivio, Yiping Li, Bastian Gaardsvig Kjeldsen, Jakob Leffland Reimers, Minqiang Bu, Dang Duong Bang, Anders Wolff, A simple and effective method for on-chip storage of reagents: towards lab-on-a-chip system for point-of-care genetic diagnostics, in: Proceedings of MicroTAS'07, Paris, France, October 11-14, 2007, accepted
- Minqiang Bu, Troels B. Chirstensen, Kristian Smistrup, Mikkel F. Hansen, Anders Wolff, A high-throughput SU-8 microfluidic magnetic bead separator, in: Proceedings of Transducers'07, Lyon, France, June 10-14, 2007, pp. 1773-1776.
- Minqiang Bu, Kristian Smistrup, Mikkel F. Hansen, Anders Wolff, Design and FEM simulation of a microfluidic magnetic bead separator, in: Proceedings of Micro and Nano Engineering (MNE2006), Barcelona, Spain, September 17-20, 2006, pp. 431-432.
- María Agirregabiria, Dolores Verdoy, Javier Berganzo, Luis J.Fernandez, Garbiñe Olabarria, Josu Berganza, María Pascual de Zulueta, Paloma Aldamiz-Echevarria, Kepa Mayora and Jesús Miguel Ruano-López. Concentration, purification, lysis and real-time PCR on a single SU-8 microchamber for rapid detection of Salmonella spp. In faeces. The 11th International Conference on Miniaturized Systems for Chemistry and Life Sciences, microTAS 2007 Conference, 7-11October, Paris (France)
- María Tijero, Luis José Fernández, Jesús Miguel Ruano-López, Kepa Mayora, María Velia Rodríguez, and Jorge Elizalde. Magnetic microvalve with biocompatible surfaces using electrochemical deposition and passivation. The 11th International Conference on Miniaturized Sstems for Chemistry and Life Sciences, microTAS 2007 Conference, 7-11 October, Paris (France).
- G.Olabarria, A.Voigt, M.F.Lagerros, A.Wolff, J.Dziuban, I.Minguez, E.Pérez-Trallero, M.Wimplinger, D.D.Bnag, J.M.Ruano-López. Mass Produced Optical Diagnostic Labcards Based on Micro and Nano SU8 Layers (OPTOLABCARD). MINOS-EURONET Conference (Invited Speaker), Bucharest, 24th May 2007
- R. Walczak, J. A. Dziuban, A. Wolff, J. Koszur, Optical DNA detection In nanograms range for Real-time PCR metod realized by the use of lab-on-chip techique: firs approach toward cheap and mass-produced ford pathoigens detection (Optyczna detekcja DNA w zakresie nanogramów dla metody real-time PCR realizowanej z wykorzystaniem techniki lab-on-chip: pierwszy krok w kierunku taniego i masowego detektora skażeń żywności), regular presentation, Proc. of the I National Nanotechnology Conference, 26-26 April 2007, Wrocław, Poland
- R. Walczak, S. Bargiel, J. A. Dziuban, J. Koszur, Fluorescence DNA analyzer based on silicon-glass nanovolume detector and optical microspectrometer

(Fluorescencyjny analizator DNA bazujący na krzemowo-szklanym czujniku nanoobjętości i mikrospektrometrze optycznym), regular presentation, Proc. of the I National Nanotechnology Conference, 26-26 April 2007, Wrocław, Poland

- R. Walczak, J. A.Dziuban, J. Koszur, J. M. Ruano-López, A. Wolff, F. Zembok, Detection of food pathogens DNA by novel optical system applied in cheap and mass produced lab-on-a-card: first approach, invited lecture, Proc. of the IX Conference on Electron Technology ELTE 2007, 3-7 September 2007, Kraków, Poland
- S. Bargiel, A. Grzegorska. R. Walczak, A. Górecka-Drzazga, J. Dziuban, Microsensor for the investigation of biochemical analytes in the nano-volume region, poster presentation, Proc. of the IX Conference on Electron Technology ELTE 2007, 3-7 September 2007, Kraków, Poland
- P. Szczepańska, R. Walczak, J. Dziuban, J. Koszur, A. Wolff, M. Bu, SU8 waveguides for integraed PCR DNA analyzer, poster presentation, Proc. of the IX Conference on Electron Technology ELTE 2007, 3-7 September 2007, Kraków, Poland
- R. Walczak, J. A. Dziuban, D. . Bang, J. M. Ruano-Lopez, A. Wolff. M. Bu, Optical readout system for DNA food pathogens detection with disposable RT-PCR SU-8/glass chip, to be publicated in the 33rd International Conference on Micro- and Nano-Engineering 23-26 September 2007, Copenhagen Denmark

Other:

Presentation on FP6 EC projects realized by Institute of Electron Technology for the Polish Ministry of Science and Higher Education (exhibition of posters, presentation of results).

Diffusion in a Scientific Magazine Call CIC Networks.

Diffusion of a Basque Research Leaflet