

Genetic analysis of single cancer cell using a multiplexed DNA amplification strategy coupled to 64-electrode PCB sensor arrays for detection

J. Ll. Acero Sanchez¹, H. Joda¹, O. Y. F. Henry¹, B. Werne Solnestam², L. Kvastad², E. Johansson², J. Lundeberg², N. Lladach³, P. Salvo^{4,5}, K. Dhaenens^{4,5}, A. Gielen^{4,5}, J. Vanfleteren^{4,5}, D. Latta⁶, I. Riley⁷ and C. K. O'Sullivan^{1,8}

¹ Universitat Rovira i Virgili, Departament de Enginyeria Química Av. Països Catalans 26, 43007 Tarragona, Spain.

² KTH Royal Institute of Technology, Science for Life Laboratory (SciLifeLab Stockholm), School of Biotechnology, Division of Gene Technology, SE-171 65 Solna, Sweden.

³ MRC Holland, Willem Schoutenstraat 6, 1057 DN Amsterdam, The Netherlands

⁴ University of Ghent, Centre for Microsystems Technology (CMST), Faculty of Engineering, 914A Technologiepark, B-9052 Ghent-Zwijnaarde, Belgium.

⁵ Interuniversity Microelectronics Centre (IMEC), 3001 Leuven, Belgium.

⁶ Fraunhofer (ICT-IMM), Carl-Zeiss-Str. 18-20, 55129 Mainz

⁷ Labman Automation Ltd., Seamer Hill, Seamer, Stokesley, North Yorkshire, TS9 5NQ, United Kingdom.

⁸ Institució Catalana de Recerca i Estudis Avançats, Passeig Lluís Companys 23, 08010 Barcelona, Spain.

ciara.osullivan@urv.cat; josepluis.acero@urv.cat

Abstract

Novel breakthrough in the understanding of metastasis and the formulation of new cancer models have motivated the development of novel integrated analytical technologies capable of isolating and identifying single cancer cells. However, the complexity of circulating cancer cells requires deeper analysis to provide sufficient insight into the real nature and therefore malignancy of the cell. Genetic profiling can undoubtedly provide the level of information required.

We report on a novel strategy towards the multiplexed genetic profiling of single tumour cell using a combination of multiplexed ligation-dependant probe amplification (MLPA) coupled to sensitive detection using electrode microarrays manufactured on standard printed circuit board (PCB) substrates. Single cancer cells were isolated by laser capture, lysed and the mRNA extracted and transcribed into DNA. Then, various relevant regions were amplified by MLPA technique. The MLPA reaction allows for multiplex amplification of multiple targets with a single primer pair. Novel synthetic MLPA probes were designed to include a unique barcode sequence in each amplified gene which acts as capture probe for microarray analysis. Capture probes complementary to each of the barcode sequences were immobilised on the electrode array surface and exposed to single-stranded MLPA products. The surface bound DNA duplexes were then detected with a secondary DNA probe bearing a HRP molecule using fast electrochemical pulse amperometry.

The approach presented is simple, rapid, flexible, relatively inexpensive, capable of quantifying 7 genetic markers for breast cancer with single tumor cell sensitivity and provides an elegant methodology towards the development of integrated "amplification-to-detection" instrumentation.

References

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