

BIOCHIPS :

1- Introduction to DNA microarray technology and application to comparative genomic hybridization

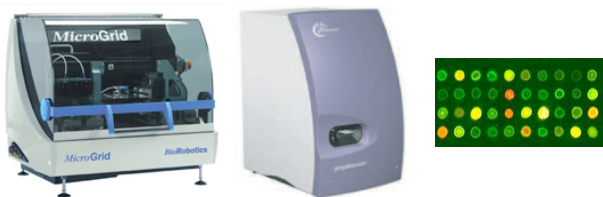
2- Introduction to protein microarray technology and application to antigen-antibody interaction using SPRI

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DNA microarrays

DNA chips are high-density arrays of oligonucleotides or small DNA fragments (probes) immobilized on a surface. Hybridization of fluorescent-labeled target DNA to complementary probes on the surface can be visualized by fluorescence imaging. DNA chips are nowadays widely used to identify gene alleles, pathogen species and to study gene expression and eukaryote gene editing.

This practical combines several well-employed technologies in the field of micro- and nano-technologies applied to biology. The lab work entails all steps of standard DNA microarray technology, from DNA probe spotting to comparative DNA hybridization analysis. Students will carry out hands-on experiments on organic macromolecules covalent coupling on an inorganic substrate, DNA preparation and fluorescent labeling, hybridization and washings, fluorescence microscopy, quantitative image analysis.



Left : MicroGrid Microarrayer ; Center : Genewave AmpliReader ; Right : Example of DNA array hybridized with two target DNA preparations labeled with green and red fluorophores.

Protein microarrays

Peptide microarrays can be used for high-throughput screening of binding partners in complex biological fluids like blood, for example. In this practical we will use gold-layered prism surfaces with defined arrays of polypyrrole-coupled peptides and Surface Plasmon Resonance imaging (SPRI) for studying antigenic peptide-antibody interactions. The SPRI signal reflects local mass changes at the surface of the prism and does not require previous labeling of the interacting antibodies. We will demonstrate that the signal produced by peptide-antibody binding is highly specific and can be quantified.



Left : SPRI-Lab+™ from Horiba Jobin Yvon and gold-layered prisms (right)

The practical schedule is as following:

Day 1 (8h) : DNA probe covalent grafting on silanized high-sensitivity reflective glass plates (AmpliSlides, Genewave). Probes will be spotted with a Microgrid microarrayer (BioRobotics). Purification of plasmid DNA from different strains of bacteria. Insert amplification by Polymerase Chain Reaction and target DNA labeling with AlexaFluor 546 or AlexaFluor 647. Hybridization with fluorescently labeled target DNA.

Day 2 (4h): Comparative fluorescence analysis with a CCD-based fluorescence reader (AmpliReader, Genewave). Quantitative analysis of fluorescence signals. The specificity and reproducibility of the technique will be studied. The influence of probe-target mismatches on the strength of the hybridization signal will be demonstrated.

Day 2 (4h): Analysis of the interaction between antigenic peptides grafted on a SPR prism and a mixture of antibodies present in different sera. The interaction specificity and quantity will be analyzed using SPR in the imaging mode. Customized prisms will be readily available and an SPRI-Lab+™ system from Horiba Jobin Yvon will be used.