Consiglio Nazionale delle Ricerche
Istituto di Biostrutture e Bioimmagini

CNR-MYRMEX Public-Private Laboratory

Technologies development and generation of a technological platform in order to evaluate drugs efficacy

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Introduction

Collaboration between public and private research groups gives them the opportunity to get the best from both, including cost/profit/timing/target without losing the fundamental research. In this contest, a Sicilian Public-Private Laboratory has been set up from the collaboration of two organizations, both present in our region, CNR-IBB and Wyeth Lederle S.p.A., now owned by MYRMEX S.p.A.
The MIUR funding, provided for the setting up of the Public-Private Laboratory, was obtained in 2006 (D.M. 2629 30-11-2006 "Public/private laboratory for developing technologies and technological platforms in order to evaluate the drugs efficacy").
The laboratory has been located inside the MYRMEX R&D Center in Catania.

Aims

This public-private collaboration has a double purpose:

- **Research**: Evaluation of drugs efficacy and biomarkers discovery by means of Genomic and Proteomic Analysis;
- **Training activity**: formation of professionals in Biotechnology and Bioinformatic fields.

Scientific Team

MYRMEX : E. Tendi, M. Cultrera, D. Scionti, M. Guarnaccia, G. Gentile.

CNR-IBB: G. Pappalardo, A. Copani, D. Milardi, G. Di Natale.

Methods

DNA microarray technology applied for gene expression profiling, identification of toxicity pathways, genotyping assay, detection of biomarkers, target selection of new compounds.

Mass spectrometry applied for targets identification, biomarkers discovery, proteins differential expression, top-down proteomics analysis, investigation of post-translational modifications, establishing of protein-protein interactions.

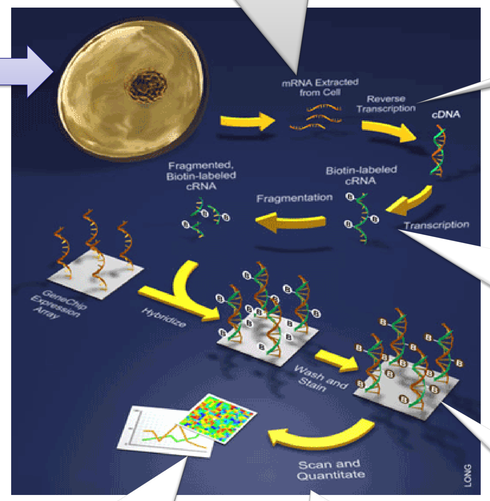
Bioinformatic approach used to collect, storage, interpret and predict complex multivariable data obtained from both genomic and proteomic studies.

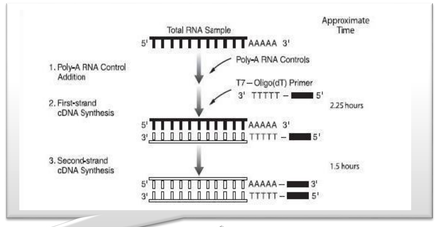
Genomic analysis

Total RNA is extracted by means of **RNeasy® Mini Kit QIAGEN**.

RNA molecules are electrostatically bound to the stationary phase of spin columns, purified in different buffer conditions and finally eluited by water.

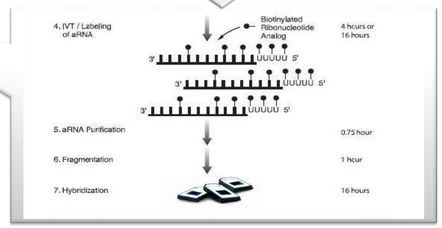
Control sample Vs Treated sample



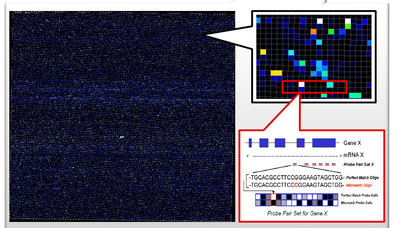


GeneChip® 3' IVT Express Kit Labeling Assay

Affymetrix



GeneChip® Fluidics Station 450 is used for washing and staining operations of Affymetrix GeneChip® arrays. Namely, staining solution is used for the amplification of fluorescent signal.



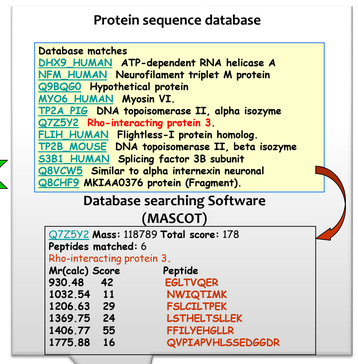
GeneChip® Scanner 7G allows to scan 48 high-density oligonucleotidic microarrays chips.

GeneChip® data analysis shows the differentially gene expression of the sample investigated.

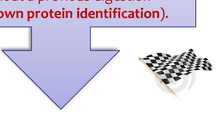
Proteomic analysis

Two procedures, PMF (Peptide Mass Fingerprint) and searches with non-interpreted peptide fragmentation spectra, constitute the most methodology used in protein identification.
In PMF, special programs compare the experimental mass spectrum taken from a protein digest, with the theoretical spectra which would be produced if the proteins in the database were subjected to the same digestion conditions.

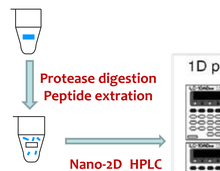
Peptide fragmentation by Collision Induced Dissociation (CID) are developed to identify proteins using fragmentation spectra of their peptides.



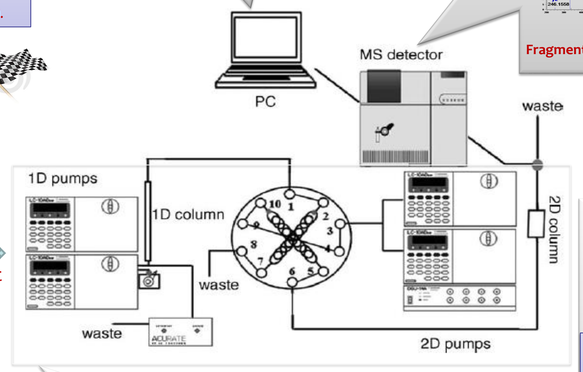
In a typical proteomics experiment, a digested protein mixture is separated by HPLC and then MS and MS/MS spectra are analyzed (**bottom up proteomics**).
Protein extract may be also analyzed without a previous digestion (**top-down protein identification**).

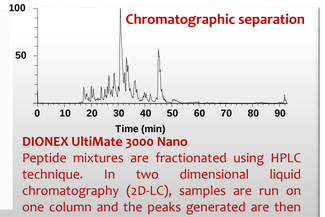


Protease digestion
Peptide extration



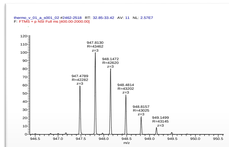
Nano-2D_HPLC



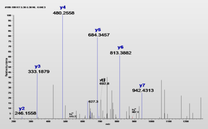


Chromatographic separation

DIONEX UltiMate 3000 Nano
Peptide mixtures are fractionated using HPLC technique. In two dimensional liquid chromatography (2D-LC), samples are run on one column and the peaks generated are then separated on a second column.

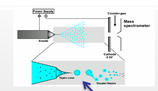


MS
Exact mass of proteins/peptides



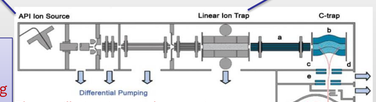
MS-MS
Fragmentation spectrum of each peptide

ThermoFisher-LTQ Orbitrap hybrid mass spectrometer provides identification, characterization and quantification of complex protein/peptide mixtures with an accurate mass measurement and high resolution spectra.

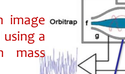


Electrospray is produced by applying a strong electric field to a liquid passing through a capillary tube.

Ions stored in the **Linear Trap** are axially ejected and trapped in the **C-trap**……



The oscillating ions induce an image current which can be detected using a differential amplifier. Each mass generate a sine wave signal.



….then, they are squeezed into a small cloud and injected into the **Orbitrap** where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation.