Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

Consortium leader

PETER PAZMANY CATHOLIC UNIVERSITY

Consortium members

SEMMELWEIS UNIVERSITY, DIALOG CAMPUS PUBLISHER

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

***A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

Neurobiológia alapjai - Módszerek

BASICS OF NEUROBIOLOGY - Methods

By Imre Kalló

METHODS IN NEUROBIOLOGY V.

Molecular biological techniques

Imre Kalló
Pázmány Péter Catholic University, Faculty of Information Technology

I. Histology techniques: light microscopic studies
II. Applications using fluorescent dyes
III. Histology techniques: electron microscopic studies
IV. Techniques to map neuronal connections
V. Molecular biological techniques
VI. Living experimental models
VII. Electrophysiological approaches
VIII. Behavioral studies
IX. Dissection, virtual dissection, imaging techniques

STUDIES ON GENE EXPRESSION

Northern blot

It is a technique, which examines gene expression via RNA samples blotted onto membranes.

- low sensitivity
- it is well quantifiable

Polimerase **C**hain **R**eaction (PCR)

It is a DNA amplification technique, which is capable to multiply a single or a few copies of a piece of DNA by several orders generating thousands to millions of copies.

- very high sensitivity
- quantification only after very strict calibration

Quantitative **R**eal-**T**ime-PCR

Expression of only a few genes is examined in a sample:

"low -throughput"

STUDIES ON GENE EXPRESSION

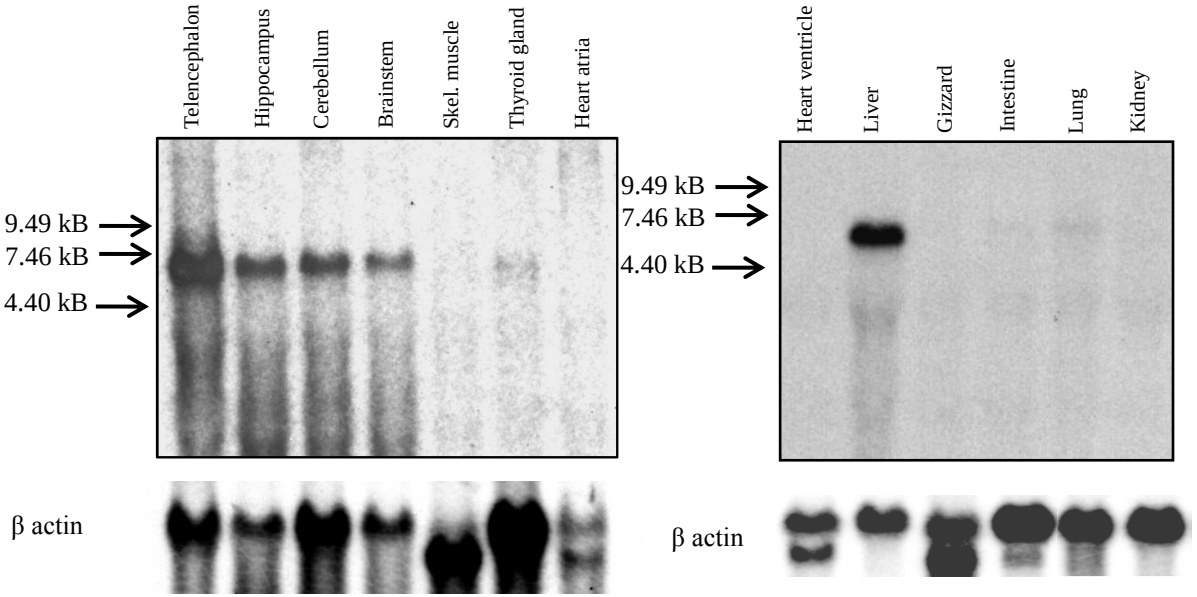
Northern blot

- isolation of total or mRNA
- size-dependent separation in gel
- membrane-blotting
- hybridization on membrane – with probes labelled isotopically or non-isotopically
- re-hybridization with other probes is limited

Polimerase **C**hain **R**eaction (PCR)

- isolation of total RNA

  synthesis of cDNA with reverse trascriptase
- amplification with heat-resistant polimerase (Taq, Vent etc.) in the presence of gene-specific oligonucleotides
- size-dependent separation of the reaction product in agarose gel

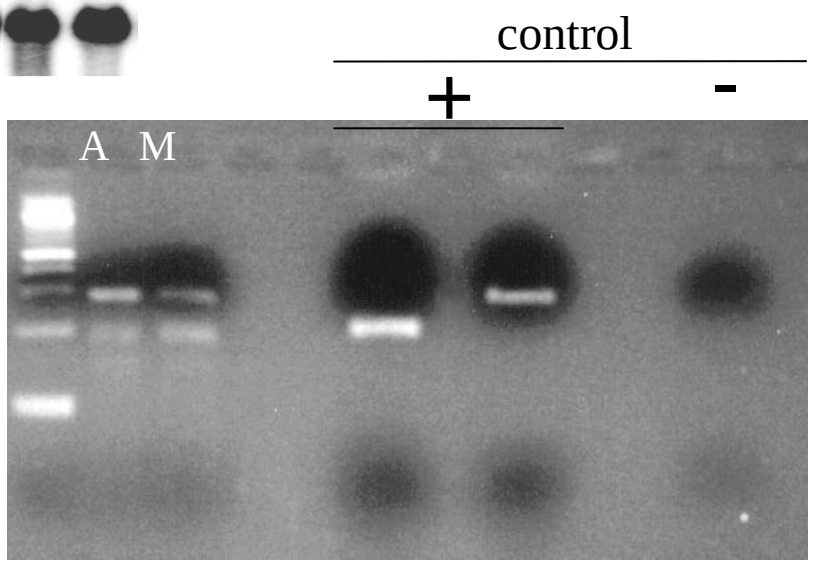


D2 expression in tissues (Northern blot)

Gereben et al. J. Biol. Chem. (1999) 274:13768-13776

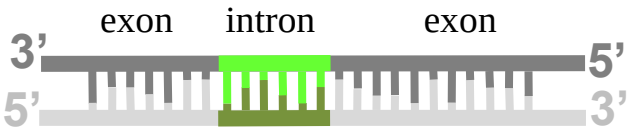
D2 expression in the brain and liver (PCR)

Gereben et al. Mol. Endocrinology (2002 16(7):1667-79



RT-PCR (reverse transcriptase PCR)

gene= a stretch of DNA
encoding a protein or RNA



antisense strand

RNAP II.

primary transcript
(heteronuclear) mRNA

processing

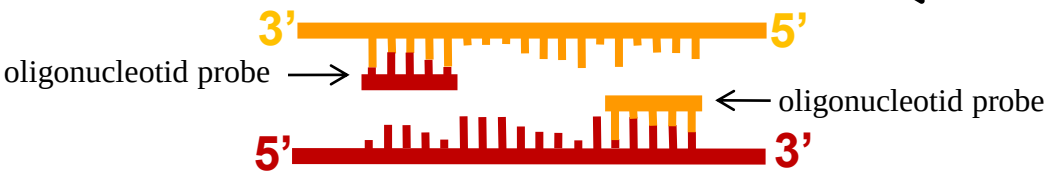
mRNA – **sense** = protein coding
sequence for translation

Reverse trasciptase

cDNA – **antisense strand**
synthesized

DNA polimerase
(heat resistant)

cloning of cDNA – **antisense and sense strands** multiplicated



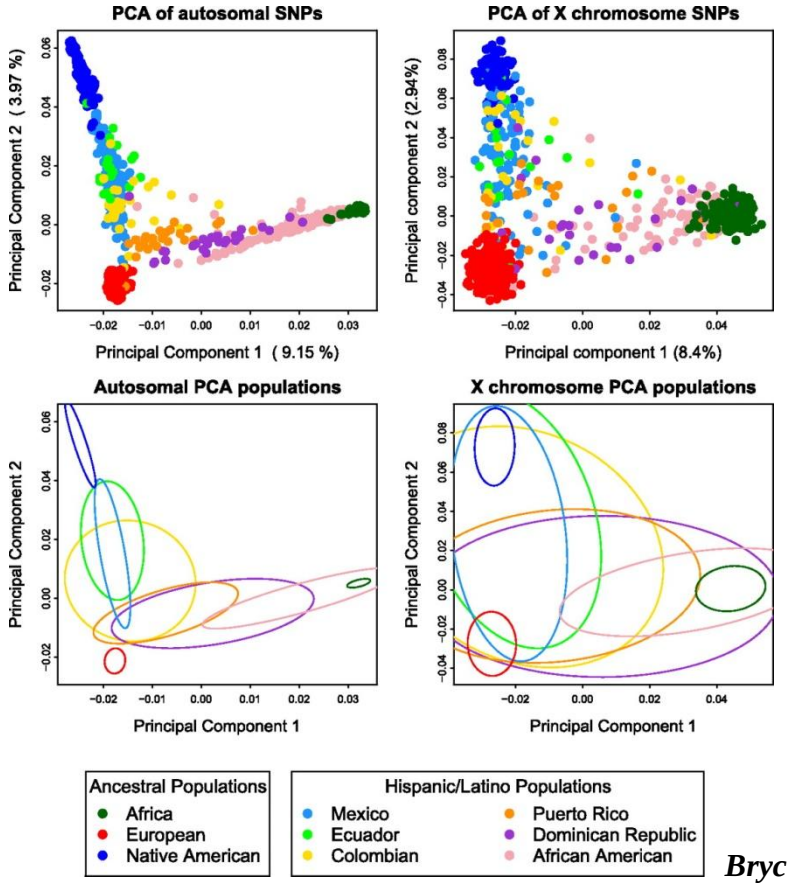
STUDIES ON GENE EXPRESSION – DNA CHIP TECHNOLOGY (DNA MICROARRAY)

- isolation of total RNA (maybe mRNA selection)
- synthesis of cDNA labeled with fluorochrome by reverse transcriptase
- hybridization of labeled probe with the chip
- computer-based evaluation of the fluorescence signal

Comparison of expression pattern of different samples by concurrent examination of several thousands of genes:

"high -throughput"

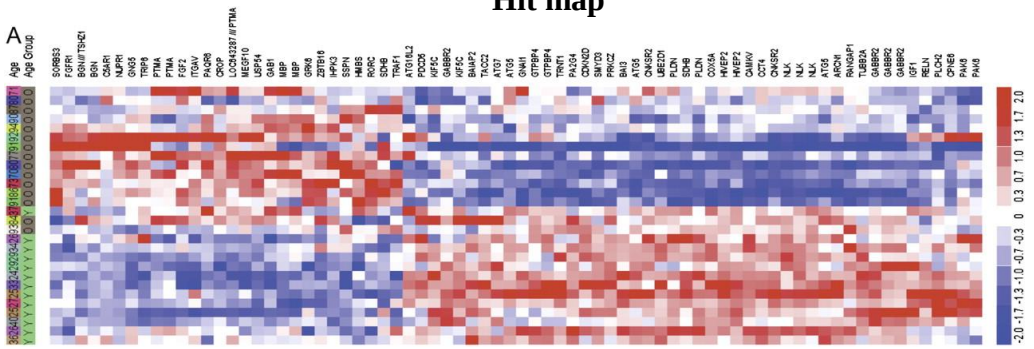
STUDIES ON GENE EXPRESSION – DNA CHIP TECHNOLOGY (DNA MICROARRAY)



Principal component analysis results

Presentation types of results

Hit map



Lipinski M M et al. PNAS 2010;107:14164-14169

Bryc K et al. PNAS 2010;107:8954-8961

GENE SEQUENCING- NEXT GENERATION SEQUENCING (NGS)

Gene **sequencing** refers to methods, by which the order of nucleotide bases – adenine, guanine, cytosine, and thymine – in the DNA molecule can be determined.

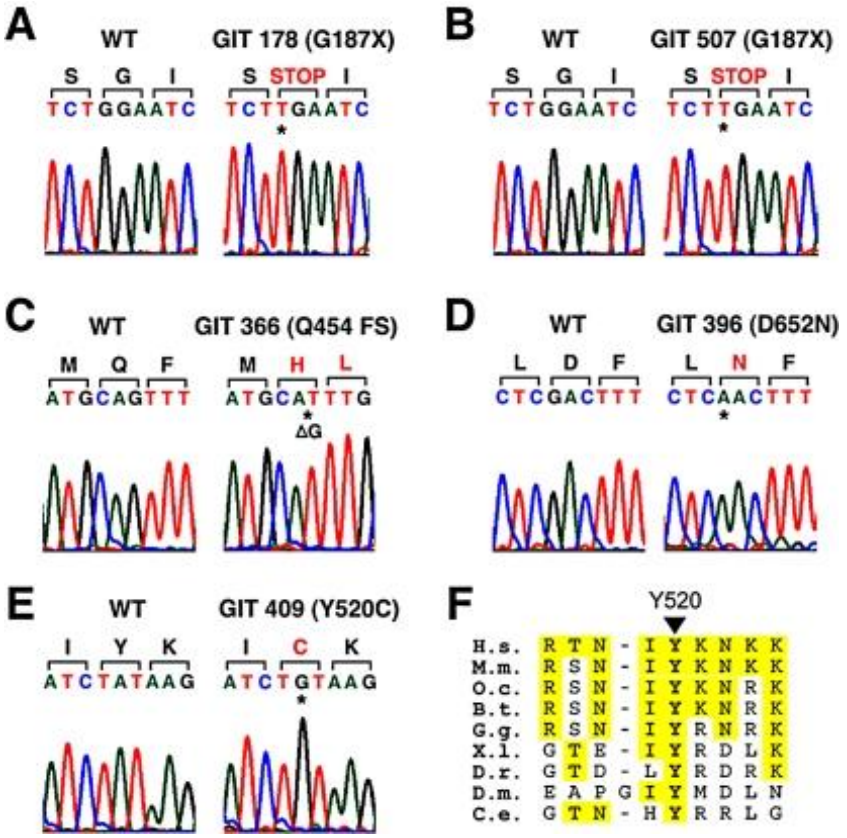
Human Genom Projekt – START in 1990

International Human Genome Sequencing Consortium Initial Sequencing and Analysis of the Human Genome. *Nature* /Feb 15/ 409, 860-921 (2001)

Celera Genomics The Sequence of the Human Genome *Science* /Feb 16/ 291(5507) 1304-51 (2001)

NGS – includes several new approaches to reduce the time required for sequencing.

Archon X Prize - intending to award $10 million to "the first Team that can build a device and use it to sequence 100 human genomes within 10 days or less, with an accuracy of no more than one error in every 100,000 bases sequenced, with sequences accurately covering at least 98% of the genome, and at a recurring cost of no more than $10,000 (US) per genome."



Choi M et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci U S A. 2009 Nov 10;106(45):19096-101.

STUDIES OF PROTEINS

Western blot

It is a technique, which examines proteins separated by electrophoresis and blotted onto membranes.

- sensitivity is dependent on antibodies available
- it is quantifiable

“low -throughput”

Proteomics

*It is a technique, which examines proteins separated by electrophoresis and analysed by **M**atrix-**A**ssisted **L**aser **D**esorption / **I**onization (MALDI) and **T**ime-**O**f-**F**light mass spectrometry.*

- high sensitivity
- critical point is control selection

“high -throughput”

STUDIES OF PROTEINS - PROTEOMICS

Genomics
Genome

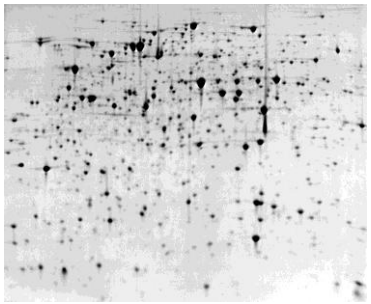
→

Proteomics
Proteome

GENOME of an organism's is more or less constant, the PROTEOME differs from cell to cell and from time to time!

Separation methods required to distinguish:

SDS 2D gel



1. proteins undergone post-translational modifications
   - phosphorilation – dephosphorilation
   - ubiquitination
   - methylation, acetylation, glycosylation, oxidation and nitrosylation.

2. proteolysis

BRAIN MAPS-mRNAs and PROTEINS

http://www.brain-map.org/

„The Allen **Human Brain Atlas** is a unique multi-modal atlas that integrates anatomic data (MRI,DTI, histology) and gene expression data (microarray, in situ hybridization).”

„The Allen **Mouse Brain Atlas** is an interactive, genome-wide image database of gene expression. ”

„The Allen **Developing Mouse Brain Atlas** provides ISH data across seven new developmental stages.”

Further projects:

- Developing human brain
- Non-human primate
- Mouse diversity
- Mouse spinal cord
- Glioblastoma
- Sleep