Q.B. 2001
(6)

YEAST ODISSEY IN MOLECULAR GENETICS

Theoretical and Practical Course

University of Buenos Aires, Argentina

Theoretical Section
March 08 - 10, 2001

Organizers:
Silvia Moreno, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
Carlo Bruschi: ICGEB, Trieste, Italy

THURSDAY, 08 March

09:30- 12:30

Preliminary talks to level the background of the assistants

In charge of Johan Thevelein's group from the Catholic University of Leuven, Belgium

Everything you should know before starting to work with yeast. **Johan Thevelein**

Expression analysis in yeast using differential display. **Katleen Lemaire**

Expression analysis in yeast using gene arrays. **An Tanghe**

Identification of protein-protein interactions by yeast two-hybrid analysis. **Barbara Leyman**

Analysis of the dual function of the yeast Tps1protein: regulator of glycolysis and trehalose biosynthesis. **Patrick Van Dijck**

Theoretical Section

14:45-15:45
The odyssey of *Saccharomyces cerevisiae*. From ancient fermentation to future nanotechnology. ***Carlo V. Bruschi***, *ICGEB, Trieste, ITALY.*

15:45-16:45
Five year of post-genomic life with 5555 yeast genes. ***André Goffeau,*** *Chaire Blaise Pascal, Ecole Normale Superieure, Paris, FRANCE.*

17:15-18:15
Functional Genomics: from genome to metabolome. ***Steve Oliver,*** *University of Manchester, UK.*

FRIDAY, 09 March

08:30-09:30
How many genes in genomes ? The yeast paradigm and the importance of comparative genomics. ***Bernard Dujon,*** *Pasteur Institute, Paris, FRANCE*

09:30-10:30
Quantitative approaches to the analysis of yeast gene function. ***S. Oliver***

11:00-12:00
Gap1 and the world of unknown functions in proteins with known function. ***Johan Thevelein***, *Katholieke Universiteit Leuven, Leuven-Heverlee, Flanders, BELGIUM*

14:30-15:30
Post-genomic analysis of multidrug resistance in *S. cerevisiae*. ***A. Goffeau***

15:30-16:30
Higher-order DNA structure of the recombinational yeast genome. ***C. V. Bruschi***

17:00-18:00
Yeast biotechnology. ***Patrick Van Dijck***, Catholic University of Leuven, BELGIUM

SATURDAY, 10 March

08:30-09:30
How to work with gene families ? The EUROFAN attempts and results. ***B. Dujon***

09:30-10:30
Genomic manipulation by selective chromosome loss via centromere knockout in yeast: a model for eukaryotic therapy of aneuploidy. ***C.V. Bruschi***

11:00-12:00
Sensing of glucose, "yeast's most preferred sugar". ***J. Thevelein***

14:30-15:30
Phylogenetic classification of 1001 membrane proteins from 15 hemiascomycetae.
A. Goffeau

15:30-16:30
Is there a lesson to learn from genomic maps ? Comparative genomics from yeasts. ***B. Dujon***

17:00-18:00
What makes a yeast species? Redundancy, recombination and reproductive isolation.
S. Oliver

Practical Section
March 12 - 23 , 2001

Organizers:

Silvia Moreno
Paula Portela
From Departamento Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Johan Thevelein
Patrick Van Dijck
From Catholic University of Leuven, Leuven, Belgium

Local teaching assistants:
Paula Portela
Silvia Rossi
Adalí Pecci
Susana Correa

Belgian teaching assistants:
Patrick Van Dijck
Barbara Leyman
Katleen Lemaire
An Tanghe

Technical assistance
Martine De Jonge

Daily work: 9-18 h

Experiment 1: DIFFERENTIAL DISPLAY

DD will be used to analyse differences in gene expression between yeast cells grown at 25°C, followed by an adaptation period at 37°C before shifted to 42°C. Also cells immediately shifted from 25°C to 42°C will be analysed.

Techniques that will be learned

- Isolation of mRNA
- Reverse transcription
- PCR analysis
- Running sequencing gels
- Northern blot
- Reverse northern blot
- Cycle sequencing

Students can bring their own material for this experiment
Outcome: two students brought their own material (from plant cells grown under different conditions, and from selected part of brain of rats trained for long memory assessment), plus yeast cells submitted to heat shock. The results with the plant material were highly successful.

Experiment 2: MICRO ARRAY ANALYSIS

In this experiment the commercially available 'Yeast Index Genefilters' (Research Genetics) will be used. These low-density arrays represent 6144 PCR-amplified ORFs of the baker's yeast genome on two nylon membranes. The same conditions or strains as for the differential display experiment will be used. This gives then a good opportunity to compare both techniques.

Techniques that will be learned

- RNA isolation
- cDNA preparation
- DNA hybridisaton
- Phosphorimager analysis
- Working with Microarray analysis softwares
- Northern blot analysis

Students can bring their own material for this experiment

Outcome: one student brought RNA from a yeast strain under two growth conditions of her interest; another brought RNA from a strain of Pseudomona under two growth conditions and brought membranes with array of E.coli genes (this experiment is not finished yet); another student brought the results of an array he had performed to analyse in the course; the rest of the students used a yeast strain submitted to heat shock. All of the arrays were successful, and every student had the chance to manipulate the information with the software.

Experiment 3- TWO HYBRID ANALYSIS

In this course the two-hybrid system will be used to screen a library of fusion proteins (fused to the AD domain) to find proteins that interact with a known protein that has been cloned in frame with the BD domain on a plasmid. Positive controls for each of the bait fusion proteins will be provided.

Techniques that will be learned

- Yeast transformation
- Different selection methods
- Recovery of plasmid from yeast strain

Students can bring their own gene of interest cloned to be used as a bait

Outcome: No student brought its own material. The belgian team brought 4 different baits of interest, and a library from restricted genomic DNA fused to the Gal4 activation domain. Confirmed positives were obtained with the four baits, although they were not sequenced. The results from one of the baits remained for the Argentine organizers.

Experiment 4- Random PCR mutagenesis

PCR mutagenesis is a very simple and fast method to isolate point mutations in a certain gene that can then be screened for a certain phenotype. This can be very interesting to find domains in a certain gene that are important for a certain characteristic of the protein.
In the course the mutagenesis protocol will be applied to a gene for which different screenings can be done in order to characterise the protein more in detail. In this way amino acids that are important for a certain phenotype are expected to be found.

Techniques that will be learned

- PCR analysis
- Yeast transformation
- Restriction digest
- Gel extraction
- Methods depending on the screening that is performed (replica-plating, heat stress experiments)

Outcome: the belgian group brought a gene of interest coding for trehalose phosphate synthase 1, in order to mutagenize randomly, looking for mutations that could separate the catalytic and regulatory regions of the protein; the screening was performed looking for different phenotypes. Several interesting positives were obtained.

SEMINARS

Each of the students must prepare a 20' seminar, in english, of his own work, results or projects.

FINAL ASSESSMENT

On the last day the students will be assessed formally in order to evaluate whether they have a comprehension of the whole course and the concepts acquired are correct.