General informations on transcriptome experiments with the “Complete Arabidopsis Transcriptome MicroArray”

General informations and conditions

The CATMA microarray is available through collaborations between the URGV and other laboratories. The partner needs to send a request to Jean Pierre Renou (URGV) via e-mail (renou@evry.inra.fr). The microarray production, labelling and hybridizations of the samples will be performed by the URGV team (Transcriptome Group). The URGV provides the know-how in this collaboration but does not have specific funding for that purpose. Therefore the consumables costs (230€ /hybridization, taxes not included) will be paid by the partner.
Specific tools for transcriptome analysis, including specific statistical methods are available at URGV. As soon as the analyses are performed a CD-rom containing the raw and analyzed data is sent to the partner. Then the transcriptome results will be integrated in the database developed at the URGV: CATdb (MIAME compliant: Brazma et al., 2001. Nat Genet. 29(4):365-71). The data will also be transferred in GeOmnibus (NCBI).
The data will be automatically released for public access after one year from the end of the project and their introduction into the databases.

The CATMA microarray

The CATMA microarray contains 34600 spots (version 5). The probes are 150 to 600 bp amplicons specific of each gene (Gene Specific Tags) based on the EuGene annotation. All the information related to the GSTs are available through FLAGdb++:

Sample preparation

The labelling process includes an in-vitro transcription step (T7 pol.) so the RNA purity is the most critical factor of the success. Therefore the RNAs have to be extracted with a column extraction kit (such as Qiagen or Promega kits for example). The samples will be controlled for quality and quantity by the Agilent bioanalyzer and ribogreen at URGV. Generally, 10µg of total RNA per sample are sufficient for running the experiment. If “difficult” samples, such as seeds for example, have to be used, please contact us for extra precautions.

Repetitions

Biological repetitions: At least two independant biological replicates are necessary.
Technical repetitions: We perform systematically one dye-swap per biological replicate:

Ex: - slide 1: Control Cy3 – Treatment Cy5
    - slide 2: Treatment Cy3 – Control Cy5

Therefore each comparison (samples1 versus sample2) will need four hybridizations.
Procedure

The protocols are available at http://www-urgv.versailles.inra.fr/

Summary of the protocol:

- Checking of the total RNA (Bioanalyser Agilent and ribogreen).
- Reverse Transcription (1µg total RNA) with an oligoT-T7promoter.
- Purification and in vitro transcription (T7 polymerase): production of 80µg of aRNA or so.
- Purification and RT (5µg of aRNA) with Cy3 or Cy5-dCTP.
- Purification, quantification of the fluorochromes, 30 pmoles of each labelled sample are used per slide.
- Prehybridization of the slides after « post-processing ». Hybridization at 42° O/N with formamide.
- Washing and drying of the slides.
- Scanning with constant PMT (photomultiplicators): the data normalization is performed at the statistical analysis step.

After the statistical analysis the gene lists contain the log2 normalized intensities per samples, the log2 normalized ratios, and the p-values (Bonferroni or FDR correction) which provide the threshold for the identification of the differentially expressed genes.

Publication of the results

In this scientific collaboration the URGV provides the know-how and expertise in transcriptome analysis including statistics, and advice in experimental design if necessary. It also provides the possibility to compare your results with the whole CATMA resources of URGV as a help to identify your favorites genes. Only the costs of the chemicals are paid for by the partner. For this reason at least one member of our team will be co-author of the first paper reporting these transcriptome results.

Document to be sent with the request

We need a short document containing:

1 – PROJECT TITLE
2 – Name address, tel number and e-mail address of the partner
3 – Description the project (briefly)
4 – Experimental design: nb of comparisons
5 – Number of slides, samples description (organ, harvesting stage according to Boyes et al. Plant Cell 2001, treatment…)
6 – Expected date of sample shipping

When the project will commence the collaborators must fill the document (submission_colleague.html, downloadable from this website) describing the samples needed for database submission.