Microarray and sequencing platform

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PRODUCT SHEET: TRANSCRIPTOME ANALYSIS ON AFFYMETRIX MICROARRAYS

1 Affymetrix microarrays

Affymetrix GeneChips are high density oligonucleotide arrays used to monitor gene expression through a one-color hybridization setting, where each sample to be compared is hybridized on a separate microarray. On the chip, each gene is typically represented by one or several probe set(s) consisting of 25 mer oligonucleotides covering features of the transcribed region of that gene. Different microarray types are available depending on the total number of oligonucleotides representing one gene and on the region these probes target on the transcripts.

1.1 3' arrays

On these first generation arrays existing for more than 20 species, each transcript is questioned by a set of 11-16 distinct oligonucleotide pairs preferentially directed against the 3' end of mRNA, close to the polyA tail. Each probe pair consists of a "perfect match" complementary to the sequence of interest and an oligonucleotide "mismatch" identical to the corresponding "perfect match" oligonucleotide with the exception of the central base which has been mutated. The oligonucleotide "mismatch" is utilized to detect and eliminate nonspecific signal.

1.2 Gene arrays

On these whole-transcript arrays available for more than 20 model organisms, each transcript is interrogated by a set of 17-26 probes "perfect-match". These oligonucleotides are distributed over the entire length of the genomic locus. On the latest version of those chips (2.0 ST), probes question the known long intergenic noncoding RNAs (LincRNAs) in addition to mRNA.

1.3 Exon arrays

These arrays, available only for human, mouse and rat, were designed for expression profiling at gene-level as well as at exon-level for alternative splicing analysis. Most exons of a size > 100 b are represented by at least one probe set consisting of four oligonucleotides "perfect match", with a total of > 30 probes for the majority of transcripts.

1.4 XTA arrays

On these high-resolution arrays, available only for human, mouse and rat, probes cover coding and non-coding transcripts as well as known exon-exon junctions. Each gene is interrogated on average by a set of 109 probes (70 on rat array), each exon by a set of 10 probes and each junction by 4 probes.

1.5 Clariom S arrays

These arrays of last generation exist only for human, mouse and rat. Built on the model of Gene arrays but with an updated content, they aim at covering all the genes very well annotated and listed this day by the following databases: HUGO (HUMAN), MGI (Mouse), RGD (rat).

1.6 Clariom D arrays

These arrays, available only for human, mouse and rat, represent the last high-resolution version of Affymetrix GeneChip with a design similar to XTA arrays. The content of Clariom D arrays for mouse and rat are similar to that of MTA 1.0 and RTA 1.0 arrays, respectively. On the other hand, the Clariom D array for human is a

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major evolution of the HTA v2.0 array. It presents in particular an advanced content for interrogating Lin-cRNAs and a content, at present hidden but which will be accessible in the year 2017, for the analysis of more than 600 gene fusions.

2 Target preparation protocols

Affymetrix GeneChips use biotinylated cRNA or cDNA as target. Different target preparation protocols are currently in use on the platform. The choice of the most appropriate method for a project mainly depends on the amount of starting total RNA and the type of microarray selected, as described in following table.

Kit used	Total RNA quantity		Array types
	Minimal	Optimal	
MessageAmp™ Premier RNA Amplification Kit, Ambion	100 ng	600 ng	3' arrays
WT Expression Kit, Ambion	100 ng	600 ng	Gene, Exon and XTA arrays
GeneChip® WT PLUS Reagent Kit, Affymetrix	100 ng	600 ng	Clariom arrays
GeneChip® Pico Kit, Affymetrix	100 pg	50 ng	Clariom arrays
Ovation Pico WTA System v2, NuGEN	500 pg	20 ng	All array types

If you want to compare your data with a previously generated dataset, we recommend choosing the same protocol and the same array type if available.

3 Services provided

1. Sample validation: quantification and quality check by spectrophotometry (Varioskan) and/or capillary electrophoresis (Bioanalyzer).
2. Biotinylated target preparation using protocols listed above.
3. Hybridization, washing and scanning of the arrays following Affymetrix recommendations.
4. Data extraction, normalization and summarization of signal value using Expression Console software from Affymetrix.

4 RNA samples preparation by the project manager

The project manager prepares total RNA samples. The quality of microarray results is closely related to the initial sample quality. Thus, precautions must be taken to avoid any contamination (Phenol, DEPC, genomic DNA, etc.) or degradation.

It is also very important to include biological replicates (>3) in your experimental plan. A randomized and balanced design is recommended. We also encourage preparing all samples to be compared at the same time, to reduce batch effects. If needed, our team offers to assist you in every step of establishing up your project.

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Characteristics of total RNA to be provided	
Quantity	Depends on the sample preparation protocol.
Quality	OD260/OD280 ≥ 1.8. No degradation on agarose gel or RIN ≥ 7 on a Bioanalyzer profile.
Shipping conditions	In solution in water, on dry ice. Tubes must be clearly identified and provided with a document describing the concentration and total volume of each sample.

5 Quality controls performed by the platform

Results arising from quality controls listed below are sent by e-mail to the project manager after each experimental step.

1. Sample validation	
Quantity	> minimal required quantity. Depends on the protocol used.
Quality	OD260/OD280 ≥ 1.8. RIN ≥ 7 on a Bioanalyzer profile.
2. Biotinylated target validation	
Target yield	≥ 20 µg for starting RNA quantity ≥ 100 ng. ≥ 5µg for starting RNA quantity ≤ 20 ng.
Target size	Peak ≥ 1000-1500 bp for starting RNA quantity ≥ 100 ng. Peak ≥ 500-900 bp for starting RNA quantity ≤ 20 ng.
3. Hybridization quality on 3' arrays	
BioB hybridization spike	"Present" in ≥ 50% of the samples of a project.
BioC, BioD, Cre hybridization spikes	"Present" in 100% of the samples of a project.
Average background	≤ 150.
Noise (pixel to pixel variation)	≤ 4.
Percentage of gene detected	≥ 20-30% and varying less than 10 to 15% between equivalent biological samples.
Global array intensity (scaling factor)	Varying less than 2 to 3 times between equivalent biological samples.
4. Hybridization quality on Gene, Exon, XTA and Clariom arrays	
Intensity of control polyA spikes added to starting total RNA samples (optional)**for**	Ilys<Iphe<Ithr<Idap.
Intensity of hybridization spikes**' a**	IBioB< IBioC<IBioD<ICre.
Pos vs Neg auc	≥ 0.80.

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[all probe set rle mean]max – [all probe set rle mean]min	≤ 0.3 between equivalent biological samples.
All probeset mad-residual mean	≤ 0.65.
Percentage of gene detected (only for Exon arrays)	≥ 20-30% and varying less than 10 to 15% between equivalent biological samples.

6 Results delivery

The project manager is informed by email of the availability of his microarray data on an IGBMC FTP server. The following data are provided for each project:

- Affymetrix raw data files (ARR and CEL files).
- Normalized and summarized expression results (CHP files) in TXT or XLS format.
- A copy of all quality controls performed during the project.
- A copy of the reference protocols used as well as a brief "Materials and Methods" for publication purpose.

According to the "Microarray and Sequencing Platform terms and conditions of business", the project manager is responsible for saving and archiving his data on his own. Following the delivery, our Platform can guarantee the conservation of raw data only for a limited period of six months.

7 Downstream analysis (optional)

Differential gene expression analysis or alternative splicing study are not part of the standard microarray services but can be done in collaboration with members of the platform. We recommend the project managers who would like to collaborate for data analysis to contact us before starting their experiment so that we can define the analyses that best fit their needs.