PLATFORM' FUNCTIONING AND RECOMMENDATIONS

The Biochips Platform can carry out your experiments from probes spotting on glass slides up to the analysis of generated images and put at your disposal some of its equipment. Before beginning any project, it is required to contact the manager Véronique Le Berre (leberre@insa-toulouse.fr).

In order to better answer your needs, we first define together the content of your project, i.e. all the experiments that will be conducted. After discussion, you need to fill the part of the project form that lists the experiments that you want us to carry out, sign it, and send it to us along with your samples.

The first step will be the control of your RNA samples. If they are correct, you will be notified with a quotation. If they are not correct, your will be informed about the problem (e.g. RNA degradation, DNA contamination…) and we will ask you to send new samples that should guarantee the success of your project.

Once this step is committed, you have to send us the purchase form for definitive proof of payment. Your experiments are then scheduled and conducted, and you are sent the results. Obviously, if at a certain stage we meet difficulties, you will be contacted and we will both discuss to find the best solution for the remaining tasks.

Chips confection

Different DNA chips bearing whole genomes of biological systems are already at your disposal at the Platform (e.g. yeast, rat, Ralstonia, etc…).

We may also elaborate your own customized chips after defining their content, the biological system, the probe types, the spotting number, the spotting buffer, type and amount of chips to produce.

Alternatively, you can provide the probes to spot, as well as the support slides for the chips specific to your studied organism.

The spotting machine used for the spotting of oligonucleotides or cDNA on functionalized glass slides is the « QArray mini » from Genetix. It is equipped with 48 pins maximum, empty (Telechem SMP_3) or flat (Telechem SSP015).The slides used are a bar-coded allowing a unique identification of each slide and an orientation of the slide.

Typically, slides are spotted with the barcode down (facing the floor).

The « *.gal » file (Spotting plan of the probes on the chip) is available and downloadable on the Platform website.

Its exact address is indicated on the shipping receipt, along with the slides when sent. For all inquiries about the « *.gal » file, please contact the Production manager Lidwine Trouilh (lidwine.trouilh@insa-toulouse.fr) or the informatics manager Delphine Labourdette (delphine.labourdette@insa-toulouse.fr) of the Platform.

Quality control of the spotting

Quality of the spotting is determined by:

* A first scan right after deposition. This scan is done on each slide before and after reloading of the pins

* A Panomer test that allows checking for the presence of oligos, the global intensity, the shape of spots, as well as the absence of signals from negative spots (no oligos) and the background intensity. It consists in hybridizing the last slide of the batch with the « Panomer™ 9 random oligodeoxynucleotide, Alexa Fluor® 555 conjugate » (Ref. P21687 from Invitrogen).

According to the quality control test defined above, the batch of slides is validated when 95% of the expected spots are present.

The last slide of the batch is stored at the Platform in order to carry out additional control tests if needed.

Slides storing

We recommend that you store the slides in a dessiccator at ambient temperature and use them within the year upon reception.

RNA Extraction and quality control

We do not extract RNAs ourselves but you can find on the Platform website some tested protocols that are in current use by various laboratories on different biological systems. RNAs have to be shipped frozen in water.

Quality controls made on « RNA 6000 Lab-on-Chip » chips from Agilent (Bioanalyzer) and on the « Nanodrop™ » allow qualitative and quantitative validation of RNAs.

RNAs are first controlled on the « Nanodrop™ ». The minimum requirements are :

* **260nm/230nm and 260nm/280nm ratios > or equal to 1.8**

* **A minimal concentration of sample** required for the chosen labelling (indicated in the usage recommendation of the kit provider)

If the RNAs pass the «Nanodrop™» test, they are then controlled with the « Bioanalyzer » on AGILENT « RNA 6000 Lab-on-Chip » chips.

A standard quality RNA, useable in a biochip experiment must have :

* **A 28S/18S ratio > or equal to 1.7 for Eukaryotes or a 23S/16S ratio > or equal to 1.2 for Prokaryotes**

* A **RIN (RNA Integrity Number) > or equal to 9 for cells and > or equal to 8 for tissues** (true only for Eukaryotes)

Labelling and labelling control

We use the «ChipShot™ Direct Labeling and Clean-Up System» (Ref. Z4100 from Promega) and « QuickAmp Labeling Kit» (Ref. 5190-0444 from Agilent) Kits.

For Promega Labelling, an amount of 5 µg (i.e. a minimum concentration of 300ng/µl) is required. Agilent labelling features an amplification step and is effective only for polyA RNAs (for Eukaryotes), requiring a minimal amount of 300ng of RNA.

Each labelling is controlled using the « Nanodrop™ » in order to determine the quantity of produced cDNA (or cRNA) quantity and the quantity of incorporated fluorochrome.

Hybridization

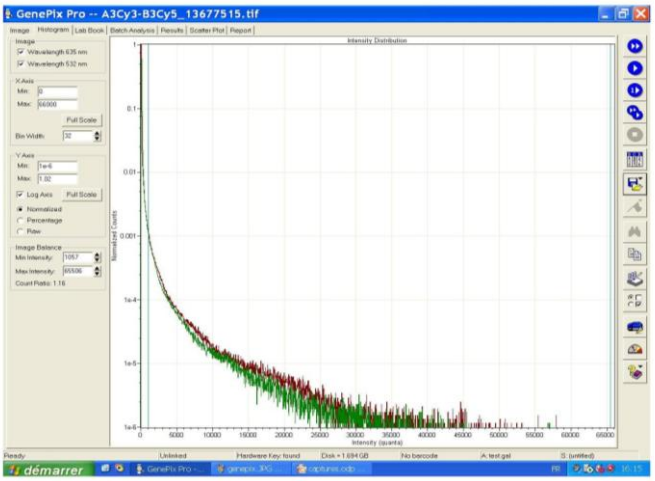
Hybridizations are carried out at the Plateform in automatic hybridization chamber (« Discovery » from Ventana) or Agilent oven following the specifications of these manufacturers.

If you wish to carry out yourself the hybridizations in your laboratory, please consult the instruction manual relative to the labelling kit you are using.

Scanning

We recommend the scanning of slides choosing scanner PMTs in a way that intensity curves in the red channel and the green channel are as closely superposed as they can be and that these curves « die off » around 50000 on the x-axis so that any saturation effect is avoided, with the widest dynamics panel possible.

Superposition of curves :



PLATFORM' FUNCTIONING AND RECOMMENDATIONS

Image analysis

Image analyses can be done at the Platform by the Informatics manager Delphine Labourdette (delphine.labourdette@insa-toulouse.fr) or by yourself, after training with Delphine Labourdette. Softwares at your disposal are either GenePixPro (Axon), Mapix (Innopsys), FeatureExtraction (Agilent), or XDotReader (Cose) for membranes. The outputs are results' files and images with .jpg extension, that can be read by the software of your choice. These data can also be pre-formatted for interpretation using softwares BioPlot/Bioclust available on the Platform website.

Important notes

- Samples are stored on the Platform during project duration, in « -20°C » freezers and coolers. The person in charge of the project is invited to get the samples back at the end of the project, unless they will be stored for maximally a month and then thrown away
- The temperature of our freezers and coolers is raised weekly, but they are not connected to an alarm system.
- Our micro-pipettes are annually checked by an independant company.
- In average, from the time when RNA quality control is validated, experiments are carried out within 4 weeks.
- Storing duration of scanning data and image analyses is respectively 1 year and 5 years for BioPlot data. Beyond, data will be archived on DVD.
- Thank you to confirm that you have received you data and/or samples.
- Thank you to have a look at the emergency map.