Gentyane

Genotyping, sequencing and optical mapping platform in Auvergne

Presentation of our services

Presentation of the platform

The platform offers a wide range of technologies, from Microsatellite Genotyping (SSR) with ABI 3730XL 96 capillary sequencer, to Single Nucleotide Polymorphism (SNP) genotyping, in simplex (KASPar chemistry on LC480 Roche, in microfluidics on the Biomark Standard Biotools), or in multiplex (Thermoscientific Axiom chip hybridization technology and AgriSeq technology). The other part of its activity is dedicated to NGS Sequencing, with the PacBio Sequel II sequencers that allows the sequencing of HiFi reads around 15 kb, full-length RNA (Iso-Seq), microbial multiplexes or amplicon pools. Recently the platform acquired the Saphyr of Bionanogenomics for the realization of optical mapping.

Critical equipment has been identified. They are under maintenance contract, or maintenance is scheduled and is performed by authorized platform personnel.

Description of the service

A discussion with the manager makes it possible to optimize the analysis strategy according to the needs of the client and the available tools. This exchange leads to a service request form which serves as a basis for an estimate.

After acceptance of the quote, a sheet of recommendations for the preparation and sending of the samples is sent to the client in order to inform him of the precautions to be respected.

A code is assigned to the service and is noted on the transfer form and on the tube / plate at each stage. This project code is to be recalled in the subject of each of your email. A member of the platform team is identified as responsible for the analysis. The expertise of the staff of the platform allows them to carry out a first level control of the results.

At the end of the service, a report of the operations is carried out by the team and a pre-invoice is established. This pre-invoice is sent to the customer who will send us a corresponding order form.

In order to constantly optimize our services, a customer satisfaction questionnaire will be sent annually.

Services

DNA extraction for plant sample

DNA extraction **DNA extraction from blood or animal tissues**

1. DNA extraction from fresh or frozen blood
2. DNA extraction from animal tissues

SNP genotyping

1. KASPar chemistry read on the LC480 Roche
2. KASPar chemistry read on the Biomark Standard Biotools
3. Hybridation on Axiom Thermoscientific
4. Genotyping by sequencing : AgriSeq Thermo

SSR genotyping

SSR genotyping on ABI3730XL

Sequencing

Pacific Biosciences Sequel II

Expression study on fluidigm chip

Optical mapping on BionanoGenomics Saphyr

Provision of platform hardware

How to access platform material for permanent and non-permanent staff of the unit.

DNA extraction

Plant DNA extraction

We control the plant material by ensuring that it meets the requirements of the platform. If it does not comply, we contact the customer to define the course of action.

Shearing

The plant material is mechanically sheared to obtain a very fine powder necessary for the DNA extraction. If it is fresh material, we work in liquid nitrogen. For freeze-dried plant material, we work at room temperature. It is important to note if wells have broken wells during grinding because they will not produce results and will be considered as missing data. This information is reported to the customer who decides to send us back material, or to continue the extraction.

Extraction

DNA extraction from fresh or freeze-dried plant material is performed using the sbeadex Livestock kit or NucleoMag® plant Macherey Nagel. We proceed to the cell lysis step by adding the lysis buffer into the samples. The extraction is completely automated (i7 Beckman or Vantage Hamilton). The volumes of reagents are controlled. The DNA binds to the magnetic beads, undergoes wash cycles and is then eluted in the elution plate. Then we proceed to the DNA assay, this allows us to quantify the samples. The DNA quantification can be done in two ways:

- In absorbance on a nanoquant
- Fluorimetry using a fluorescent molecule (Hoechst: DNA intercalator)

The reading of the optical densities is done using a spectrophotometer plate, the Tecan SPARK (under maintenance contract).
Following the assay, samples with a low amount of DNA are checked on agarose gel.
We then normalize the DNA to the desired concentration according to the technique used.

Blood or animal tissue DNA extraction

We control the material by ensuring that it meets the requirements of the platform. If it does not comply, we contact the customer to define the course of action.

Extraction

For blood, we use the Genfind kit from Beckman Coulter. We proceed to the cell lysis step with a lysis buffer containing proteinase K. The samples are then placed on Beckman's I7. The extraction is completely automated. The volumes of reagents are controlled. The DNA binds to the magnetic beads, undergoes wash cycles and is then eluted in the elution plate.
For animal tissues, we use the DNAdvance kit from Beckman Coulter or the Macherey Nagel NucleoMag® tissus kit. We proceed to the cell lysis step from a lysis buffer containing proteinase K and DTT. The samples are then placed on Beckman's I7. The extraction is completely automated. The volumes of reagents are controlled. The DNA binds to the magnetic beads, undergoes wash cycles and is then eluted in the elution plate.
Then we proceed to the DNA assay to quantify the samples. It can be done in 2 ways:

- In absorbance on a nanoquant
- Fluorimetry using a fluorescent molecule (Hoechst: DNA intercalator)

The reading of the optical densities is done using a spectrophotometer plate, the Tecan SPARK (under maintenance contract).
Following the assay, samples with a low amount of DNA are checked on agarose gel.
We then normalize the DNA to the desired concentration according to the technique used.

SNP genotyping

Genotyping by KASPar Chemistry coupled with LC480 reading

Description

KASPar chemistry allows SNP genotyping in simplex. It is based on the use of assays comprising 3 primers: 2 being specific alleles and the other common. The specific amplification of each allele is then coupled to a particular fluorescence emission. Fluorescence is detected by Roche's Light Cycler 480 via an end-point reading protocol to acquire genotyping data.

Manipulation

Reception of the samples

When the samples are received, a visual and / or technical control of the conditioning of the samples and the samples themselves is done. In the case of non-extracted material, the correspondence between the plate plans previously supplied and that observed for the material received is evaluated (correspondence with the empty wells). We also visually assess the quantity and quality of the material received.

For samples where the DNA is already extracted, we check the volume of DNA contained in the plates (visually and by pipetting if necessary). A message is always sent to inform the customer of the sample reception, and if one of the criteria is not respected, we then contact the customer to agree together on the procedure to follow: either a new shipment will be made or we continue handling.

DNA distribution in 384 plates

The DNA is distributed in white plates 384 with a pipetting platform.

Preparation and distribution of PCR mix

Primer mixes and PCR mixes are manually prepared and then distributed into the plates containing the DNA in a robotic manner.

PCR

The amplification is carried out on thermocyclers according to a predefined program.

LC480 reading

The acquisition of fluorescence data takes place on Roche's LC480. We visualize the profiles obtained to determine if it is necessary to add cycles. If the results do not seem satisfactory to us, the customer will then be contacted to define the course of action to be taken. PCR plates are discarded once the link is forwarded to the customer.

Genotyping with the KASPar chemistry coupled with a reading on the Biomark or Biomark X Standard Biotools

Description

KASPar chemistry allows SNP genotyping in simplex. It is based on the use of assays comprising 3 primers: 2 being specific alleles and the other constant. The specific amplification of each allele is then coupled to a particular fluorescence emission. The DNA and reagents necessary for amplification are distributed in IFCs (Integrated Fluidic Circuits) available in 2 formats (48 x 48 or 96 x 96). The amplification and detection of the fluorescence signal are carried out in the Fluidigm Biomark.

Manipulation

Reception of the samples

When we receive the samples, we make sure that they meet the requirements defined in the "recommendation sheet for the preparation and sending of samples" by carrying out a visual and / or technical control of the conditioning of the samples and the samples themselves. We also check that the plate contains an empty well which will serve as a negative control. In the case of non-extracted plant material, the correspondence between the plate plans previously supplied and that observed for the material received is evaluated (correspondence between the empty wells). We also visually assess the quantity and quality of the material received.

For samples where the DNA is already extracted, we check the volume of DNA contained in the plates (visually and by pipetting if necessary) and quantification of several samples taken at random in the plate (usually on 1 / 6th samples) by a UV dosage. If one of the criteria is not respected, we then contact the customer to agree together on the procedure to follow: either a new shipment will be made, or we continue handling.

Mix preparation

The different mixes are prepared manually in a plate 96.

Chip preparation

After verification and oil injection, the chip is pressurized.

The chip is either used within one hour to be charged, or it can be stored 24h at 4 ° C. In the latter case, it is pressurized again before use.

The different mixes are distributed into the chip. No bubbles should have been generated, these are sources of missing data.

PCR

Amplification is performed on the Biomark or Biomark X (Standard Biotools).

Before launching the chip, we clean it to avoid the presence of dust, hindering reading.

Reading on the Biomark

Fluorescence data acquisition takes place on the Biomark or Biomark X.

We then visualize the profiles obtained to determine if the amplification worked well, considering the fluorescence signal intensity and the good separation of the clusters obtained for the samples and that obtained for the "negative" control well. If the results do not seem satisfactory (poor separation of clusters), the client will be contacted to define the course of action.

The chips are discarded after reading.

Ultra High Speed SNP genotyping Axiom Thermoscientific Genetitan reading

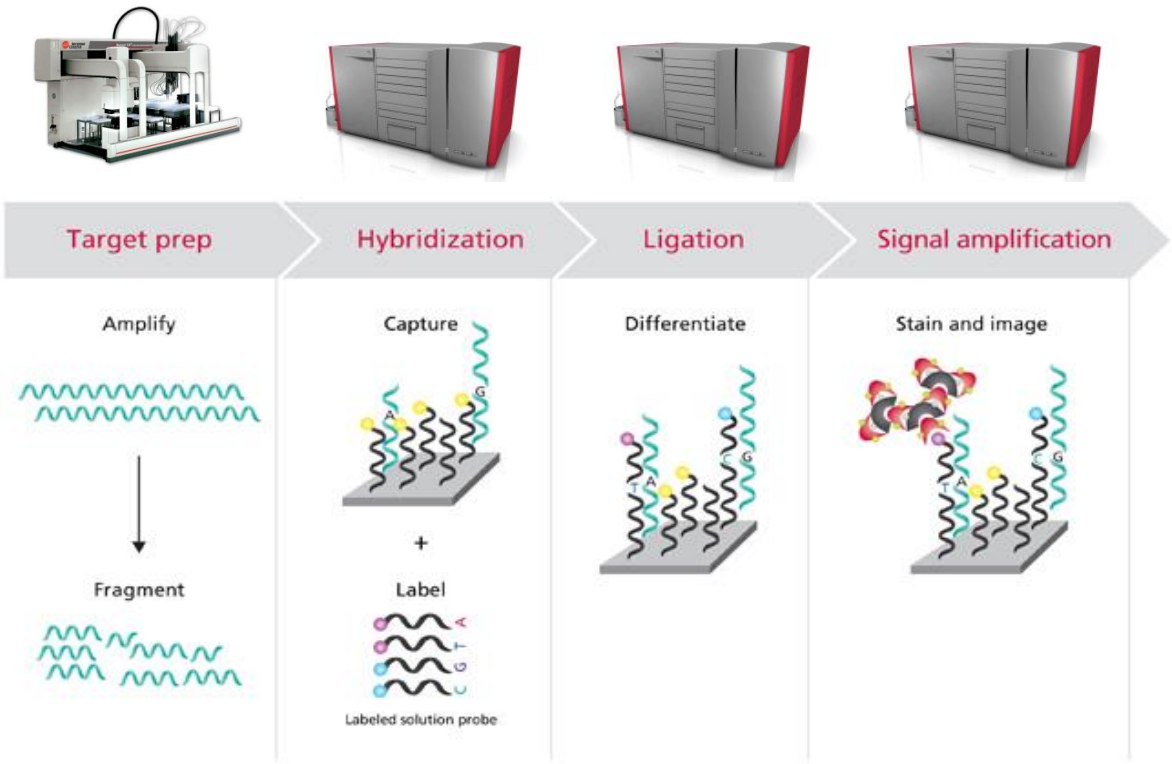
Description

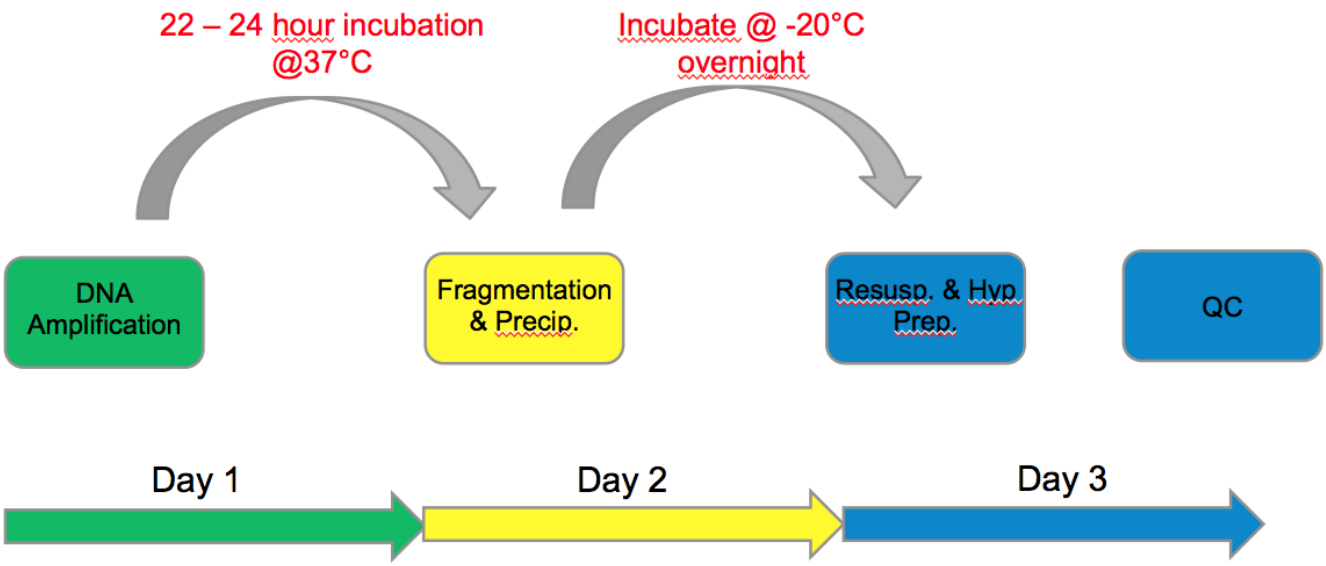
Axiom technology enables SNP genotyping by on-chip hybridization. It relies on the use of GeneChip provided by

Thermo Fisher, each depends on the species. Thermo Fisher has a catalog of chips but also allows the synthesis of microchip. Two chip formats are available, 96 and 384 individuals genotyped per chip and 1500 to 2.6 million SNPs per individual. The chips are read on Genetitan ™ at a rate of 3072 samples per week. The preparation of the samples before hybridization is automated on the FxP (Beckman).

With the support of Thermoscientific application engineer, we offer the training to our customers for the analysis of their results. The analysis is done on Axiom analysis suite.

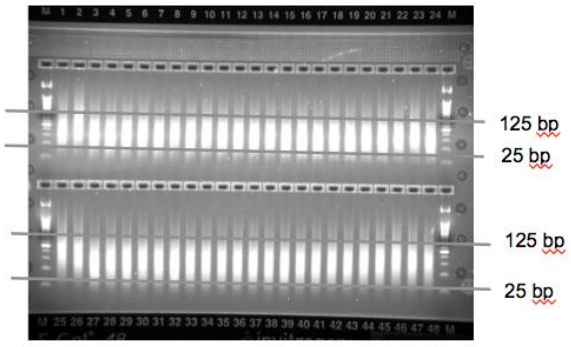
Manipulation





✓ DNA Fragmentation

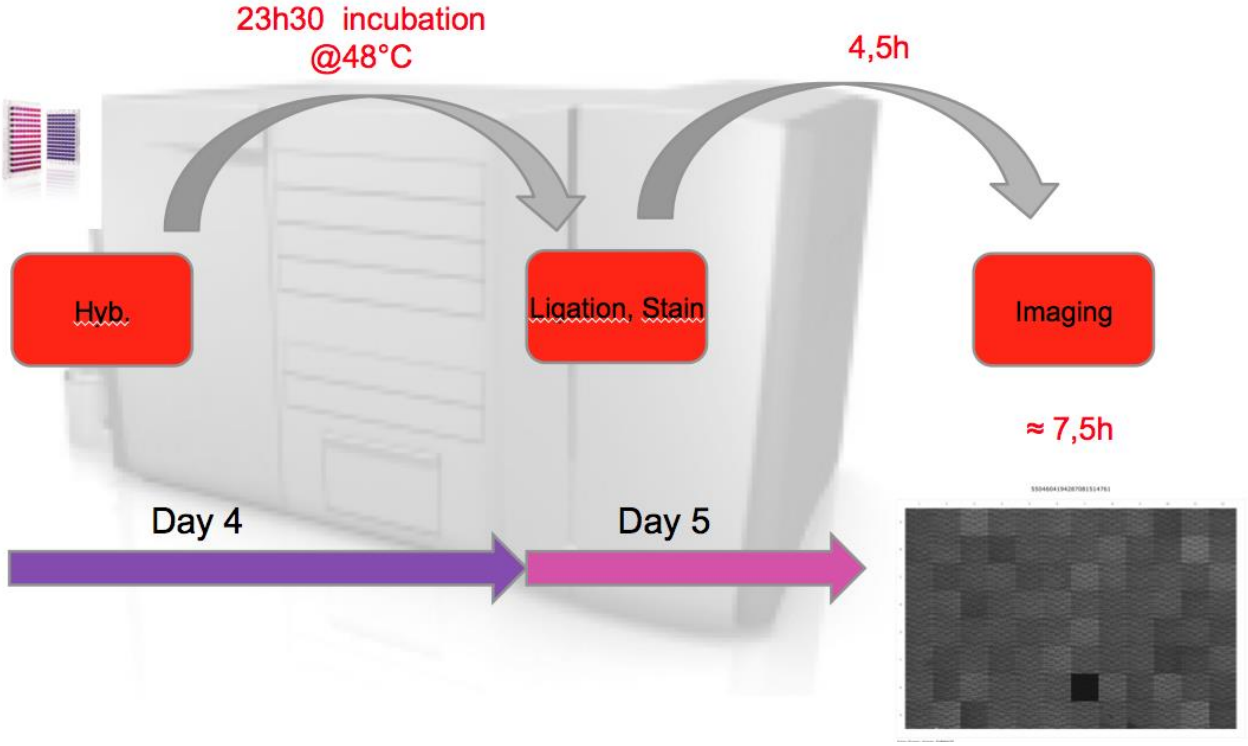
Use 4% E-gel, DNA should be between 25bp – 125 bp



✓ DNA Yield

OD 260 nm, 280 nm and 320 nm on Tecan Infinite 1000

The yield must be > 1200µg


23h30 incubation
@48°C
4,5h
Hyb.
Ligation, Stain
Imaging
≈ 7,5h
Day 4
Day 5

Genotyping by sequencing : AgriSeq ThermoFisher

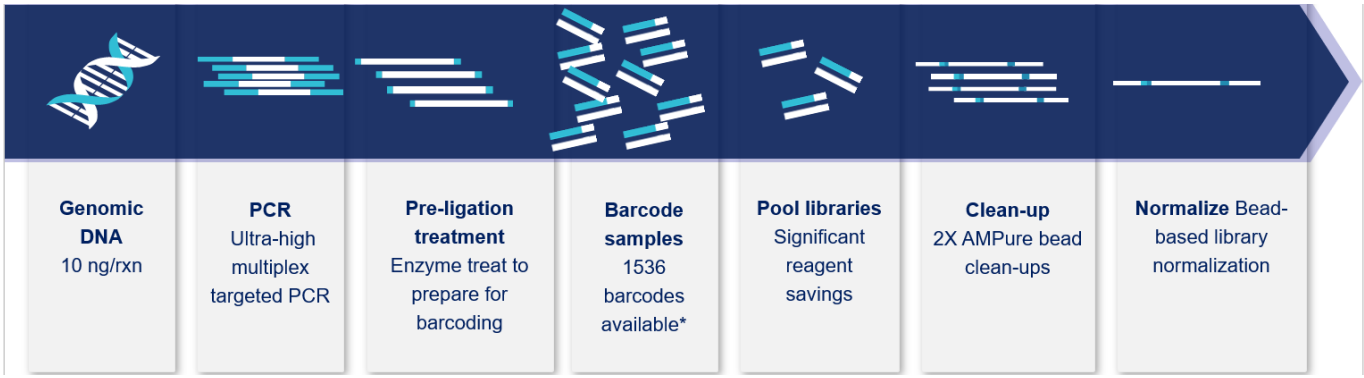
Description of the technology

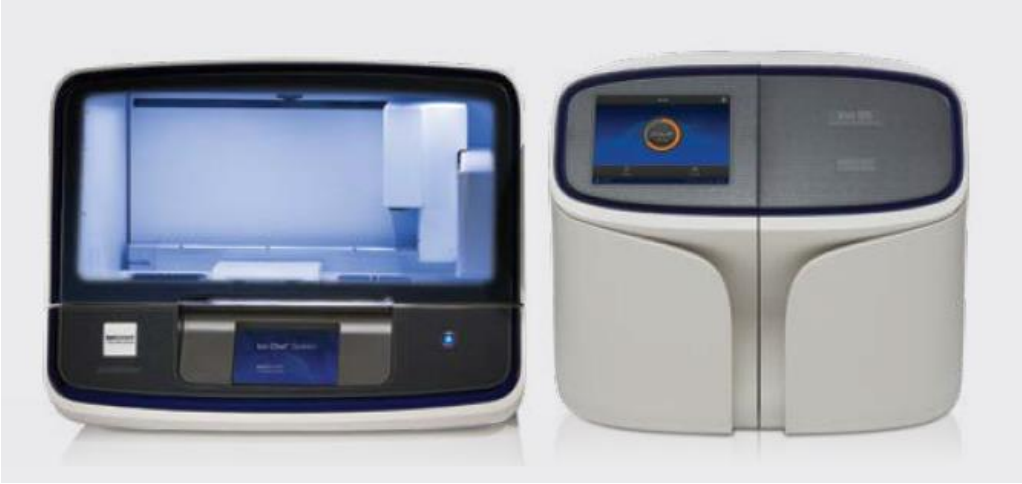
AgriSeq technology enables SNP genotyping by sequencing. After sending the genome sequence of interest to Thermo, a panel (from 150 to 5000 markers) of oligos is synthesized for 6000 samples. The maximum number of samples genotyped simultaneously is 1536 (number of possible barcodes). The samples are amplified in multiplex (amplification from 125 to 400bp) then barcoded and pooled and the library is prepared on the Ion Chef and sequenced with the GeneStudio S5 Prime.

The analysis of the results is done with the AgriSum software, and the genotyping matrix is provided to the customer.

Operations

Steps of the library preparation and sequencing





Data analysis is done with AgriSum Toolkit

SSR genotyping

Direct or M13 labeling in 384 plate

Dilution plates preparation

For SSR labeling, the DNAs must be at the concentration of 10 ng / µl and will be diluted or concentrated by evaporation if necessary.
If we carry out the extraction, the samples are systematically measured in absorbance.
If the samples are supplied by the customer, they are dosed on demand.

Preparation of work plates in 384

The work plates are prepared on the robot which distributes the samples in the desired order and noted with the date and the delivery number. We check that the volume is homogeneous otherwise we restart the distribution.

Preparation and distribution of the PCR mix

The PCR mix is prepared according to the technique used, either in fluo M13 or direct marking. This mix is distributed by the robot according to the selected program. We control the volume distributed, if all the wells are filled, if not we correct the volume. The plate is placed in a thermocycler to perform a DNA amplification that takes place in several stages:

- denaturation of the DNA: this is the separation of the two strands of DNA, obtained by raising the temperature to 95 ° C.
- Hybridization: by lowering the temperature to a temperature specific to the primers, the specific primers hybridize on the single-stranded DNA molecules. The primers consist of short DNA sequences complementary to the sequence of the DNA to be amplified.
- elongation: at 72 ° C. It is the synthesis of the complementary strand. A polymerase enzyme (Taq polymerase) adds at the end of the primer nucleotides (dNTPs) present in the reaction medium. At the end of the cycle the plate is diluted with water to be read by the capillary sequencer. The migration is verified using a size indicator introduced into the plate.

Reception, preparation of the deposit plates and launch of the sequencer

At the reception of the PCR plates, we control different points:
- The presence of the transfer sheet, the physical state of the plates, visual inspection of the homogeneity of the filling (10µl of minimum PCR product. In case of insufficient volume, we add a volume of Milli-Q water).

- These different control points are then reported in a control sheet. The customer is warned in case of an anomaly.

We then prepare the sequencing depot plates 384:
- We check the correspondence between the plate names and the plate layout ,
- We set up the pipetting robot, according to the program suitable for the type of deposit:

o In the case of PCR plates 96, chronological positioning according to the destination in the dilution plate 384 (per quarter: A1, B1, A2, B2)
o Positioning of the dilution and deposit plates
o Distribution according to the dilution chosen by the client
o Deposit of this dilution in the run plate, containing a mixture of formamide and size marker (defined with the client)

- We create the roadmap (s) according to the plan (s) received from the customer.

- We import the roadmaps associated with these deposit plates, and check the correspondence between the name of the sheet and the plate.
- The plates are then loaded into the sequencer for genotyping.
- The identity of the plates, the date, the operator and the service number are plotted.
- The verification of the results released from the sequencer, is made according to the following criteria:

o Quality of the size marker (aspect and intensity)
o Quality of genotyped markers (definition and intensity)

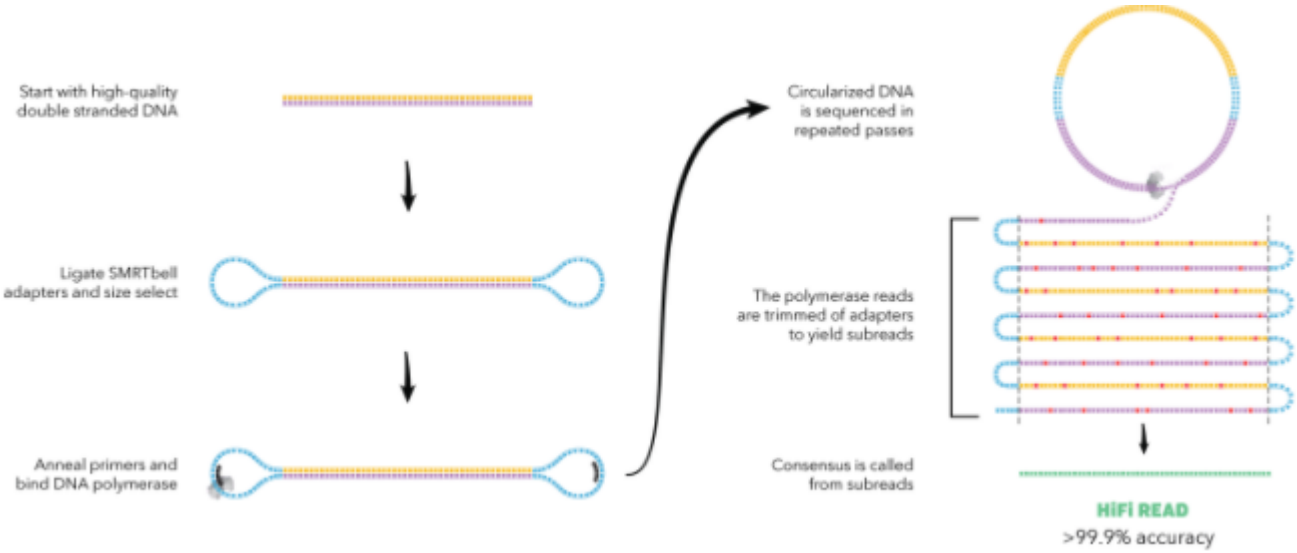
- This data is compressed, and deposited on a server, a download link is sent to the customer who then has 9 days to recover these results.

SEQUENCING

Sequel II PacBio

Description

Sequel II Pacbio are sequencers that obtain long HiFi sequences (N50 at 17 Kb and about 30Gb of CCS (Circular consensus sequences) by SMRTCell at Q30, 99.9% accuracy) to facilitate the assembly of complex genomes.



Chemistry and data processing software are constantly evolving to increase lengths and yields. The initial qualities and quantities of DNA are defined between our specialist NGS Véronique Gautier and the client in accordance with the PacBio recommendations.

DNA or RNA are routinely controlled upon reception (UV and Qubit assays, capillary electrophoresis deposition Fragment Analyzer or Agilent Femto Pulse).
Each application on the Sequel gives rise to a feasibility study of the platform and a precise estimate according to the specificity of the request. We are certified as PacBio Service Provider and we benefit from PacBio application support for setting up new protocols.

Results

The results are sent, either without treatment (only CCS data) to the customer who wishes to do the analysis himself, or after a first assembly with the quality criteria of this assembly in accordance with the sequencing responsible and bioinformatician.

Expression study on fluidigm chips

Description

The goal is to quantify gene expression using the EvaGreen DNA intercalator on the Biomark (Standard Biotools).

Manipulation

Reception of the samples

Upon receipt of the samples (cDNAs and primers), we make sure that they comply with the requirements defined in the "recommendation sheet for the preparation and sending of samples" by performing a visual and / or technical inspection of the sample conditioning and samples themselves. We also verify that the cDNA plate contains six empty wells that will be used for internal controls and that the primers plate contains 1 empty well for primer RNAse P control. If one of the criteria is not respected, we then contact the customer to agree together on the procedure to follow: either a new shipment will be made or we continue handling.

Mix preparation

The different mixes are prepared manually in a plate 96.

Chip preparation

After verification and oil injection, the chip is pressurized.
The chip is either used within one hour to be charged, or it can be stored 24h at 4 ° C. In the latter case, it is pressurized again before use.
use.
The different mixes are injected into the chip. No bubbles should have been generated, these are sources of missing data.

Quantitative PCR

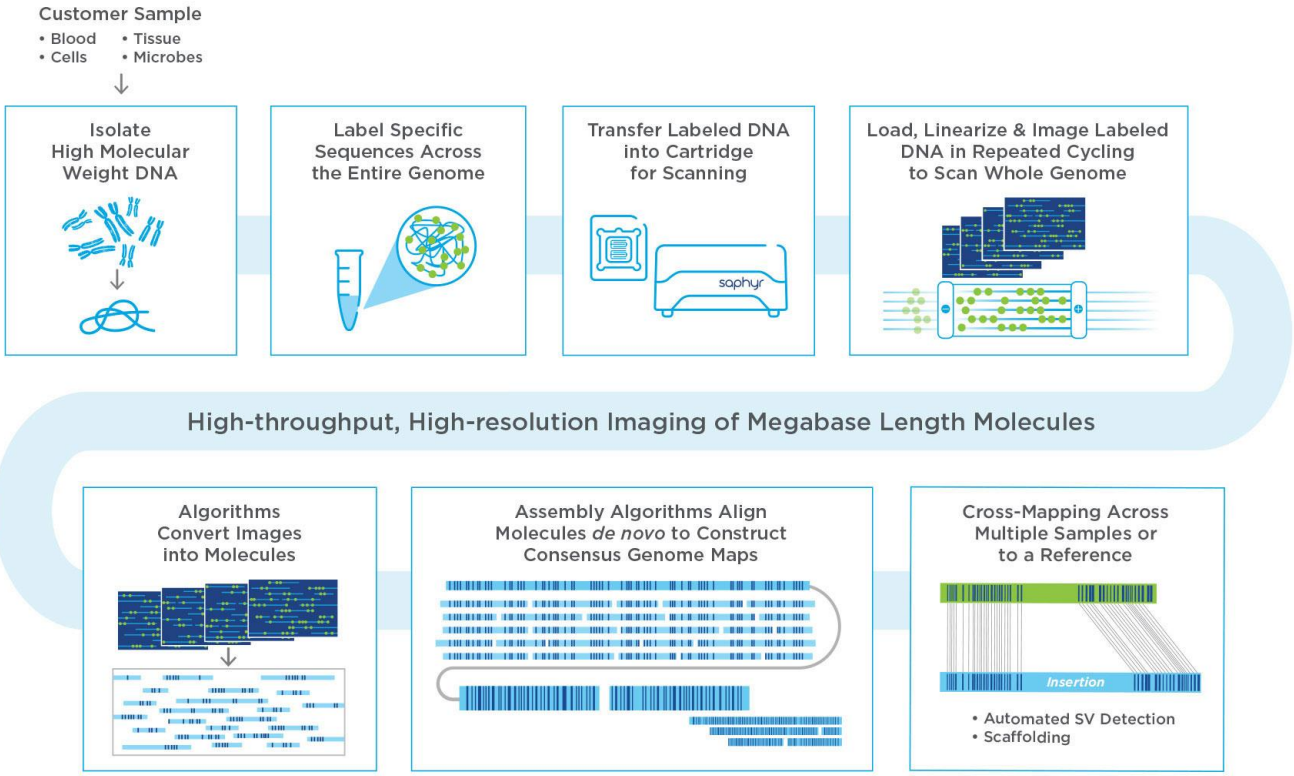
The amplification is done on the Biomark (Standard Biotools).
Before launching the chip, we clean it to avoid the presence of dust, hindering reading.

Reading on the Biomark

Fluorescence data acquisition takes place on the Biomark (Standard Biotools). We then visualize the profiles obtained to determine if the amplification worked well. If the results do not seem satisfactory (internal control ADNg and primer RNAse P), the client will be contacted to define the course of action. The chips are discarded after reading.

Optical mapping on the Saphyr BionanoGenomics

Since September 2018, the platform offers optical mapping services on the Saphyr marketed by BionanoGenomics. The first step is to perform a DNA extraction of very high molecular weight on fresh or frozen samples (plants or animals). Bionano Genomics protocols provide DNA strands greater than 150kb. The DNAs are then labeled on fluorescence-specific sequence motifs using a Direct Label and Stain (DLS) reaction.



The labeled DNA is deposited on the chip. It is divided into three cells (flowcell) to process three samples in parallel. The DNA passes through nano-channels allowing their linearization and the images are recorded by the high-resolution camera of Saphyr. The raw image data is converted to digital representations. The Bionano Solve ™ data analysis software then collates *de novo* data to recreate a complete genome map assembly.

Transmission of results for all technologies

The raw results are deposited on a computer server, the link is then transmitted to the customer by email. The data is then downloadable within 15 days.

Data and samples storage

The preservation of the samples is defined by our protocols. The shelf life of the samples is defined with the customer in the service request form (standard retention of 3 months without guarantee) in accordance with the protocol.

- Fresh plant samples are stored at -80°C. Plant samples sent in dry ice are also stored at -80°C
- Fresh tissue samples and blood samples are stored at +4°C
- Lyophilized samples are stored at room temperature
- Genomic DNA is stored at +4°C in a cold room or at -20°C if received frozen
- PCR products are stored at +4°C
- The PCR product, formamide and size marker mixtures are stored at +4°C
- RNA is stored at -80°C
- Amplicons and cDNA are stored at -20°C

Regarding the storage of computer data, these will be kept in standard 2 months (without warranty).

Provision of material

Organization

Before each arrival of new personnel on the platform, a form for requesting the use of the platform must be completed and sent to the platform manager.

From this request, a training date is decided, and the program defined according to needs.

The training is traced and followed on the "Evaluation of Training" form. An evaluation of the trainee is carried out and recorded on this sheet. If he meets the evaluation criteria, he is entitled to handle the material for which he was trained.

Equipments

The facilities available are:

For the extraction room: a grinder, a chemical hood

For the technological development room: a Qubit dosing system, capillary electrophoresis DNA or RNA control systems (Fragment Analyzer and Femto Pulse Agilent).

For the PCR mixing room: two pipetting robots, the ultra-pure water system, the automatic sealer, 4 centrifuges, a spectrofluorimeter.

For the DNA room: a pipetting robot.

For the PCR room : a real-time PCR (Roche LC 480), thermocyclers

For the post-PCR room: a pipetting robot, a centrifuge, two real-time PCRs.

In all these rooms, the pipettes are made available. These pipettes are controlled each year.