Personalized Medicine & Genetics

Services

1) DNA SEQUENCING AND FRAGMENT ANALYSIS

1.1) DNA SEQUENCING (Sanger method)

Sanger sequencing by capillary electrophoresis is the gold-standard DNA sequencing technique that is used in a number of experimental workflows in life sciences laboratories.

The automatic DNA sequencing uses the methodology of Sanger, which is based on the selective incorporation of chain-terminating dideoxynucleotides during the PCR (polymerase chain reaction).

The product of the reactions is separated using a capillary electrophoresis. Fragments are labelled using 4 fluorochromes (one per nucleotide). These fluorochromes can be incorporated either into the primer, “dye primer kit”, o into the ddNTPs o terminators, "dye terminator kit".

The DNA to be sequenced must have the highest quality, and it could have been obtained from: PCR products, plasmids or phages. The primers for the cyclic sequencing can be either those specific primers for each PCR product or the universal primers of plasmids or phages.

EQUIPMENT: Genetic analyzer ABI 3130XL using the advanced chemical BigDye Terminator 3.1.

Applications:

- *De novo* sequencing (to obtain the primary genetic sequence of a particular organism)
For *de novo* sequencing using capillary electrophoresis, the target DNA is fragmented and cloned into a viral or plasmid vector. Cloning provides amplification of the target DNA (by bacterial growth) and allows sequencing primers to bind to known sequence in the vector and extend the sequence into the unknown target DNA.

- Targeted DNA sequencing
Identifying heterozygous base positions or small insertions or deletions in genomic DNA is often employed to locate mutations or polymorphisms in diploid organisms, detect genetic rearrangements, and uncover rare variants.

- Next-generation sequencing validation
To confirm results obtained from next-generation sequencing (NGS) data.

- Mitochondrial sequencing
Mitochondrial DNA sequencing is a useful tool for researchers studying human diseases such as diabetes, certain cancers, and mechanisms of aging. Mitochondrial DNA sequencing is also used in population genetics and biodiversity assessments. Targeted mitochondrial DNA sequencing can be used to detect mutations present in some copies of the mitochondrial genome (heteroplasmic mutations). Finally, mitochondrial DNA sequencing is important for human identification and forensics applications.

1.2) DNA FRAGMENT ANALYSIS

Fragment analysis generates a size estimate for DNA fragments relative to a size standard of DNA fragments with known lengths. The size standard is combined with the sample of interest and co-injected on the capillary electrophoresis system.

This service includes:

- Amplification and labelling of the PCR product.

- Electrophoresis of the samples.

- Analysis of the results with Gene Mapper software.

Applications:

- Microsatellite Analysis Application
Microsatellite genotyping is the genotyping of tandem repeats such as Short Tandem Repeats (STRs) or Variable Nucleotide Tandem Repeats (VNTRs). STRs and VNTRs are polymorphic DNA loci present throughout the genome. Microsatellite genotyping is a widely accepted tool for a variety of applications such as paternity testing, linkage mapping studies, association studies, and identification of organisms.

- Amplified Fragment Length Polymorphism (AFLP) Mapping
Amplified fragment length polymorphism (AFLP®) analysis is a genetic mapping technique that uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprint for a particular genome. The power of AFLP analysis derives from its ability to quickly generate large numbers of marker fragments for any organism, without prior knowledge of the genomic sequence.

- SNP genotyping

SNP genotyping identifies single nucleotide polymorphisms (SNPs) that are common DNA variants present across the human genome. SNPS have been shown to be responsible for differences in genetic traits, susceptibility to disease and response to drug therapies. Genotyping of SNPs has become extremely important to researchers working on understanding and treating disease.

- Single Stranded Conformation Polymorphism (SSCP) Analysis

Many researchers in the field of genomics need to be able to reliably identify allelic variants of genetic traits. Single-stranded conformation polymorphism (SSCP) is a widely used technique that can be an effective screening tool to find variants in a large number of samples, in everything from micro-organisms to humans. The SSCP analysis method detects point mutations by analyzing their unique electrophoretic mobility that results from small changes in the variant's nucleotide sequences.

2) HIGH THROUGHPUT GENOTYPING

The high throughput low cost genotyping system is based on a microarray format which allows the analysis of thousands of SNPs (Single-Nucleotide Polymorphisms) in several samples simultaneously using nanovolumes of sample.

SNP Genotyping Assay collection includes over 4.5 million genotyping assays. Of these, there are 3.5 million HapMap SNPs and 2600 drug metabolizing enzyme (DME) genotyping assays. Additionally, researchers can create Custom TaqMan SNP Genotyping Assays for any genome by submitting their target SNP sequences.

This service includes:

- Training and support for the design of customized assays.

- Preparation, loading and running of samples in the designed plate.

- Analysis of the result obtained using specific software.

Applications:

- Pharmacogenetics

- Diagnostic

3) CNVs ANALYSIS

Gene deletion and gene duplication are genomic variations that can also affect protein function or phenotype. Gene copy number Variations (CNVs) can be readily quantitated using real-time quantitative PCR. The method involves relative quantification of the gene of interest versus s reference gene known to be single copy.

Copy number variations (**CNVs**) are ≥ 1Kb segments whose copy number is variable compared with the reference genome. CNVs have been associated with different diseases and the response to certain drugs.

This service includes:

- Preparation, loading and running of samples.

- Analysis of the results with copy caller software.

Applications:

- Pharmacogenetics

- Susceptibility or resistance to disease.

4) GENE EXPRESSION ANALYSIS

We offer high-throughput gene expression analysis using a microarray format that allows the design of customized arrays depending on the number of genes and samples to analyze. This technology allows to reduce the cost and the volume of sample required.

This service includes:

- Training and support for the design of customized assays.

- Preparation, loading and running of samples in the designed plate.

- Analysis of the results.

Applications:

- Human cancer panel
Targets 624 validated genes related to DNA repair, angiogenesis, cell adhesion, and ECM, as well as

genes involved in the cell cycle and apoptosis, and many of the genes encoding kinases and transcription factors that have been found to be differentially expressed in early cancer and metastatic disease.

- Human Stem Cell Panel
Panel that covers 609 validated genes for the characterization of undifferentiated stem cells or their differentiated derivatives. The gene content resulted directly from the work of the I.S.C.I consortium and characterization of human embryonic stem cell lines by the International Stem Cell Initiative.

- Human Kinome Panel
This panel targets 772 well-defined genes and is intended to capture the full set of protein kinases in the human genome.

- Human Inflammation Panel
This panel covers 586 genes that have been studied as targets for a range of inflammatory diseases for quantitative gene expression analysis of human inflammation genes important in drug discovery.

- Human Signal Trasduction Panel
This panel was developed to identify differentially expressed genes involved in major signaling pathways. Contains 573 TaqMan® assays specific to signal transduction-related genes including JAK-STAT, NFκB, Akt, GPCR, cAMP, and MAP kinase pathways.

- Mouse Inflammation Panel
This panel covers 632 genes that that have been studied as targets for a range of inflammatory diseases for quantitative gene expression analysis of human inflammation genes important in drug discovery.

5) MICRORNAS ANALYSIS

We have a highly efficient platform for profiling human and rodent micro RNAs (miRNAS). This means that in one full run you can profile nine individual samples for 754 miRNas in just less than 3 hours.

We have a panel which includes validated microRNAs form the Sanger miRBase v14 database. It is also possible to perform a customized design for a specific project with pre-designed or customized probes.

This service includes:

- Training and support for the design of customized assays.

- Preparation, loading and running of samples in the designed plate.
- Analysis of the results.

6) DIGITAL PCR

Digital PCR is a new approach to nucleic acid detection and quantification, which is a different method of absolute quantification and rare allele detection relative to conventional qPCR.

Digital PCR works by partitioning a sample into many individual real-time PCR reactions; some portion of these reactions contain the target molecule (positive) while others do not (negative). Following PCR analysis, the fraction negative answers is used to generate an absolute answer for the exact number of target molecules in the sample, without reference to standards or endogenous controls.

This service includes:

- Preparation, loading and running of samples in the designed plate.

- Analysis of the result obtained.

Applications:

- Absolute quantification of pathogens (eg viral load)

- Detecting rare alleles
- Determining fetal sex from maternal blood
- Detecting tumor cells with sensitivity under 0.1%

7) CAST PCR

The Competitive Allele-Specific TaqMan PCR (castPCR) is a highly specific and sensitive method of detecting and quantifying rare mutations in sample that contains large amounts of wild-type genomic DNA. The technology uses a probe which blocks the wild type allele amplification and a second probe which allows the mutant allele amplification.

This service includes:

- Preparation, loading and running of samples in the designed plate.

- Analysis and results.

Applications:

- Early detection of somatic mutations in genes associated with different types of cancer such as KRAS, FRAF KIT and JAK2 among others.

8) SAMPLE QUANTIFICATION

Concentration and purity of DNA, RNA or protein samples are measured with high accuracy and reproducibility using 1 µl of sample. It uses an innovative technology based on the superficial tension to keep the sample in the place of measurement, which avoids the use of cuvettes. Moreover no dilutions are required. The software automatically calculates the concentration and the sample purity ratios (260/280 nm and 260/230nm).

EQUIPMENT: NanoDrop 2000 UV-Vis Spectrophotometer

9) ADVISING AND TRAINING

AB-BIOTICS Laboratory offers advising and training on the methodologies and techniques included in our services.

For each I+D project we offer:

- Design of the project to optimize cost and efficiency.
- Selection of the most suitable format for high-throughput analysis depending on the number of genes or variants to analyze.
- Design of the study with predesigned or custom assays.
- Support and advice during the different stages or the project.

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MANAGEMENT SYSTEM