GENOMICA IIBm

GENOMICS CORE FACILITY

NEW USERS HANDBOOK

INTRODUCTION

Genomics Core Facility at the IIB founded 1994 provides its services to IIB and external users in order to support their research and diagnosis.

Service is under UNE-EN ISO 9001:2000 certification.

Service belongs to the Laboratory Net of the Madrid Autonomous Community (CAM) under register number 281. http://www.madrimasd.org/laboratorios.

LOCATION

Ground floor IIBm-1.

CONTACT

Phone 91 585 44 70/ 44 74 - Fax 91 585 44 01 – Email address: genomica@iib.uam.es

OPENNING HOURS

9:00 to 17:00 h

SUMMER OPENNING HOURS (15th June - 15th September)

8:00 to 15:00 h

GENOMICA IIBm

GENOMICS CORE FACILITY SERVICES

This handbook tries to help all new Genomics Core Facility users providing information about services and sample requirements.

SERVICES

GENOMICA IIBm

PCR BASED MOUSE GENOTYPING

Sigma's REDExtract-N-Amp™ Tissue PCR kit provides all the necessary reagents to extract and amplify genomic DNA in as little as 20 minutes. DNA can be extracted from mouse tails, other animal tissues, buccal swabs, hair shaft and saliva using the Extract-N-Amp™ technology. The kit also includes a specially formulated hot start PCR reaction mix for direct amplification from the extract. PCR products are then analyzed in agarose gels and data obtained are emailed to the user.

Sample requirements for Mouse genotyping:

Mouse tails should be brought in 1.5 ml eppendorf tubes properly labelled with animal identification. Tubes should be kept on ice.

50 µl of 100 µM PCR oligos should be provided. Please keep oligos on ice.

Information concerning oligonucleotides sequences, PCR conditions (if known), PCR fragments size… is requested. Please provide control samples for the different expected genotypes.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services).

For further information please contact: genomica@iib.uam.es.

GENOMICA IIBm

PLASMID DNA PURIFICATION
DNA OR RNA PURIFICATION FROM CELLS, TISSUES, BLOOD, SALIVA, FFPE SAMPLES...

Genomic Core Facility uses QIAcube® technology to process Qiagen® spin columns for fully automated RNA or DNA purification from different sources. Up to 12 samples can be processed per run. Innovative QIAcube® components include a centrifuge, heated shaker, pipetting system, and robotic gripper.

Up to now we have used Qiacube® to process: human blood, mouse and rat chocleas, mouse and human brain biopsies, human saliva, mouse pancreas, mouse tails or stools, cultured cell lines, FFPE samples (mouse tissues: kidney, testis, skin, brain, pancreas, heart, prostate, liver, lung, spleen…). This has enabled us to fully automate more than 20 Qiagen® different protocols.

The QIAcube® together with the QIAprep Spin Miniprep Kit enables fully automated purification of plasmid DNA. Purified DNA is suitable for use in routine molecular biology applications, such as fluorescent Sanger sequencing, cloning, or cells transfection. Minipreps will be done on Tuesdays so please provide cultures on Mondays.

RNA or DNA obtained is either spectrophometricallly (Nanodrop) or fluorimetrically (Qubit®) quantified.

Sample requirements:

For plasmid DNA:	1ml of inoculate. Please provide: vector name, insert size, selection media…
For cell lines DNA:	please provide 3-5 10^6 suspension cells or adherent cells from a confluent P60 in 200 µl of PBS in 2.0 ml tubes.
For blood DNA:	200 or 400 µl of blood in 2.0 ml tubes.
For blood RNA:	600 µl of lysed leukocytes
For brain/adipose tissue RNA:	< 100 mg of starting tissue
Other tissues RNA:	< 30 mg of starting tissue
FFPE RNA:	1-2 or 3-4 10 µM sections

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

You will receive an email when your results are available so you can come and pick up your samples. Attach to the email you will find information concerning: purification method used, sample volume, sample concentration, RNA quality (if required)…

GENOMICA IIBm

NUCLEIC ACIDS QUANTIFICATION

UV-Vis spectrometry allows us to confirm DNA or RNA quantity and quality for further analysis as Real Time qPCR, SNPs detection or DNA Sanger sequencing.

Nanodrop ND1000 and ND8000 (8 samples at one time) allow UV-Vis measurements to be made from 1-1.5 µl of sample with no cuvettes or dilutions. Using fiber optic technology and surface tension the sample is held in place between two optical surfaces that define the path length in a vertical orientation. Absorbance measurements are then faster and easier.

DNA dynamic range: 2-3700 ng/µl. RNA dynamic range: 2-3000 ng/µl.

The Qubit® 2.0 Fluorometer is designed to work with highly specific and sensitive Qubit® DNA, RNA, and protein quantitation assays. Specifically designed fluorometric technology uses Molecular Probe® dyes to quantitate biomolecules of interest. These fluorescent dyes emit signals ONLY when bound to specific target molecules, even at low concentrations.

Qubit® 2.0 Fluorometer dynamic range for different RNA and DNA assays:

Kit	Rango
dsDNA HS assay	20 pg/µl – 100 ng/µl
dsDNA BR assay	100 pg/µl – 1000 ng/µl
dsRNA HS assay	250 pg/µl – 100 ng/µl
dsRNA BR assay	1 ng/µl – 1000 ng/µl

Sample requirements:

Users should bring 3 µl of RNA or DNA (10 -2000 ng/µl) in 1.5 ml tubes

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

After your samples have been quantified you will receive an email with the results: sample concentration, 260/280 and 260/230 ratios…

GENOMICA IIBm

RNA INTEGRITY ANALYSIS

The Agilent 2100 Bioanalyzer system provides sizing, quantitation and quality control of DNA or RNA samples. The electrophoretic assays are based on traditional gel electrophoresis principles that have been transferred to a chip format. The chip format dramatically reduces separation time as well as sample and reagent consumption. The system provides automated sizing and quantitation information in a digital format.

For DNA assays, quantitation is done using the upper marker. The area under the upper marker peak is compared with the sample peak areas. Because the concentration of the upper marker is known, the concentration for each sample can be calculated.

For RNA assays, quantitation is done with the help of the ladder area. The area under the ladder is compared with the sum of the sample peak areas. For total RNA assays, the ribosomal ratio is determined, giving an indication on the integrity of the RNA sample. Additionally, the RNA integrity number (RIN 1 to 10) can be used to estimate the integrity of total RNA samples based on the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products. RNA integrity is completely necessary for gene expression analysis either with microarrays or through Real Time PCR.

SPECIFICATIONS

NANOCHIPS

Quantitative range	Total RNA: 25-500 ng/µl mRNA: 25-500 ng/µl
Qualitative range	Total RNA: 5-500 ng/µl mRNA: 25-500 ng/µl

PICOCHIPS

Qualitative range	Total RNA: 50-5000 pg/µl mRNA: 250-5000 pg/µl

Sample requirements:

Users should bring 3 µl of RNA or DNA (10 -2000 ng/µl) in 1.5 ml tubes. 1 µl will be used for Nanodrop quantification and 1 µl for Bioanalyzer analysis. If you do not have enough sample, please contact us.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

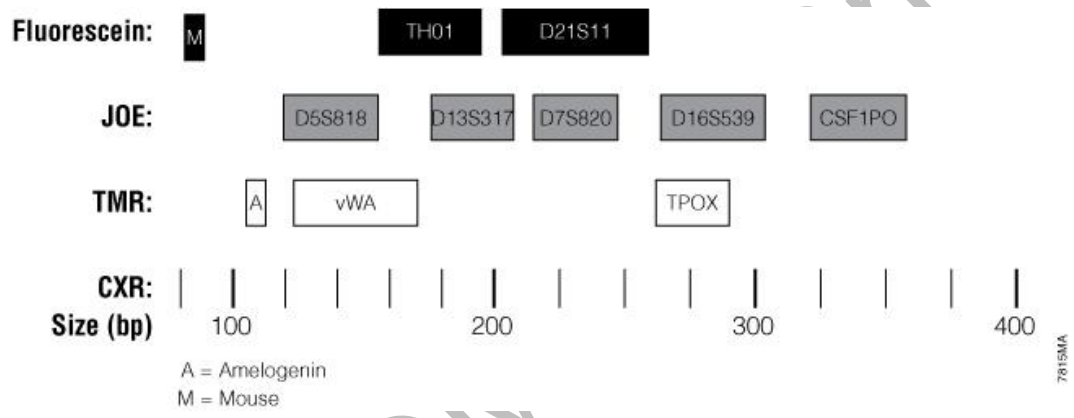
After your samples have been processed you will receive an email with the results (sample concentration, 260/280 and 260/230 ratios) together with RIN profiles pdfs.

GENOMICA IIBm

HUMAN CELL LINES AUTHENTICATION

The GenePrint® 10 System allows co-amplification and three-color detection of ten human loci (TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317, D5S818 and D21S11) for human cell line authentication. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92×10^9 and exceed standard ASN-0002 issued by the American Tissue Culture Collection Standards Development Organization Workgroup for cell line authentication. GenePrint® 10 amplified products are run in our Applied Biosystems 3130xl Genetic Analyzer and analyzed with Gene Mapper® software. Profiles obtained are checked against public data bases like ATCC or DSMZ.

For mouse cross contamination detection please contact us.



Sample requirements:

Please provide 3-5 10^6 suspension cells or adherent cells from a confluent P60 in 200 µl of PBS in 2.0 ml tubes.

After DNA purification with the Qiacube extractor and DNA quantification with Qubit® fluorometer, the microsatellites will be PCR amplified and analyzed.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

After your samples have been processed you will receive an Excel document with the different microsatellite polymorphic data and data bases information along with profiles pdfs.

DNA SANGER SEQUENCING

Volumen:	6µl
ADN:	250-300 ng
PCR:	10-20 ng/100 pb
Primer:	3,2 pmoles
Tubos:	0,2 ml

DNA AND *PRIMERS* REQUIREMENTS

PLASMID DNA

QUALITY

NO: Phenol, chloroform, proteins, chromosomal DNA, RNA, ethanol, salts, detergents, PEG, EDTA (TE).

YES: Column purified plasmid DNA.
DNA in H_2O.
¡¡¡¡¡¡¡ MIND SECONDARY STRUCTURES!!!!!!!!!

QUANTITY (300ng)

YES: Spectrophotometric sample quantification (Nanodrop).

PCR PRODUCT

QUALITY

NO: Nucleotides, *primer*s, dNTPs or PCR amplification enzymes.

YES: Column purified DNA, exo I y SAP treated samples or PCR product dilution.

QUANTITY 10-20 ng/100pb

YES: Spectrophotometric sample quantification (Nanodrop).

PRIMERS

QUANTITY 3,2 pmoles

QUALITY Avoid *primers* with 3 or 4 Gs ó Cs in a row.
Primers should be at least 18 bases long to avoid non specific hybridization. If possible anchor *primers* with GCs in 3´.
Avoid secondary structures in *primers* sequences, dimmers and mismatches.
Primers Tm should be over 50 ^{0}C.

Tm =	2 x (A+T) + 4 x (G+C)
Ta =	Tm - 5°C

GENOMICA IIBm

SANGER SEQUENCING TROUBLESHOOTING

1. NO REACTION

- No DNA o less than needed.
- EDTA or other polymerase inhibitors, sample contamination.
- Sequencing *primer* with secondary structures.
- No *primer* or concentration below 3.2 pmoles.
- Sequencing *primer* does not hybridize.
- Tm *primer* below 50ºC.

2. NOISY ELECTROPHEROGRAM

- Weak sequencing signal due to low DNA amount, contaminants, low primer concentration…

3. SEQUENCING SIGNAL GOES DOWN

- Sample contaminants: EDTA, salts…
- DNA and primer quantities are not compensated.

4. DOBLE SEQUENCE ELECTROPHEROGRAM

- More than one DNA template.
- More than one *primer* priming sites.
- *Primer* contamination or unproper purification.
- PCR product unproperly purified

5. SEQUENCING SIGNAL STOPS ABRUPTLY

- DNA is broken or digested at that level.
- Strong DNA secondary structure.

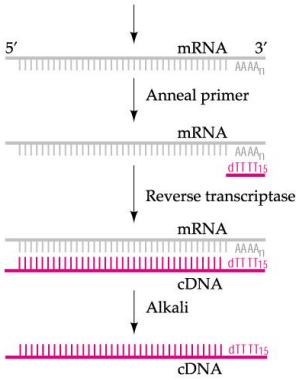
6. DOUBLE SEQUENCE AFTER POLI-A OR POLI-T

- DNA polymerase slippage.

GENOMICA IIBm

RNA RETROTRANSCRIPTION

Reverse transcription polymerase chain reaction (RT-PCR) is commonly used in molecular biology to detect RNA expression levels. In RT-PCR, the RNA template is first converted into complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR.



The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems®) delivers extremely high-quality, single-stranded cDNA from 0.02 to 2 µg total RNA. Quantitative first-strand synthesis of all RNA species is achieved with the use of random primers. Downstream applications include real-time PCR, standard PCR, and microarrays.

Sample requirements:

Users should bring 10 µl of RNA samples (100 ng/µl) in 0.2 ml tubes. RNA should not be degraded. To check RNA integrity samples will be bioanalyzed using nano or picochips in 2100 Bioanalyzer.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

You will receive an email when your results are available so you can come and pick up your samples.

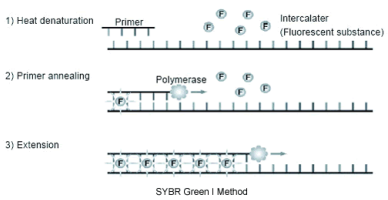
GENOMICA IIBm

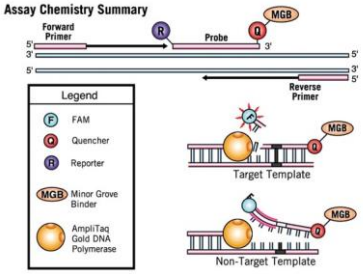
REAL TIME PCR FOR GENE EXPRESSION

Real-time PCR —also known as quantitative reverse transcription PCR (RT-qPCR), and quantitative PCR (qPCR)—is one of the most powerful and sensitive gene analysis techniques available. It is used for a broad range of applications including quantitative gene expression analysis, genotyping, copy number, drug target validation, biomarker discovery, pathogen detection, and measuring RNA interference. Real-time PCR measures PCR amplification as it occurs, so that it is possible to determine the starting concentration of nucleic acid. In traditional PCR, which is based on end-point detection, results are collected after the reaction is complete, making it impossible to determine the starting concentration of nucleic acid. Every real-time PCR contains a fluorescent reporter molecule—a TaqMan®probe or SYBR®Green dye, for example—to monitor the accumulation of PCR product. As the quantity of target amplicon increases, so does the amount of fluorescence emitted from the fluorophore.

An important difference between the TaqMan®probes and SYBR®Green I dye chemistries is that the SYBR®Green I dye chemistry binds all double-stranded DNA, including nonspecific reaction products. A well-optimized reaction is therefore essential for accurate results.

.





Sample requirements:

RNA: 100ng/µl
Volume: 10µl
Tubes: 0.2ml

Primer: 5µM
Volume: 100µl
Tubes: 1.5ml

RNA together with Taqman assays and/or oligos should be brought on ice. Samples and oligos should be properly labelled (name and concentration). Please contact responsible technician in the lab for samples reception and endogenous and calibration sample identification.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

You will receive an email with your results attached when available. Please come back soon and pick up your samples, oligos, dyes.... Real Time gene expression data can be analyzed using Integromics Statminer ®qPCR Software. Please contact if interested.

GENOMICA IIBm

REAL TIME PCR ENDOGENOUS GENES

There are several human and mouse endogenous genes available for Real Time experiments at the Genomics Core Facility. Please ask.

TAQMAN ASSAYS

- 18S (Hs99999901_s1)
- TBP (Hs00427621_m1)
- RPLP0 (Hs99999902_m1)
- RPLP0 (Mm01974474_gH)
- HPRT1 (Hs99999909_m1)
- HPRT1 (Mm00446968_m1)
- ACTB (Hs99999903_m1)
- ACTB (Mm00607939_s1)
- GUSB (Hs99999908_m1)
- GUSB (Mm01197698_m1)

***SYBR GREEN* OLIGONUCLEOTIDES**

- 18S
  - Forward: 5' CCA GTA AGT GCG GGT CAT AAG C 3'
  - Reverse: 5' CCT CAC TAA ACC ATC CAA TCG G 3'

TAQMAN ASSAYS NOMENCLATURE

The first two positions designate the species

Hs = *Homo sapiens* Mm = *Mus musculus* Rn = *Rattus norvegicus*

The second-to-last position contains a letter that means the following:

Assay Suffix	Assay placement
_m	Probe spans exon-exon junction and the assay will not detect genomic DNA
_s	Primer and probe are designed within a single exon and the assay will detect genomic DNA
_g	Probe spans exon-exon junction but the assay may detect genomic DNA
_mH, _sH, _gH	The assay was designed to a transcript belonging to a gene family with high sequende homology

GENOMICA IIBm

REAL TIME PCR FOR ALLELIC DISCRIMINATION

TaqMan® 5'-nuclease assay chemistry provides a fast and simple way to get single nucleotide polymorphism (SNP) genotyping results. Each predesigned TaqMan® SNP Genotyping Assay two allele-specific TaqMan® MGB probes containing distinct fluorescent dyes and a PCR primer pair to detect specific SNP targets. These TaqMan® probe and primer sets (assays) uniquely align with the genome to provide unmatched specificity for the allele of interest.

SNP Genotyping Assays contain VIC®dye-labeled probe, FAM™ dye-labeled probe,and two target specific primers. TaqMan®probes incorporate MGB technology at the3' end to deliver superior allelic discrimination. The MGB molecule binds to the DNA helix minor groove, improving hybridization based assays by stabilizing the MGBprobe-template complex. This increased binding stability permits the use of probes as short as 13 bases for improved mismatch discrimination and greater flexibility when designing assays for difficult or variable sequences. All MGB probes also include a non-fluorescent quencher (NFQ) that virtually eliminates the background fluorescence and provides excellent signal-to-noise ratio for superior assay sensitivity.

Detected fluorescence	Means….
VIC	Homozygous for allele X
FAM	Homozygous for allele Y
Both	Heterozygous

GENOMICA IIBm

Sample requirements:

Liophilized DNA genomic samples can be brought in 96 or 384 plates. Total sample concentration should be 5-10 ng. An Excel file with sample names should be filled in and emailed to genomica@iib.uam.es

Users should bring 10 µl of RNA samples (100 ng/µl) in 0.2 ml tubes. RNA should not be degraded. To check RNA integrity samples will be bioanalyzed using nano or picochips in 2100 Bioanalyzer.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

As soon as your samples have been processed you will receive an Excel file with your haplotype results.

REAL TIME PCR STOOL *HELICOBACTER* DETECTION

Several species of helicobacter have been identified from naturally infected laboratory mice, most commonly *H. bilis*, *H. hepaticus*, *H. muridarum*, *H. rodentium*, and *H. typhlonius*. Recent reviews described the significant effects of these bacteria upon research, including their association with hepatic neoplasia, intestinal neoplasia, and chronic proliferative enteritis in mice.

The most sensitive and widely used method for detecting helicobacter infections is PCR targeting of a genus-specific, conserved region of 16S rRNA. For speciation, this has been followed by restriction enzyme digestion of the amplicons for identification of species-specific fragment lengths. At IIBm Genomics Core Facility optimize PCR conditions that would simultaneously detect and speciate five of the more common helicobacter mouse species without the need for restriction enzyme analyses is used.

See "Relation between the severity of hepatitis C virus...". World of Gastroenterol. Vol 7; No 12 (45): 7278-7284. 2006) or “Differential detection of five mouse-infecting...”. Clinical and diagnostic laboratory immunology. Vol 12, No 4: 531-536. 2005.

Sample requirements:

Users should bring 180-220 mg of mice stools in 2.0 ml tubes.

Order number for IIBm users is compulsory. Order number can be purchased through the Lab Store Department web page (look for Genomics Services). For further information please contact: genomica@iib.uam.es

As soon as your samples have been processed you will receive an Excel file with your results.

GENOMICA IIBm