
Biomarkers and Precision Medicine Unit
MEDICAL RESEARCH INSTITUTE HOSPITAL LA FE

Instituto de Investigación Sanitaria La Fe

Unidad Epigenómica
INSTITUTO DE INVESTIGACIÓN SANITARIA LA FE

# Unidad de epigenómica y H2020

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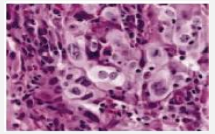
Investigador Contratado Miguel Servet

Director de Epigenomics core facility

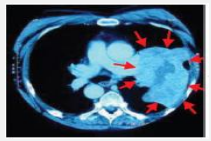
Investigador de la Unidad de biomarcadores y medicina de precisión

# Aplicación de la biología molecular a la medicina

## Técnicas standard de diagnóstico

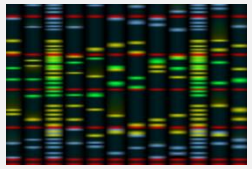

HISTOLOGIA

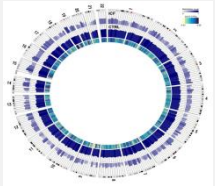

ANALITICAS

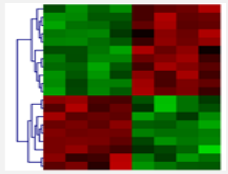


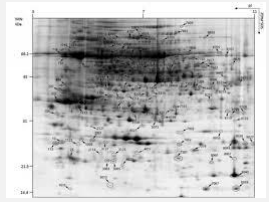
IMAGEN

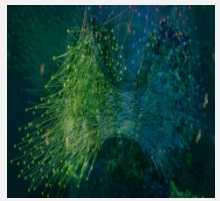
## Técnicas de última generación (BIOLOGIA MOLECULAR)


GENOMA


EPIGENOMA


TRANSCRIPTOMA


PROTEOMA


METABOLOMA


MICROBIOMA


TRATAMIENTO

DIAGNÓSTICO

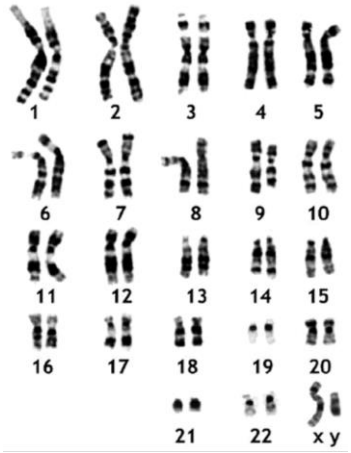
# HUMAN GENOME

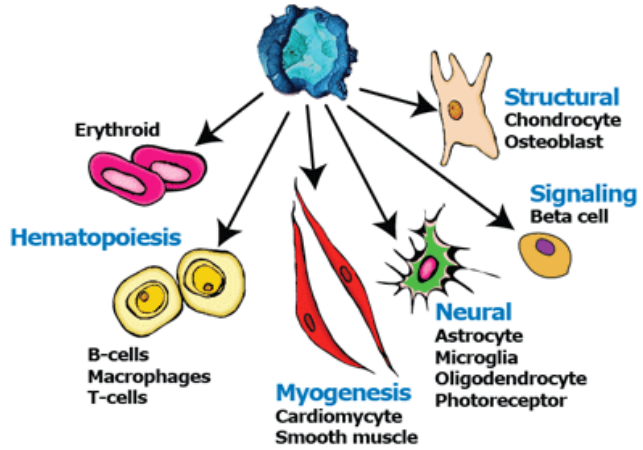


- 3,3 Gb (3,137,144,693)
- ± 22.000 genes
- 99,9% humans
- 98,77% *Pan troglodytes*

- The lack of identified genetic determinants that fully explain the heritability of complex traits.
- Inability to pinpoint causative genetic effects in some complex diseases.

Suggest possible epigenetic explanations for this missing information.

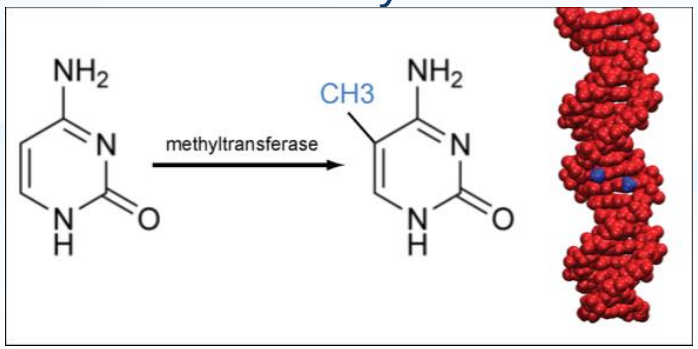


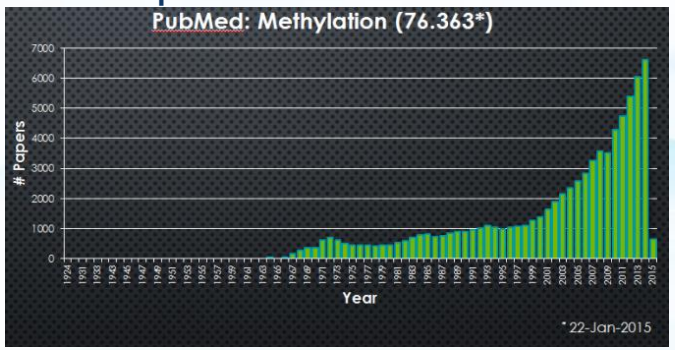
# Epigenetics

Epigenetics: heritable changes in gene function that are not associated with changes in the DNA sequence.

## DNA methylation

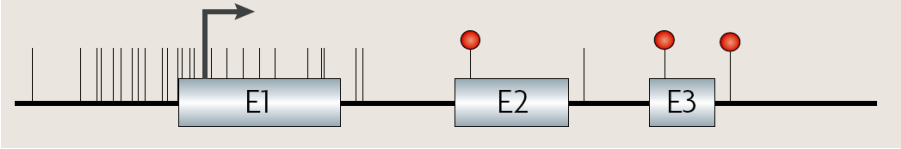



## Exponential evolution




## Dogma in epigenétics and cancer

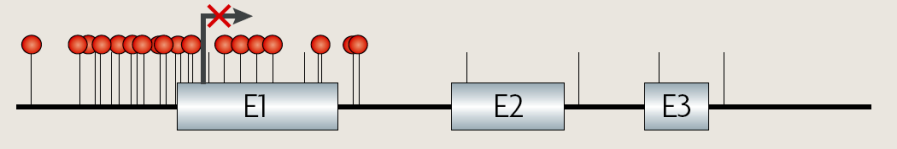
### Healthy cell




Tumor suppressor gene with CpG island in promoter

Open chromatin (gene expression)

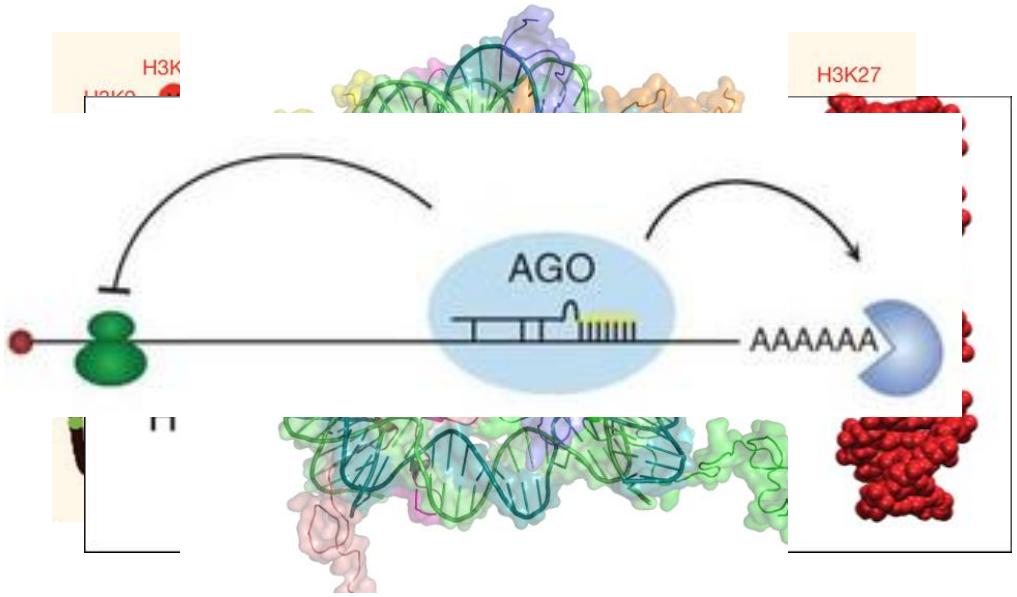
### Cancer cell




Hypermethylation on CpG island in promoter

Closed chromatin (gene silenging)

# Factores Epigenéticos




- Metilación del DNA
- Modificaciones de las histonas
- RNAs no codificantes (microRNAs)
- Estructura tridimensional de la cromatina

# Perfil epigenómico de metilación del DNA
## (Golden gate, infinium 27K, infinium 450K y **INFINIUM 850K )**

SYSCOL
SYSTEMS BIOLOGY OF COLORECTAL CANCER

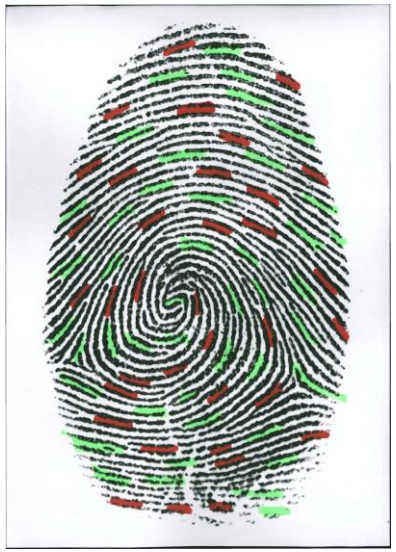

NEXT-GENERATION SEQUENCER
HiScanSQ, Illumina®

Infinium HumanMethylation
450K BeadChip Kits, Illumina®

✓aprox. 850,000 methylation sites per sample at single-nucleotide resolution

✓ coverage of all designable RefSeq genes

- CpG islands and shores
- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells
- Differentially methylated sites identified in tumor versus normal (multiple forms of cancer) and across several tissue types
- CpG islands outside of coding regions
- miRNA promoter regions
- Disease-associated regions identified through GWAS



*Sandoval J, Genetic engeneering and biotechnology news 2011*

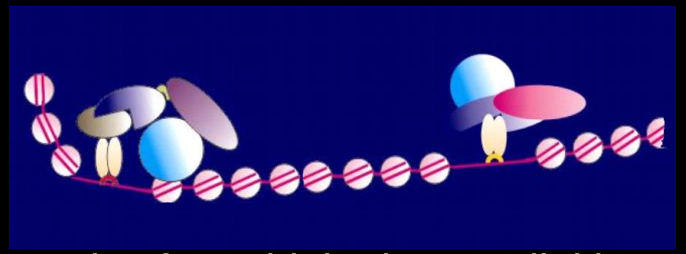
# Pyrosequencing for DNA methyaltion analysis


~ 6h | ~ 2h | ~ 15 min | ~ 10-60 min

| gDNA Isolation | Bisulfite Conversion | Assay Design | PCR | Sample Prep | Pyrosequencing |
|---|---|---|---|---|---|
| QIAamp Kits | EpiTect Bisulfite Kits | PyroMark Assay Design SW 2.0 | PyroMark PCR Kit | PyroMark Q24 Vacuum Workstation | PyroMark Q24<br>PyroMark Q24 SW<br>PyroMark Gold Reagents |
| DNAeasy Kits | EpiTect 96 Bisulfite Kits | PyroMark Pre-designed Assays | | PyroMark Q96 Vacuum Workstation | PyroMark Q96 ID<br>PyroMark Q96 MD<br>PyroMark Gold Reagents<br>PyroMark CpG SW |
| QIAcube/EZ1 | | | | | |
| QIAsymphony | | | | | |

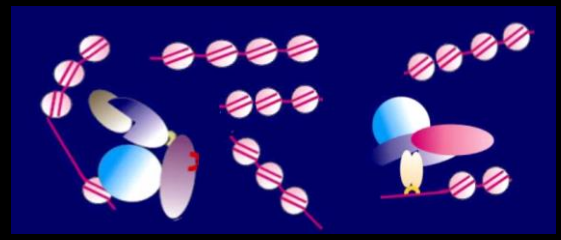
Pyrosequencing is the method of choice whenever single base methylation levels need to be analyzed with high precision and in a quantitative manner. Moreover it is feasible for a daily basis analysis in hospitals.

# Chromatin immunoprecipitation (histone modifications)



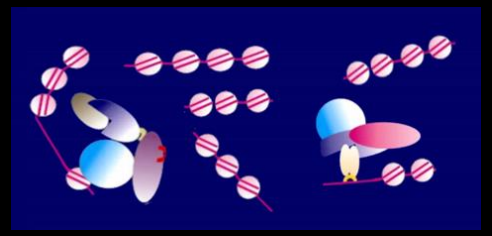
In vivo formaldehyde crosslinking

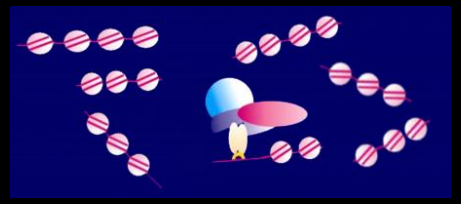
Sonication



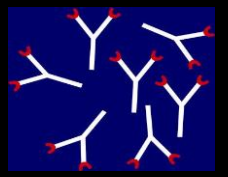
Pool of crosslinked chromatin fragments

INPUT



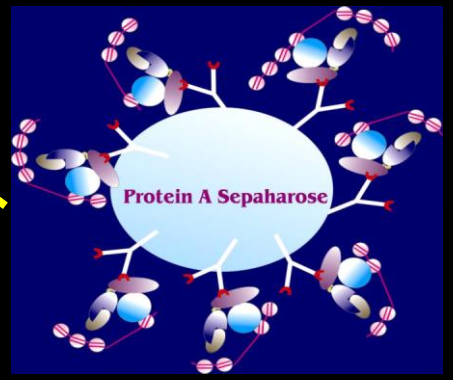


Protein A sepharose

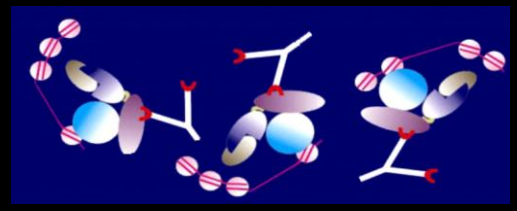


Specific Ab

washes




INPUT

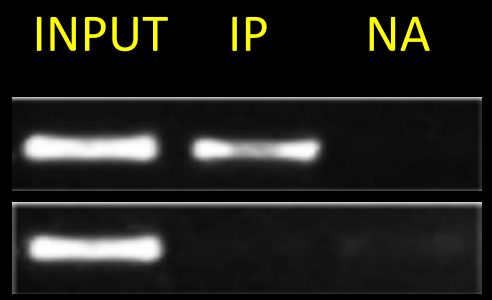


IP

DNA purification and PCR

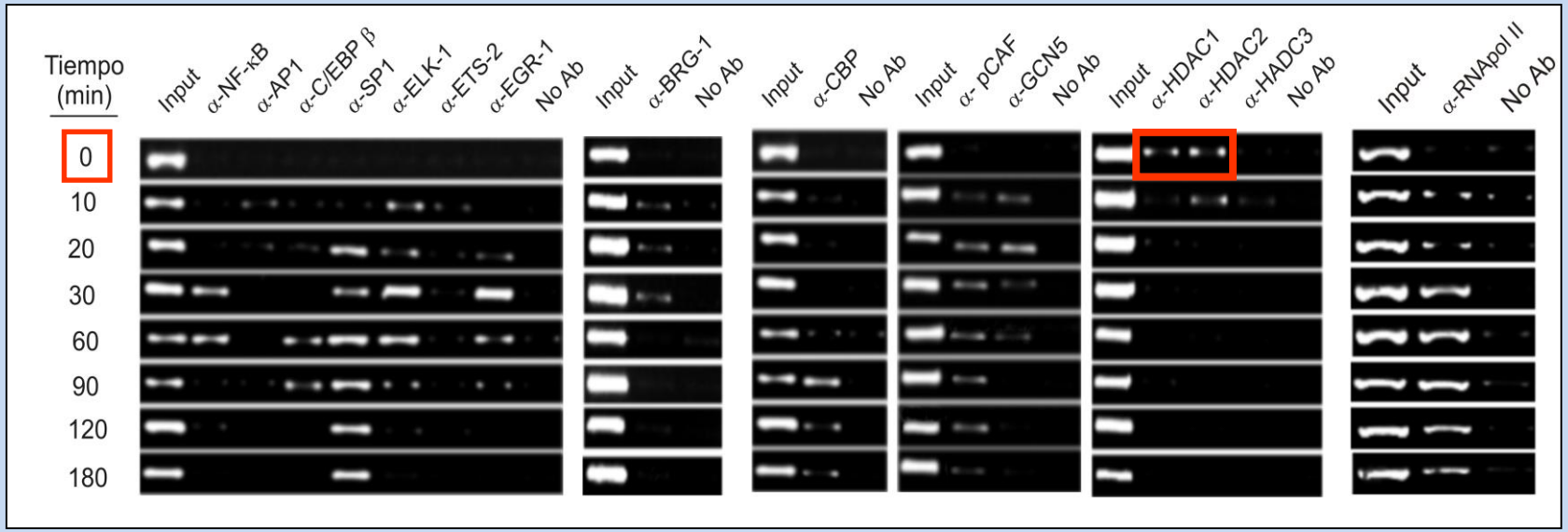
With specific primers

Gel electroforesis

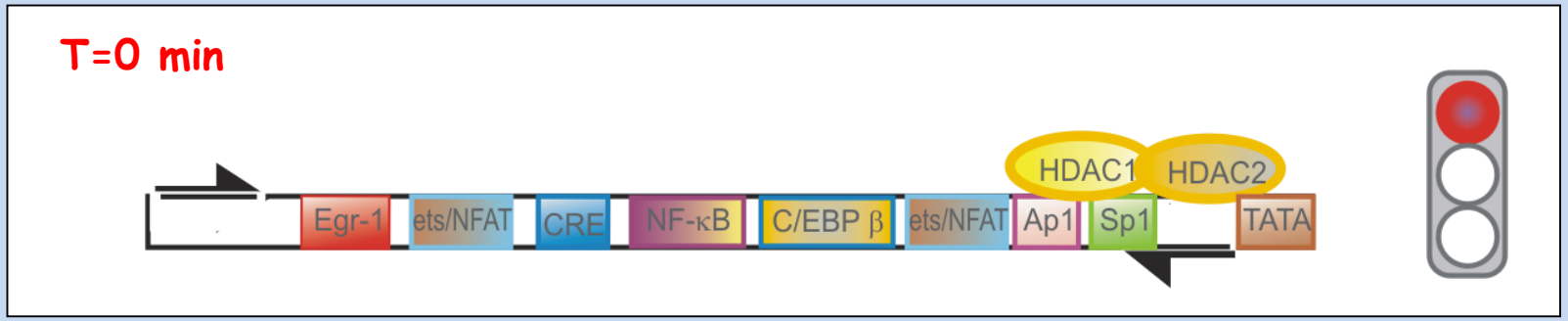


# Estudio temporal de unión de factores y complejos reguladores en el promotor de *tnf-α* durante la pancreatitis aguda

## *tnfα promoter*




## *Model of activation of tnfα*



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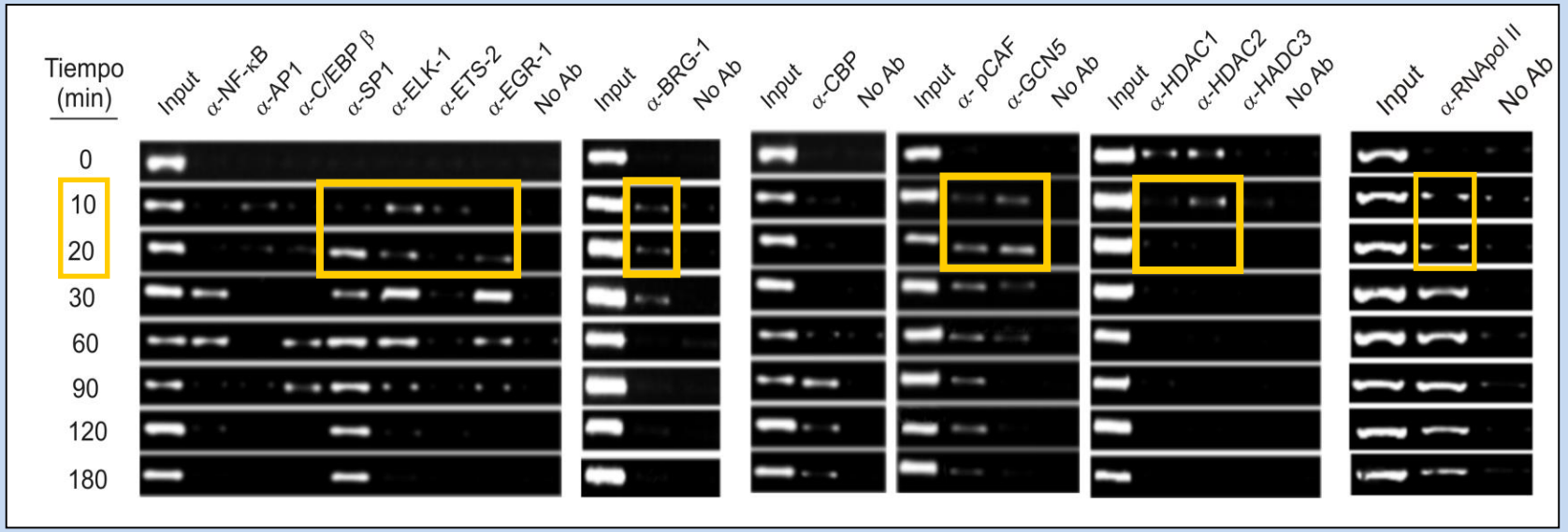
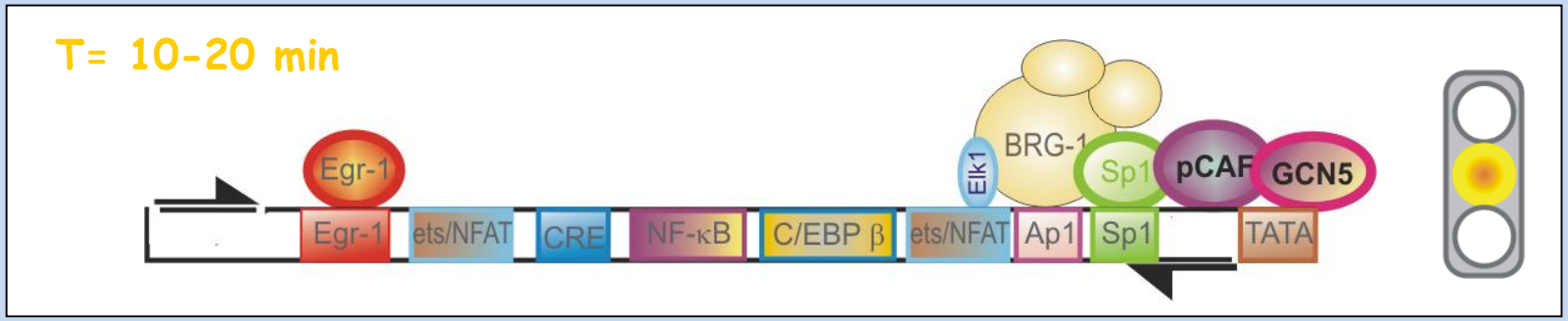
*tnfα promoter*




*Model of activation of tnfα*



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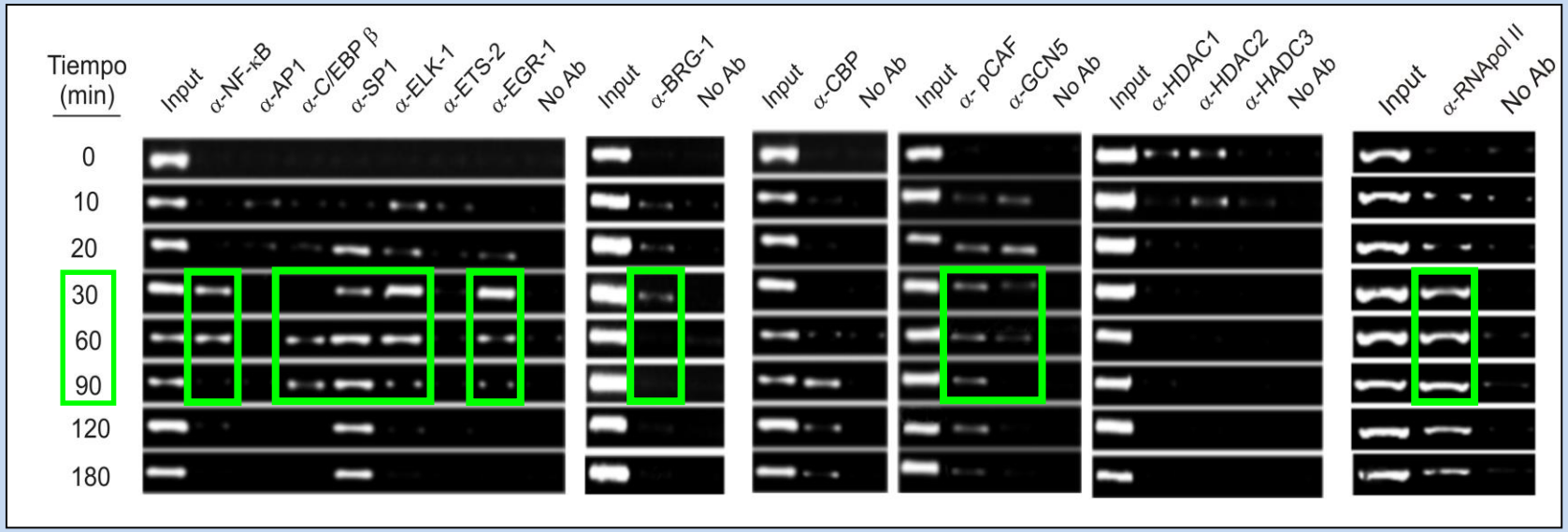


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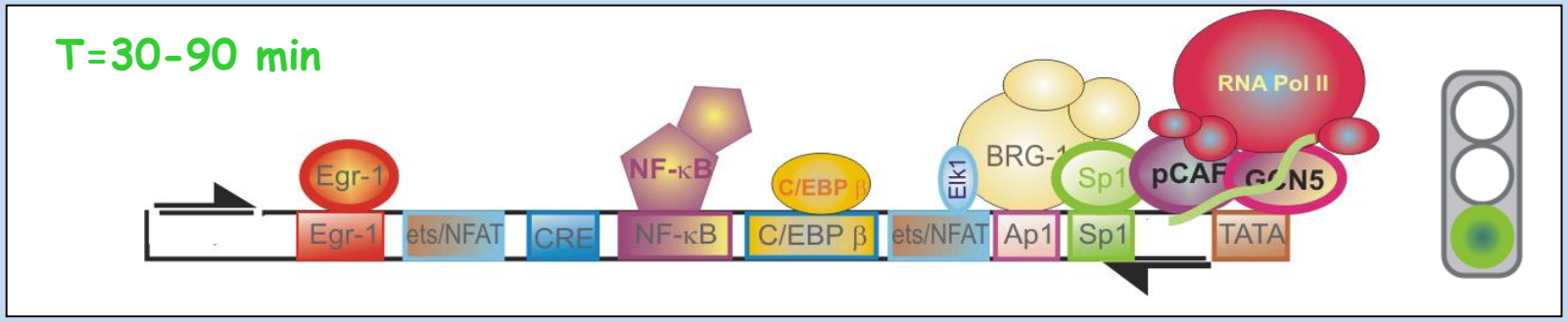
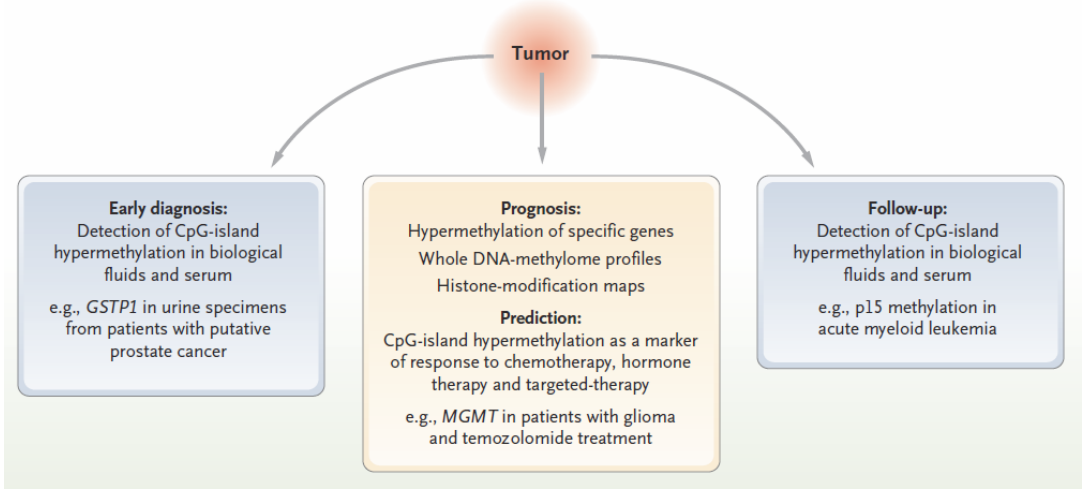





## APLICACIONES DE LA EPIGENETICA EN EL MANEJO DEL CANCER

- ✓ ***Perfiles epigenómicos como <u>marcadores de screening</u>***
- ✓ ***Perfiles epigenómicos como marcadores diagnóstico (GSTP1, p16INK4a, MGMT, MLH1)***
- ✓ ***Perfiles epigenómicos como <u>marcadores de pronóstico</u> (DAPK, EMP3)***
- ✓ ***Farmacoepigenética: epigenómica como <u>marcador de respuesta</u> a quimioterapia (MGMT)***
- ✓ ***Marcas epigenómica como <u>dianas terapéuticas</u> (HDAC inhibitors; DNA demethylation agents)***

# Epigenomics core facility

## Usuarios en el IISLaFe

- Cardiopatías Familiares, Muerte Súbita y Mecanismos de Enfermedad
- Regeneración y trasplante cardiaco
- Disfunción miocárdica y trasplante cardíaco
- Enfermedad inflamatoria intestinal
- Biomedicina molecular, celular y genómica
- Investigación clínica y traslacional en cáncer
- Unidad de biomarcadores y medicina de precisión (UBYMP)
- Perinatología
- Farmacogenética
- Grupo de investigación en neurociencia
- Urología
- Dermatología y regeneración tisular

## Usuarios a nivel nacional


## Usuarios a nivel internacional


IIS La Fe

Data Science

Biostatistics & Bioinformatics

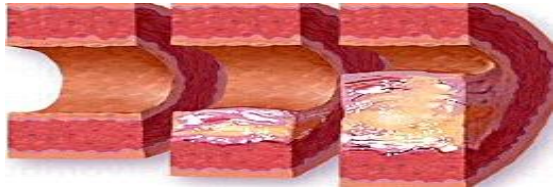
## Milestones

| 6 Papers | 7 Funded Projects | 1 Patent | 1 Prize |
|---|---|---|---|
| 2 published<br>1 in press<br>3 in preparation | 2 FIS<br>1 Hospital manises<br>1 prometeo<br>3 private agencies | European patent (lung cancer) in colaboration with Bemygene S.L. | Best oral communication in Congress Asociación Española Pediatría |

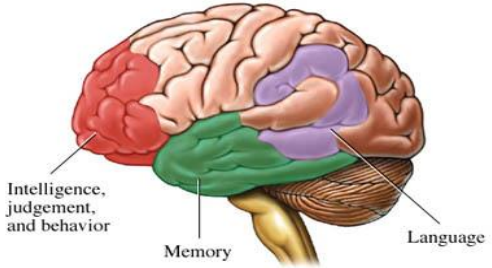
# Epigenómica y enfermedad



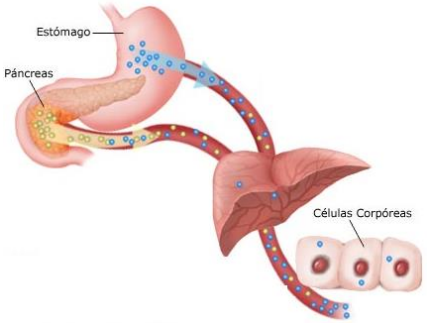
- Enfermedades cardiovasculares (ej. atherosclerosis)



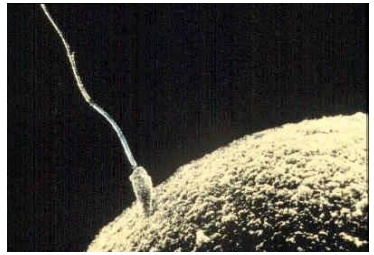
- Enfermedades neurológicas (ej. Alzheimer)




- Enfermedades autoinmunes (ej. Diabetes tipo I)




- Infertilidad



*Sandoval J et al, br j hematology 2013*
*Sandoval J, Krausz C et al, Plos one 2013*
*Heyn H, Sandoval J et al, Plos one 2013*
*Lopez-Serra P, Sandoval J et al, Nat commun 2014*
*Ongen H, Sandoval J et al, Nature 2014*

# Aplicaciones en calls H2020

## Calls

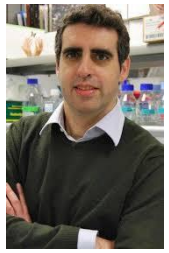
- 1.1 Personalized medicine
- 1.2 Innovative health and care industry
- 1.3 Infections diseases and improving global health
- 1.5 Decoding the role of environment for health and well being

# Experiencia en 3 proyectos europeos FP7

CURELUNG Determining (epi)genetic therapeutic signatures for improving lung cancer prognosis

CURELUNG
LUNG CANCER RESEARCH

Coordinadores
2011-2014
3.489.117 €
IP: Manel Esteller



**Publicaciones**

Journal clinical oncology
Clinical cancer research

SYSCOL: systems biology of colorectal cancer


Work package leaders
2011-2015
16.615.047 €
IP: Jusi Taipale

**Publicaciones**

International Journal of cancer
**Nature**
Cell reports

TArgeted therapy in Renal cell cancer: GEnetic and Tumour related biomarkers for response and toxicity

Euro TARGET

Work package leaders
2011-2015
7.187.700 €
IP: Mark Diederich

**Publicaciones**

En preparación


'M
YOUR
MAN