Nanotecnología: concepto, aplicaciones industriales. Estado del arte y tendencias en el área de alimentación y medioambiente

Nanotecnología Aplicada a la Valorización de Recursos Marinos

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Environment monitoring, security and food quality control research area
International Iberian Nanotechnology Laboratory (INL)

Environment monitoring, security and food quality control

MOLECULAR BIOLOGY AND NANOMATERIALS FOR FOOD ANALYSIS

DNA based analysis have become a very useful instrument to assess the safety and quality of the food chain. DNA testing has a broad range of applications such as detection of adulteration of food products (e.g. replacement of highly priced meat or fish species by similar but cheaper species, presence of horsemeat in food products…), identification of microorganisms that cause food-borne diseases, detection of genetically modified organisms (GMOs) or identification of allergenic ingredients among others.

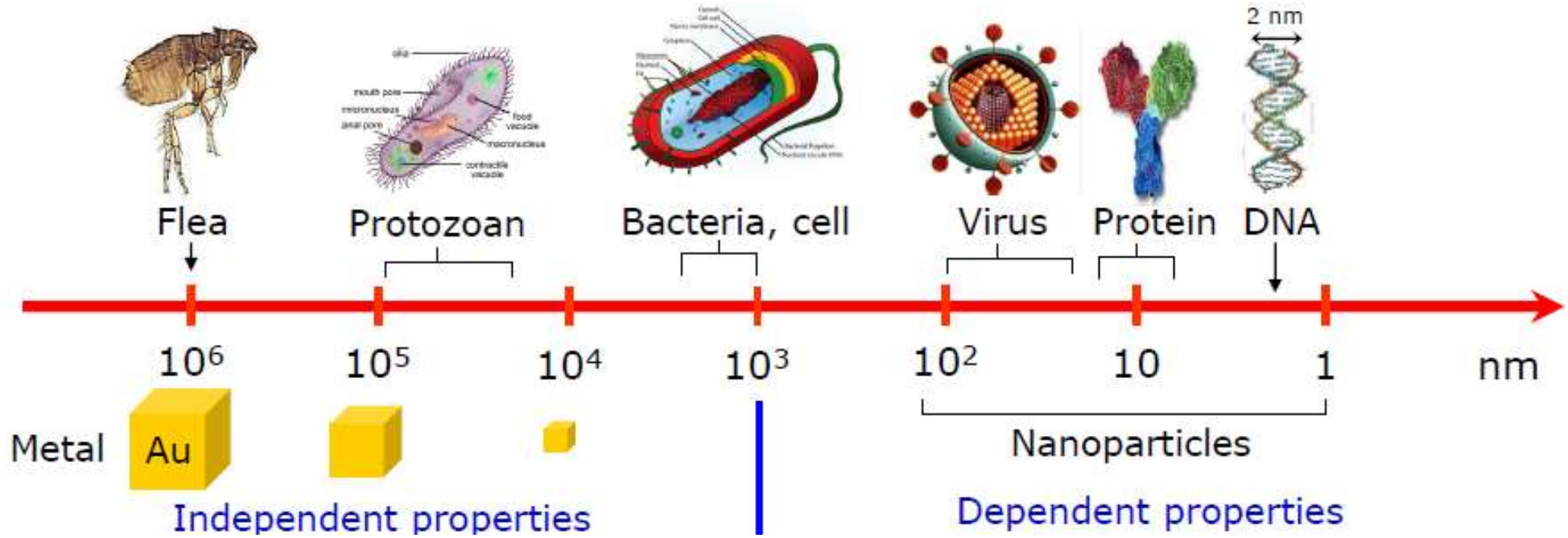
DNA testing involves several steps from the food product until the final result including: (i) DNA extraction/purification from the food product, (ii) frequently the amplification of a DNA sequence through an enzymatic process that will allow us to unequivocally identify the organism(s) of interest, and (iii) the detection and/or quantification of this DNA sequence. Each of this steps can be improved with the help of nanomaterials in order to develop faster, cheaper, multiplexed and/or more sensitive methodology of analysis.

The goal of the research in this area is to combine molecular biology and nanomaterials to improve each of the steps of DNA analysis with the focus on very specific applications in food analysis. We evaluate its fitness-for-purpose and its advantages when compared with conventional methodology, in order to develop fully validated and integrated DNA based analytical methods to provide control laboratories and food industry with efficient tools for food monitoring.

- Licenciatura en Ciencia y Tecnología de los Alimentos– 1999
- Doctorado Biología Molecular y Análisis de Alimentos– 2005
- Oficial Científico: Joint Research Center, EC (Geel, Belgium)-2005-2010
- Investigadora Laboratorio Ibérico Internacional de Nanotecnología (INL) (Braga, Portugal)

Contenido:

1. Nanotechnología: Concepto y aplicaciones
2. Laboratorio Ibérico Internacional de Nanotecnología (INL)
   a. Infrastructuras y áreas de investigación
   b. Medioambiente y alimentación
3. Biología molecular y nanomateriales para el análisis de alimentos
   a. ADN para nálisis de alimentos
   b. Extracción/purificación de ADN utilizando microfluídica



(Fuente: Campbell K, 2014)

¿Qué es la Nanotecnología?:
La manipulación de la materia con al menos una dimensión del tamaño de entre 1 a 100 nanómetros

¿Por qué es interesante?:
Estos materiales a escala nanometrica presentan propiedades diferentes a las que presentan estos mismos materiales de mayor tamaño (p. ej.: mayor reactividad química debido a su mayor área superficial)

RECOMENDACIÓN DE LA COMISIÓN de 18 de octubre de 2011 relativa a la definición de nanomaterial 2011/696/UE

Por «nanomaterial» se entiende un material natural, secundario o fabricado que contenga partículas, sueltas o formando un agregado o aglomerado y en el que el 50 % o más de las partículas en la granulometría numérica presente una o más dimensiones externas en el intervalo de tamaños comprendido entre 1 nm y 100 nm.

CE: “Las nanotecnologías tienen un gran potencial para lograr un crecimiento sostenible, mejorar la competitividad de la industria europea, proteger el medio ambiente, crear empleos altamente cualificados y mejorar nuestra calidad de vida.”

“La UE ha invertido mucho dinero en investigación y desarrollo de las nanotecnologías. Ahora deben crearse las condiciones adecuadas para aprovechar todo su potencial.”
http://ec.europa.eu/health/nanotechnology/policy/index_es.htm

Tecnología clave

Electrónica/ICT

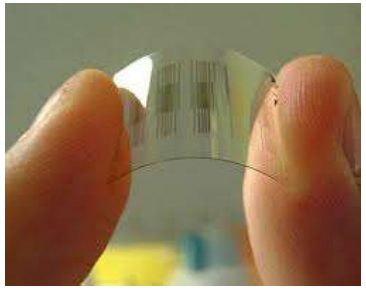
Componentes electrónicos, microprocesadores, semiconductores…

Energía y Medioambiente

paneles solares con mayor eficiencia, baterias con mayor capacidad
Nanomembranas y otros nanomateriales para purificación y eliminación de contaminantes

Salud

Diagnóstico, terapia (hipertermia, nanoencapsulación…), medicina regenerativa



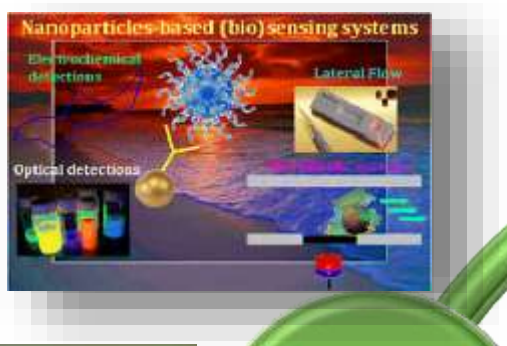
Textiles

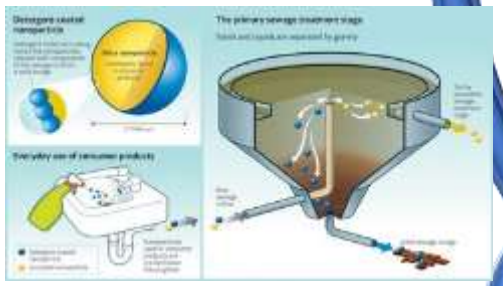
UV Block, antibacterianos, antihumedad, sustancias ignífugas

Otras aplicaciones

Sensores integrados, nanorobótica, cosméticos, envasado inteligente…

Ejemplos de aplicaciones de la nanotecnología





Envases de alimentos

Aditivos

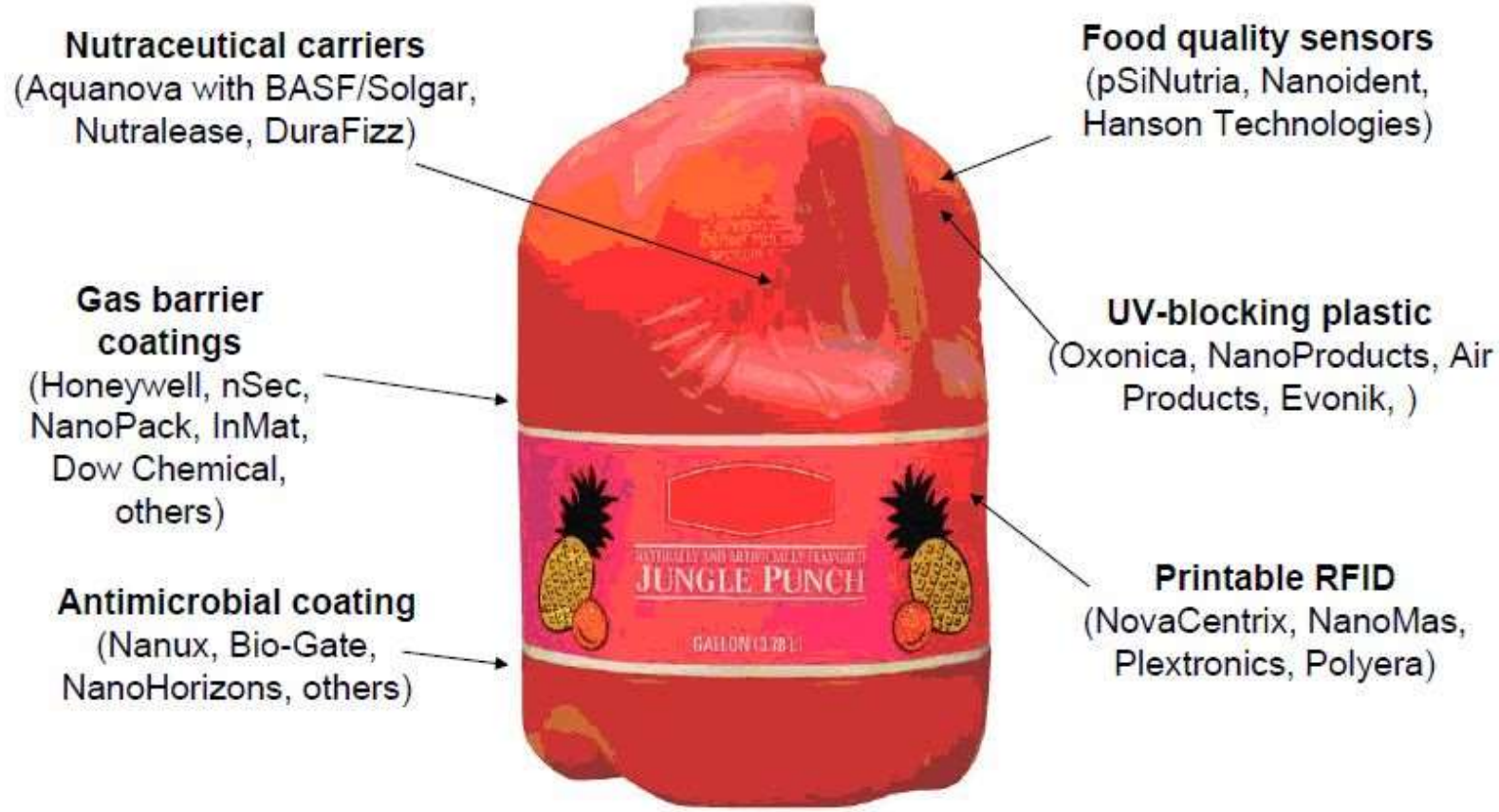
Actividad Antimicrobiana

Tratamiento de aguas

Sensing

Nanomateriales en alimentación y medioambiente

Ejemplos de produtos comerciales basados en la utilización de nanopartículas



Source: http://www.observatorynano.eu/project/document/2077/

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International Iberian Nanotechnology Laboratory numbers

1) Location: **Braga- Portugal**

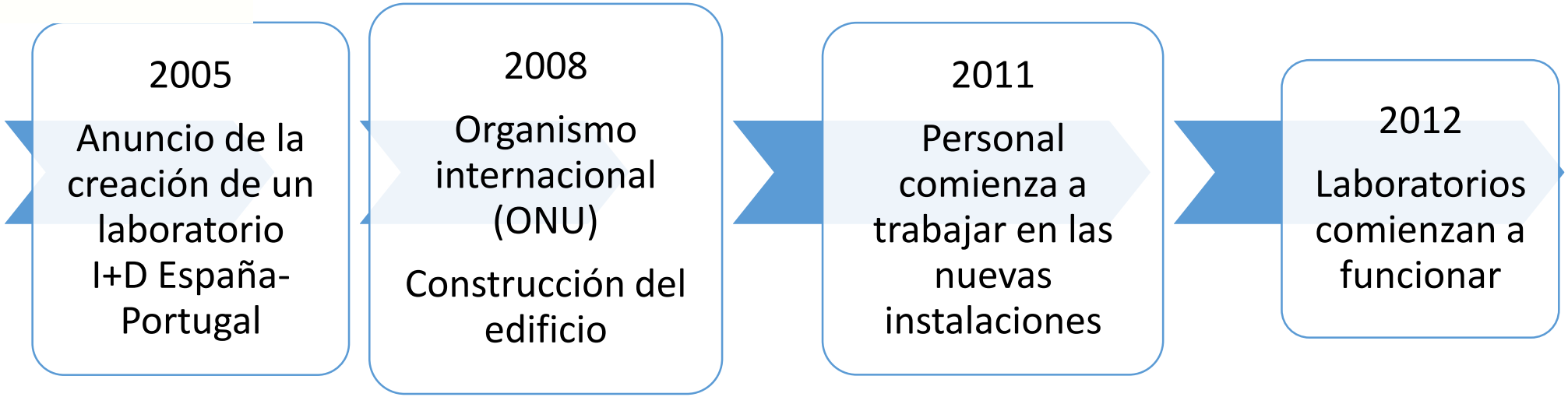
2) Status: **Intergovernmental Organization**

3) Projected Researchers:**~200 postdoctoral scientists**

4) Projected Total Staff: **~ 400 people**

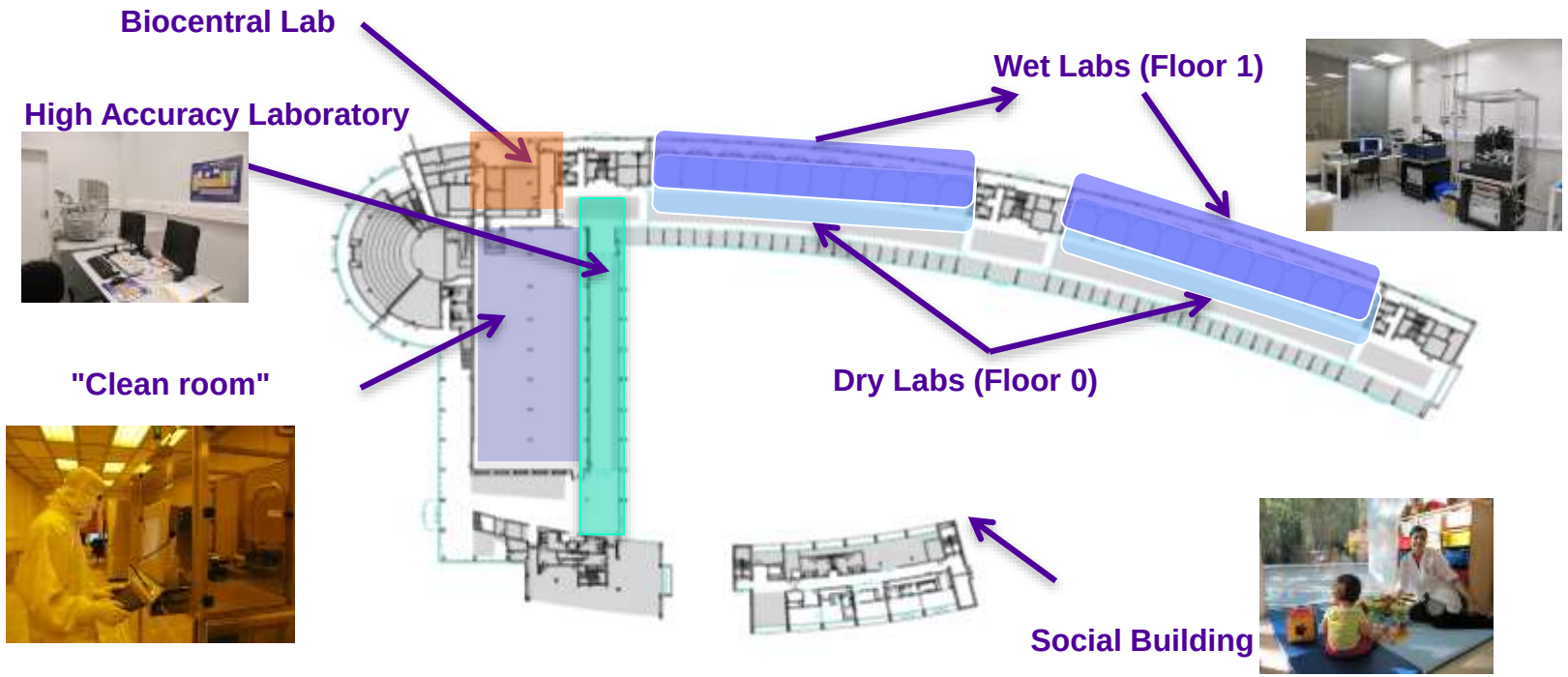
5) Research Space: **40 Principal Investigators labs**

INL
INTERNATIONAL IBERIAN
NANOTECHNOLOGY
LABORATORY



- The scientific infrastructure comprises central laboratories (providing services for the INL resident research personnel and visiting scientists) and specialized laboratories associated with individual Principal Investigators (PIs) or research groups and research topics.

- TheScientific building includes: Cleanroom, High Accuracy Laboratory, Wet and Dry PI laboratories, Biochemistry laboratory and other support labs.

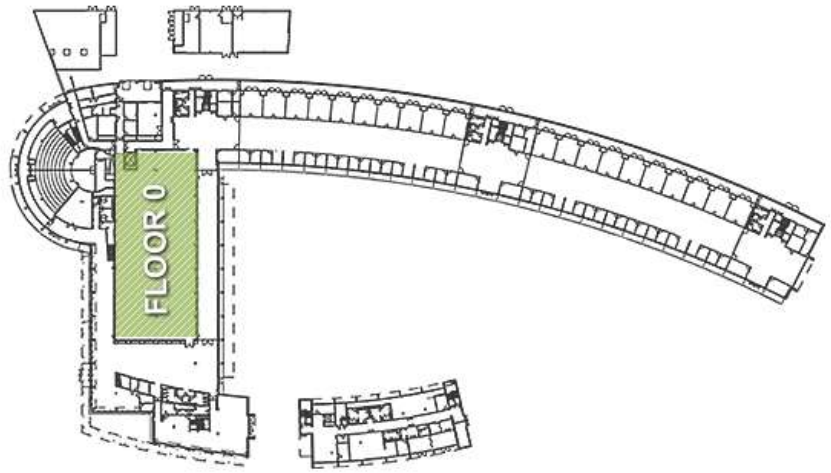


CLEANROOM

HIGHT ACCURACY LAB.

WET & DRY PI LABS.

BIOCHEMISTRY & RFID LAB.



The INL Cleanroom:

- 7 bays
- It comprises approximately 450 m2 of Class 1000 and 150 m2 of Class 100.
- Micro-and Nanofabrication solutions for 200 mm wafers down to small-sized sample pieces.

Nanolithography
Photolithography
Wet Process
Hot Process & Planarization
Reactive Ion Etching and Deposition
Cleanroom Biology and Biochemistry bay
Packaging Laboratory

CLEANROOM

HIGHT ACCURACY LAB.

WET & DRY PI LABS.

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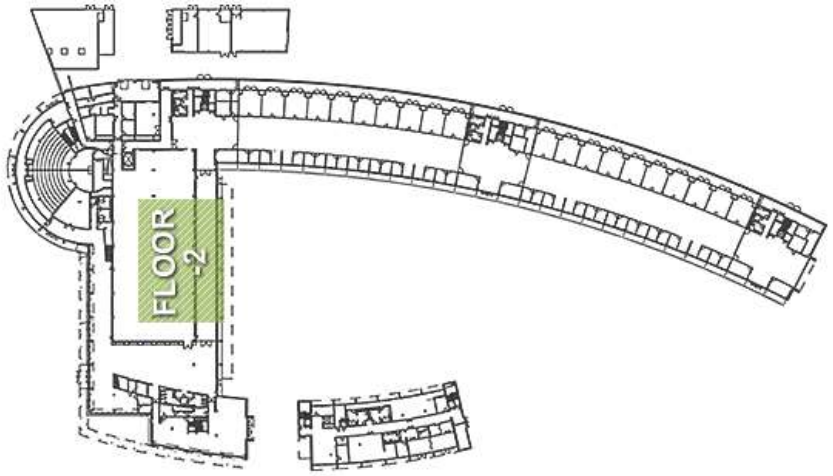

Advanced instrumentation for in-depth characterization of nano-materials and nanostructures

X-Ray Photoelectron Spectroscopy
Dual FIB with UHREM SEM
X-ray Thin Film/Reflectometry
Atomic Force Microscope - Materials
Environmental Scanning Electron Microscope (ESEM)
Small Angle X-ray Scattering (SAXS)
Atomic Force Microscope - BioScience
Probe Corrected HRTEM

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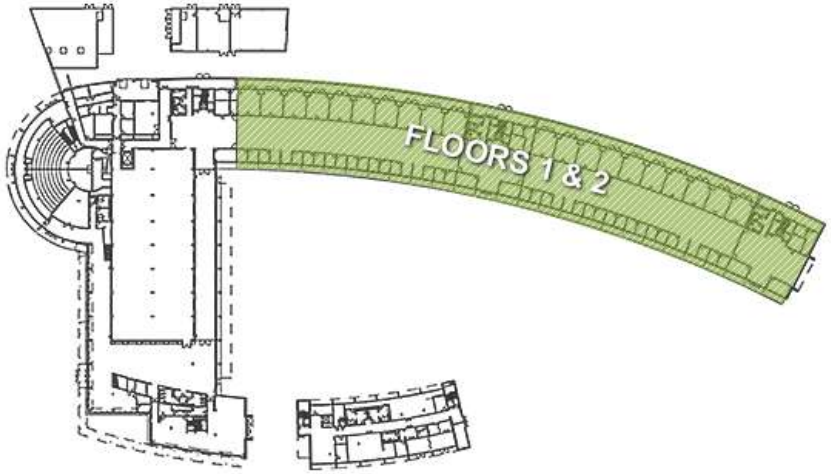

Wet Labs are laboratory spaces closer to group office space, available for PIs and their research teams to install and use equipment for synthesis, catalysis, and other wet chemistry experiments. Dry Labs are laboratories also available for PIs and their research teams to install and use equipment for characterization and dry experiment techniques

SQUID Magnetometry

Spectral Imaging Ellipsometry

Raman Confocal Microscopy

DLS

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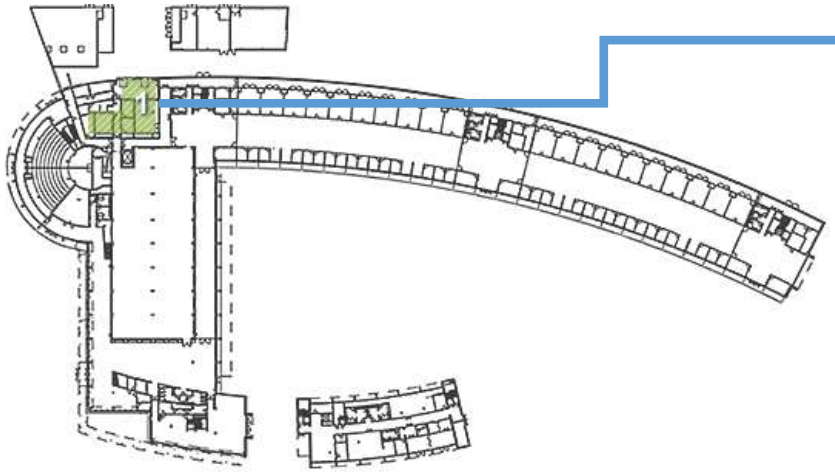

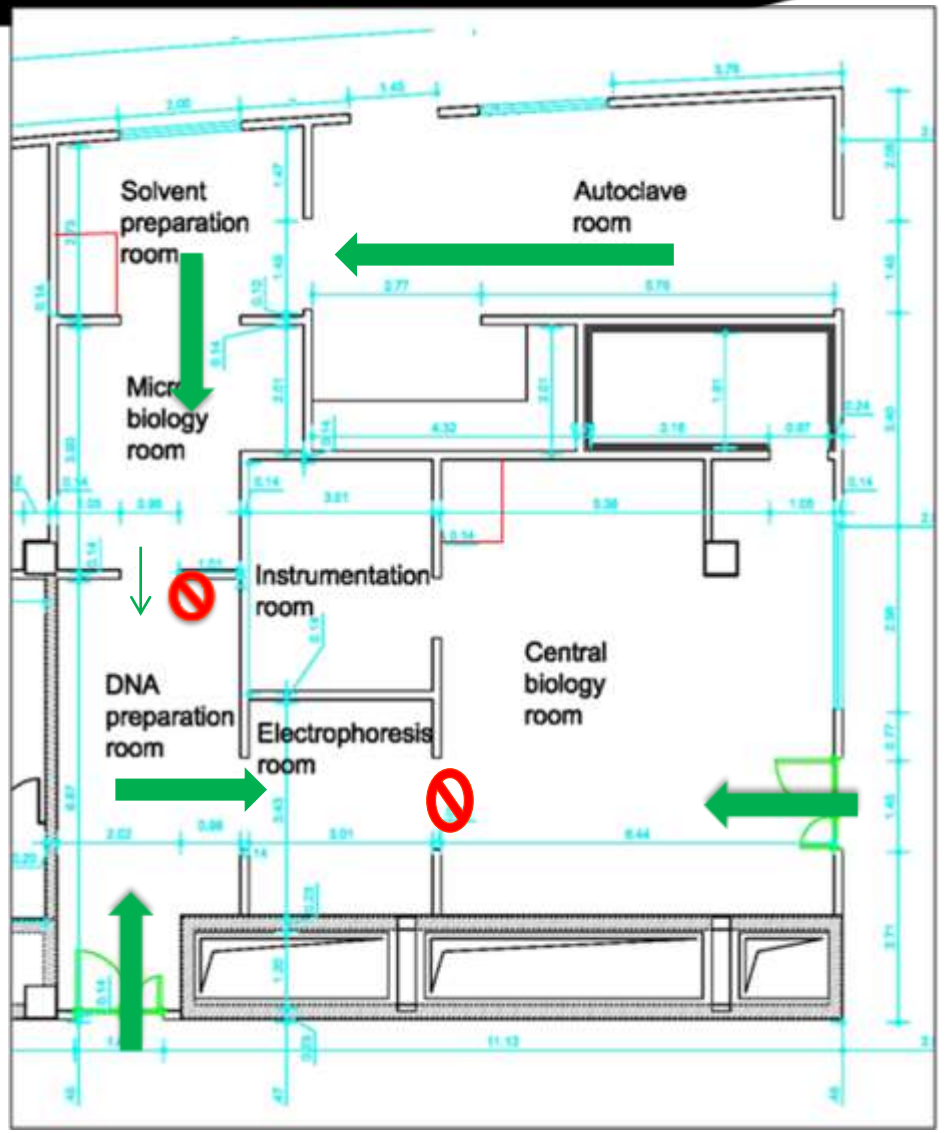

Central Biology Biochemistry Laboratory: Equipment for studies in the interface between biology and chemistry including all the necessary instrumentation for sample preparation, luminescence, absorbance and fluorescence measurements and imaging.

Instrumentation Room: Life Science Confocal Microscope and Multi-functional Inv Microscope for Life Sciences

Microbiology: basic microbiology equipment

Cell culture Lab: in separated area

Molecular biology area: dedicated areas for DNA/RNA extraction and quantification, DNA amplification (PCR and qPCR) and amplicon analysis.

1)NANOMEDICINE:

Drug Delivery systems, molecular diagnosis systems and chips, cell therapies, imaging solutions, regenerative materials, biomolecular labels, synaptic process monitoring, tissue engineering, etc

2) ENVIRONMENTAL AND FOOD CONTROL:

Nanotechnology applied to Food industry, food safety and environmental control. Water and Soil control, air pollution monitoring, artificial nanopore sensors, lab-on-a-chip technologies, Smart Packaging and labels, food control process, biosensing technologies,

3) NANOELECTRONICS:

NEMS/MEMS, Spintronics, Photonics, Nanofluidics, Molecular electronics, Organic electronics, Nanotechnologies to support the previous research areas

4) NANOMANIPULATION:

Single molecule/atom manipulation, molecular motors, nanotwezzers, Self-assembly controlled processes of building blocks for nanodevices.

Proyectos en el área de Medioambiente y Alimentos

- Detection of food-borne pathogens using a magnetoresistive platform

Maria Teresa Fernandez-Argüelles

- Electrochemical biosensor for Paralytic Shellfish Toxins detection

Raquel Queirós

- Metal organic frameworks for toxin encapsulation
- Magnetoresistive-based biosensor for fresh water biotoxins detection

Begoña Espiña

Proyectos en el área de Medioambiente y Alimentos

- Bacteriophages-driven *Campylobacter spp* detection using Magnetoresistive Biosensor

Carla Carvalho

- Smart-Nanohydrogels as Phage-Delivery Systems for the Control of Foodborne Pathogens

Pablo Fuciños

Proyectos en el área de Medioambiente y Alimentos

- Nanoparticle assisted PCR for food and environmental control applications

- Development and in-house validation of qPCR methods for detection of allergenic ingredients in food

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- DNA extraction from oil samples for PCR applications through microscale solid phase extraction

- Development of a biosensing device for olive oil authentication, combining Gold Nanoparticles and Ligation Chain Reaction

Karola Böhme

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Biología molecular y nanomateriales para análisis de alimentos

Environment monitoring, security and food quality control

MOLECULAR BIOLOGY AND NANOMATERIALS FOR FOOD ANALYSIS

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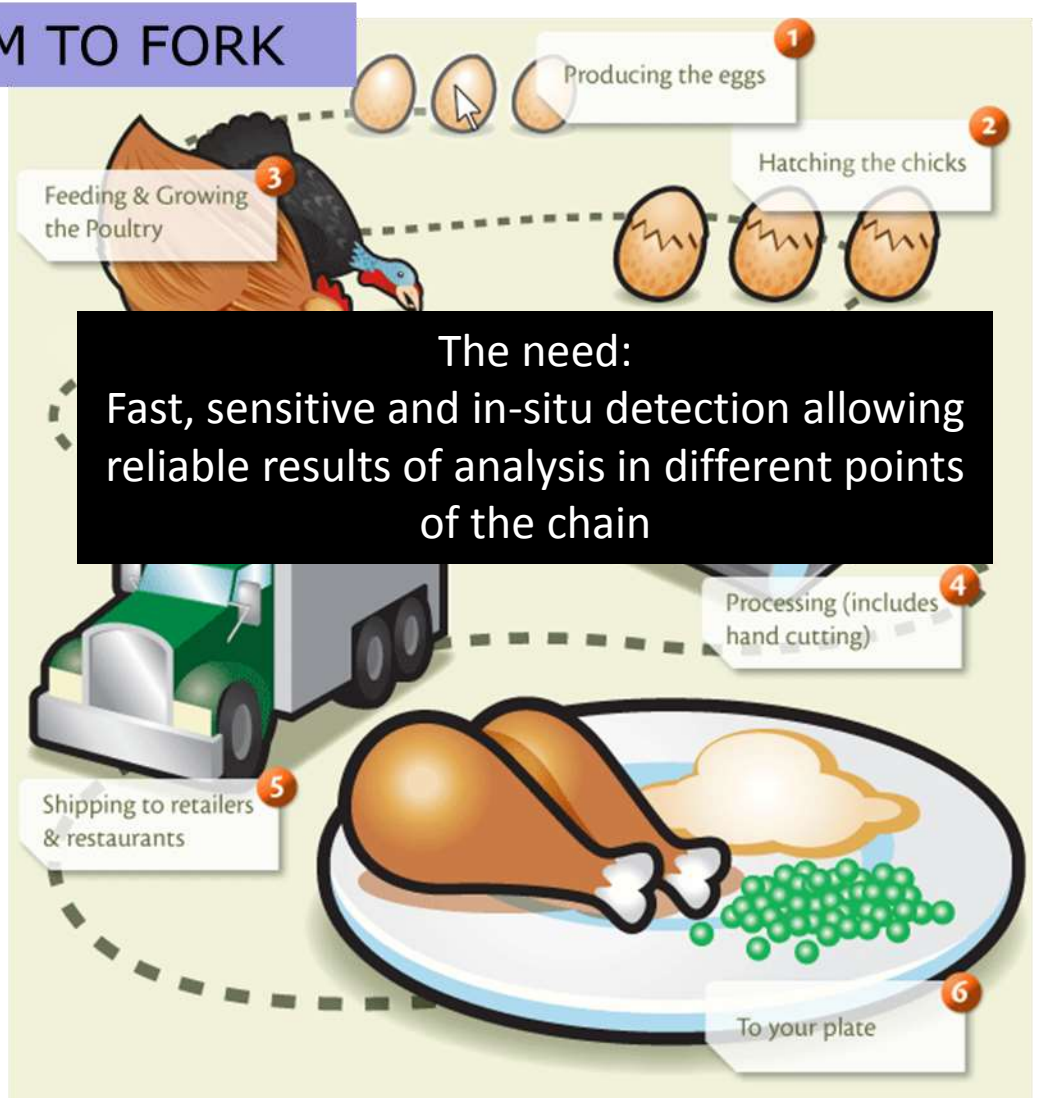
Nanotecnología para el control de la calidad y seguridade de los alimentos

FARM TO FORK



The need:
Fast, sensitive and in-situ detection allowing reliable results of analysis in different points of the chain

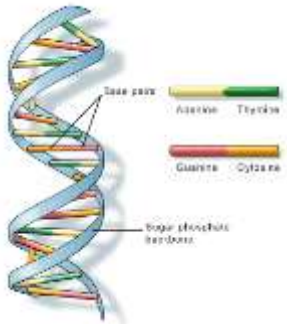
Relatively fast process

Many steps in the chain

Methods of analysis

- Slow
- Expensive
- Complicated

DNA for food and environmental analysis



Very specific: DNA sequence

Ubiquity: blood, muscle, bones

Not affected by variability of phenotype

Stability: mummified tissues, bones, forensic samples, processed products

Pathogenic microorganisms

GMOs

Authenticity:

- Basmati rice
- Fish & shrimp species determination
- Meat and meat products
- Olive oil

Allergenic ingredients in food products

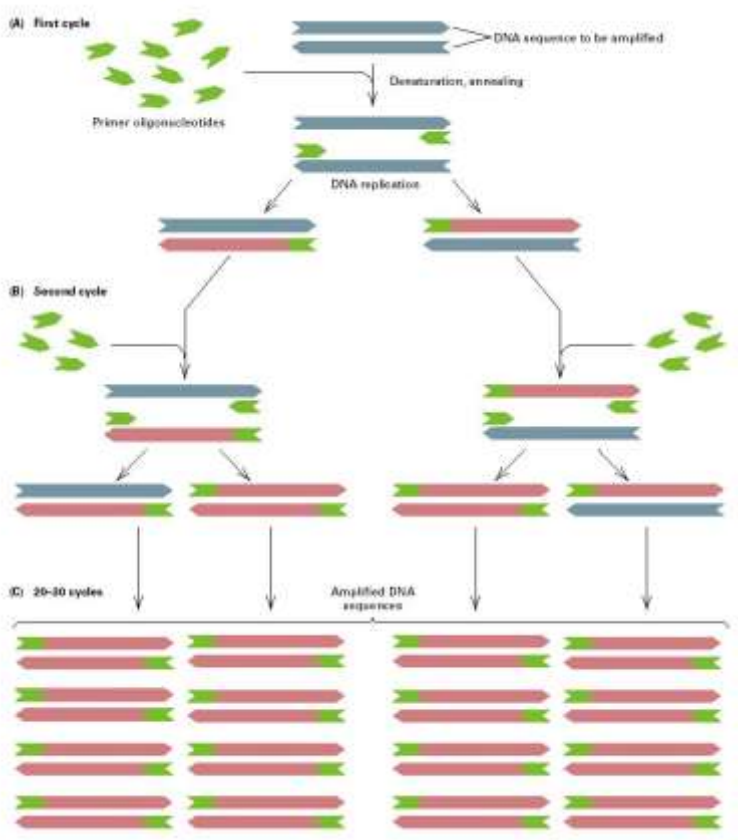
Nanomaterials
optical, electronic, magnetic and catalytic properties

+

=

- Increase the sensitivity of analysis
- Allow new detection approaches
  - Multiplexing possibilities
  - New tools for DNA analysis

Polymerase Chain Reaction (PCR) for food analysis

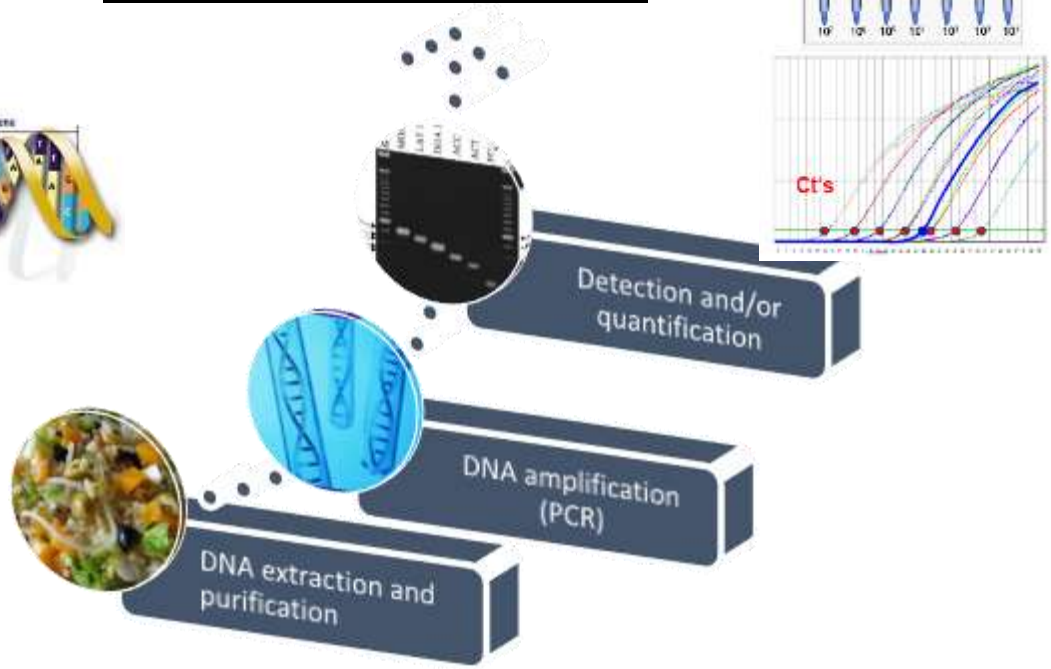


Enables detection of target sequence

PCR workflow for food analysis

Analysis of results and reporting

qPCR



- Requires experience personnel
- Due to its sensitivity might be influenced by false positives
- It is relatively time consuming

- DNA extraction from oil samples for PCR applications through microscale solid phase extraction (µSPE)

DNA extraction and purification

- Development and in-house validation of qPCR methods for detection of allergenic ingredients in food
- Evaluation of the effect of NPs on PCR: NP assisted PCR

Amplification of DNA sequences

- New detection/quantification approaches using NPs

Detection and/or quantification

Objetivo final: miniaturizar sistemas de detección, rapidez, sensibilidad

- DNA extraction from oil samples for PCR applications through microscale solid phase extraction (µSPE)

DNA extraction and purification

Interes en análisis de ADN para muestras de aceite:

Autenticidad (aceite de oliva)

Detección de ingredientes alergénicos

Dificultades para extraer ADN amplificable:

Bajo contenido en ADN

ADN altamente degradado

Inhibidores de la PCR

Rendimiento de AND muy variable (Diferentes métodos de procesado del aceite, tiempo de almacenamiento…)

Ventajas

El aceite es una matriz líquida

Es una buena prueba de concepto del sistema

Commercial DNA extraction/purification systems:

Purpose of DNA extraction:

To obtain DNA in a relatively purified form

2 main parts:

- A technique to lyse the cells gently and solubilize the DNA
- Enzymatic, physical or chemical methods to remove contaminating proteins, RNA, or macromolecules

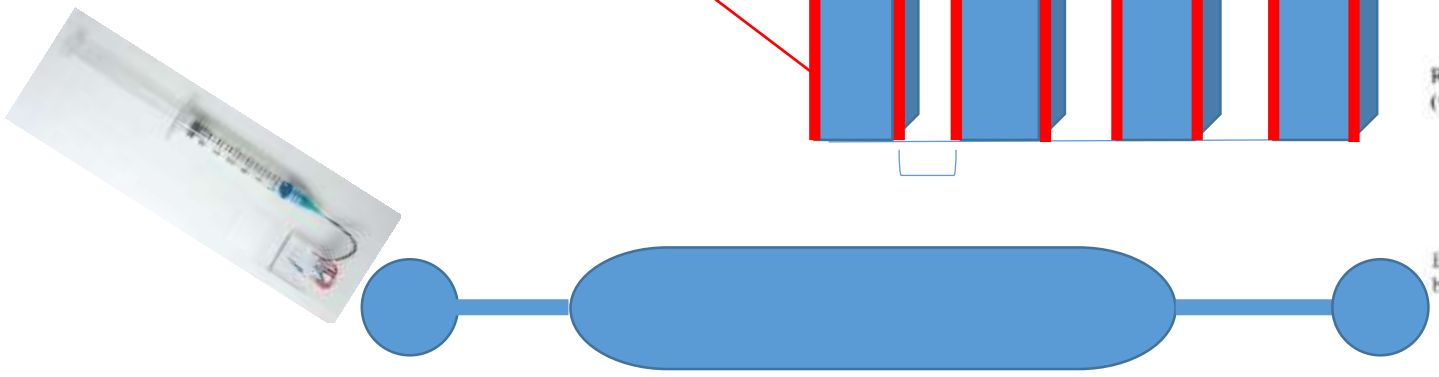
Most common traditional and commercial available methods:

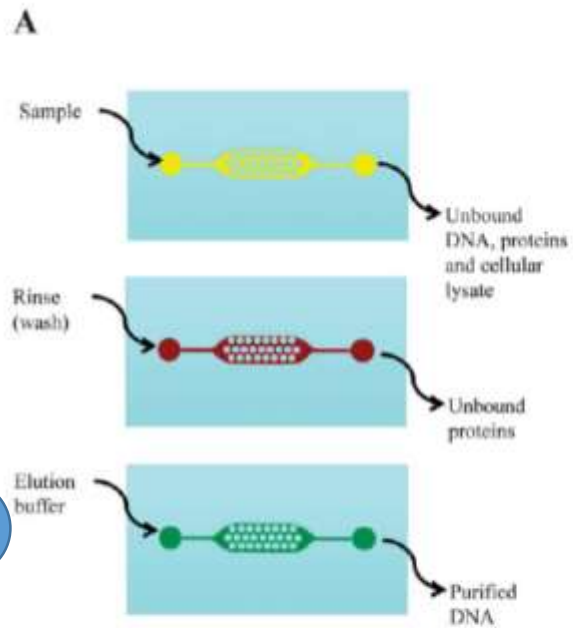
- Phenol/Chloroform extraction
- Silica membranes extraction
- Magnetic beads extraction

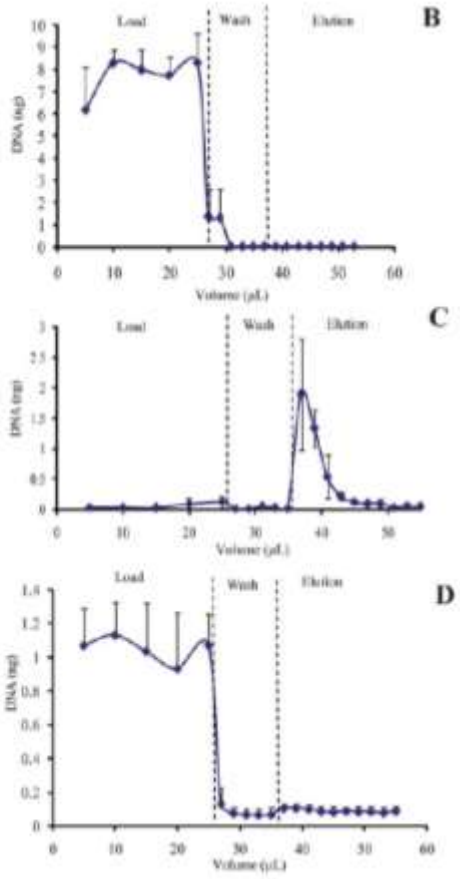
DNA extraction from oil samples for PCR applications through microscale solid phase extraction (µSPE):

Model 1: **coated pillars**

coating for DNA capture and release



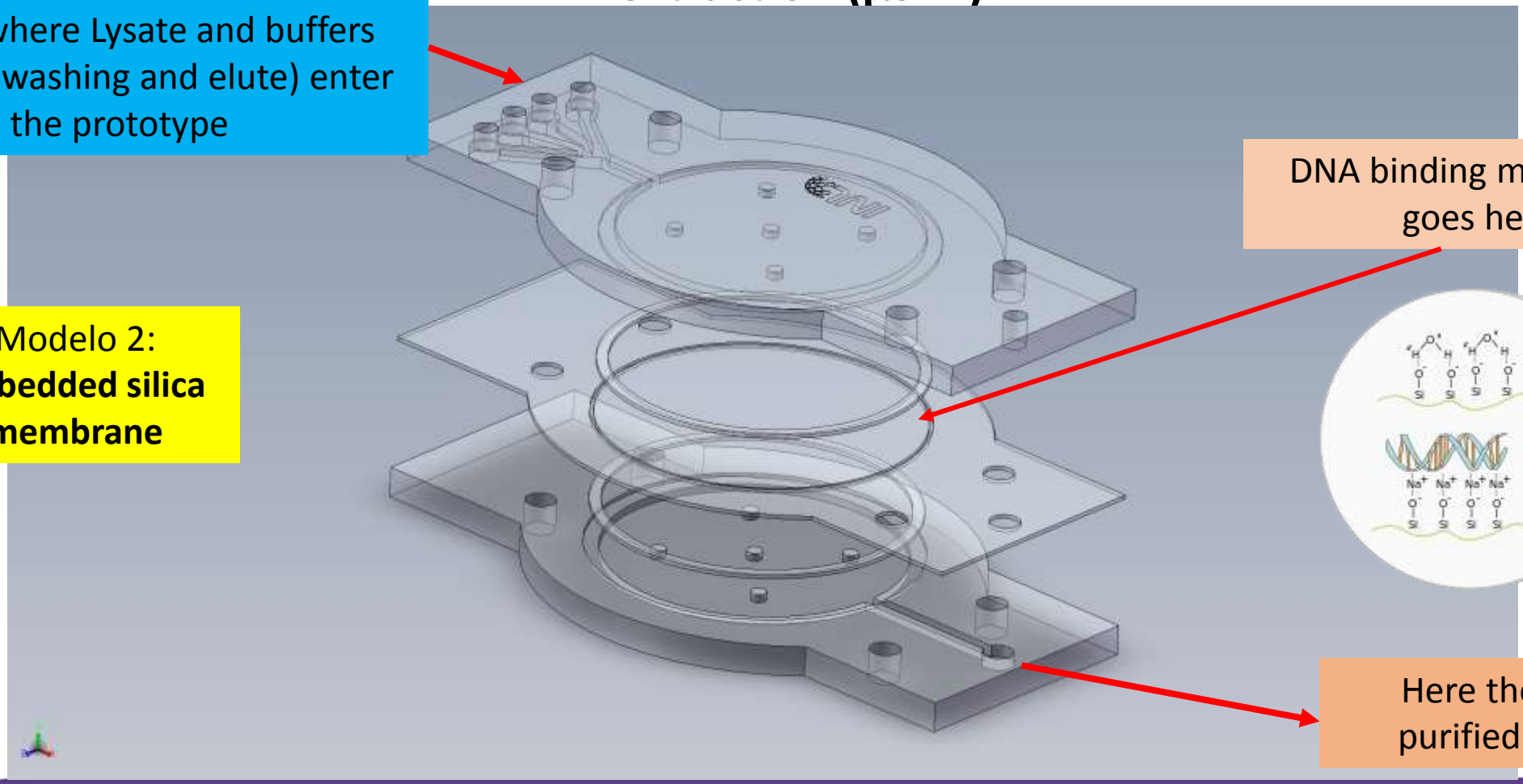




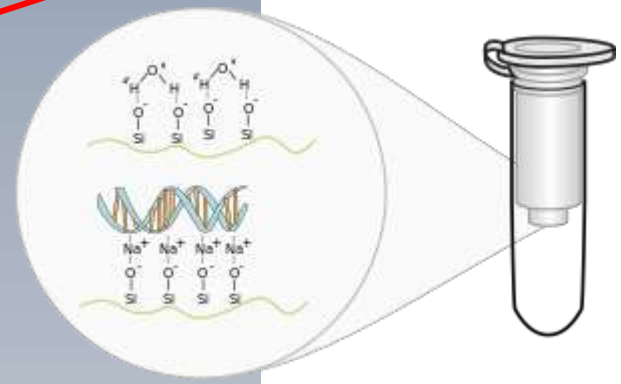
DNA extraction from oil samples for PCR applications through microscale solid phase extraction (µSPE):

Here is where Lysate and buffers (binding , washing and elute) enter the prototype

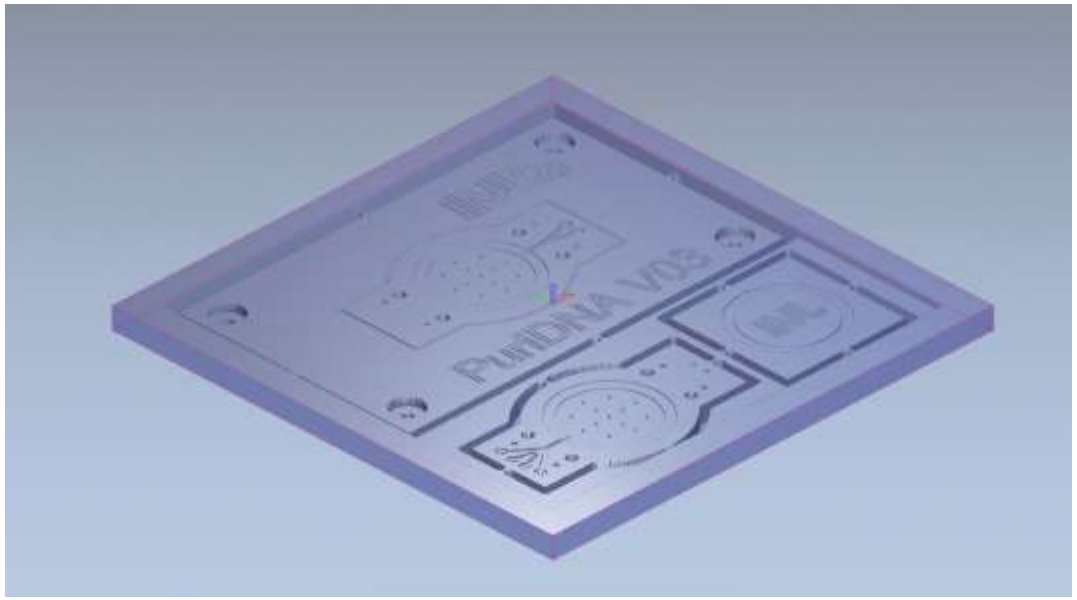
Modelo 2: **embedded silica membrane**

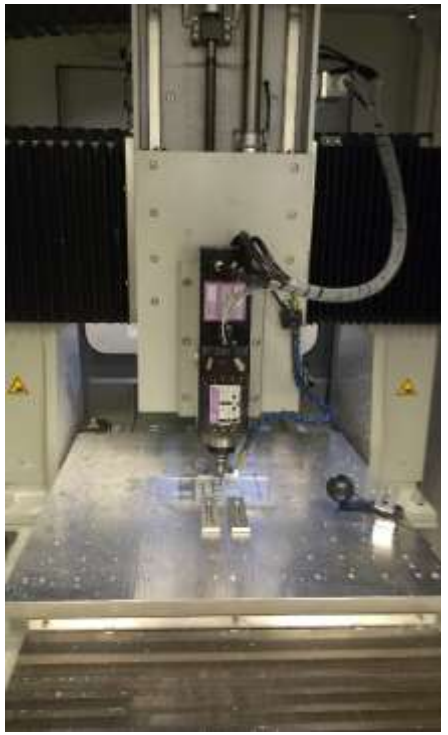


DNA binding membrane goes here



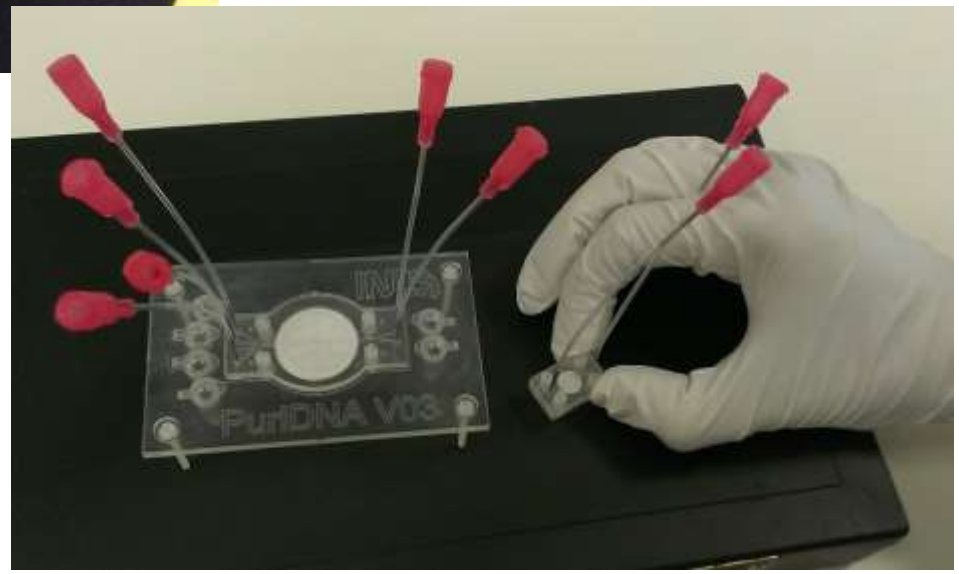
Here the waste and purified DNA go out





Computer Numerical Control (CNC) Mill for fabrication of pieces in PMMA

“PuriDNA” prototypes



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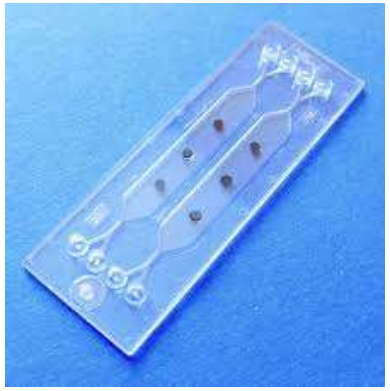
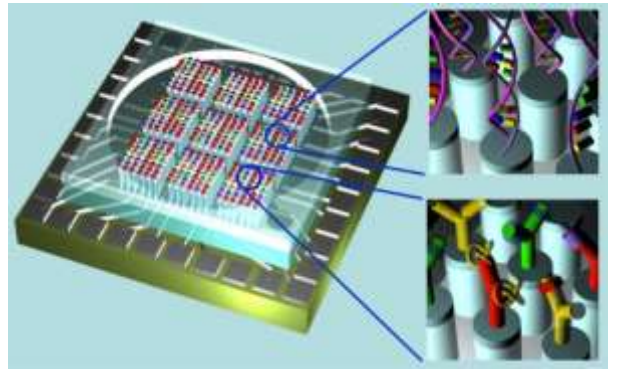


Palm PCR™
Portable High-Speed PCR System




THANK YOU
OBRIGADA
GRACIAS