29. Proyecto ANGELAB

Nombre del Proyecto	ANGELAB - A new genetic laboratory for non-invasive prenatal diagnosis		
Resumen del proyecto (1 frase)	Desarrollo de un nuevo sistema de diagnóstico prenatal no-invasivo basado en formato Lab-on-a-Chip.		
Fecha de comienzo del proyecto	1/10/2012	**Fecha de fin del proyecto**	30/09/2016
Organización líder o coordinadora	IK4-IKERLAN. Además de ser el líder del proyecto, es el líder del paquete de trabajo 4, de diseño, integración y fabricación de un componente microfluídico para trabajar con varias muestras, del paquete de trabajo 9 para la fabricación del LabonaChip, así como del paquete 16, de gestión del proyecto.		
Entidades participantes	**Organización**	**Contribución principal al proyecto**	
	HAHN-SCHICKARD-GESELLSCHAFT FUER ANGEWANDTE FORSCHUNG E.v. (Alemania)	SOCIO. Responsable del desarrollo de la unidad de chip para la PCR digital para la identificación de mutaciones (paquetes de trabajo 4 y 7). Participa junto con Ikerlan en la definición de los requerimientos en el paquete de trabajo 1 y en los aspectos integradores de la unidad de control del LabonaChip (paquetes de trabajo 8, 9 y 10).	
	ADEMTECH SA (Francia)	SOCIO. Se centra en el desarrollo de reactivos (partículas magnéticas) y protocolos para la preparación de las muestras. Sus tareas principales están dentro de los paquetes de trabajo 1 y 2.	
	SVS-OSAKIDETZA (entidad vasca)	SOCIO. El grupo de Genética del Hospital Universitario Basurto es el líder del paquete de trabajo 2, donde se ponen a punto los protocolos en tubo de las reacciones que luego se van a desarrollar en los chips de diagnóstico, del paquete de trabajo 14, sobre los aspectos éticos del proyecto, y líder de una de las tareas del paquete de trabajo 11, para la verificación técnica de uno de los sistemas desarrollados.	
	FUNDACION RIOJA SALUD	SOCIO. Está involucrado en todas las actividades del proyecto relacionadas con las tareas BIO (paquetes 2, 3, 6 y 7). Es el líder del paquete de trabajo 3, extracción de ADN fetal.	

	POLITECHNIKA WROCLAWSKA (Polonia)	SOCIO. Su principal contribución al proyecto es dentro del paquete de trabajo 8, para el desarrollo del sistema óptico de lectura de los chips.
	THE CYPRUS FOUNDATION FOR MUSCULAR DYSTROPHY RESEARCH (Chipre)	SOCIO. Es el líder de los paquetes de trabajo 1 y 11, para la definición de las especificaciones y los parámetros necesarios para el desarrollo del sistema, así como el responsable de testar y validar el sistema ANGELAB2.
	NIPD GENETICS LIMITED (Chipre)	SOCIO. Es el líder del paquete de trabajo 5, para el desarrollo y validación del módulo de PCR a tiempo real para la identificación de aneuploidías en los cromosomas 13, 18, 21 y X en la plataforma ANGELAB2. También es el líder del paquete de trabajo 14, de explotación, regulación y diseminación de los resultados.
	DNA DATA SLP (empresa vasca)	SOCIO. Es el líder de una de las tareas del paquete de trabajo 11, para la verificación del chip de diagnóstico de la fibrosis quística, atrofia muscular espinal y enfermedades ligadas al cromosoma X.
	BIOPHARMA TECHNOLOGY LTD (Reino Unido)	SOCIO. Se encarga de investigar los parámetros necesarios para la liofilización de los reactivos de PCR y DNA. También investigará la producción a pequeña escala del prototipo y el escalado del proceso.
	EV GROUP E. THALLNER GMBH (Austria)	SOCIO. Su principal tarea es la definición de los requerimientos del mercado para la fabricación modular y a gran volumen de los chips microfluídicos, así como el desarrollo de los módulos para el sistema. Es el líder del paquete de trabajo 12, cuyo objetivo principal es implementar, fabricar e instalar una línea de producción piloto.
	GAIKER-IK4 (entidad vasca)	SOCIO. Participa en el paquete de trabajo 2 de puesta punto de los protocolos en tubo de las reacciones, y es el líder de dos de las tareas del paquete de trabajo 9, de evaluación de las posibles alternativas de reciclaje de los chips de diagnóstico, y de evaluación del ciclo de vida económico y ambiental a escala de laboratorio del dispositivo.

	ASOCIACION INSTITUTO BIODONOSTIA (entidad vasca)	SOCIO. El grupo de Genética del Hospital Universitario Donostia es el líder del paquete de trabajo 13, de validación del nuevo sistema de diagnóstico en un escenario real, comparándolo con la técnica habitual.
	Centrum fur Angewandte Nanotechnologie (CAN) GmbH (Alemania)	SOCIO. Es el líder del paquete de trabajo 8, para el desarrollo de los sistemas de detección.
	POC MICROSOLUTIONS SL (empresa vasca)	SOCIO. Es el líder del paquete de trabajo 10, para el diseño del sistema de diagnóstico y su integración.

Presupuesto del Proyecto (miles euros)	**Año**	**Presupuesto Total**	**Participación vasca**
	2012-2016	10.955.292,00€	4.354.227,00€

Fuentes de financiación de la participación vasca (miles euros)	**Año**	**Financiación 1: FP7**	**Financiación 2: Socios del proyecto**	**Otras Ayudas públicas**
	2012-2016	3.320.782,00€	1.033.445,00€	

Ámbito de actuación	**Áreas prioritarias estratégicas** Marcar con una X		
	Fabricación Avanzada	Energía	Biosanitaria
			X

	Territorios de Oportunidad Marcar con una X			
	Alimentación	Hábitat Urbano	Ecosistemas	Ind. Cultural y Creativas

Descripción resumida del Proyecto: principales objetivos y resultados a desarrollar, retos a los que responde, impacto potencial económico y social, etc.

Existing gold standards for fetal genetic diagnosis are invasive techniques (CVS, amniocentesis). These techniques are risky and expensive while current non invasive alternatives (pre-screening tests) have low sensitivities (80-90%) and specificities (around 95%).

There is not any non-invasive alternative in the market yet. Along 2012, three companies (Sequenom, NIPD Genetics and DNAData) have foreseen the launching of non-invasive techniques done on test tube using fetal DNA extracted from mother's blood/plasma. Their main inconveniences are the very limited set diseases and uses (sex determination and trisomy 21), the complexity of the process, the need of delivering the samples to a specialised laboratory (specific equipment and trained technicians), and their high cost (300€ per sex determination and ~1.500 € per trisomy 21). Therefore, it is not possible to offer these emerging solutions to all pregnancies since it is not a cost effective screening solution.

Against the above mentioned methods, ANGELAB project aims at developing new highly reliable, irrefutable and cost-effective NIPD systems by transferring advanced on tube techniques belonging to the consortium partners to LabonaChip. The intrinsic tube difficulty and its invasive alternatives are the reasons why we actually chose this application to be transferred to a LabonaChip. This project will deliver a set of non-invasive genetic diagnostic systems with a CE mark for hospital labs covering the prenatal genetic diseases models. This set consists of 3 systems (see next figure):

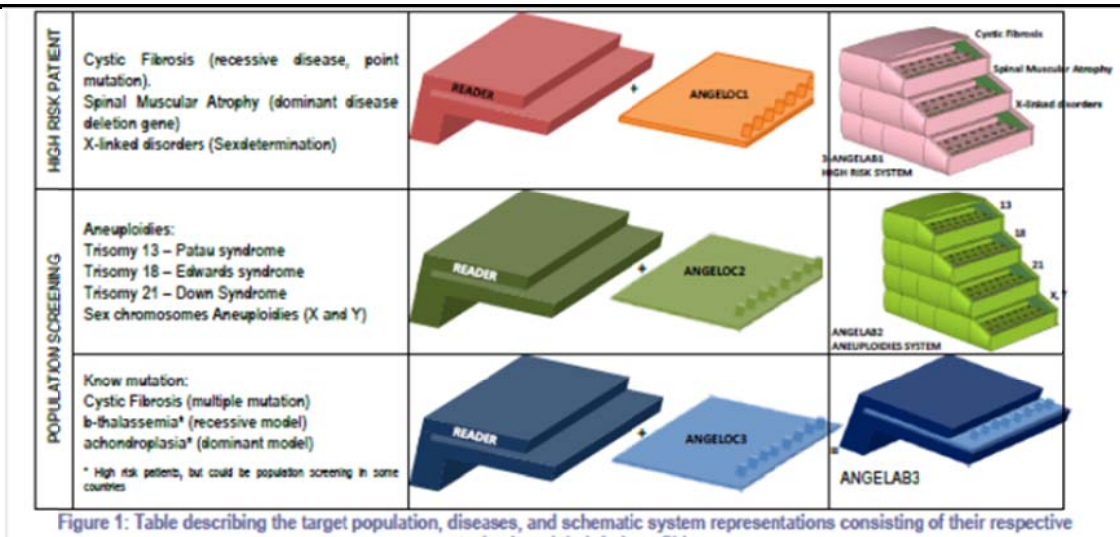

Figure 1: Table describing the target population, diseases, and schematic system representations consisting of their respective control unit and their LabonaChip.

- **ANGELAB1**. This system will use a LabonaChip to extract fetal DNA from the mother´s plasma based on differences in methylated pattern between fetus and mother and captured by specific by magnetophoresis plus qPCR for monogenic diseases with a known mutation: SMA, CF, or X-linked disorders. This system will be used only on high risk population with 8 samples at a time.
- **ANGELAB2**. This system will use a LabonaChip to carry out immunoprecipitation (MeDIP) for fetal DNA extraction from mother´s blood and real time qPCR for Aneuploidies of chromosomes 13, 18, 21, X and Y (using epigenetic differences between fetal and mother DNA). This system will be used for population screening purposes for 8 samples at a time.
- **ANGELAB3**. This system will use the DNA sample provided from ANGELAB1 or ANGELAB2 and it will carry out digital PCR on a LabonaChip to detect multiple mutations in: CF, β - thalassemia and achondroplasia. This system will be used for screening purposes for 8 samples at a time.

The consortium will also develop and integrate a LabonaChip Pilot Production Line (LPPL) in order to demonstrate the feasibility of the solution even at a manufacturing scale:

- **LPPL**: This LabonaChip Pilot Production Line will provide a sustainable and economic LabonaChip manufacturing. Materials, processes, and its life cycle will be considered. The environmental assessment will be carried out following the general requirements of ISO 14044. Quality control tools will be integrated along the entire production chain of LabonaChips (dimensional, surface coating, reagents dispensing, sealing quality). Furthermore, the Intelligent Manufacturing Systems (IMS) program will be followed in this task through the fabrication of 1000 LabonaChips (300 for testing, development and verification purposes and 700 for the mentioned technical validation).

This project has been conceived from its very first steps to fulfil the market needs. The perspective of end users (hospital labs) has been taken into account in order to maximize the project results and developing a close to market solution. In addition, the project will end with the implementation of these three diagnostic systems as pilot routines in two hospitals through a technical validation of 700 pregnancies. In order to be able to attract health technology assessment committees (e.g. OSTEBA), our developed systems will go through an extensive technical validation fulfilling CE standards.

The main objective of the project is to replace Invasive Prenatal Diagnostics methods by extracting and analysing fetal DNA from maternal blood using a LabonaChip strategy. Patented molecular tube techniques will be transferred to also unique LabonaChip designs creating systems. These systems will give a unique world position to the only European supply chain that has their own patent portfolio to sell prenatal diagnostics based on fetal DNA from maternal blood. The other two competitors are USA based

companies: Sequenom and Verinata Health. This commercially oriented goal has an incredible challenge since it requires integrated systems for sensitive, specific and multiparametric in vitro analysis under a cost effective model in real scenarios. In fact it has been never attempted before. This driving idea is represented in the Figure 10.

The **main objective of ANGELAB project** is to develop the first highly reliable, conclusive and cost-effective NIPD systems based on the extraction and analysis of fetal DNA from mother's blood/plasma, by transferring advanced in test tube techniques to LabonaChip. This is scientifically and technologically a huge and risky challenge. To achieve this objective, we will develop, consolidate and exploit a set of technologies that will revolutionise the In Vitro Diagnostics based on LabonaChip since there is nothing like it. To help the reader to quantify the scientific objectives related to each ANGELAB system, we have split the scientific objectives to the systems to be developed. The next table not only summarizes and quantifies the objectives of each system, but it also gives an idea about the ambitious objectives of the project comparing the expected results with the current invasive gold standards:

PROJECT OBJECTIVES VERSUS EXISTING COMMERCIAL GOLD STANDARDS				
PROJECT OBJECTIVES				Gold Standard
Features	ANGELAB1	ANGELAB2	ANGELAB3	AMNIO / CVS
Disease model	Known mutation	Aneuploidies	Multiplemutation	Known mutation, Aneuploidies,.
Disease number per system?	3 diseases	NA	3 diseases	NA
Result delivery time to patient	1 week	1 week	1 Week	3-4 weeks
Week of the analysis	8-10 week	8-10 week	8-10 Week	13-18 week
Minimum amount of samples per test?	Up to 8 patients	Up to 8 patients	Up to 8 patients	1
Result elaboration time?	2 hours	2 hours	1– 2 hours	3-4 days/3 weeks **
Automatic or Manual	Auto	Auto	Auto	Manual
General population screening	NO NEED	YES	YES	NO
Sample volume needed?	1 ml (plasma)	1ml blood	Few µl of DNA	20ml Amniotic fluid
Fetal DNA Purification ratio obtained?	>80%	50%	NA	NA
Sensitivity	>99%	>99.9%	>99.9%	>99%
Specificity	>99%	>99.9%	>99.9%	>99%
False Negative Ratio	<1%	<1%	< 1%	<1%
Miscarriage risk	0%	0%	0%	1-2%
Minimum size needed of the system?	PC size	PC size	PC size	Several rooms
Price per test/disease?	100€	100€	100€	875€
Manufacturing cost of the control unit?	1500€	1500€	500€	Several expensive equipment needed
How many +/- controls required?	1/1	0/3	1/1	NA
Connectivity (telemaintenance…)	Yes	Yes	Yes	No
Calibration by housekeeping gen?	Yes	No	NA	No
Shelf life and Temperature.	1 year/24ºC	1 year/24ºC	1 year/24ºC	NA
DNA contamination after amplification?	No risk	No risk	No risk	Risk
The maximum T° QD must withstand?	95ºC	95ºC	95ºC	NA
How many QD parameter detection?*	5	5	5	1
Sensitivity /Wavelengths**	3-8/0,01nM	3-8/0,01nM	3/0,01nM	NA
Multiparameter QD Fluorescence sensor	0,5 x 3 x 1 cm	0,5 x 3 x 1 cm	0,5 x 3 x 1 cm	NA

Figure 11: List of the objectives and their quantification according to the proposed systems.** We will have a higher theoretical number by combining colours. Culture is required. NA means Non applicable.

SOCIAL, ECONOMIC AND ENVIRONMENTAL OBJECTIVES:
This project has three main concise social objectives:

- To substitute at medium term the invasive techniques used for prenatal diagnosis by the safe new cost effective solutions developed in ANGELAB project
- To translate the benefits of the research effort carried out in this project to two hospitals and its patients in the form of three NIPD systems contributing to their future implementation in European Health Care Systems.
- To demonstrate to Health Care Technology Assessment providers the social benefits of a sensitive

and specific Non Invasive Prenatal Diagnostics (NIPD).

This project has three main economic objectives:

- To develop cost effective solutions (test prices of around 100€) that make the tests affordable to the general health system of every country, so that every pregnant woman can benefit from them.
- To put the European research organizations of NIPD LabonaChip automatic systems in leading positions in the world, improving the competitiveness of the European industry and contributing to attract new investments.
- To add value to society in the form of job creation and wealth by SME consolidation and Intellectual Property creation within the actors involved in the added value chain of NIPD In-Vitro Diagnostics.

This project has four main Environmental objectives:

- To carry out an environmental evaluation using a Life Cycle Assessment (LCA) and guidance ISO 14040 and ISO 14044 series, and the ILCD Handbook.
- To develop an innovative in-vitro diagnostic system achieving sustainability related advantages since our project will consider adaptation of a biodegradable material to the specific product requirement and functionality at a sustainable cost.
- To evaluate material recovery options, while avoiding special treatment requirements associated to conventional systems, and ensuring compliance with legislation.
- To apply this analysis to the fabrication equipment to be developed (LPPL).

This project has one main Medical objective:

To substitute a very risky invasive procedure for the life of the fetus with an automated, fast, simple and accurate non-invasive prenatal diagnosis with absolutely no risk for the fetus.