29. ANGELAB proiektua

Proiektuaren izena	ANGELAB - A new genetic laboratory for non-invasive prenatal diagnosis		
Proiektuaren laburpena (esaldi 1)	Lab on a Chip formatua oinarri hartuta jaio aurreko diagnostikorako sistema ez-inbaditzaile berri bat garatzea.		
Proiektuaren hasiera-data	2012/10/01	**Proiektuaren amaiera-data**	2016/09/30
Erakunde nagusia edo koordinatzailea	IK4-IKERLAN Proiektuko burua izateaz gain, 4. lan-paketearen (hainbat laginekin lan egiteko osagai mikrofluidiko bat diseinatu, integratu eta fabrikatzea), 9. lan-paketearen (LabonaChip fabrikatzea zein 16. lan-paketearen (proiektua kudeatzea) liderra ere bada.		
Erakunde parte-hartzaileak	**Erakundea**	**Proiektuari egindako ekarpen nagusia**	
	HAHN-SCHICKARD-GESELLSCHAFT FUER ANGEWANDTE FORSCHUNG E.v. (Alemania)	BAZKIDEA. Mutazioak identifikatzeko PCR digitalerako txip-unitatea garatzeaz arduratzen da (4. eta 7. paketeak). Ikerlanekin batera errekerimenduak definitzen ditu 1. lan-paketean eta LabonaChiparen kontrol-unitatea osatzen duten alderdietan (8., 9. eta 10. lan-paketeak).	
	ADEMTECH SA (Frantzia)	BAZKIDEA. Laginak prestatzeko erreaktiboak (partikula magnetikoak) eta protokoloak garatzen ditu batez ere. Haren eginkizun nagusiak 1. eta 2. lan-paketeen barruan daude.	
	OSAKIDETZA (EAEko erakundea)	BAZKIDEA. Basurtuko Unibertsitate Ospitaleko Genetikako taldea 2. lan-paketearen liderra da. Pakete horretan ondoren diagnostiko-txipetan garatu behar dituzten erreakzioen hodiko protokoloak prestatzen dituzte. Horrez gain, liderra ere bada 14. lan-paketean (proiektuaren alderdi etikoak) eta 11. lan-paketearen zeregin batean (garatutako sistemetako bat teknikoki egiaztatzea).	
	FUNDACION RIOJA SALUD	BAZKIDEA. BIO zereginekin zerikusia duten proiektuaren jarduera guztietan nahasita dago (2., 3., 6. eta 7. paketeak). 3. lan-paketearen liderra da (fetuaren DNA ateratzea).	
	POLITECHNIKA WROCLAWSKA (Polonia)	BAZKIDEA. Proiektuari egindako ekarpen nagusia 8. lan-paketean da (txipak irakurtzeko sistema optikoa garatzea).	
	THE CYPRUS FOUNDATION FOR MUSCULAR DYSTROPHY RESEARCH (Zipre)	BAZKIDEA. 1. eta 11. lan-paketeen liderra da (sistema garatzeko beharrezko diren zehaztapenak eta	

			parametroak definitzea), bai eta ANGELAB2 sistema testatu eta balidatzeaz arduratzen dena ere.
	NIPD GENETICS LIMITED (Zipre)		BAZKIDEA. Liderra da 5. lan-paketean (ANGELAB2 plataforman PCR-ko modulua denbora errealean garatu eta balidatzea 13, 18, 21 eta X kromosometan aneuploidiak identifikatzeko). Liderra ere bada 14-lan-paketean (emaitzak ustiatu, arautu eta barreiatzea).
	DNA DATA SLP (EAEko enpresa)		BAZKIDEA. Liderra da 11. lan-paketearen zeregin batean (fibrosi kistikoaren, muskulu-atrofia espinalaren eta X kromosomari lotutako gaixotasunen diagnostiko-txipa egiaztatzea).
	BIOPHARMA TECHNOLOGY LTD (Erresuma Batua)		BAZKIDEA. PCR eta DNA erreaktiboak liofilizatzeko beharrezko diren parametroak ikertzen ditu. Prototipoa eskala txikian ekoiztea eta prozesuaren eskalatzea ere ikertuko ditu.
	EV GROUP E. THALLNER GMBH (Austria)		BAZKIDEA. Txip mikrofluidikoen fabrikazio modularra eta bolumen handitan egiteko merkatuan zer eskakizun dauden definitzea da haren zeregin nagusia, baita sistemarako moduluak garatzea ere. Liderra da 12. lan-paketean (ekoizpen-linea pilotua inplementatu, fabrikatu eta instalatzea du helburu nagusitzat).
	GAIKER-IK4 (EAEko erakundea)		BAZKIDEA. Erreakzioen hodiko protokoloak prestatzeari buruzko 2. lan-paketean parte hartzen du, eta liderra da 9. lan-paketearen bi zereginetan (diagnostiko-txipak birziklatzeko aukerak ebaluatzea eta gailuaren bizi-zikloa ekonomiko eta anbientala laborategiko eskalan ebaluatzea).
	BIODONOSTIA INSTITUTUA ELKARTEA (EAEko erakundea)		BAZKIDEA. Donostia Unibertsitate Ospitaleko Genetikako taldea liderra da 13. lan-paketean (diagnostiko-sistema berria eszenario errealean balidatzea ohiko teknikarekin alderatuta).
	Centrum fur Angewandte Nanotechnologie (CAN) GmbH (Alemania)		BAZKIDEA. Liderra da 8. lan-paketean (hautemateko sistemak garatzea).
	POC MICROSOLUTIONS SL (EAEko enpresa)		BAZKIDEA. Liderra da 10. lan-paketean (diagnostiko-sistema diseinatzea eta integratzea).
Proiektuaren	**Urtea**	**Aurrekontua guztira**	**EAEren parte-hartzea**

<table>
<tr><td>aurrekontua (milaka euro)</td><td>2012-2016</td><td colspan="2">10.955.292,00 €</td><td>4.354.227,00 €</td></tr>
<tr><td rowspan="2">EAEren parte-hartzearen finantzaketa-iturriak (mila euro)</td><td>Urtea</td><td>1. finantzaketa: FP7</td><td>2. finantzaketa: Proiektuaren bazkideak</td><td>Bestelako laguntza publikoak</td></tr>
<tr><td>2012-2016</td><td>3.320.782,00 €</td><td>1.033.445,00 €</td><td></td></tr>
<tr><td rowspan="5">Jardun-eremua</td><td colspan="4">Lehentasunezko arlo estrategikoak Markatu X batekin</td></tr>
<tr><td colspan="2">Fabrikazio aurreratua</td><td>Energia</td><td>Biosanitarioa</td></tr>
<tr><td colspan="2"></td><td></td><td>X</td></tr>
<tr><td colspan="4">Aukera-esparruak Markatu X batekin</td></tr>
<tr><td>Elikadura</td><td>Hiri-habitata</td><td>Ekosistemak</td><td>Kulturaren eta sormenaren arloko industria</td></tr>
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Proiektuaren deskribapen laburtua: helburu nagusiak eta garatu beharreko emaitzak, zer erronkari erantzuten dion, ekonomian eta gizartean izan dezakeen inpaktua, eta abar.

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):

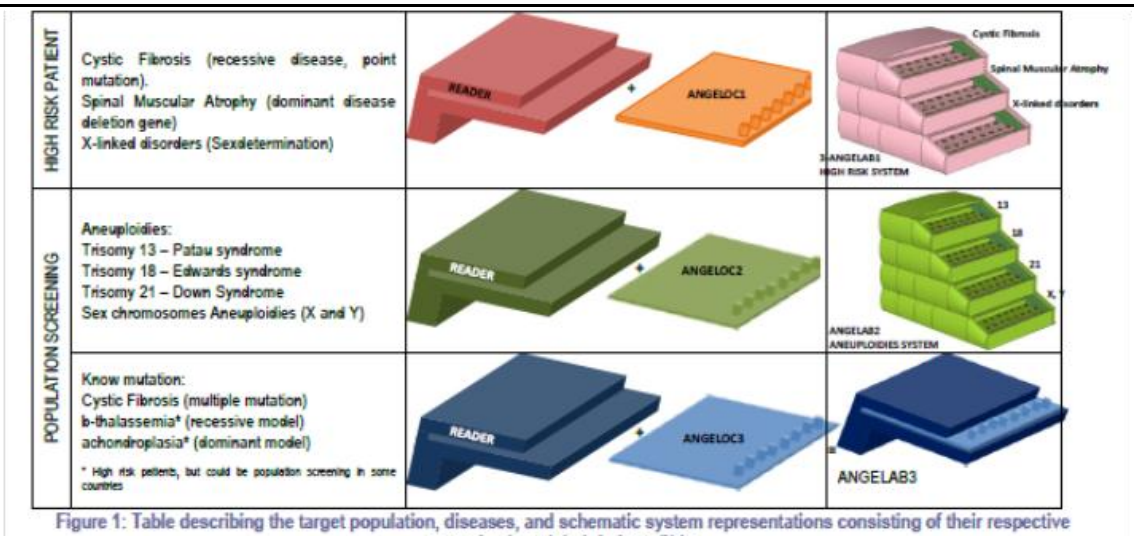

Figure 1: Table describing the target population, diseases, and schematic system representations consisting of their respective control unit and their LabonaChip.

- **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 Tª 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.