

# Development of a system of early detection of zebra mussel through analysis of eDNA

Fast monitoring of the presence of IAS on river basins



***Marta Prado Rodríguez***

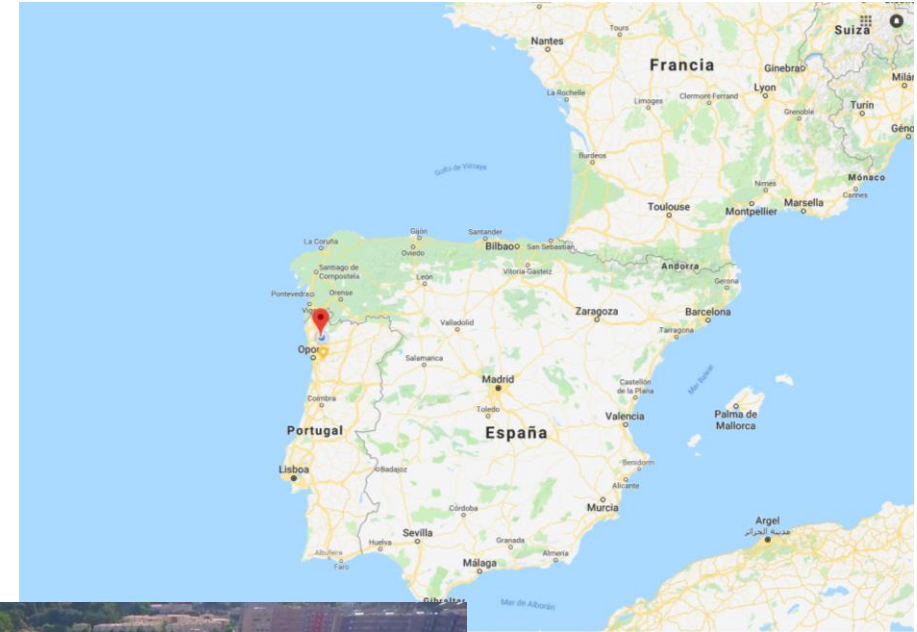
Dep. of Life Sciences/Nano4Food Unit/Food Quality and Safety Research Group

- ✓ Presentation
- ✓ What is environmental DNA and Why we use it?
- ✓ Our approach to detect eDNA
  - ✓ DNA purification module
  - ✓ DNA amplification module and protocol
  - ✓ DNA detection module
  - ✓ Data acquisition
- ✓ Final considerations

## ABOUT INL

The INL International Iberian Nanotechnology Laboratory, located in Braga (North of Portugal) was founded by the governments of Portugal and Spain under an international legal framework to perform interdisciplinary research, deploy and articulate nanotechnology for the benefit of society. INL aims to become the world-wide hub for nanotechnology addressing society's grand challenges.

The INL research programme comprises four strategic fields of application of nanoscience and nanotechnology: Food and Environment monitoring, ICT, Renewable Energy and Health.



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### MISSION & VISION

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#### MISSION

Perform interdisciplinary cutting edge research, deploy and articulate nanotechnology for the benefit of Society.



#### VISION

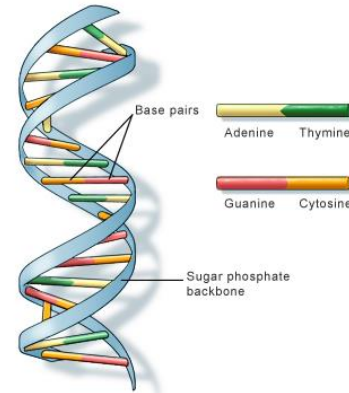
Become the worldwide hub for nanotechnology deployment, addressing society's grand challenges.



# What is environmental DNA and Why we use it?

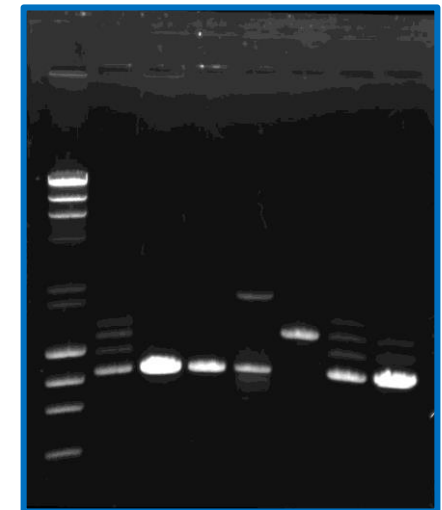
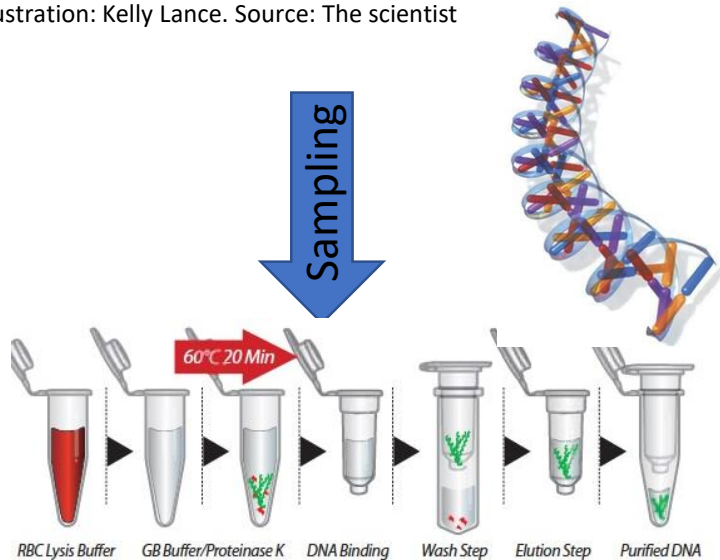


Illustration: Kelly Lance. Source: The scientist



Detection of the presence of zebra mussel before they produce noticeable harm

**Conventional or "bench-top" approach**



**eDNA extraction and concentration**

**Species-specific DNA amplification**

**DNA detection**



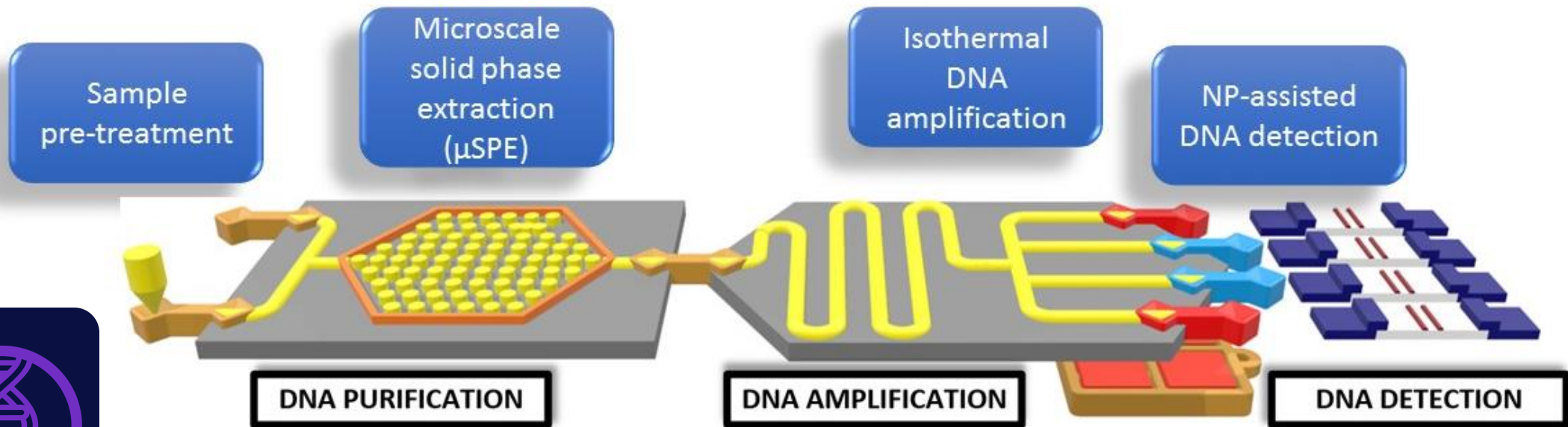
# Our approach to detect eDNA

Cooperation agreement INL-CHG for the development of system of early detection of zebra mussel through analysis of eDNA



## DNA NANO.SCREEN

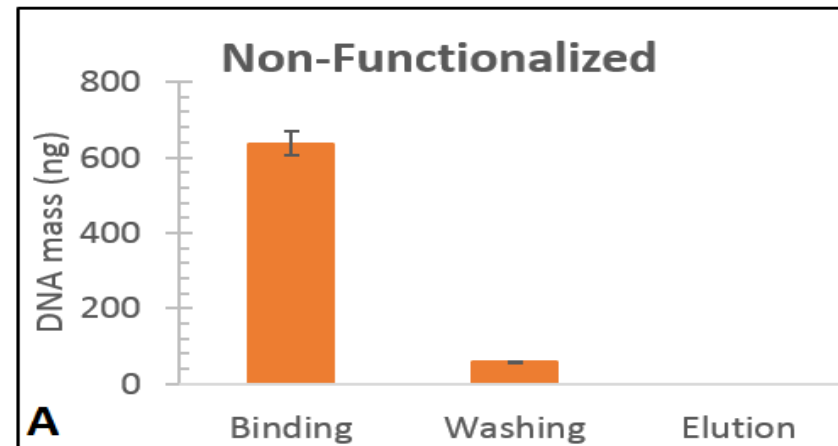
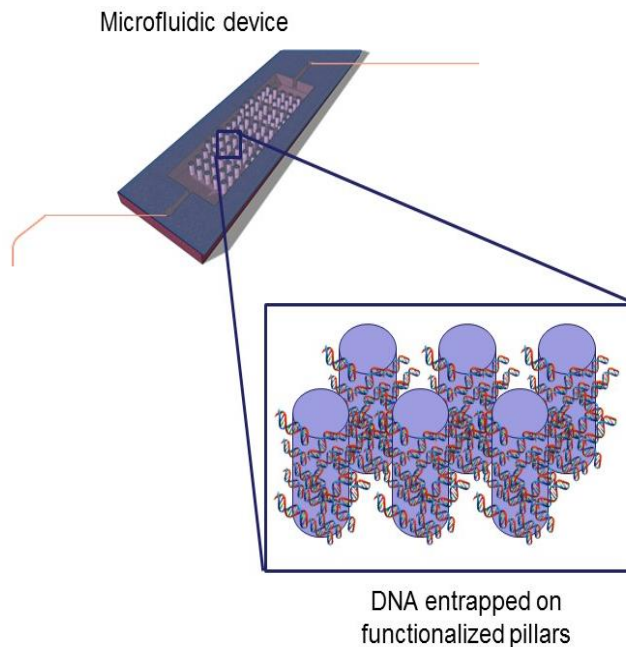
**We use a modular design and evaluation to develop reliable analytical solutions**



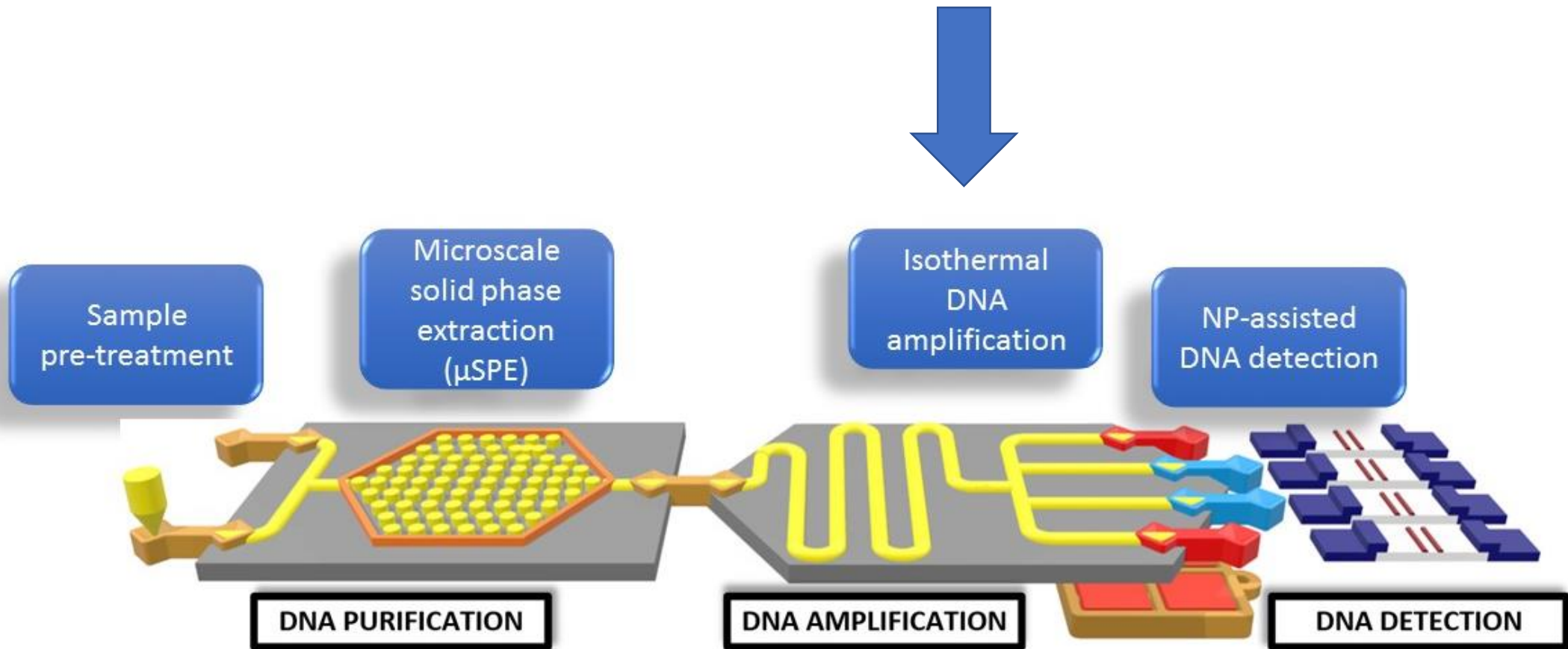


**Fig 1.** Microfluidic device for DNA purification

## *High efficient DNA extraction and purification from complex matrixes combining micro Solid Phase Extraction ( $\mu$ SPE) and microfluidics*



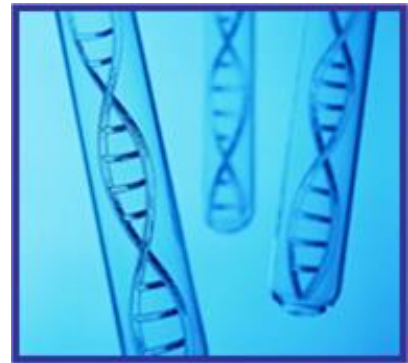
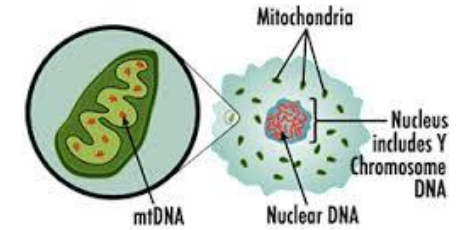
DNA purification with a non-functionalized device (A) and a functionalized device (B) using the same pH-induced DNA capture/release protocol.



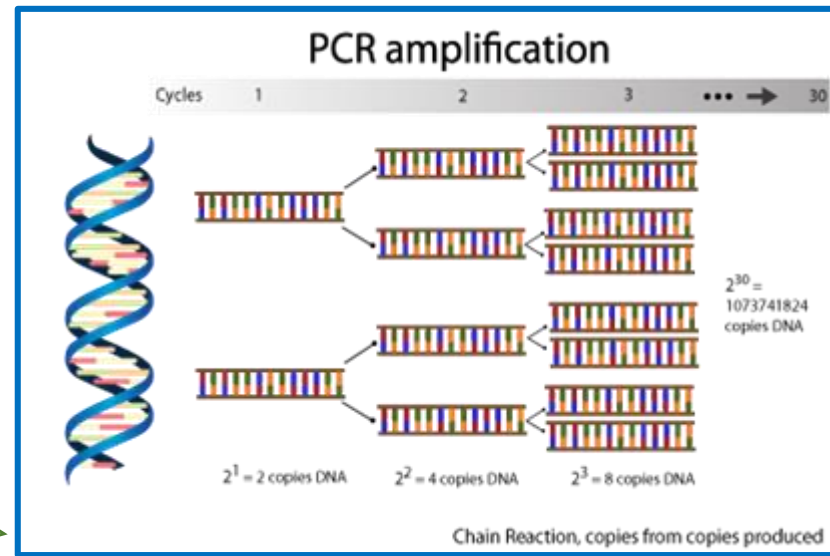


# DNA amplification module and protocol

- **2 specific methods developed and tested:**
  - qPCR based approach for the specific amplification and detection of zebra mussel
  - LAMP based approach (isothermal amplification)

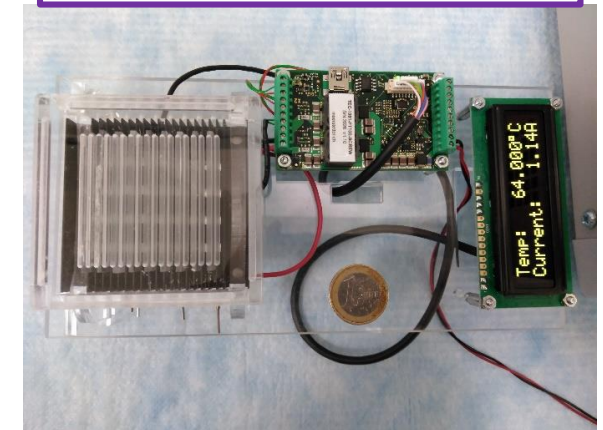


Total DNA extract



**Polymerase chain reaction (PCR):** exponentially amplify a single copy or a few copies of a specific segment of DNA to generate thousands to millions of copies of a particular DNA sequence

Miniaturized isothermal amplification device with integrated thermal control



Protocols tested with:

17 well characterized autochthonous species from the Guadalquivir (Guadalictio/ Alphanus Research Group. U Córdoba) and Asian clam from Loire river (USC)

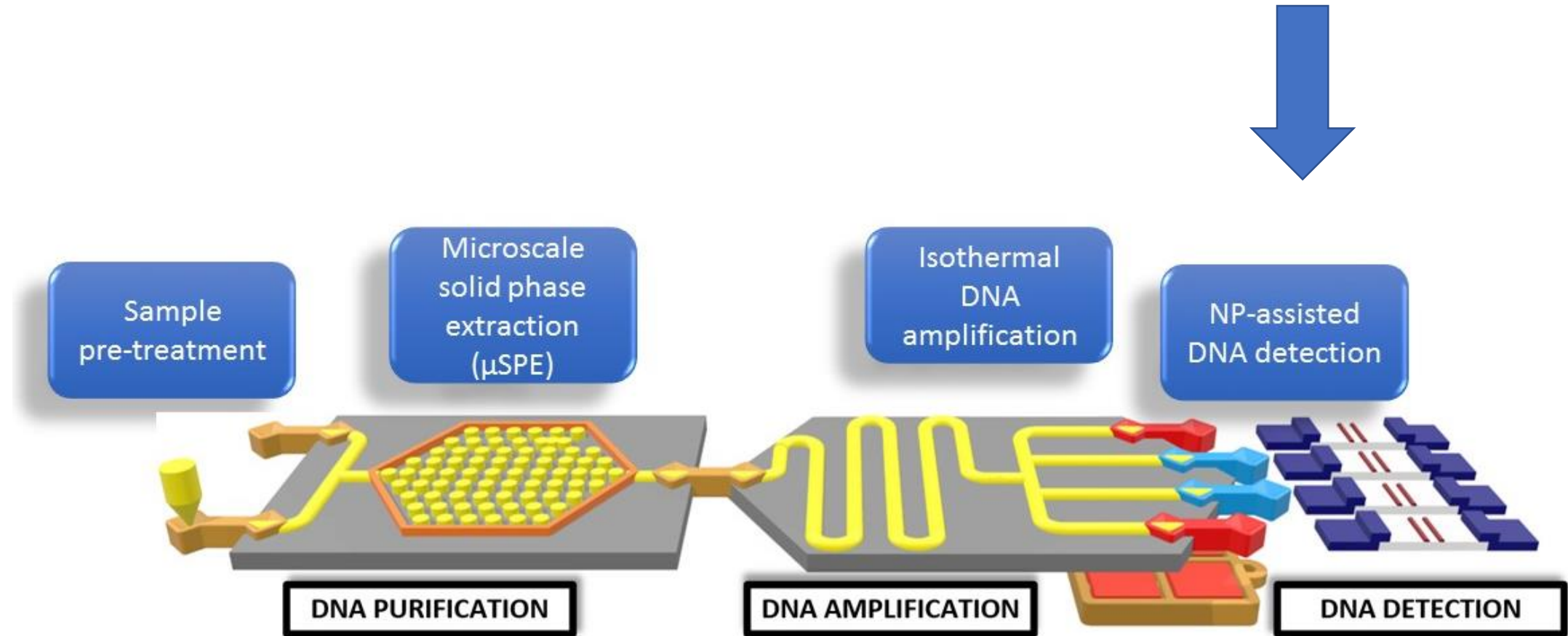
Sensitivity:

PCR up to 0.056 pg/mL

LAMP up to 0.56 pg /mL

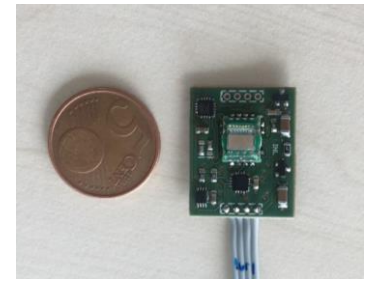
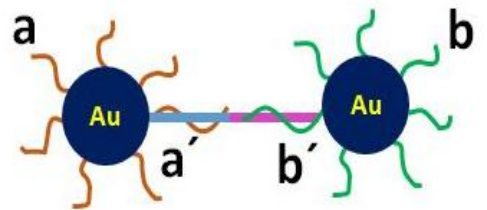
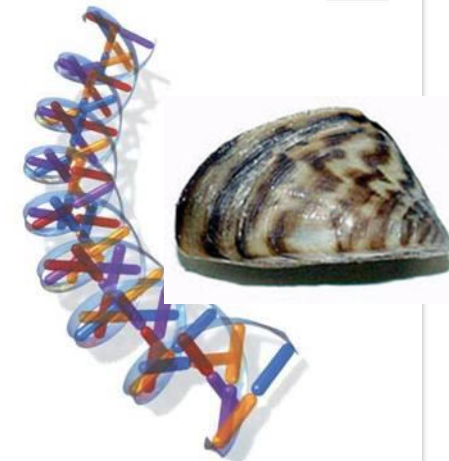
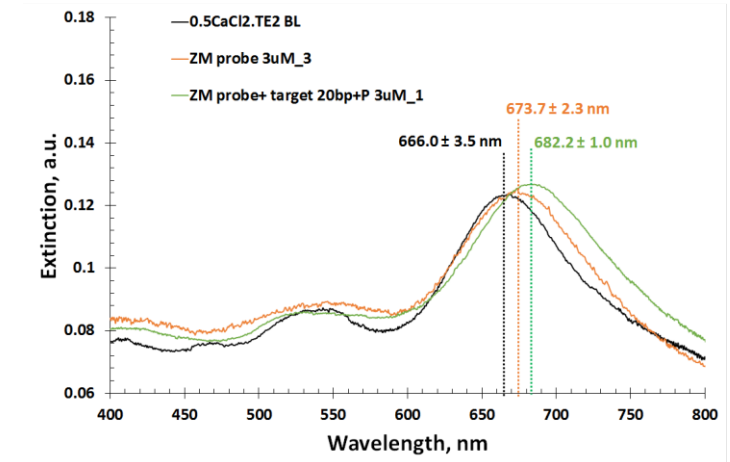
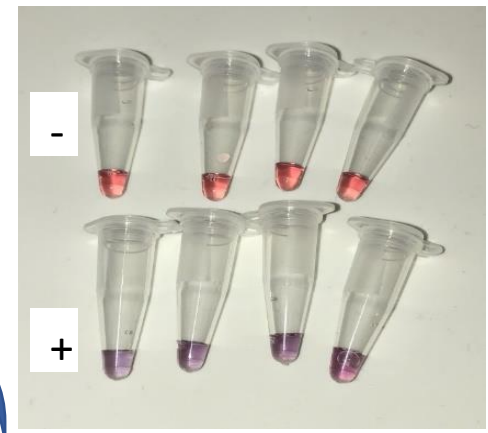
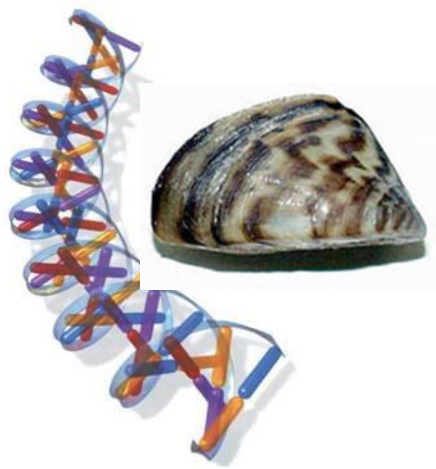
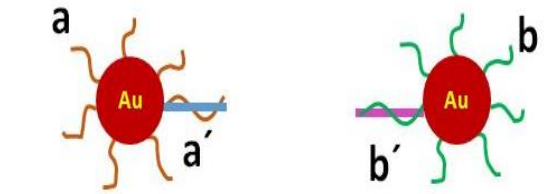


		SAMPLE	STANDARD INTERNATIONAL NAME	NR. OF SAMPLES	SAMPLING LOCATION	qPCR		qLAMP
						Hydrolysis Probe	F3/B3	
NEGATIVE SAMPLES	Fish	Luciobarbus sclateri	Southern Iberian barbel	3	Guadalquivir river (Spain)	-	-	-
		Pseudochondrostoma willkommii	Southern straight-mouth nase	2		-	-	-
		Squalius alburnoides complex.	Calandino	3		-	-	-
		Squalius pirenais	Southern Iberian Chub	3		-	-	-
		Cobitis poaludica	Southern Iberian spined-loach	3		-	-	-
		Iberochondrostoma lemmingii	Iberian arched-mouth nase	3		-	-	-
		Cyprinus carpio	Carp	1		-	-	-
		Carassius gibelio	Prussian carp	3		-	-	-
		Lepomis gibbosus	Pumpkinseed	3		-	-	-
		Micropterus salmoides	Largemouth Black-bass	3		-	-	-
		Gambusia holbrooki	Eastern mosquitofish	3		-	-	-
	Alburnus alburnus	Bleak	3	-	-	-		
	Mollusks	Corbicula fluminea	Asian clam	1	Loire river (France)	-	-	-
		Physa acuta	Freshwater snail (general)	3	Guadalquivir river (Spain)	-	-	-
Ancylus fluviatilis		Limpet (general)	3	-		-	-	
fam. Unionidae		Freshwater mussel (general)	4	-		-	-	
Crustacean	Procambarus clarkii	red swamp crayfish	1	Guadalquivir river (Spain)	-	-	-	
POSITIVE SAMPLES	Meat Samples	D. polymorpha 1	Zebra mussel	1	Guadalquivir river (Spain)	+	+	+
		D. polymorpha 2		1		+	+	+
		D. polymorpha 3		1		+	+	+
		D. polymorpha 4		1		+	+	+
		D. polymorpha 5		1		+	+	+
	Positive Water Samples	D.p. transport water 1	**	1	Guadalquivir river (Spain)	+	+	+
		D.p. transport water 2		1		+	+	+
		D.p. transport water 3		1		+	+	+
		D.p. transport water 4		1		+	+	+
		D.p. transport water 5		1		+	+	+
		D.p. transport water 6		1		+	+	+
Surface river water 1 (Los Bermejales, December 2016)		1		-		-	-	
Surface river water 2 (Los Bermejales, May 2017)	1	+	+	+				



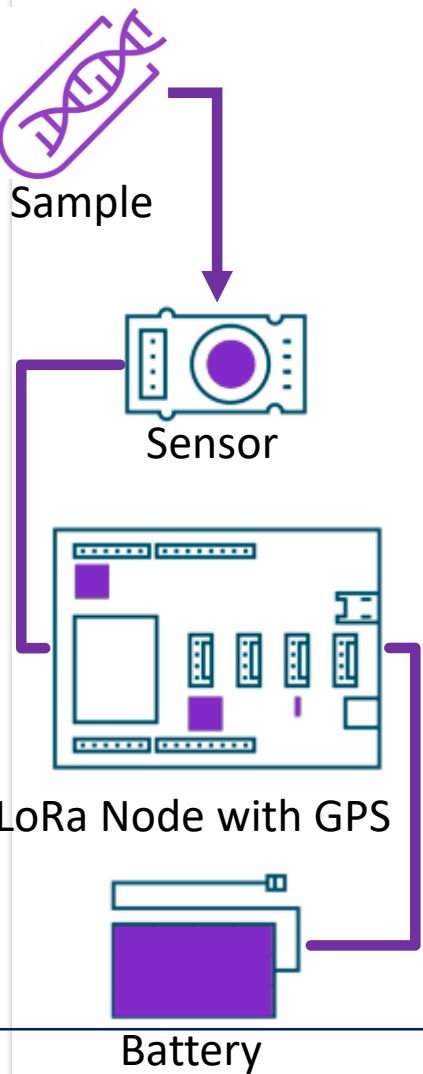


# DNA detection module



# Data acquisition

## In the River

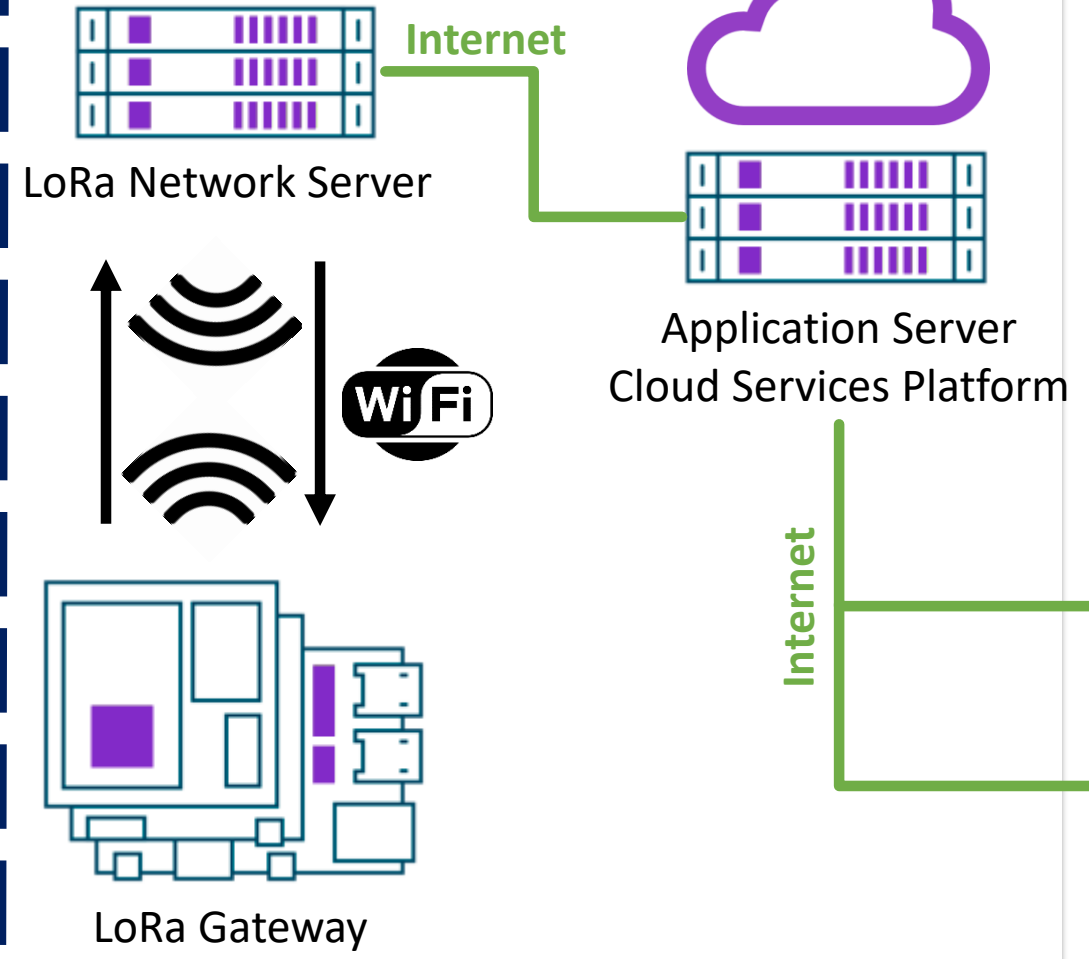


The data is collected by the **Sensor**, sent by the **Node** to the **Gateway** and eventually to the **Cloud**, from where is accessible to end customers on a web page and a mobile app



Radio transmission Up to 10 Km Range

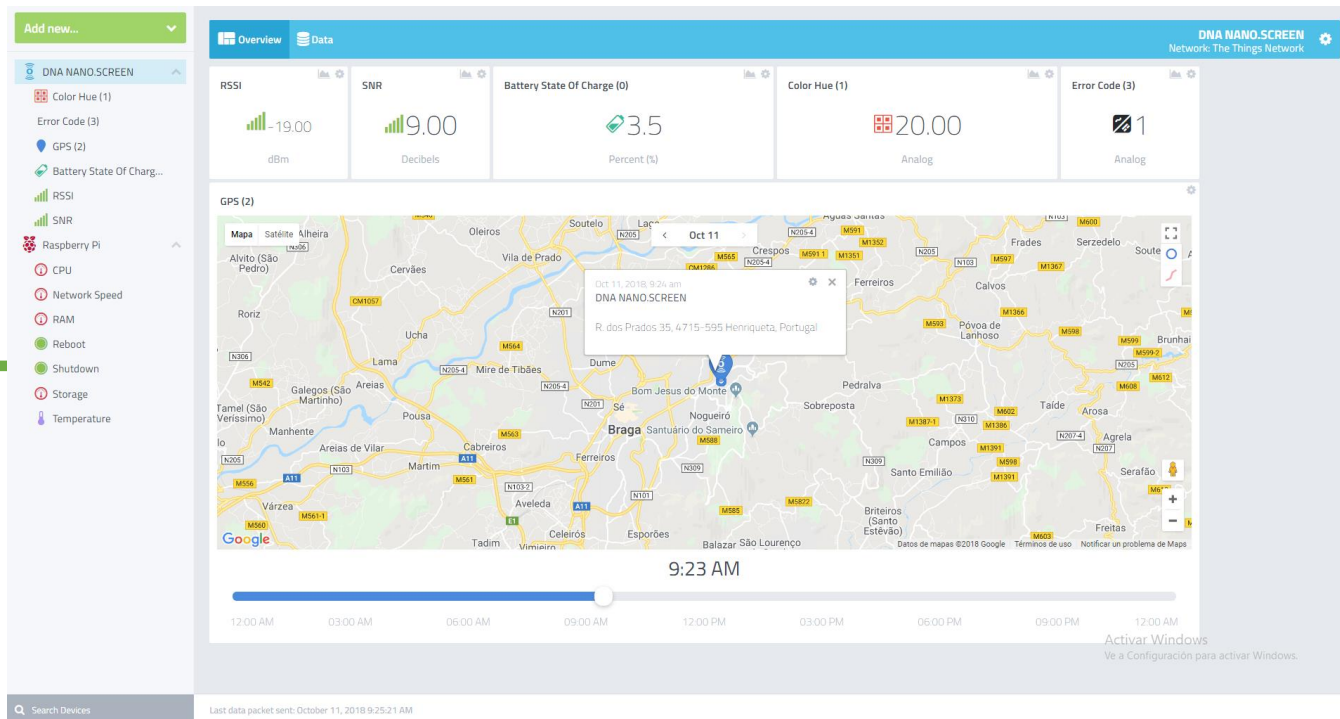
## In the Office



# Data acquisition

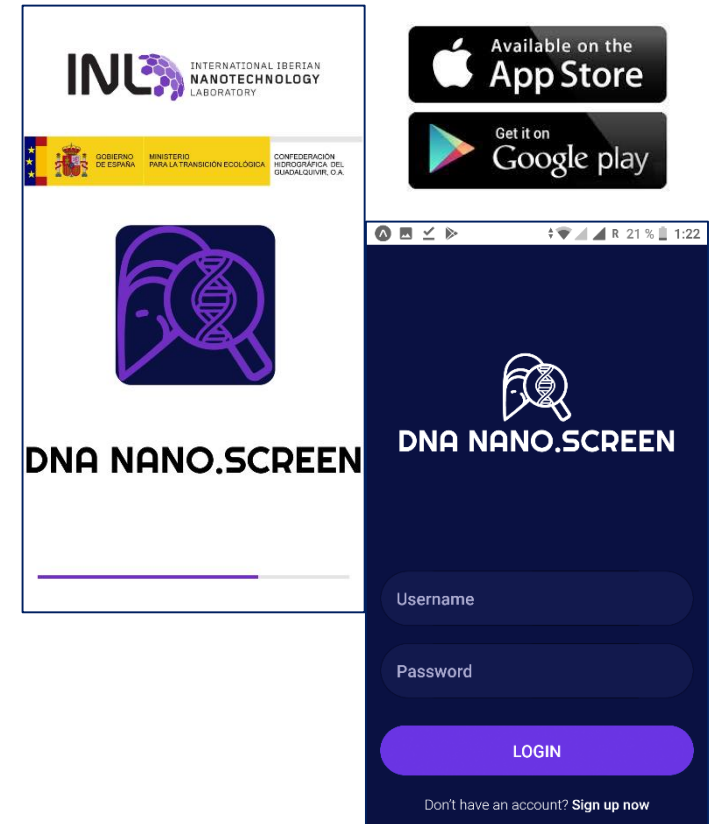
In the Office

On a web page accessing from my PC



Everywhere.....

With an app at my smartphone



INL INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

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- Evaluation of each individual module allow us to develop the most efficient approach in each case
- Integration of the independent modules will allow the development of automated in-situ monitoring system
- Real time sample data adquissition, transmission and accesibility together with the GPS location will allow the early identification of the posible presence of zebra mussel and geographical traceability of the results



Thanks for your attention

Joana Carvalho

Dr. Alejandro Garrido

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Monisha Elumalai

Dr. Andrey Ipatov

Dr. Lorena Diéguez

Yosbel Toledo



DNA  
NANO SCREEN

Verónica Gros Giraldo (CHG)

Guadalictio/ Alphanus Research  
Group. (U Córdoba)

Aguas de Córdoba

Dr. Manuel Rey Méndez (USC)  
Dr Jorge Barros-Velázquez (USC)

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*Thank you for listening!*

***Marta Prado***

Dep. of Life Sciences/Nano4Food Unit/Food Quality and Safety Research Group

