"Enhancing Collaboration between the CEE and Central Asia's Centres of Excellence to Address the Key Drivers of Biodiversity Loss and Maintain Human, Crop and Livestock Health"

Workshop on Laboratory Identification of Species, Screening of Living Modified Organisms and Detection of Plant Pathogens
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ENVIRONMENTAL DNA (eDNA): A REVOLUTIONARY APPROACH TO STUDY BIODIVERSITY OF ANY ENVIRONMENT

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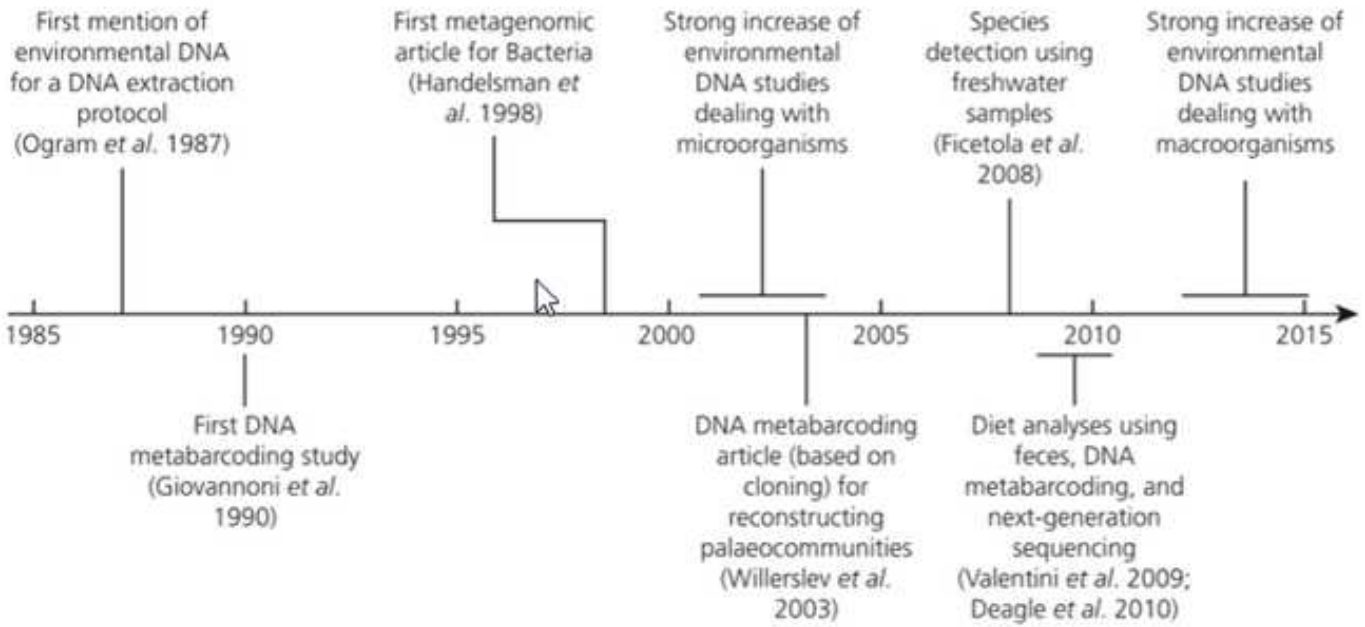
Environmental DNA (eDNA)

- **Nuclear or mitochondrial DNA discharged to the environment** by the organisms (faeces, urine, mucus, epidermal cells, gamets, corpses...)
- Usually short, degraded fragemts shorter than 500 bp
- Intracellular and extracellular DNA
- The rate of degradation varies depending on different environmental and biological factors

A brief history of eDNA



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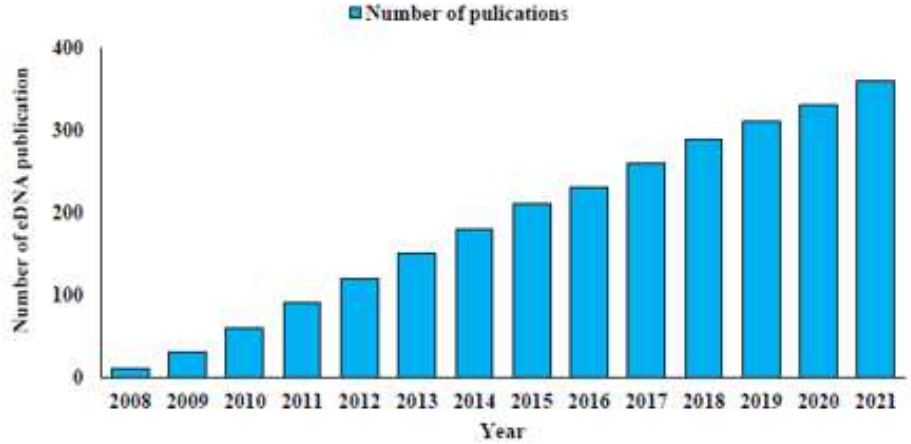
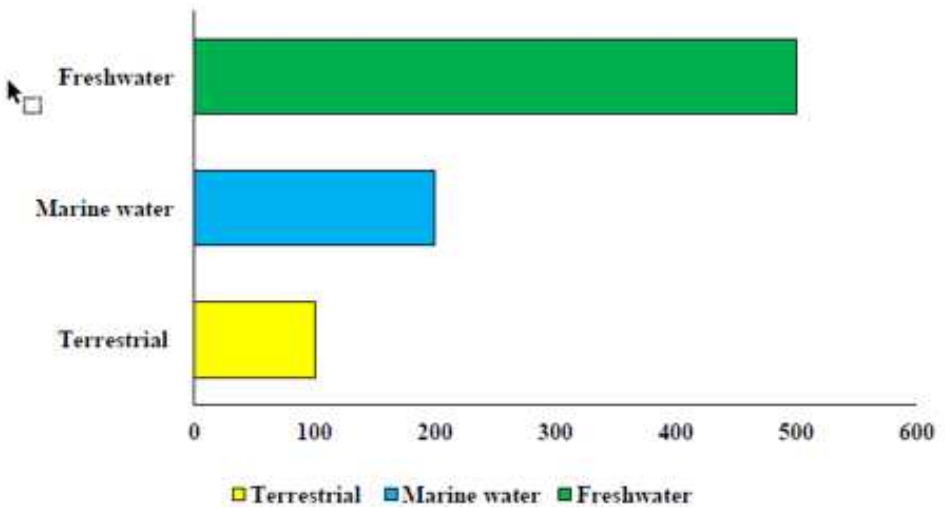
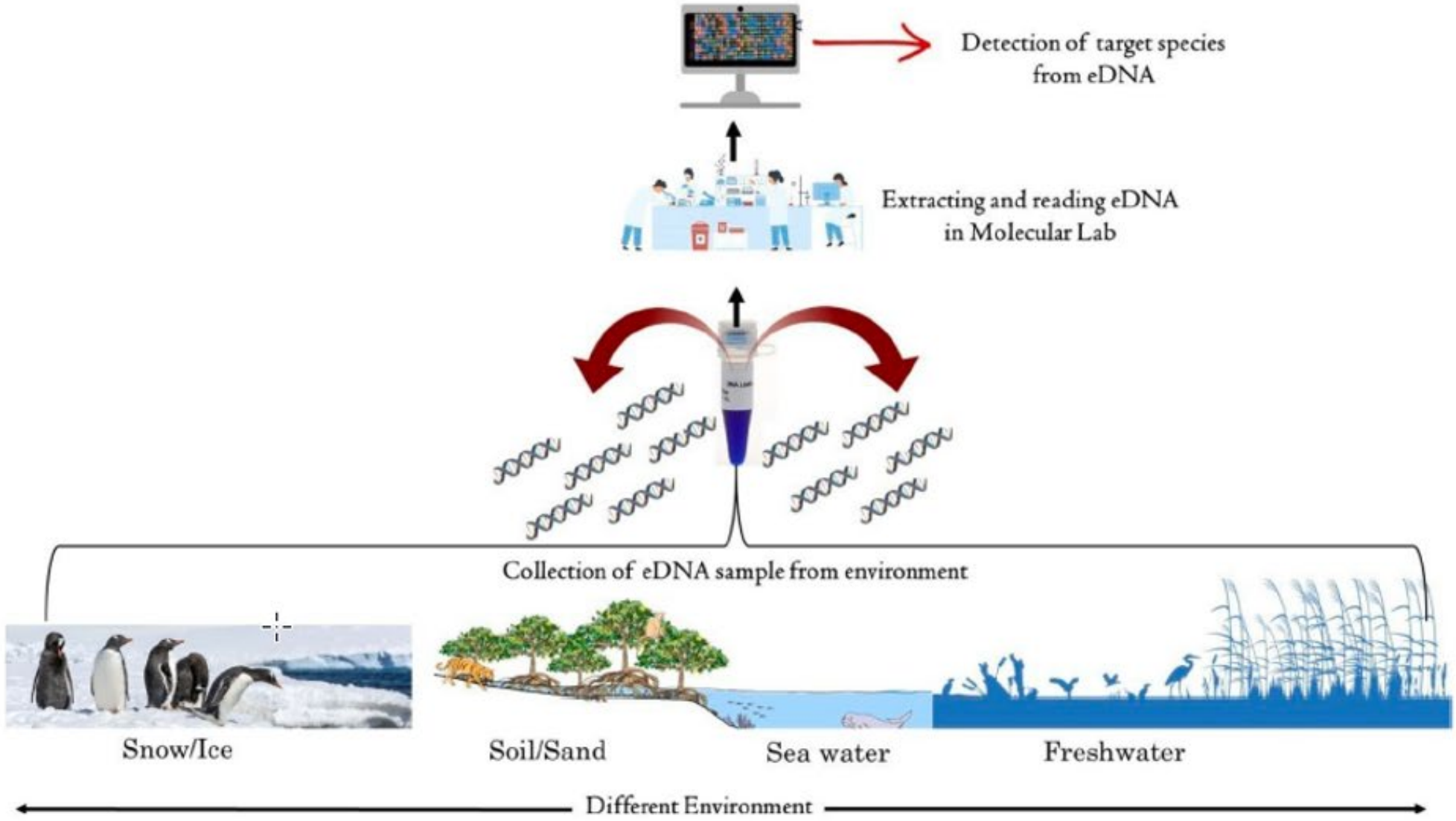


Number of studies using environmental DNA (eDNA) increasing day by day between 2008 and 2021.



Number of studies using environmental DNA (eDNA) in different environments

eDNA workflow



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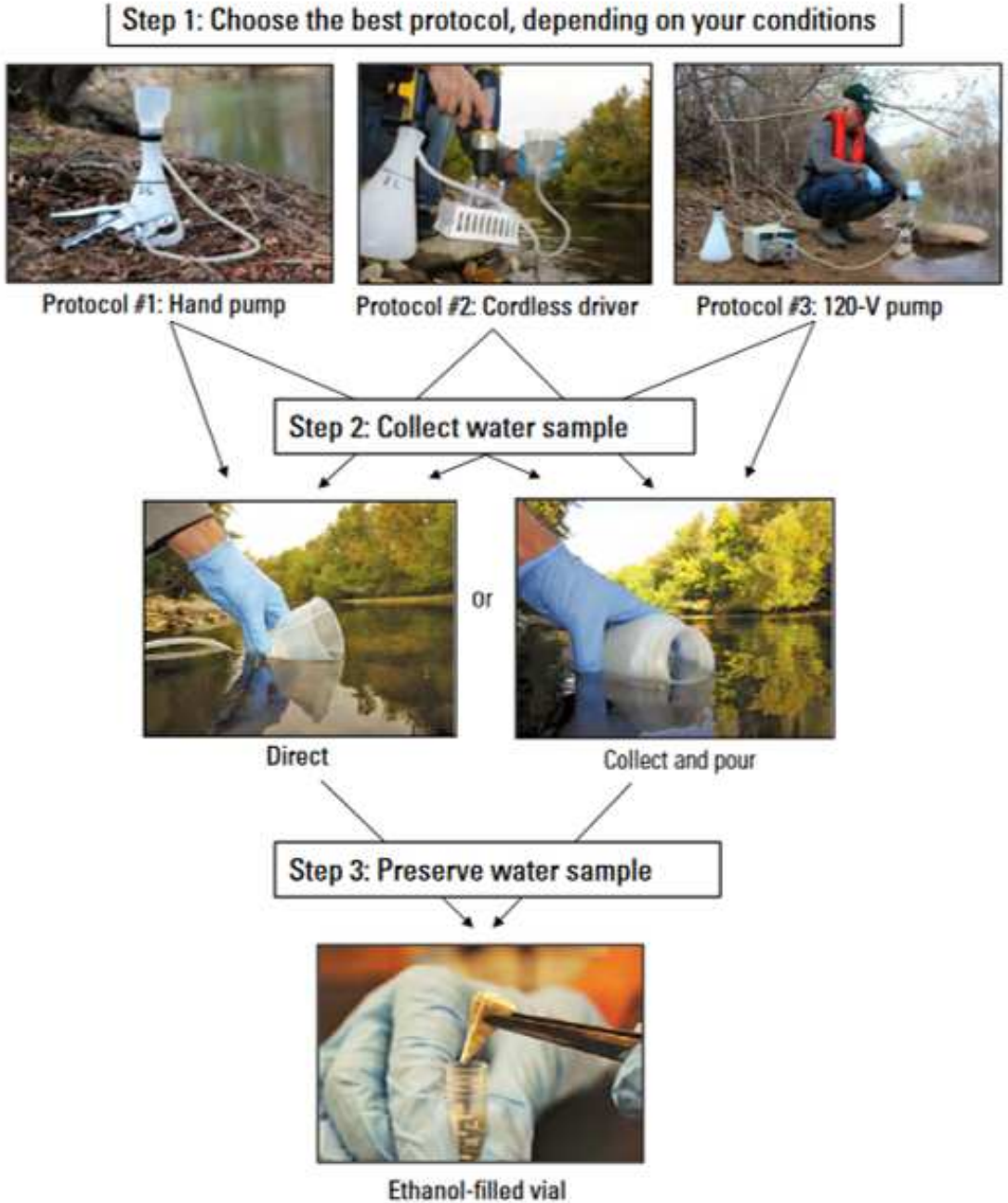
eDNA workflow

- **eDNA extraction**
  - Water, air, soil, sediment...
  - Grab or composite sampling?
  - Sterilization of the equipment to avoid contamination!

- **eDNA capture**
  - Filtration (glass fiber, nitrocellulose, PES)
  - Precipitation (ethanol/isopropanol + sodium acetate)
  - Centrifugation

- **eDNA extraction**
  - Commercial extraction kits

- **Analysis**



https://ednaresources.science/field-methods

Analysis aproaches

- **eDNA barcoding**
  - Target species analysis
  - PCR with species-specific primers
  - Conventional PCR methods

Single Species PCR

- **eDNA metabarcoding**
  - To study multiple species and community dynamics
  - High-throughput sequencing methods
  - Selection of a target region

Metabarcoding

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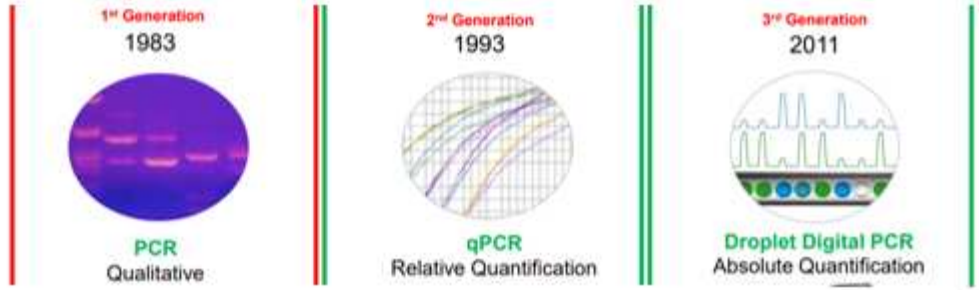
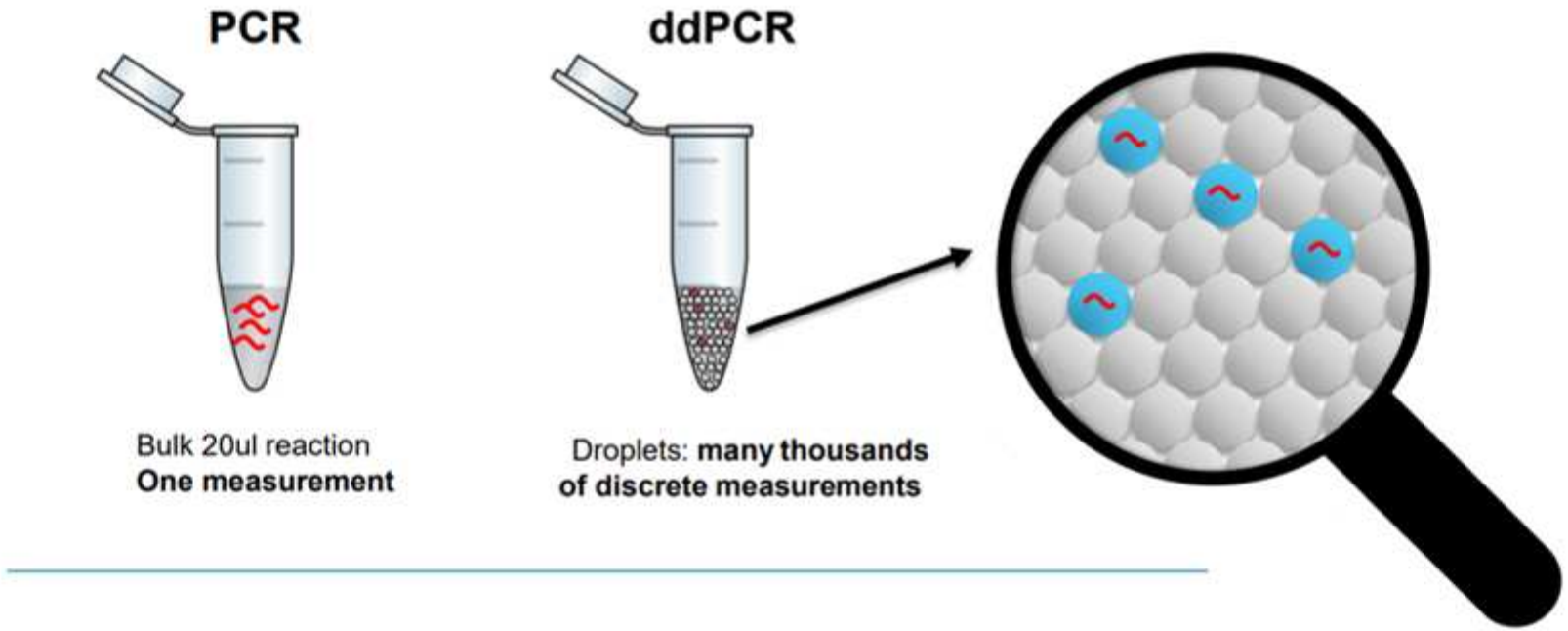
Droplet digital PCR (ddPCR)

- 3rd generation PCR (2011)
- Absolute quantification





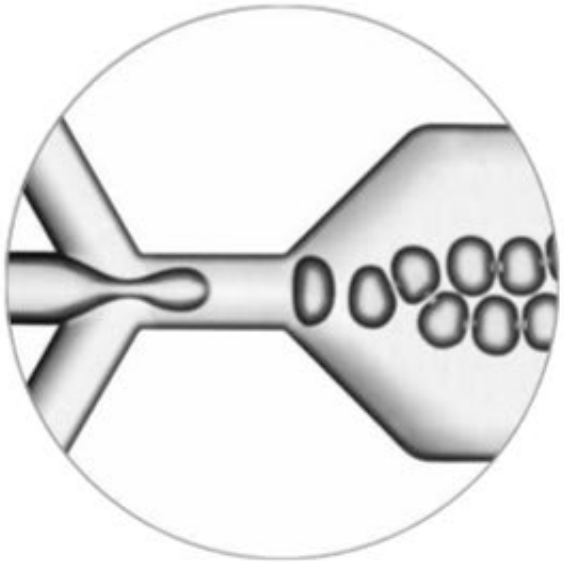
Key advantages of ddPCR

- Absolute quantification (no standard curve required)
- Endpoint method
- Less sensitive to PCR inhibition
- Reduced intra- and interlaboratory variation
- Quantitate low input concentration of DNA target
- Quantitate a rare target in large backgrounds

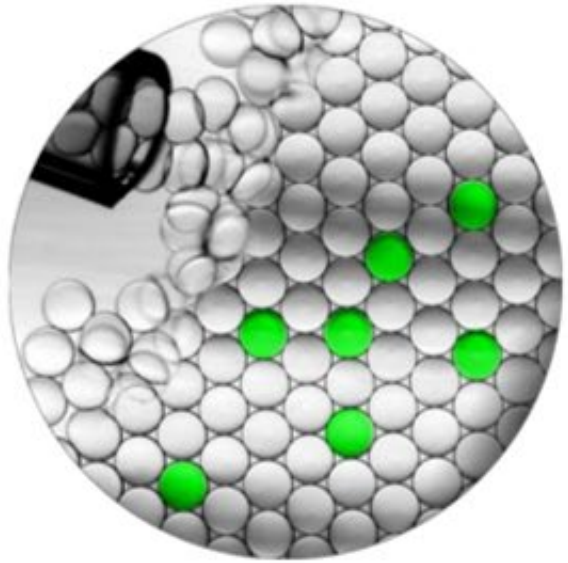
Possible uses of ddPCR

ddPCR workflow

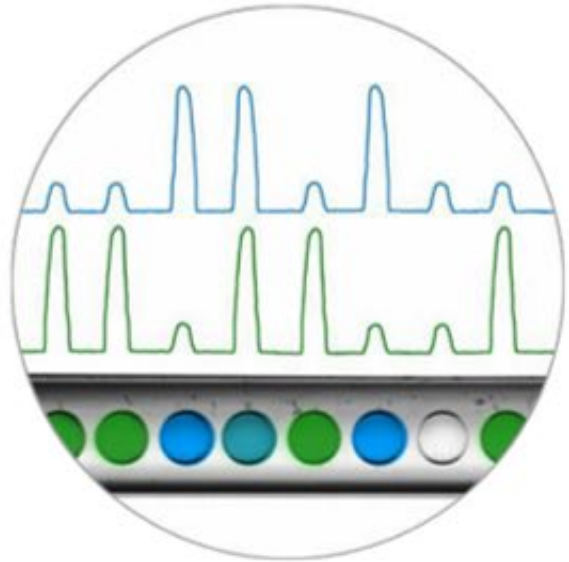
Partition sample into Droplets



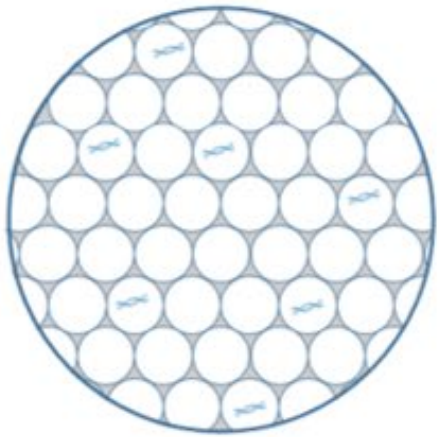
Cycle Droplets

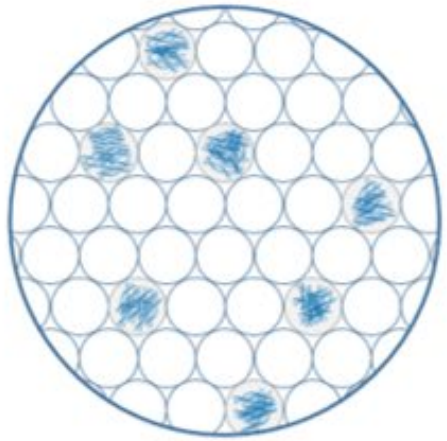


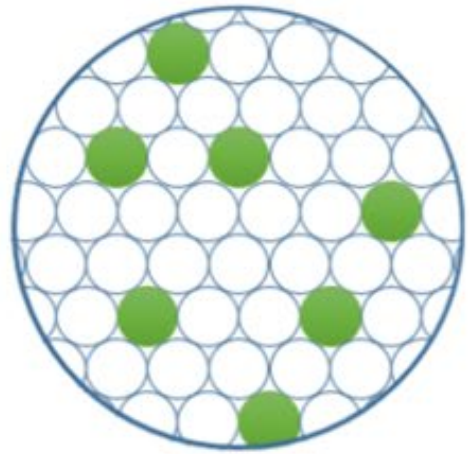
Read Droplets



ddPCR workflow

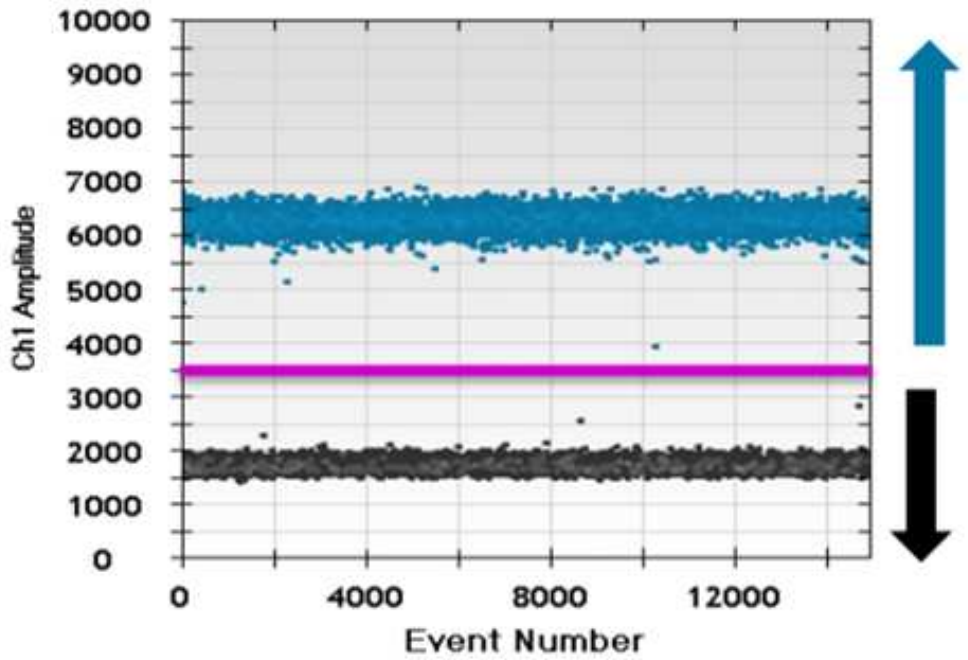






Interpretation of the results

- Positive droplets contain at least one copy of target DNA or cDNA
- Increased fluorescence in positive *vs.* negative droplets
- Software measures the number of positive and negative droplets per fluorophore per sample



Each positive counted as 1

Threshold

Each negative counted as 0

What we've been doing so far

- The TransNational Monitoring Network is an important tool under the Danube River Protection Convention (1996)

- Aims to provide a well-balanced overall view of pollution and long-term trends in water quality and pollution loads in the Danube and its major tributaries

- The EU Water Framework Directive (WFD) requires that countries in the Danube River Basin periodically assess certain water characteristics in their territory.

- JDS1 (2001), JDS2 (2006), JDS3 (2013), **JDS4 (2019)**

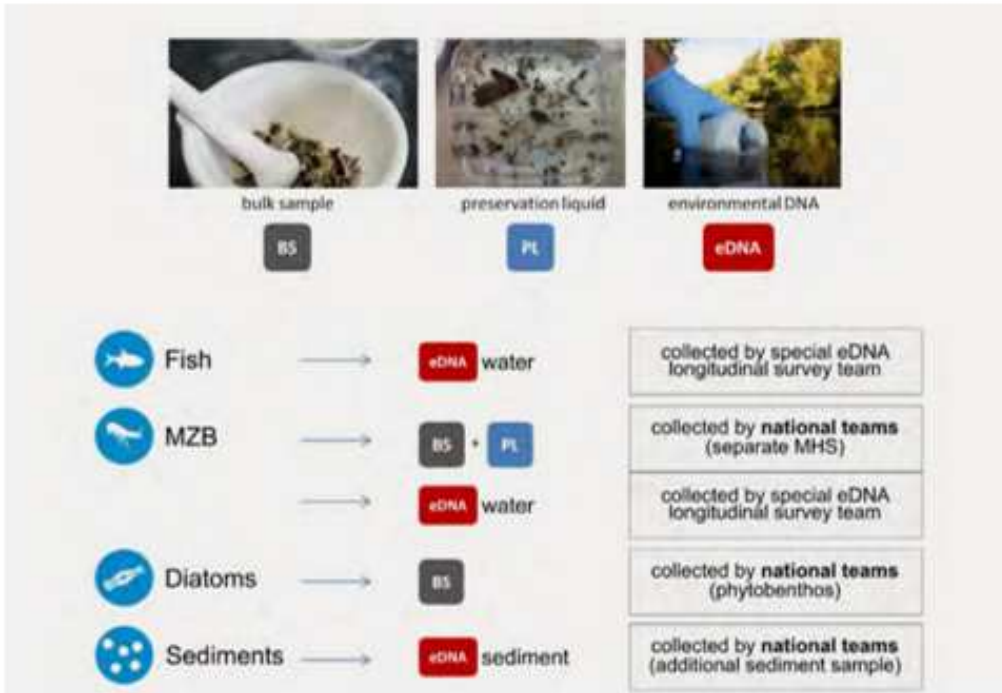
https://www.danubesurvey.org/jds4/publications/scientific-report

What we've been doing so far

- With JDS4, eDNA-based metabarcoding approach included for the first time in the extensive analytical program of the Joint Danube Survey
- Fish, macrozoobenthos, phytobenthos and sediment comunity
- 18S, COI, rbcL, 12S

- Promising potential of the eDNA metabarcoding approach recognised
- Coherent results when compared to traditional capture methods
- Significant increase in taxonomic resolution
- The detection of hard to observe species and developmental stages enabled

- Stil can be improved and standardised, JDS5 is being planed at the moment...



What we've been doing so far

- **Wastewater based epidemiology approach**

- Bilateral cooperation agreement with Belarus: IBISS + The Republican Research and Practical Center for Epidemiology and Microbiology (Minsk)
- Commercial application of ddPCR in wastewater-based epidemiology

- Development and implementation of common algorithm for monitoring SARS-CoV2 based on wastewater epidemiology

- Development of **early warning system** which, based on the presence of SARS-CoV-2 RNA in wastewater, would indicate the presence of infected persons in the population

- Wastewater, surface water or tissues of filtering-feeding sessile benthic animals as a sample?

https://www.ibiss.bg.ac.rs/index.php/en/present/national/item/2637-ddpcr

What we've been doing so far

- **Wastewater based epidemiology approach: Belgrade**

- Samples: RNA extracts from wastewaters; RNA extracts from digestive gland of *Sinanodonta woodiana*

- RNeasy mini kit (Qiagen, Cat. No. 74104)

- PREvalence ddPCR SARS-CoV-2 Wastewater Quantification
- Multiplex: N2 and E regions

Laka Janja, 2023, MSc thesis

For further cooperation, please contact:
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Thank you for your attention!
Спасибо за внимание!

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Suggested resources to start with ☺

https://ednaresources.science/

https://www.bio-rad.com/en-rs/life-science/droplet-digital-pcr

https://doi.org/10.1007/s10531-020-01980-0

https://doi.org/10.1016/j.jnc.2022.126325