eDNA – Linking biomarkers / mechanisms of toxicity and effects in the environment

eDNA – Kopplingen mellan biomarkörer / mekanismer för toxicitet och effekter i miljön

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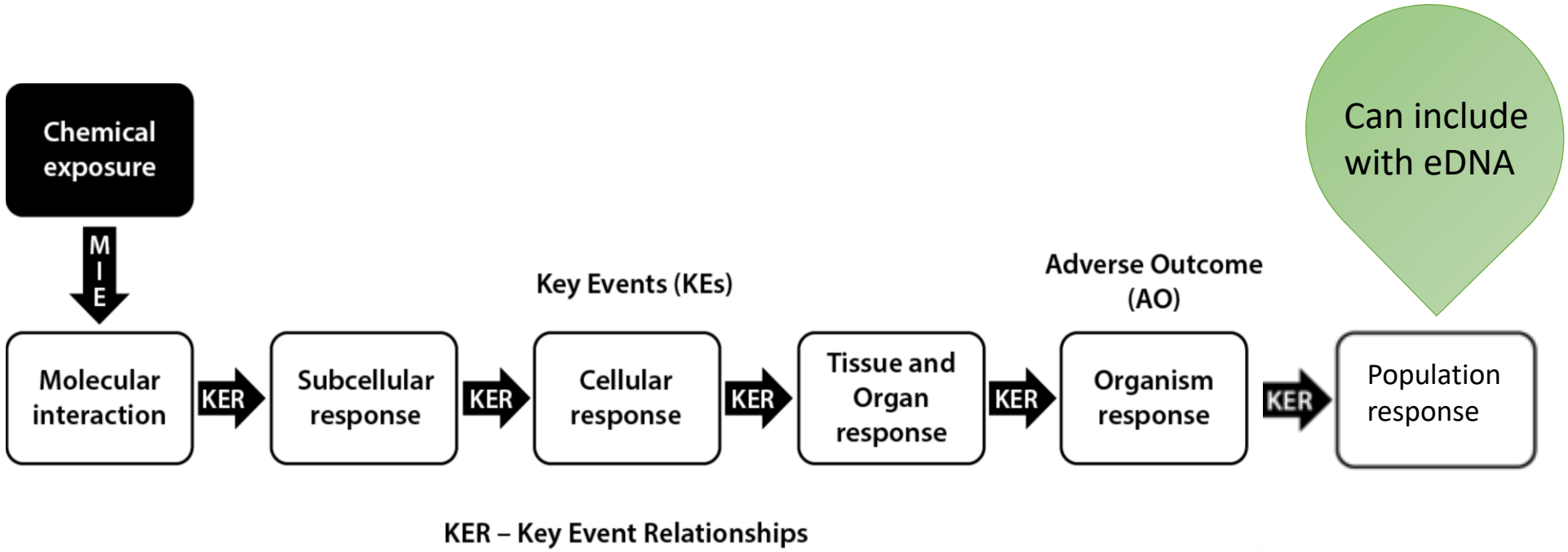
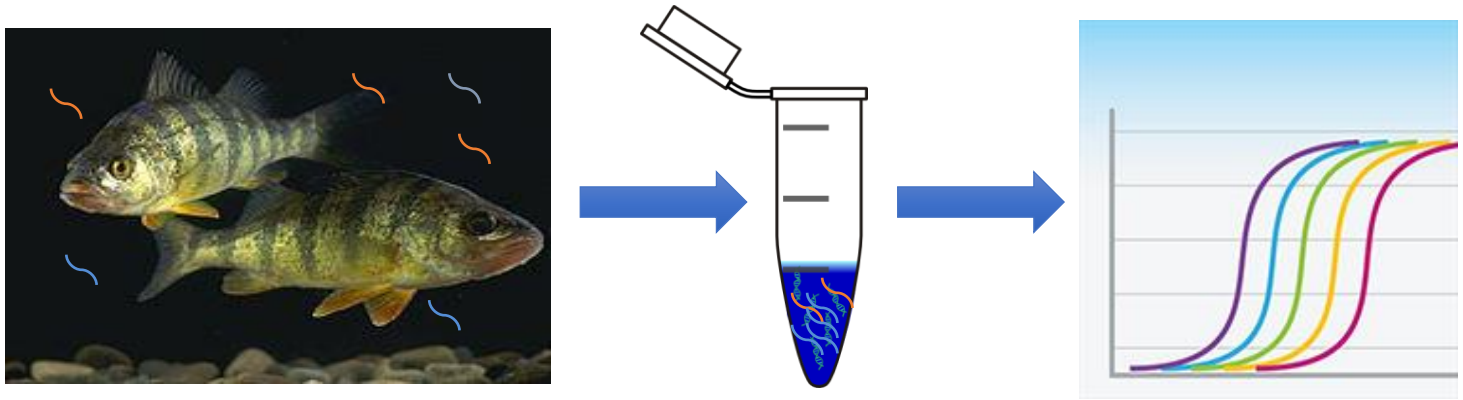
Adverse outcome pathway



Gene regulation cell organ individual population

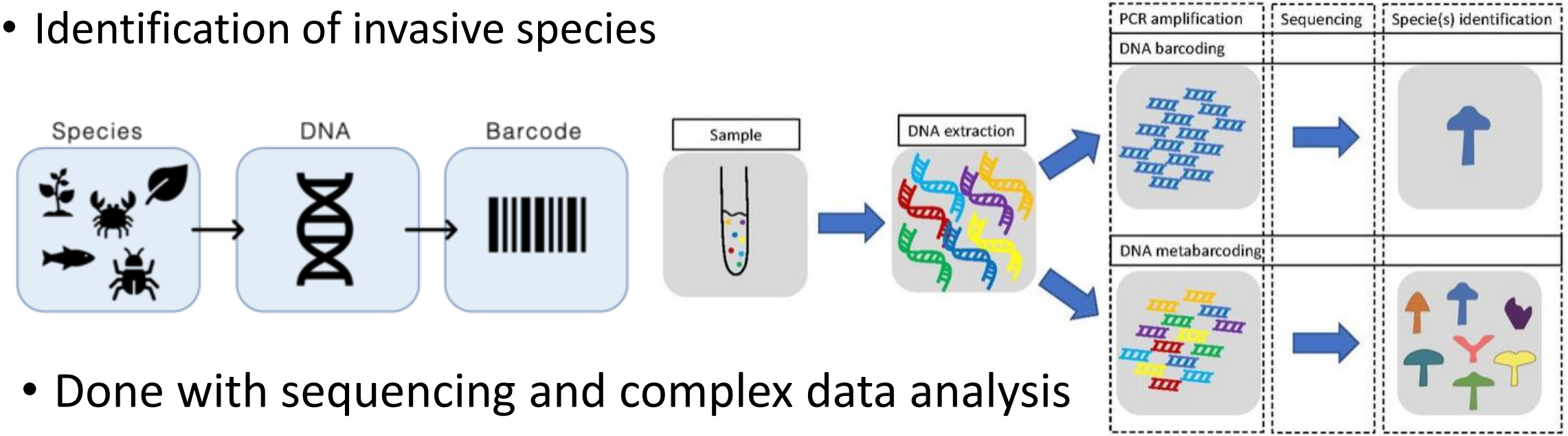
Environmental DNA (eDNA)

- The DNA left behind by an organism in an environment
- Animals shed cells so the DNA from these can be detected – Mitochondria DNA
- Amount of eDNA present is related to the amount of animals



eDNA applications

- Taxonomic studies
- Indicator organisms – presence or absence of species
- Identification of invasive species



- Done with sequencing and complex data analysis

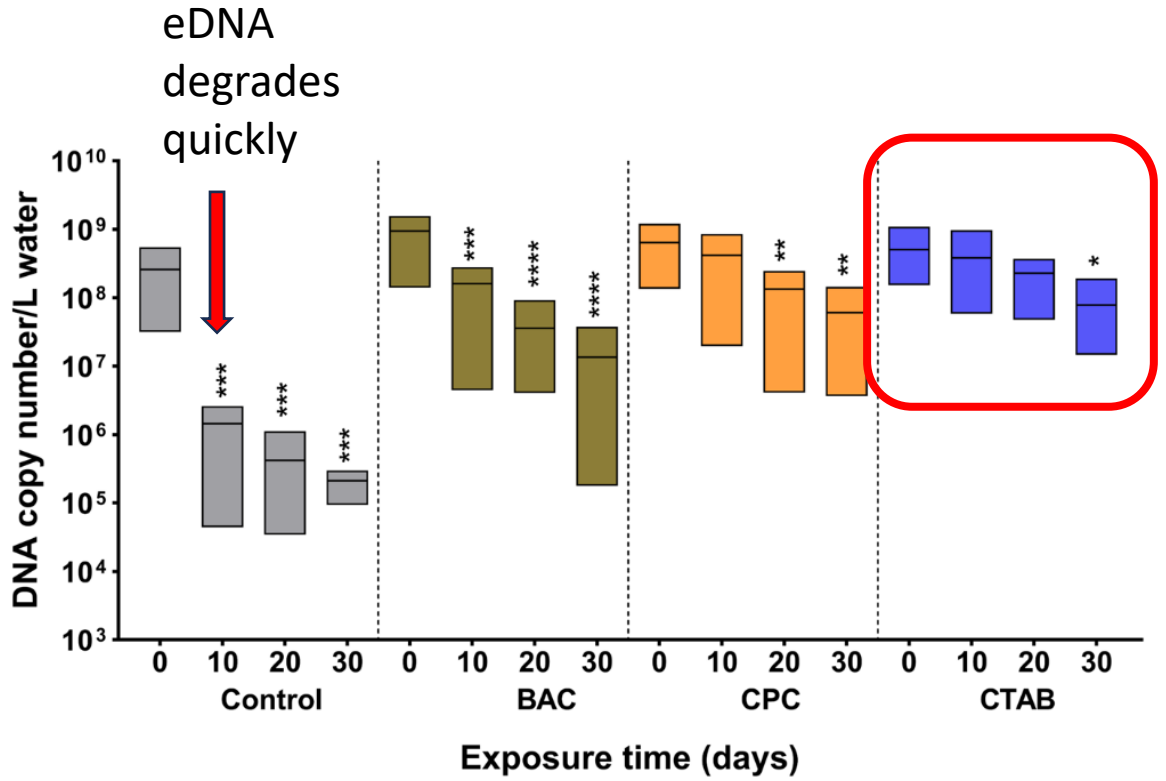
From Wikipedia

Current eDNA sample collection

- Samples (water / soil) collected on site
- Filtration – pumps and filtering apparatus for water
- Cold-chain – requires fridge/freezer storage
- Increased risk of cross-contamination
- Logistics of storage and transport of samples
- Reagents (ethanol, sodium acetate, lysis buffer)

Developed eDNA stabilization method

- DNA must be stabilized to carry out precise extraction and analysis in the laboratory
- We developed a method based on detergents that stabilizes DNA for up to 30 days
- All preparation and analysis can be done in the laboratory



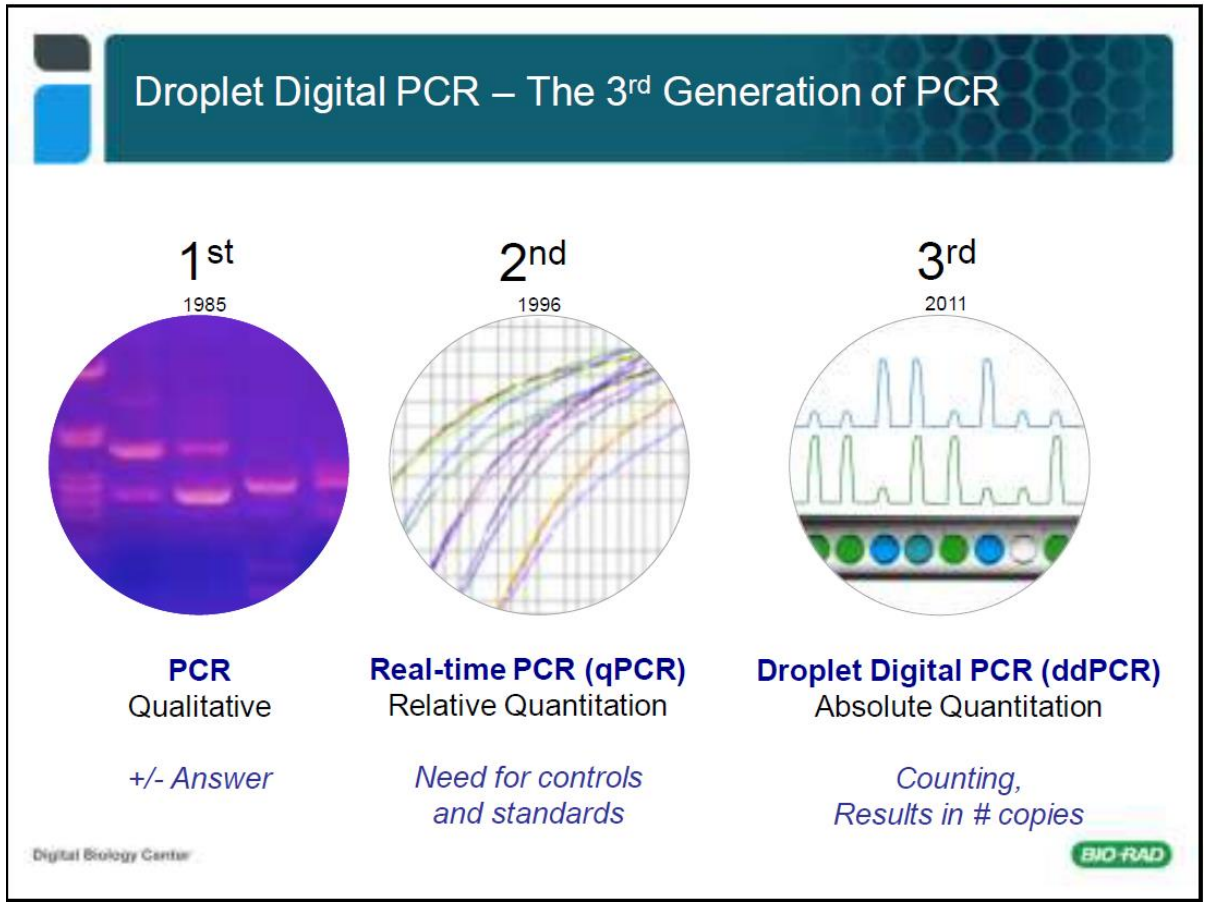
Current protocol for eDNA analysis

- eDNA is amplified by PCR followed by sequencing.
- All genetic material in the sample is analyzed.

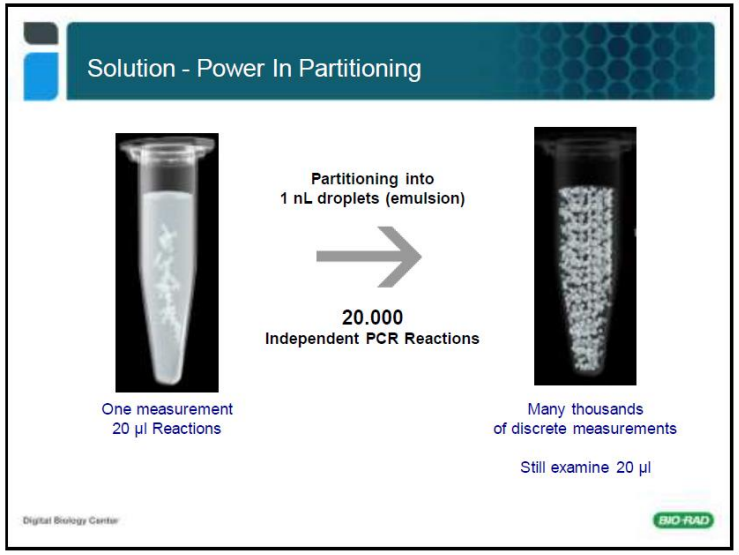
- Metabarcoding
- Identifies species through sequencing.
- Requires the sequences to be species specific.
- Species must be in a database
- It is not certain that an accurate quantification of species can be performed with sequencing.

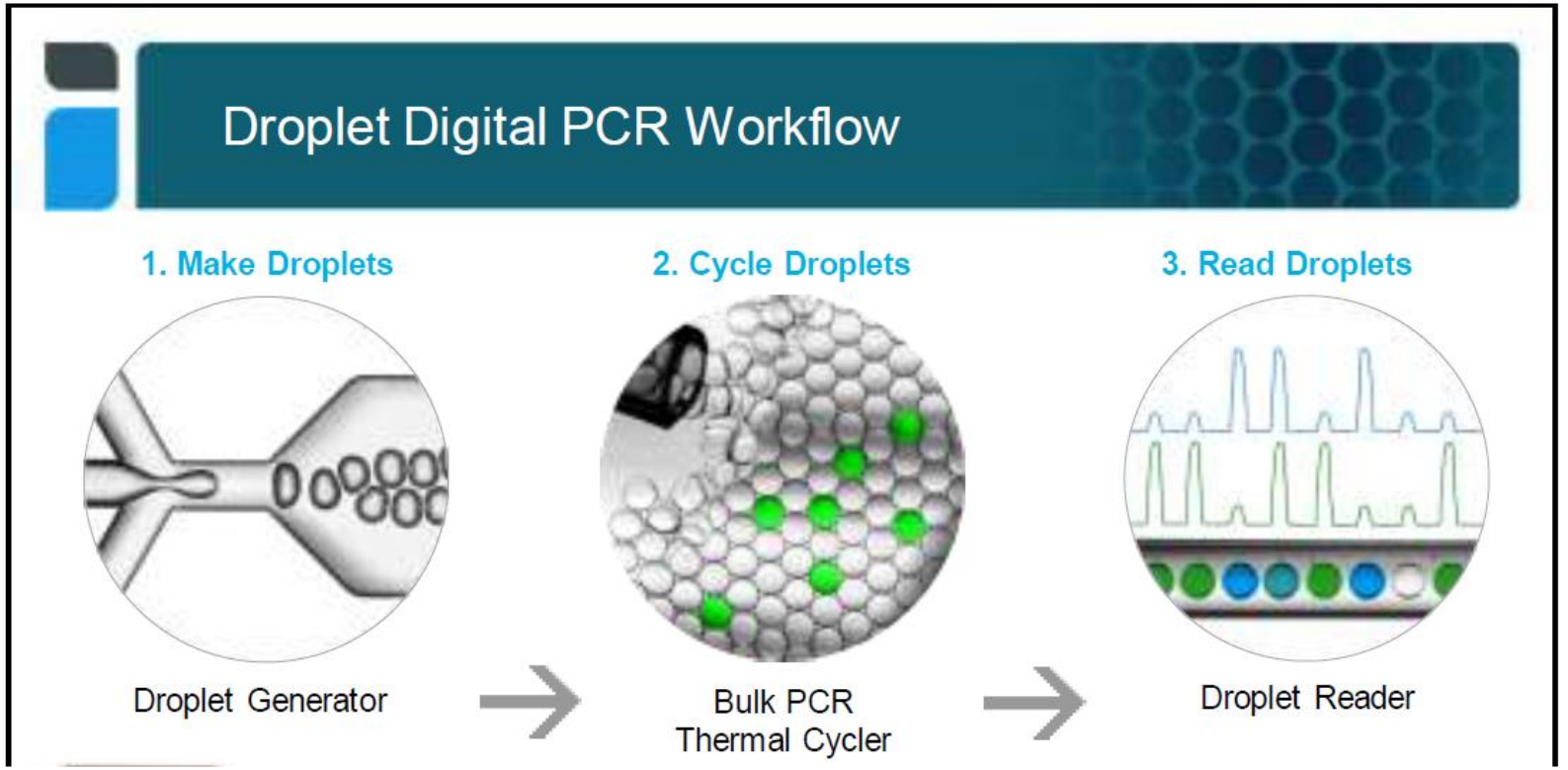
ddPCR / qPCR instead of barcoding

- Droplet digital PCR
  - 3rd generation PCR
  - amplification with absolute quantification
- Extremely high sensitivity
- Resistant to inhibitory effects of impurities
  - PCR and qPCR are sensitive to impurities



Droplet digital PCR





Absolute quantification of DNA

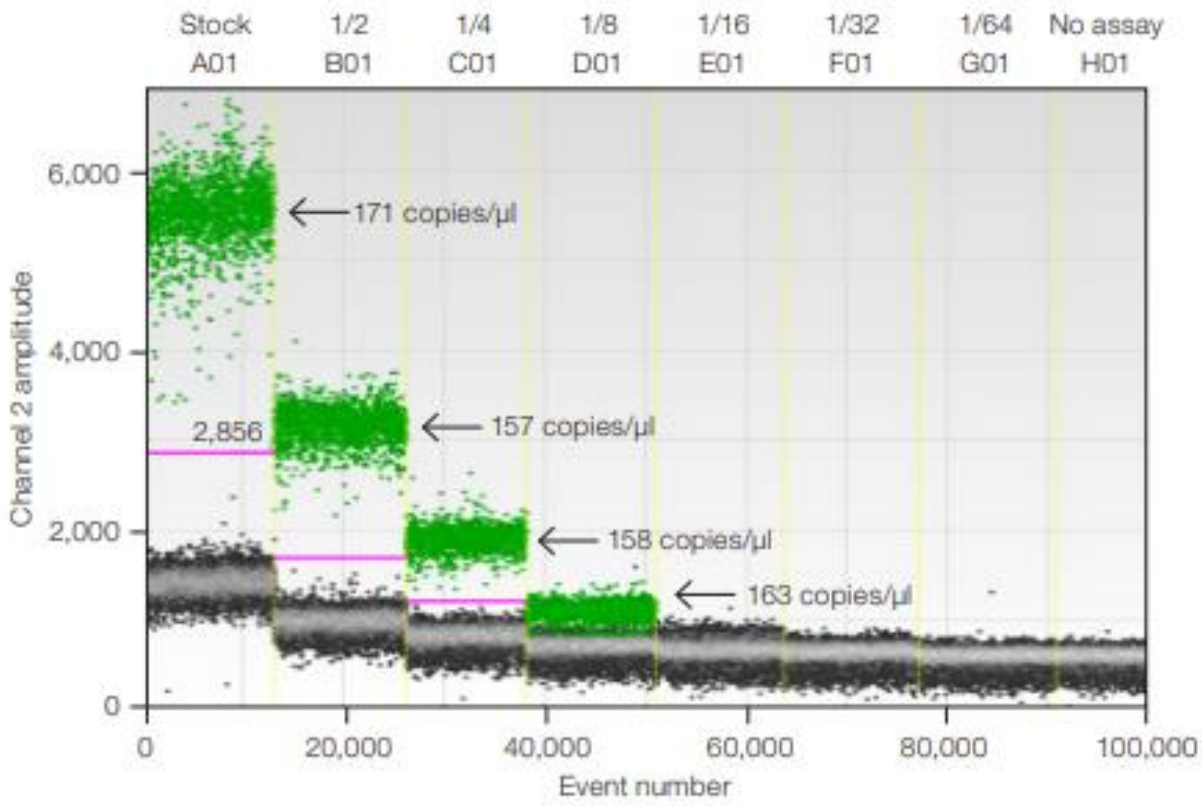
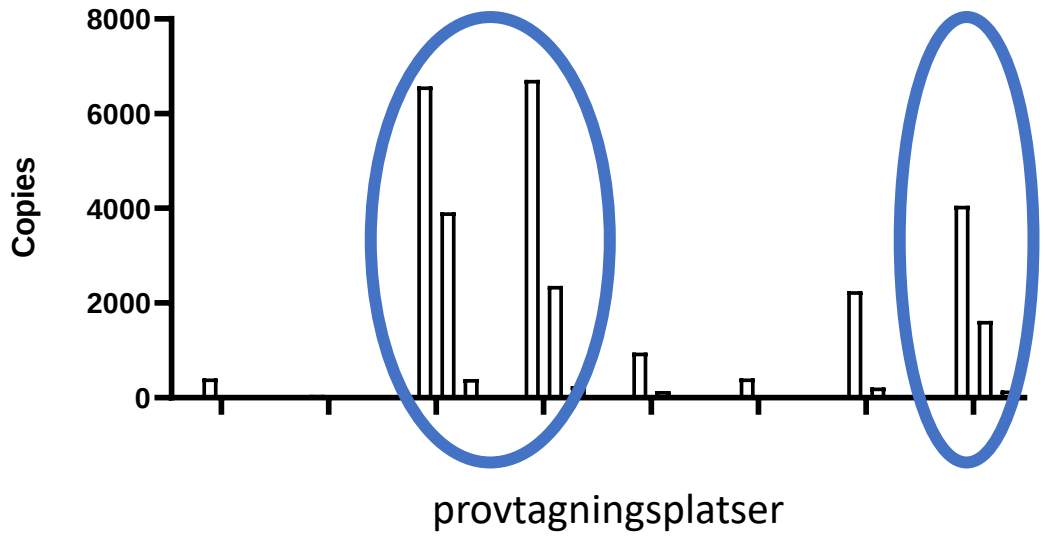


Fig. 1. Effect of assay dilution on fluorescence amplitude. Diluting the assay enables modification of the fluorescence intensity of the positive droplets while retaining the ability to quantify the targets with great reliability.

Environmental forensics

Problem: leakage or misconnections between sewage pipes and stormwater pipes

- Human – specific primers
- Detect low levels of human eDNA in water
- Track faulty connections in stormwater networks where wastewater enters storm water pipes



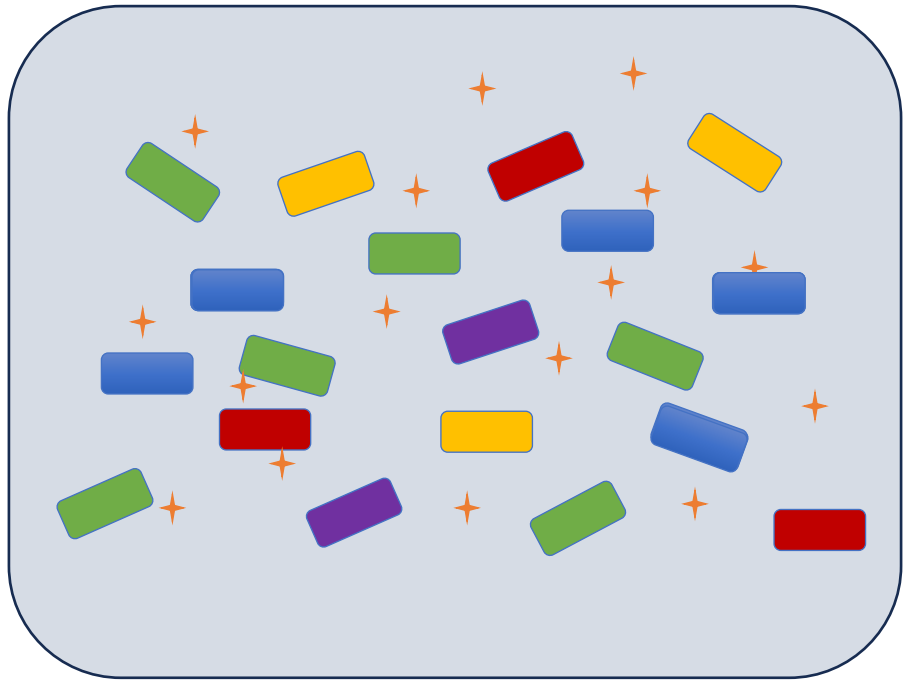
Future directions for eDNA and ddPCR

- Rapid quantification of individual organisms in a water or soil

- Ability to analyze functional biodiversity in soils
  - A change in microbial composition to detect disorders
  - Presence of microbial detoxifying systems and/or pump out toxins
  - Analysis for genes targeting detoxification mechanisms can be a future method for risk analysis of soils

Functional biodiversity

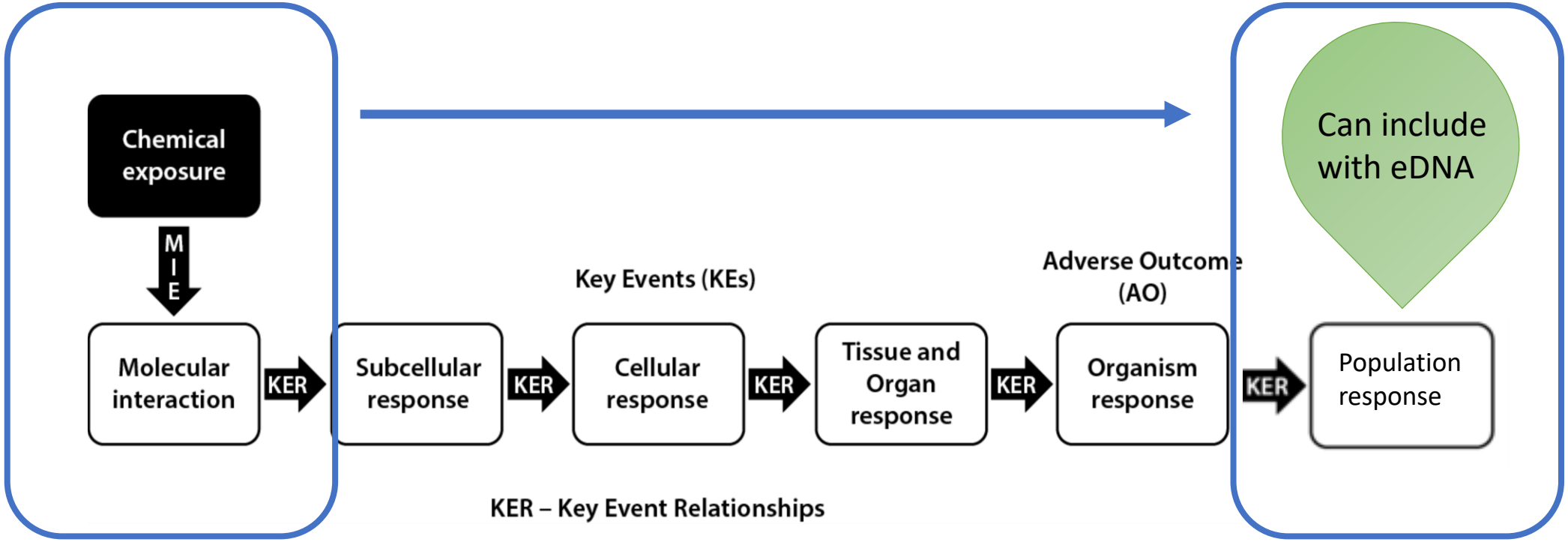
- Microbial degradation of organic compounds
  - Transformation
- Detoxification mechanisms
  - Eflux pumps
  - Prevention of uptake
  - Reduction
  - Adsorption
- Population dynamics



Future opportunities for eDNA and ddPCR

- Currently DNA from mitochondria is used for analysis of organisms.
  - Pros: Mitochondria protects DNA
  - Cons: Mitochondrial genes are conserved, lacks sufficient resolution for closely related organisms
- Stabilizing eRNA using the same method we developed for eDNA can increase resolution
  - The half-life of eDNA is short - hours
  - The half-life of eRNA is even shorter - minutes
  - Greater differences in RNA sequences
  - RNA from e.g. ribosomes and other highly expressed genes

Adverse outcome pathway



BioImpakt

- BioImpakt AB utför genanalyser baserat på den nya tekniska specifikationen, SIS-CEN/TS 17883:2022

- I samarbete med Örebro universitet utvecklar vi nu nya eDNA och eRNA metoder.

- Hemsida: https://www.bioimpakt.com

THANK YOU FOR LISTENING

QUESTIONS?