



European Partnership on
Animal Health and Welfare



FRIEDRICH-LOEFFLER-INSTITUT



Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health



Animal &
Plant Health
Agency



The EraBSE project: general description and focus on the specific genetic activity

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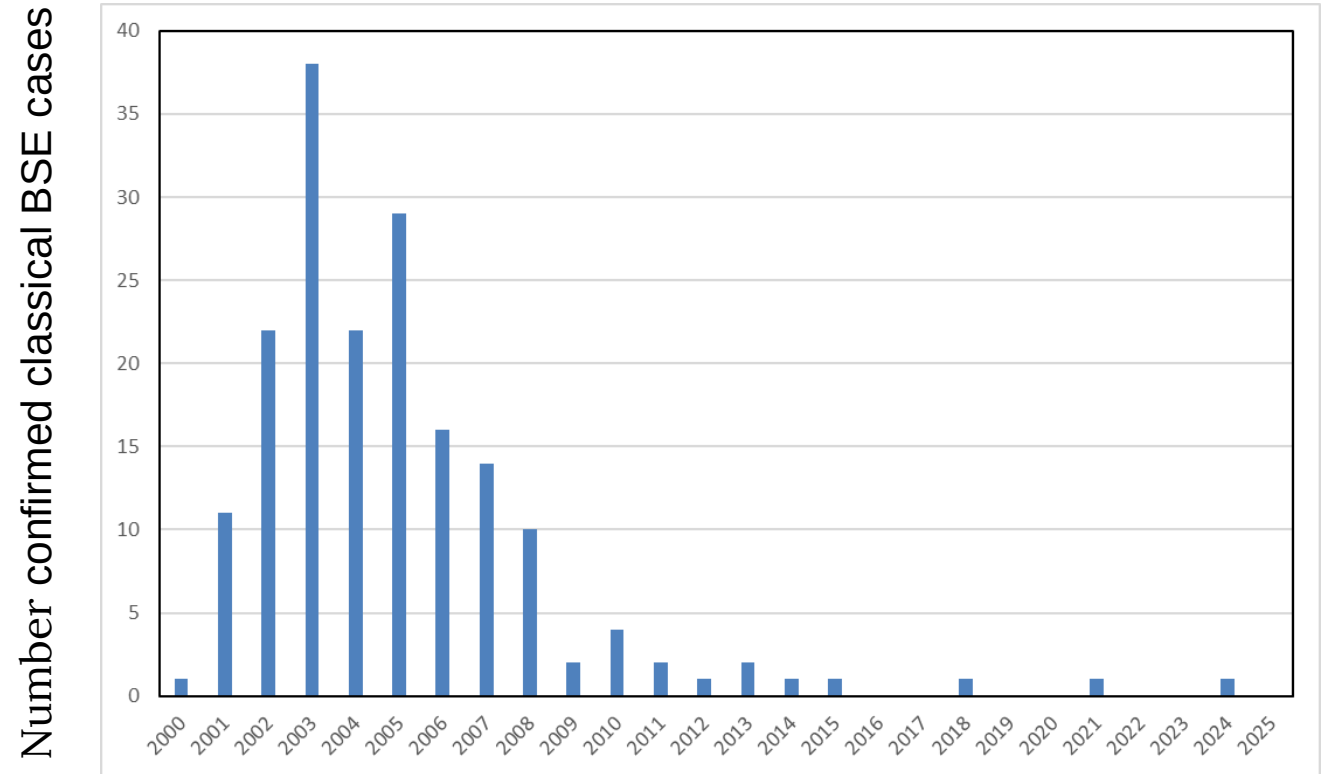
Project background

- ❖ BSE emerged as a novel prion disease in the 1980s, with huge impacts on livestock industries.
- ❖ BSE has been effectively controlled by comprehensive bans on feeding animal-derived protein to livestock and by EU eradication measures.

However ongoing surveillance continues to identify occasional cases Born After Reinforced Ban (BARB)

From 2000-2025:

	Number of cases
Classical BSE	179
Atypical BSE	10
Total	189



Source: <https://www.gov.uk/government/collections/tse-disease-surveillance-statistics>

Origin of BARB cases?

Bovine spongiform encephalopathy (BSE) cases born after the total feed ban

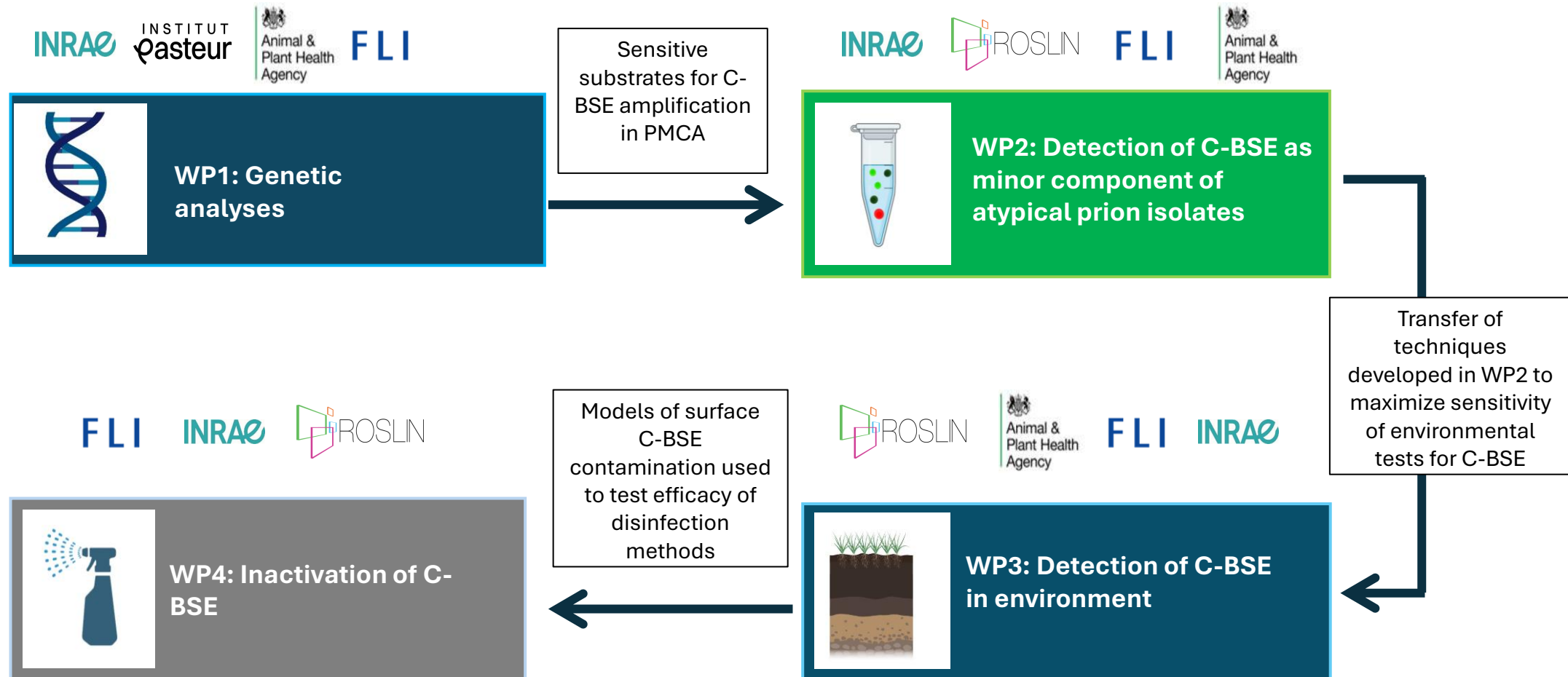
EFSA Panel on Biological Hazards (BIOHAZ),
Antonia Ricci, Ana Allende, Declan Bolton, Marianne Chemaly, Robert Davies,
Pablo Salvador Fernández Escámez, Rosina Gironés, Lieve Herman, Kostas Koutsoumanis,
Roland Lindqvist, Birgit Nørrung, Lucy Robertson, Moez Sanaa, Marion Simmons,
Panagiotis Skandamis, Emma Snary, Niko Speybroeck, Benno Ter Kuile, John Threlfall,
Helene Wahlström, Amie Adkin, Aline De Koeijer, Christian Ducrot, John Griffin,
Angel Ortiz Pelaez, Francesca Latronico and Giuseppe Ru

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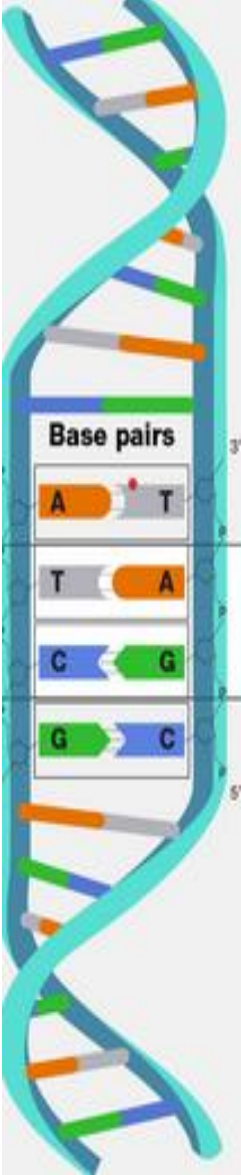
- ❖ Investigation of **60 BARB cases** (across several EU countries) to provide opinion on most likely origin of disease
- ❖ Considered **alternative sources of infection** (maternal, environmental, iatrogenic) and **genetic** factors
- ❖ **High level of uncertainty about the origin** of these cases; feed-borne exposure could not be excluded

Project overview

Project aim: to accelerate progress towards final eradication of C-BSE, through greater understanding of factors contributing to its persistence, and development of tools that can be strategically deployed for cost-effective disease detection, control and management of emerging/re-emerging risks.



Genetic analysis of C-BSE BARB cases



- ❖ **Whole Genome Sequencing (100% of the genome)**

- ❖ **Coverage 30X**

~3 Gb Bovine Genome ~90 billion bases sequenced

Limitations of WGS

- ❖ **Data Storage & computing**

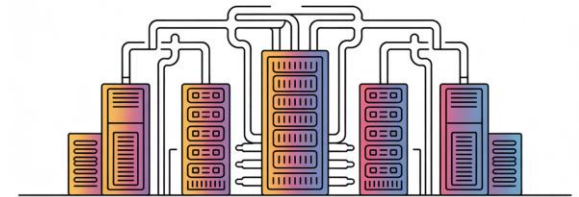
Demands high performance computing infrastructure

- ❖ **Variant Interpretation**

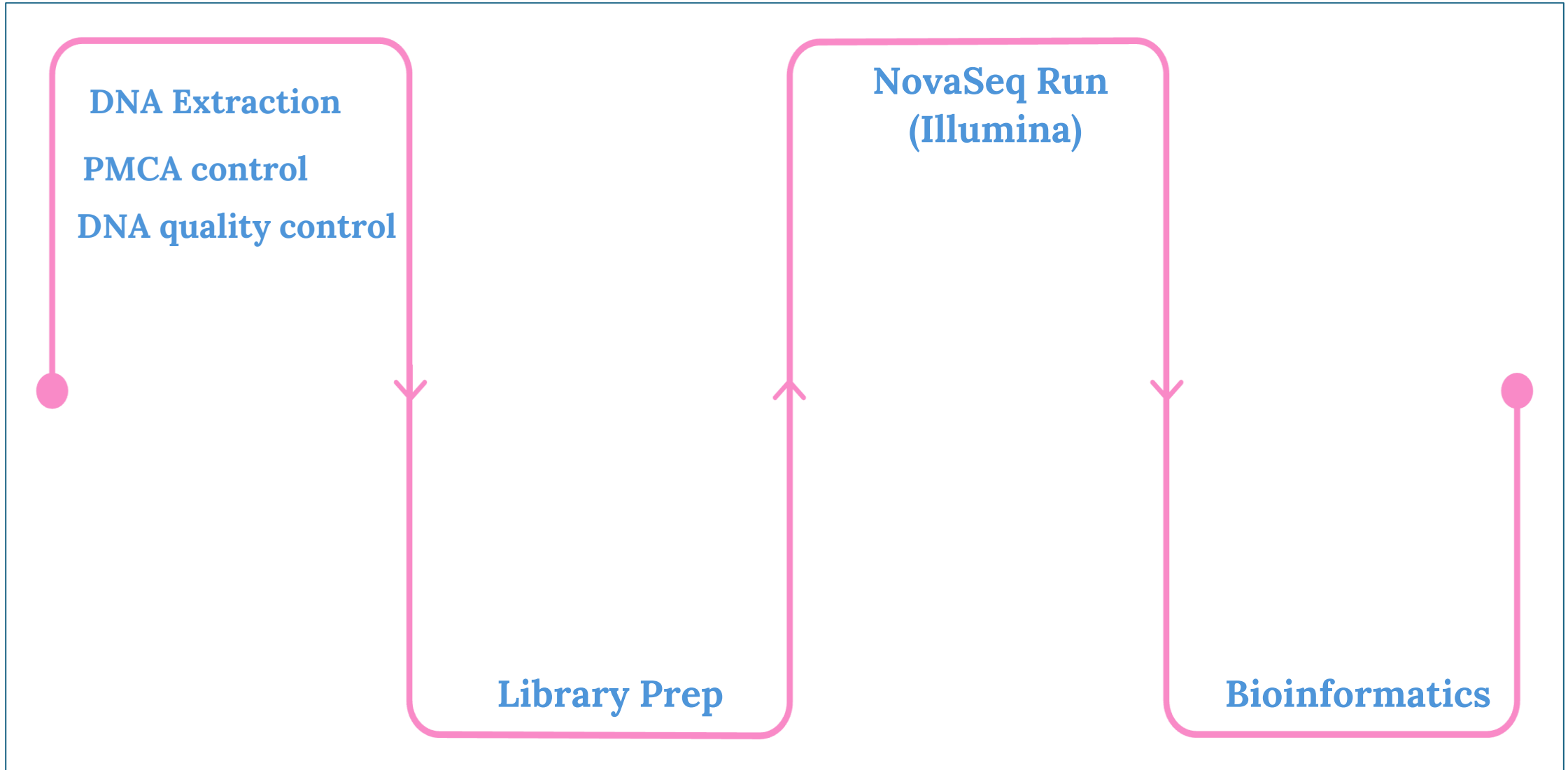
Distinguishing pathogenic from benign variants is a persistent challenge, requiring curated databases and functional evidence

- ❖ **Financial Cost**

Reagent, sequencing, and computational costs



WGS Workflow



Samples

In short term, we are going to study samples from the United Kingdom, Germany and France. Those from UK have been identified and are provided by the Animal and Plant Health Agency.

❖ 42 BARB cases

6 breeds (N=18)

10 crossbreeds (N=22)

unknown (N=2)

❖ 39 controls

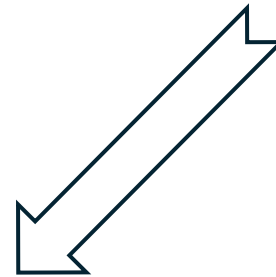
sample of various types

❖ Frozen brainstem

❖ Brainstem Homogenate

❖ Brainstem Wax blocks (FFPE)

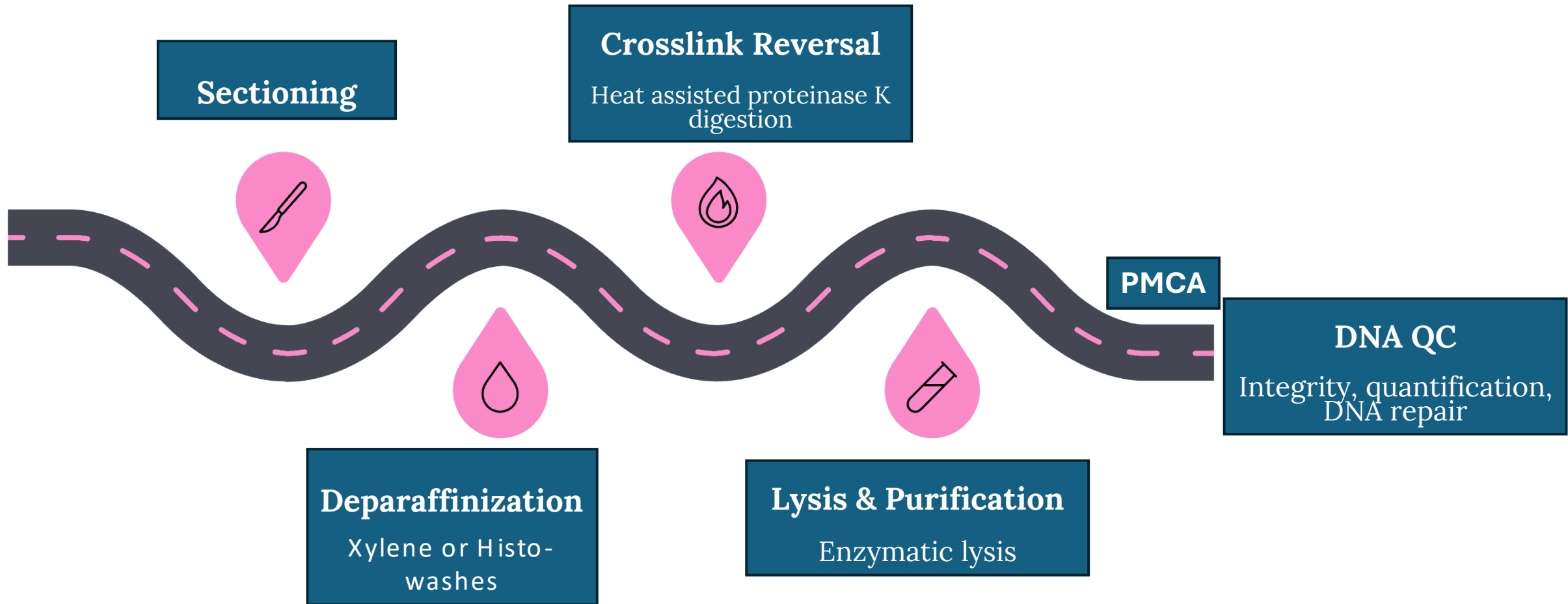
❖ Blood



Need to **improve** DNA extraction techniques for each type of sample
(stored in the same way and for the same duration before beginning)

DNA Extraction from FFPE Brain Tissue

The degree of fragmentation depends on the type and age of the sample and the conditions used for fixation



DNA Extraction from FFPE Brain Tissue is a challenging task

Brain =Lipid-rich tissue

Interference of Lipids with Extraction Reagents

- **Hydrophobic Nature of Lipids** that can form aggregates or micelles in solution
- **Enzyme Inhibition**

Paraffin Embedding: Contamination and Deparaffinization Challenges

- Incomplete deparaffinisation can interfere with DNA purification or introduce PCR inhibitors

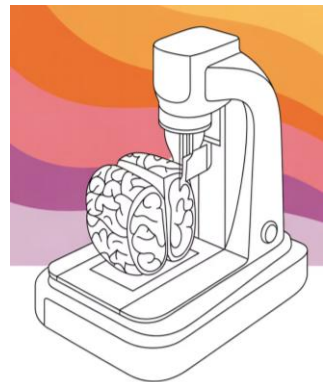
Formalin Crosslinks

- **Formation of Methylene Bridges** between proteins and nucleic acids, between lipid molecules and proteins
- **DNA Fragmentation**

Prolonged formalin fixation can also fragment DNA
(Fragments typically 100–300 bp)

Low Yield

- Expected : 5–50 ng/μL
- multiple sections often required



Request

We can still add some more samples.

Could you please contact us if you would be willing to provide us with additional BARB cases and associated controls (Katayoun.Goudarzi@inrae.fr; Christine.Fast@fli.de)

The key details we need are :

- ❖ TSE status/ case type
- ❖ Animal ID
- ❖ Country
- ❖ Breed
- ❖ Date of Birth
- ❖ Date of Death
- ❖ Sex
- ❖ County/regions
- ❖ Type of sample

Negative controls should be :

- ❖ same breed (and, if possible, related)
- ❖ same sex

Ideally

- ❖ same age,
- ❖ same farm or region
- ❖ sampled in the same year

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