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DNA microsatellite analysis step-by-step

Our Presious Transbounadry Waters

Work package 2 Brown trout of Pasvik river

Before analysis and sample storage

Long-term storage in freezer at -20 C.

Short-term storage in fridge.

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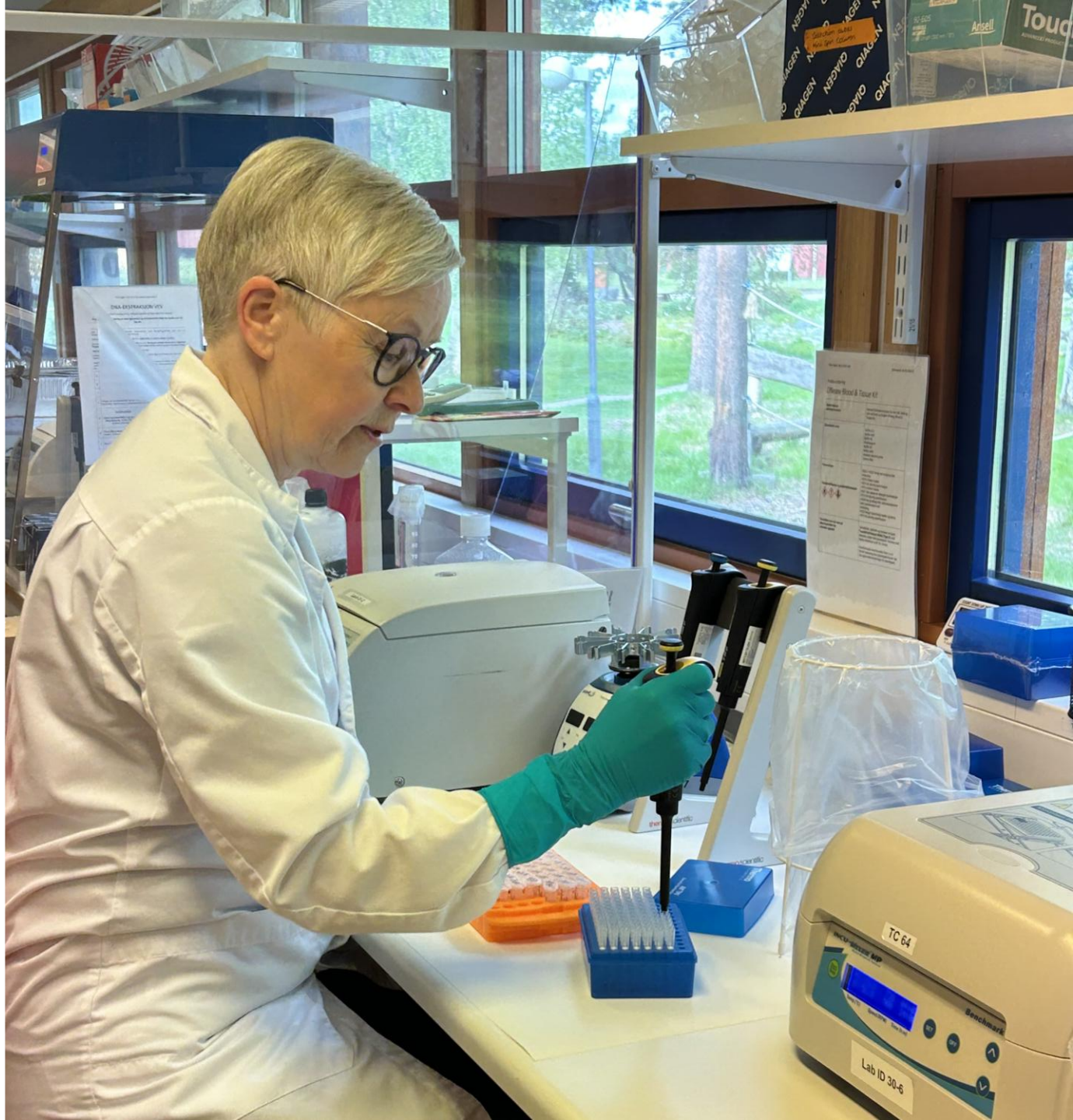
Preparing for PCR analyses

Samples are prepared by pipetting for PCR analysis.

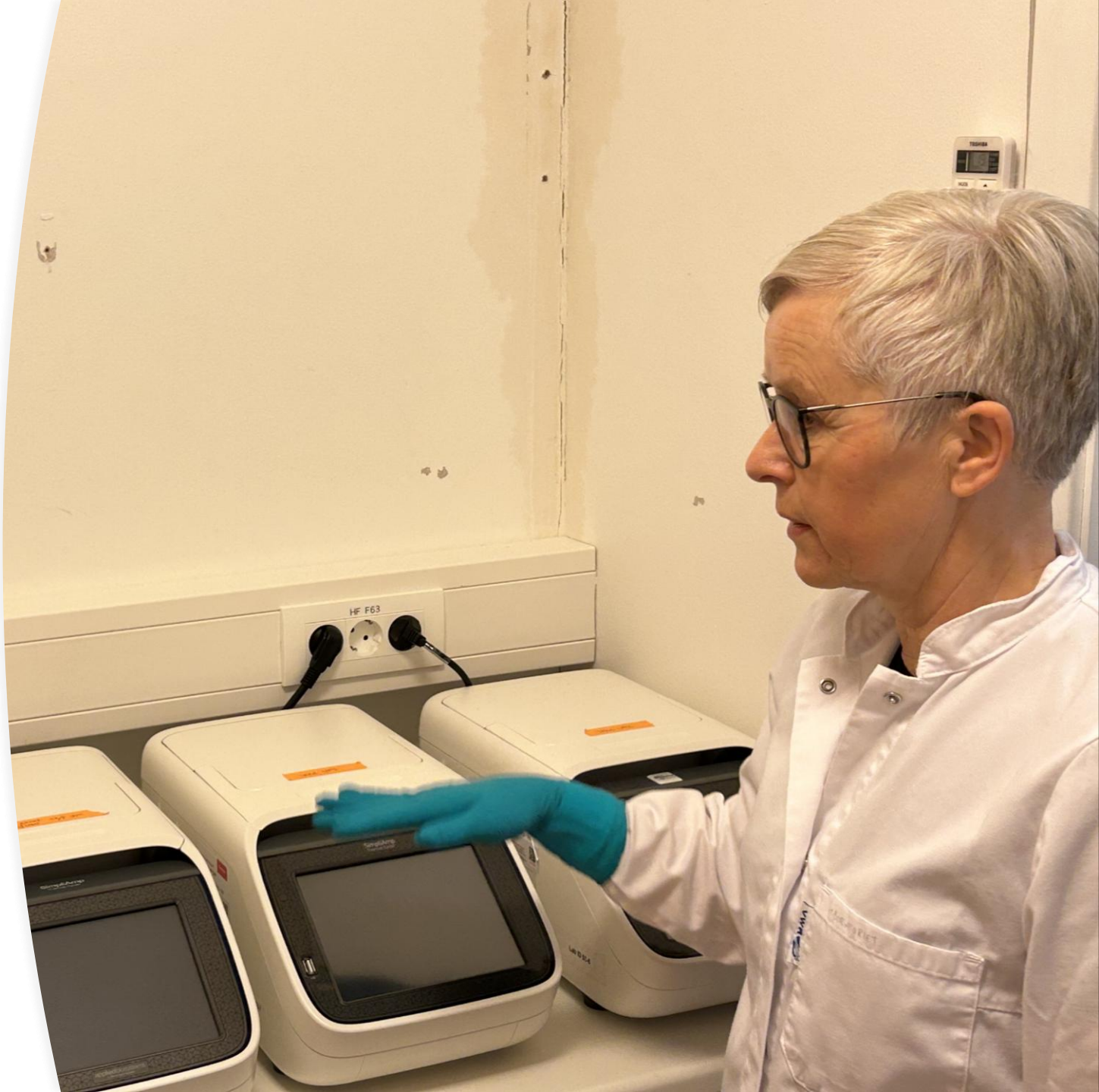
Polymerase chain reaction (abbreviated PCR) is a laboratory technique for rapidly producing (amplifying) millions to billions of copies of a specific segment of DNA, which can then be studied in greater detail.



Analysis

Several PCR machines are visible. These are used to perform the reaction to amplify a target fragment from the genome.

In this case, microsatellites; each locus is labelled with a different fluorescent dye – this is done to be able to analyze several microsatellite loci (= several different places in the genome) at the same time.



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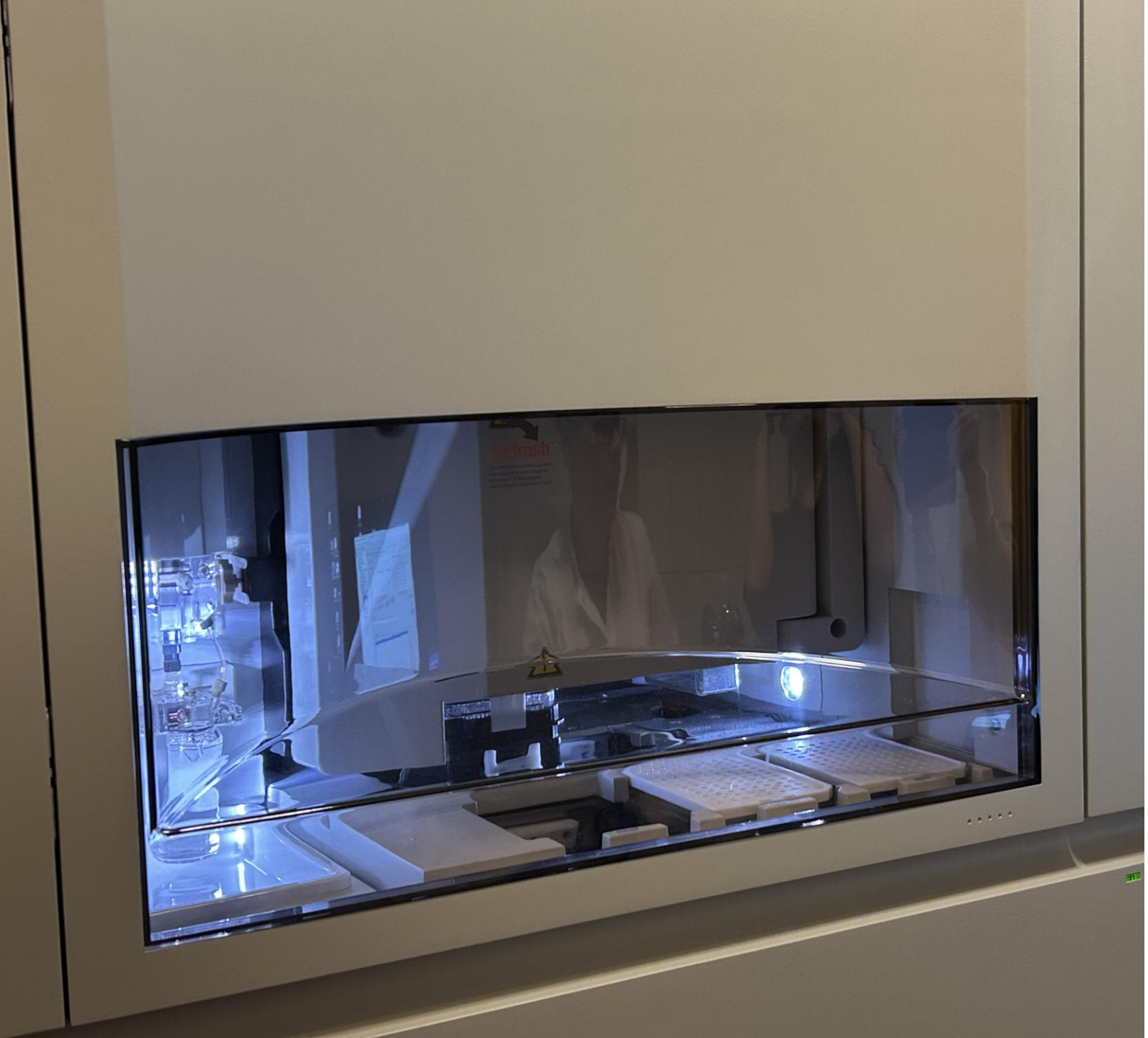
Microsatellite analysis

Preparation of the ABI machine for analysis by putting the analysis parameters in.



Microsatellite analysis – ABI machine

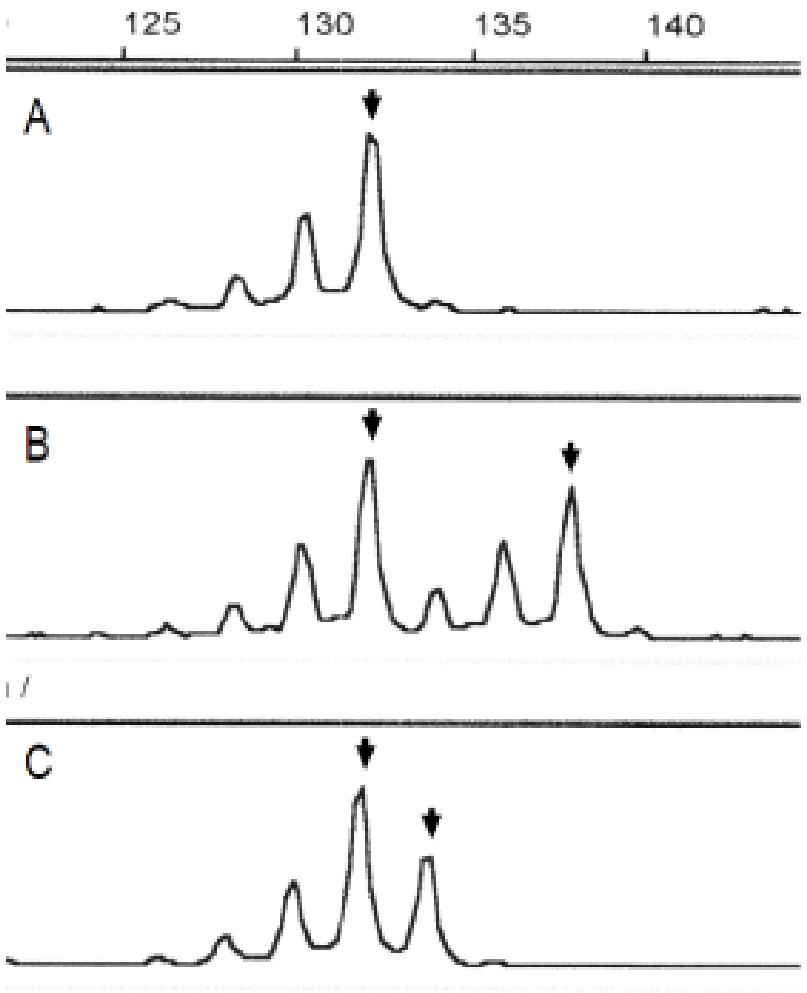
In the ABI, fragments are separated by capillary electrophoresis.

Different fragment sizes (= alleles) are subsequently statistically analyzed to retrieve information about

- sex
- relatedness of individuals within a population
- relatedness between population (=genetic differentiation)

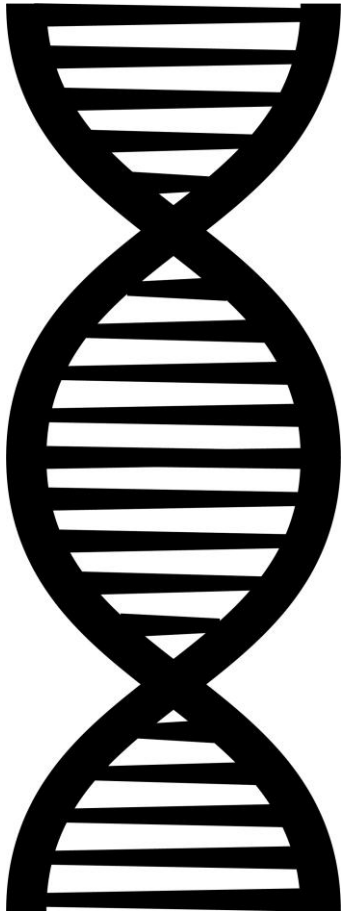
Microsatellites

- Short Tandem repeats (e.g., CACACA)
- Highly polymorphic
- High resolution over shorter time periods because of high mutation rate
- Neutral genetic variation
- Short fragments – analysis of difficult samples



In example A, there is one peak = homozygote
In example B and C, two peaks = heterozygote

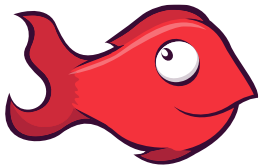
What is a mutation/SNP?

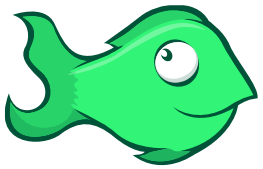


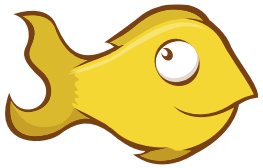
AGTCCTGGATGTCTACGT

AGTCCTGGATGTCAACGT

AGTGCTGGATGTCAACGT







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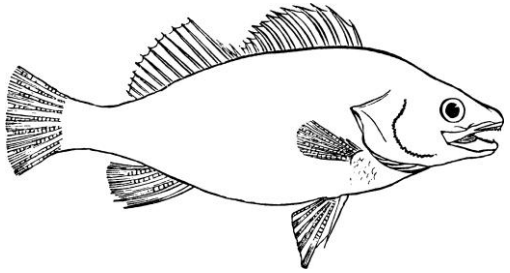
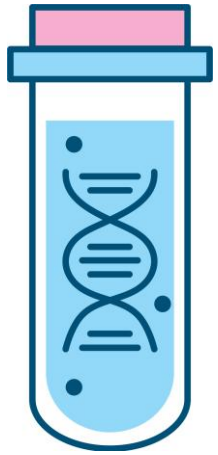
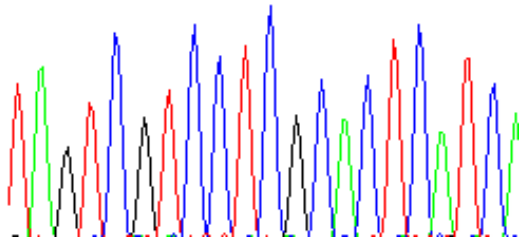
These mutations or SNPs (Single Nucleotide Polymorphisms) can be under selection, meaning that they change characteristics in the fish that may be advantageous or disadvantageous

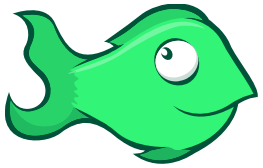
Molecular analysis

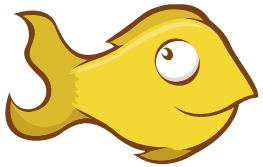






Sample 1: ATCTATATTTAA
Sample 2: ATCTGTATTCAA





SNPs can be detected by sequencing and subsequently can be analysed by targeted amplification similar to microsatellites but the machine is called FLUIDIGM.

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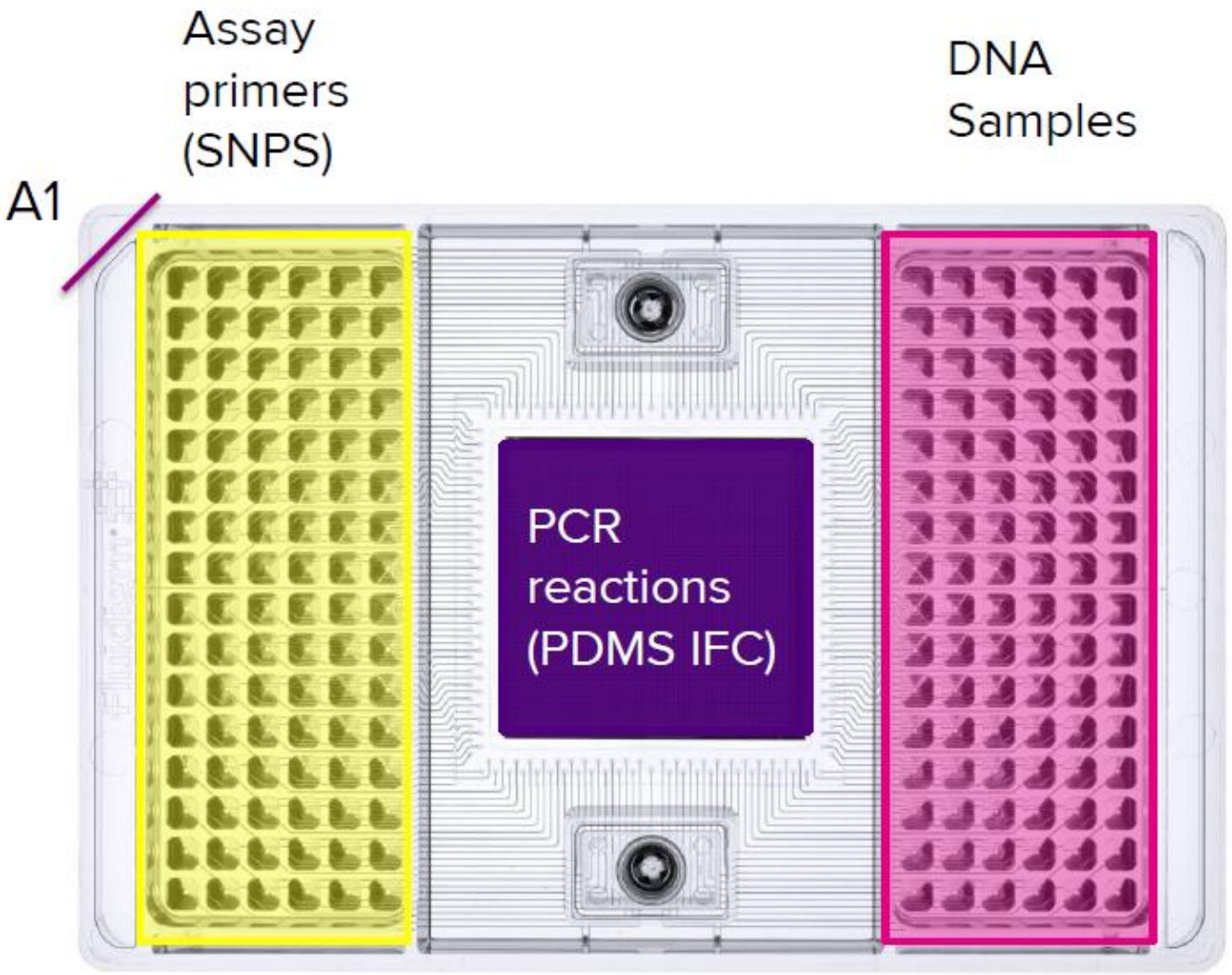

- Development of a set of SNPs (= assay)
- This assay can be analyzed for a large number of samples
- Detection of adaptive variation

Principle of SNP analysis. Source: FLUIDIGM training material.