Genomics of farm animals/ Sequencing demonstration

Laboratory examples

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Modul no. 1: Animal genetics

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Sequencing procedure

1. Sample preparation :
    a) cloned DNA (vector+examined DNA-insert)
    b) PCR product

    1.1. Sample cleaning (cleaning in solution or gel isolation)
    1.2. Determination of DNA template concentration
2. Sequencing reaction - cyclical
    primer (universal from vector or direct PCR), only 1!
    polymerase (Taq DNA FS, does not have 5´→3´ exonuclease activity),
    dNTP,
    labelled ddNTP,
    buffer (+Mg^{2+})
3. Sequencing mixture purification (EtOH+NaAc, columns, absorption) – elimination of free ddNTP
4. Capillary electrophoresis on the sequencer
5. (Automatic) evaluation - sequence determination

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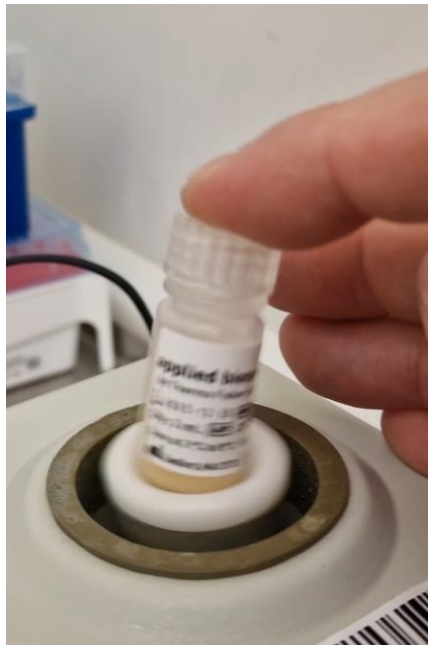
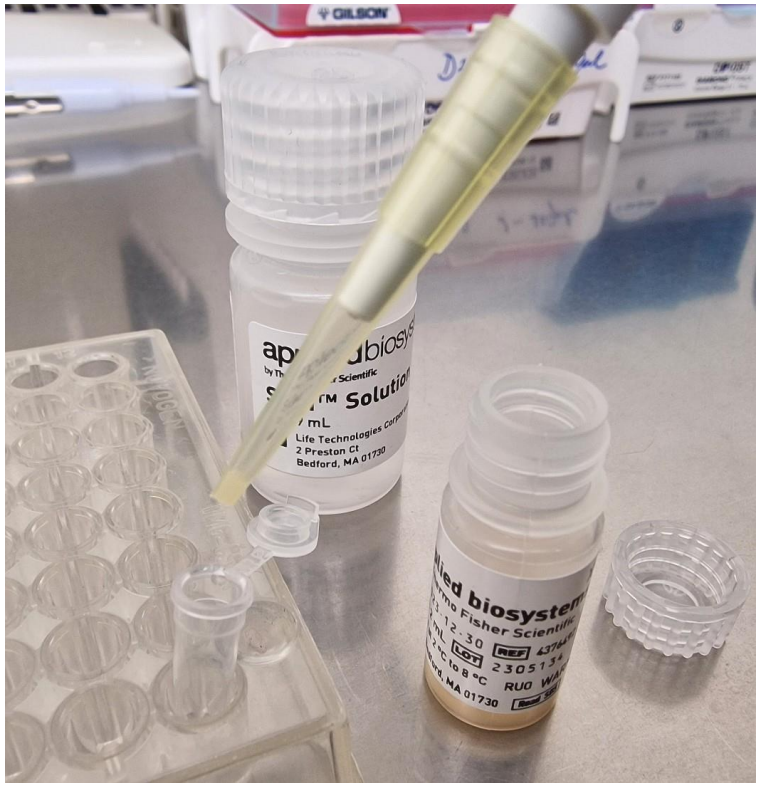
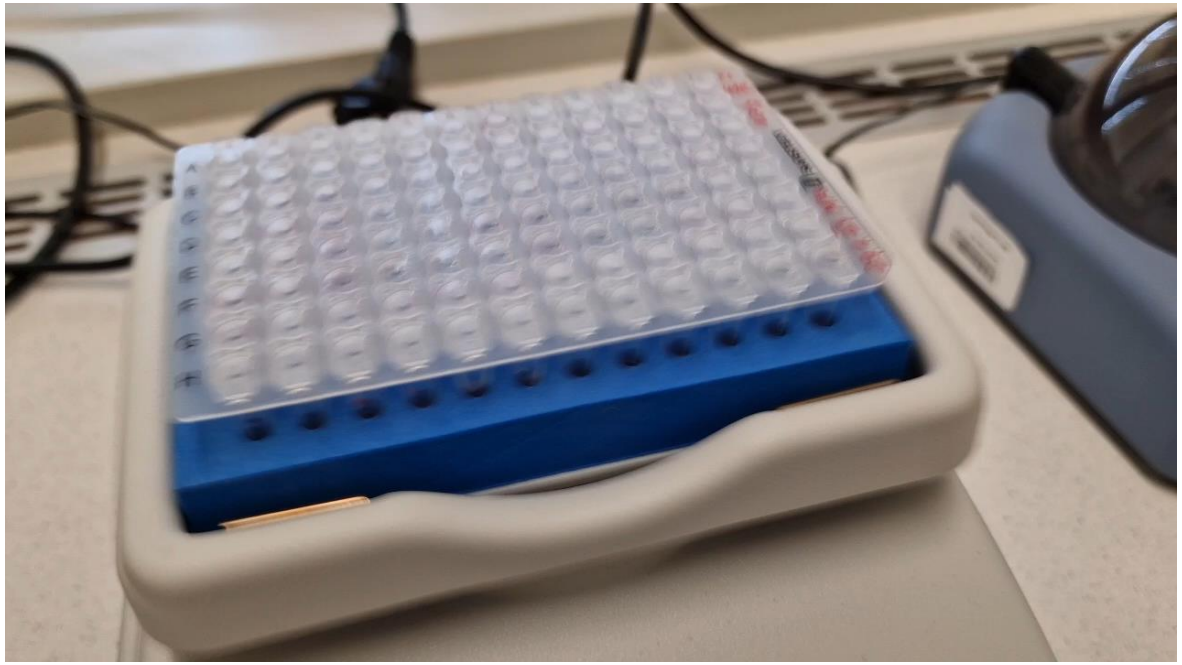
Sequencing mixture purification

Capture of free nucleotides and terminators by porous reagent emulsion (30 min shaking)







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Genetic analyzer (sequencer)

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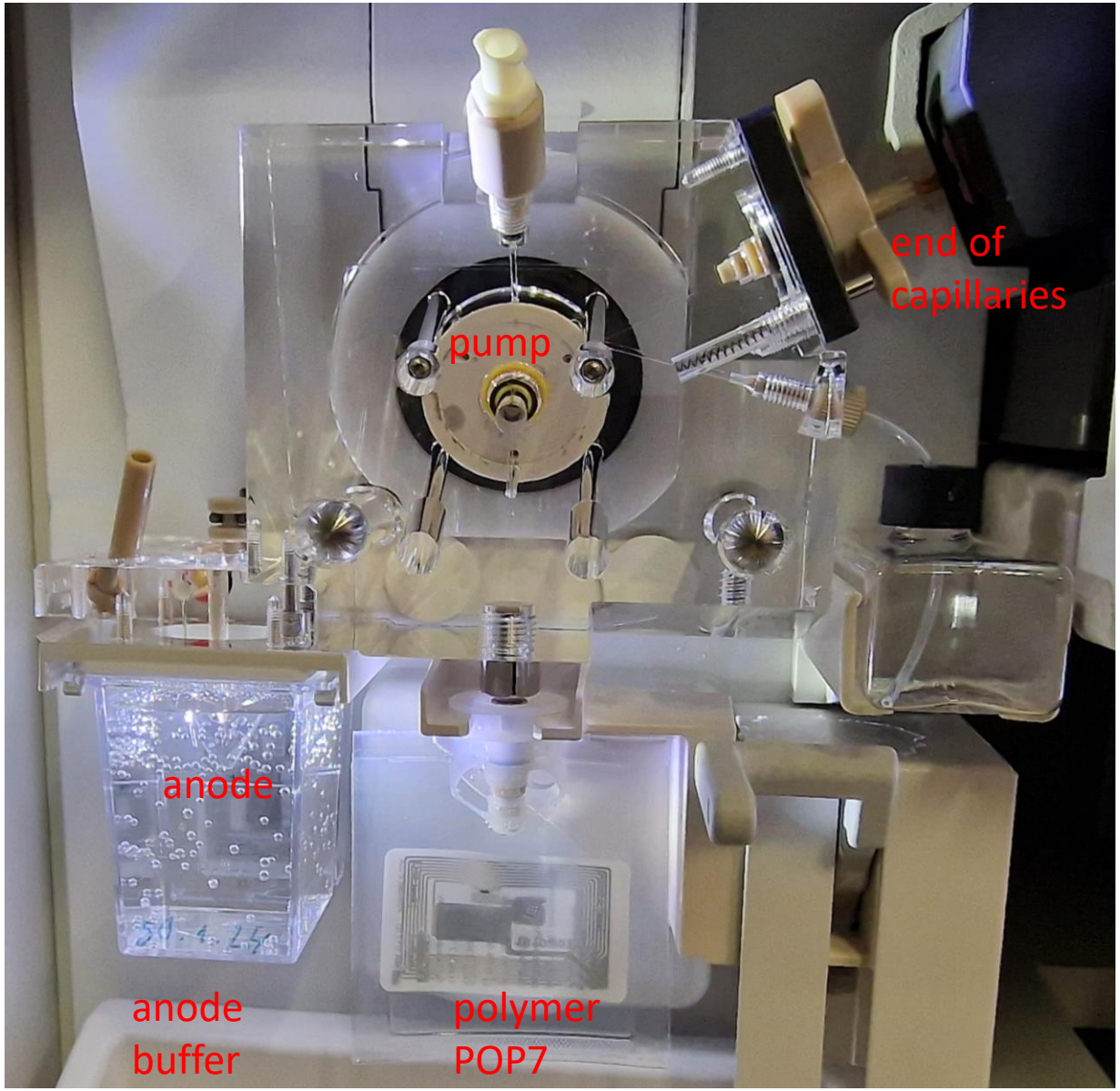
Anode area of sequencer



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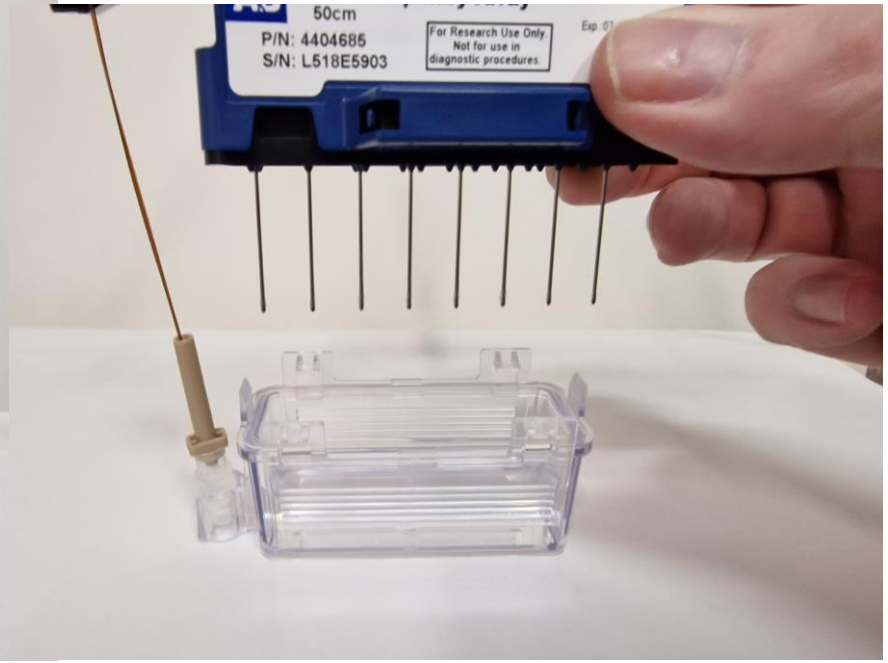
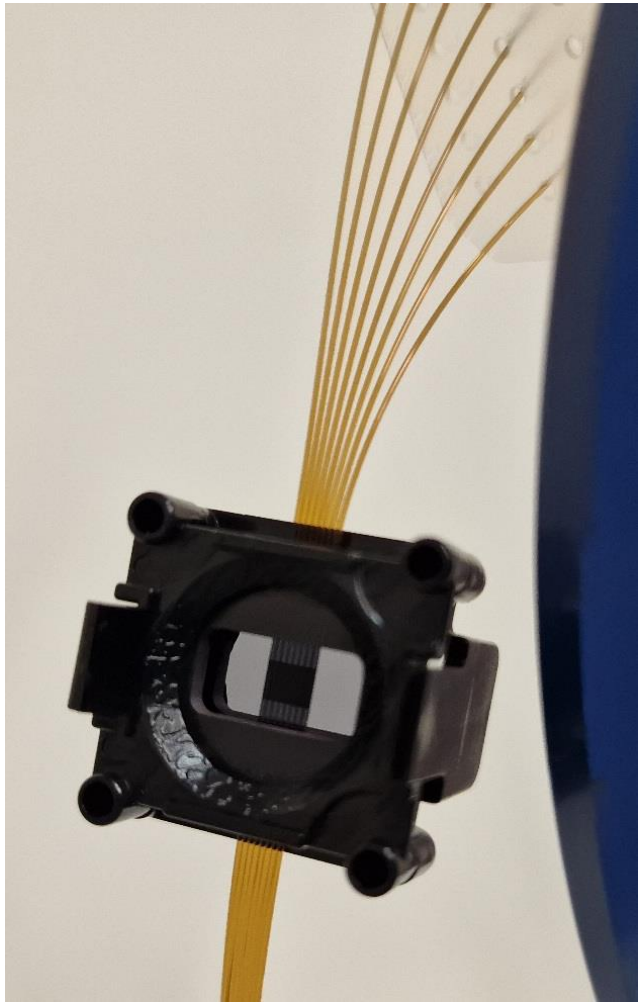
Capillary Array

8 capillaries



platinum electrodes



window for fluorescent signal detection

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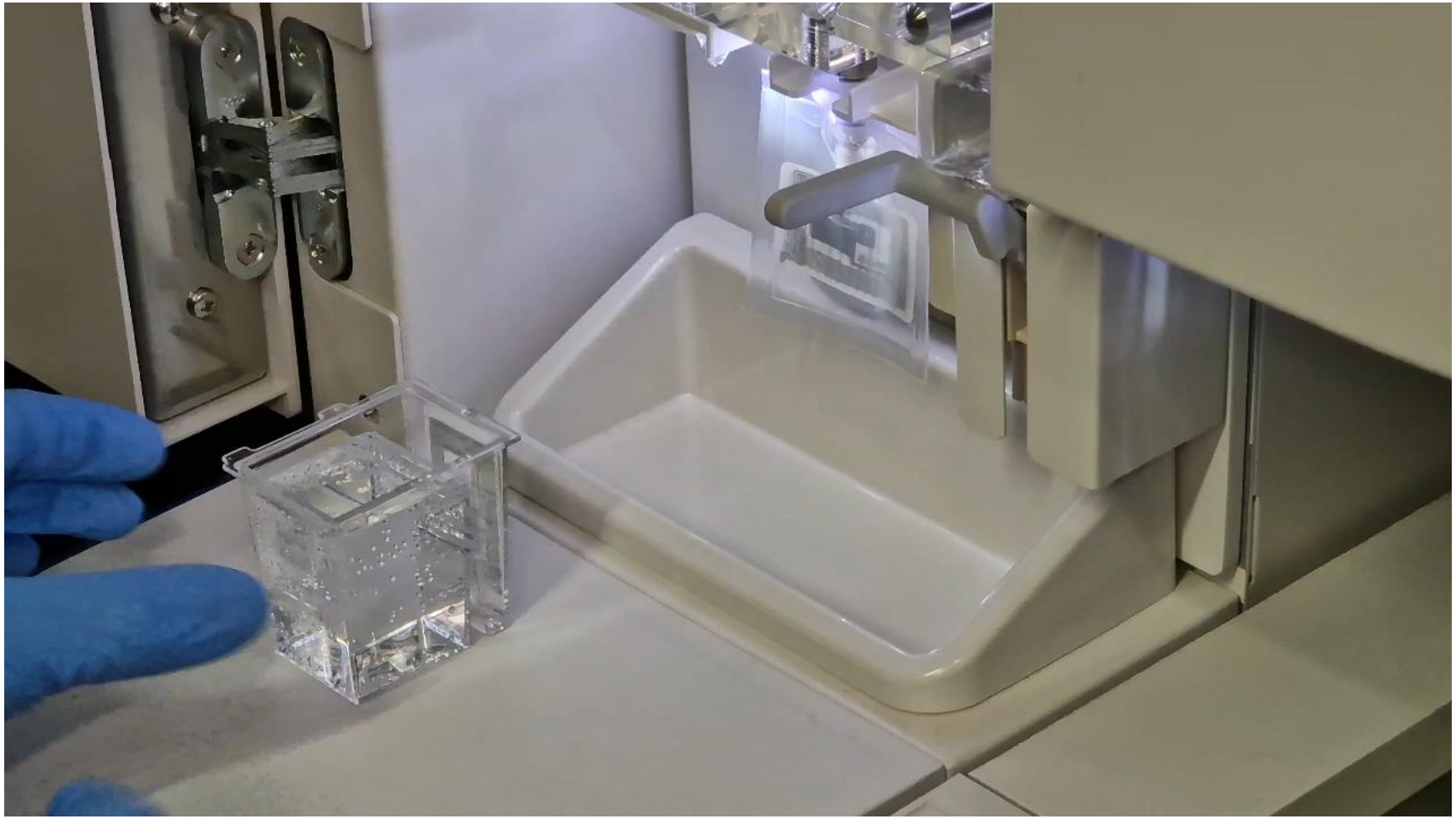
Sequencing procedure

Insertion of anode buffer



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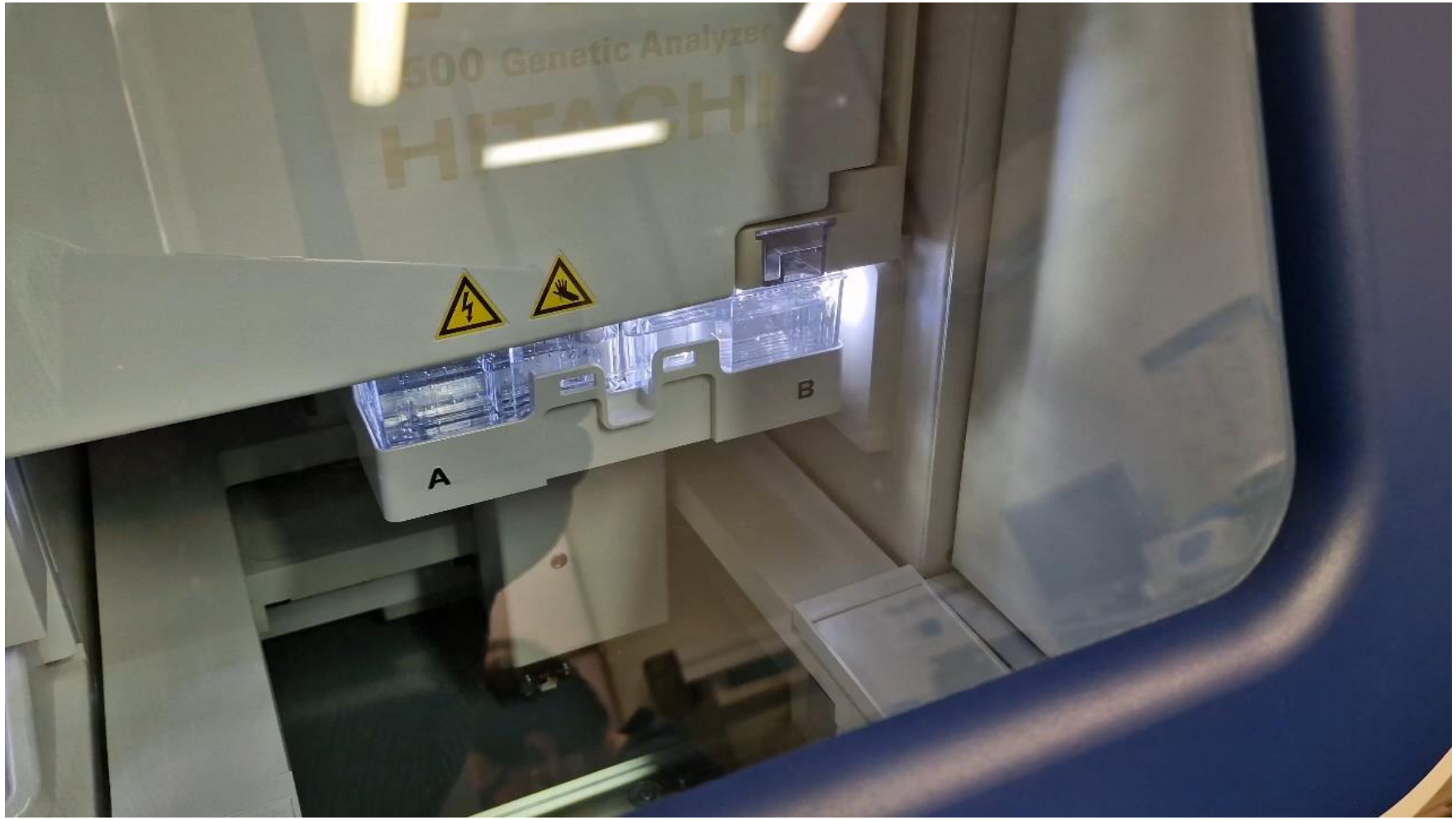
Sequencing procedure

Preparing the sequencer for loading samples



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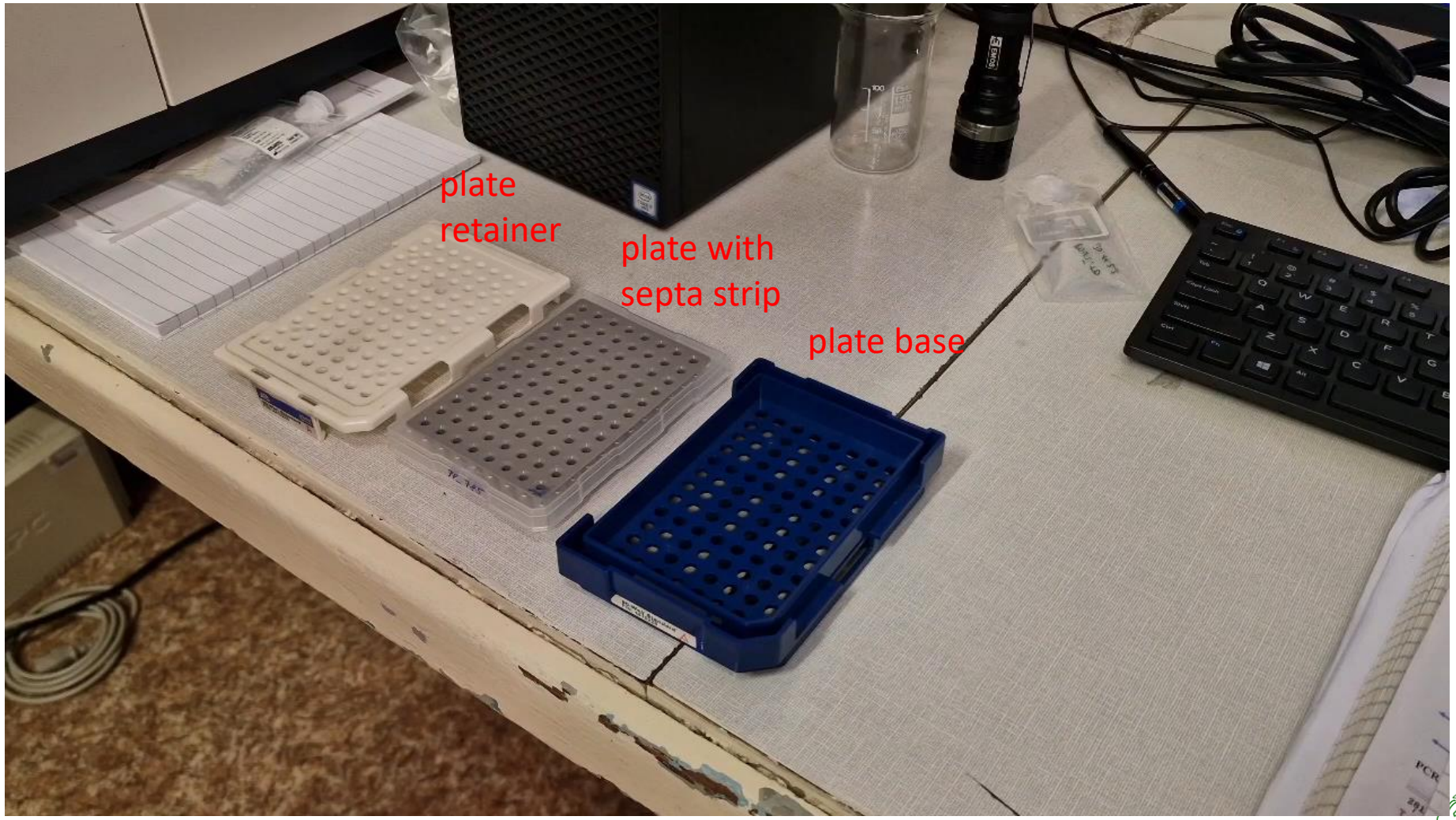
Sequencing procedure

Plate assembly preparation



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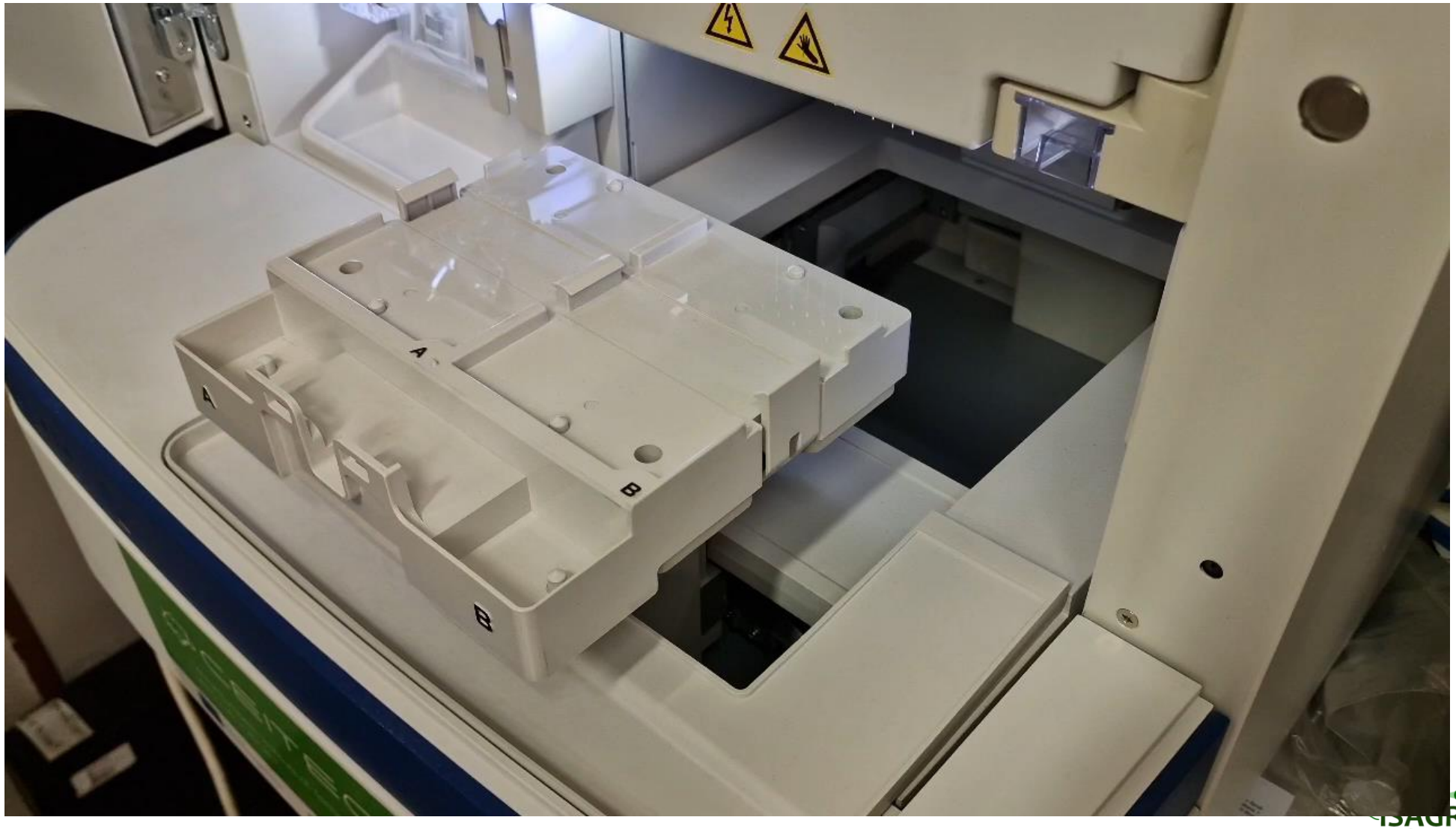
Sequencing procedure

Insertion of the sample plate



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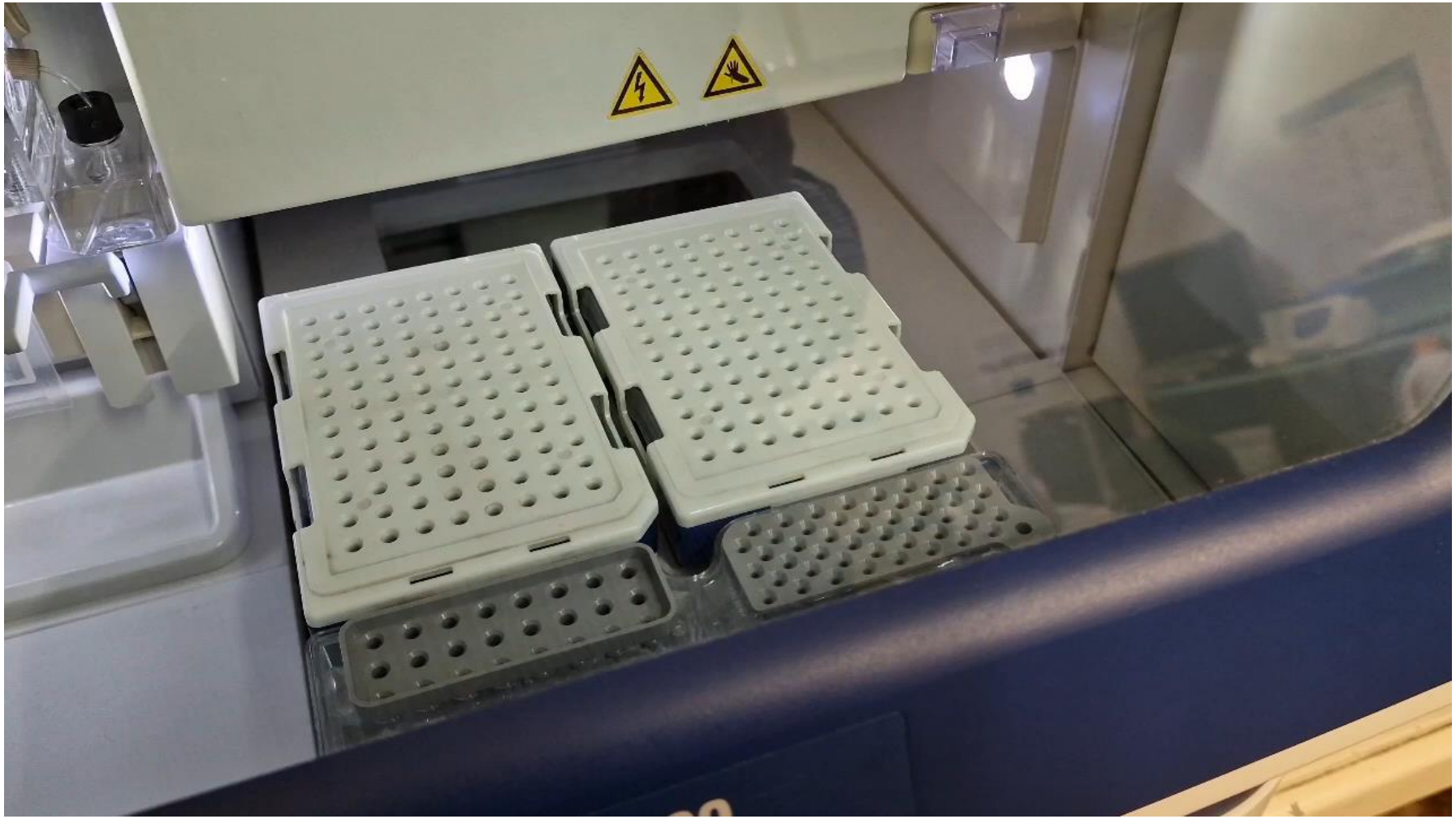
Sequencing procedure

Samples stored in both plates (2 x 96 samples)



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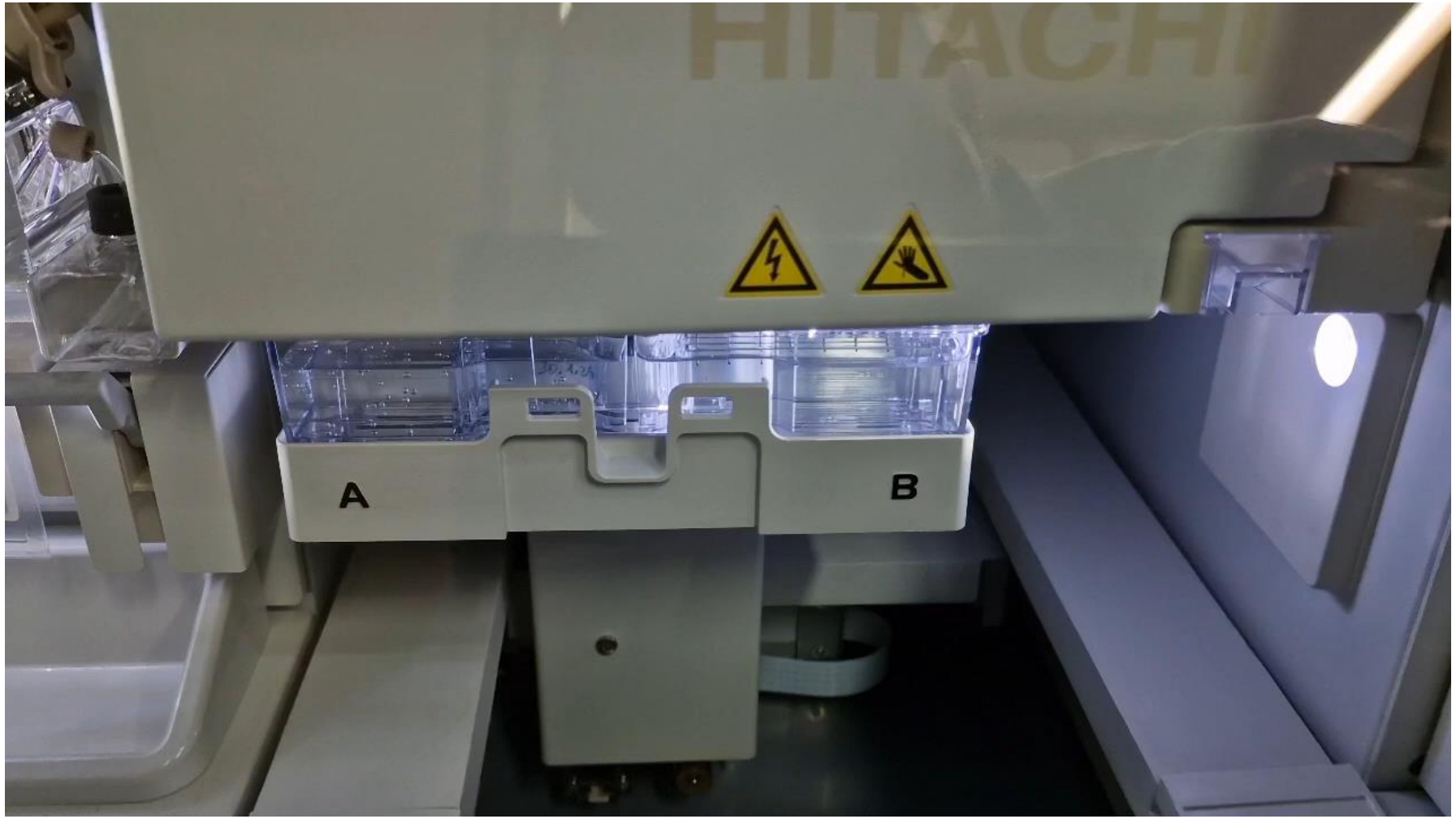
Sequencing procedure

Injection of samples into capillaries: electroinjection



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Sequencing procedure

The reading of the fluorescent signal takes place gradually as the relevant fragments travel to the sensor

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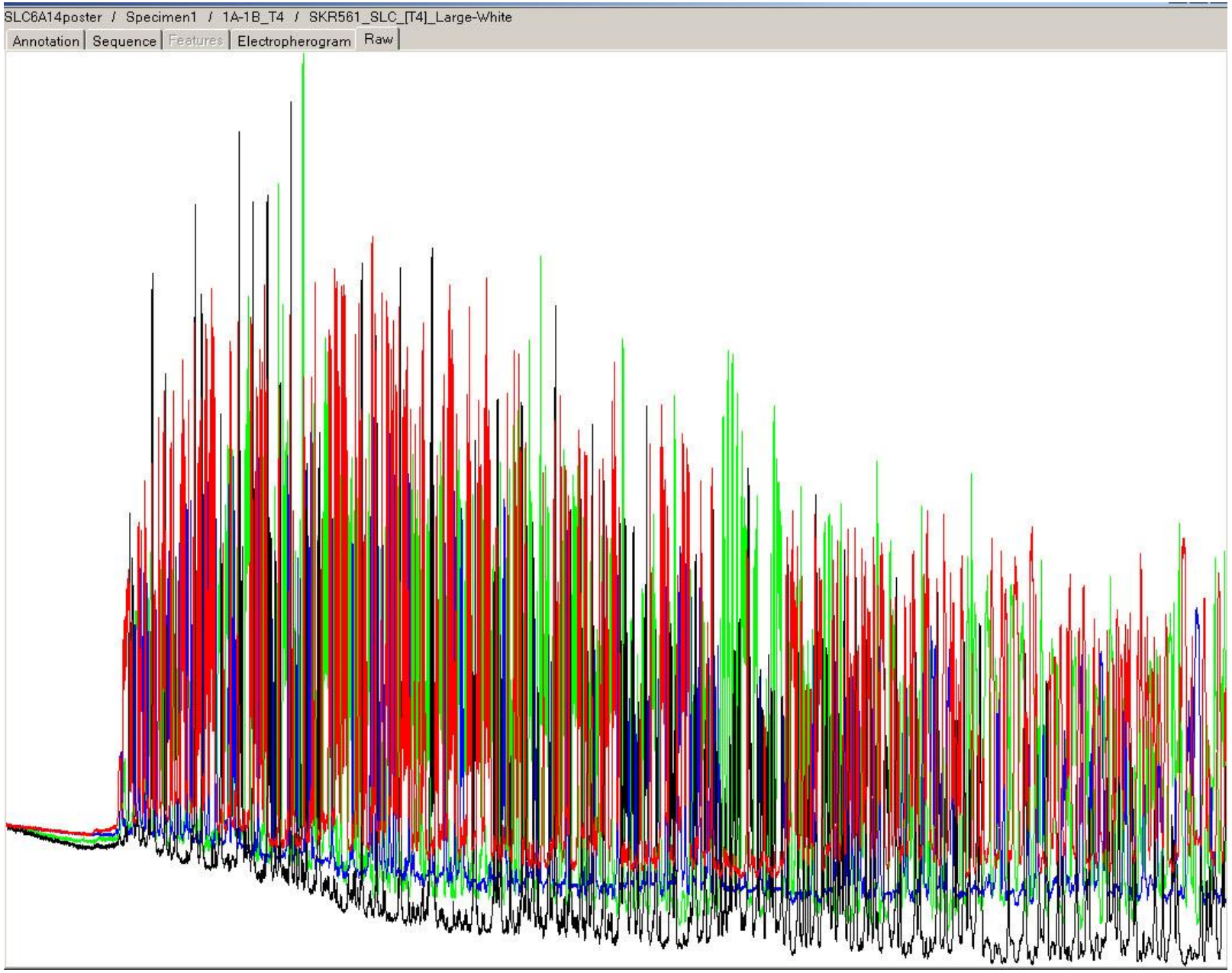
Raw data



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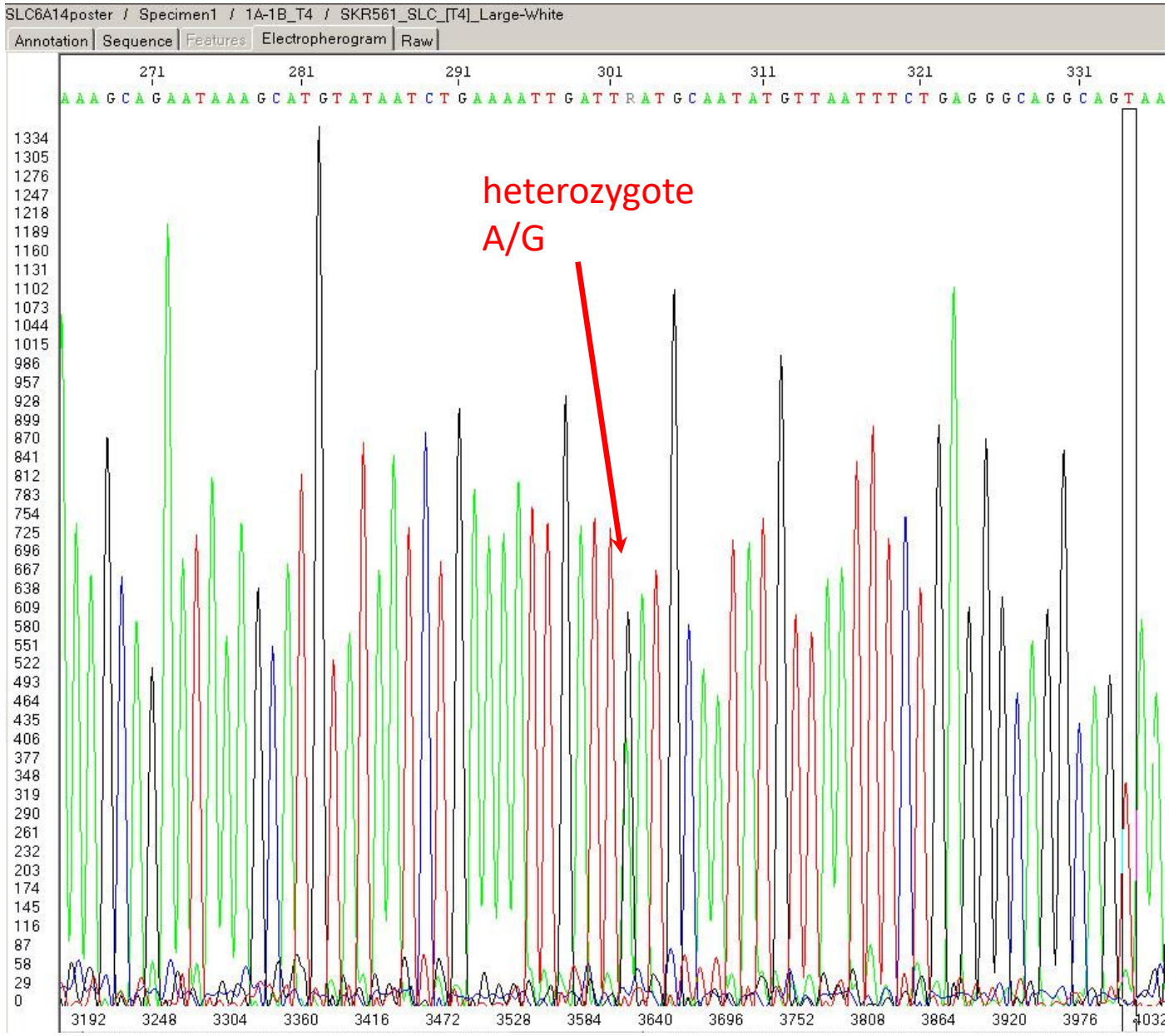
Evaluated electrophoretogram (heterozygote)



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Sequence comparison

Seqscape software: example of polymorphisms in mt DNA in honeybee

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Sequence export

Fasta format

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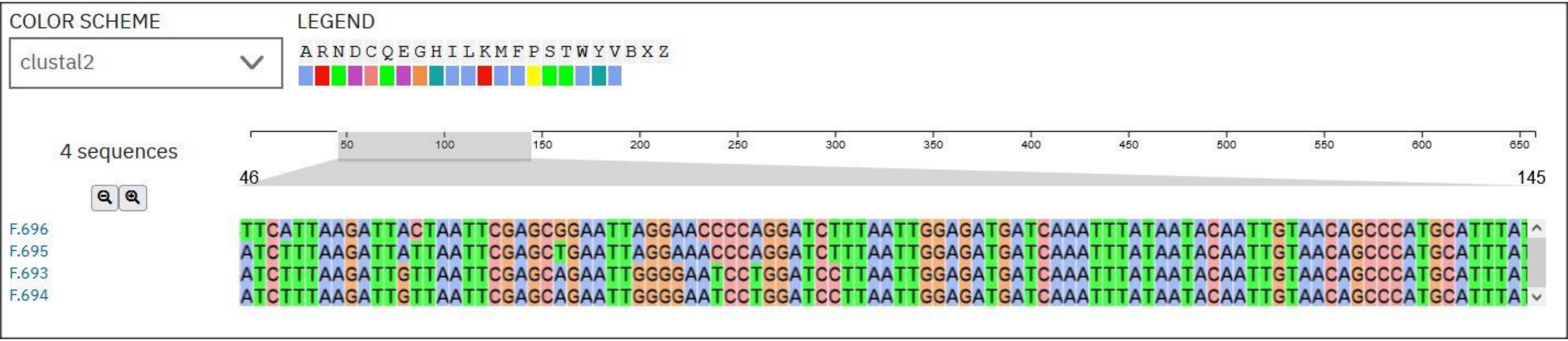
Comparison Clustal

https://www.ebi.ac.uk/jdispatcher/msa/clustalo



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Thank you for your attention!

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