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A ROBOTIZED DNA EXTRACTION PROTOCOL FROM BUCCAL SWABS FOR DATABASING PURPOSES, USING PROMEGA'S DNA IQ SYSTEM

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Our laboratory processes several thousands of buccal swab samples per year, for human DNA databasing purposes. To undertake this comission it was absolutely necessary to automate the workflow. The robot performs the protocol with a high level of confidence and uniformity, in a reliable manner, overcoming thus the –inevitable– human mistakes. The labour process consists of a DNA extraction phase, followed by DNA concentration measurement, and a PCR setup.

To set up a robotized protocol, we chose the TECAN Genesis RSP 150 liquid handling platform with the Te-MO option.

Promega's DNA IQ System proved to be the DNA extraction kit of choice, most suitable for robotization. The kit uses paramagnetic resin to capture DNA from the lysed samples, then applying a magnetic stand, DNA is immobilized on the walls of the plate during pipetting steps (wash, elution).

Because of the limited positions in the carousel of the robotic table, it was important to limit the number of pipet tip racks to the minimum.

We set up a protocol, where the DNA extraction from a whole 96-well plate is carried out using one rack of pipet tips. The pipet tips are individually separated in a special rack, and washed between the steps in distilled water.

The DNA isolation protocol is performed in 50 minutes.

Although the Promega's DNA IQ System kit is adjusted to yield a limited amount of DNA, our experience is that measuring the DNA concentration, and bringing the samples to the same concentration is inevitable in order to achieve uniform results.

We measure DNA concentration using PicoGreen DNA quantitation reagent on a SpectraMax Gemini spectrofluorimeter, as an external step of the robotized protocol.
On the base of the measured values the amount of water is calculated, which added to a given amount of DNA dilutes this to the desired concentration.

The distribution of the individual amounts of water is performed –on the base of a robotic script exported from the resulting excel file– by the RSP 150 part of the robot, which works with four pipet tips.

Finally, the PCR reaction is set up by adding the diluted DNA to the AmpFlSTR Master mix distributed in advance in a 96-well PCR plate.