A COMPARISON OF PROTOCOLS FOR THE AUTOMATED EXTRACTION OF DNA FROM REFERENCE BUCCAL SAMPLES

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Historically, the California DOJ Offender Databank program has used whole blood as its source for reference DNA. Although blood is an excellent source of genomic DNA for STR genotyping, its collection is invasive and expensive, and its handling exposes collectors and analysts to potential blood-borne pathogens. As alternatives that minimize these problems, we have developed two automated protocols for extracting reference DNA from buccal samples. These protocols, developed on a Tecan Genesis 150 Robotic Workstation, have been based on the following DNA extraction technologies: Promega's DNA IQ™ magnetic-bead protocol and Epicentre's BuccalAmp™/QuickExtract™ protocol.

Our automated DNA IQ™ protocol can be summarized as: (1) lysis of the swabs in 350uL of Lysis Buffer (with DTT) in a 96-deepwell plate format for times ranging from 1 to 16 hours and at temperatures ranging from room temperature to 70° C; (2) isolation of the lysate by plate centrifugation/filtration; (3) automated binding of DNA to the bead resin, bead-washing, and elution, all using a Tecan TeMagS magnetic-bead handler. Promega has designed the DNA IQ™ extraction technology to give nominal DNA yields of 1ng/uL, if sufficient DNA is present in the initial lysate, and if the lysate and magnetic bead resin are well mixed for efficient initial binding. With our automated protocol, we have found that the success rate for DNA extraction is very dependent upon the age of the swabs, the lysis conditions, and the efficiency of lysate-resin mixing in the initial bead-binding step. For *freshly sampled swabs* (*ca.* 1 week-old or less), we have seen uniformly successful extractions; human DNA yields ranged from 0.4-4 ng/uL, as measured by the QuantiBlot™ technique. For these freshly sampled swabs, mild lysis conditions (1 hr at room temperature) gave more uniform DNA yields (0.8-1.6 ng/uL of human DNA) than did more vigorous lysis conditions. However, for older swabs (*ca.* 1 month- to 12 month-old), *mild* lysis conditions were not successful; adequate yields (>0.3 ng/uL) of human DNA were seen for less than 15% of the samples. This success rate increased to *ca.* 50% when the lysis temperature was increased to 60° C. With a set of swabs that had been stored for approximately one year, our success rate for extraction was increased to *ca.* 80% by using stronger lysis conditions (2 hours at 70° C) and by vigorously tip-mixing the initial lysate and magnetic bead resin to increase the DNA binding efficiency.

Our Epicentre QuickExtract™ protocol can be summarized as: (1) lysis of the swabs in 250-400uL of QuickExtract™ solution for 1 hour at 68° C; (2) isolation of the lysate by plate centrifugation/filtration; (3) vortexing, then re-heating of the lysate at 65° C for 30 minutes; (4) vortexing, then re-heating of the lysate at 98° C for 30 minutes, followed by a final vortexing of the plate. The resulting plate contains extracted DNA that is PCR-ready for STR amplification, if the human DNA concentration exceeds *ca.* 0.4 ng/uL. (At lower concentrations, inhibition of AmpF*l*STR® Profiler Plus® amplification is observed.) With this protocol, using swabs that had been stored for approximately one year, we obtained human DNA yields ranging from 0.5 – 2.5 ng/uL. An STR profile was readily obtained for each swab sample. With freshly prepared swabs, the Epicentre QuickExtract™ protocol gave yields ranging from 2-5 ng/uL.