AUTOMATION

DNA Extraction Using the Tecan Freedom EVO® 200 and DNA IQ™ System

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INTRODUCTION

In our laboratory we process about 15000 samples per year using the Tecan Freedom EVO® 200, including reference samples (mainly oral swabs but also dried blood, liquid blood and even muscle tissue, nails, etc.), shed cells on clothing, differentially extracted samples, blood stains, cigarette butts, hairs, etc. Before purchasing the Tecan Freedom EVO® 200 robot, we evaluated various DNA extraction kits and decided to use the DNA IQ™ System[a] based on the results obtained and ease of automation. Here we summarize our results with the Tecan Freedom EVO® 200 robot and DNA IQ™ System.

We summarize our DNA extraction results from samples, including oral swabs, dried blood, liquid blood, shed cells on clothing, differentially extracted samples, blood stains, cigarette butts, hair and even muscle tissue and nails.

DECK CONFIGURATION

The Freedom EVO® configuration for the automated DNA IQ™ method is basic and includes a 200 cm deck with an 8-channel liquid-handling (LiHa) arm, robotic RoMa arm to move plates, plate shaker, heating block, magnetic separation device, 100 ml reservoirs, disposable tips, 96-well plates, racks for 1.5 ml tubes, plate racks and a refrigerated zone. Our system is surrounded by a methacrylate cover and has a UV lamp to minimize cross-contamination. A plate sealer with compressor, temperature regulator and plate-cooling system were ordered as options on our platform.

RESULTS

We found that performance varied depending on the sample type and was generally better with organic DNA extraction than with the DNA IQ™ System, but in most cases, DNA IQ™ performance was still sufficient for subsequent STR analysis. Table 1 summarizes our success rates in generating full profiles from samples processed using the DNA IQ™ System on our custom automated system from May 2006 to April 2007. We found that success rates depended on the sample type and type of support material, with the best results from liquid blood and blood samples on different supports, such as gauze, swabs, paper, knives or other weapons, and even soil. The samples that gave the worst results were those with shed cells collected from clothing (collars of shirts, interior of gloves, etc.). These samples contained low quantities of DNA, and in many cases the DNA was degraded.

Saliva samples on different supports generally gave good success rates: about 70% for swabs, 84% for paper and 72% for cigarettes. This contrasts with the low performance success rates from bottles, ski masks and other types of samples containing shed cells. Chewing gum gave an intermediate success rate of 55%. However, chewing gum requires specific processing, as the DNA IQ™ Lysis Buffer and gum form a gel that impedes DNA extraction and adheres to pipette tips, leading to potential contamination of other samples. For hairs, proteinase K digestion is required prior to DNA extraction. For these "difficult" samples (hair, chewing gum and shed cells), we used the same lysis buffer used for organic extractions and subsequently added the DNA IQ™ Lysis Buffer, centrifuged the samples and collected the supernatants.

Results with sperm samples varied; 52% of female fractions and 64% of male fractions were positive. We do not know if the lower efficacy is caused by the DNA extraction system or differential lysis. This is a sample type where analysis can be improved.

Editor's Note: *Promega scientists have extracted DNA from 100 mg of chewing gum successfully with no gel formation. It is possible that a gel will form when larger amounts of chewing gum are processed.*

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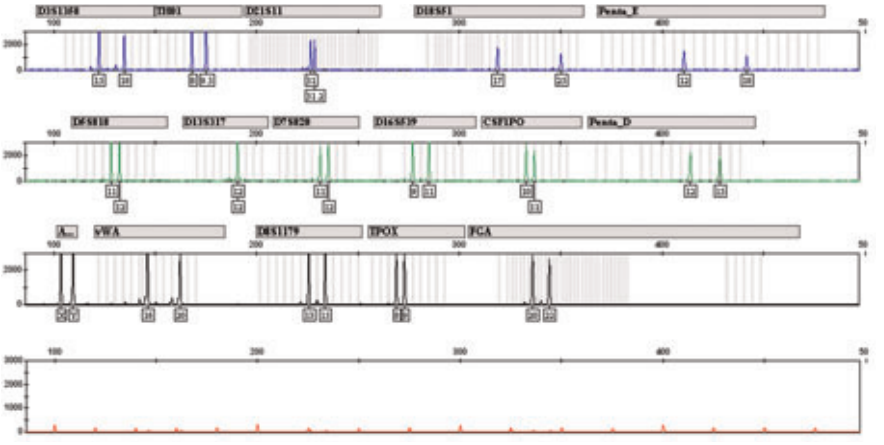

Figure 1. Representative STR analysis results. DNA was extracted from a blood swab, then amplified using the PowerPlex® 16 System and analyzed using an Applied Biosystems 3130 Genetic Analyzer.

HOW THE ROBOT IMPROVES OUR DAILY WORK

Using the Tecan Freedom EVO® to extract DNA benefits our laboratory in several ways:

- Improved working conditions. Automation allows analysts to avoid handling toxic substances such as phenol-chloroform and reduces routine work, like manually moving samples, changing pipettes, etc.
- Time savings during sample processing. The instrument helps prepare samples for quantification and dilution prior to amplification. This results in higher efficiency, by freeing up the analyst's time for calculations and manual dilutions.
- Better monitoring of sample status during DNA extraction, quantification and dilution. Automation also facilitates the incorporation of laboratory information management systems (LIMS).
- Lower risk of errors when managing samples through the use of barcodes to identify samples.

The cost savings brought about by automation are not clear, since large quantities of expensive, consumable materials are needed. Currently, we are working with a tip-saving system to reduce expenses.

FUTURE GOALS

Future goals for automation in our laboratory include:

- Automating all pre-PCR processes and capillary pre-electrophoresis. We have acquired another robot, a Freedom EVO® 75, for automated capillary pre-electrophoresis.
- Improving overall system performance, and improving performance with some specific samples.
- Implementing the same robotic equipment in laboratories at Valencia and La Coruña. In May, the laboratory of Seville began to implement automation.

Table 1. Results of DNA Extractions Using the Tecan Freedom EVO® 200.

Sample Type	Total Number of Samples	Number of Positive Samples[1]	Success Rate (%)
Reference Samples			
Buccal swab	3019	2926	97
Blood swab	173	162	94
Liquid blood	38	36	95
Muscle tissue	2	2	100
Forensic Samples			
Toothbrush	2	2	100
Chewing gum	22	12	55
Cigarettes	1494	1073	72
Saliva on bottles	49	18	37
Buccal swab	182	125	69
Saliva on paper	31	26	84
Rolled cigarettes (joints)	72	65	90
Cigars	2	1	50
Saliva on a ski mask	3	1	33
Blood on glasses	44	42	95
Blood on knives	55	46	84
Blood on scales	69	63	91
Blood on gauze	6	6	100
Blood swabs	3080	2733	89
Blood cards	192	151	79
Blood on clothes	1546	1284	83
Blood in soil	1	1	100
Differential extraction (female fraction)[2]	102	53	52
Differential extraction (male fraction)[2]	185	118	64
Hair	15	12	80
Biological remains	2304	742	32

[1]Samples were considered positive if a genetic profile was obtained following DNA extraction and STR analysis.

[2]Differentially extracted samples were processed using the Differex™ System.