Flocked Swabs, a New State of the Art for Forensic DNA applications

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Copan Innovations

Company workshop

Forensica 2008

April 25th 2008, Prague, Czech republic

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History of DNA sampling for forensic purposes

Lynda Mann †1983
Dawn Ashworth †1986

15-years old girls raped and murdered

Colin Pitchfork

1st mass screening

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General requirements for reference DNA sampling

- Easy to use
- Sample well preserved during the transport
- Compatible with current DNA techniques
- Non-invasive
- Non-intimate

Buccal swabs

Leriche A., Vaněk D., Schmitter H. at al. (1998) Final report of the INTERPOL European Working Party on DNA Profiling. Proceedings from the Second European Symposium on Human Identification 48-54, Promega Corporation

Buccal swabs

Sufficient amount of DNA for down-stream DNA identification applications

General requirements for crime-scene DNA sampling

- Easy use, efficient sampling
- Sample well preserved during the transport
- Security during the transport
- Compatible with current forensic genetics techniques
- Maximum DNA recovery
- Human DNA-free, PCR inhibitor-free, DNase-free

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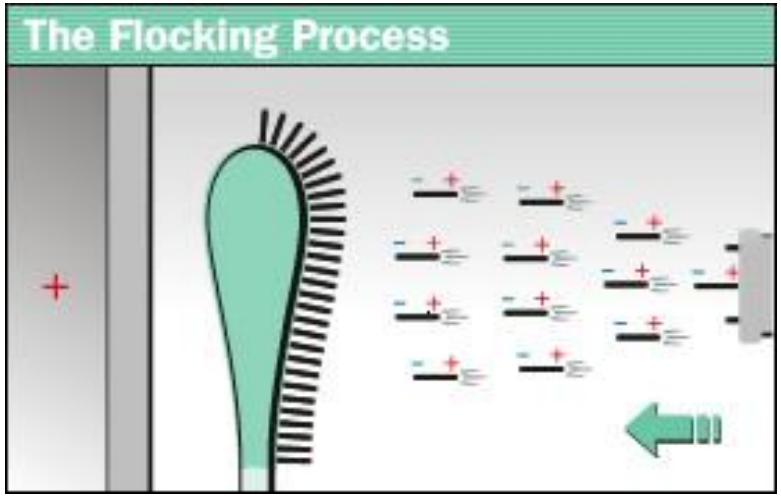
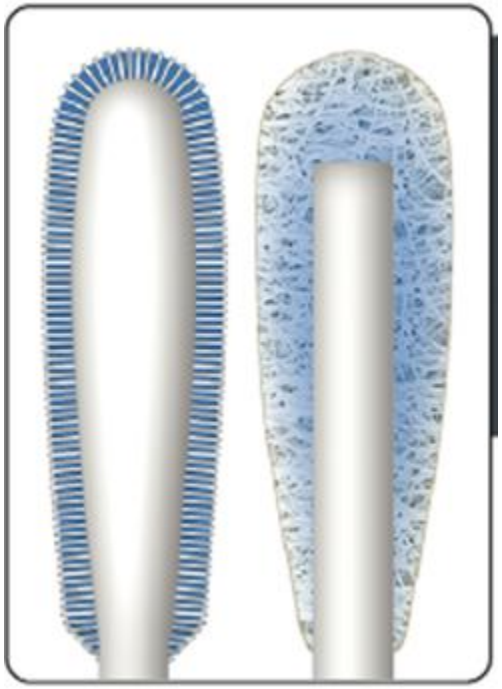
Flocked Swabs, a New State of the Art for Forensic DNA applications

New and unique nylon **flocked swab** technology for DNA sample collection





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Flocked swabs

Ready to use

4n6 DNA swabs in 3 different formats

-**3520CS01 4n6** flock swab with breaking point in sterile paper-plastic pouch, for forensic use.

- **3520CA 4n6** flock swab with breaking point and 2ml Eppendorf DNA LoBind tube, in sterile paper-plastic pouch, for forensic use - laboratory sampling.

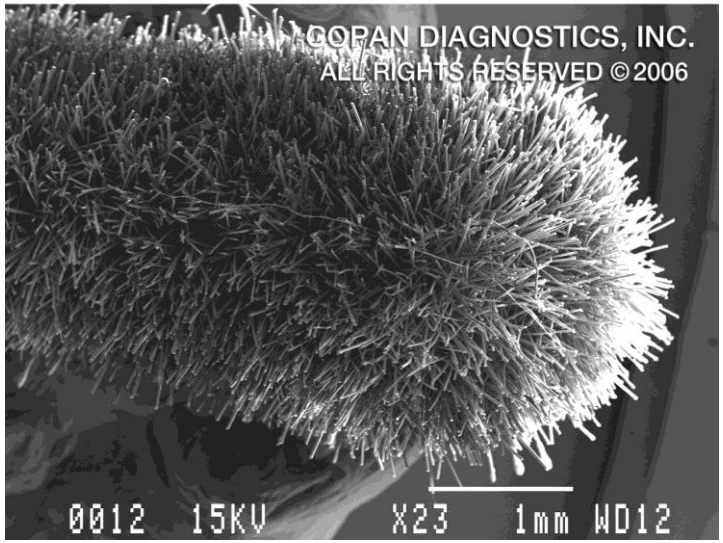
- **3520CF 4n6** flock swab with breaking point and 2ml Eppendorf DNA LoBind tube with evaporation duct, in sterile paper-plastic pouch, for forensic use - crime scene sampling.

4n6 DNA swabs

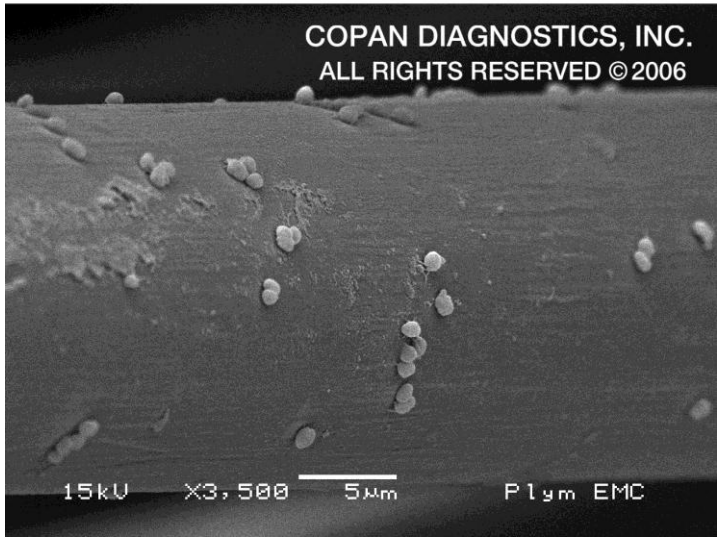
Unique design of flocked swabs

Rapid absorption: sprayed-on fibers arranged in a uniform perpendicular fashion results in tremendous hydraulics which rapidly absorbs the sample

Electron Microscope photograph of a nylon flocked swab.



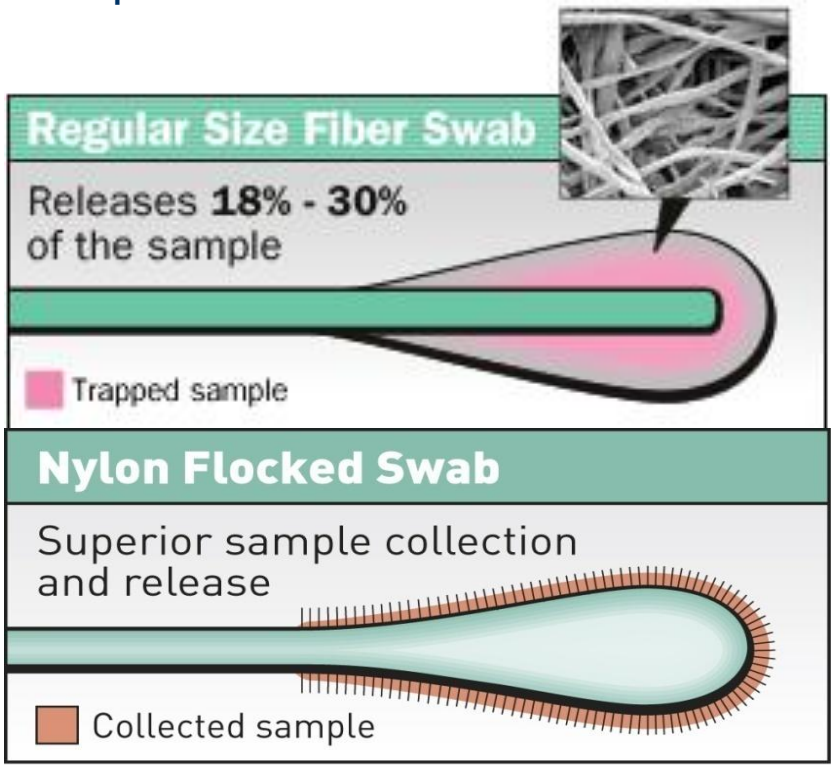
Neisseria gonorrhoeae sitting on the surface of a single strand of nylon.



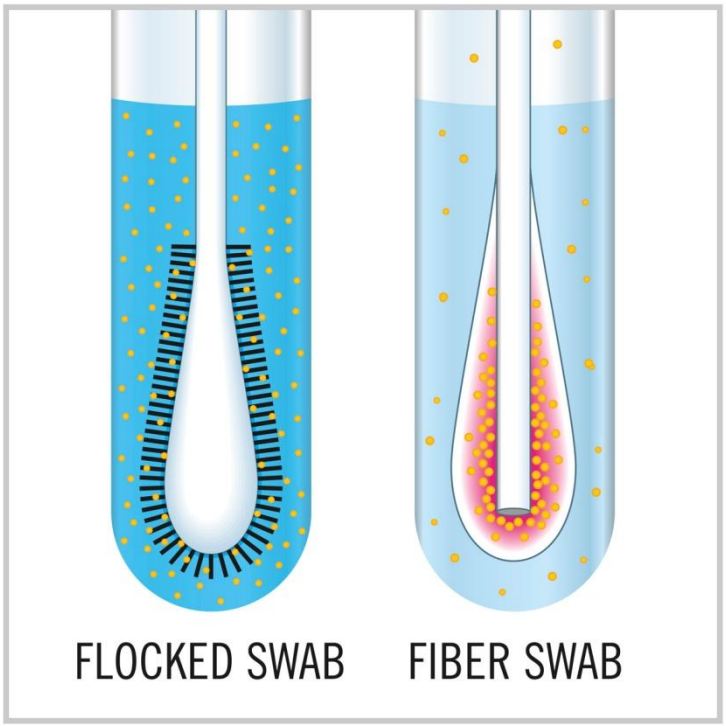
4n6 DNA swabs

Unique design of flocked swabs

Superior sample release: open fibre structure means no sample entrapment as occurs with traditional mattress wound swabs.



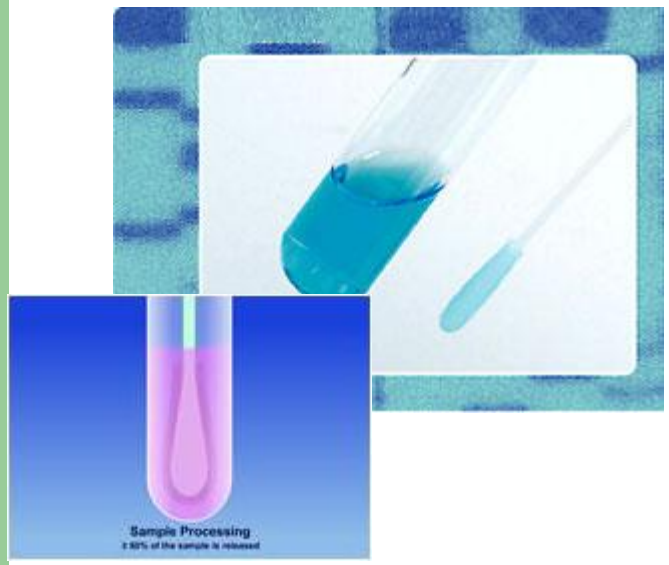
> 80% of the sample analyte released*



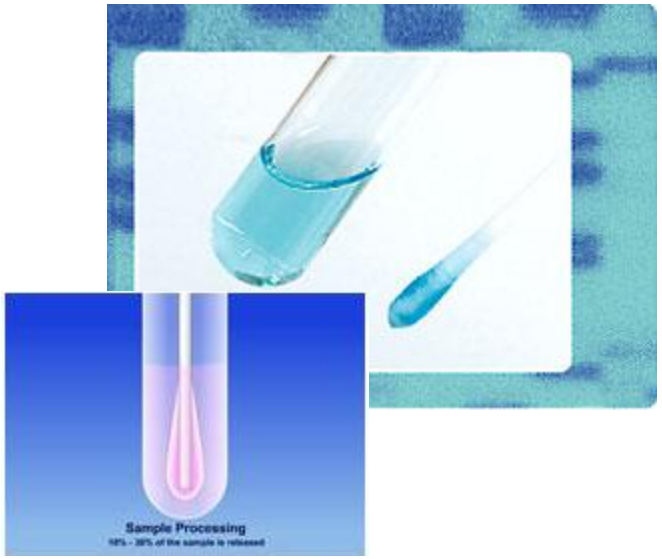
What You Swab Is What You Get

Increased assay sensitivity: 4n6 DNA Swabs are proven to elute >90% of the original sample rapidly and easily resulting in improved assay sensitivity.

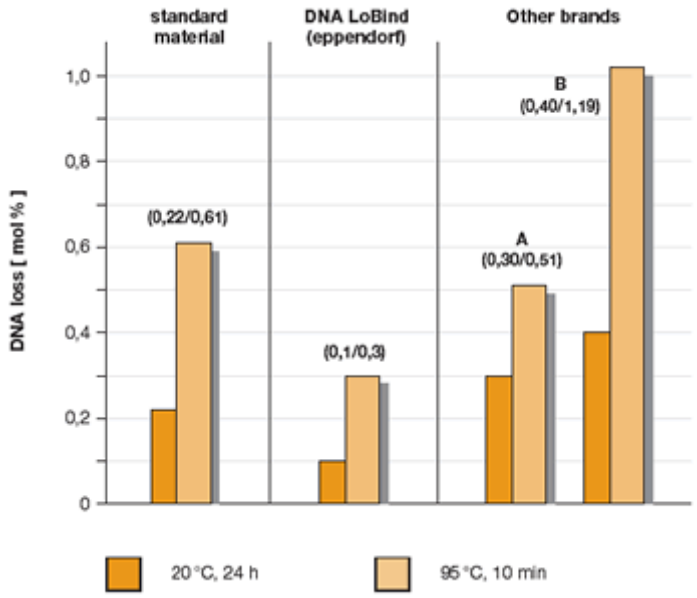
Flocked swab



Traditional fiber swab



Meeting criteria for forensic use



1. DNase-free
2. RNase-free
3. Human DNA-free
4. PCR-inhibitor free
5. *DNA profiles of workers*

+ DNA LoBind system (Eppendorf)

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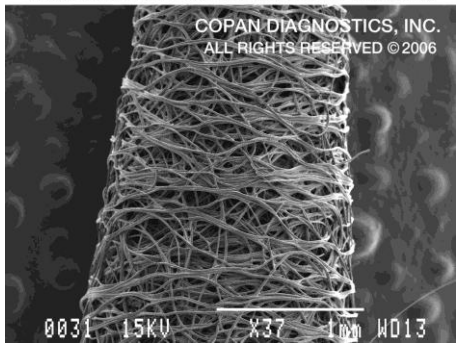
Certified for forensic use

regular swab

Cotton, ryon, fiber winded

Electron Microscope photograph of traditional fiber winded swab.



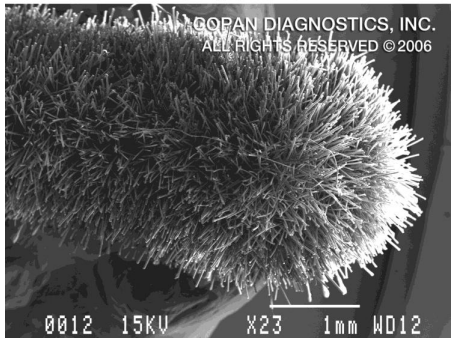
sterile

flocked swabs

Nylon technology

DNase-free
RNase-free
Human DNA-free
PCR-inhibitor free

Electron Microscope photograph of a nylon flocked swab.



Feel the difference

4n6 DNA swabs

Ready for automation

Minimal fiber release during extraction =
No problems with liquid handling

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The core features of the DNA-ID sampling kit for reference sample

- Efficient swabbing
- Efficient sample release
- Contamination controlled manufacturing (inc. DNA profiles of workers)
- Automation friendly format
- PCR inhibitor free certificate
- Possible bar-coding (1-2 dimensional)
- Security during transport (safety seal)
- Stable during transport in unfriendly environmental conditions (humidity, temperature)
- Competitive price

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Spontaneous release of cells from the 4N6 flocked swabs during 2 minutes incubation in different extraction buffers

Assay description:

- DNA extraction from mouth swabs (4N6 flocked swabs, Copan)
- DNA IQ (Promega), ChargeSwitch (Invitrogen), DNA Micro kit (Qiagen) extraction chemistry

Serie a) all swabs incubates 2 minutes in the respective lysis buffer, swab removed (A), extraction continued with supernatant

Serie b) Swab from (A) placed to a new tube, new extraction buffer added, and samples processed accordingly to the extraction protocol (B) + spin baskets

All extractions were performed accordingly to the standard protocol for the respective chemistry qRT PCR - SYBR/ALU – Mastercycler ep *realplex* (Eppendorf)

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Sample release efficiency

Assay	Ct SYBR	ng DNA/ ul
Copan_DNA IQ_1a	9,43	0,42789738
Copan_DNA IQ_1b	9,17	0,505024731
Copan_DNA IQ_2a	9,52	0,404041421
Copan_DNA IQ_2b	9,67	0,367200273
Copan_DNA IQ_3a	10,44	0,224777135
Copan_DNA IQ_3b	9,86	0,325317426
Copan_DNA IQ_4a	10,36	0,236536233
Copan_DNA IQ_4b	10,43	0,22621444

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Sample release efficiency

Assay	Ct SYBR	ng DNA/ ul
Copan_ChSw_1a	9,68	0,364867181
Copan_ChSw_1b	9,47	0,417125611
Copan_ChSw_2a	10,46	0,221929863
Copan_ChSw_2b	10,41	0,229116682
Copan_ChSw_3a	16,44	0,004907229
Copan_ChSw_3b	18,72	0,00114733
Copan_ChSw_4a	12,06	0,080039591
Copan_ChSw_4b	16,57	0,004516997

Sample release efficiency

Assay	Ct SYBR	ng DNA/ ul
Copan_Qiagen_1a	3,81	15,38381714
Copan_Qiagen_1b	5,01	7,159471189
Copan_Qiagen_2a	3,39	20,1061175
Copan_Qiagen_2b	4,28	11,40142243
Copan_Qiagen_3a	4,36	10,83461522
Copan_Qiagen_3b	4,53	9,721968533
Copan_Qiagen_4a	4,11	12,70627698
Copan_Qiagen_4b	4,62	9,179953341

Sample release efficiency –summary

Spontaneous release of cells from the 4N6 flocked swabs during 2 minutes incubation in different extraction buffers

- Approximately **1/2 of the biological material** (DNA) captured on the flocked swab **is released during 2 minutes** of incubation.
- It is not necessary to perform the centrifugation step, the preparation of samples is faster.
- 2 minute incubation of flocked swabs without centrifugation in the spin baskets is used as a standard operation procedure for DNA extraction from the mouth swabs.
- **The described process of extraction provides sufficient amount of DNA necessary for all down stream ID applications.**

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Compatibility with extraction chemistries

- QiaAmp (Qiagen)
- DNA IQ (Promega)
- ChargeSwitch (Invitrogen)
- Chelex
- Phenol/Chlorophorm
- …….

Compatibility with qPRC and PCR chemistries

- Quantifiler (Applied Biosystems)
- SYBR green (Bio-Rad)
- Identifiler (Applied Biosystems)
- Y-filer (Applied Biosystems)
- PowerPlex Y(Promega)
- PowerPlex 16(Promega)

4N6 flocked swabs related forensic applications

- 4N6 XC test (environmental control)
- Automated DNA extraction on Eppendorf epMotion 5075 LH
  - DNA IQ (Promega Corp.)
  - ChargeSwitch (Invitrogen)

- Numerous applications is medical, food and veterinary sector (flocked swab studies)

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4N6 XC test

Novel testing system for monitoring of background contamination in DNA laboratories

Saskova L.(1), Giambra A.(2), Pospisek M.(3), and Vanek D.(1)

(1) Forensic DNA service, Prague, Czech Republic; (2) Copan Innovation Group, Italy; (3) Faculty of Science, Charles University, Prague, Czech Republic

Introduction

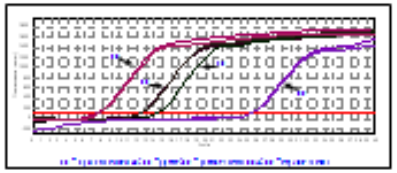
According to ISO 17025 requirements DNA laboratories shall ensure that environmental conditions do not invalidate the results or adversely affect the required quality of DNA typing. All areas in which DNA is worked with must be regularly and systematically monitored both to check for DNA contamination and, when detected, to confirm its removal after appropriate decontamination. Presented 4N6 XC–test system is a new, faster and less expensive approach of monitoring the degree of contamination in DNA laboratories.

Testing process

Sample collection → DNA isolation → DNA quantification + STR typing in one step → Capillary electrophoresis

1. Real-time PCR quantification and STR typing assay amplify three human nuclear DNA target sequences and the sex identification amelogenin marker to assess DNA contamination in laboratories (Figure 1, Table 1).

Fig. 1. Amplification curves for selected samples. All samples were assayed in duplicates.



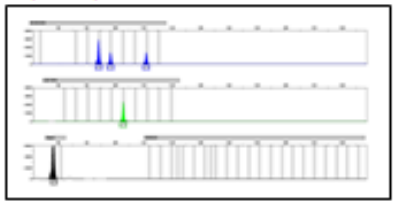
Tab. 1. Summary of qPCR quantification results.

Source of DNA*	Cт**	cDNA (ng/µl)
Epilation 5075 LH	14.36	0.008
Pipette (post-PCR room)	12.43	0.036
Positive control DNA	7.53	1.000
Negative control	25.26	---

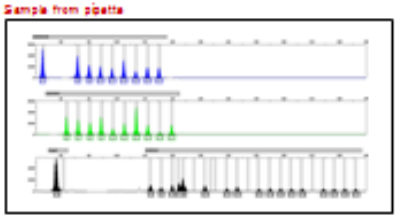
2. Visualization of amplified STR alleles and identification of contamination source (Figure 2).

Fig. 2. Comparison of results obtained from different contaminated surfaces and control samples. Peak labels are allele calls; peak heights are in relative fluorescence units.

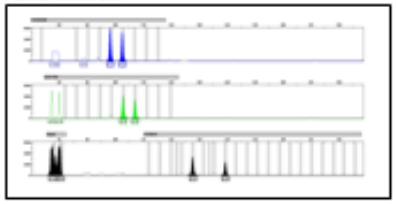
Sample from Epilation 5075 LH



Sample from pipette



Positive control DNA



Negative control

Methods

Sample collection
Swabs were taken from surfaces of an epilation 5075LH, automated liquid handling workstation; PCR box; pipettes from isolation and post-PCR rooms, and ABI 310 Genetic analyzer. The total surface areas (100 cm²) were sampled with 4N6 DNA Swabs (Copan).

DNA isolation
DNA from samples was extracted via standard ChargeSwitch procedure (Invitrogen) using epilation 5075 LH automated liquid handling workstation (Eppendorf) (see Poster FG10).

Real-time PCR quantification and STR typing assay design
PCR reactions were carried out in MastarCycler ep realplex S instrument (Eppendorf). DNA from samples was quantified and labelled in one reaction using the Sybr Green Supermix system (Bio-Rad) and fluorescence labelled primers of selected STR markers (Table 2).

Tab. 2. STR markers definition.

STR locus	Dye label	STR size (pb)
D18S51	Fam	81-121
CSF1PO	Joe	88-129
D18S51	Tamra	113-313
Amelogenin	Tamra	77-82

Capillary electrophoresis
Fluorescently labelled STR alleles were diluted immediately and separated on ABI 310 Genetic analyzer. Samples were injected – 3 kV injections– for 5 s (tested samples) or 2 s (positive control DNA). Data were analyzed using GeneMapper ID (V 3.2) software with a 150 RFU analysis treshold.

Conclusions

4N6 XC testing system is a new valuable tool for fast and inexpensive monitoring of one of the critical factors in DNA laboratories – cross-contamination – and even identification of the contamination source. By changing the primers the XC-test can be used for monitoring of non-human DNA too.

Acknowledgements
This work was supported in part by Research Grant No. 1H-PK/27 from the Ministry of Industry and Trade, Czech Republic.

Automated DNA extraction on Eppendorf epMotion 5075 LH

Applications

Note 141 | February 2007

Automated DNA Extraction of forensic samples with the DNA IQ™ System on the epMotion® 5075 LH

Jana Hajkova[1], Daniel Wehrhahn[2], Martin Pospisek[3], Daniel Vanek[1]

Abstract

Forensic DNA laboratories are experiencing rapidly growing demand to process large numbers of evidence samples. In an effort to meet the rising needs for DNA analysis, liquid handling workstations are utilized for the automation of the liquid handling tasks. The epMotion 5075 LH (Eppendorf AG) is a flexible and extremely accurate automated pipetting system capable to extract DNA from forensic casework and reference samples. This study demonstrates that the epMotion 5075 LH used in combination with the DNA IQ System (Promega Corporation) is versatile enough to accommodate the whole spectra of samples encountered by a crime laboratory. The performance of the epMotion LH 5075/DNA IQ System with regard to DNA yields and potential cross-contamination for different sample types was evaluated.

Introduction

In order to automate DNA extraction it is necessary to use a suitable purification method. Organic extraction (phenol/chloroform) not only utilizes hazardous chemicals but also requires multiple centrifugation steps. Chelex extraction is a rapid and relatively cheap method but it can leave PCR inhibitors in the final extract. Purification on silica matrices seems to be the best candidate for automation as the extraction process does not require centrifugation, gives high yield and possible PCR inhibitors are efficiently removed (Greenspoon, 2004).

Promega's DNA IQ system has been chosen for the automation on the Eppendorf epMotion platform due to its ability to rapidly purify small quantities of DNA. It becomes more efficient with samples containing small amounts of DNA (less than 20 pg). The DNA IQ system is widely used for the manual extraction of a broad range of forensic samples, including buccal swabs, blood stains, cigarette butts, sexual assault samples and various types of tissues (Promega Technical Bulletin #TB295).

The procedure for automated DNA extraction using DNA IQ system described below is based on the standard Promega extraction protocol and was adapted for the Eppendorf epMotion 5075 LH automated liquid handling system. Buccal swabs and swabs of dry blood stains were taken using novel swabs introduced by MicroRheologics (Copan Innovation Group). Those swabs were specially designed and certified for forensic use. Magnetic resin employed by the DNA IQ System has a defined DNA capacity in the presence of excess DNA and does only bind a specific amount of DNA. The resulting DNA extracts from both manual and automated extractions were compared using a human specific qPCR. A contamination study revealed no signs of well-to-well contamination during the automated process (extraction blank and checkerboard tests).

Materials and Methods

Consumables

- 4N6 DNA Swab, 4N6 DNA Kit (MicroRheologics, Copan Innovation Group)
- 1.5 ml Eppendorf DNA LoBind tubes
- 2.0 ml Eppendorf DNA LoBind tubes
- Promega DNA IQ System
- Promega DNA IQ Spin baskets
- Absolute ethanol, isopropyl alcohol, DTT

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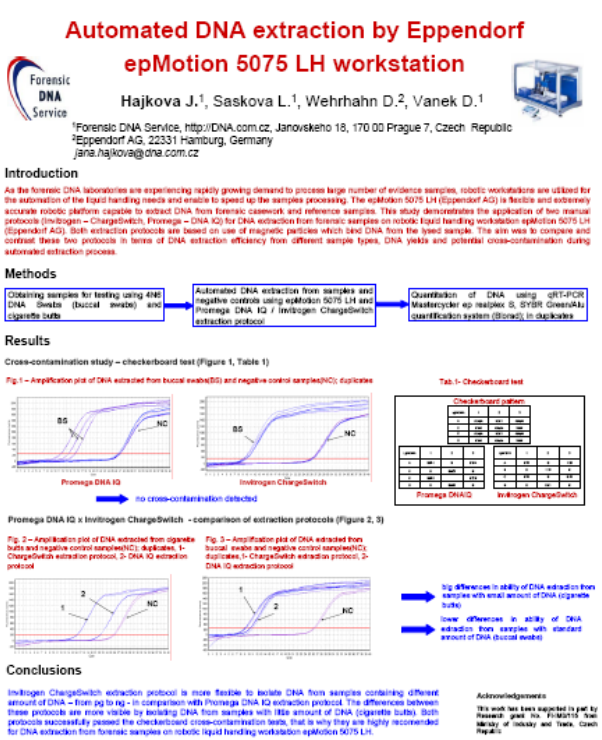
2) RNDr. Martin Pospisek, Geologica, http://www.biologicals.cz, Sramkova 316, 251 01 Ricany, Czech Republic
3) Dr. Daniel Wehrhahn, Eppendorf AG, Hamburg, Germany

eppendorf



Automated DNA extraction by Eppendorf epMotion 5075 LH workstation

Forensic DNA Service

Hajkova J.[1], Saskova L.[1], Wehrhahn D.[2], Vanek D.[1]

[1]Forensic DNA Service, http://DNA.com.cz, Janovskeho 18, 170 00 Prague 7, Czech Republic
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Introduction

As the forensic DNA laboratories are experiencing rapidly growing demand to process large number of evidence samples, robotic workstations are utilized for the automation of the liquid handling needs and enable to speed up the samples processing. The epMotion 5075 LH (Eppendorf AG) is flexible and extremely accurate robotic platform capable to extract DNA from forensic casework and reference samples. This study demonstrates the application of two manual protocols (Invitrogen – ChargeSwitch, Promega – DNA IQ) for DNA extraction from forensic samples on robotic liquid handling workstation epMotion 5075 LH (Eppendorf AG). Both extraction protocols are based on use of magnetic particles which bind DNA from the lysed sample. The aim was to compare and contrast these two protocols in terms of DNA extraction efficiency from different sample types, DNA yields and potential cross-contamination during automated extraction process.

Methods

Obtaining samples for testing using 4N6 DNA Swabs (buccal swabs) and cigarette butts → Automated DNA extraction from samples and negative controls using epMotion 5075 LH and Promega DNA IQ / Invitrogen ChargeSwitch extraction protocol → Quantification of DNA using qRT-PCR Mastercycler ep realplex S, SYBR GreenAlu quantification system (Bonaldi) in duplicates

Results

Cross-contamination study – checkerboard test (Figure 1, Table 1)

Fig.1 – Amplification plot of DNA extracted from buccal swabs(BS) and negative control samples(NC); duplicates

Tab.1- Checkerboard test

Checkerboard pattern

Promega DNA IQ

Invitrogen ChargeSwitch

no cross-contamination detected

Promega DNA IQ x Invitrogen ChargeSwitch - comparison of extraction protocols (Figure 2, 3)

Fig. 2 – Amplification plot of DNA extracted from cigarette butts and negative control samples(NC); duplicates. 1- ChargeSwitch extraction protocol, 2- DNA IQ extraction protocol

Fig. 3 – Amplification plot of DNA extracted from buccal swabs and negative control samples(NC); duplicates.1- ChargeSwitch extraction protocol, 2- DNA IQ extraction protocol

big differences in ability of DNA extraction from samples with small amount of DNA (cigarette butts)

lower differences in ability of DNA extraction from samples with standard amount of DNA (buccal swabs)

Conclusions

Invitrogen ChargeSwitch extraction protocol is more flexible to isolate DNA from samples containing different amount of DNA – from pg to ng. - In comparison with Promega DNA IQ extraction protocol. The differences between these protocols are more visible by isolating DNA from samples with little amount of DNA (cigarette butts). Both protocols successfully passed the checkerboard cross-contamination tests, that is why they are highly recomended for DNA extraction from forensic samples on robotic liquid handling workstation epMotion 5075 LH.

Acknowledgements

This work has been supported in part by Research grant No. 1H-MS115 from Ministry of Industry and Trade, Czech Republic

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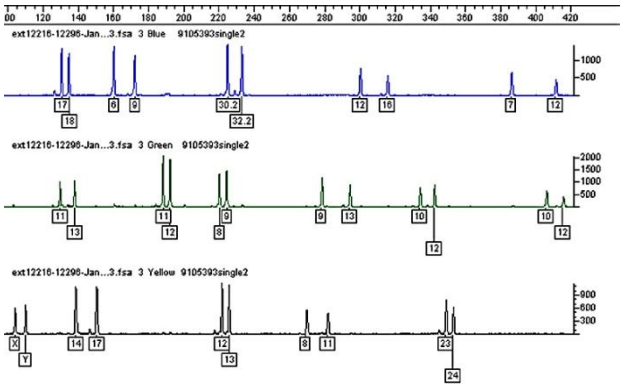
FLOCKED SWAB STUDIES

Flocked Swab Studies - 2008			
	Comparison of Nylon Swabs versus Cotton Swabs for the Diagnosis of Herpes Simplex Virus	Paula Väre, Klaus Hedman, Maija Lappalainen	ESCV 2008 Clinical Virology Annual Meeting Saariselkä, Lapland, Finland 12-15 March 2008
	Comparison of different sampling types for the detection of rhinovirus infections using quantitative RT-PCR	M. Waris, V. Peltola, R. Österback, T. Vuorinen, O. Ruuskanen	ESCV 2008 Clinical Virology Annual Meeting Saariselkä, Lapland, Finland 12-15 March 2008
	Sampling of Human Papilloma Viruses and Chlamydia trachomatis: Novel Flocked Swabs Increase Detection Rates significantly.	Thomas Krech, Santina Castriciano, and Max Chernesky	ESCV 2008 Clinical Virology Annual Meeting Saariselkä, Lapland, Finland 12-15 March 2008
	Orthomyxo-, paramyxo- and flavivirus infections in wild waterfowl in Finland	Erika Lindh, Anita Huovilainen, Osmo Ratti, Christine Ek-Kommonen, Tarja Sironen, Eili Huhtamo, Hannu Poysa, Antti Vaheri, Olli Vapalahti	Virology Journal 2008 28 February 2008
	Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children	K.H. Chan, J.S.M. Peiris, W. Lim, J.M. Nicholls, S.S. Chiu	Journal of Clinical Virology (2008) 5 December 2007
Flocked Swab Studies - 2007			
	Comparison of Three Nasal Collection Specimen Methods for the Detection of	Paul Walsh, Christina Lim Overmyer, Larisa Gofman, Lisa DeSalvia, Diana	Association of Molecular Pathlogy (AMP)

Visit: www.copanswabs.com/studies/flocked.php

DNA sampling of new-borns

- Breast milk contains mothers cells
- Flocked swabs efficiently rub-off epithelial cells
- No mother´s DNA profile in new-borns buccal swab



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Different flocked swab shapes

Vaginal swabs, finger-nail scrapes, etc..

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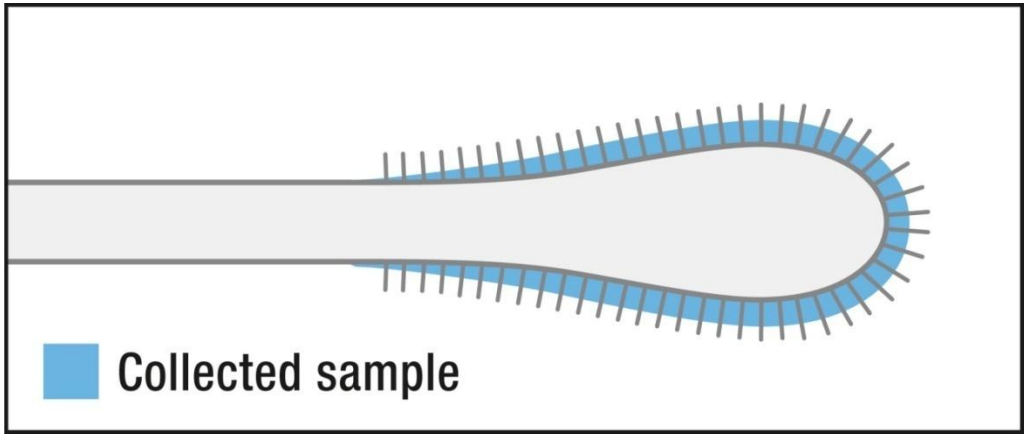
Current R&D projects with flocked swabs

- Twin head swabs for reference sampling
- Super-robust swabs for unfriendly environment
- Crime scene wetting buffer
- Different swab shapes

ISO 9001 CERTIFIED CE 0124 DEKRA R&D driven by customers needs

Crime scene wetting buffer

- Limited liquid intake
- Speeds up drying
- Stops microbial growth
- Higher efficiency for biolipids (greasy stains)



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Inter-laboratory comparative study

- Compare the suitability of your currently used swabs with 4N6 **flocked swabs**
- Sign-in now and get free samples of flocked swabs for the study

COPAN
OUR STRENGTH IS YOURS

Conclusion -  flocked swabs

- Novel design that fits the forensic needs
  - DNA-free, PCR inhibitor-free, DNase-free
  - ISO 17025 requirements for sampling
  - Friendly format
  - Swabbing efficiency, maximum DNA recovery
- Numerous applications

Visit the company workshop, guess and win a special prize

QUIZ:

How many nylon fibers are on average sprayed on the flocked swab?

Name:

Your qualified estimate: