Novel testing system for monitoring of background contamination in DNA laboratories

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Introduction

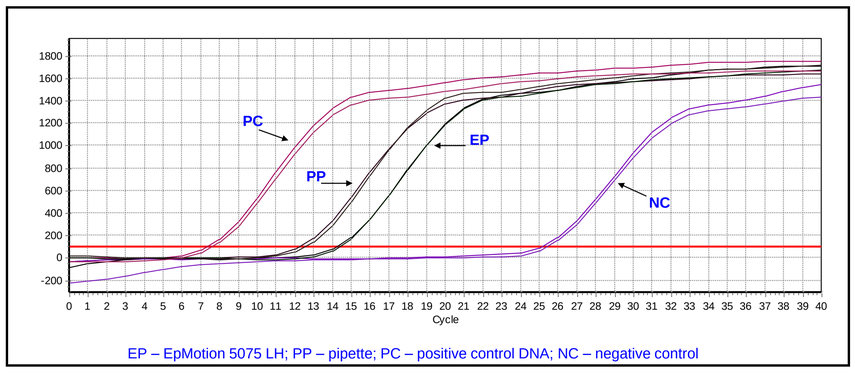
According to ISO 17025 requirements DNA laboratories shall ensure that environmental conditions do not invalidate the results or adversely affect the reguired quality of DNA typing. All areas in which DNA is worked with must be regularly and systematically monitored both to check for DNA contamination 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.



EP – EpMotion 5075 LH; PP – pipette; PC – positive control DNA; NC – negative control

Tab. 1. Summary of qPCR quantification results.

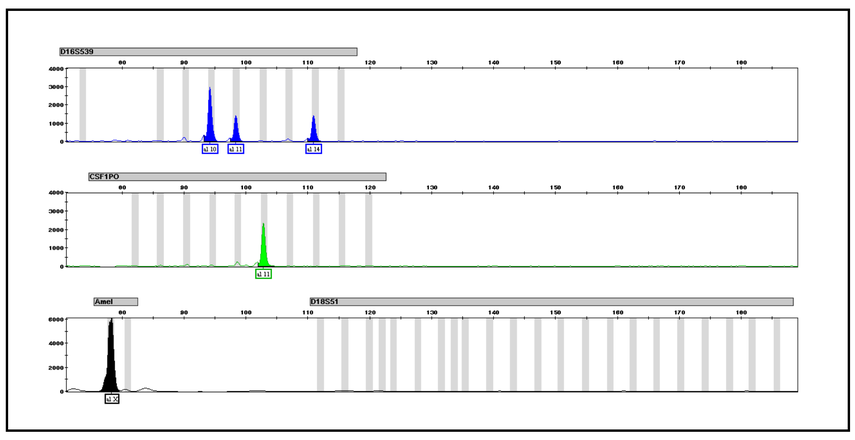
Source of DNA*	C_T**	cDNA (ng/μl)
EpMotion 5075 LH	14.36	0.008
Pipette (post-PCR room)	12.43	0.036
Positive control DNA	7.53	1.000
Negative control	25.26	---

* see Methods
* * Cycle treshold

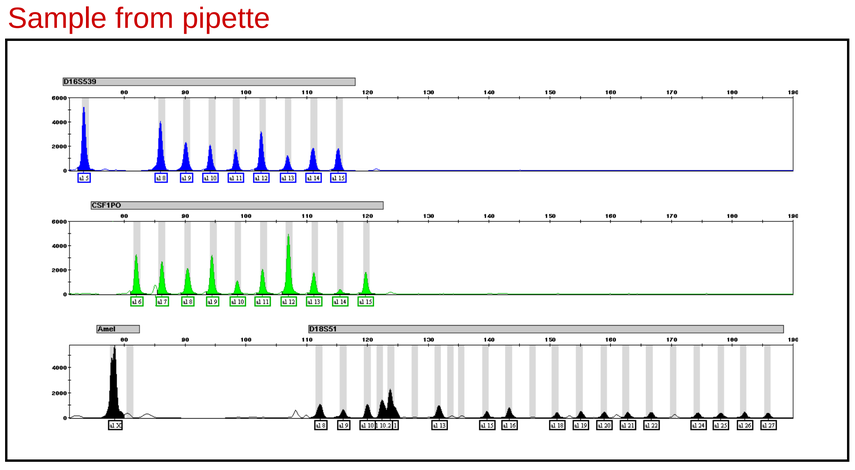
2. Visualization of amplified STR alleles and identification of contamination source (Figure 2).

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.

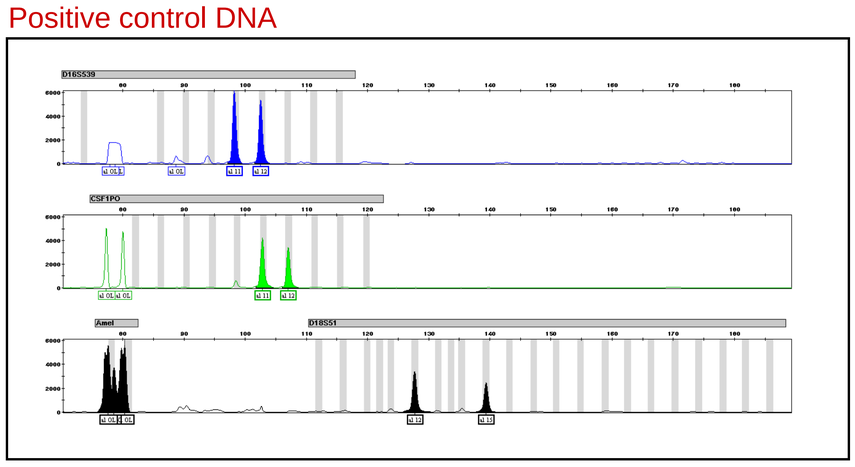
Sample from EpMotion 5075 LH



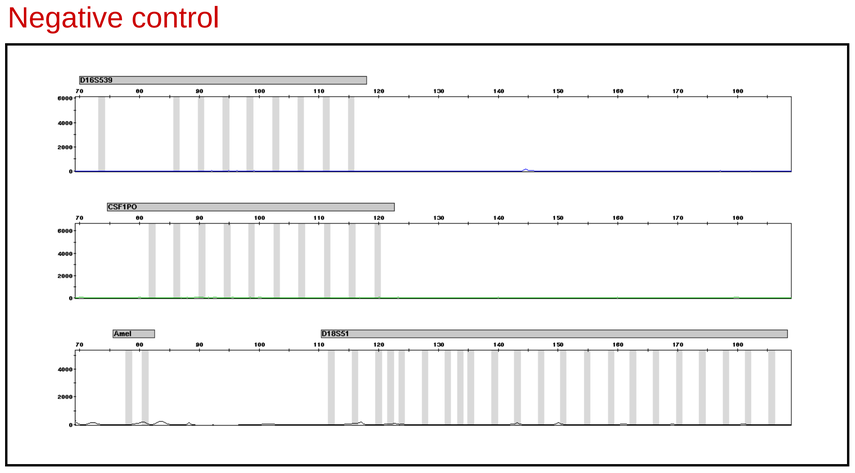
Sample from pipette



Positive control DNA



Negative control



Methods

Sample collection
Swabs were taken from surfaces of an epMotion 5075LH automated liquid handling workstation; PCR box; pipettes from isolation and post-PCR rooms, and ABI 310 Genetic analyzer. The total surface areas (100 cm^2) were sampled with 4N6 DNA Swabs (Copan).

DNA isolation
DNA from samples was extracted via standard ChargeSwitch procedure (Invitrogen) using epMotion 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 MasterCycler 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)
D16S539	Fluo	81-121
CSF1PO	Joe	89-129
D18S51	Tamra	113-193
Amelogenin	Tamra	77-82

Capillary electrophoresis
Fluorescently labelled STR alleles were diluted immediately and separated on ABI 310 Genetic analyzer. Samples were injected – 5 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.

Acknowledgments
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