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Test for contamination in connection with renovation of post-PCR laboratories

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ABSTRACT

The Teilum building housing the Department of Forensic Medicine at the University of Copenhagen was renovated in 2021/22. All windows were replaced, and the heating system was upgraded. During the renovation, the usual measures to prevent PCR products from escaping the post-PCR laboratories could not be maintained, since construction workers had to move in and out of the rooms carrying tools and debris. Instead, new measures were introduced, that included 1) the construction of a changing room for the workers with immediate access to the post-PCR laboratories, 2) clothing and shoes for the workers, that should only be worn inside the post-PCR laboratories, and 3) strict limitations on the areas the workers could enter, while renovating the post-PCR laboratories. Samples were taken before, during and after the renovation to monitor the possible spread of PCR products from the post-PCR areas. Mixtures of gDNA and PCR products were detected in only three of the 303 samples. All three samples were collected from the post-PCR areas prior to the renovation began, which indicated that the renovation did not cause wide-spread contamination of PCR products.

1. Introduction

At the Section of Forensic Genetics, several measures are in place to prevent PCR products from escaping the post-PCR laboratories and contaminating other areas in the building, primarily the pre-PCR laboratories. First, the post-PCR laboratories (one for trace and reference samples, respectively) and the airlocks leading into the rooms have reduced air pressure compared to the hallway and the rest of the Department. Second, doors leading into the airlocks and the post-PCR laboratories cannot be open at the same time. Third, laboratory staff change lab coats and shoes in the airlock and are not allowed to return to the pre-PCR laboratories after they have been inside one of the post-PCR laboratories.

During the renovation of the Teilum building housing the Department of Forensic Medicine, the post-PCR laboratories were not operational, and the laboratory work was moved to temporary buildings on the nearby parking lot. Obviously, the lower air pressure could not be maintained in the vacated post-PCR laboratories, and construction workers had to move in and out of the rooms. To minimize the possible spread of PCR products, a changing room was built in the hallway outside one of the airlocks and the construction workers were instructed to change to boiler suits and shoes that should only be worn in the post-PCR rooms. Furthermore, the workers should leave the Department via the elevator next to the changing room, and they were not allowed to enter other areas than the elevator, the changing room, and the post-PCR laboratories during this part of the renovation.

2. Materials and methods

A total of 285 samples were collected with cotton swabs before, during and after the renovation from 1) surfaces in the post-PCR laboratories, airlocks, and hallway, 2) boiler suits, shoes, and surfaces in the changing room, and 3) buttons, rails, and the wall of the nearby elevator. In addition, 18 samples were collected from surfaces in the pre-PCR laboratories.

Samples were extracted using the QIAamp DNA Investigator Kit (Qiagen) and the DNA was eluted in 50 µL water. All samples were quantified in duplicate using the Quantifiler™ Trio DNA Quantification Kit and the AB7500 real-time PCR system (Thermo Fisher Scientific) as recommended by the manufacturer.

A total of 15 µL sample was used as target for the PCR using the GlobalFiler™ Express PCR Amplification Kit (Thermo Fisher Scientific).

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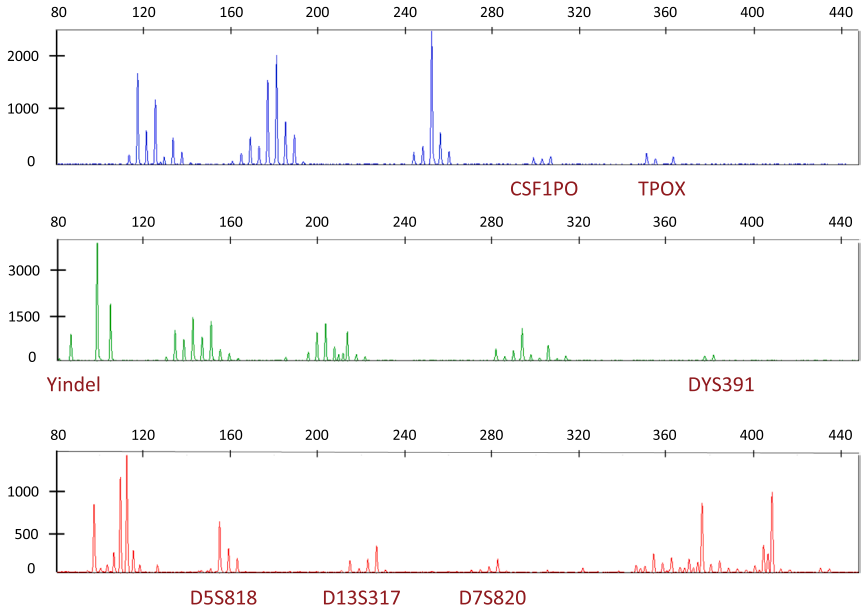

Fig. 1. Electropherogram. The best example of a mixture of gDNA and NGM Select PCR products amplified with the GlobalFiler kit. The sample was collected from a surface in the trace post-PCR laboratory. The six STRs and one Yindel (indicated in red), that were not amplified in Fig. S1B, showed reduced signals, indicating that a large part of the sampled DNA was PCR products.

One positive and one negative PCR control (water) was included in every experiment. The PCRs were conducted according to the recommended protocol using 29 PCR cycles (Thermo Fisher Scientific) and a GeneAmp™ PCR System 9700 (Thermo Fisher Scientific). The PCR products were analyzed using a 3500xL Genetic Analyzer and the GeneMapper™ ID-X 1.4 software (Thermo Fisher Scientific).

3. Results and conclusions

The AmpFLSTR™ NGM SElect™ PCR Amplification Kit (Thermo Fisher Scientific) has been the preferred STR kit in our laboratory since 2012. In order to identify PCR products amplified with the NGM Select kit and distinguish them from genomic DNA, the GlobalFiler™ Express PCR Amplification Kit was used to amplify the collected samples. Fig. S1 shows that 6 STRs (CSF1PO, D13S317, D5S818, D7S820, TPOX, DYS391) and one Yindel could not be amplified from dilutions of a positive control amplified with the NGM Select kit, whereas all other loci were successfully amplified and detected. Thus, mixtures of gDNA and NGM Select PCR products amplified with the GlobalFiler kit would have lower signals in these loci (example in Fig. 1).

- Of the 303 collected samples, 227 were mixtures, 13 contained DNA from a single individual, and 63 had no or weak florescent signals in CE. Eleven of the 13 single source profiles matched the profile of one of the construction workers, one profile matched the profile of an employee, and one profile did not match a known individual.
- None of the single source samples contained NGM Select PCR products.
- Mixtures of gDNA and NGM Select PCR products were detected in only three samples: two from the post-PCR laboratories and one from an airlock. These samples were collected prior to the construction began, and shortly after PCR-CE analyses of STRs had ceased in the laboratories.
- DNA concentrations ranged from 0.000 ng/μL to 2.123 ng/μL (Average: 0.054 ng/μL). Degradation indices ranged from 0.6 to 13.6 (Average: 1.7).
- The highest concentrations of DNA were found in the samples from the bench in the changing room and the shoes (samples were collected from the sole) worn by the construction workers. Some build-up of DNA was indicated on the bench, the shoes, and the boiler suits (samples were collected from the sleeves).
- The lowest concentrations of DNA were found in the samples collected from the elevator (button, rail, and wall) and the door handles in the changing room.
- The DNA concentrations of the samples collected after the renovation were generally lower than in the samples collected prior to the renovation.
- In conclusion, contamination of surfaces or clothes with PCR products from the post-PCR laboratories was not observed during or after the renovation.

Conflict of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigss.2022.09.002.