Experience with Fluorescent STR Multiplex Analysis in a Belgian Center. Population Database with New Alleles, use in Paternity and Chimerism Testing

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Short tandem repeat (STR) loci consist of tandemly repeated 3 to 7 bp sequence motifs. Many of these loci are highly polymorphic and are therefore useful for human identification purposes. Since the PCR generated products are very short (150-400bp long) analysis is even possible on partially degraded samples.

Moreover, the fluorescent-labeled PCR products, automated detection and accurate sizing by means of an internal standard (alleleic ladder) represents a reproducible and fast way of analysis. Due to these characteristics, STR analysis is used increasingly for forensic applications.

We studied 8 STR loci in two quadriplex reactions (quadriplex 1 or CTTv; vWA, TH01, TPOX, CSF1PO and quadriplex II or FFFL: LPL, F13B, FESFPS, F13A01). Reagents were obtained from Promega (Madison, WI, USA). The fluorescent-labeled PCR generated products were analysed on a Pharmacia A.L.F. DNA sequencer. Beside a positive and negative control, each run contained an allelic ladder at the beginning and at the end of the gel allowing accurate determination of alleles.

More than 200 unrelated individuals were typed for all 8 loci. Allele frequencies as well as the number of homozygous and heterozygous individuals were calculated. No major differences were noted compared to the frequencies in other Caucasian populations. However, several new or uncommon alleles were found: vWA-12, F13B-5, F13A01-17. The routine typing of these unrelated individuals did not reveal major problems although a few times an artifact band one base shorter than expected (no terminal addition) was seen. Fluorescent PCR products can at least be analysed four weeks after amplification showing stability of the products (when stored in the dark at 4°C).

The applicability of STR analysis was retrospectively evaluated in 25 paternity cases. The conclusion was identical as obtained with prior extensive bloodgroup and HLA Class I and II determinations or RFLP (probe M13) analysis.

Another interesting application is the monitoring of patient/donor chimerism after allogeneic bone marrow transplantation. Indeed, follow-up of chimerism is essential for the study of both the take of the graft or recurrence of the patients disease. Peripheral blood samples of eight patients were analysed before and after allo-BMT. The number of informative alleles varied between 3 and 9. Quantitative assessment was derived using informative allele peak areas. Six cases had 100% engraftment with 10 and 20% mixed chimerism in the remaining two cases. The detection level for mixed chimerism detection is approximately 1%. Interlaboratory concordance of results was also obtained with a 10 marker panel (Profiler, ABI) using the ABI 377 automated sequencer (Perkin-Elmer). In conclusion, the approach for detection of chimerism by automated STR analysis is both quantitative and informative.

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