## B144 A Single Multiplex Polymerase Chain Reaction (PCR) Assay of Rapidly Mutating (RM) Y-Chromosomal Short Tandem Repeat (Y-STR) Loci to Complement Current Sets of Markers Used in Forensic Y-Chromosome Analysis

*Daniela Lacerenza, PhD, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, c.so Galileo Galilei #22, Torino 10126, ITALY; Giancarlo Di Vella, MD, PhD*, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, Corso Galileo Galilei 22, Torino 10126, ITALY; and Carlo Robino, 22, Torino, TO 10126, ITALY*

The goal of this presentation is to introduce a set of RM Y-STR markers that can complement commercially available multiplex kits.

This presentation will impact the forensic science community by highlighting the ability of additional RM Y-STR loci to resolve paternal lineages and discriminate closely related male suspects.

Male-specific Y-STR loci are especially useful in cases of sexual assault, where mixed stains that contain abundant DNA from the female victim together with trace DNA from the male assailant are analyzed. A general drawback of Y-STRs is that, compared with autosomal STRs, their ability to identify single individuals is limited. Since mutation is the only driving force of variation in the human Y chromosome, and currently used Y-STRs have low mutation rates (in the order of 1 x 10-3), groups of paternally related men, and in particular closely related males, cannot be easily differentiated. To improve the power of discrimination of conventional Y-STRs, it is therefore necessary to complement them with additional markers with much higher mutation rates. Through a systematic study, it was possible to identify a subset of 13 RM Y-STRs displaying a few mutations per marker every 100 generations.[1] Part of these markers have subsequently been included in commercial kits such as the PowerPlex® Y23 system by Promega® (DYS570, DYS576) and Y Filer® Plus by Life Technologies™ (DYS570, DYS576, DYS627, DYS518, DYS449, and DYF387S1).

In a forensic context, minimization of analytical steps is necessary to draw as much information as possible from minute amounts of DNA isolated from stains; however, amplification of the RM Y-STR markers (DYF399S1, DYF403S1ab, DYF404S1, DYS526ab, DYS547, DYS612, and DYS626) not already included in the Y Filer® Plus panel, which currently represents the most comprehensive commercial set available for both conventional and RM Y-STR loci, was originally described in three separate reactions.[2] The goal of this project was to combine these reactions in a single compact multiplex PCR assay.

PCR primers and amplification conditions for the seven RM Y-STRs included in the multiplex are those described by Robino et al., with the exception of a modification of the dye label for locus DYS626 (from 6-FAM to TAMRA) in order to avoid overlapping between marker-specific fluorescent signals.[3] Developmental validation of the assay included a sensitive study of serial dilutions of male control DNA (2800M) and a mixture study, in which the serial dilutions of male DNA were mixed 1:1 with a female control DNA sample (200ng/µl).

The sensitivity study showed that full RM Y-STR profiles were obtained from as little as 62.5pg of template male DNA, whereas partial profiles, including drop-out artefacts at the multi-copy markers DYF399S1, DYF403S1a, and DYF404S1, could be observed at lower concentrations. Results were not affected by the concurrent presence of female DNA. Though the Y Filer® Plus panel has been shown to greatly improve the resolution of paternal lineages, it does not achieve complete differentiation, especially in more isolated/endogamous populations.[4] The described multiplex can therefore complement Y Filer® Plus data in selected cases requiring further power of discrimination, in particular when closely related male suspects are involved. The main concern regarding the use of additional RM Y-STRs in trace DNA analysis is that the occurrence of allelic drop-out and drop-in must be considered in multi-copy markers with a non-fixed number of alleles.





*Presenting Author

**Reference(s):**

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3. Robino C., Ralf A., Pasino S., De Marchi M.R., Ballantyne K.N., et al. Development of an Italian RM Y-STR haplotype database: results of the 2013 GEFI collaborative exercise. *Forensic Sci. Int. Genet*. 15 (2015): 56-63.
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**Sexual Assault, Y Chromosome, Rapidly Mutating Y-STRs**