Taxonomic identification of forensic samples using SPInDel

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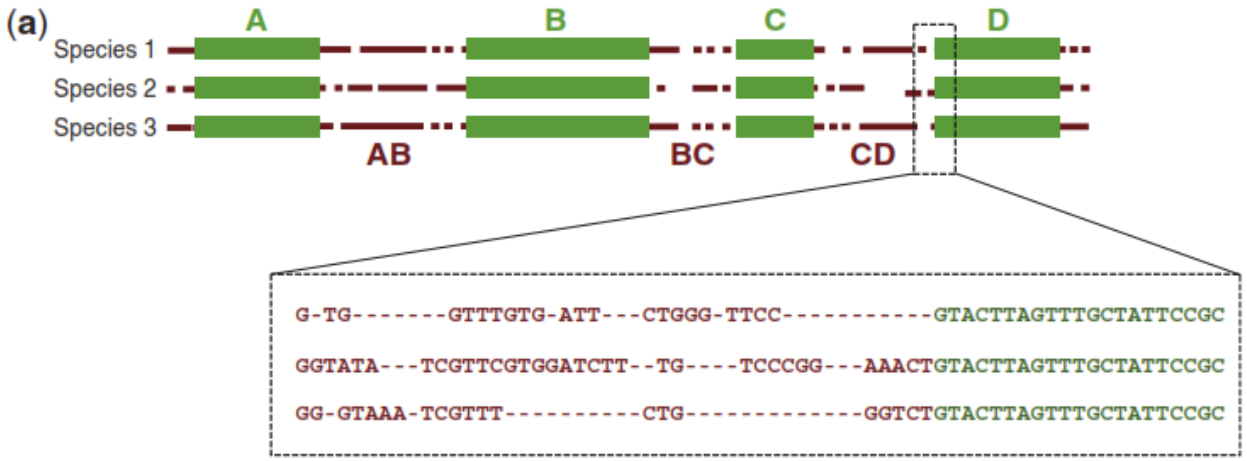
SPInDel Concept



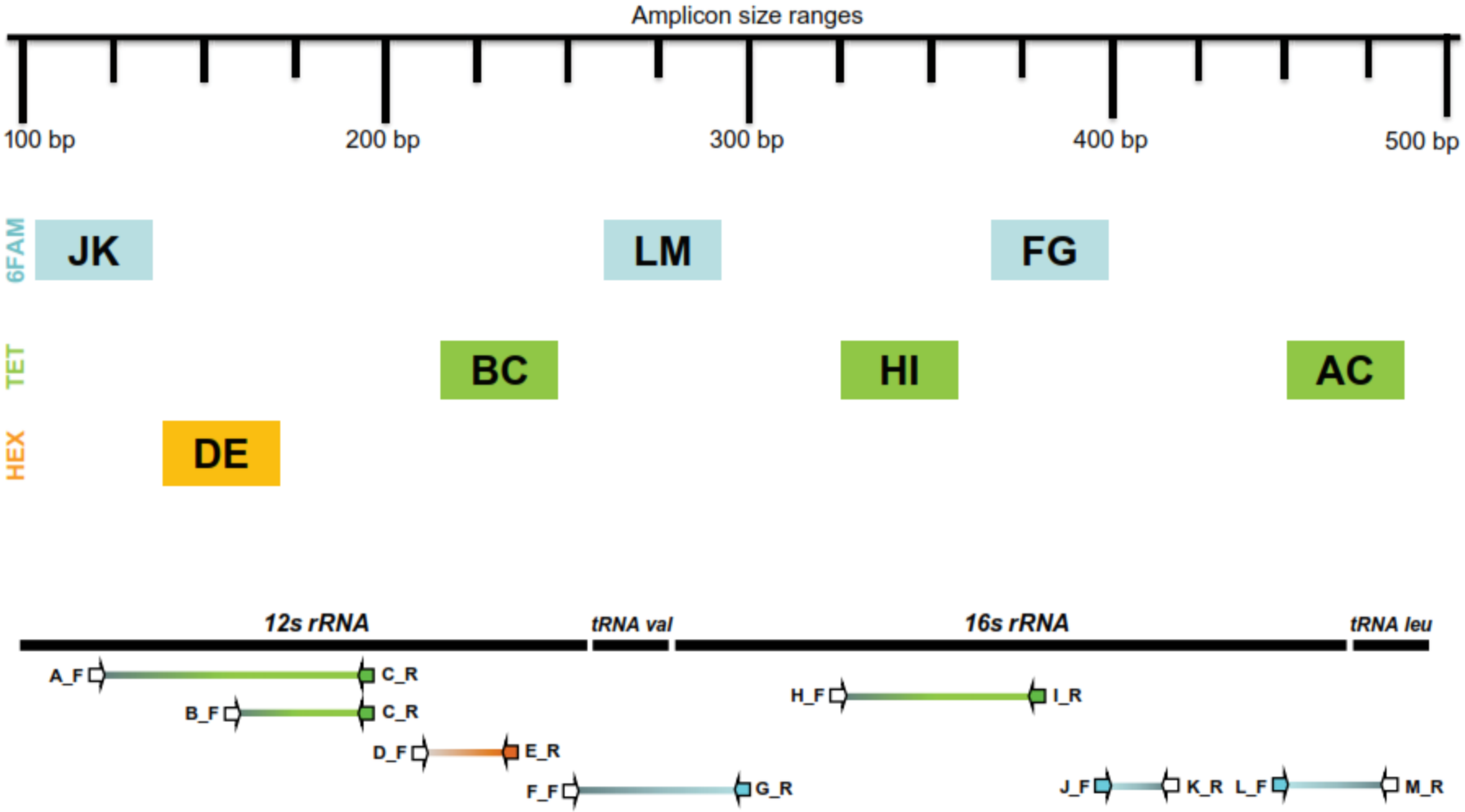
(b)

	AB	BC	CD
Species 1	176	101	119
Species 2	181	96	132
Species 3	204	101	130

Figure 1. Schematic illustration of the strategy used in the species identification by the insertions/deletions (SPInDel) method. (**a**) Illustration of the sequence alignment for three hypothetical species. Four conserved regions (green boxes) define three hypervariable domains (dotted brown lines). A section of the alignment is magnified to show the presence of multiple gaps in hypervariable regions. (**b**) Each species is identified by a numeric profile resulting from the combination of lengths in hypervariable regions.

ORIGINAL

Supplementary Figure S10. Schematic representation of size ranges and dye labels used in designing the SPInDel assay. The profiling kit uses three spectrally distinguishable fluorescent dyes in the filter set C: 6–FAM (blue), TET (green), and HEX (yellow). We devised a simple way of retrieving all information enclosed in two contiguous hypervariable regions by multiplex PCR, as exemplified for hypervariable regions AB and BC (bottom image). Instead of using a reverse primer for conserved region B to amplify amplicon AB (B_R would be complementary to B_F used to amplify BC), we used the same reverse primer (C_R) for both regions, which meant that region AC was used instead of AB. In this way, we avoided the problem of indels that eliminate size differences in large regions.



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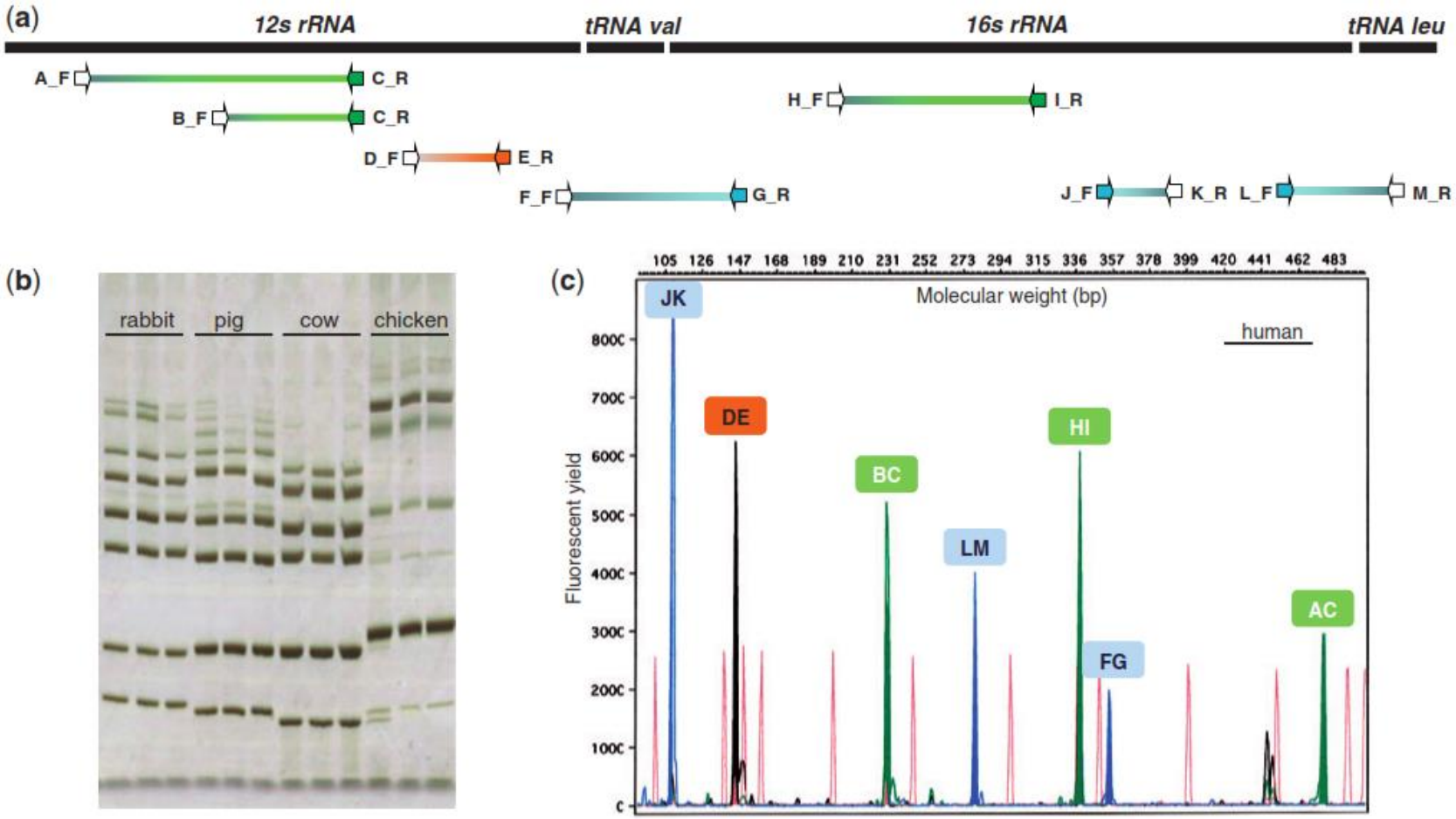
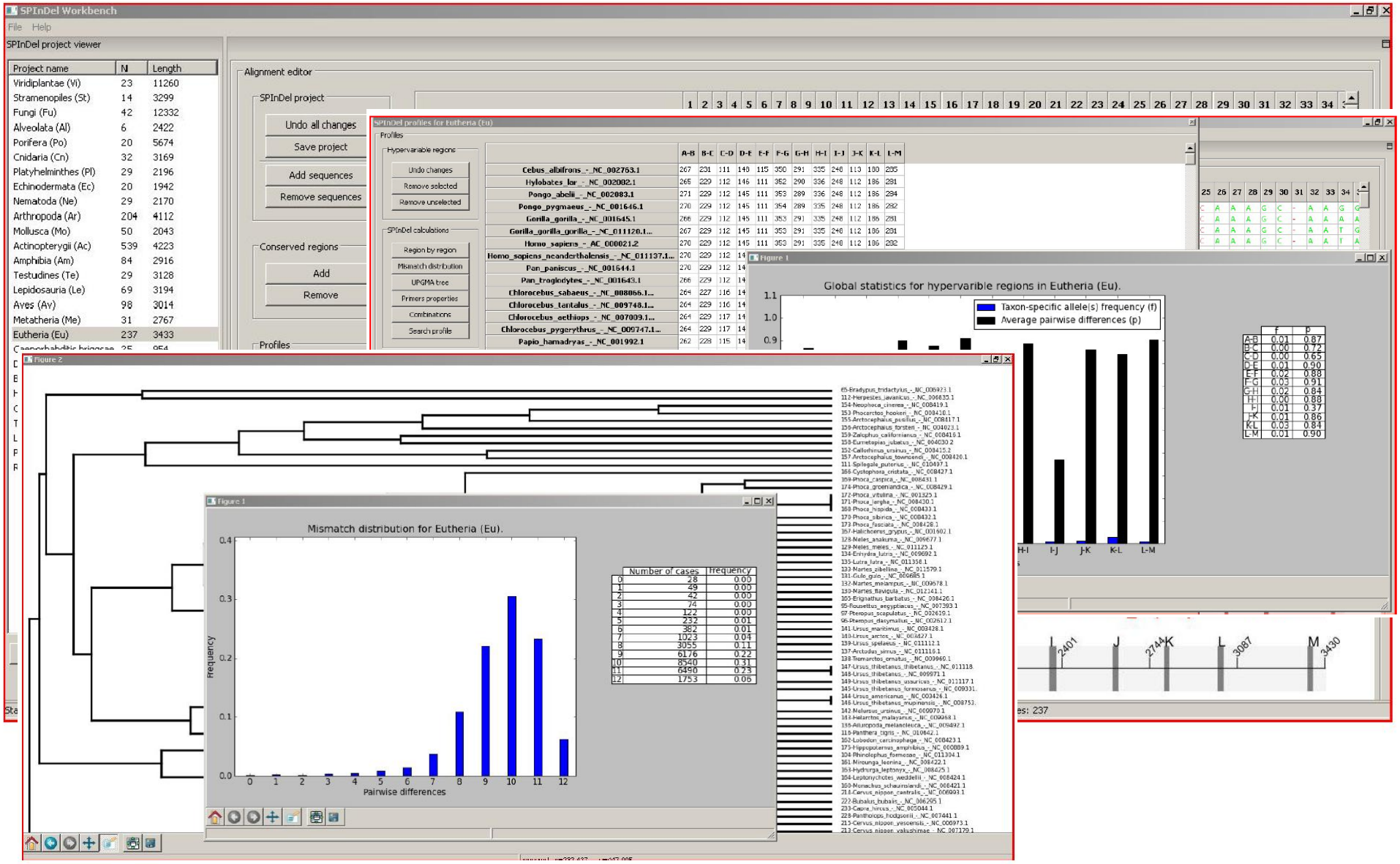


Figure 5. Experimental application and validation of a SPInDel profiling kit for identification of eutherian species. (**a**) Graphical representation of the seven ribosomal RNA hypervariable regions amplified by multiplex PCR. PCR primers (arrows) were named using letters A to M, their orientation (F, for forward, R for reverse) and labeling with fluorescent dyes (blue, orange and green arrows). (**b**) The products of multiplex PCRs of three eutherian and one avian species are shown on a silver stained polyacrylamide gel. Each species has a unique pattern of migration because of differences in the length of amplicons (hypervariable regions). Identification of species using such gels is only possible by comparing the banding pattern of the target sample with those of reference samples analyzed with the same procedure. (**c**) Electropherogram illustrating a SPInDel profile from a human reference sample obtained by capillary electrophoresis with multidye fluorescence detection. The profile is displayed in a four-color fluorescent system, in which green, blue and yellow channels were used for detection of amplified products and red was used for a size marker. The species identification is achieved by running the SPInDel numeric profile of the target sample against a reference database (SPInDel workbench).

SPInDel workbench

http://www.portugene.com/SPInDel/SPInDel_web.html



SPInDel v.3

New project design for mammalian/domesticates

Minimum number of primers

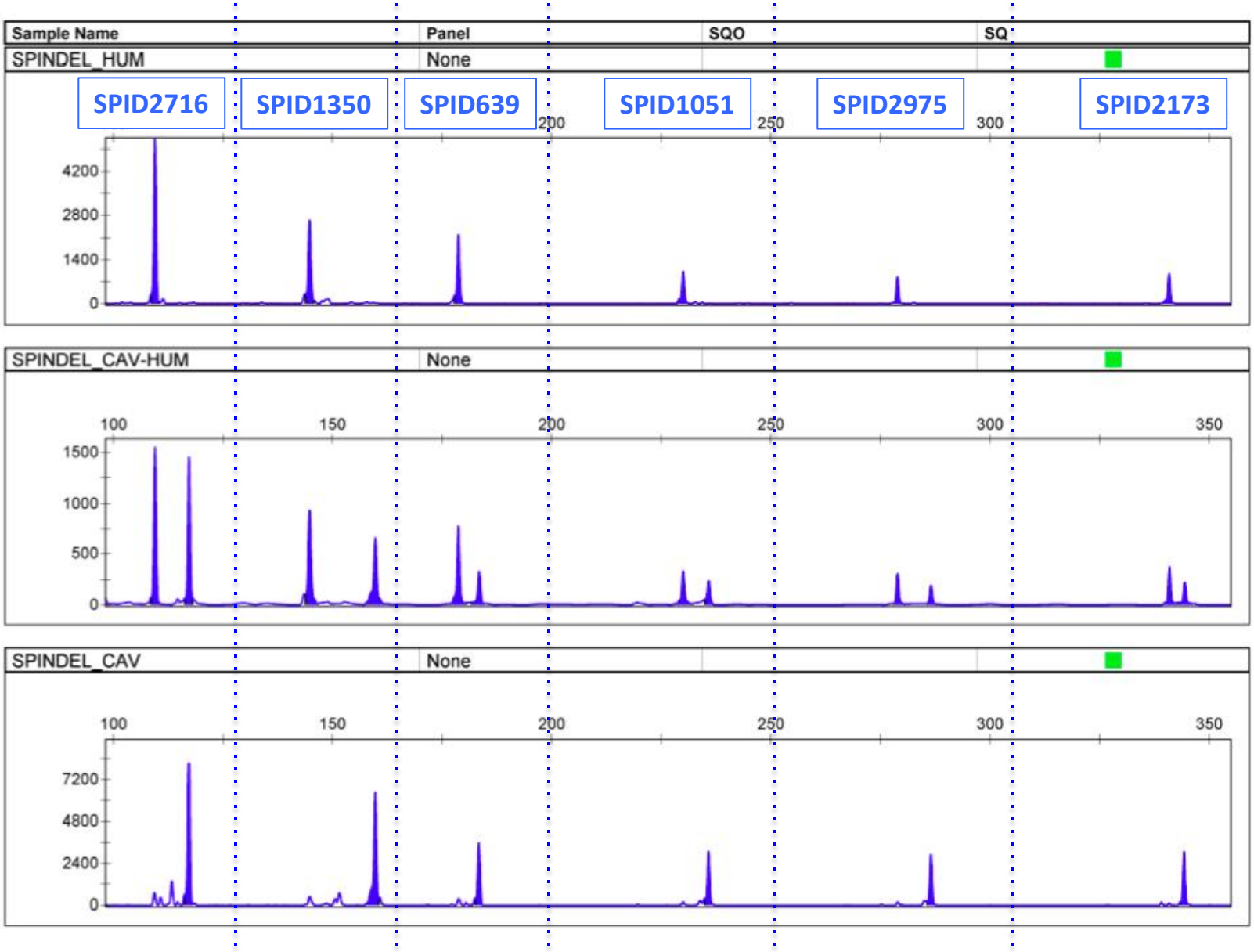
Monochromatic

SPInDel v.3

Human

Mix Human/Horse

Horse



Aims

Evaluation of SPInDel as an exploratory/investigative tool in preliminary forensic casework analysis

Through

- Test sample analysis (distributed along QC?) with SPInDel
- Primers v3 distributed
- Report using SPIndel platform