Development and validation of Y-miniSTR multiplex systems for the use on the ancient DNA and for genetic genealogy purposes

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Introduction

The aim of this work was to develope a "non-core" loci containing Y-miniSTR multiplex systems that offer an extremely high discrimination potential and are robust enough for use in both degraded DNA samples and genetic genealogy. The newly designed multiplexes include 8 "non-core" Y-STR loci DYS388, DYS426, DYS444, DYS446, DYS447, DYS449, DYS459, DYS481 and an additional 2 Y-STR loci DYS392 and DYS438 of Y-filer kit. Primers for all selected loci were designed to produce amplicon sizes as small as possible.

Results

Fig. 1. Sensitivity and specificity of the Y-miniSTR multiplex systems. Marker names are abbreviated (e. g. DYS446 is listed as 446); peak heights are in relative fluorescence units.

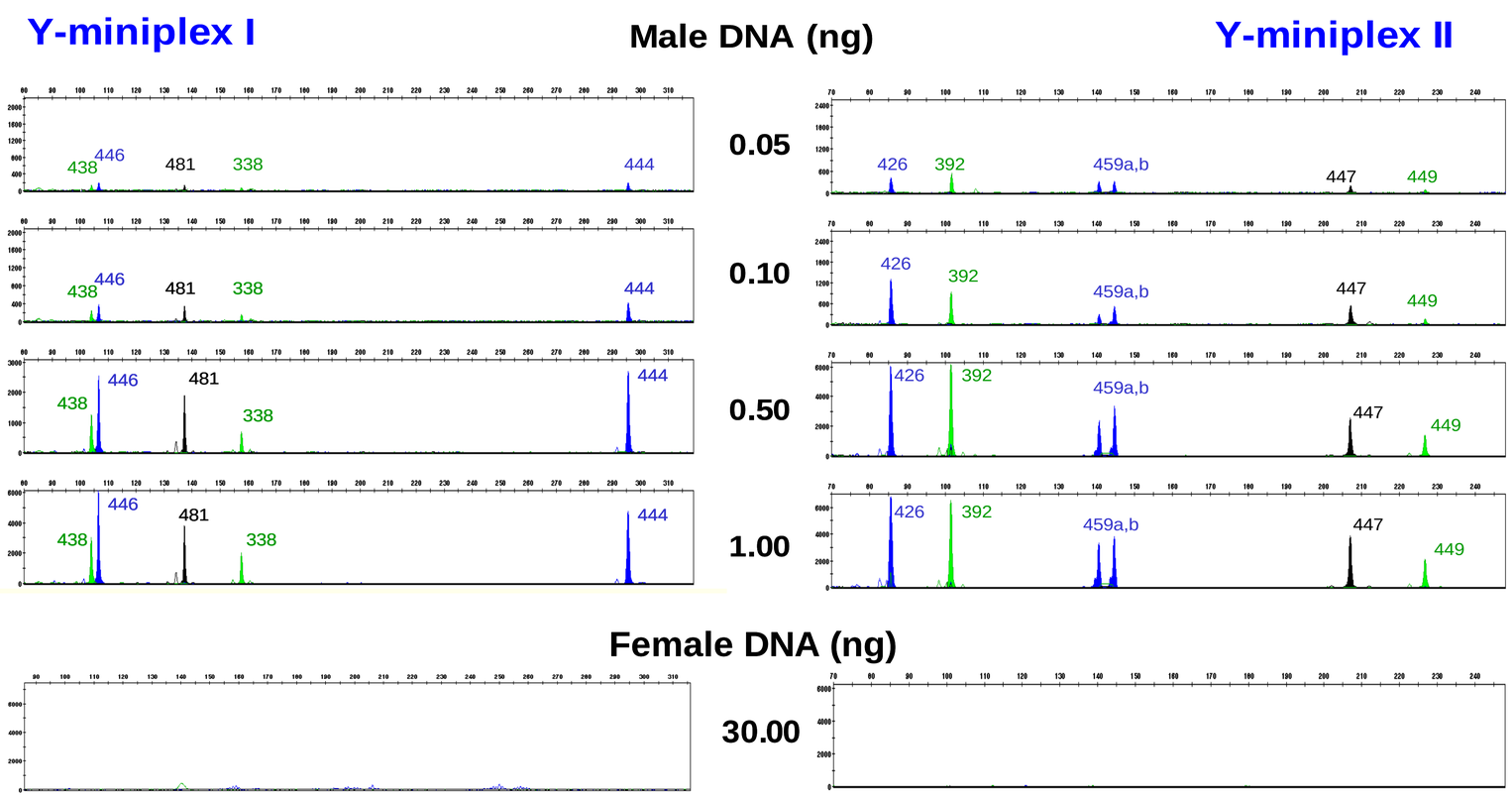
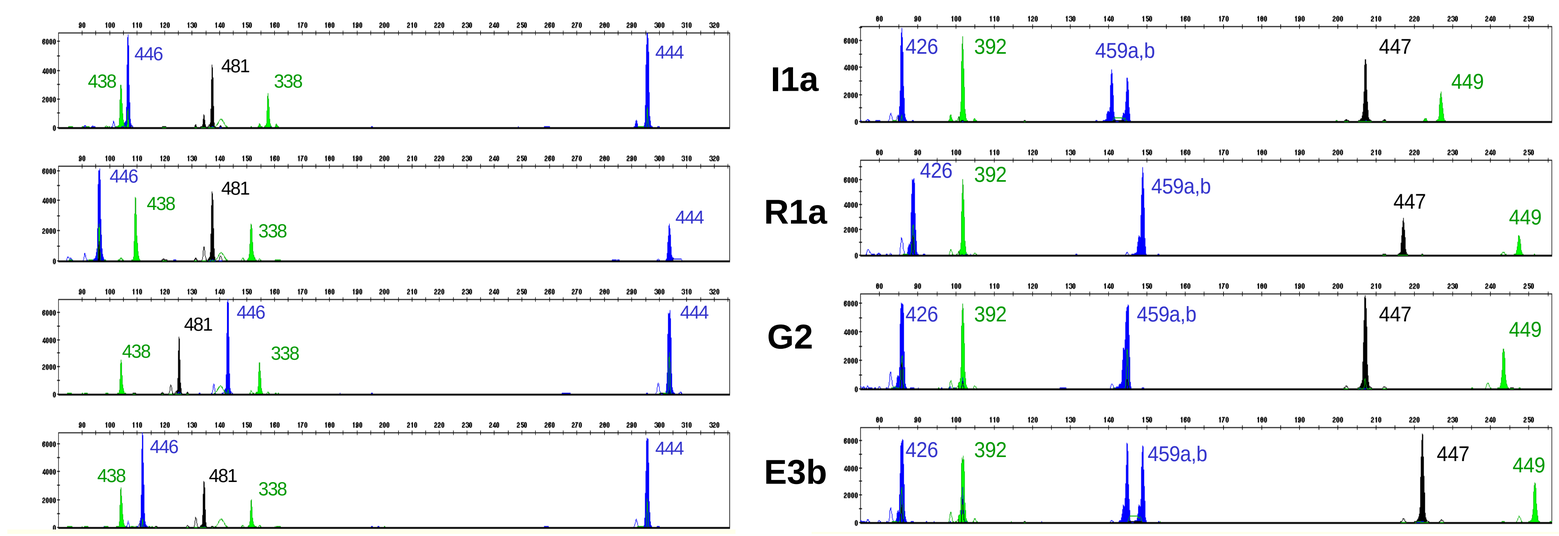


Fig. 2. Discrimination potential of the Y-miniSTR multiplex systems. Marker names are abbreviated (e. g. DYS446 is listed as 446); peak heights are in relative fluorescence units.



Tab. 1. Diversity value of selected loci.

Y-STR locus	Diversity value
DYS446	0.72
DYS444	0.61
DYS438	0.66
DYS388	0.33
DYS481	0.84

Y-STR locus	Diversity value
DYS426	0.50
DYS459 a, b	0.75
DYS392	0.6
DYS449	0.84
DYS447	0.77

Methods

Sample collection
Buccal samples were collected from volunteers using sterile 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).

Primer design paramters

- primer size from 20 to 26 nucleotides
- primer *Tm* values from 55°C to 64°C
- PCR amplicon as small as posible
- forward primers fluorescently labelled.

Tab. 2. Y-miniSTR markers definition.

Y-STR locus	Repeat motif	Allele range	Product size (bp)	Avg % Stutter
Y-miniplex I				
DYS446	(TCTCT)n	8-23	85-160	6.97
DYS444	(ATAG)n	9-16	287-315	9.16
DYS438	(TTTTC)n	7-16	95-140	3.65
DYS388	(ATT)n	10-15	151-166	9.47
DYS481	(CTT)n	18-31	115-158	20.10
Y-miniplex II				
DYS426	(GTT)n	9-13	85-110	13.32
DYS459	(ATTT)n	6-10	136-156	5.88
DYS392	(TAT)n	6-18	94-130	9.46
DYS449	(TTTC)n (N)n (TTTC)n	26-37	222-262	13.47
DYS447	(TAATA)n (TAAAA)n	22-29	200-245	3.87

Capillary electrophoresis
Fluorescently labelled Y-miniSTR alleles were separated on ABI 310 Genetic analyzer. Samples were injected – 5 kV injections- for 5 . Data were analyzed using GeneMapper ID (V 3.2) software with a 50 RFU analysis treshold.

Conclusions

We designed and developed a highly discriminating Y-miniSTR multiplex systems that use loci that are not present in any of the commercially available Y-STR kits. A sensitivity test using serially diluted standard male DNA showed that all the values of Y-STR loci in the Y-miniplexes are reliable at template concentration as low as 50 pg.

Reduced-size amplicons also produce a high success rate with highly degraded forensic or anthropological samples.

Acknowledgments
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