The development of reduced size Y chromosomal STR for genotyping of degraded DNA

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DNA Use in Human Identification

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Historical investigations
- Missing persons investigations
- Mass disasters -- **putting pieces back together**
- Convicted felon DNA databases

Y Chromosomal STRs

STR Markers
DYS19
DYS389I/II
DYS390
DYS391
DYS392
DYS393
DYS385
DYS388
DYS434
DYS435
DYS436
DYS437
DYS438
DYS439
⋮

- Applications
  - forensic investigation
  - genealogical purpose
  - evolutionary studies
- Advantages to Human Identity Testing
  - male component isolated without differential extraction
  - paternal lineages

Sources of Biological Evidence

- Blood
- Semen
- Saliva
- Urine
- Hair
- Teeth
- Bone
- Tissue

DNA degradation:

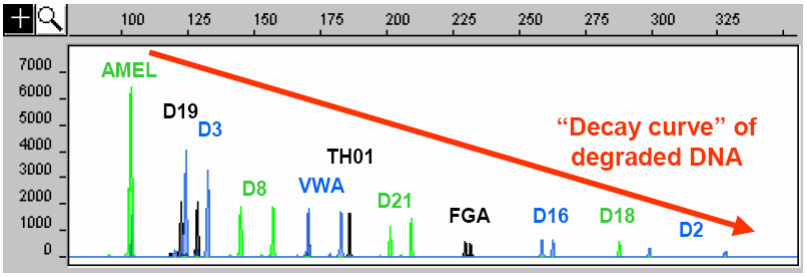
- If DNA exposed to external environmental for any length of time, degradation can occur due to **bacterial, biochemical** or **oxidative** process
- Environmental contaminants can also be commingled with the forensic samples

Approaches for Degraded Samples

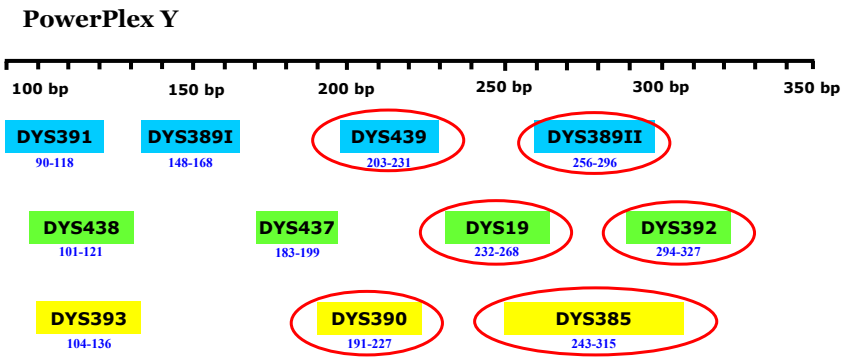
- mtDNA - high copy number per cell
  - maternal lineages
  - time-consuming process, low discriminatory power
- SNPs - small size, lower mutation rate
  - Easier data interpretation (no microvariants or stutter)
  - How many SNPs = STR
  - Databases, Platform for SNP typing?
- **STRs** - **Highly variable , higher discriminatory power**
  - **Rapid processing is attainable**

STRs Use in Degraded Samples

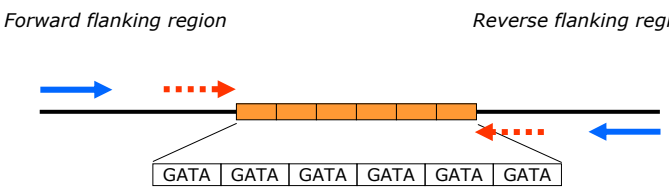
- Poor amplification of the larger sized loci of >200-250 bp
- The yield of complete target fragment is greatly reduced
  - "Decay Curve"



Commercial Y-STRs kit



Redesign of Y-STR Maker



Advantages of Approach:

- Retains same marker information (database compatibility)
- Uses highly polymorphic STR loci (high discriminatory power)

Materials and Methods

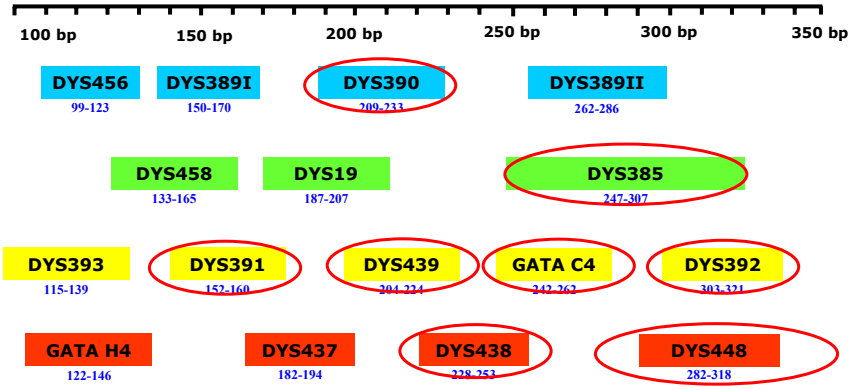
- **New PCR primer design**
  - DYS390, DYS391, DYS392, DYS385a/b, DYS438, DYS439, DYS448 GATA C4 (DYS635) (according to Yfiler) additional DYS446, DYS449
  - Reference sequences: GeneBank (http://www.ncbi.nlm.nih.gov)
  - Primer 3: the PCR product size was made as small as possible
- **DNA samples**
  - **Concordance**: 100 buccal swabs samples form unrelated Koreans
  - **Sensitivity**: 1ng, 500pg, 250pg, 125pg, 62pg, 31pg, 15pg of control 9948 male DNA (promega, Madison, MA)
  - **Degraded DNA**: A blood DNA treated with 0.01U of DNase I (NEB, Beverly, MA) for 0 min, 2 min, 5 min, 10min, 15 min, 20min, 30min
  - **Real cases**: DNA (>0.01 ng/μl) extracted from 10 50-year old bones by modification of the QIAamp protocol by Yang *et al.* (Am J Phys Anthropol, 105: 539-43)

Materials and Methods

- **Multiplex PCR**
  - Total 10 μl PCR reaction: 1 μl of DNA, 1.6 μl of Gold STR buffer 2.0 U of AmpliTaq Gold polymerase and primers
  - Cycling condition: 95°C 11'; 96°C 1'; 94°C 30", 60°C 30", 70°C 45" x 10; 90°C 30" 60°C 30" 70°C 1' x 20-25; 60°C 30' using the GeneAmp 9600
- **Detection System**
  - ABI prism 310 genetic analyzer, Gene Scan software 3.1, and Genotyper 2.5 software (Applied Biosystems, CA, USA)

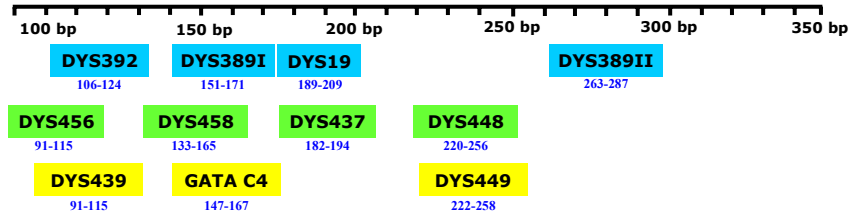
Commercial Y-STRs kit

AmpFlSTR Yfiler

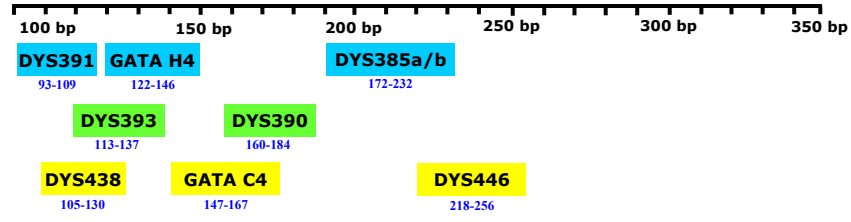


Reduced size Y-STRs

Multiplex I



Multiplex II



Y-STRs information in this study

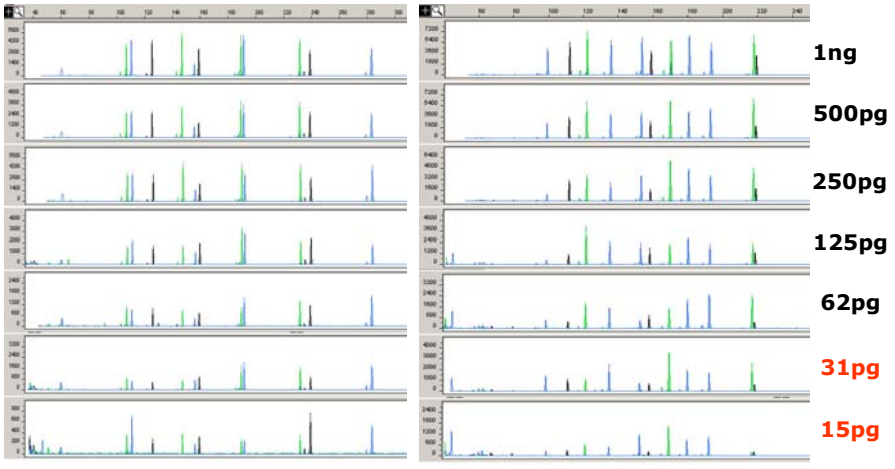
Multiplex II

Y-STR locus	GenBank Accession	GenBank Allele	Allele Range	Allele Spread	Product Size Yfiler	Product Size Mini-Y set	Size Reduction
DYS391	AC011302	11	7-9	16 bp	152-168 bp	93-109 bp	59
GATAH4	AC011751	12	8-14	24 bp	122-146 bp	122-146 bp	
DYS385	Z93950	10	8-23	60 bp	247-307 bp	172-232 bp	75
DYS393	AC006152	12	10-16	24 bp	115-139 bp	113-137 bp	
DYS390	AC011289	24	21-27	24 bp	209-233 bp	160-184 bp	49
DYS438	AC002531	10	9-14	25 bp	228-253 bp	105-130 bp	123
DYS446*	AC006152	14	11-19	40 bp	294-334 bp	218-258 bp	76
DYS448	AC025227	19	17-23	36 bp	282-318 bp	220-256 bp	62
DYS439	AC002992	13	9-14	20 bp	204-224 bp	117-137 bp	87
GATAC4 (DYS635)	G42673	21	19-24	20 bp	242-262bp	147-167 bp	95
DYS449*	AC051663	29	26-35	36 bp	344-380 bp	222-258 bp	122

* Compared to previously reported product size

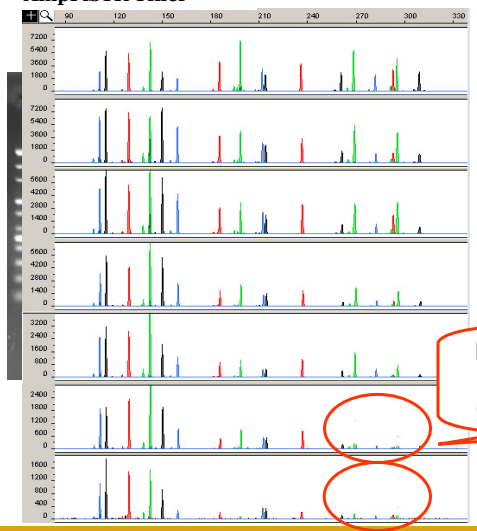
Sensitivity

Multiplex I **Multiplex II**



Amplification in Artificially Degraded DNA

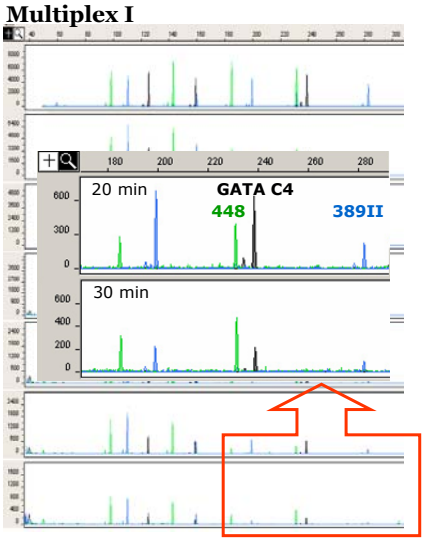
AmpFlSTR Yfiler



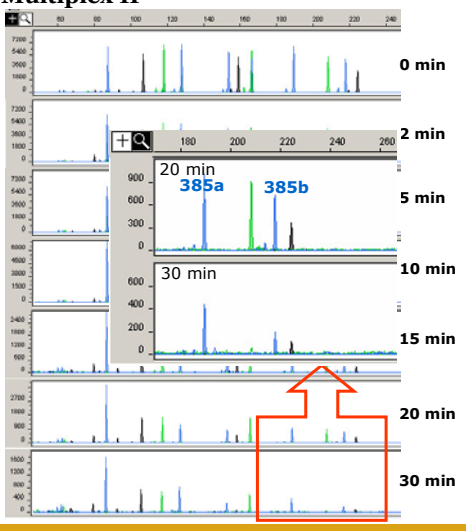
Larger sized allele bounded
DYS389II, DYS385a/b,
GATA C4(DYS635), DYS448

Amplification in Artificially Degraded DNA

Multiplex I

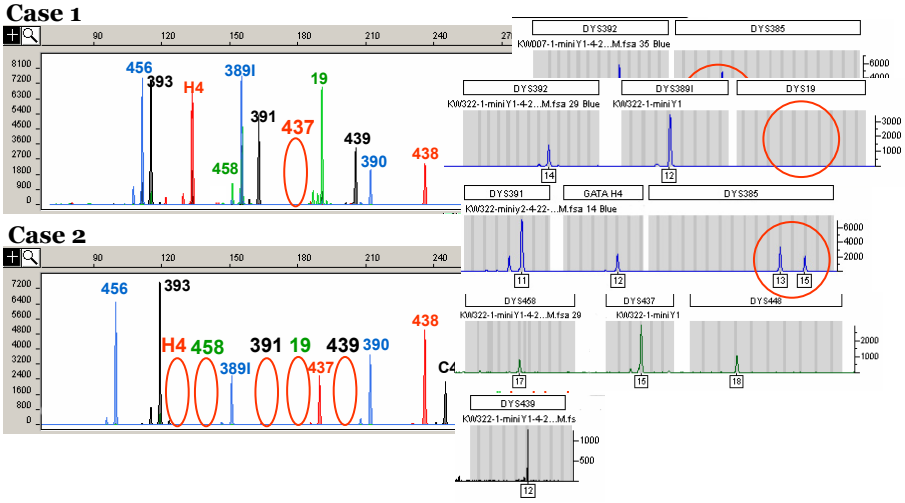


Multiplex II



Amplification in the Bone DNA

AmpFlSTR Yfiler | **Mini Y-STR set**



Y STR Profiling Results in 10 Bone DNAs

	Yfiler (size)	MiniY (Size)	Yfiler (%)	Mini-Y (%)	Drop in
DYS392	303-321	106-124	3 (30%)	9 (90%)	0
DYS438	228-253	105-130	6 (60%)	8 (80%)	1
GATA C4	242-262	147-167	7 (70%)	9 (90%)	0
DYS439	204-224	117-137	7 (70%)	9 (90%)	0
DYS385a/b	247-307	172-232	3 (30%)	7 (70%)	1
DYS448	282-318	220-256	6 (60%)	8 (80%)	0
	AmpFlSTR Yfiler		**Mini-Y set**		**Yfiler+Mini-Y set**
Amplified Loci	108 (67.5 %)		135(83.1 %)		140 (87.5 %)
Total Loci	160		160		160
DYS458	133-165	133-165	7 (70%)	9 (90%)	4
DYS437	182-194	182-194	8 (80%)	9 (90%)	1
DYS389I	150-170	150-170	9 (90%)	8 (80%)	1
DYS19	187-207	188-208	6 (60%)	7 (70%)	1
DYS389II	262-286	262-286	8 (80%)	7 (70%)	0

Conclusion

- **Redesigned Y-STRs Primer sets, in which amplicon size is kept at minimum, provide an effective tool for degraded forensic samples, as seen from the sensitive study, enzymatically degradation study, and the real case samples.**
- **The use of these primer sets with commercial kits will increase the probability that degraded samples can be typed.**
- **Also they provide a check for the presence of allele drop out due to problem with primer binding of commercial STR sets**
- **Development of new additional mini Y-STRs or Y-SNPs is required because a few Y STR loci cannot be made into more smaller amplicon**