Forensic evaluation of nine miniSTR loci to aid analysis of degraded DNA in Koreans

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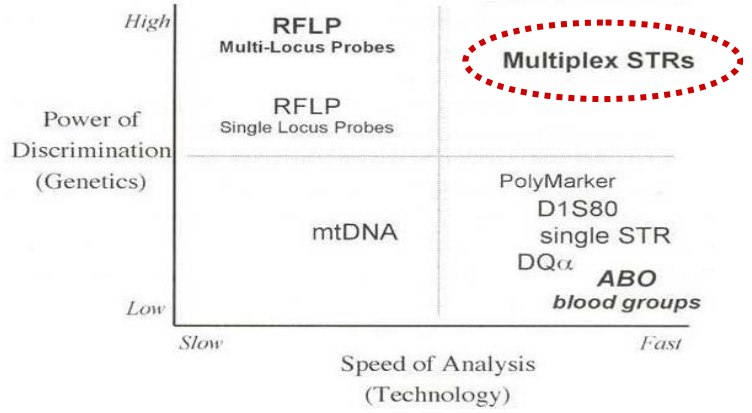
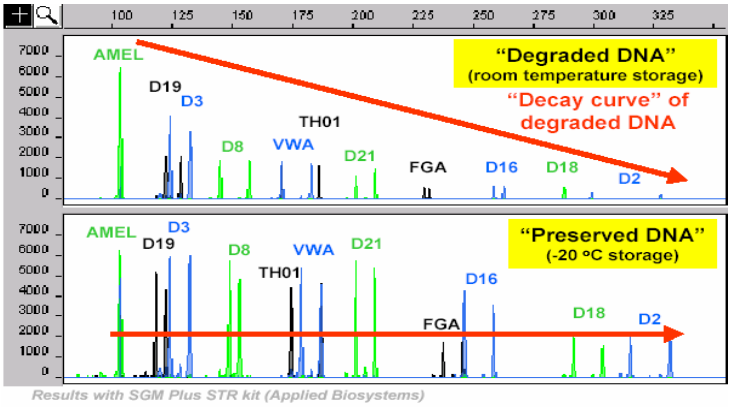
Markers used for human identification



Power of discrimination
PowerPlex ™ 16 (Promega): 1 in 2 x 10^{17}

PCR pattern of degraded DNA

Loss of Signal for Lager PCR Products



Data from a study done at NIST in May 2001

Size reduction of STR amplicon

Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

Advantages of Approach:

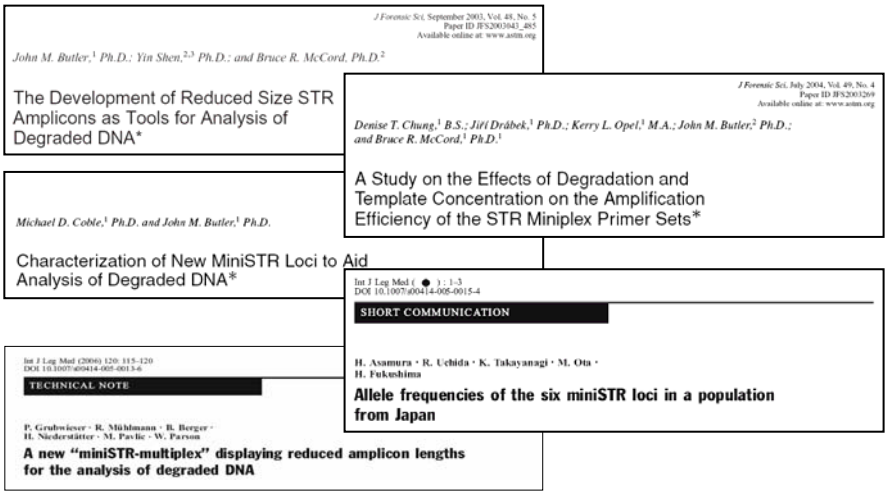
- Size reduction enhances success rate with degraded DNA
- Retains same marker information (database compatibility)

Needs for new miniSTRs

- A few of CODIS loci cannot be made into smaller amplicons.
- Additional miniSTR markers can improve the power of discrimination.

New PCR primers were designed and tested for the NC01 (non-CODIS miniSTR 01) and NC02

STRs with reduced size amplicon



Characterization of new miniSTRs

- Evaluation of usefulness in degraded DNA analysis
- Population analysis for forensic application

Selection of new miniSTR loci

Locus	Chromosome Location	Chromosome bp Position	GenBank Accession	GenBank Allele	Repeat Motif
NC01					
D10S1248	10q26.3	130,566,908	G08820	13	$[GGAA]_n$
D14S1434	14q32.3	93,298,432	G27275	13	$[GATA]_m[GACA]_n$
D22S1045	22q12.3	35,779,369	G08085	13	$[TAA]_n$
NC02					
D4S2364	4q22.3	93,975,767	G08326	9	$[GAAT][GGAT][GAAT]_n$
D2S441	2p14	68,213,613	G08184	12	$[TCTA]_n$
D1S1677	1q23.3	160,747,193	G09926	15	$[GGAA]_n$
NC03					
D3S3053	3q26	173,233,666	G08294	9	$[GATA]_n$
D6S474	6q16	112,985,899	G08540	16	$[AGTA]_m[GATA]_n$
D20S482	20p11.2	4,454,338	G08052	14	$[GATA]_n$

Primers of NC03 were designed by web-based Primer3

PCR conditions

Reaction mix

Total volume 10 μl
1.0 μl 10X Gold STR Buffer (Promega)
10X Primer Mix
1.0 U AmpliTaq Gold DNA Polymerase (Applied Biosystems)
Template DNA 1 ng

Thermal cycling

Initial denaturation	95 °C for 11min	
Denaturation	94 °C for 1min	30 cycles
Annealing	55 °C for 1min	
Extension	72 °C for 1min	
Final extension	60 °C for 45min	

Electrophoresis and Genotyping

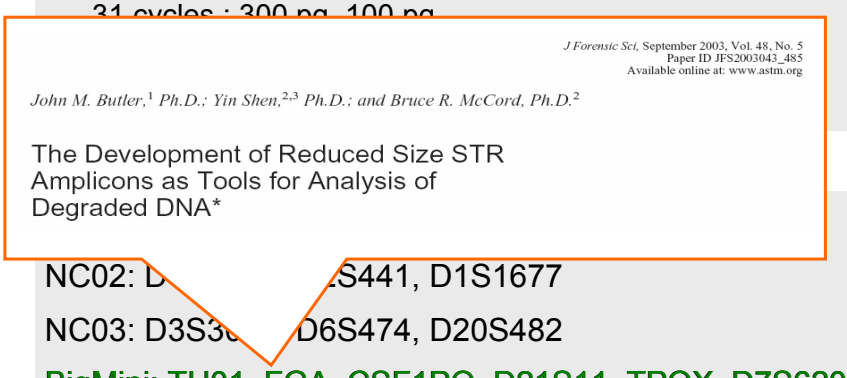
- The PCR products were separated by capillary electrophoresis using an **ABI PRISM 310 genetic analyzer** (Applied Biosystems).
- **Allelic ladder** was constructed by combining all observed alleles at each locus.
- Genotyping at each locus was performed using **Genotyper 2.5 software** (Applied Biosystems).

Sensitivity test

Template : Standard 9948 DNA (Promega)

29 cycles : 1000 pg, 500 pg

31 cycles : 300 pg, 100 pg



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The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

NC02: D S441, D1S1677

NC03: D3S3 D6S474, D20S482

BigMini: TH01, FGA, CSF1PO, D21S11, TPOX, D7S620

Efficiency test

Artificially degraded DNA

Degradation of 3.0 μg blood DNA using 0.02 U DNase I (NEB) for several time periods: 0(control), 5, 10, 20, 30 and 40 min

DNA from old skeletal remains

DNA extracted from 30 old skeletal remains
Total of 20 μl PCR reaction volume
35 thermal cycling

Primer sets

NC01, NC02, NC03 and BigMini

DNA samples for population study

- 300 unrelated Koreans
- Mucosal epithelium of oral cavity
- DNA was extracted using QIAamp DNA Mini Kit (Qiagen)

Statistical analysis

PowerStat Excel Template

Allele Frequency
Polymorphism Information Content (PIC)
Power of Discrimination (PD)

Arlequin Statistical Analysis Package Version 2.000

Observed Heterozygosity
Expected Heterozygosity
Hardy-Weinberg Equilibrium (HWE) test

Mean Exclusion Chance (MEC)

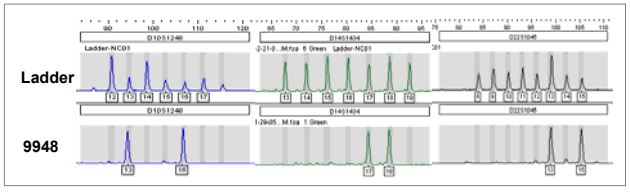
$$MEC_T = \sum_{i=1}^{k} p_i(1-p_i)^2 + \sum_{i<j}^{k} (p_i p_j)^2 (3p_i + 3p_j - 4)$$

Primer sequences and concentrations

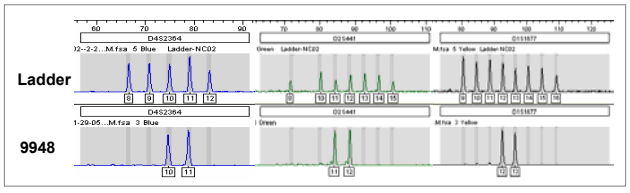
Locus	Label Dye	Primer Sequence (5' to 3')	From Repeat(bp)	Final Conc.(μM)
NC01				
D10S1248	FAM	F- TTAATGAATTGAACAAATGAGTGAG	1	1.20
		R- GCAACTCTGGTTGTATTGTCTTCAT	0	1.20
D14S1434	HEX	F- TGTAATAACTCTACGACTGTCTGTCTG	-11	1.30
		R- GAATAGGAGGTGGATGGATGG	0	1.30
D22S1045	NED	F- ATTTTCCCCGATGATAGTAGTCT	3	1.15
		R- GCGAATGTATGATTGGCAATATTTTT	6	1.15
NC02				
D4S2364	FAM	F- CTAGGAGATCATGTGGGTATGATT	2	1.30
		R- GCAGTGAATAAATGAACGAATGGA	-7	1.30
D2S441	HEX	F- CTGTGGCTCATCTATGAAAACTT	0	1.00
		R- GAAGTGGCTGTGGTGTTATGAT	0	1.00
D1S1677	NED	F- TTCTGTTGGTATAGAGCAGTGTTT	0	1.30
		R- GTGACAGGAAGGACGGAATG	0	1.30
NC03				
D20S482	FAM	F- GAGACACCGAACCAATAAGAGA	-2	0.23
		R- GCCACATGAATCAATTCCTATAATAAA	4	0.23
D3S3053	HEX	F- TGATAATGAACCCACTCAGATAGA	22	1.30
		R- GTGAGGTCTTTGCTCTCATGAAT	-6	1.30
D6S474	NED	F- GGTTTTCCAAGAGATAGACCAAT	1	0.25
		R- GCCTCTCATAAATCCCTACTCATATCT	6	0.25

Multiplex PCR of new miniSTRs

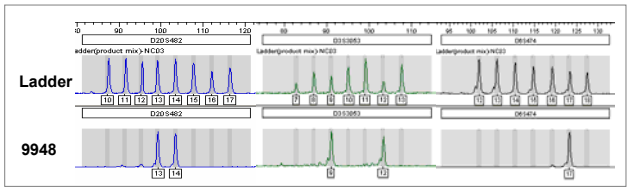
NC01



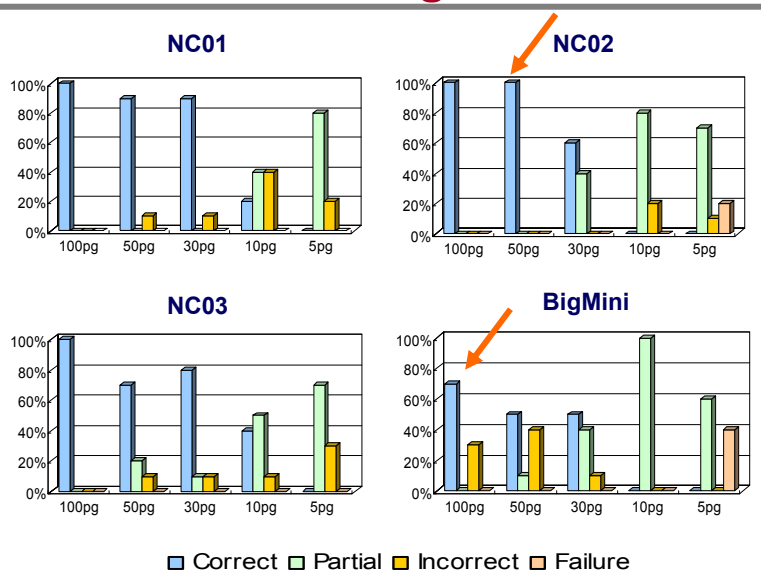
NC02



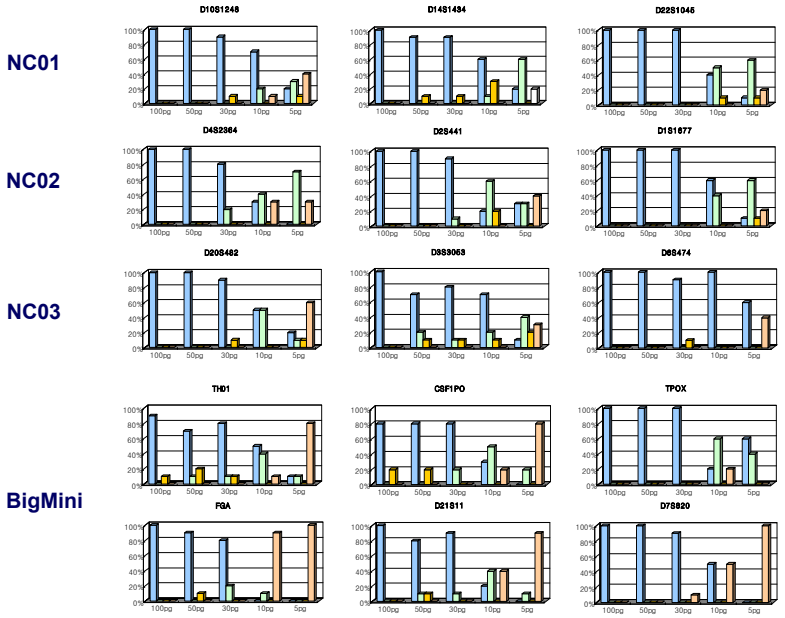
NC03



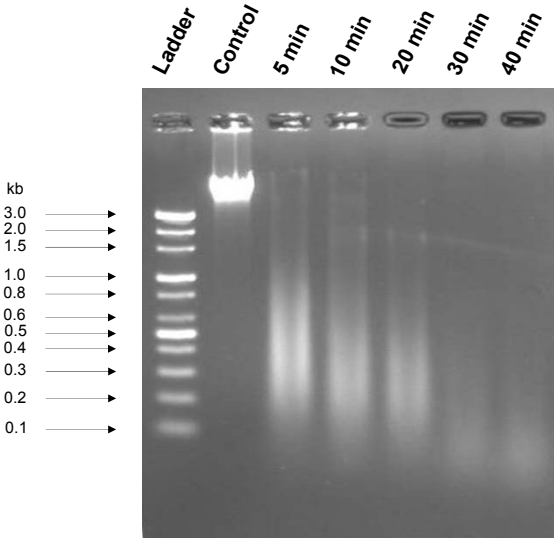
Sensitivity comparison between NCs and BigMini



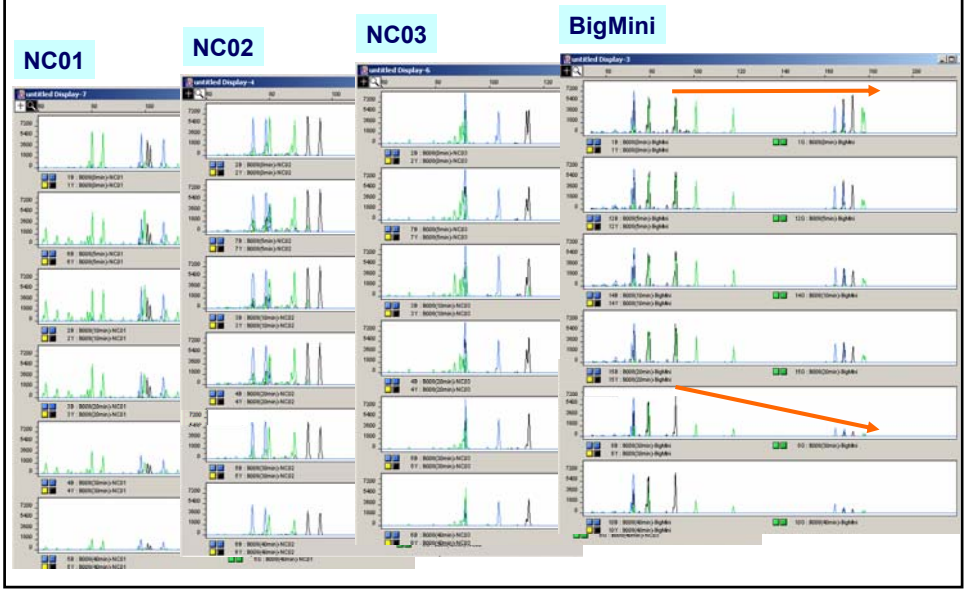
Locus-specific sensitivity comparison



Gel electrophoresis pattern of enzymatically degraded DNA



PCR amplifications of NCs and BigMini with artificially degraded DNA



PCR success rates of NCs and BigMini with DNA from 30 old skeletal remains

Marker	Multiplex Set	Consensus profile	Profile with drop in	Failure
D4S2364	***NC02***	**26 (86.7%)**	0 (0.0%)	4 (13.3%)
D3S3053	***NC03***	**24 (80.0%)**	2 (6.7%)	4 (13.3%)
D14S1434	***NC01***	**21 (70.0%)**	0 (0.0%)	9 (30.0%)
D1S1677	***NC02***	**20 (66.7%)**	0 (0.0%)	10 (33.3%)
TPOX	BigMini Set 1	18 (60.0%)	6 (20.0%)	6 (20.0%)
D2S441	NC02	18 (60.0%)	2 (6.7%)	10 (33.3%)
D10S1248	NC01	17 (56.7%)	0 (0.0%)	13 (43.3%)
D20S482	NC03	17 (56.7%)	1 (3.3%)	12 (40.0%)
D6S474	NC03	15 (50.0%)	0 (0.0%)	15 (50.0%)
TH01	BigMini Set 1	12 (40.0%)	0 (0.0%)	18 (60.0%)
CSF1PO	BigMini Set 1	9 (30.0%)	0 (0.0%)	21 (70.0%)
D22S1045	NC01	3 (10.0%)	0 (0.0%)	27 (90.0%)
D7S820	BigMini Set 2	0 (0.0%)	0 (0.0%)	30 (100.0%)
D21S11	BigMini Set 2	0 (0.0%)	0 (0.0%)	30 (100.0%)
FGA	BigMini Set 2	0 (0.0%)	0 (0.0%)	30 (100.0%)

Allele frequency distributions of NC01, NC02 and NC03 STRs in Koreans

Allele	D1S1677	D2S441	D3S3053	D4S2364	D6S474	D10S1248	D14S1434	D20S482	D22S1045
7			0.003						
8		0.002	0.008	0.030					0.185
9	0.008	-	**0.368**	0.208					0.003
10	0.003	0.235	0.090	**0.445**				0.017	0.003
11	0.013	**0.375**	0.367	0.315				0.010	0.005
11.3		0.028							
12	0.125	0.185	0.153	0.002		0.005		0.048	**0.308**
13	**0.472**	0.038	0.010		0.005	0.082	0.002	0.270	0.263
14	0.318	0.122			**0.357**	**0.345**	0.127	**0.453**	0.205
15	0.050	0.015			0.347	0.245	0.168	0.153	0.025
16	0.010				0.127	0.210	0.045	0.045	0.002
17					0.100	0.088	0.262	0.003	
18					0.063	0.025	**0.370**		
19					0.002		0.027		

Statistical parameters

	D1S1677	D2S441	D3S3053	D4S2364	D6S474	D10S1248	D14S1434	D20S482	D22S1045
p-value[a]	0.124	0.000	0.508	0.053	0.883	0.599	0.161	0.252	0.985
Obs-H[b]	0.657	0.620	0.737	**0.607**	0.737	**0.783**	0.770	0.660	0.760
Exp-H[c]	0.659	0.754	0.702	**0.660**	0.725	**0.763**	0.745	0.695	0.761
PD[d]	**0.824**	0.902	0.842	0.825	0.879	0.900	0.888	0862	**0.901**
PIC[e]	0.600	0.720	0.640	**0.590**	0.680	**0.720**	0.710	0.650	**0.720**
MEC[f]	0.402	0.535	0.444	**0.383**	0.486	**0.545**	0.525	0.456	0.526

[a] HWE p-values
[b] observed heterozygosity
[c] expected heterozygosity
[d] power of discrimination
[e] polymorphism information content
[f] mean exclusion chance

PD and MEC of CODIS and new miniSTRs

Power of Discrimination	Locus	Multiplex Set	**Mean Exclusion Chance**	Locus	Multiplex Set
0.996	FGA		0.846	FGA	
0.959	D18S51		0.829	D18S51	
0.954	D8S1179		0.816	D8S1179	
0.932	D13S317		0.774	D13S317	
0.929	D21S11		0.761	D21S11	
0.924	VWA		0.758	VWA	
0.921	D16S539		0.75	D5S818	
0.920	D5S818		0.576	D16S539	
0.915	D7S820		0.562	D7S820	
0.902	**D2S441**	*NC02*	0.545	**D10S1248**	***NC01***
0.901	**D22S1045**	***NC01***	0.535	**D2S441**	*NC02*
0.900	**D10S1248**	***NC01***	0.526	**D22S1045**	***NC01***
0.888	**D14S1434**	***NC01***	0.525	**D14S1434**	***NC01***
0.879	**D6S474**	*NC03*	0.486	**D6S474**	*NC03*
0.865	D3S1358		0.477	CSF1PO	
0.864	CSF1PO		0.456	D20S482	*NC03*
0.862	D20S482	*NC03*	0.452	D3S1358	
0.842	D3S3054	*NC03*	0.444	D3S3054	*NC03*
0.837	TH01		0.415	TH01	
0.825	D4S2364	*NC02*	0.402	D1S1677	*NC02*
0.824	D1S1677	*NC02*	0.386	TPOX	
0.811	TPOX		0.383	D4S2364	*NC02*

Conclusions

- We constructed three new miniplex sets of **NC01** (D10S1248, D14S1434, D22S1045), **NC02** (D1S1677, D2S441, D4S2364) and **NC03** (D3S3053, D6S474, D20S482).
- Most of 9 miniSTRs showed reliable genotyping results with **50 pg** DNA and some had good sensitivity even with **30 pg** DNA.
- **D4S2364**, **D3S3053**, **D14S1434** and **D1S1677** produced more useful STR profiles for degraded samples.
- Population study demonstrated that **D10S1248, D2S441, D22S1045, D14S1434 and D6S474** are as highly informative as CODIS STRs.