Development of novel YSTR assays for application to genealogical research (male lineage reconstruction).

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Megan Marie Shuey

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Dr. Hans-Werner Herrmann
Arizona Research Laboratories

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Development of novel YSTR assays for application to genealogical research (male lineage reconstruction).

Megan Marie Shuey

Undergraduate Research Assistant

Human Origins Genotyping Laboratory

Abstract

I developed multiplex polymerase chain reaction (PCR) assays utilizing 22 Y chromosome short tandem repeats (STRs) to be used in male lineage reconstruction. The PCR consists of two panels containing a combination of previously described DYS and DYF markers: DYS462, DYS476, DYS480, DYS494, DYS497, DYS580, DYS568, DYS569, DYS583, DYS632, DYS709, DYS453, DYS488, DYS491, DYS508, DYS525, DYS540, DYS567, DYS575, DYS618, DYS721, and DYF390s1. I tested these multiplexes on a global panel compiled of samples from the major Y chromosome haplogroups to determine allelic range, frequency, and diversity. Only one marker, DYS569, was monomorphic with the other marker diversities ranging from .178 to .816. These markers should prove useful for genealogic reconstruction.

Introduction

The earliest experiments of genetic inheritance came from Gregor Mendel's pea plant cross breading (Mendel 1865). At the time of his experimentation the specific units of genetic inheritance had not been described but were proposed to be genes. For many years genes

were suspected to be part of proteins, however, Oswald Avery's 1944 experiments on Pneumococcus verified the gene's relation to DNA (Avery et al 1944). His experiments using R and S strain Pneumococcus showed that removal of proteins from the S strain bacteria using protease and their subsequent addition to a mixture of R strain remnants could still induce R strain transformation to S. The experiment was repeated using deoxyribonuclease instead of protease to remove DNA in the S strain. The result of this experimentation was: no transformed R strain remnants indicating that DNA is the necessary component for genetic transformation (Avery et al 1944).

Acknowledgment of DNA's role in cellular inheritance resulted in additional research into its structure. The structure of DNA was proposed in1953 as helical with two twisting chains formed by a phosphate-sugar backbone and interior connections made through nucleotide bonding (Watson and Crick 1953). Four nucleotide bases; adenine, guanine, thymine, and cytosine vary repetition in combination to create the genetic instruction for all living organisms. DNA is passed on from parents to offspring in two forms: mitochondrial DNA (mtDNA) from only the mother and nuclear DNA. Nuclear DNA is located in the nucleus of cell. It is inherited from both the mother and father and combines as chromosomes. In humans, chromosomes come in two forms; the autosomal, non-sex linked chromosomes that come in pairs, and the allosome or sex-linked chromosome. The allosome is denoted by X or Y. Females have two copies of the X chromosome denoted XX and males have a copy of the X and Y chromosome denoted XY.

As DNA is replicated mutations from recombination, addition, and deletion of nucleotides can occur. The inheritance pattern of DNA makes it a useful tool for ancestral research, such as forensic and genealogic testing. In forensic testing, DNA is used to create

a fingerprint of individuals using a group of unique loci where the likelihood of another individual sharing the same unique combination is minuscule. Genealogic testing is a way for individuals to trace their ancestry from their most recent to most distant relatives by looking at common mutations within a group of loci that are found within individuals of a particular haplotype- a combination of alleles forming a group on a particular chromosome that are inherited as an unit.

Genetic genealogic testing found its origins in the profiling experiments performed by Sir Alec Jeffreys in the 1980s. Jeffrey's experiments came from a realization of genetic similarities in the DNA of family members. His recognition of tandem repeats "minisatellite" regions proved to be highly variable and can provide an individual "fingerprint" (Jeffreys et al 1985). This fingerprinting technique commonly used in forensics was modified for early genealogic testing. A partnership between Dr. Karl Skorecki and Dr. Michael Hammer who's experiment to determine if the Kohanim Jews were descendents from a single man (Skorecki et. al 1997) revolutionized the field by focusing their research on the Y-chromosome. The field grew slowly until Bryan Sykes's publication of "The Seven Daughters of Eve" which brought the foundation of the seven major haplogroups of mtDNA to the public (Sykes 2001). The book shone light on the growing field of genealogic testing as well as provided support for the methodology that established the public market for genealogic testing companies.

Family Tree DNA (FTDNA) was the first genealogic testing facility in the United States. It's origins lies in the work of Dr. Michael Hammer of the University of Arizona whose surname research of the Y-chromosome appealed to Bennett Greenspan, a genealogist from New York. Greenspan was interested in testing his own lineage and

Matthew Kaplan, a research assistant in Dr. Hammer's lab, preformed the initial testing using twelve Y-STR markers on 25 samples obtained from Greenspan of his assumed relatives. The results spurred Greenspan to found FTDNA; a company who provides genetic testing to their clients to create genetic family trees. The genetic testing originally preformed by M. Kaplan at the University of Arizona resulted in the formation of the Human Origins Genotyping Laboratory (HOGL) at the University of Arizona. Today HOGL tests for 67 Y-STR markers through FTDNA as well as mitochondrial DNA sequencing.

Currently, multiple other public genealogic testing companies exist all around the world including Sorenson Genetics, DNA Heritage, and Oxford Ancestors which test for over 100 different Y STR markers. In 2005 Family Tree DNA and the University of Arizona partnered with National Geographic and IBM for the "Genographic Project" . The Genographic Project is a five-year project established to map the genetic history of modern human origins and migratory patterns. This has brought further exposure to the utility of using DNA to trace our ancestral past.

While all DNA is inherited, regions of DNA that recombine are not necessarily useful tools for genealogical testing. The X-chromosome and autosomes have multiple ancestors due to recombination while Y-chromosome DNA has only one paternal ancestor. MtDNA, likewise, does not recombine and is inherited only from the mother making it useful for testing matrilineal past. Genetic testing can be preformed on Y DNA through observation on single nucleotide polymorphisms (SNPs) or microsatellites or short tandem repeats (STRs). Single nucleotide polymorphisms represent small changes that occur in an individual's genetic sequence resulting in a single nucleotide replacement, e.g. C replaces

T. In 2003 the Ensemble database listed approximately 28,650 Y specific SNP's, however the difficulties in determining whether a SNP is based on an inheritance mutation or a remnant from recombination with a different chromosome has resulted in questioning of their validity (Jobling, Tyler-Smith 2003).

Microsatellites or short tandem repeats (STRs) have faster mutation rates corresponding to more recent common ancestry. As such, they have become the common tool for genealogic testing. STRs located on the Y-chromosome contain nucleotides that occur in a repeating pattern or motif. STRs typically have motifs of 2-6 nucleotides that are labeled as di-, tri-, tetra-nucleotide based on the number of nucleotides within a given motif; with dinucleotides containing a two nucleotide motif (AT) or a trinucleotide containing a three nucleotide motif (ATA). Their repeat range is also determined to provide length estimates for testing. STR variability differs amongst loci based on the repeat length and nucleotide repeat within the motif. A comprehensive survey of the Y chromosome resulted in the identification of at least 417 STR loci that may be informative for genealogic testing (Hanson, Ballantyne 2005).

STR motifs can be simple, complex, or compound in form (Butler et al 2008). Simple motifs are STRs whose nucleotide pattern occurs in a consecutive order; such as, ATA ATA ATA with the ATA representing the motif repeated three times. A compound STR motif is a motif made up of adjacent repeating nucleotide patterns of the same length; such as, ATA ATA ATA TTA TTA TTA with $(ATA)_n(TTA)_n$ representing the motifs . A complex STR motif consists of nonconsensus repeated nucleotides of varying length; such as, ATA ATA ATA TTA TTA TTA AC AC AC with $(ATA)_n(TTA)_n(AC)_n$ representing the motifs. Motifs may also contain non-variable or non-repetitive parts of the sequence

between regions of an identical motif; such as, ATA ATA ATA CGTGCTG ATA ATA ATA where the sequence is $(ATA)_nN_n(ATA)_n$. The motif can vary from two to six nucleotides in length (Ayub et al 2000). The number of motifs varies amongst individuals due to mutation. On average, in every 500 generations a mutation will occur that adds or subtracts one of the repeating units (Walsh 2001). The estimated average mutation rate per generation is $2x\ 10^{-3}$ (Zhivotovsky et al 2004). The described regions of nucleotide repeats, called markers or loci, are used in combination for genealogic testing. The combined state of multiple STRs adds power to predicting haplotypes (Jobling, Tyler-Smith1995).

A multiplex polymerase chain reaction (PCR) combines multiple STR markers in to a single reaction that allows for multiple regions DNA to be amplified simultaneously. This process has streamlined genetic testing however it remains a greater challenge than uniplexing because each primer must work under competitive conditions for results to be observed (Butler et al 2003). PCR works by the denaturation of DNA by temperature and the building of complementary DNA strands at a specific region or locus. This occurs by binding pre-established primers at the beginning and end of the DNA, the 3' or 5' end, determined region and the subsequent addition of nucleotides by a polymerase to create the complimentary DNA strand. The development of a standardized multiple marker PCR is thus the corner stone of genealogic testing. To maintain consistency and control for errors the Human Origins Genotyping Laboratory performs multiplex testing on standard reference material (SRM) and universally distributed cell line controls (Coriell Institute, NJ) so resultant information can be standardized amongst companies and projects. The desire for standardization is further emphasized by the increase in information sharing amongst laboratories (Butler et al 2008).

With the support of the Human Origins Genotyping Laboratory my project was to create a new multiplex for their genetic testing service partnership with Family Tree DNA. The initial goal of the project was to increase the number of tested Y-STR markers within the laboratory from 67 to 100.

Materials and Methods

Loci Determination

I compiled candidate Y STR loci based on use by genealogic research companies and availability of information in published literature (Butler et al. 2008, Kayser et al. 2004, Leat et. al, Genome Data Base, AAAT-DYS453, SRM 2395, Huang et al. 2001, Hao et al. 2006, as well as unpublished sources). I removed markers currently used in the Human Origins Genotyping Laboratory genealogic testing from the candidate loci list. I collected information on candidate markers including previously determined forward and reverse primer sequences, repeat motif, repeat pattern, repeat variance, length, and diversity. I determined the expected amplicon length by NCBI-BLAST if not available in literature. I ordered unlabeled forward and reverse primers for all loci. I ordered reverse primers with the addition of a pigtail sequence, GTTTCTT, at the 5' end to encourage taq's adenylation of the 3' end of the forward sequence (Brownstein et al 1996).

Loci Optimization

I determined optimal PCR conditions for all loci using a temperature and MgCl gradient (Figure 1). I preformed optimization PCRs to determine the range of reaction conditions for which each locus could be used to create multiplexes. I made a master mix

containing .10 units platinum taq polymerase (Invitrogen, Inc., Carlsbad, CA), dNTPs (10mM), and primers (.4M) forward and reverse. Mini master mixes containing DNA were made in the wells in the first row of the PCR plate with variable $MgCl_2$ concentrations. $MgCl_2$ concentrations were varied from 1.0-4.5 mM with constant tris base 100mM pH 8.3 and KCl. I ran the completed PCR plate in a DNA Tetrad 2 thermocycler (BioRad, Inc., Hercules,CA) with a gradient profile of 35 cycles of three minutes 94°C (initial denature), 30 seconds at 94°C (denature), 30 seconds at an extension temperature gradient between 50° and 62° C (annealing), 30 seconds at 72° C (extension), and 3 minutes at 72° C (final extension).

$MgCl_2$ (mM)	Temps (°C) 50.0	50.3	51.0	52.0	53.4	55.1	57.2	58.9	60.2	61.1	61.8
1.0											
1.5											
2.0											
2.5											
3.0											
3.5											
4.0											
4.5											

Figure 1 Optimization PCR Layout: From the first row's master mixes the remaining plate was filled using a serial addition procedure. I designed the 96-well PCR plate temperature gradient and buffer concentration optimization to remove three columns so as optimized for gel electrophoresis steps that will follow.

I visualized PCR products using a 2% agarose gel and determined ideal conditions based on amplification of a single band within the targeted length range. I considered conditions resulting in multiple bands or suspect amplicon length were considered to likely represent mispriming. If a gel exhibited no resultant bands the PCR was reran or the gel was reran with larger volume of PCR product.

Multiplex PCR Optimization

I compiled the data collected from loci specific optimization PCRs to determine the range of conditions for which each marker amplified. I used extension temperatures and MgCl concentration variances to compile possible multiplex combinations. I also ranked the marker's utility based on their repeat pattern (simple/complex) and published diversity. I ranked markers with little or no diversity lower priority and I ranked markers with trinucleotide repeats or higher and with simple motifs greater priority because of their decreased ambiguity in scoring by fragment analysis. I also gave preference to markers based on their current use by genealogical testing services other than FTDNA. The markers were ranked in to four categories of utility with any marker outside of these rankings being dropped from further testing.

I matched the markers in the top four rankings with others indicating the same ideal temperature and buffer conditions from the compiled data (Appendix2). I gave preference to PCR conditions yielding the majority of the markers over those that yielded fewer. I laid out all of the markers determined to have worked under the chosen condition by base pair range on a spreadsheet to determine possible overlap in fragment analysis. I labeled markers that overlapped in range with a different fluorescent dyes for differentiation in fragment analysis. I ordered the forward primers for chosen loci from Applied Biosystems with the addition of a specific fluorescent dye to the 5' end- NED, HEX, FAM-6, or VIC.

Multiplex Development

I first ran the determined loci for a given multiplex as uniplex PCRs on 32 samples including the SRMs A-E (NIST, Gaithersburg, MD), 26 Coriell Cell lines (Coriell Institute, NJ), and a female sample and water as controls (Table 1). This initial uniplexing was used to verify locus variability. Loci with observed variability were then combined within an initial multiplex with .4 M forward and reverse primers. Starting with the same concentration of forward and 5' labeled pigtailed reverse primers for all markers allowed for a level base for future multiplex PCR optimization. I designed a 10µl PCR master mix containing .10 units platinum taq polymerase (Invitrogen, Inc., Carlsbad, CA), dNTPs (10mM), MgCl buffer containing KCL and tris base 100mM pH 8.3 and loci specific primers (.4M) forward-labeled and reverse to be added to 10 ng of DNA from 32 samples. Temperature extension and $MgCl_2$ buffer concentration varied between the three multiplexes. I submitted PCR product to University Arizona Genomic Core (UAGC) for fragment analysis on the 3730 DNA Analyzer (Applied Biosystems, Inc, Foster, CA) and I analyzed results using GeneMarker V1.71 (SoftGenetics, Llc, State College, Pa).

Sample Name	Population	Y-Haplogroup
SRM_A	Caucasian	R-M207
SRM_B	Caucasian	J2-M172
SRM_C	African American	E3a-M2
SRM_D	African American	G-M201
SRM_F	Female Caucasian	n/a
TAY	Male North American	I-1B
GM03043	!Kung	A2
JK736	Biaka Pygmy	B2b4b
JK741	Biaka Pygmy	B2b4b
JK1029	Mbuti Pygmy	E2b1
JK1031	Mbuti Pygmy	B2b
JK1033	Mbuti Pygmy	E3a
JK965	Melanesian	K1
JK971	Melanesian	M2a
JK1370	Karitiana tribe	Q3
JK2935	Atayal (Aboriginal Tribe From Taiwan)	C3
JK3159	Adygei (Russian)	G2
JK3168	Adygei (Russian)	J2
JK3490	Adygei (Russian)	R1a1
JK3496	Russian-speaking Russian	R1b1c
JK3517	Russian-speaking Russian	I1b
MK	Male North American	J2
BARB	Female North American	n/a
H20	n/a	n/a

Table 1: SRM and Coriell Cell line Haplogroups

The process of PCR and analysis allowed for primer concentration adjustments. I ran successive trial multiplex PCRs until I observed all markers at a relative fluorescent unit (RFU within in 500-5000) without magnification. I adjusted primer specific concentrations based on the amplification of the targeted peak in relation to the others in subsequent PCRs. I also assessed the primer concentration to account for noise in the electropherogram profile from mispriming and formation of primer-dimers due to the primers of multiple loci annealing to each other in the reaction. I adjusted PCR conditions by adding or removing specific primer sets or adjusting primer concentrations to balance the reaction (Figure 2).

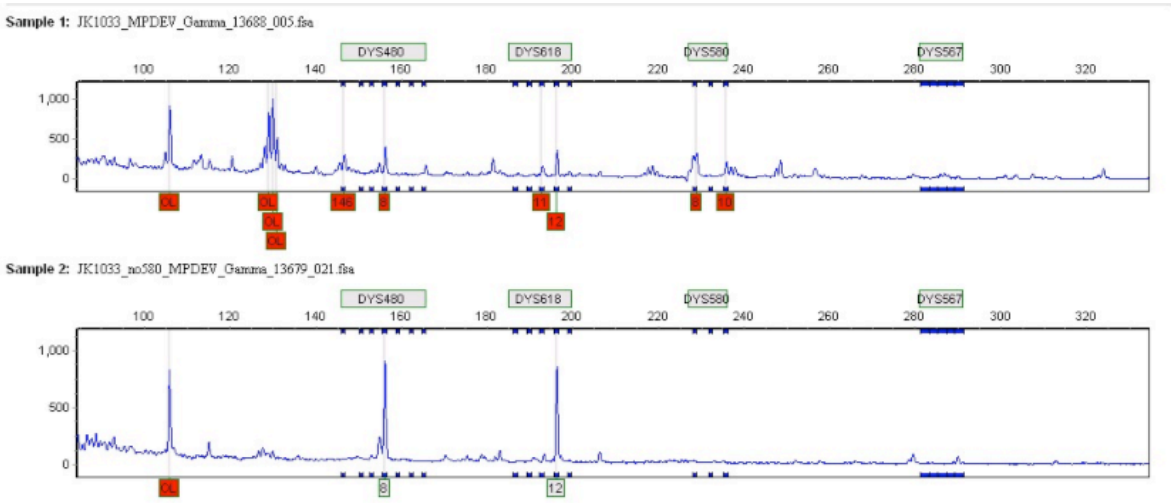

Figure 2 Blue Lane Genemarker Data for sample JK1033: Sample one contains data for JK1033 and shows a lot of peaks throughout the lane indicative of primer mispriming. Sample two contains data for JK1033 with primers for DYS580 removed. With the primer removed fewer mispriming peaks are observed with less amplitude than with its presence indicating the primers for DYS580 interact within the PCR mixture resulting in mispriming.

Kit specific PCR testing

I also used a Qiagen Multiplex PCR kit (Qiagen, Inc., Valencia, CA) simultaneously with initial multiplex development for a comparison of results. Primers for

this multiplex were same as aforementioned. The multiplexes using the kit followed standard protocols and were run with and without the provided Q-solution.

Sequencing

I ran each marker as a uniplex with unlabeled primers on the SRM samples plus three in house samples to determine repeat length and verify repeat motif. I submitted the PCR product to the University of Arizona Genomic Core (UAGC) to be run on an AB Prism 3730 XL DNA Analyser (Applied Biosystems, Inc, Foster, CA) to generate sequence data for each locus. I analyzed results using Sequence Navigator 1.0.1 (Applied Biosystems, Inc, Foster, CA).

Descriptive Statistics

I used the results from the uniplex PCRs to create bins for scoring on GeneMarker V1.71 (SoftGenetics, Llc, State College, Pa) that reflected the repeat ranges determined by confirmation of sequence data. I ran the multiplex PCRs on a global panel of 24 samples including a water and female control sample to determine the frequency of the alleles. The marker scores from the test panel were used to determine allele frequency and gene diversity. I determined Allele frequency by counting the number of repeats provided by the sequence data. Allele diversity was calculated using the equation: (Nei, 1987)

$$h = n\frac{\left(1 - \sum x^2\right)}{n-1}$$

h is the gene diversity, n is the number of individuals, and x is the allele frequency in the tested population sample.

Standard deviations for the marker diversity were calculated using the equation: (Nei, 1987)

$$S.E. = \left\{ 2 \frac{\left\{ \sum x^3 - \left(\sum x^2 \right)^2 \right\}}{n} \right\}^{1/2}$$

S.E. represents the standard errors or standard deviation, *x* is the allele frequency and *n* is the number of individuals.

Results

Loci Determination

I collected information of the initial 65 markers including previously determined forward and reverse primer sequences, repeat motif, repeat pattern, repeat variance, length, and diversity. (Appendix 1)

Loci Optimization

PCR products were isolated for the markers using a 2% agarose gel that was analyzed to determine what conditions worked best for individual markers (Figure 3). From the gels I determined optimal PCR conditions for all loci based on temperature and $MgCl_2$ gradients visualized on a 2% agarose gel (Appendix 2).

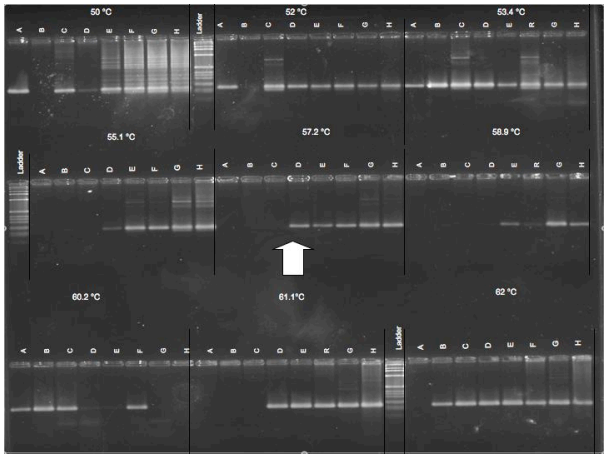

Figure 3: DYS408 Optimization Gel
Using the ladders located column 9 row 1, column 1 row 2, and column 17 row 3, single bands located in the 200 bp range were visualized on a 2% Agarose gel. Each column represented a specific buffer concentration and annealing temperature. Columns possessing a single band were determined to represent ideal PCR conditions for this marker (arrow).

Multiplex PCR Optimization

I ranked the markers in to four categories based on utility (Appendix 1). I gave PCR conditions yielding the majority of the markers preference in multiplex panel condition development. I aligned desired markers in a single PCR by expected allelic range with overlapping markers labeled with different fluorescent dyes for fragment analysis (Figure 4).

Figure 4: Loci position and dye lanes

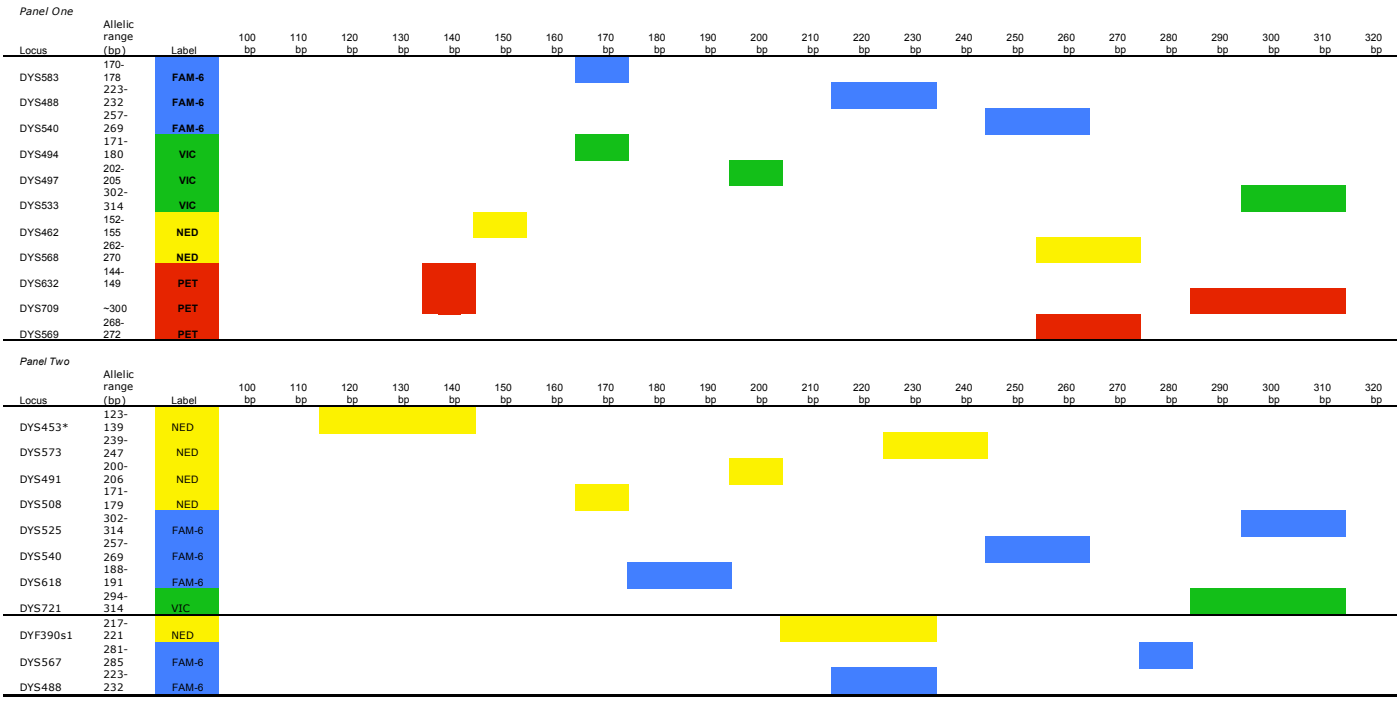

Figure 4: The 22 chosen loci laid out by base pair in the two subsequent PCR panels. The loci show bp range and the chosen fluorescent dye lables.

Multiplex Development

The loci were initially divided in to three PCR multiplexes that were further divided in to two panels of 22 markers that are novel to HOGL and Family Tree DNA. The first panel is run using a 1.5 mM $MgCl_2$ Buffer solution at an annealing temperature of 57°C. The second panel consists of two multiplexes the first run with the Qiagen kit, Q solution and an annealing temperature of 57°C with the second multiplex of three primers

run with a 1.0 mM $MgCl_2$ and an annealing temperature of 60°C (Appendices 3 and 4). The PCR products of the two multiplexes for the second panel are combined post PCR for submission and run through fragment analysis.

I used the determined scores of the final 22 markers run on a global population test panel to determine allelic range, allele frequency (Appendix 5) and allele diversity (Tables 2 and 3).

Marker Name	Dye Lane	Repeat Motif	Allelic Range	Diversity	S.E.
DYS462	NED	(TATG)	8-12	0.256	0.104
DYS476	VIC	(TGA)	11-12	0.178	0.086
DYS480	VIC	(TTA)	7-10	0.449	0.098
DYS494	VIC	(TAT)	8-10	0.357	0.103
DYS497	VIC	(TTA)	13-16	0.622	0.047
DYS530	NED	(AAAC)	139-144*	0.255	0.097
DYS568	NED	(AAAT)	10-13	0.541	0.093
DYS569	PET	(ATTT)	11**	0	0
DYS583	FAM-6	(AAAC)	8-9	0.245	0.095
DYS632	PET	(CATT)	8-9	0.261	0.095
DYS709	PET	(CTTT)	9-17	0.816	0.03

Table 2: Panel One consists of 11 markers. **DYS569 showed no diversity in the test panel however there was multiple dropout samples for this marker. *DYS530 diversity, frequency, and standard of error (S.E.) calculations were done based on approximated allelic range values as sequencing at time of submission had not been determined.

Marker Name	Dye Lane	Repeat Motif	Allelic Range	Diversity	S.E.
DYF390s1	NED	(TTTA)	8-11	0.345	0.059
DYS453*	NED	(AAAT)			
DYS488	FAM-6	(ATA)	11-13	0.458	0.036
DYS491	NED	(ATA)	12-14	0.426	0.081
DYS508	NED	(TATC)	9-14	0.691	0.046
DYS525	FAM-6	(AGAT)	10-15**	0.679	0.056
DYS540	FAM-6	(TTAT	11-13	0.71	0.041
DYS567	FAM-6	(ATAA)	10-12	0.518	0.045
DYS573	NED	(TTTA)	9-12	0.66	0.04
DYS618	FAM-6	(TAT)	10-13	0.493	0.085
DYS721	VIC	(AAGGG)N(AAGGG)N(AAGCA)	9-11	0.632	0.026

Table 3: Panel 2 consists of 11 markers. DYS453* was not sequenced prior to submission so allelic range, diversity, and s.e. values were not available. **DYS 525 sequence data showed an alternative motif nomenclature; according to NIST guidelines the motif should be noted as TAGA not AGAT. Microalleles were found for and were counted as the allele higher or lower depending if it was scored a .1 or .3. DYF390s1 was treated as a diploid for its diversity and frequency estimates.

Sequencing

At the time of submission three of the markers had incomplete data to determine repeat range scores. While, specific repeat range could not be determined, the data provided by GeneMarker V1.71 (SoftGenetics, Llc, Stat College, Pa) binning was used to determine allele frequency and gene diversity.

Discussion

At the time of submission, only results for global test panels had been completed. Further experimentation using closely related relatives is necessary to determine the multiplexes' usefulness in distinguishing lineage amongst individuals of a closely related population. The diversity and frequency of the alleles thus represent those corresponding to global populations.

I was able to compile a list of approximately 100 loci from published papers providing diversity estimates and primer sequences for these markers. Kayser's 2004 paper provided a substantial amount of the Y-STRs for initial testing. From these loci I ran uniplexes to flush out desired loci conditions. I analyzed the loci based on criteria deemed most important for genealogic analysis. The criteria was based on standard preferences for genealogic research such as diversity as well as HOGL preferences based on experience in multiplex production as well as analysis such as preference to simple motifs for their ease in data analysis. Ultimately, I combined 22 loci in to two panels with allele diversity ranging from .178 to .816.

Due to time constraints I was unable to ascertain additional data concerning DYS569 to determine if its monomorphic appearance is accurate or if the results were

based on a too limited population analysis. Similarly, I was unable to provide allelic range and diversity results for DYS453 due to poor sequence data and unreliable pherogram results at time of submission. For both of these markers additional testing is required to determine their value in genealogic testing.

The increasing public interest in genealogic research has resulted in a growing field of research. This expansion is at the heart of my research. It is my hope that these additional multiplexes can be combined with the current multiplexes run by genealogic research companies to create a larger base of information for haplogroup assignment and origin studies.

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Appendix

Appendix 1:Initial 65 Markers' Information

Loci	Primer	Motif		Nucleotide motif	Length (bp)	Competitor (y/n)	Ranking	Literature
DYF406S1	FWD:CCTGGGTGACACAGTGAGACT REV:gtttcttTCCACCAAAATTCCATGACA	(TATC)10**	simple	tetranucleotide	226-241	N	1	Kayser
DYS525	FWD:ATTCACACCATTGCACTCCA REV:gtttcttCCATCTGTTTATCTTCCCATCA	(AGAT)9-12	simple	tetranucleotide	302-314	N	1	Kayser
DYS540	FWD:GACCGTGTACTCTGGCCAAT REV:gtttcttCAGGAGGCTAGCTCAGGAGA	(TTAT)10-13	simple	tetranucleotide	257-269	N	1	Kayser
DYS508	FWD:ACAATGGCAATCCCAAATTC REV:gtttcttGAACAAATAAGGTGGGATGGAT	(TATC)11**	simple	tetranucleotide	171-179	N	1	Kayser
DYS573	FWD:GGGGGAGAAAAAGTTTGGTG REV:gtttcttAAAAATGGGGAGGTGGAAAT	(TTTA)9-11	simple	tetranucleotide	239-247	N	1	Kayser
DYS488	FWD:GGGGAGGGATAGCATTAGGA REV:gtttcttTACCCTGGTCCACTTCAACC	(ATA)13-16	simple	trinucleotide	223-232	N	1	Kayser
DYS618	FWD:CCCATACCCTTGGTGTTGTC REV:gtttcttGAGGGCTATGGGAGGGATAG	(TAT)11-12	simple	trinucleotide	188-191	N	1	Kayser
DYS567	FWD:GGAAGCTGAGGAAGGAGGAG REV:gtttcttTTATGACCGGGATCAAGTGC	(ATAA)10-11	simple	tetranucleotide	281-285	N	1	Kayser
DYS497	FWD:AACATGTGCGTTTTCAACCA REV:gtttcttGCATGTTGTGCACATGTAACC	(TTA)14-15	simple	trinucleotide	202-205	N	1	Kayser
DYS643	FWD:AAGCCATGCCTGGTTAAACT REV:gtttcttTGTAACCAAACACCACCCATT	(CTTTT)8-13	simple	pentanucleotide	132-159	Y	2	Kayser
DYS589	FWD:CATCCACATTGTTGCAAAGG REV:gtttcttTGACGAGTTAGTGGGTGCAG	(TTATT)13**	simple	pentanucleotide	271-286	Y	2	Kayser
DYS522	FWD:CCTTTGAAATCATTCATAATGC REV:gtttcttTCATAAACAGAGGGTTCTGG	(ATAG)9-13	simple	tetranucleotide	350-366	Y	2	Kayser
DYS495	FWD:CCCAGCTATTCAGGAGGTTG REV:gtttcttGCCAGAAAGTGTGAGTCATCC	(AAT)14-17	simple	trinucleotide	211-220	Y	2	Kayser
DYS556	FWD:TGCTGTCACATCACCAATGA REV:gtttcttTTTGGTTGCTGAAGCATTGA	(AAAT)8-12	simple	tetranucleotide	198-214	Y	2	Kayser
DYS505	FWD:TCTGGCGAAGTAACCCAAAC REV:gtttcttTCGAGTCAGTTCACCAGAAGG	(TCCT)10-13	simple	tetranucleotide	164-176	Y	2	Kayser
DYS549	FWD:AACCAAATTCAGGGATGTACTGA REV:gtttcttGTCCCCTTTTCCATTTGTGA	(GATA)10-14	simple	tetranucleotide	229-245	Y	2	Kayser
DYS485	FWD:AAAGCAGACTTCGCCACTACA REV:gtttcttAAAAATTAGCTGGGCCTGGT	(TTA)12-16	simple	trinucleotide	270-282	Y	2	Kayser
DYS533	FWD:CATCTAACATCTTTGTCATCTACC REV:gtttcttTGATCAGTTCTTAACTCAACCA	(TATC)9-12	simple	tetranucleotide	302-314	Y	2	Kayser
DYS638	FWD:ACAATTTCCCTTGGGGCTAC REV:gtttcttCATGGTGGTAGGCACCTGTA	(TTTA)10-12	simple	tetranucleotide	256-264	Y	2	Kayser
DYS494	FWD:TTGCAACACTGTTCATTTGGA REV:gtttcttAACAAACCTGCATGTTCTTCAA	(TAT)10**	simple	trinucleotide	171-180	Y	2	Kayser
DYS575	FWD:GGTGGTGGACATCCGTAATC REV:gtttcttAGTAATGGGATGCTGGGTCA	(AAAT)8-12	simple	tetranucleotide	215-231	Y	2	Kayser
DYS636	FWD:AATCCCATTGCATTTAGCAGA REV:gtttcttTGACACGTTAGTGGGTGCAG	(TTTA)9-12	simple	tetranucleotide	246-258	Y	2	Kayser
DYS461	FWD:AGGCAGAGGATAGATGATATGGAT REV:gtttcttTTCAGGTAAATTGTCCAGTAGTGA	(TAGA)11**	simple	tetranucleotide	178-186	Y	2	Kayser
DYS568	FWD:GTGGCAGACAAAACCCAGTT REV:gtttcttTTGAAAAGGGATGGGACTCA	(AAAT)10-12	simple	tetranucleotide	262-270	Y	3	Kayser
DYS436	FWD:CCAGGAGAGCACACACAAAA REV:gtttcttGCAATCCAACTTCAGCCAAT	(GTT)8-12	simple	trinucleotide	122-134	Y	3	Kayser
DYS434	FWD:CACTCCCTGAGTGCTGGATT REV:gtttcttCCCAGCTATTCAGGAGGTTG	(ATCT)9-11	simple	tetranucleotide	114-122	Y	3	Kayser
DYS462	FWD:TGTGCTGTACCAGTTGCCTA REV:gtttcttCCAGCCTGAGCAAGAGAGTA	(TATG)n	simple	tetranucleotide	152-155	Y	3	
DYS435	FWD:AGCATCTCCACACAGCACAC REV:gtttcttTTCTCTCTCCCCCTCCTCTC	(TGGA)11	simple	tetranucleotide	220	Y	4	Kayser
DYF385s1	FWD:CACCTCCAAATAAGTAAACATGGA REV:gtttcttTCTGCCAACTGAAAGGAAAAA	(TTA)10	simple	trinucleotide	204	Y	4	Kayser
DYS583	FWD:GCAGGAAAATTGCTTGAACC REV:gtttcttCCTCATCCAATAGCTCTTCCT	(AAAC)7-9	simple	tetranucleotide	170-178	N	4	Kayser
DYS476	FWD:CGACTATGATTTGGGCTGTG REV:gtttcttAGCTGGGAAGTACTCAATGCTC	(TGA)11**	simple	trinucleotide	118-124	N	4	Kayser
DYS491	FWD:GGAATGGGGAGGGATAACAT REV:gtttcttGGAGAAAATTCAATGCAGATACC	(ATA)11-13	simple	trinucleotide	200-206	N	4	Kayser
DYS569	FWD:TCCATGGGATATGATGAGCA REV:gtttcttGGCAGCCTGTAGGACAGAGA	(ATTT)10-11	simple	tetranucleotide	268-272	N	4	Kayser
DYF390S1	FWD:AGCATTCCCTTTCTCATTGC REV:gtttcttTGACGAGTTAGTGGGTGCAG	(TTTA)10**	simple	tetranucleotide	217-221	N	4	Kayser
DYS530	FWD:CAGGGTCAAAATCACCTTCC REV:gtttcttCTGCGGGACAATGAAACAC	(AAAC)9-10	simple	tetranucleotide	137-141	N	4	Kayser

Loci	Primer	Motif		Nucleotide motif	Length (bp)	Competitor (y/n)	Ranking	Literature
DYS580	FWD:GCAGTGAGCCGAGATCAGG REV:gtttcttGGAGCAAACACTGCAATTTCC	(AATA)8-10	simple	tetranucleotide	224-232	N	4	Kayser
DYS632	FWD:GGCCGTTGCAAAATAAACTG REV:gtttcttTCTGGGCAACAGAAGGAGAC	(CATT)8-9	simple	tetranucleotide	144-149	N	4	Kayser
DYF408	FWD:GAGGCCAGCCTGAGTAACAC REV:gtttcttATACAGTGGTGCCATCGTCA	(TATC)14**	simple	tetranucleotide	n. r.	Y	4	Kayser
DYF397	FWD:TTTCAGCCTTTTCAATTTCCA REV:gtttcttGACTGCTCAACTGCACTCCA	(TAT)14**	simple	trinucleotide	n.r.	Y	4	Kayser
DYS441	FWD:ATGTACCTGTAGCCCCAGTGAAC REV:gtttcttAAGTTGCAGTGAGCGAAGATTG	(CCTT)n	simple	tetranucleotide	338-362	Y	4	Kiesler
DYS716	FWD:TAAATCAGAATTCCTTTCCAATCCA REV:gtttcttTCTGGGTTTCAGAGTGGGATAATTT	(CACTC)6(CATTC)11	simple	pentanucleotide		Y	4	Decker
DYS452	FWD:CATTGGTGGTGTTCTGATGAGGATAAT REV:gtttcttGAGTTTTACATGATGTAGCAAATAGGTT	(YATAC)10	simple	pentanucleotide	226-267	Y	4	Leat
DYS445	FWD:AGTTAAGAGCCCCACCTTCCTG REV:gtttcttTTTGGTGGCATAATCTCAGCTC	(TTTA)n	simple	tetranucleotide		Y	4	Kiesler
DYS579	FWD:GCCAGCAGTAGACCCAGACT REV:gtttcttAGGCAGAGGTTGCAGTGAGT	(TATT)9**	simple	tetranucleotide	225-229	N	4	Kayser
DYS480	FWD:CCAGCACCTAGGTTGAGGTA REV:gtttcttCAGCACTCCAAAATGACAGA	(TTA)7-8	simple	trinucleotide	152-155	N	4	Kayser
DYS453	FWD:GGGTAACAGAACAAGACAGT REV:gtttcttCTAAAAGTATGGATATTCTTCG	(AAAT)n	simple	tetranucleotide	123-139	N	4	Geneloc
DYS709	FWD:TTTTTGCCTGCCATATTGGT REV:gtttcttTGACGAGTTAATGGGTGCAG	(CTTT)13-16	simple	tetranucleotide		N	4	SRM2395
DYF399	FWD:GGGTTTTCACCAGTTTGCAT REV:gtttcttCCATGTTTTGGGACATTCCT	(GAAA)20**	complex	tetranucleotide	n.r.	Y	4	Kayser
DYF401	FWD:TCGCAAACATAGCACTTCAG REV:gtttcttTTCTAGGAAGATTAGCCACAACA	(AAAG)13**	complex	tetranucleotide	n.r.	Y	4	Kayser
DYF411	FWD:TGTGTACTGGAGGAGACCATTTT REV:gtttcttGACTGTGTTTGCACTTTCACG	(CTTT)2(CCTTT)11**	complex		n. r.	Y	4	Kayser
DYS710	FWD:GAGGTCAAGGCTGCAAGAATCTATGA REV:gtttcttGCATACTCTCTCCCTCCCTCTCTTTTTTC	[AAAG]n [AG]n [AAAG]	complex		197-255	Y	4	Leat
DYS714	FWD:GTATTAGGCATCTTGCCAGC REV:gtttcttTTTTCTACCTATGATGCCCTTTG	[TTTCT]n[CTTCT]n[TTTCT]n [CTTCT]n[TTTCT]n	complex		152-205	Y	4	Leat
Y-GATA-A10	FWD:CCTGCCATCTCTATTTATCTTGCATATA REV:gtttcttATAAATGGAGATAGTGGGTGGATT	(TTTC)2(TCT)1(CCAT)2(ATCT)12**	complex		159-171	Y	4	Kayser
DYS717	FWD:GCCATGGATGGTGGTAGAAC REV:gtttcttTTGTTCAGGTGAGGAATATGCT	TGTAT [TGTAC]n [TGTAT]n	complex			Y	4	SRM2395
DXYS156	FWD:GTAGTGGTCTTTTGCCTCC REV:gtttcttCAGATACCAAGGTGAGAATC	(TAAAA)n	simple	pentanucleotide	203-218	Y	4	Kim
GATAC4	FWD:AGTGTCTCACTTCAAGCACCAAGCAC REV:gtttcttGCAGCAAAATTCACAGTTGGAAAAATGT	[TCTA]$_4$(TGTA)$_2$[TCTA]$_2$(TGTA)$_2$[TCTA]$_2$(TGTA)$_2$ [TCTA]$_5$ TC-A [TCTA]$_2$	complex			Y	4	Butler2006
DYS463	FWD:AATTCTAGGTTTGAGCAAAGACA REV:gtttcttATGAGGTTGTGTGACTTGACTG	(AARGG)n	simple	pentanucleotide	224-269	Y	4	Butler2006
DYS725	FWD:TAGAGAGCCCAGAAAGAGACC REV:gtttcttGGTAACCAATTATTCTAGCACC					Y	4	GDB
DYS726	FWD:GAATGACAGACCAAGACTCTCTC REV:gtttcttGGGTAAACCTCTGAAGACCATAC					Y	4	Hao2008
GGAAT1B07						Y	4	
DYS614	FWD:GTGGCGATGTTGTGAGTGTT REV:gtttcttGCCACCAAAAGGTTTTCAGA	(CTT)2(CCT)3(CTT)3(CTTCTTT)2(CTT)2(CTG)1-(CTT)2(CTGCTT)2(CTGCT)1(CTT)18**	complex		n.r.	N	4	Kayser
DYS626	FWD:GCAAGACCCCATAGCAAAAG REV:gtttcttAAGAAGAATTTTGGGACATGTTT	(GAAA)16-27(GGAA)5-6	complex		223-266	N	4	Kayser
DYS644	FWD:GGGTAGTTCCAGGCCCTAATTCAT REV:gtttcttGTTGTGTCACTGACCTCCAACCT	(TTTTA)11... TTTA (TTTTA)11...	complex		137-208	N	4	Kayser
DYS443	FWD:GAGTTCATGCTGATGACAAGC REV:gtttcttTCATTGGCCACCTGACATTA	(TTCC)13(CTT)3**	complex		n.r.	N	4	Kayser
DYF392S1	FWD:TTGAAGGCACAGATTTGCAC REV:gtttcttTTGGTGGCAACTTGAAGACA	(TTAT)8**	simple	tetranucleotide	n.r.	N	4	Kayser
DYS532	FWD:TTGGTTTTATGCCTTTCACT REV:gtttcttTAGGTGACAGAGCAGGATTC	(TTCT)3(TTCC)1(TTCT)9-15	complex		448-472	N	4	Kayser
DYS504	FWD:TCTACACCACTGTGCCAAGC REV:gtttcttGGCAACAGAGCAACCCTCT	(CTTC)11-16	complex		268-288	N	4	Kayser
DYS557	FWD:TTTTCTGTGCCAAGCCTACA REV:gtttcttTCTAATGCACCTTGAGGGATG	(TTTC)4(TTCTC)1(TTTC)4(TTC)1(TTTC)14-23	complex		190-226	N	4	Kayser
DYS612	FWD:CCCCCATGCCAGTAAGAATA REV:gtttcttTGAGGGAAGGCAAAAGAAAA	(CCT)5(CTT)1(TCT)4(CCT)1(TCT)25-29	complex		200-212	N	4	Kayser
DYS715	FWD:TGATAGATGGATAAATGGATGAATG REV:gtttcttTCTATCTCATCTTTGTCTCTTTCTGC	[TAGA]n N$_{20}$ [TGGA]n	complex			N	4	SRM2395
DYF386S1	FWD:GACTGCTCAACTGCACTCCA REV:gtttcttCCAATGTTACTCACTATGCTGCTT	(AAT)14**	simple	trinucleotide	119-122	N	4	Kayser
DYS645	FWD:GGTTACGGGTGGCAATCATA REV:gtttcttACTGCCAGACTCACACATGG	(TGTTT)8-9(GAG)2	complex		268-273	N	4	Kayser
DYF380	FWD:AGCCATGTGGATTCACCACT REV:gtttcttGACAAACCCATCCTGTCTCC	(AAT)10	simple	trinucleotide	190	N	4	Kayser
DYS652	FWD:AGGACATGCCTGTGCTACAA REV:gtttcttGGGAGGGGAAGTACATGGAA	[TAATA]n TAAAA [TAATA]n TAAAA [TAATA]	complex	pentanucleotide		N	4	SRM 2395
DYS650	FWD:TCACATGCTACAGTCACAGC REV:gtttcttCTCTTTCTCCCTTCCCACCT	(AAGG)13-17	complex	tetranucleotide		N	4	SRM2395
DYS527	FWD:TCGCAACATAGCACTTCAG REV:gtttcttTTCTAGGAAGATTAGCCACAACA	(GGAA)3(GGAA)2(GGAA)2(GGAA)3(GGAA)4(GGAA)3(GAAA)n(GGAA)n	complex			N	4	Huang
DYS721	FWD:GGGTGATAGAGGGAGGCTTCT REV:gtttcttCGGGCATGAGCTATTGAGTC	(AAGGG)n(AAGGG)n(AAGCA)n	complex			N	4	Decker
DYS712	FWD:GCAAGAACAGCCTGGGTAACAGTG REV:gtttcttTTATATGGTACAGCCCATGAACACTT	(AGAT)(AGAC)19-26	complex			N	4	SRM2395
DYS719						N	4	
DYS723	FWD:GACAGGTGGATGCATAAATGG REV:gtttcttCCTATCTGGCATCTGTCTGC	(GATA)10GAT(GATA)1GAT(GATA)7				N	4	Decker

Appendix 2:Optimal Buffer and Temperature Conditions

Temperature (50 C)

A	B	C	D	E	F	G	H
DYS533	DYS726	DYS726	DYS726	DXYS156	DXYS156	DYS408	DXYS156
DYS435	DYS533	DYS533	DYF392s1	DYF392s1	DYS452	DYF392s1	DYS452
DYF380	DYS540	DYS540	DYS533	DYS533	DYS408	DYS533	DYS408
DYF386s1	DYS556	DYS556	DYS540	DYS540	DYF392s1	DYS522	DYF392s1
DYS436	DYS636	DYS568	DYS556	DYS556	DYS533	DYS540	DYS533
DYS476	DYF380	DYS569	DYS569	DYS638	DYS540	DYS556	DYS522
DYS480	DYS476	DYS505	DYS505	DYS568	DYS556	DYS638	DYS556
DYS488	DYS480	DYS636	DYS636	DYS569	DYS638	DYS568	DYS638
DYS491	DYS491	DYF397	DYS434	DYS636	DYS568	DYS569	DYS568
DYS497	DYS495	DYS476	DYF397	DYS462	DYS569	DYS505	DYS569
DYS495	DYS709	DYS480	DYS476	DYS434	DYS505	DYS434	DYS505
DYS535	DYS715	DYS495	DYS480	DYF385S1	DYS636	DYF385S1	DYS636
DYS618	DYS719	DYS719	DYS495	DYF397	DYS462	DYF397	DYS434
DYS709	DYS723	DYS494	DYS612	DYS476	DYS434	DYS476	DYF385S1
DYS715	DYS644	DYS723	DYS715	DYS480	DYF397	DYS480	DYF397
DYS719	DYS508	DYS508	DYS719	DYS495	DYS476	DYS612	DYS461
DYS723			DYS494	DYS626	DYS480	DYS494	DYS476
DYS508			DYS723	DYS716	DYS716	DYS723	DYS480
DYS556			Y-GATA-A10	DYS494	DYS494	DYS504	DYS495
			DYS508	DYS723	DYS723	DYS632	DYS527
				DYS504	DYS504		DYS612
				DYF399	DYF399		DYS650
							DYS494
							DYS723
							DYS504
							DYF399

Temperature (52 C)

A	B	C	D	E	F	G	H
DXYS156	DYS726	DYS589	DYS589	DXYS156	DYS589	DXYS156	DYS726
DYS452	DXYS156	DYS726	DYS726	DYS452	DYS726	DYS452	DXYS156
DYF392S1	DYS452	DXYS156	DXYS156	DYS463	DXYS156	DYS463	DYS452
DYS533	DYF392S1	DYS452	DYS452	DYF408	DYS452	DYF408	DYS463
DYS522	DYF406S1	DYF408	DYS463	DYF392S1	DYS463	DYF392S1	DYF408
DYS556	DYS575	DYF392S1	DYF408	DYS575	DYF408	DYS540	DYF392S1
DYS569	DYS533	DYF406S1	DYF392S1	DYS533	DYF392S1	DYS556	DYS533
DYS505	DYS522	DYS575	DYS525	DYS522	DYS575	DYS580	DYS522
DYF390S1	DYS556	DYS533	DYS575	DYS540	DYS533	DYS530	DYS540
DYS580	DYS568	DYS522	DYS533	DYS556	DYS522	DYF385S1	DYS556
DYS434	DYS569	DYS540	DYS522	DYS638	DYS540	DYF397	DYS638
DYF380	DYS505	DYS556	DYS540	DYS568	DYS556	DYS495	DYS569
DYS434	DYF390S1	DYS568	DYS556	DYS569	DYS638	DYS532	DYF390S1
DYF385S1	DYS580	DYS569	DYS638	DYF390S1	DYS569	DYS612	DYS530
DYF397	DYS530	DYS505	DYS569	DYS530	DYF390S1	DYS494	DYF385S1
DYS436	DYS434	DYF390S1	DYS505	DYS434	DYS530	DYS723	DYF397
DYS476	DYF380	DYF390S1	DYF390S1	DYS434	DYS434	DYS644	DYS573
DYS480	DYS434	DYS580	DYS580	DYF385S1	DYS434	Y-GATA-A10	DYS443
DYS488	DYF385S1	DYS530	DYS530	DYF397	DYF385S1		DYS495
DYS491	DYF386S1	DYS434	DYS434	DYS495	DYF397		DYS532
DYS495	DYF397	DYS434	DYS434	DYS534	DYS495		DYS645
DYS497	DYS443	DYF385S1	DYF385S1	DYS612	DYS532		DYS650
DYS534	DYS480	DYF397	DYF397	DYS716	DYS534		DYS715
DYS535	DYS491	DYS495	DYS495	DYS494	DYS612		DYS723
DYS612	DYS495	DYS534	DYS534	DYS723	DYS716		DYS504
DYS618	DYS534	DYS612	DYS612	DYS644	DYS494		DYS721
DYS626	DYS612	DYS715	DYS716	DYF399	DYS723		Y-GATA-A10
DYS709	DYS626	DYS716	DYS719	DYS721	Y-GATA-A10		
DYS715	DYS709	DYS719	DYS494				
DYS719	DYS715	DYS494	DYS723				
DYF399	DYS716	DYS723	DYF399				
	DYS719	DYF399					
	DYS494	DYS721					
	DYS723	Y-GATA-A10					
	DYF399						

Temperature (53.4 C)

A	B	C	D	E	F	G	H
DYS726	DYS589	DYS726	DXYS156	DXYS156	DXYS156	DXYS156	DXYS156
DXYS156	DYS726	DXYS156	DYS452	DYS452	DYS452	DYS452	DYS452
DYS452	DXYS156	DYS452	DYF408	DYF408	DYS463	DYF408	DYS463
DYS463	DYS452	DYS463	DYF392S1	DYF392S1	DYF408	DYF392S1	DYF408
DYF392S1	DYS463	DYF392S1	DYS525	DYS525	DYF392S1	DYS525	DYF392S1
DYS533	DYF392S1	DYS525	DYS533	DYS533	DYS525	DYS533	DYS575
DYS556	DYS533	DYS533	DYS522	DYS522	DYS533	DYS522	DYS533
DYS568	DYS556	DYS522	DYS556	DYS556	DYS522	DYS556	DYS522
DYS569	DYS568	DYS556	DYS638	DYS638	DYS556	DYS638	DYS556
DYS505	DYS569	DYS568	DYS568	DYS568	DYS638	DYS568	DYS638
DYF380	DYS505	DYS569	DYS569	DYS569	DYS568	DYS569	DYS568
DYF385S1	DYS583	DYS505	DYS505	DYS505	DYS569	DYS505	DYS569
DYF386S1	DYF380	DYS583	DYS567	DYS567	DYS505	DYS567	DYS505
DYF397	DYF385S1	DYS463	DYS463	DYS463	DYS636	DYS463	DYS636
DYS436	DYF397	DYF385S1	DYF385S1	DYS434	DYS567	DYS434	DYS567
DYS476	DYS480	DYS461	DYS461	DYF385S1	DYS463	DYF385S1	DYS463
DYS480	DYS488	DYS491	DYS491	DYS461	DYS434	DYS461	DYF385S1
DYS488	DYS491	DYS495	DYS495	DYS495	DYF385S1	DYS495	DYS461
DYS491	DYS495	DYS626	DYS612	DYS534	DYS461	DYS534	DYS495
DYS495	DYS535	DYS652	DYS626	DYS626	DYS495	DYS612	DYS645
DYS497	DYS618	DYS715	DYS716	DYS716	DYS534	DYS645	DYS715
DYS535	DYS626	DYS716	DYS719	DYS719	DYS716	DYS650	DYS494
DYS618	DYS709	DYS719	DYS494	DYS494	DYS494	DYS719	DYS723
DYS626	DYS716	DYS494	DYS723	DYS723	DYS723	DYS494	DYS504
DYS709	DYS719	DYS723	DYS504	DYS504	DYS504	DYS723	DYS721
DYS712	DYS494	DYS504	DYF399	DYF399	GATA-C4	DYS504	GATA-C4
DYS715	DYS723	DYF399	GATA-C4	DYS721	DYS508	DYS721	DYS508
DYS719	DYS504	Y-GATA-A10	DYS508	GATA-C4		GATA-C4	
DYS494	DYF399	GATA-C4		DYS508		DYS508	
DYS723	Y-GATA-A10	DYS508					
DYS614	GATA-C4						
DYF399	DYS508						
Y-GATA-A10	DYS632						

GATA-C4
DYS508
DYS632

Temperature (55.1 C)

A	B	C	D	E	F	G	H
DYS643	DYS643	DYS643	DYS643	DYS643	DYS643	DYS643	DYS643
DXYS156	DYS726	DYS589	DYS726	DYS726	DYS726	DYS589	DYS726
DYS452	DXYS156	DYS726	DXYS156	DXYS156	DXYS156	DYS726	DXYS156
DYS461	DYS452	DXYS156	DYS452	DYS452	DYS452	DXYS156	DYS452
DYS575	DYS575	DYS452	DYF392S1	DYF08	DYS461	DYS452	DYS461
DYS533	DYS533	DYS461	DYS525	DYF392S1	DYF08	DYF08	DYF08
DYS556	DYS522	DYS525	DYS575	DYS525	DYF392S1	DYF392S1	DYF392S1
DYS638	DYS556	DYS575	DYS575	DYS575	DYS525	DYS525	DYS525
DYS568	DYS638	DYS533	DYS533	DYS533	DYS575	DYS575	DYS575
DYS569	DYS569	DYS522	DYS556	DYS522	DYS533	DYS533	DYS533
DYS505	DYS505	DYS556	DYS638	DYS556	DYS522	DYS522	DYS522
DYS583	DYS583	DYS638	DYS569	DYS638	DYS556	DYS556	DYS556
DYS380	DYS462	DYS638	DYS505	DYS569	DYS638	DYS638	DYS638
DYF385S1	DYS434	DYS569	DYS583	DYS505	DYS569	DYS569	DYS569
DYF386S1	DYS380	DYS505	DYS462	DYS583	DYS505	DYS505	DYS505
DYF397	DYF385S1	DYS583	DYS530	DYS462	DYS583	DYS583	DYS583
DYS436	DYF386S1	DYS462	DYS434	DYS530	DYS462	DYS462	DYS462
DYS476	DYF397	DYS434	DYS380	DYS434	DYS530	DYS530	DYS530
DYS480	DYS436	DYF385S1	DYF385S1	DYF385S1	DYS434	DYS434	DYS434
DYS488	DYS476	DYF386S1	DYF386S1	DYF386S1	DYF385S1	DYF385S1	DYF385S1
DYS491	DYS480	DYF397	DYF397	DYF397	DYF386S1	DYF386S1	DYF386S1
DYS497	DYS488	DYS461	DYS461	DYS573	DYF397	DYF397	DYF397
DYS535	DYS491	DYS480	DYS491	DYS461	DYS461	DYS461	DYS573
DYS612	DYS497	DYS491	DYS495	DYS534	DYS527	DYS534	DYS461
DYS618	DYS527	DYS534	DYS534	DYS612	DYS534	DYS612	DYS534
DYS650	DYS534	DYS612	DYS612	DYS618	DYS612	DYS618	DYS612
DYS652	DYS535	DYS618	DYS618	DYS626	DYS618	DYS626	DYS618
DYS709	DYS612	DYS626	DYS626	DYS652	DYS626	DYS709	DYS709
DYS712	DYS618	DYS645	DYS652	DYS709	DYS645	DYS715	DYS715
DYS715	DYS626	DYS709	DYS709	DYS716	DYS709	DYS494	DYS494
DYS719	DYS645	DYS716	DYS715	DYS719	DYS715	DYS723	DYS723
DYS494	DYS652	DYS719	DYS716	DYS494	DYS716	GATA-C4	DYS725
DYS723	DYS709	DYS494	DYS719	DYS723	DYS494	DYS508	GATA-C4
DYS504	DYS715	DYS723	DYS494	DYS725	DYS723		DYS508
DYS721	DYS716	DYS725	DYS723	DYS644	DYS725		
GATA-C4	DYS719	DYS644	DYS644	DYF399	Y-GATA-A10		
DYS632	DYS494	DYF399	DYF399	GATA-C4	GATA-C4		
	DYS723	Y-GATA-A10	GATA-C4	DYS508	DYS508		
	DYS644	GATA-C4	DYS508				
	DYF399	DYS632	DYS632				
	Y-GATA-A10						
	GATA-C4						
	DYS632						

Temperature (57.2 C)

A	B	C	D	E	F	G	H
DYS589	DYS643	DYS643	DYS643	DYS643	DYS643	DYS643	DYS643
DXYS156	DYS589	DYS589	DYS589	DYS589	DYS589	DYS589	DYS589
DYS452	DYS726	DYS726	DYS726	DYS726	DYS726	DYS726	DYS726
DYS463	DXYS156	DXYS156	DYS452	DXYS156	DXYS156	DXYS156	DXYS156
DYS408	DYS452	DYS452	DYS408	DYS452	DYS452	DYS452	DYS452
DYF392S1	DYS408	DYS408	DYF392S1	DYS408	DYS463	DYF392S1	DYF392S1
DYS575	DYF392S1	DYF392S1	DYS525	DYF392S1	DYS408	DYF406S1	DYF406S1
DYS533	DYS525	DYS525	DYS575	DYS525	DYF392S1	DYS525	DYS575
DYS540	DYS575	DYS575	DYS533	DYS575	DYS525	DYS575	DYS533
DYS556	DYS533	DYS533	DYS540	DYS533	DYS575	DYS533	DYS522
DYS638	DYS522	DYS540	DYS556	DYS556	DYS533	DYS556	DYS556
DYS568	DYS540	DYS556	DYS568	DYS568	DYS522	DYS568	DYS568
DYS569	DYS556	DYS638	DYS569	DYS569	DYS556	DYS569	DYS569
DYS505	DYS638	DYS568	DYS505	DYS505	DYS568	DYS505	DYS505
DYF30S1	DYS568	DYS569	DYS567	DYS636	DYS569	DYS636	DYS636
DYS567	DYS569	DYS505	DYS583	DYS567	DYS505	DYS567	DYS583
DYS583	DYS505	DYS636	DYS462	DYS579	DYS636	DYS583	DYS462
DYS462	DYS636	DYS567	DYS580	DYS583	DYS567	DYS462	DYS580
DYS380	DYS567	DYS579	DYS530	DYS462	DYS583	DYS580	DYS530
DYF385	DYS579	DYS583	DYS380	DYS580	DYS462	DYS530	DYS380
DYF386S1	DYS583	DYS462	DYF385	DYS530	DYS580	DYS434	DYF385
DYF401	DYS462	DYS580	DYF386S1	DYS434	DYS530	DYS380	DYF386S1
DYF397	DYS580	DYS380	DYF401	DYS380	DYS434	DYF385	DYF397
DYS436	DYS380	DYF385	DYF397	DYF385	DYS380	DYF386S1	DYS573
DYS476	DYF385	DYF386S1	DYS480	DYF386S1	DYF385	DYF397	DYS443
DYS480	DYF386S1	DYF401	DYS491	DYF401	DYF386S1	DYS527	DYS612
DYS488	DYF401	DYF397	DYS495	DYF397	DYF401	DYS534	DYS709
DYS491	DYF397	DYS480	DYS527	DYS573	DYF397	DYS612	DYS719
DYS497	DYS436	DYS491	DYS612	DYS480	DYS527	DYS709	DYS494
DYS535	DYS476	DYS497	DYS626	DYS491	DYS534	DYS715	DYS725
DYS618	DYS480	DYS527	DYS709	DYS527	DYS612	DYS719	Y-GATA-A10
DYS612	DYS488	DYS532	DYS716	DYS534	DYS709	DYS494	GATA-C4
DYS650	DYS491	DYS535	DYS719	DYS612	DYS715	Y-GATA-A10	DYS508
DYS709	DYS497	DYS612	DYS494	DYS645	DYS716	GATA-C4	
DYS712	DYS535	DYS626	DYS725	DYS709	DYS719	DYS508	
DYS715	DYS612	DYS652	DYS644	DYS716	DYS494	DYS632	
DYS716	DYS626	DYS709	DYF399	DYS719	DYS725		
DYS494	DYS709	DYS716	Y-GATA-A10	DYS494	Y-GATA-A10		
DYS723	DYS715	DYS719	GATA-C4	DYS725	GATA-C4		
DYF399	DYS716	DYS494	DYS508	DYF399	DYS508		
GATA-C4	DYS719	DYS723	DYS632	Y-GATA-A10	DYS632		
DYS632	DYS494	DYS725		GATA-C4			
	DYS723	DYS504		DYS508			
	DYS644	DYS644		DYS632			
	DYF399	DYF399					
	DYS721	Y-GATA-A10					
	Y-GATA-A10	GATA-C4					
	GATA-C4	DYS632					
	DYS632						

Temperature (58.9 C)

A	B	C	D	E	F	G	H
DYS589	DYS726	DYS589	DYS589	DYS643	DYS643	DYS643	DYS643
DYS726	DXYS156	DYS726	DYS726	DYS589	DYS726	DYS726	DXYS156
DXYS156	DYS452	DXYS156	DXYS156	DYS726	DXYS156	DXYS156	DYS452
DYS452	DYS408	DYS452	DYS452	DXYS156	DYS452	DYS452	DYS463
DYS408	DYF392S1	DYS408	DYS408	DYS452	DYS463	DYS463	DYS408
DYF392S1	DYF406S1	DYF392S1	DYF392S1	DYS463	DYS408	DYS408	DYF392S1
DYF406S1	DYS533	DYF406S1	DYF406S1	DYS408	DYF392S1	DYF392S1	DYF406S1
DYS533	DYS522	DYS533	DYS533	DYF392S1	DYF406S1	DYF406S1	DYS575
DYS540	DYS540	DYS556	DYS522	DYF406S1	DYS575	DYS575	DYS533
DYS556	DYS556	DYS568	DYS540	DYS575	DYS533	DYS533	DYS522
DYS568	DYS568	DYS569	DYS556	DYS533	DYS522	DYS522	DYS556
DYS505	DYS505	DYS505	DYS568	DYS522	DYS556	DYS556	DYS569
DYS462	DYS505	DYS636	DYS569	DYS540	DYS568	DYS569	DYS462
DYS580	DYS636	DYS462	DYS505	DYS556	DYS569	DYS462	DYS530
DYS530	DYF390S1	DYS580	DYS567	DYS569	DYS462	DYS530	DYS434
DYS434	DYS462	DYS530	DYS462	DYS462	DYS580	DYS434	DYS435
DYS435	DYS580	DYS434	DYS580	DYS580	DYS530	DYS435	DYF380
DYF380	DYS530	DYS435	DYS530	DYS530	DYS434	DYF380	DYS434
DYS434	DYS434	DYF380	DYS434	DYS434	DYS435	DYS434	DYF385S1
DYF385S1	DYS435	DYS434	DYS435	DYS435	DYF380	DYF385S1	DYF386S1
DYF386S1	DYF380	DYF385S1	DYF380	DYF380	DYS434	DYF386S1	DYF397
DYF397	DYS434	DYF386S1	DYS434	DYS434	DYF385S1	DYF397	DYS461
DYS436	DYF385S1	DYF397	DYF385S1	DYF385S1	DYF386S1	DYS461	DYS497
DYS443	DYF386S1	DYS443	DYF386S1	DYF386S1	DYF397	DYS497	DYS527
DYS461	DYF397	DYS461	DYF397	DYF397	DYS443	DYS527	DYS532
DYS476	DYS436	DYS476	DYS443	DYS443	DYS461	DYS532	DYS534
DYS480	DYS443	DYS480	DYS461	DYS461	DYS497	DYS534	DYS612
DYS485	DYS461	DYS491	DYS476	DYS480	DYS527	DYS612	DYS645
DYS488	DYS476	DYS497	DYS480	DYS491	DYS532	DYS652	DYS652
DYS491	DYS480	DYS527	DYS491	DYS527	DYS534	DYS709	DYS709
DYS497	DYS488	DYS534	DYS497	DYS534	DYS652	DYS715	DYS715
DYS534	DYS491	DYS535	DYS534	DYS626	DYS709	DYS719	DYS719
DYS535	DYS497	DYS626	DYS535	DYS652	DYS715	DYS494	DYS723
DYS618	DYS534	DYS645	DYS645	DYS709	DYS719	DYS723	DYS399
DYS626	DYS535	DYS652	DYS652	DYS716	DYS494	DYS504	Y-GATA-A10
DYS650	DYS618	DYS652	DYS709	DYS719	DYS723	DYS399	GATA-C4
DYS709	DYS626	DYS709	DYS716	DYS723	DYS504	Y-GATA-A10	DYS508
DYS712	DYS645	DYS712	DYS719	DYS504	DYS399	GATA-C4	
DYS715	DYS650	DYS716	DYS494	DYS399	Y-GATA-A10	DYS508	
DYS494	DYS652	DYS719	DYS723	Y-GATA-A10	GATA-C4	DYS632	
DYS723	DYS709	DYS494	DYS644	GATA-C4	DYS508		
DYS399	DYS712	DYS723	DYS399	DYS508			
DYS632	DYS715	DYS644	Y-GATA-A10	DYS632			
	DYS494	DYS399	GATA-C4				
	DYS723	Y-GATA-A10	DYS632				
	DYS644	GATA-C4					
	DYS399	DYS632					
	DYS721						
	GATA-C4						
	DYS632						

Temperature (60.2 C)

A	B	C	D	E	F	G	H
DYS726	DYS726	DYS726	DYS726	DYS726	DYS726	DYS726	DYS643
DXYS156	DXYS156	DXYS156	DXYS156	DXYS156	DXYS156	DYS452	DYS726
DYS452	DYS452	DYS452	DYS452	DYS452	DYS452	DYS463	DYS452
DYF408	DYF408	DYF408	DYS463	DYF408	DYS463	DYF408	DYS463
DYF392S1	DYF392S1	DYF392S1	DYF408	DYF392S1	DYF408	DYF392S1	DYF408
DYF406S1	DYF406S1	DYF406S1	DYF392S1	DYF406S1	DYF392S1	DYF406S1	DYF406S1
DYS533	DYS533	DYS533	DYF406S1	DYS575	DYF406S1	DYS575	DYS575
DYS556	DYS556	DYS556	DYS533	DYS533	DYS575	DYS533	DYS533
DYS505	DYS568	DYS568	DYS556	DYS522	DYS533	DYS522	DYS522
DYF390s1	DYS505	DYS569	DYS638	DYS556	DYS522	DYS556	DYS556
DYS462	DYF390s1	DYS505	DYS568	DYS638	DYS556	DYS638	DYS638
DYS580	DYS462	DYF390s1	DYS569	DYS568	DYS638	DYS569	DYS568
DYS530	DYS580	DYS462	DYS505	DYS569	DYS568	DYF390s1	DYS569
DYF380	DYS530	DYS580	DYF390s1	DYF390s1	DYS569	DYS567	DYF390s1
DYF386s1	DYF380	DYS530	DYS462	DYS462	DYF390s1	DYS462	DYS567
DYS436	DYF386s1	DYF380	DYS580	DYS530	DYS567	DYS530	DYS579
DYS476	DYF397	DYF386s1	DYF380	DYF380	DYS579	DYF385s1	DYS462
DYS480	DYS436	DYF397	DYF397	DYF385s1	DYS462	DYF397	DYF385s1
DYS488	DYS443	DYS436	DYS436	DYF397	DYF385s1	DYS443	DYF397
DYS491	DYS461	DYS476	DYS476	DYS436	DYF401	DYS461	DYS443
DYS497	DYS476	DYS480	DYS480	DYS443	DYF397	DYS480	DYS461
DYS535	DYS480	DYS535	DYS497	DYS461	DYS443	DYS527	DYS480
DYS618	DYS491	DYS626	DYS535	DYS480	DYS461	DYS534	DYS527
DYS626	DYS495	DYS645	DYS626	DYS497	DYS480	DYS626	DYS534
DYS645	DYS497	DYS650	DYS645	DYS527	DYS495	DYS645	DYS626
DYS650	DYS535	DYS709	DYS652	DYS626	DYS534	DYS652	DYS645
DYS712	DYS618	DYS716	DYS709	DYS650	DYS612	DYS715	DYS715
DYS715	DYS626	DYS719	DYS712	DYS652	DYS626	DYS716	DYS716
DYS494	DYS645	DYS494	DYS715	DYS709	DYS645	DYS719	DYS719
DYS723	DYS712	DYS723	DYS716	DYS716	DYS650	DYS494	DYS494
DYF399	DYS715	DYF399	DYS719	DYS719	DYS652	DYS504	DYF399
DYS508	DYS716	DYS721	DYS494	DYS494	DYS709	DYF399	Y-GATA-A10
DYS632	DYS494	GATA-C4	DYS723	DYS504	DYS715	DYS721	
	DYS723	DYS508	DYF399	DYF399	DYS716	Y-GATA-A10	
	DYF399		GATA-C4	DYS721	DYS719	DYS632	
	GATA-C4		DYS508	Y-GATA-A10	DYS494		
	DYS508			GATA-C4	DYS504		
	DYS632			DYS508	DYF399		
				DYS632	DYS508		

Temperature (61.1 C)

A	B	C	D	E	F	G	H
DYS463	DYS726	DYS726	DYS726	DYS726	DYS726	DYS726	DYS726
DYS408	DYS452	DYS452	DXYS156	DXYS156	DYS452	DXYS156	DYS452
DYF392s1	DYS408	DYS568	DYS452	DYS452	DYF392s1	DYS452	DYS533
DYS575	DYF392s1	DYS569	DYS556	DYS556	DYS533	DYF392s1	DYS556
DYS533	DYS533	DYS505	DYS569	DYS568	DYS556	DYS533	DYS569
DYS568	DYS556	DYS636	DYS505	DYS569	DYS568	DYS556	DYF390s1
DYS505	DYS568	DYF390s1	DYS636	DYS505	DYS569	DYS569	DYS462
DYS636	DYS505	DYS530	DYF390s1	DYS636	DYS505	DYS569	DYF385s1
DYF390s1	DYS636	DYS435	DYS530	DYF390s1	DYS636	DYF390s1	DYF401
DYS435	DYF390s1	DYS380	DYS580	DYS435	DYF390s1	DYS462	DYF397
DYS380	DYS580	DYF386s1	DYS435	DYS380	DYS462	DYS530	DYS443
					DYS580	DYF385s1	

DYF386s1	DYS435	DYF401	DYF401	DYS435	DYS435	DYF401	DYS461
DYF401	DYS380	DYF397	DYF397	DYS380	DYF401	DYF397	DYS480
DYS436	DYF386s1	DYS436	DYS436	DYF401	DYF397	DYS443	DYS527
DYS476	DYF397	DYS476	DYS476	DYF397	DYS443	DYS461	DYS534
DYS480	DYS436	DYS480	DYS480	DYS436	DYS461	DYS480	DYS535
DYS488	DYS476	DYS495	DYS495	DYS443	DYS480	DYS534	DYS612
DYS491	DYS480	DYS497	DYS497	DYS461	DYS534	DYS535	DYS645
DYS497	DYS491	DYS535	DYS535	DYS480	DYS535	DYS626	DYS715
DYS535	DYS497	DYS618	DYS626	DYS495	DYS626	DYS652	DYS716
DYS618	DYS535	DYS626	DYS645	DYS532	DYS652	DYS715	DYS719
DYS709	DYS618	DYS709	DYS652	DYS535	DYS715	DYS716	DYS494
DYS712	DYS626	DYS715	DYS709	DYS626	DYS716	DYS719	DYF399
DYS715	DYS709	DYS719	DYS715	DYS645	DYS719	DYS494	DYS508
DYS723	DYS712	DYS494	DYS716	DYS652	DYS494	DYS504	
DYF399	DYS715	DYS723	DYS719	DYS716	DYS504	DYF399	
	DYS494	DYF399	DYS494	DYS719	DYF399	DYS508	
	DYS723	G-ATA-C4	DYS723	DYS494	DYS508		
	DYS644		DYS644	DYS723			
	DYF399		DYF399	DYS504			
	G-ATA-C4		G-ATA-C4	DYS644			
			DYS508	DYF399			
			DYS632	G-ATA-C4			
				DYS508			

Temperature (62 C)

A	B	C	D	E	F	G	H
DYF408	DYS452	DXYS156	DXYS156	DXYS156	DYS452	DYS452	DYS452
DYF392	DYF408	DYS452	DYS452	DYS452	DYS575	/DYF406s1	/DYF406s1
DYS636	DYF392	DYF408	DYF408	DYF408	DYS533	DYS575	DYS533
DYF390s1	DYS533	DYF392	DYF392	DYF392	DYS556	DYS533	DYS556
DYS435	DYS556	DYS568	DYS533	DYS533	DYS568	DYS556	DYS638
DYF380	DYS568	DYS569	DYS569	DYS556	DYS569	DYS569	DYS568
DYF386	DYS505	DYS505	DYS505	DYS568	DYS462	DYS462	DYS569
DYS436	DYS636	DYF390s1	DYS530	DYS569	DYS530	DYS530	DYS462
DYS476	DYS435	DYS435	DYS435	DYS505	DYS435	DYS435	DYS530
DYS480	DYF380	DYF380	DYF380	DYF390s1	DYF397	DYF397	DYF397
DYS488	DYF386	DYF386	DYF386	DYS462	DYS480	DYS436	DYS443
DYS491	DYS436	DYF397	DYF397	DYS530	DYS497	DYS480	DYS461
DYS497	DYS476	DYS436	DYS436	DYS435	DYS535	DYS497	DYS480
DYS535	DYS480	DYS476	DYS476	DYF380	DYS612	DYS532	DYS497
DYS612	DYS485	DYS480	DYS480	DYF397	DYS626	DYS535	DYS535
DYS618	DYS491	DYS485	DYS485	DYS436	DYS709	DYS709	DYS612
DYS712	DYS497	DYS491	DYS497	DYS476	DYS715	DYS715	DYS626
DYS716	DYS535	DYS497	DYS535	DYS480	DYS716	DYS716	DYS645
DYS719	DYS612	DYS535	DYS612	DYS497	DYS719	DYS719	DYS709
DYS723	DYS618	DYS612	DYS709	DYS535	DYS494	DYS494	DYS715
	DYS709	DYS618	DYS712	DYS612	DYS504	DYS504	DYS716
	DYS712	DYS709	DYS715	DYS626	DYF399	DYS644	DYS719
	DYS715	DYS712	DYS716	DYS645	DYS508	DYF399	DYS494
	DYS716	DYS716	DYS719	DYS709	DYS632	DYS508	DYS644
	DYS719	DYS719	DYS494	DYS716		DYS632	DYF399
	DYS723	DYS494	DYS723	DYS719			DYS508
	GATA-C4	DYS723	GATA-C4	DYS494			DYS632
	DYS508	GATA-C4	DYS508	DYS723			
		DYS508	DYS632	DYS504			
				GATA-C4			
				DYS508			
				DYS632			

Appendix 3: Multiplex loci results

Multiplex Alpha

Markers	*$MgCl_2$ Buffer (mM)*	*Annealing Temp (℃)*
DYS494	4.0	57
DYS508	4.0	57
DYS522	4.0	57
DYS525	4.0	57
DYS530	4.0	57
DYS556	4.0	57
DYS573	4.0	57
DYS643	4.0	57
DYS491	4.0	57
DYS533	4.0	57

Multiplex Beta

Markers	*$MgCl_2$ Buffer (mM)*	*Annealing Temp (℃)*
DYS436	1.5	57
DYS462	1.5	57
DYS476	1.5	57
DYS494	1.5	57
DYS497	1.5	57
DYS568	1.5	57
DYS569	1.5	57
DYS583	1.5	57
DYS525	1.5	57
DYS632	1.5	57

Multiplex Gamma

Markers	*$MgCl_2$ Buffer (mM)*	*Annealing Temp (℃)*
DYF385S1	2.5	60
DYF386s1	2.5	60
DYF390S1	2.5	60
DYS485	2.5	60
DYS435	2.5	60
DYS441	2.5	60
DYS461	2.5	60
DYS480	2.5	60
DYS567	2.5	60
DYS575	2.5	60
DYS580	2.5	60
DYS618	2.5	60
DYS721	2.5	60

Appendix 4: Final PCR loci

Panel1

Markers	*Dye Lane*	*MgCl2 Buffer (mM)*	*Annealing Temp (°C)*
DYS462	NED	1.5	57
DYS476	VIC	1.5	57
DYS480	VIC	1.5	57
DYS494	VIC	1.5	57
DYS497	VIC	1.5	57
DYS530	NED	1.5	57
DYS568	NED	1.5	57
DYS569	PET	1.5	57
DYS583	FAM-6	1.5	57
DYS632	PET	1.5	57
DYS709	PET	1.5	57

Panel2

Markers	*Dye Lane*	*MgCl2 Buffer (mM)*	*Annealing Temp (°C)*
DYF390s1	NED	1.0	60
DYS453	NED	Qiagen Kit [c]/ Q solution	57
DYS488	FAM-6	1.0	60
DYS491	NED	Qiagen Kit [c]/ Q solution	57
DYS508	NED	Qiagen Kit [c]/ Q solution	57
DYS525	FAM-6	Qiagen Kit [c]/ Q solution	57
DYS540	FAM-6	Qiagen Kit [c]/ Q solution	57
DYS567	FAM-6	1.0	60
DYS573	NED	Qiagen Kit [c]/ Q solution	57
DYS618	FAM-6	Qiagen Kit [c]/ Q solution	57
DYS721	VIC	Qiagen Kit [c]/ Q solution	57

Appendix 5: Allele Frequency Panel 1 and 2

Panel 1			**Panel 2**		
Marker Name	*Alleles*	*Frequency*	*Marker Name*	*Alleles*	*Frequency*
DYS462	8	0.071	DYF390s1	8	0.091
	9	0.000		9	0.000
	10	0.000		10	0.796
	11	0.071		11	0.114
	12	0.857	DYS453		
DYS476	11	0.769	DYS488	11	0.046
	12	0.231		12	0.273
DYS480	7	0.143		13	0.682
	8	0.714	DYS491	12	0.722
	9	0.000		13	0.222
	10	0.143		14	0.056
DYS494	8	0.071	DYS508	9	0.444
	9	0.786		10	0.278
	10	0.143		11	0.167
DYS497	13	0.071		12	0.000
	14	0.429		13	0.056
	15	0.429		14	0.056
	16	0.071	DYS525	10	0.056
DYS530	139	0.849		11	0.056
	144	0.154		12	0.444
DYS568	10	0.143		13	0.333
	11	0.643		14	0.056
	12	0.143		15	0.056
	13	0.071	DYS540	10	0.056
DYS569	11	1.000		11	0.333
DYS583	8	0.857		12	0.389
	9	0.143		13	0.111
DYS632	8	0.846		14	0.111
	9	0.154	DYS567	10	0.364
DYS709	9	0.071		11	0.591
	10	0.000		12	0.046
	11	0.000	DYS573	9	0.167
	12	0.071		10	0.445
	13	0.071		11	0.333
	14	0.143		12	0.056
	15	0.214	DYS618	10	0.053
	16	0.286		11	0.158
	17	0.143		12	0.684
				13	0.105
			DYS721	9	0.409
				10	0.409
				11	0.182