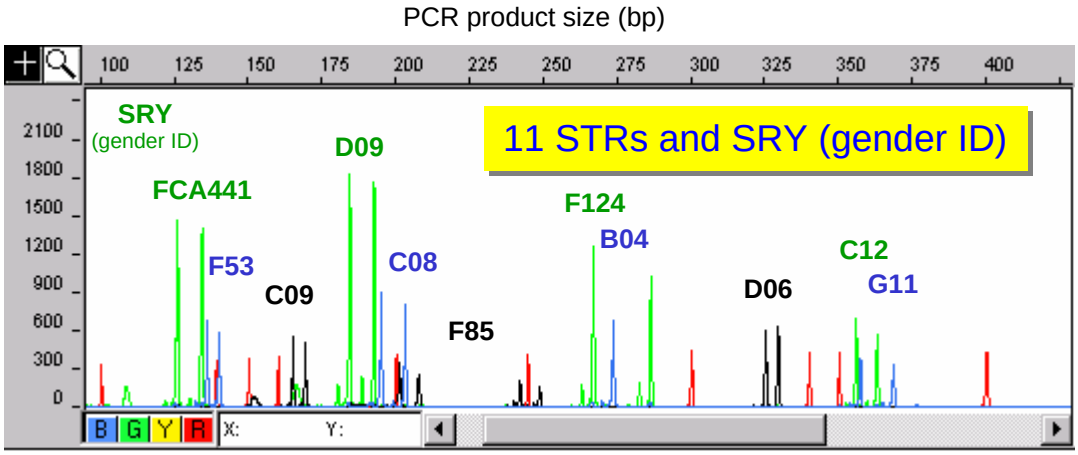
Information on the Cat STR Multiplex Developed at NIST and a Protocol for Its Use on the ABI 310 and ABI 3100 Instruments



4 ng female cat DNA

Project Funded by the National Institute of Justice

Based on research conducted by
John Butler
(NIST)

June 7, 2002

Outline of Protocol Instructions and Information

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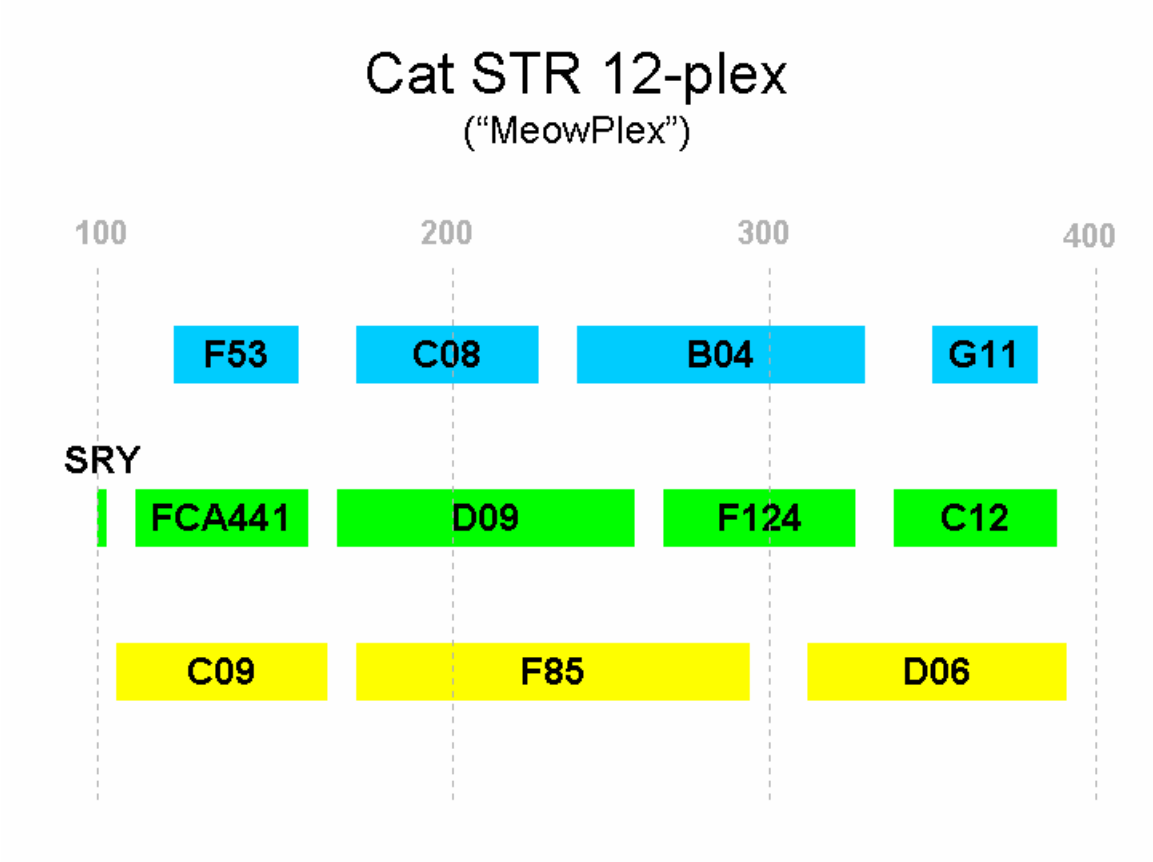
For more information on development of this cat STR multiplex, contact Dr. John Butler, National Institute of Standards and Technology, Biotechnology Division, 100 Bureau Drive MS 8311, Gaithersburg, MD 20899; 301-975-4049; john.butler@nist.gov



Expected Allele Sizes

	STR Locus	Size Range (bp)	Allele Spread (bp)	PCR Product Size of Sequenced Allele	Reference Allele	Allele Range
1	F53	154-186	32	164	17	14--22
2	C08	178-230	52	199	28	23--36
3	B04	194-280	86	221	24	17--39
4	**G11**	**324-344**	**20**	**368 bp**	**???**	**???**
	SRY	101 bp	N/A	101 bp		
5	FCA441	152-178	26	159	10	8--15
6	D09	198-280	82	219	25	20--40
7	F124	211-265	54	223	30	27--41
8	C12	219-263	44	240	38	33--44
9	C09	123-175	52	149	18	11--25
10	F85	216-316	100	274	31	16--42
11	D06	199-265	66	207	16	14--31

Repeat structures of STR loci

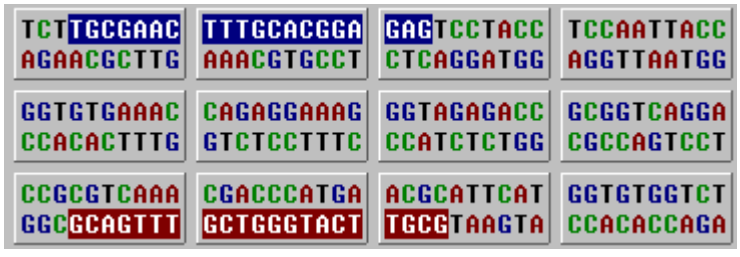
Locus	Repeat Region for Reference Sequence (from NCI)	Allele Designation
F53	[AAGA]17	17
C08	[ATA(C/G)]28	28
B04	[AAGG]9 [AAAG]15	24
G11	***??? (complex with duplication)***	**???**
SRY (gender ID)	------	-----
FCA441	[ATAG]10	10
D09	[ACAT]9 [ACAC]2 [ATAG]14	25
F124	[AGGA]12 AGGG AGGA [AGAA]16	30
C12	[AGAT]10 ACAT [AGAT]2 ACAT [AGAT]2 ACAT AGAT AGAA AGAT T [AGAT]7 GGAT [AGAT]4 AGAA AGAT AGAA [AGAT]3	38
C09	[CTTT]3 CTT [CTTT]11 TT [CTTT]2 TT [CTTT]2	18
F85	[TTTC]11 [TCTC]4 TCTT[TTTC]15	31
D06	[TATC]11 [TATA] [TCTA] [TCTG]TC[TATC]3	17

Final Primer Sequences and Concentrations

STR Marker		Dye	Primer Sequence (5'-3')	primer mix µM	Final Conc. µM
F53	F	**6FAM**	CCTATGTTGGGAGTAGAGATCACCT	4.5	**0.90**
	R2		*GTGTCTT*GAGTGGCTGTGGCATTTCC	4.5	**0.90**
C08	F		GATCCATCAATAGGTAAATGGATAAAGAAGATG	4.5	**0.90**
	R	**6FAM**	TGGCTGAGTAATATTCCACTGTCTCTC	4.5	**0.90**
B04	F	**6FAM**	TGAAGGCTAAGGCACGATAGATAGTC	4.5	**0.90**
	R2		*GTGTCTT*CCACCCAGGTGTCCTGCTTC	4.5	**0.90**
G11new	F	**6FAM**	ATCCATCTGTCCATCCATCTATT	7.2	**1.44**
	R		GGTCAGCATCTCCACTTGAGG	7.2	**1.44**
SRY	F	**VIC**	TGCGAACTTTGCACGGAGAG	0.2	**0.04**
	R		GCGTTCATGGGTCGTTTGACG	0.2	**0.04**
FCA441	F2		*GTGTCTT*GATCGGTAGGTAGGTAGATATAG	3.0	**0.60**
	R	**VIC**	ATATGGCATAAGCCTTGAAGCAAA	3.0	**0.60**
D09	F	**VIC**	CCGAGCTCTGTTCTGGGTATGAA	0.8	**0.16**
	R2		*GTGTCTT*TCTAGTTGGTCGGTCTGTCTATCTG	0.8	**0.16**
F124	F	**VIC**	TGTGCTGGGTATGAAGCCTACTG	3.0	**0.60**
	R2		*GTGTCTT*CCATGCCCATAAAGGCTCTGA	3.0	**0.60**
C12	F	**VIC**	GAGGAGCTTACTTAAGAGCATGCGTTC	3.0	**0.60**
	R2		*GTGTCTT*AAACCTATATTCGGATTGTGCCTGCT	3.0	**0.60**
C09	F	**NED**	AAATTTCAATGTCTTGACAACGCATAAG	6.0	**1.20**
	R2		*GTGTCTT*CCAGGAACACCATGTTGGGCTA	6.0	**1.20**
F85	F2	**NED**	TAAATCTGGTCCTCACGTTTTC	7.2	**1.44**
	R		*G*CCTGAAAATGTATCCATCACTTCAGAT	7.2	**1.44**
D06	F	**NED**	CCAAGGAGCTCTGTGATGCAAA	6.0	**1.20**
	R		*G*TTCCCACAGGTAAACATCAACCAA	6.0	**1.20**

Position of G11*new* primers

Position of SRY primers in provided sequence (amplifies a 101 bp section of male cat DNA)



PCR Amplification

Amplifications may be performed in reaction volumes of 20 µL. The PCR mix contains 1X Gold buffer (Applied Biosystems), 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates (Life Technologies, dNTPs: dATP, dCTP, dGTP, dTTP), and 0.16 mg/mL bovine serum albumin (BSA; Sigma). The preparation of a master mix when setting up a set of PCR reactions should include 2 units of AmpliTaq Gold® DNA polymerase (Applied Biosystems) for each reaction. An aliquot of 4 uL of cat STR primer mix should be added to each reaction tube or mixed in with the other components of the master mix. Optimal DNA quantities with the standard 28 cycle PCR conditions appear to be 2-4 ng genomic DNA.

Thermal Cycling

Thermal cycling was performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e., ramp speeds of 1 ^{o}C/s):

95 ^{o}C for 10 minutes
28 cycles: 94 ^{o}C for 1 minute, 59 ^{o}C for 1 minute, 72 ^{o}C for 1 minute
60 ^{o}C for 45 minutes
25 ^{o}C forever

Full adenylation of all peaks should be seen under these PCR conditions.

Clean up of PCR Products (Optional)

An aliquot from the 20 µL PCR reaction may be filtered to remove free dye molecules that generate the so-called “dye blobs” in CE electropherograms that can interfere with data interpretation and allele designations. The free dye removal is accomplished using Edge Performa™ DTR Gel Filtration Cartridges (Catalog # 42453) from Edge BioSystems (Gaithersburg, MD) and following the manufacturer’s protocol.

There are 3 simple steps in the Edge spin column protocol. First, centrifuge the Gel Filtration Cartridge as supplied for 2 minutes at 750 x g. This removes the storage liquid in the cartridge. Second, transfer the cartridge to a clean microcentrifuge tube provided with the Edge kit and add the 15 uL to the center of the slanted gel bed surface. It is critical that the sample be placed correctly onto the Edge cartridge gel material. Third, close the cap and centrifuge for 2 minutes at 750 x g. Retain the eluate as it contains the purified PCR product. The Gel Filtration Cartridge contains the free dye impurities and may be discarded. Use 1 µL of the eluate for ABI 310 or 3100 sample preparation.

Residual dye artifacts may be seen around 78 bp with 6FAM (blue dye), 82 bp and 130 bp with VIC (green dye), and approximately 115 bp with NED (yellow dye). These dyes can interfere with allele calls in the FCA441 locus.

Detection of PCR Products

The ABI 310 and 3100 testing may be performed with the same POP4 polymer, capillaries, and buffer used for STR typing with commercial kits. The primary difference is the matrix file that must be setup on the instruments for the cat STR system, which uses the following dyes: 6FAM (blue), VIC (green), NED (yellow), and ROX (red) dyes.

ABI 310

The ABI Prism® 310 Genetic Analyzer is used with **filter set F** (same as Profiler Plus and COfiler kits use). An appropriate matrix needs to be established with matrix standards for the 4 dyes **6FAM**, **VIC**, **NED**, and **ROX** (Applied Biosystems). ABI does not supply a matrix standard for these 4 dyes so materials from **matrix standards set DS-33** (P/N 4318159) and **DS-32** (P/N 4312131) must be combined. The 6FAM, VIC, and NED standards come from DS-33 and the ROX standard comes from DS-32. Follow the standard methods outlined in the ABI 310 User's Manual (pages 3-42 to 3-45) for creation of a 4-dye matrix file.

Each sample for analysis on the 310 is prepared by adding **1 µL PCR product** to **19 µL** of **Hi-Di™ formamide** (Applied Biosystems, P/N 4311320) containing **0.75 µL GS500 ROX** (P/N 401734). Samples do not need to be heat denatured nor snap cooled primer to running them. Samples are injected for 5 s at 15,000 volts and separated at 15,000 volts for 24 minutes with a run temperature of 60 °C. Standard electrophoretic conditions are used including 310 Genetic Analyzer POP™-4 (P/N 402838), 1X Genetic Analyzer Buffer with EDTA, and a 47 cm x 50 µm capillary (P/N 402839).

ABI 3100

Prior to running any samples with the cat STR system on the ABI 3100, a 4 dye matrix needs to be established under the "**Z filter**" with the dyes 6FAM (blue), VIC (green), NED (yellow), and ROX (red) using **matrix standard set DS-30** (P/N 4316100) and substituting the **VIC matrix standard** (P/N 4323022) for HEX. Samples are typically prepared with **9 µL Hi-Di™ formamide** (Applied Biosystems, P/N 4311320), **0.6 µL GS500 ROX** (P/N 401734), and with either **1 µL PCR product** (filtered or unfiltered). Again, no heat denaturation nor snap cooling is required prior to injection on the ABI 3100.

The samples may be run using the default module **GeneScan36_POP4DefaultModule**, which performs an electrokinetic injection onto the 16-capillary array for 10 s at 3,000 volts. The STR alleles are then separated at 15,000 volts for approximately 30 minutes with a run temperature of 60 °C using the 3100 POP™-4 sieving polymer (Applied Biosystems, P/N 4316355), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and a 36 cm array (P/N 4315931).

Data Analysis

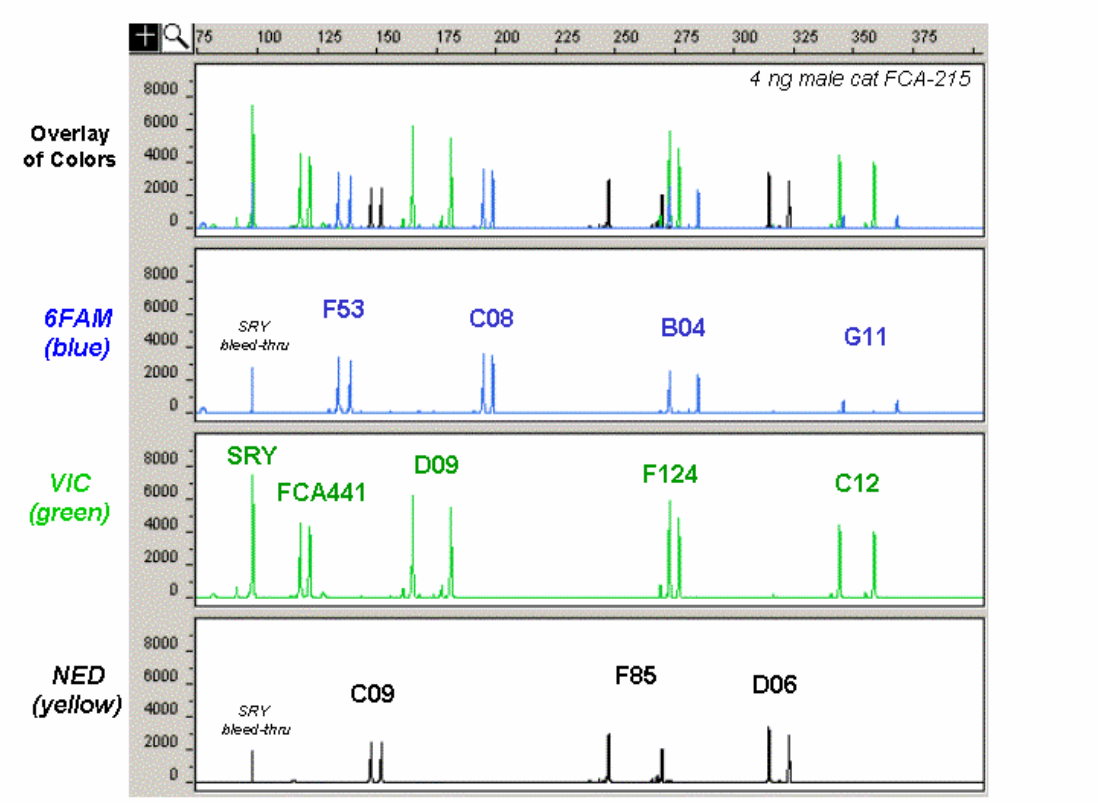
GeneScan

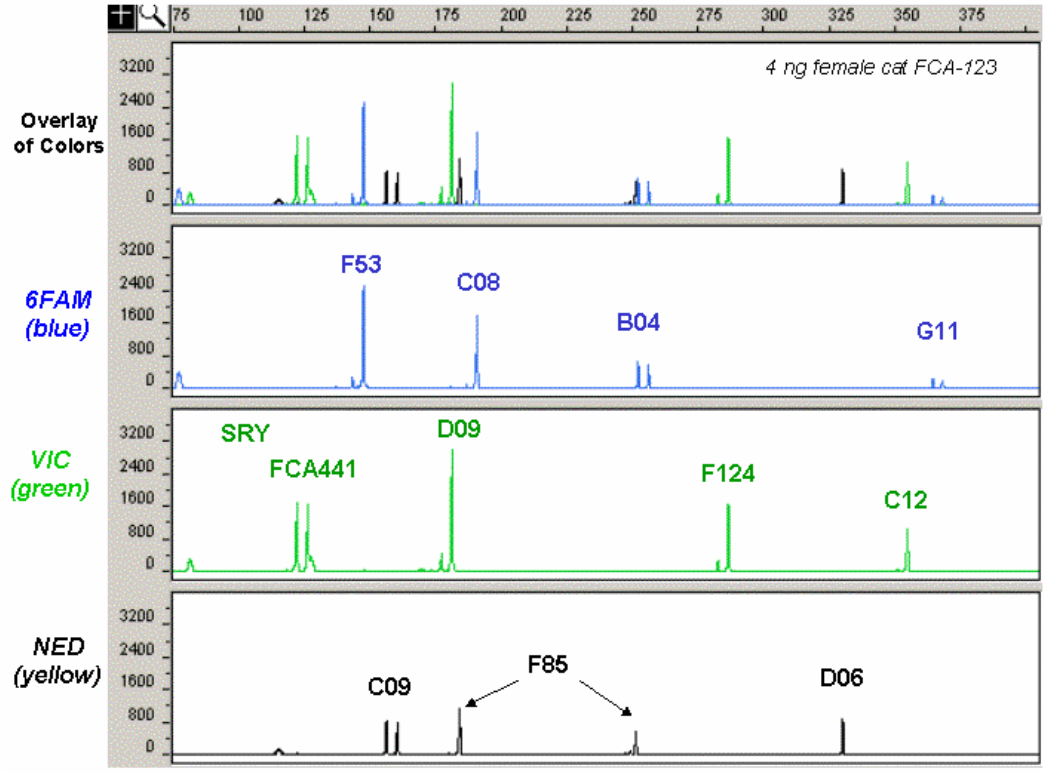
Samples must first be processed in Genescan® 3.1 (for Macintosh) or GeneScan 3.7 (for Windows NT) to define the peaks for the STR alleles through applying the matrix for dye color spectral separation and to size the STR alleles properly against the GS500 ROX internal size standard. Check the allelic ladder to make sure that all alleles are designated as peaks. Some of the G11 allele peaks may fall below the detection threshold if your instrument sensitivity is poor.

Genotyper

Dye blobs may impact allele calls but should be able to be removed during sample editing. A genotyping macro may be developed in the future to call alleles based on a sequenced allelic ladders or sizing information.

EXAMPLE DATA





The provided primers for the cat STR multiplex system contain free, unattached dye molecules that are a by-product of the primer synthesis procedure. These free dyes create what are referred to as “dye blobs” when samples are injected onto a capillary electrophoresis system. Dye blobs are seen as peaks that are usually much wider than the STR alleles. However, they can fall in regions of the electropherogram that may interfere with allele calling. Our current fix for the dye blob problem is to remove the free dye molecules through post-PCR filtration using Gel Filtration Columns from Edge Biosystems (Gaithersburg, MD). The figure below illustrates the difference obtained on an ABI 310 when dye blobs have been removed from a Big Mini PCR sample.