HIGH THROUGHPUT DNA ISOLATION AND STR ANALYSIS FOR ANIMAL IDENTIFICATION: FORENSIC CASEWORK AND DNA DATABASE APPLICATIONS IN ANIMAL GENETICS

PAOLO GAROFANO, ROBERTA VENERONI, SIMONA MOLINARI, MARGHERITA BONUGLIA, PAOLO DE IULIIS, MARCO MONTANA
Forensic Genetics Division UNIRELAB s.r.l. Settimo Milanese Italy.

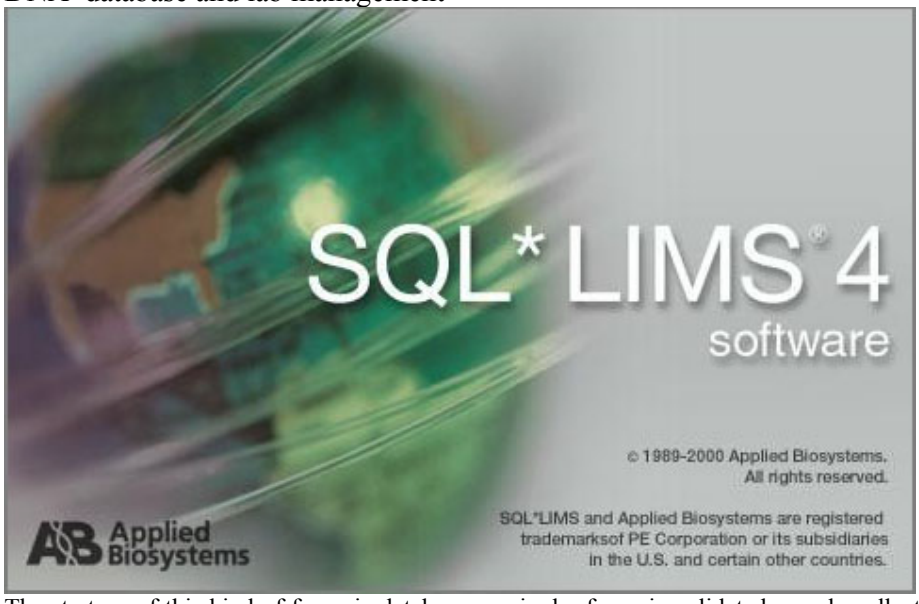
Introduction

In Italy in 2002 legislation became effective to establish "Horse Competition Court Prosecutors" involved in investigations about "crimes" committed in the "Racing Horse World" essentially due to frauds about bets (horse's substitution during competitions and reproduction, doping etc.), and the necessity occurred to set up a "forensic lab" divided in two Divisions:

1. Toxicology Division in charge of about 40.000 samples per year
2. Genetics Division in charge of 20.000 samples per year

The Genetics Division needed to start up a national centralized DNA database with a fast-automated procedure to analyze all samples of interest for genetic identification, paternity test, kinship tests and future comparisons.

DNA database and lab management



The start up of this kind of forensic database required a forensic validated sample collection procedure and a forensic validated DNA storage method for future analysis. For this purpose, a solid support such as FTA® Paper Whatman has been chosen to have a relatively inexpensive and easy to consult DNA database. A new 17 loci validated genotyping kit using Short Tandem Repeats (STR) suggested by I.S.A.G. produced by Applied Biosystems, was the best choice in order to increase the discrimination power of the analysis for paternity tests and to achieve the identification of each subject from the genetic profile. Forensic validated kits and protocols were seletced for DNA extraction too.

All the process from sample registration to final result were managed by SQL* LIMS® Forensic software by Applied Biosystems. SQL* LIMS® Forensic is a powerful "laboratory information management system" designed for use in the laboratory environment for data storage and retrieval. This system, in our case, contain all the general information about Italian Competition Horses (phenotypical aspect, , parents profile and genealogy, race, toxicology tests, DNA profiles etc.) and is able to interface with all the analysis software and all the laboratory instruments.

In this way, for the first time, forensic procedures have been applied on animals genetic identity to the entire process, from sample collection to final diagnosis.

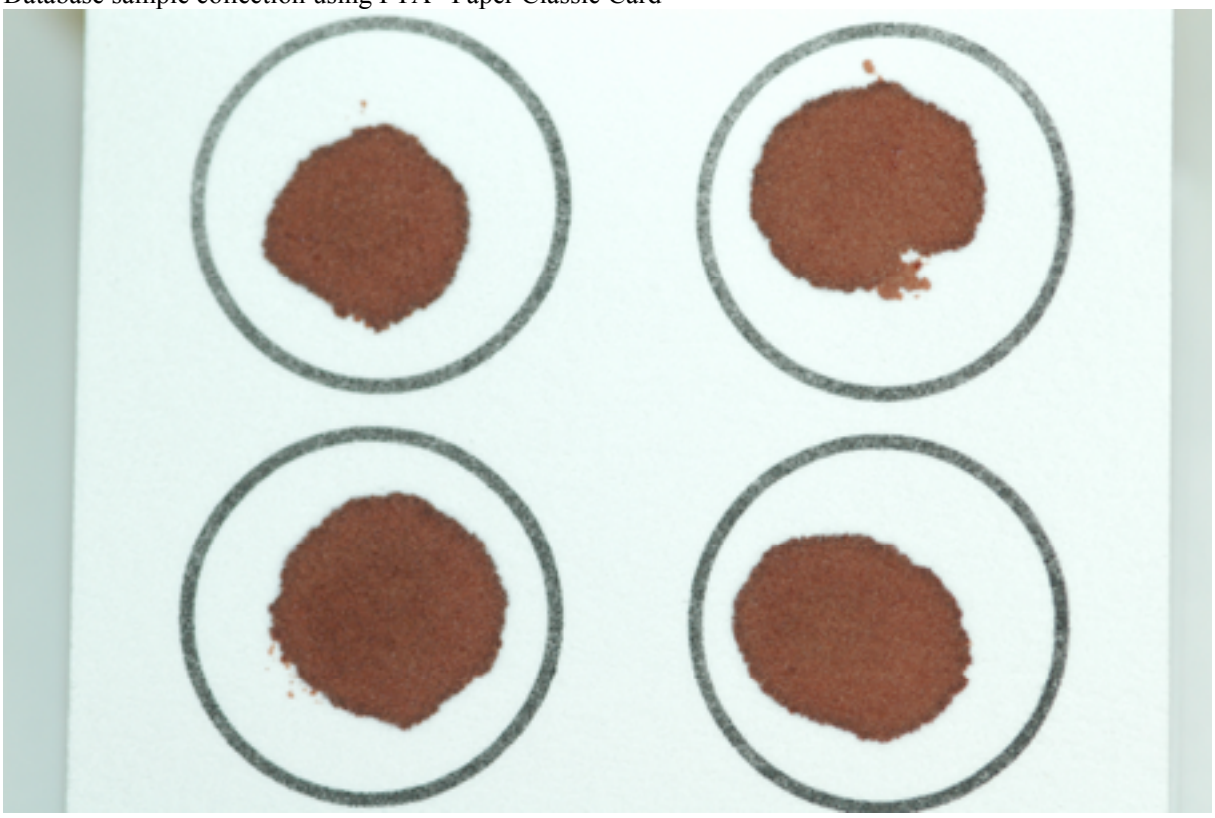
Automation

Two automatic workstations have been set up with different protocols, able to isolate DNA from several kind of sample like FTA®, hair roots, blood, urine, sperm etc. After DNA extraction, on the same line, they are able to prepare samples and reagents for PCR and electrophoresis analysis.
The robotic workstations are:

1. TECAN GENESIS RSP 150
2. HAMILTON MICROLAB® STAR

Both of them are extremely versatile and they are able to execute every laboratory protocol with high accuracy and high speed with no possibilities of human errors and contaminations.
HAMILTON'S MICROLAB® STAR, 4-8-12-16 independent pipetting channels, (is a highly modular and flexible system. It can easily be adapted to various processes of molecular biology applications. It is the first robot that doesn't use peristaltic pump for liquid handling, it is a fully automated hand-free processing with built-in robotic plate-handler (iSWAP) for a fully validate system. Hamilton's technology with a dual liquid level detection system is able to moves each single channel with an accuracy of 0.1mm in all axes, and is able to pipetting 0.1µl in aspiration and injection.
Exact efficient liquid handling, minimal reaction volume, 10-fold increase in throughput compared to manual processing, are just some of the main characteristics of this robot. The mechanical arm can be equipped with fixed tips, low volume tips, disposable tips and filter tips.
Interfacing these Robots with thermalcyclers, genetic analyzers and analysis software, through SQL LIMS® forensic management is possible to easily process about 1000 samples per day with just one operator for each robot.
All the procedures are completely executed from automatic work stations with a report of all the analytical process step by step.

Database sample collection using FTA® Paper Classic Card



FTA technology provides a method that is designed to simplify the collection, shipment, archiving and purification of nucleic acids. FTA card are impregnated with a patented chemical formula that lyses cell membranes and denatures proteins upon contact. Nucleic acid are physically entrapped, immobilized and stabilized for storage at room temperature.

A procedure for collection of sample has been improved by UNIRELAB s.r.l. to obtain certified collected samples.

During the first six months of life, every competition horse is examined by a Veterinary Doctor that compiles the "Racing Passport card" with all the classical identification data, He inserts a transponder and collect blood too.

A small amount of it (about 125µl) is immediately dispensed on a sterile foam and then through this process, placed onto the FTA® sterile Classic Card in order to avoid the pudding of the liquid sample which should overload the chemicals on the card. The sample is let to dry at room temperature for few minutes.

The microchip number is transformed in a bare code sticker and applied onto FTA® card shell. the FTA® card is sent to laboratory.

Database DNA isolation protocol using FTA® Paper Classic Card

When samples and reports arrive to our lab, they are registered into SQL* LIMS®.
SQL* LIMS® generates working sheet composed by 94 samples which are directly communicated for the first step process to an automatic puncher equipped with a barcode reader. This instrument read again the barcode to control the sample, cut and dispenses a disc of FTA Paper (1.2mm) into 96-well microplates; two wells are used for negative and positive control for PCR analysis.
The microplates are transferred on the robotic workstations and the worksheet is communicated to start the next step: DNA extraction.
For every sample, 200µl of FTA® Purification Reagent are added; after an incubation for about 5 minutes at room temperature all spent FTA® Purification Reagent is removed. This step is repeated twice for a total of three washes. Then 200µl of TE Buffer (10mM tris-HCl, 0.1mM EDTA, pH8.0) are added incubated for 5 minutes at room temperature, removed and discarded. This step is repeated once more time. At last the FTA punches are washed with molecular grade distilled water and then they are dried at 60°C for 30. Now the FTA punch is ready-to-use for PCR applications.

DNA extraction protocol from different kind of sample

Differently from FTA procedure, Promega's DNA IQ ™ System is used for DNA isolation from hair roots, blood, urine, sperm, tissues, stains, DNA or stain on FTA paper etc.
Promega's DNA IQ™ is a magnetic beads based extraction system including two steps; for biological material on solid supports, the first step provides an easy, rapid, efficient and almost universal DNA extraction method. This step is unnecessary for liquid samples. The second step uses a specific paramagnetic resin that purifies the DNA without requiring extensive washing to remove the lysis reagent; extensive washing may cause a significant loss of material. This system is designed to rapidly purify small quantities of DNA, approximately 100ng or less.
The paramagnetic resin is used to capture a consistent amount of DNA, and it has a fixed binding DNA capacity, also in presence of excess DNA, as a result can be bypassed the quantitation step after purification procedure.
Horse hair follicles are the sample that arrives in laboratory more frequently. Horse hair follicles are drayed by veterinaries paying attention to take entire follicles. For DNA extraction three follicles placed in a 96-well microplates

are used; 25 µl of Incubation Buffer/ Proteinase K Solution (Incubation Buffer, supplied with the DNA IQ ™ System kit, 0.1M DTT and 1.8mg/ml ProteinaseK) are added at each sample, mixed and incubated at 56°C for 1 hour. After incubation sample are disposed at room temperature and added 2 volumes of Lysis Buffer adds with 1:100 DTT 1M, and 7µl of resuspended DNA IQ ™ Resin. Then the sample are vortexed at high speed and incubated at room temperature for 5 min. After the incubation the samples are placed in a magnetic stand for the resin separation, all solution is removed paying attention to not aspirate the resin pellet.
100 µl of Lysis Buffer are added to sample and removed from the magnetic stand to be vortexed. After replacement of microplatse on the magnetic stand, all reagents are removed as the previous step. Now 100µl of Wash Buffer are added and the resin is wash for three times with the same procedure of Lysis Buffer. Then the resin is let to dry in Magnetic Stand for 5 min. At last 25µl of Elution Buffer are added, well mixed and incubated at 65°C for 5 min. After resin separation on magnetic stand, the DNA solution is transferred in a new microplate, that can be stored at 4°C for short term storage or at -20°C for long term.
A similar procedure is used to extract DNA from liquid sample, blood, sperm, urine, trace samples, but there is no need to digest the samples with Proteinase K, this samples are sufficiently disrupted with the DNA IQ ™ Lysis Buffer and thus not require pretreatment with Incubation Buffer/ Proteinase K Solution to ensure sample lysis.
The quality of template is very excellent and DNA IQ ™ system permits to use only one protocol for different kind of sample and its certified for forensic use.
The robotic workstations are able to process different kind of samples with Promega DNA IQ ™ System for DNA extraction with high troughput.

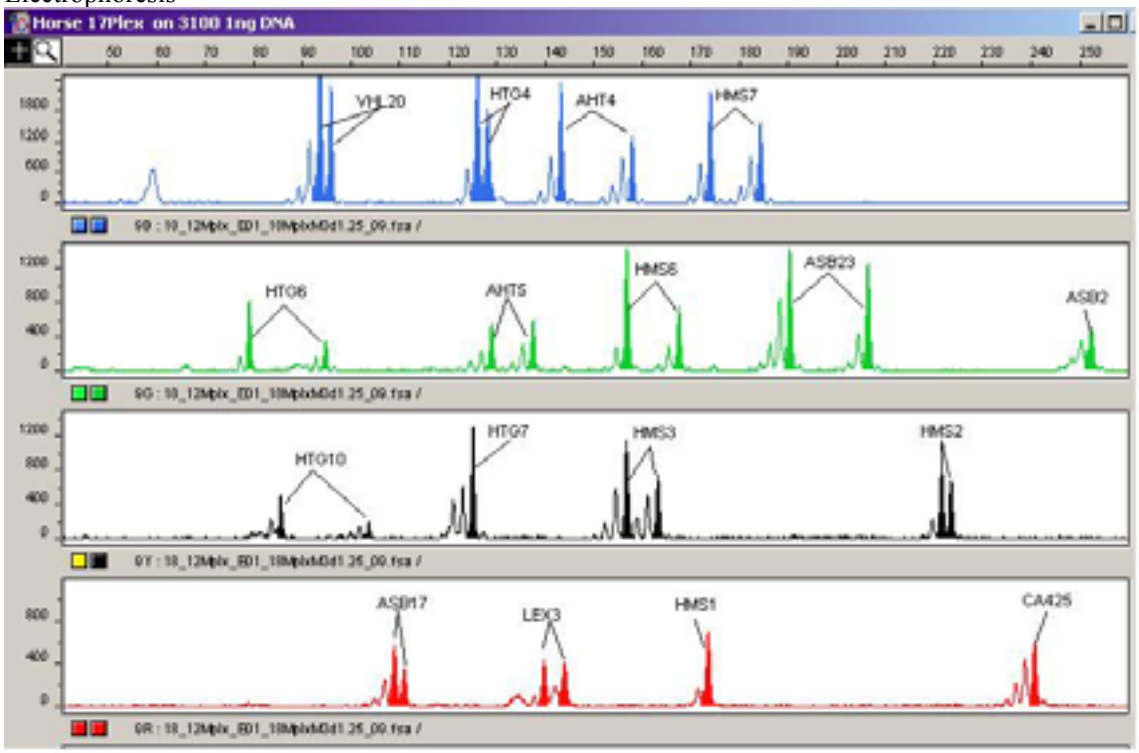
Polimerase Chain Reaction

For PCR analysis, is used. the new 17 loci 5 colors forensic validated and certified kit called Horse StockMarks® 17 plex supplied by Applied Biosystems
This genotyping kit is highly discriminative 17 equine-specific loci. The kit uses a new set of five fluorescent dyes developed by Applied Biosistems (DS-31), with four dyes used to label the forward amplification primers (6-FAM®, VIC®, NED ™, and PET®) in each primer set. The stock Marks equine kit contains five loci (ASB17, LEX3, HMS1, CA425, AND ASB23) in addition to the 12 original loci recommended by International Society of Animal Genetics (ISAG). These 17 loci are combined and amplified in a single PCR cycle, which dramatically increases the power of discrimination or inclusion for pedigree analysis while reduce the time and work required to perform such tests.
The StockMarks® 17 Horse kit, designed to improve the laboratory efficiency by genotyping more markers in shorter time, is composed by 17 primer sets labeled with new fluorescent dyes, which can be amplified in a single tube PCR and run on capillary electrophoresis genetic analyzers.
The PCR is performed on a GeneAmp® PCR System 9700. The cycling program expects an initial step at 95°C for 10min to activate the AmplyTaq Gold® DNA Polymerase, 28 cycles each of them divided into denaturing phase (95°C for 30sec), annealing phase (60°C for 30sec), and extension phase (72°C for 60sec), then two final extensions 60°C for 30sec and 72°C for 60min and a final step at 4°C hold.
The robotic workstations prepare the PCR master mix and dispense it with volume's reduction saving time and costs. Perfect results can be reached with 25% reaction recommended volume (3,5 µl). PCR mix reaction can be added directly onto FTA® or to 0,125 µl - 0.5µl of DNA extracted by IQ ™ System.

Equine specific loci analyzed with the kit StockMarks® 17 Plex

Locus	Dye	Color	Size Range (bp)
VHL20	6-FAM®	Blue	70-110
HTG4	6-FAM®	Blue	112-137
AHT4	6-FAM®	Blue	140-166
HMS7	6-FAM®	Blue	167-202
HTG6	VIC®	Green	67-110
AHT5	VIC®	Green	112-147
HMS6	VIC®	Green	150-175
ASB23	VIC®	Green	176-216
ASB2	VIC®	Green	218-268
HTG10	NED™	Yellow	80-110
HTG7	NED™	Yellow	114-141
HMS3	NED™	Yellow	145-182
HMS2	NED™	Yellow	204-254
ASB17	PET®	Red	84-130
LEX	PET®	Red	131-165
HMS1	PET®	Red	166-208
CA425	PET®	Red	216-250

Electrophoresis

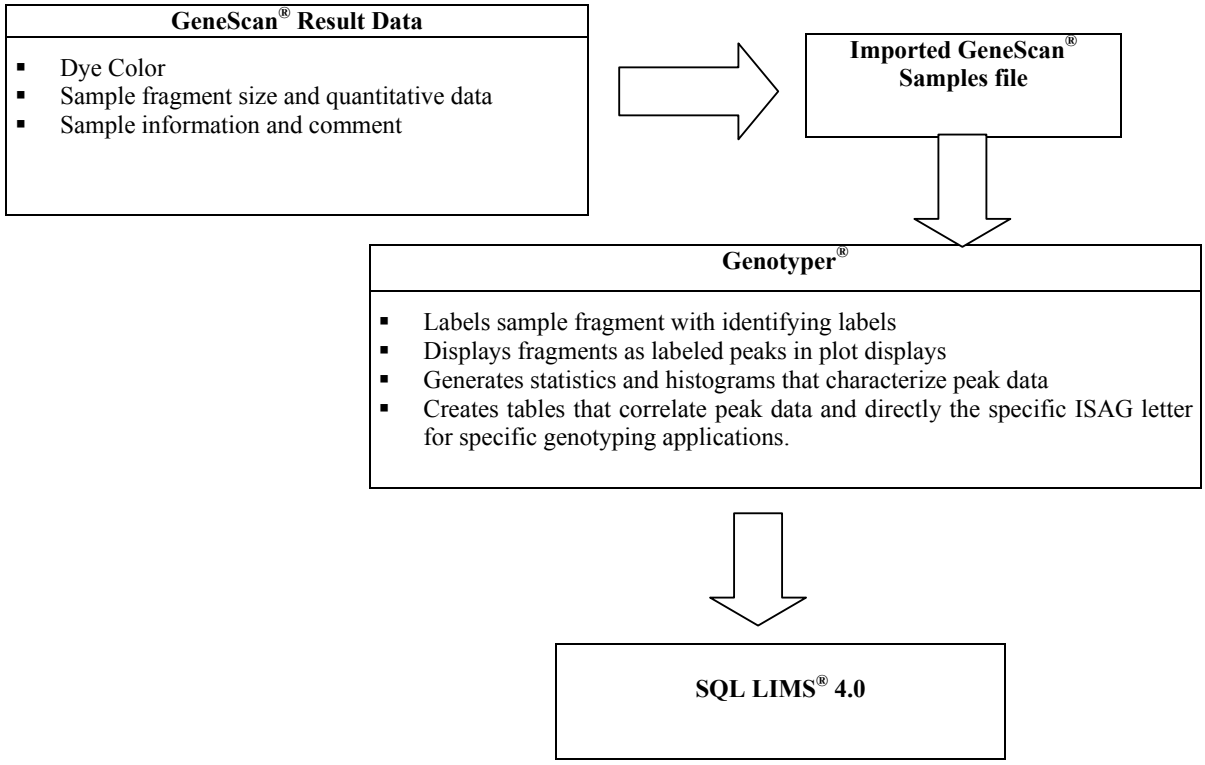


After the amplification process the size of dye-labeled PCR products is determine by running them on 310 or 3100 ABI PRISM® Genetic Analyzers.
The DNA template are denatured using a mix of Hi-Di™ Formamide and GeneScan®-500 LIZ that is the internal size standard for the ABI PRISM® Genetic Analyzer 310 or 3100. The samples are prepared with 12µl of Hi-Di™ Formamide, 0.1µl GeneScan ®-500 LIZ® Size Standard and 0.33µl of PCR product, dispensed in a new 96 well microplate. The sample are denatured in thermalcycler at 94°C for 2min and 4°C hold.
To run is used polymer syringe GS STR POP™ 4 (3100 POP-4) (Performance Optimized Polymer) with Buffer (10X) with EDTA (402824 ABI).
The samples, through the capillaries, are revealed by a fluorescence detector using Filter Set G5 and run module GeneScan36vb_POP™4 Default Module.

Genetic profile and I.S.A.G. nomenclature

The GeneScan® software collects the signal and determines size of amplified loci DNA, assigning a base pair size for each sample. GeneScan® data are exported directly to GenoTyper® or GeneMapper® software for automated genotyping of individual.
The Genotyper® software analyzes the results of a fragment analysis data collected by an ABI PRISM® instrument and generated by the GeneScan® Analysis Software. It is an application that enable to analyze and interpret nucleic acid fragment size data by converting it into user defined results.

The following figure shows how the Genotyper® software analyzes imported ABI PRISM® GeneScan® Analysis software sample files.



The Genotyper® 3.7 NT software can automatically perform all calculations, comparative analyses and peak labeling activities once you specify appropriate settings and issue the appropriate sequence of commands.
The software uses templates and macros to automate procedures for an application. The macro and templates supplied with the Genotyper® software automate procedures presumed necessary to complete particular genotyping applications.
Dye/lanes contain sample information for electrophoresed nucleic acid fragment; They provide the source data for all the Genotyper® software procedures. This is what happens in each phase of the dye/lane generation process:

1. Nucleic acid fragments are labeled with four different colors (blue, green, yellow, red) and electrophoresed in a single lane of a gel based automated DNA sequencer.
2. The GeneScan® Analysis Software extracts fragment information from capillaries and generates one GeneScan® file per lane or capillary. Each file contains size and quantity information for each dye/labeled fragment.
3. When the GeneScan® file is imported in the Genotyper® software; Genotyper® extracts file information and generates one dye/lane list entry for each dye color. Each dye/lane list entry contains size, quantity and sample information for all fragments labeled with single dye color and electrophoresed in a single lane.

It's interesting to note that we developed in collaboration with APPLERA ITALIA an horse 17 plex macro for Genotyper® able to convert base pair size with exactly corresponding ISAG letters.

File Name	Sample Info	Category	Allele 1	Allele 2	Allele 1 bp	Allele2bp
2_BATCH-115_A05_33_01.fsa	1692116C	AHT4	O	O	158	158
2_BATCH-115_A05_33_01.fsa	1692116C	AHT5	J	K	130	132
2_BATCH-115_A05_33_01.fsa	1692116C	ASB17	R	S	119	120
2_BATCH-115_A05_33_01.fsa	1692116C	ASB2	O	R	246	252
2_BATCH-115_A05_33_01.fsa	1692116C	ASB23	J	K	188	190
2_BATCH-115_A05_33_01.fsa	1692116C	CA425	I	M	231	239
2_BATCH-115_A05_33_01.fsa	1692116C	HMS1	J	J	176	176
2_BATCH-115_A05_33_01.fsa	1692116C	HMS2	P	R	232	236
2_BATCH-115_A05_33_01.fsa	1692116C	HMS3	I	P	148	162
2_BATCH-115_A05_33_01.fsa	1692116C	HMS6	P	P	167	167
2_BATCH-115_A05_33_01.fsa	1692116C	HMS7	L	L	174	174
2_BATCH-115_A05_33_01.fsa	1692116C	HTG 7	O	O	125	125
2_BATCH-115_A05_33_01.fsa	1692116C	HTG10	I	N	86	96
2_BATCH-115_A05_33_01.fsa	1692116C	HTG4	K	M	126	130
2_BATCH-115_A05_33_01.fsa	1692116C	HTG6	J	J	85	85
2_BATCH-115_A05_33_01.fsa	1692116C	LEX3	P	P	161	161
2_BATCH-115_A05_33_01.fsa	1692116C	VHL20	M	M	94	94

Profile registration and paternity test

File Name	Sample Info	Category	Allele 1	Allele 2	Allele 1 bp	Allele2bp
2_BATCH-115_A05_33_01.fsa	1692116C	AHT4	O	O	158	158
2_BATCH-115_A05_33_01.fsa	1692116C	AHT5	J	K	130	132
2_BATCH-115_A05_33_01.fsa	1692116C	ASB17	R	S	119	120
2_BATCH-115_A05_33_01.fsa	1692116C	ASB2	O	R	246	252
2_BATCH-115_A05_33_01.fsa	1692116C	ASB23	J	K	188	190
2_BATCH-115_A05_33_01.fsa	1692116C	CA425	I	M	231	239
2_BATCH-115_A05_33_01.fsa	1692116C	HMS1	J	J	176	176
2_BATCH-115_A05_33_01.fsa	1692116C	HMS2	P	R	232	236
2_BATCH-115_A05_33_01.fsa	1692116C	HMS3	I	P	148	162
2_BATCH-115_A05_33_01.fsa	1692116C	HMS6	P	P	167	167
2_BATCH-115_A05_33_01.fsa	1692116C	HMS7	L	L	174	174
2_BATCH-115_A05_33_01.fsa	1692116C	HTG 7	O	O	125	125
2_BATCH-115_A05_33_01.fsa	1692116C	HTG10	I	N	86	96
2_BATCH-115_A05_33_01.fsa	1692116C	HTG4	K	M	126	130
2_BATCH-115_A05_33_01.fsa	1692116C	HTG6	J	J	85	85
2_BATCH-115_A05_33_01.fsa	1692116C	LEX3	P	P	161	161
2_BATCH-115_A05_33_01.fsa	1692116C	VHL20	M	M	94	94

The genetic profile of each horse is inserted into SQL* LIMS Forensic and associated with parents profiles. SQL* LIMS® Forensic can compare different profiles or search if one or more unknown ones are present in the database.
The paternity tests are performed using DNA VIEW™ software by Charles Brenner.
DNA VIEW™ is the only integrated software package for DNA identification analysis in forensic genetics: this software has been customized for horse forensic genetics too and through our database
DNA data is normally supplied to the program either by importing a text file such as a Genotyper® allele table or by typing it in. The subjects are recognized from the computer by *sample* or *accession number* which in our case is the LIMS number. DNA VIEW™ has several commands to analyze cases of different kinds in different ways. Each of them operates on a selected case that has a collection of *roles* with sample numbers attached. Each horse in a case occupies a role within that case: roles are single capital letters and each role letter has a descriptive word associated with it. In our case:
"Mother" is associated with the role "M"
"Father" is associated with the role "F"
"Child" is associated with the "C".
The DNA profiles for the various individuals in the case are brought together for the analysis.

Paternity/maternity case: the typical paternity trio case, or common variation of it such as missing mother or maternity for example, are analyzed by the *Paternity case* command. It operates on a has roles designating mother (usually), colt and alleged father or fathers. Per trio performs the analysis locus by locus and prepares an informal report of the result.

Kinship: the paternity trio is one of example of an infinitude of *kinship* problem: deciding how strongly the DNA evidence favors one or another arbitrary alternate hypothesis as to how a collection of horse might be related.

Many DNA VIEW™ operations produce a report. The report resides in the "report buffer" until it is supplanted by another report. In the meantime, the report can be printed, viewed or stored as a file.

Protocols and data flow

The data flow is managed by software. SQL* LIMS® software transmit the working sheet at the automatic puncher which dispenses 1.2mm of FTA® into 96 well microplates; at the end of punching process, microplates are transfered to robots for DNA isolation and PCR set up. The mechanical arms move the microplates to the thermalcycler and back again at the end of PCR cycling protocol for electrophoresis set up. The files generated from DNA Sequencers, are managed from different and finally the genetic profiles are stored from SQL *LIMS®.

CERTIFIED SAMPLE ON FTA® WITH BARE CODE
↓
REGISTRATION OF SAMPLE IN SQ LIMS® Forensic software
↓
BSD DUET AUTOMATIC PUNCHER equipped with barcode reader
↓
Robotic Workstations
TECAN GENESIS RSP 150
and/or HAMILTON MICROLAB® STAR
DNA extraction , reaction mix and sample set up for PCR analysis
↓
THERMAL CYCLERS
GeneAmp® PCR System 9700
↓
Robotic Workstation
samples set up for electrophoresis analysis
↓
GENETIC ANALYZERS
ABI PRISM® 310/3100 Genetic Analyzers
↓
GeneScan®
↓
Genotyper® 3.7 NT
↓
SQL* LIMS®
↓
DNA VIEW™
↓
SQL* LIMS®

Results

In short time, over 50.000 whole samples have been processed comparing two kits (12 loci 4 colors and 17 loci 5 colors) and a forensic DNA database has been constituted. Because of the establishment of "Horse competition Court Prosecutors", starting from 2003, all genetic identifications and paternity tests diagnosis of Italian competition horse population are completely forensic certified and validated in all steps. Human forensic protocols and procedures for the first time has been applied to animals and high quality standards have been achieved.
Through these procedures is possible to process with just one complete robotic station easily 576 samples each day that means more than 200.000 samples per year with only two operators. Complete routine diagnosis is possible from 3 to 24 hours depending on sample and question requested (simple profile, paternity test, kinship test, forensic analysis). Because of the potential productivity of this kind of system, it has been set up for all the available animal forensic patented kits like cattle, dogs and for human identification of course.

References

- Dimsoski P. Development of a 17-plex Microsatellite Polymerase Chain Reactions Kit for Genotyping Horse. *Croatian Medical Journal 2003; 44(3):332-335*
- Clark, J.M. Novel non-templated nucleotide addition reactions catalyzed by prokaryotic and eukaryotic DNA polymarases. *Nucleic Acids Res. 1988; 16:9677-9686*
- Kwok S. and Higuchi R. Avoiding false positives with PCR. *Nature 1989; 339:237-238*
- Magnuson VL, et al. Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polimerase: Implication for PCR-based genotyping and cloning. *Bio Techniques 1996; 21:700-709*
- Mullis KB, and Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol 1987; 155:335-350.*
- Fimmers R, Schneider MP, BaurMP. Comparison of Comparison of Different Methods for the Calculation of Indices of Paternity in Advances in Forensic Haemogenetics 4, *Eds C. Rittner & PM Schneider, pp277-284, 1992*
- Morris JW, Garber RA et al. The Avuncular Index and Incest Index. *Advances in Forensic Haemogenetics 2, 1998; 607-611*
- Sosee AC, Demers D. Molecular Analysis of Genetic Identity. *Current Protocols in Human Genetics 1994; 9.71-9.7.14*
- Wier BS. Genetic Data Analysis. *Sinauer Associatees. Inc. 1996*
- Bruce Budawel et al. DNA Typing Protocols: Molecular Biology and Forensic Analysis. *A Bio Techniques Book Publication. Eaton Publishing. 2000*
- Tozaki T. et al. Population Study and Validation of Paternity Testing for Thoroughbred Horse by 15 Microsatellite Loci. *J. Vet. Med. Sci. 2001; 63(11): 1191-1197*
- Aralda et al. FTA Technology, unique Format for the Collection, Shipment, Archiving, and Processing of Biological Samples. *Poster 7th CODIS User's Conference 2001*
- Baker et al. Isolation of Genomic DNA from Blood Using a Novel Filter-Based DNA Purification Tecnology. *Bio Tecniques 2001; 31: 142-145*
- Belgrader & Marino. Automated Sample Processing Using Robotic for Genetic Typing of SRT Polymorphisms by Capillary Electrophoresis. *Lab. Robotics & Automation 1997; 9:3-7*
- Belgrader et al. Automated DNA Purification and amplification from Bloodstain Cards Using a Robotic Workstation. *Biotechniques 1995; 19: 427-432*
- Bever et al. Implementation of Laboratory Automation for the analysis of STR loci. *8th International Symposium on Human ID 1997*
- Borys et al. PCR Volume Reduction Study Using Bloodstain FTA Collection Card and Capillary Electrophoresis. *Poster: 9th Annual Int Symp. Of Human ID. Orlando, USA 1998*
- Brenner CH. Difficulties in estimating ethinic affiliation. *Am J Hum Genet 1998; 62: 1558-1560*
- Brenner CH. Probable Race of a Stain Donor. *Procedingss from the Seventh Human Symposium 1996*, Promega Corporation 48-52
- Krenke, BE, A Tereba, SJ Anderson, E Buel, S Culhane, CJ Finis, CS Tomsey, et al. Validation of a 16-locus fluorescent multiplex system. *J Forensic Sci 2002; 47(4): 773-785.*
- Tack, L & T Grunst. Automated Forensic DNA Isolation from Blood using Promega's DNAS IQ System and the MultiPROBE II Forensics DNA Workstation. *Poster 2002.:*
- Tack, L. PerkinElmer's MultiPROBE II Forensic Workstation. *Application Note: AAFS 55thAnnual Meeting, Feb 18-21, 2003, Chicago IL* .
- Promega Technical Bulletin #TB297: "DNA IQ System – Database Protocol". 6/02
- Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., et al. DNA extraction strategies for amplified fragment length polymorphism analysis. *J For Sci 1994; 39 (5): 1254-1269.*
- Promega Technical Bulletins #TMD012 and TMD006: "PowerPlex 16 System", 10/02 and "GenePrint® Fluorescent STR Systems". 6/02.