The DNA-Project of the Llama and Alpaca Registry Europe (LAREU)

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Abstract

The European registry LAREU is providing an online registration system for breeders and owners of South American camelids (SACs), free of charge. Founded in the year 2005, over 11000 animals from more than 10 European countries are stored in LAREU's database up to now, with a yearly growth of about 15%. In 2008 LAREU has suggested an international standard for DNA markers of SACs for parentage verification, which has been developed in the following years by ISAG (International Society of Animal Genetics) and is being monitored by regular ring tests since then. The standard set of 14 markers is now being used by several European laboratories collaborating with LAREU. After giving some details on the main features of the LAREU registry, the new international DNA marker set is presented and the procedure for DNA testing and checking the parentage in SACs with the new marker set within the LAREU registry is described.

Key words: camelid, registry, online, marker panel

Introduction

The transparent borders within the European Union are offering breeders of South American Camelids (SACs) great opportunities for improving the genetics of their animals, both with respect to fiber quality as well as to conformation and health. An essential factor in a successful breeding program is the detailed knowledge of the animals' ancestors, which can only be asserted by means of genetic information, on the basis of which a reliable pedigree can be built. DNA testing and parentage verification of alpacas and llamas is frequently done by national camelid associations, who have their own data bases, with no possibility (or intention) to share their animal data. They work together with DNA labs of their choice, who usually perform their DNA analyses using their favorite "private" DNA marker sets. Unless one is willing (and in a position) to retest parents and crias by the same lab, there is obviously no way to obtain reliable pedigrees for animals across associations and national borders. Another important aspect for the animal breeder of a unified European registry is the possibility to follow the pedigree of an animal, if it changes ownership and with it most likely also the national association where it was originally registered.

LAREU was founded with the intention to overcome these shortcomings. The general philosophy of LAREU is to provide just a registry, the creation and maintenance of stud books and other "quality" criteria, usually depending on the specific breeding aspects of the different camelid associations, should stay in their responsibility. The LAREU members have access only to the information on the animals which have been entered into the database by them. But they are free, of course, to extract material from the LAREU database and provide it to

their canelid association. In this respect LAREU views itself as complementary to the (many) national and regional camelid associations.
In section 2 we will describe the basic functionality of the LAREU registry and present statistical material from the LAREU database. In section 3 we present the new DNA marker set as defined by ISAG, and then describe in section 4 the procedures developed together with internationally accredited DNA laboratories to provide a transparent and reliable system for the camelid owner to obtain and evaluate the DNA data of his animals. Section 5 concludes this paper with some ideas of further plans and developments of the LAREU registry.

The LAREU Registry

LAREU was founded in 2005 as a truly European registry by a group of camelid owners from Finland, France, Germany, Italy, Sweden, Switzerland and The Netherlands. It was clear from the beginning that only a low-profile personnel structure would have a chance of a long-term reliable service. It was the idea of the founding members to create a computer-based system where the animal registration is done by the owners, using electronic input masks from a (at that time still to be created) LAREU webpage (www.lareu.org). Legally the structure of LAREU is that of a Swiss association, with seat in Brig/Glis, Vallais, Switzerland.

It was very fortunate to find a provider willing to host and support LAREU's database on an honorary basis: TASSO e.V., with seat in Germany. Otherwise this project would not be possible as it was planned. TASSO [1] is managing a large database for searching and finding lost animals, mostly cats and dogs, and is strongly engaged in animal welfare projects. All the programming for the database access and the corresponding services is done by the CEO of TASSO, together with the author of this article. The data of the animals registered with LAREU are entirely separated from the TASSO database, and the LAREU registration software has been developed independently.

Protection of privacy is an important feature of LAREU: Each animal owner creates his own account where he enters his breeder data (the mask to be filled is shown in fig. 1) and the data entered are visible only to the owner of the LAREU account.

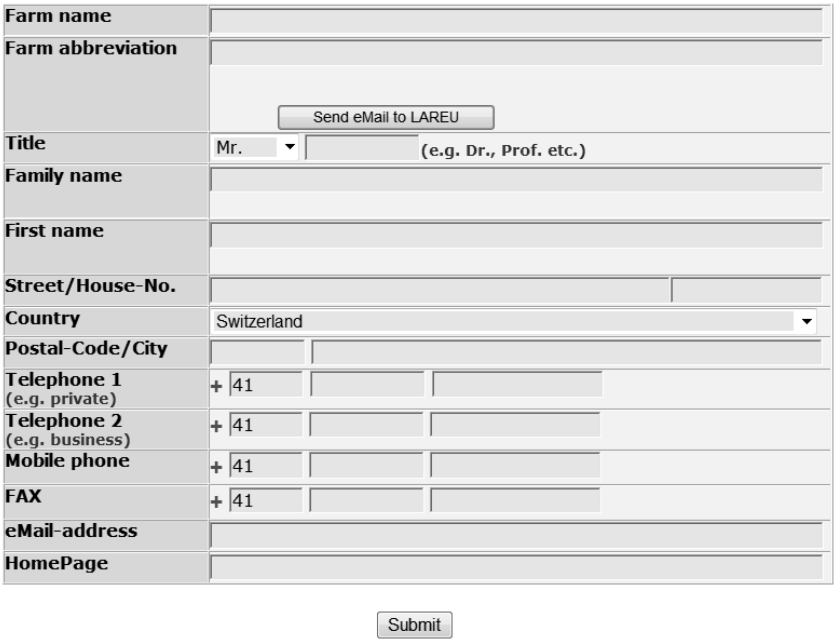


Figure 1*: Input mask for the registration of a new owner/breeder. Once all the data are entered, pressing the "submit" button will transfer the data to the LAREU database. Each action on the LAREU system is acknowledged instantaneously by an email generated automatically without human intervention. The essential input for the flawless communication between LAREU and the breeder is a valid email address.*

As one can see from fig. 1, the language chosen for the masks and for any further interaction with the LAREU registration system is English. The LAREU webpage itself (other than the registration part which is reached by a link accessible through the menu) is not yet hosted by TASSO. It is maintained by the author in ten European languages, not always consistently, with the help of a translator team. These persons also serve as national contacts for the owners and breeders registered with LAREU. Once registered with LAREU, a breeder identifies himself by a breeder number and a password, both provided in an automatic email by LAREU after the first registration action. With breeder number and password (the breeder can change his password at any time) one can login to LAREU and start registering the animals.

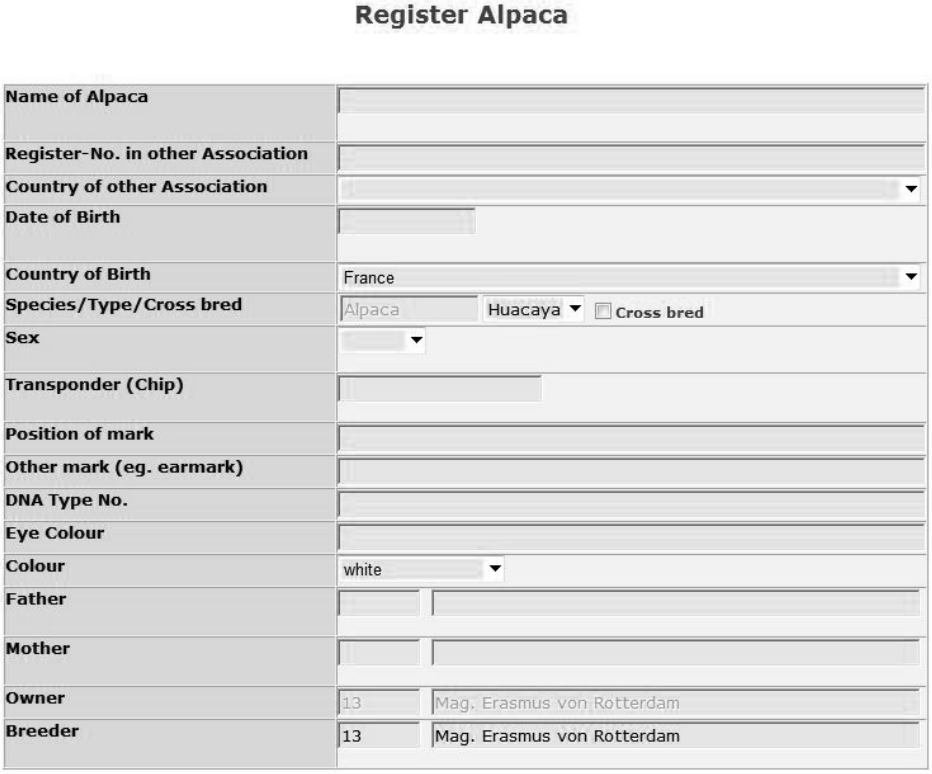


***Figure 2**: Input mask for an alpaca. There are separate masks for alpacas and llamas. For several entries (e.g. species, color) a pull-down menu is available to facilitate the input process. Alpacas and llamas are logged in separate registries.*

For the registration of his animals, a breeder has to fill another online form, as shown in fig.2 (the "submit" button on the bottom is omitted in the picture). The registration of animals should generally be done by the breeder. An owner/breeder can transfer an animal to another owner, usually when the animal has found a new home. Several herd management options, such as "View" or "Change" are implemented as well. Double registration of breeders and animals is blocked by system, checking the names and email addresses of the breeders, and the transponder numbers of the animals.

Search functions through the database, accessing areas of other owners (as is frequently implemented in commercial registries, such as in the Alpaca Registry International, ARI, in the United States), will not to be provided as a matter of principle.

At present (July 2013) over 1600 breeders and more than 11000 animals (7300 alpacas and 3900 llamas) are registered in the LAREU database, coming from more than 16 European countries. Most of the animals are registered in France and Germany, followed by Switzerland, the Netherlands and Belgium.

Development of an International DNA Marker Panel for Camelids

LAREU has recognized from the beginning the importance of a Europe-wide registry, and, for the reasons given in the Introduction, of a world-wide standard of the DNA markers used for SACs. In 2007 LAREU has contacted the "International Society of Animal Genetics

(ISAG)", responsible for the standardization of animal DNA typing, and suggested a research program with the aim of finding a suitable set of markers for SACs. During the XXXI. Conference of the International Society for Animal Genetics in Amsterdam, in spring of 2008, representatives of the LAREU board of directors met with about 50 researchers in animal genetics for a special meeting, organized and chaired by Dr. Cecilia Penedo, Veterinary Genetics Laboratory, University of California at Davis (UC Davis), USA. A working group was created and it was decided to conduct a worldwide ring test, convened by UC Davis, to establish a suitable set of DNA-markers for parentage testing in camlids. A total of 17 laboratories from Europe, the United States and Australia participated in this two-year ring test. After a second ring test for verification of the initial results, the final marker set was published in the 2012 ISAG conference. This set consists of 14 markers, which are shown in fig. 3.

ISAG RECOMMENDED MINIMUM PANEL - Llamas and Alpacas			
Locus	**Forward**	**Reverse**	**Size Range**
LCA5	GTGGTTTTTGCCCAAGCTC	ACCTCCAGTCTGGGGATTTC	178-218
LCA8	GCTGAACCACAATGCAAAGA	AATGCAGATGTGCCTCAGTT	211-261
LCA19	TAAGTCCAGCCCCACACTCA	GGTGAAGGGGCTTGATCTTC	80-122
LCA37	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG	124-174
LCA65	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG	159-193
LCA66	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA	216-266
LCA94	GTCCATTCATCCAGCACAGG	ACATTTGGCAATCTCTGGAGAA	187-213
LCA99	CAGGTATCAGGAGACGGGCT	AGCATTTATCAAGGAACACCAGC	263-297
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG	210-232
YWLL40	CACATGACCATGTCCCCTTAT	CCAGTGACAGTGTGACTAAGA	176-190
YWLL44	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA	84-136
LCA56	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG	133-171
LGU49	TCTAGGTCCATCCCTGTTGC	GTGCTGGAATAGTGCCCAGT	219-249
LGU50	CTGCTGTGCTTGTCACCCTA	AGCACCACATGCCTCTAAGT	183-201

Figure 3: *The panel of DNA markers recommended by ISAG for the DNA typing and parentage testing of llamas and alpacas. In the first column the marker names are shown, the second and third columns give the start ("forward") and stop ("reverse") sequence of the basic amino acids defining the markers on the single-stranded DNA chain. The last column contains the variation of repeats of base pairs lying (amongst other amino acid chains) between the start and stop sequences.*

The key to DNA identification, namely the variability of the number of base pairs in the markers was an important criterion in the definition of the final panel. This set is now used by the DNA laboratories working together with LAREU (see next section), which had to adapt their analysis procedures accordingly. It is very likely that other laboratories in Europe and around the world will now follow this development.

The LAREU DNA Ordering System

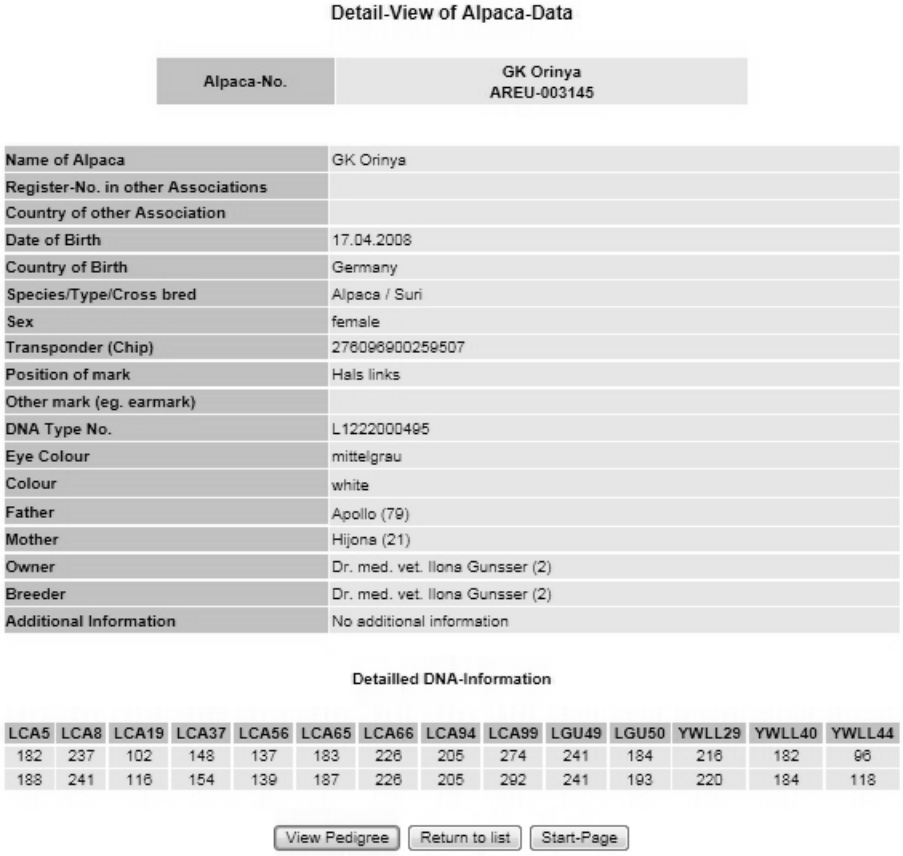
The ordering procedure for DNA typing and parentage verification as well as the delivery of the results by the DNA laboratory had to fulfill the request, as in the case for the registration process, of zero personnel involvement on the side of LAREU. All the "manual" work, such as taking the DNA sample, sending it to the DNA laboratory of his choice together with an order form specifying the requested services, is put into the hands of the breeder. The DNA laboratory executes the "manual" tasks of writing a bill for their services and transfers the DNA results to the LAREU database. Each of these steps is supported with the automatic generation of order forms, confirmation emails for the breeder and sections of the relevant animal information for the DNA laboratory. As an inevitable consequence, such an "automatic", web-based ordering system can only be created with a lot of expert programming work. This is true not only for the LAREU part, but also partly for the collaborating DNA laboratories, which have to prepare their systems to communicate with the LAREU database. Still, the programming work on the side of the laboratory is limited to the update of the animal information supplied by LAREU, adding the allele information for all the 14 legal markers. The laboratory is free to add further DNA markers beside the international standard, if it seems useful to them. Up to now, LAREU works together with three DNA laboratories, one in Germany (Certagen [2]), one in France (Genindexe [3]) and one in the Netherlands (Van Haeringen BV [4]). All of them are internationally accredited according to the ISO17025 standard. The breeder has the option to choose a laboratory through a menu provided by the LAREU system, where links to the laboratories in general and to the prices in particular are provided. The laboratories have agreed to give special price reductions to LAREU members.

In order to enable the breeder to collect the DNA material from his animals, LAREU has proposed a simple, non-invasive method, using so-called "swabs", a kind of Q-tip-like cotton stick, named Genotube Livestock (see fig. 4), produced by Prionics [5]. The swab comes in a plastic tube and can be removed from the tube by a small plastic handle. The swab is removed from the tube and introduced into the nose of the animal, some 5-6 cm deep, and rubbed vigorously up and down the nasal wall to collect the DNA material. The swab is then placed back into a plastic tube and can then be sent to the laboratory.

Figure 4*: Swab by Prionics [5] used for collecting the DNA sample from an animal. The swab can be ordered through the DNA laboratory, using an ordering form provided by the LAREU system. The swab carries a unique number for proper identification.*

The swabs can be ordered through the DNA laboratory, an ordering form in pdf format containing the breeder's return address as well as the address and the fax number of the chosen DNA laboratory is appended to the order confirmation email. The breeder only needs to enter the number of desired swabs and his signature (signed order forms were a request from the laboratories to initiate the business relation). All negotiations are therefore done between the breeder and the laboratory, without any intervention from LAREU, except for the necessary (and automatic) production of ordering forms and the animal / DNA information transfer to and from the laboratory.

The DNA typing for an animal then proceeds as follows: A list of animals without DNA typing is shown by the system and the animal to be DNA-typed is selected. The selection is confirmed by an email with two pdf-Files appended, which must be printed out. One of the pdfs contains the DNA order with the animal information, the address of the laboratory, and the breeder's address as entered it into the LAREU database. The order is in two languages: English and German for Certagen, English and French for Genindexe, and English and Dutch for Van Haeringen BV. The second page of the pdf contains the order form for the swabs (see above). On the DNA order form the breeder only has to note down the date of the DNA sampling, the serial number of the swab, and the date of the submission. With the breeder's signature he also authorizes the laboratory to transmit the DNA data to the LAREU database. The second pdf contains a bar code together with the name of the animal to be DNA-typed and its LAREU number, which the breeder has to cut out and glue onto the swab tube for unique identification in the laboratory. The same bar code is also printed onto the ordering form containing the animal data.



Detail-View of Alpaca-Data

Alpaca-No.	GK Orinya AREU-003145

Name of Alpaca	GK Orinya
Register-No. in other Associations	
Country of other Association	
Date of Birth	17.04.2008
Country of Birth	Germany
Species/Type/Cross bred	Alpaca / Suri
Sex	female
Transponder (Chip)	276096900259507
Position of mark	Hals links
Other mark (eg. earmark)	
DNA Type No.	L1222000495
Eye Colour	mittelgrau
Colour	white
Father	Apollo (79)
Mother	Hijona (21)
Owner	Dr. med. vet. Ilona Gunsser (2)
Breeder	Dr. med. vet. Ilona Gunsser (2)
Additional Information	No additional information

Detailled DNA-Information

LCA5	LCA8	LCA19	LCA37	LCA56	LCA65	LCA66	LCA94	LCA99	LGU49	LGU50	YWLL29	YWLL40	YWLL44
182	237	102	148	137	183	226	205	274	241	184	216	182	96
188	241	116	154	139	187	226	205	292	241	193	220	184	118

View Pedigree | Return to list | Start-Page

Figure 5: *Detailed view of the animal data within the LAREU system, containing also the DNA information. The DNA Type No. is generated automatically during the data upload.*

When the laboratory receives the order form, it will send an invoice to the breeder's address as given on the form. After payment is received, the DNA typing is carried out and the DNA data are uploaded to the LAREU data base. When this happens, the breeder will receive an email from LAREU stating that he can now inspect the DNA data of his animal. The DNA data established by the laboratory can be found at the bottom of the detailed view of the animal. The full DNA information is given by three lines, where the allele lengths for each marker are displayed (see fig. 5). The LAREU system also generates a DNA number ("DNA type No"), which will appear in the detailed view as well. The LAREU DNA type number has the following format: Lyyll000xxx, where yy notes the year of the test, "ll" the laboratory number, and "xxx" the internal order number within the LAREU system. A typical DNA type number will look like this: L1222000495, as can be seen in fig. 5.

In general the parents have already been DNA-typed and the breeder now wishes to verify the parentage for his cria, for which he has sampled the DNA using the swab according to the description above. DNA typing plus parentage verification can be selected in a mask by checking the boxes for "mother" and / or "father". These boxes can only be checked when the DNA-typing has already been done in the LAREU system, see fig. 6.

Place DNA order at Certagen GmbH - Germany

Animal No.	AREU-009711	
Animal Name	GK Sapiro	☑ DNA typing
Species	Alpaca	
Sex	male	
Date of Birth	27.08.2012	
Mother	Hera (37)	☐ verify Mother
Father	GK Miraculix (971)	☐ verify Father

Figure 6: *Mask for ordering DNA-typing and optionally parentage verification. Mother or father or both can be selected only, if they are already DNA-typed.*

Parentage verification can also be done in a separate step after DNA-typing. In this case no DNA-material has to be sent in. As proof of the correct verification of the parents, the LAREU DNA number will receive an appendix with "FV" if the father was verified, and "MV", if the mother was verified. A typical DNA number with both parents verified will look like this: L1223000278FVMV. In case the parentage could not be confirmed, this will be stated in the confirmation email and no appendix to the LAREU DNA number will be generated.

Conclusions

The LAREU registry is running now successfully for more than six years and the number of registrations is showing a constant increase of 15-20 % each year. New features of the registry are being added, the most recent one (and the most complex one so far concerning the informatics side) has been the DNA ordering, working closely together with three renowned DNA laboratories in Europe. Registration with LAREU has been free of charge and it will stay like this, based on the continued enthusiasm and dedication of the key players in the system development. LAREU's registry, and the DNA project in particular, has a piloting function in demonstrating the great advantages of a unified system across Europe's borders. There are still a number of further projects on the LAREU's to-do list, such as an "infinite pedigree" which would allow following the ancestry over an unlimited number of generations. Another project is to offer the registration system in several languages (English, French and German seem a natural choice to start with).

One of the important projects of the LAREU registry, which was not mentioned so far, could not be offered for free: LAREU has created, starting in the year 2006, the production of an animal passport, modeled along the lines of the equine passport. It took some years to make the value of the camelid passport evident to the breeders, but now the numbers of ordered passports is rising quite strongly. It is hoped that the conscious community of breeders and owners of alpacas and llamas will also discover soon the value of a verified ancestry.

References

[1] TASSO-Haustierzentralregister für die Bundesrepublik Deutschland e.V.,
Frankfurter Str. 20, D-65795 Hattersheim, Tel/Fax.: +49 (6190) 93 73 00,
Internet: www.tasso.net

[2] Certagen GmbH, Marie-Curie-Str.1, D-53359 Rheinbach, Tel.: +49 (0)2226 / 8716-00, Fax.: +49 (0)2226 / 8716-04, Internet: www.certagen.de

[3] GENINDEXE - Laboratoire d'Analyses Génétiques**,** 6 rue des sports, F-17000 La Rochelle, Tél : +33 (0)5.46.30.69.66, Fax : +33 (0)5.46.30.69.68, Internet : www.genindexe.com

[4] Dr. Van Haeringen Laboratorium b.v., P.O.Box 408, NL-6700 AK Wageningen, Tel: +31 (317) 416 402, Fax: +31 (317) 426 117, Internet: www.vhlgenetics.com

[5] PRIONICS AG, Wagistrasse 27A, CH8952 Schlieren, Switzerland, Phone: +41 (0)44 200 2000, Fax: +41 (0)44 200 2010, Internet: www.prionics.com

The DNA-Project of the Llama & Alpaca Registries Europe

Christian Kiesling
LAREU, Switzerland

- Short History of LAREU
- Some Statistics of the LAREU Registration
- LAREU initiative: Standard DNA marker set for SACs
- DNA ordering within the LAREU system
- Summary & Conclusions

LAREU
Llama & Alpaca Registries Europe
www.lareu.org

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Short History of LAREU

- First meeting of breeders interested in a European registry during the 1995 Camerino conference on SACs (Italy)
- Biggest problem recognized at that time:
  How to realize a European registry with minimum administrative overhead? → internet-based !
- Discussions with CEO of TASSO e.V. in 2004
  (large registry for pets, mainly dogs and cats, retrieval system for lost pets, active in animal welfare, Internet: www.tasso.net)
  Offer to host the data base for a European registry of SACs
  the break-through!
- Founding Assembly in Stettlen (CH), November 6, 2005
- Start of free online registration system on March 10, 2006

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Characteristics of the LAREU Registry

- Web-based interface for the user ("breeder")
- Login / registration only with valid email address (+ breeder nr. / pw)
- Breeder / animal data are entered by the breeder/owner only
- Name/email of the breeder and chip numbers of the animals are checked for uniqueness (no double registrations)
- Data are protected: only the breeder has access to his data
- Pedigree is represented (in principle with infinite nr. of generations)
- Registration and use of system free of charge
- Animal passport offered (charge to cover cost of production)

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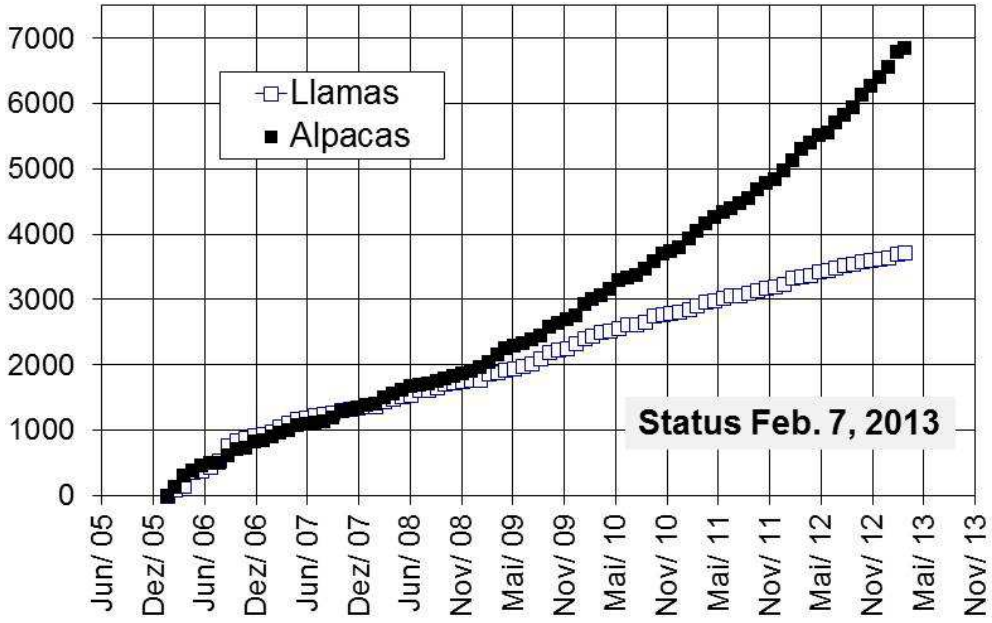
Time-Development of Registrations

Total of 10565 SACs, alpacas: 6850, llamas: 3715, yearly growth by ~ 15-20 %



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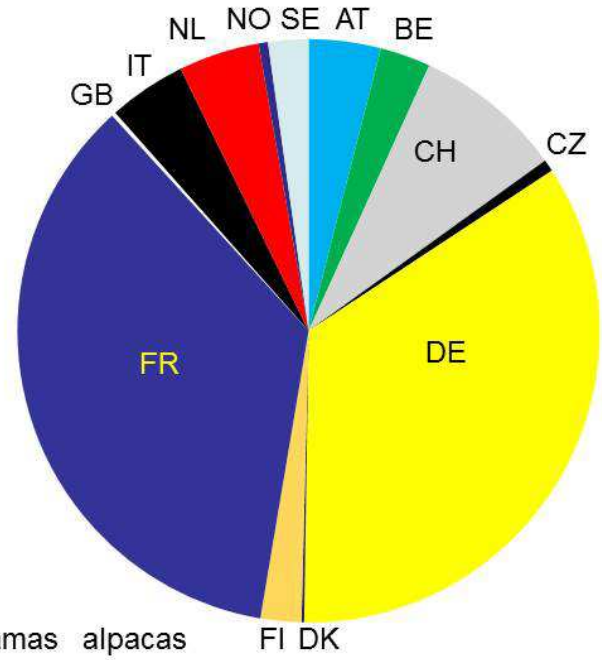
Breakdown per Country (Status Feb. 7, 2013)

Country (fract. of animals)	Anim. /Own.
AT (3.7 %)	6
BE (2.6 %)	7
CH (7.6 %)	10
CZ (0.6 %)	9
DE (31.9 %)	7
DK (0.1 %)	5
FI (2.1 %)	5
FR (32.9 %)	6
GB (0.2 %)	2
IT (4.0 %)	10
NL (4.1 %)	8
NO (0.5 %)	5
SE (2.1 %)	9



Breeders		total	llamas	alpacas
1454	7	10565	3715	6850

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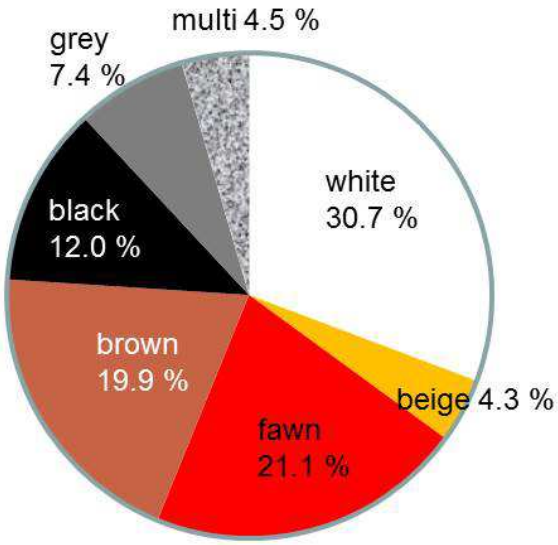
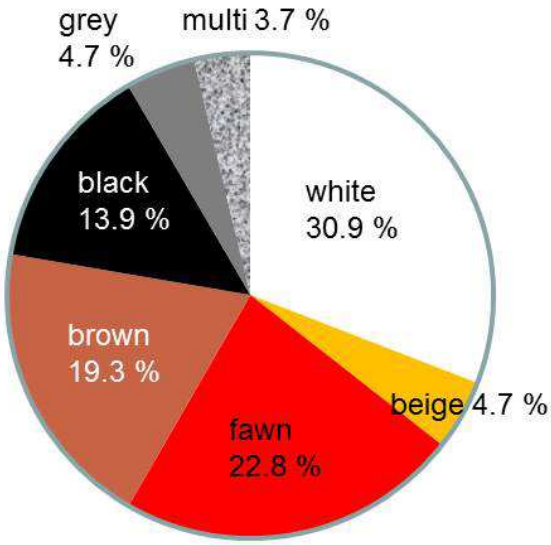
Alpacas: Breakdown by Type and Color



Huacaya (91.3 %)



Suri (8.7 %)

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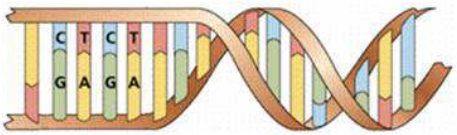
The LAREU DNA Project

- DNA testing required by several associations, but no common marker standard, no reliable pedigree check across systems
- LAREU started an initiative for a world-wide standard of markers for parentage testing in alpacas and llamas, established contacts with ISAG (International Society of Animal Genetics)
- ISAG Conference in Amsterdam (2008): first ring test agreed
- Conference in Edinburgh (2010): results published
- "Standing Committee" for Camelids founded within ISAG in 2010
- Collaboration of 3 accredited DNA labs who also participated in the ring tests, starting in spring 2012
- 2nd ring test completed in 2012



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The Official DNA Marker Panel (ISAG) for SACs

ISAG RECOMMENDED MINIMUM PANEL - Llamas and Alpacas			
Locus	**Forward**	**Reverse**	**Size Range**
LCA5	GTGGTTTTTGCCCAAGCTC	ACCTCCAGTCTGGGGATTTC	178-218
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LCA37	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG	124-174
LCA65	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG	159-193
LCA66	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA	216-266
LCA94	GTCCATTCATCCAGCACAGG	ACATTTGGCAATCTCTGGAGAA	187-213
LCA99	CAGGTATCAGGAGACGGGCT	AGCATTTATCAAGGAACACCAGC	263-297
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG	210-232
YWLL40	CACATGACCATGTCCCCTTAT	CCAGTGACAGTGTGACTAAGA	176-190
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LCA56	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG	133-171
LGU49	TCTAGGTCCATCCCTGTTGC	GTGCTGGAATAGTGCCCAGT	219-249
LGU50	CTGCTGTGCTTGTCACCCTA	AGCACCACATGCCTCTAAGT	183-201

replaced LCA24 after second ring test

range of base pair repeats

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Realization of the LAREU DNA Project

We now work with three officially accredited DNA labs:
Genindexe (France), Certagen (Germany), van Haeringen BV (The Netherlands)

Principal steps for the DNA typing / verification process at LAREU:

1. Links to the DNA labs, contains conditions and prices, order form generated by LAREU and sent via email (legal aspect: client agrees that data will be uploaded to the LAREU data base)
2. LAREU is not involved in the negotiations between lab and breeder
3. DNA-Ordering is done via the LAREU website (emails are generated)
4. The lab waits for payment, does the analysis, and uploads the DNA data. Email sent automatically to breeder / owner after upload.
5. Method of getting DNA material: „swabs“ (to be ordered at the DNA lab)

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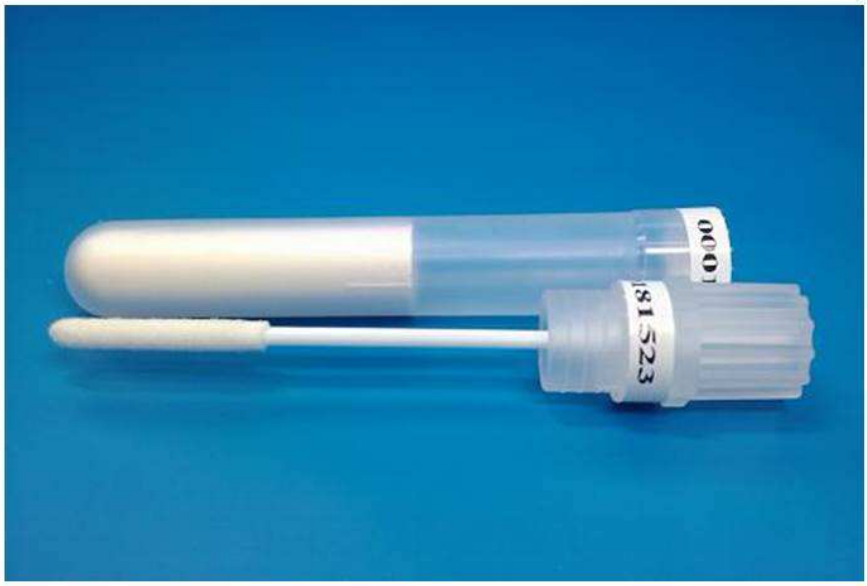
Method to collect the DNA Material



Prionics „Genotube Livestock“ -„swab“ (ordered from the DNA lab)

Method: rub against inner nasal wall (can be done by breeder / owner)

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Ordering Process for DNA Typing / Verification

Number of animals to display per page: All | Display animals without DNA-typing

You have registered 67 animals (47 Llamas and 20 Alpacas)

	Animal-No.	Animal Name	Species	Sex	Type	Date of Birth	Transponder	DNA Type No.	Order Placed
Place Order	3	Lucile de Garenne	Llama	female	light wool	31.12.1993	1F28065B63		
Place Order	21	Hijona	Alpaca	female	Suri	01.09.2002	999000000472957		order placed
Place Order	23	GK Bugato	Llama	gelding	light wool	22.05.1996	977200001439514		
Place Order	24	Djebelle	Llama	female	light wool	01.01.1993	1F4B513213		
Place Order	25	GK Cecile	Llama	female	light wool	10.03.1997			

Place DNA order at Certagen GmbH - Germany

Animal No.	AREU-009711	
Animal Name	GK Sapiro	☑ DNA typing
Species	Alpaca	
Sex	male	
Date of Birth	27.08.2012	
Mother	Hera (37)	☐ verify Mother
Father	GK Miraculix (971)	☐ verify Father

☐ I accept the terms and conditions of Certagen GmbH - Germany

Email sent to DNA lab with required animal data,

Confirmation email sent to breeder

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Certagen GmbH Marie-Curie-Str. 1 53359 Rheinbach
Tel. 02226-871600 Fax 02226-871604 Mail: Labor@certagen.de

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Order Form / Auftragsformular GenoTube Livestock

LAREU Eingang: Certagen Nr.

Address / Empfänger
Certagen GmbH
Abt. Lama+Alpaka
Marie-Curie-Str. 1
53359 Rheinbach
Germany
Fax: +49 (2226) 871609

Addresser / Auftraggeber:
Les Lamas de Paris
Mag. Erasmus von Rotterdam
Place de l'Etoile 23
80802 Paris
France
Email: christian.kiesling@cern.ch

Requested Services / Gewünschte Leistung

No of Swab Tubes to order / Anzahl Tupfer-Röhrchen zu bestellen

Date / Datum Signature / Unterschrift

Swab order form

PDFs appended to confirmation email

Certagen GmbH Marie-Curie-Str. 1 53359 Rheinbach
Tel. 02226-871600 Fax 02226-871604 Mail: Labor@certagen.de

certagen

Order Form DNA Typing / Auftragsformular DNA-Typisierung

LAREU Eingang: Certagen Nr.

Address / Empfänger
Certagen GmbH
Abt. Lama+Alpaka
Marie-Curie-Str. 1
53359 Rheinbach
Germany

Addresser / Auftraggeber:
Les Lamas de Paris
Mag. Erasmus von Rotterdam
Place de l'Etoile 23
80802 Paris
France
Email: christian.kiesling@cern.ch

☒ Alpaca / Alpaka ☐ Llama / Lama ☐ Mix Breed / Mischling Order No / AuftragNr: **LAREU-000548**

Animal Data / Tierdaten
LAREU-No.: AREU-000156 Name / Name: D'Alpi
Chip-No.: 111124762894388 Date of Birth / Geburtsdatum: 22.04.2008 Sex / Geschlecht: female

Mutter
LAREU-No.: AREU-000132 Name / Name: Bailee CVF227
Chip-No.: 977200000397859 Date of Birth / Geburtsdatum: 18.06.2005

Vater
LAREU-No.: AREU-000880 Name / Name: Bozedown Chip
Chip-No.: 985120014980358 Date of Birth / Geburtsdatum: 23.05.2002

Requested Services / Gewünschte Leistung
☒ DNA typing / DNA Typisierung
Date of DNA sample / Datum Probennahme
Parentage Verification / Verifizierung der Eltern ☐ Mother / Mutter ☐ Father / Vater
No of Swab Tube / Nr des Tupferröhrchens

I hereby authorize the laboratory Certagen GmbH to upload the DNA data of my animals to the LAREU data base. The terms and conditions in their present form apply.
Ich erteile der Certagen GmbH die Erlaubnis, die DNA-Daten der Tiere an die zentrale Datenbank von LAREU zu übermitteln. Es gelten unsere AGB in der aktuellen Fassung.

Date / Datum Signature / Unterschrift

DNA typing order form

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DNA Data Upload at the DNA Lab

After successful upload, email is sent to ordering address, stating completion of DNA order (typing and/or parentage verification)

Presentation of the DNA Data to the Breeder

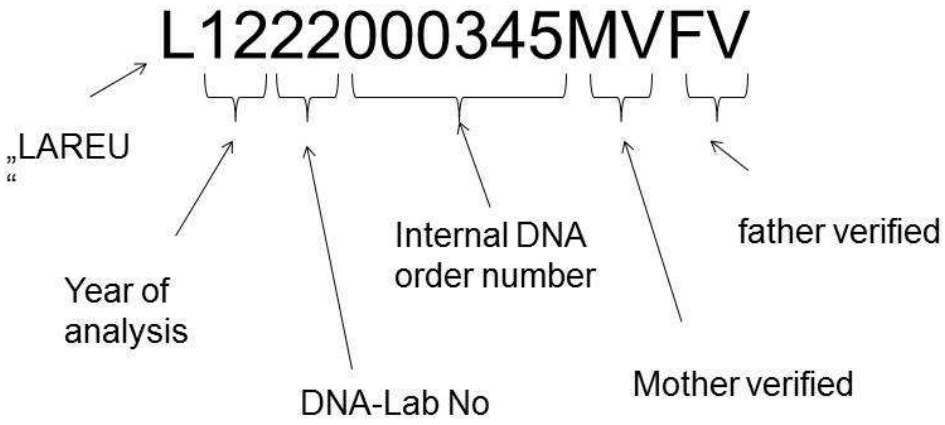
Name of Alpaca	GK Miraculix
Register-No. in other Associations	
Country of other Association	
Date of Birth	26.06.2006
Country of Birth	Germany
Species/Type/Cross bred	Alpaca / Huacaya
Sex	male
Transponder (Chip)	276096900151898
Position of mark	Hals links
Other mark (eg. earmark)	
DNA Type No.	L1222000103
Eye Colour	mittelgrau
Colour	white
Father	Houdini (39)
Mother	Hera (37)
Owner	Dr. med. vet. Ilona Gunsser (2)
Breeder	Dr. med. vet. Ilona Gunsser (2)
Additional Information	No additional information

LAREU DNA code automatically updated by upload program

Detailled DNA-Information

LCA5	LCA8	LCA19	LCA37	LCA56	LCA65	LCA66	LCA94	LCA99	LGU49	LGU50	YWLL29	YWLL40	YWLL44
204	232	100	152	133	181	223	201	288	229	187	216	186	104
206	240	100	154	141	187	241	205	292	229	187	226	186	118

LAREU DNA Code (including verified Parentage)



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Conclusions

- LAREU: professional registration system for SACs on a European level, hosted by TASSO e.V., independent of national SAC associations, based entirely on the web, protected access to his data by the user only, free of charge
- DNA verification of parents important: According to SAC associations, about 20-30 % of the parent information given by the breeders is incorrect
- LAREU initiated the definition of a world-wide standard for DNA typing under the auspices of the newly founded ISAG Standing Committee for SACs, bi-annual ring tests continuing
- LAREU has realized the new standard within its registry in collaboration with three accredited European DNA laboratories (Germany, France, The Netherlands), consistent procedure for DNA typing and parent verification across labs has been established

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Thank you for your attention
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Backup

Why a European Registry for SACs?

- At present, at total of 22 llama & alpaca associations exist in Europe. Many of them have their own registry.

  Don't we have already enough? Why „another" (European) registry?

- **Main problem:**
  no networking between the various registries, no exchange of data
  Change association → redo the registration, usually loss of information

- **Two important reasons for a unique European register:**

  - Alpacas and llamas frequently „move" across national borders

  - Pedigree cannot be reliably followed (different unrelated registers)

- (In the past) no unique European/world-wide standard to reliably cross-check pedigrees (only DNA analysis will give the answer).

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DNA Identification – Why ?

A responsible breeding program requires certainty in the pedigree

- potential stud management problems
  - relatively large time interval for end of pregnancy (± 1 m)
  - intentional re-mating with another stud
  - stud in female group by accident
- Errors in the book keeping
- geldings („fertile one last time" after late castration)
- in case of crias born at the same time and place:
  mothers may confuse their crias (actually happened !)

Proof of parentage (pedigree) is important

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Breakdown per Country (Status Feb. 7, 2013)

Country (an.fr.)	Breeders	Animals	Llamas	Alpacas	Anim. /Own.
AT (3.7 %)	69	390	182	208	6
BE (2.6 %)	40	274	37	237	7
CH (7.6 %)	83	808	210	598	10
CZ (0.6 %)	7	65	49	16	9
DE (31.9 %)	449	3367	891	2476	7
DK (0.1 %)	3	14	14	0	5
FI (2.1 %)	41	221	26	195	5
FR (32.9 %)	618	3471	1639	1832	6
GB (0.2 %)	10	22	11	11	2
IT (4.0 %)	44	421	191	230	10
NL (4.1 %)	56	438	32	406	8
NO (0.5 %)	11	51	8	43	5
SE (2.1 %)	23	218	79	139	9
	1454	10565	3715	6850	7

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Some Statistics on the Animals

Species	male	female	gelding
Alpacas	39.7 %	56.6 %	3.7 %
Llamas	38.1 %	54.7 %	7.2 %

Llamas	Light wool	Medium wool	Heavy wool	Suri
	38.0 %	24.7 %	33.8 %	3.5 %

Alpacas			Huacaya	Suri
			91.3 %	8.7 %

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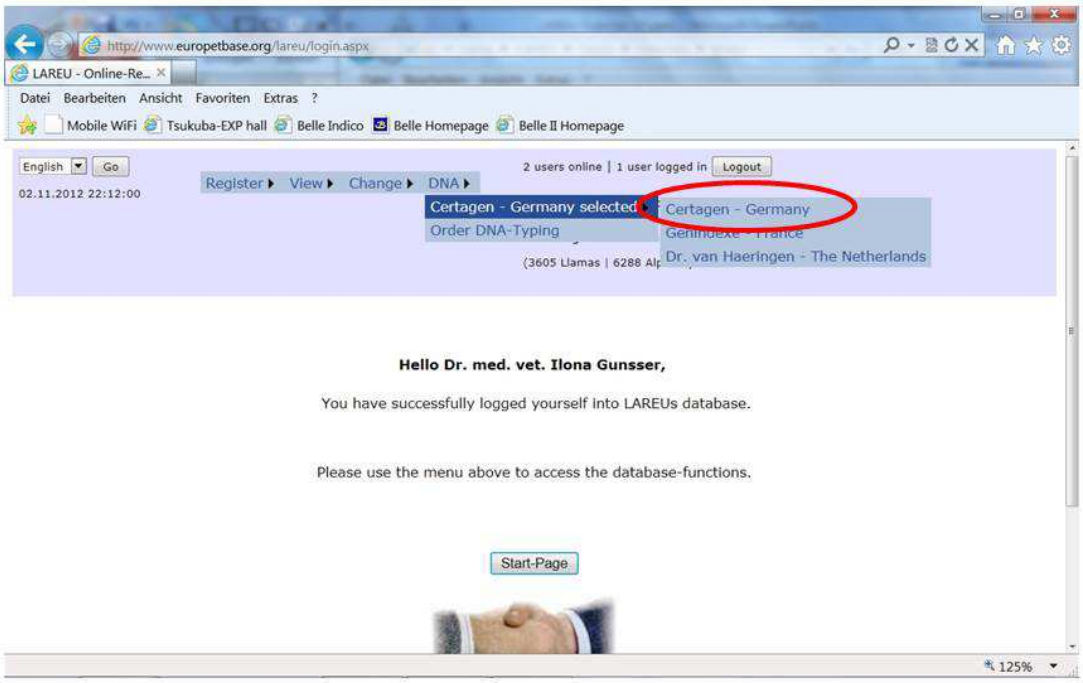
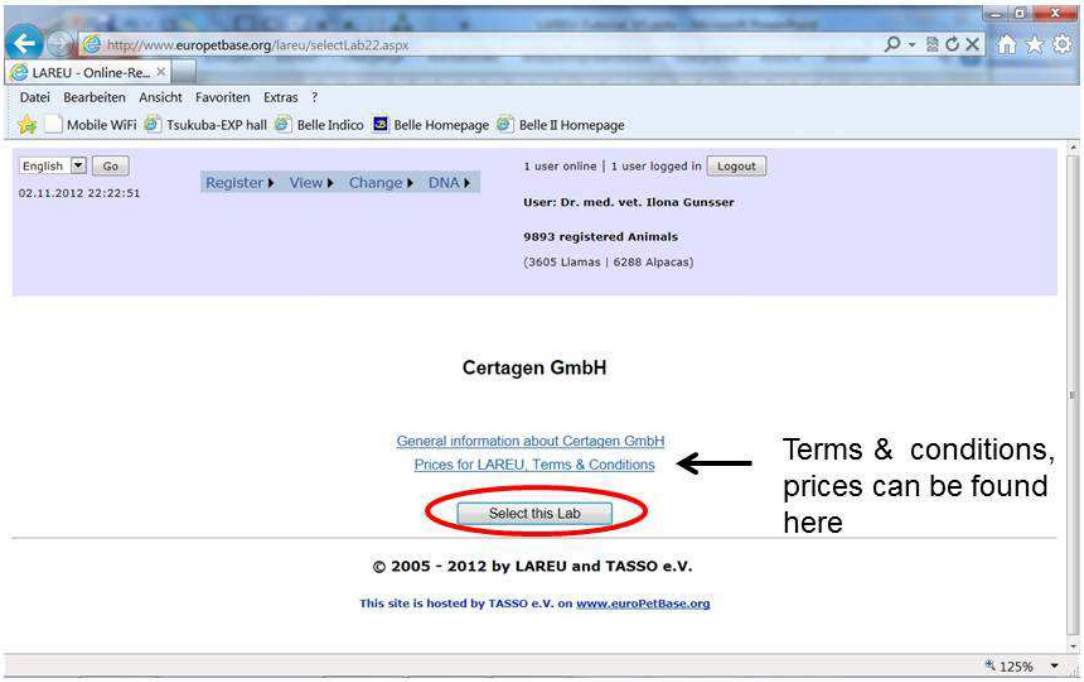
DNA-Typing with the LAREU System

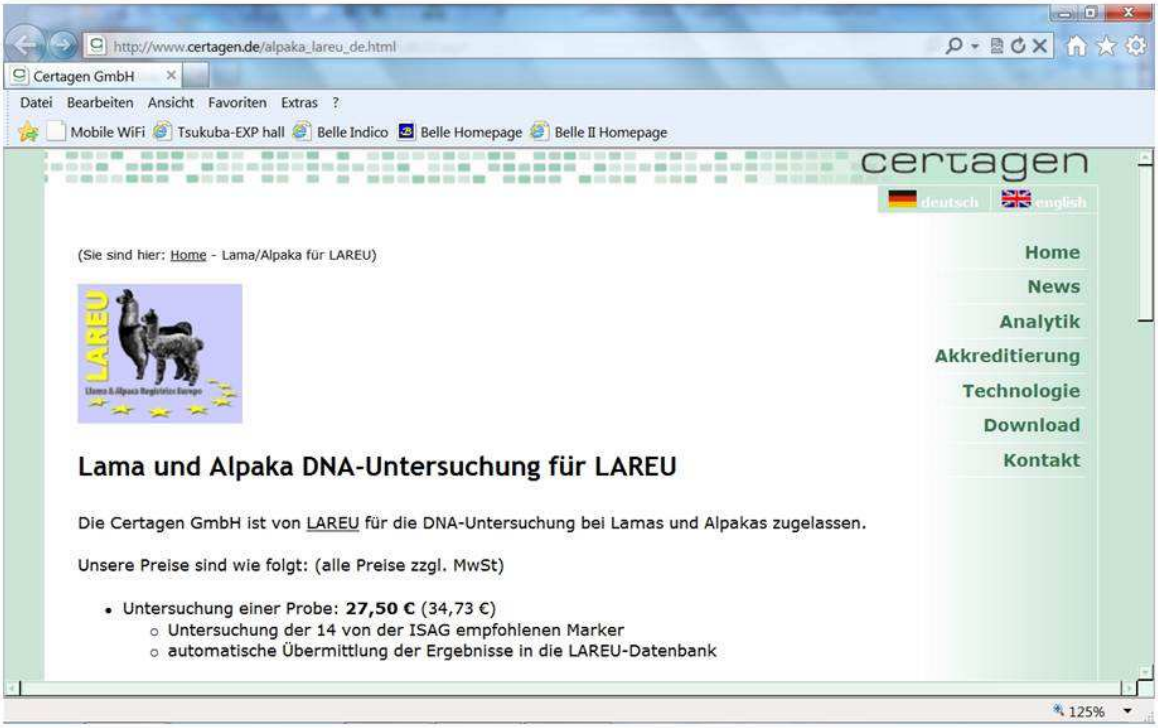
Choice of the DNA Laboratory (I)



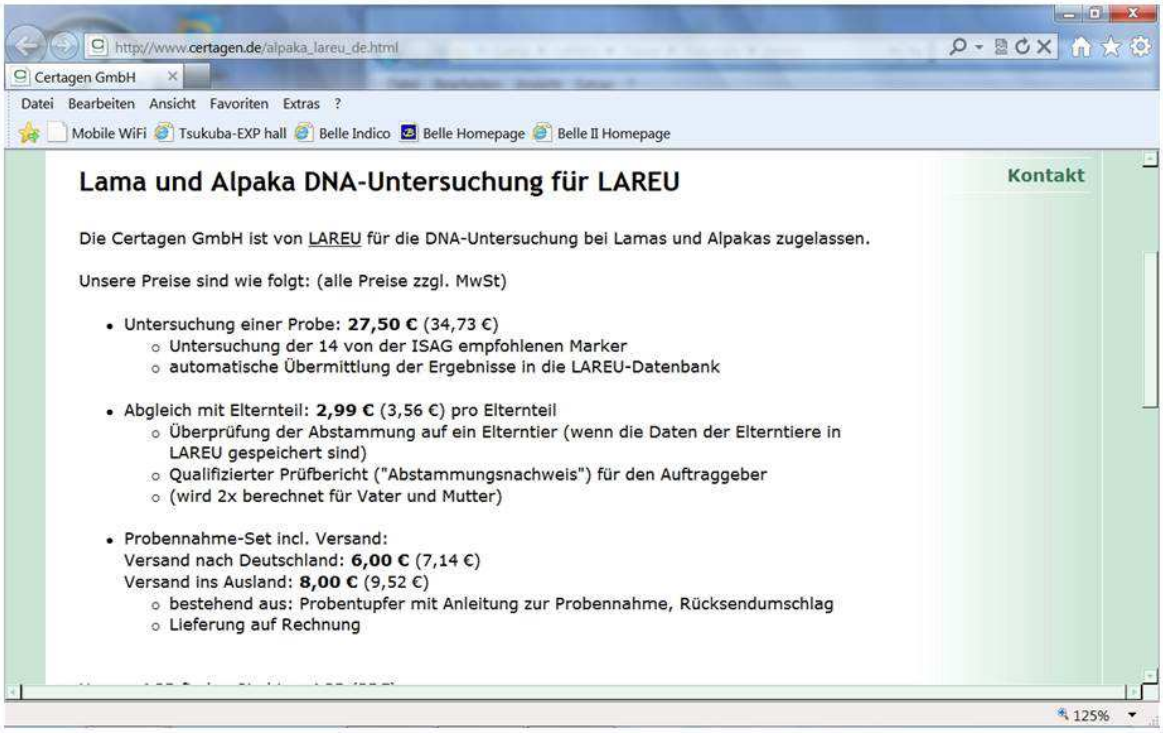
Choice of the DNA Laboratory (II)



Choice of the DNA Laboratory (III)



Choice of the DNA Laboratory (IV)



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Ordering of DNA-Typing (I)

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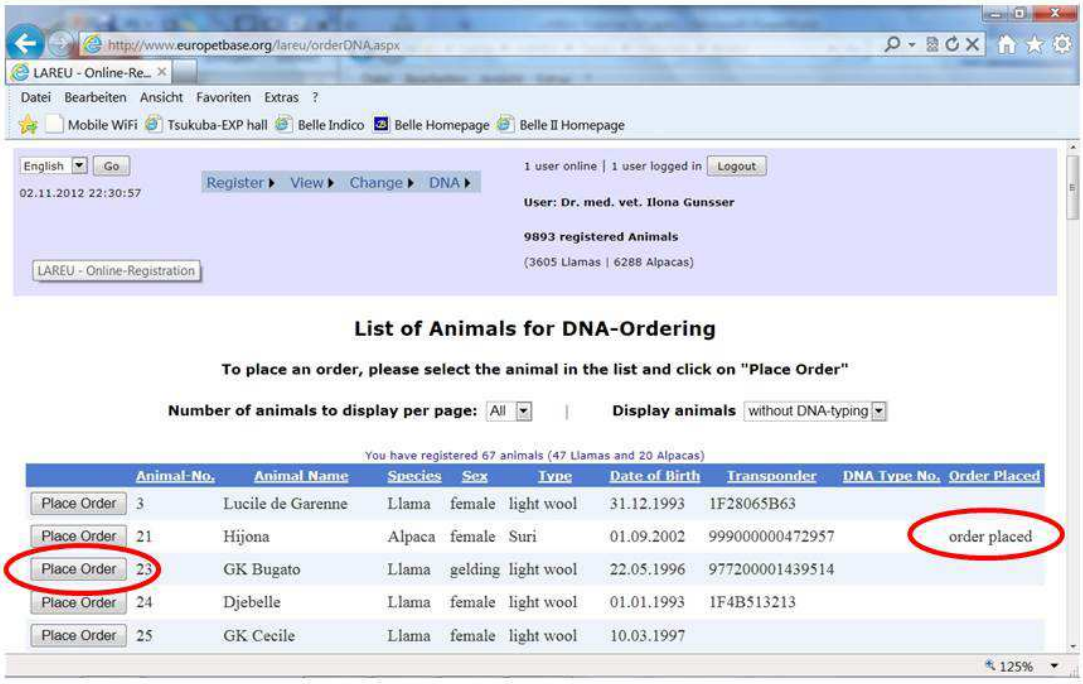
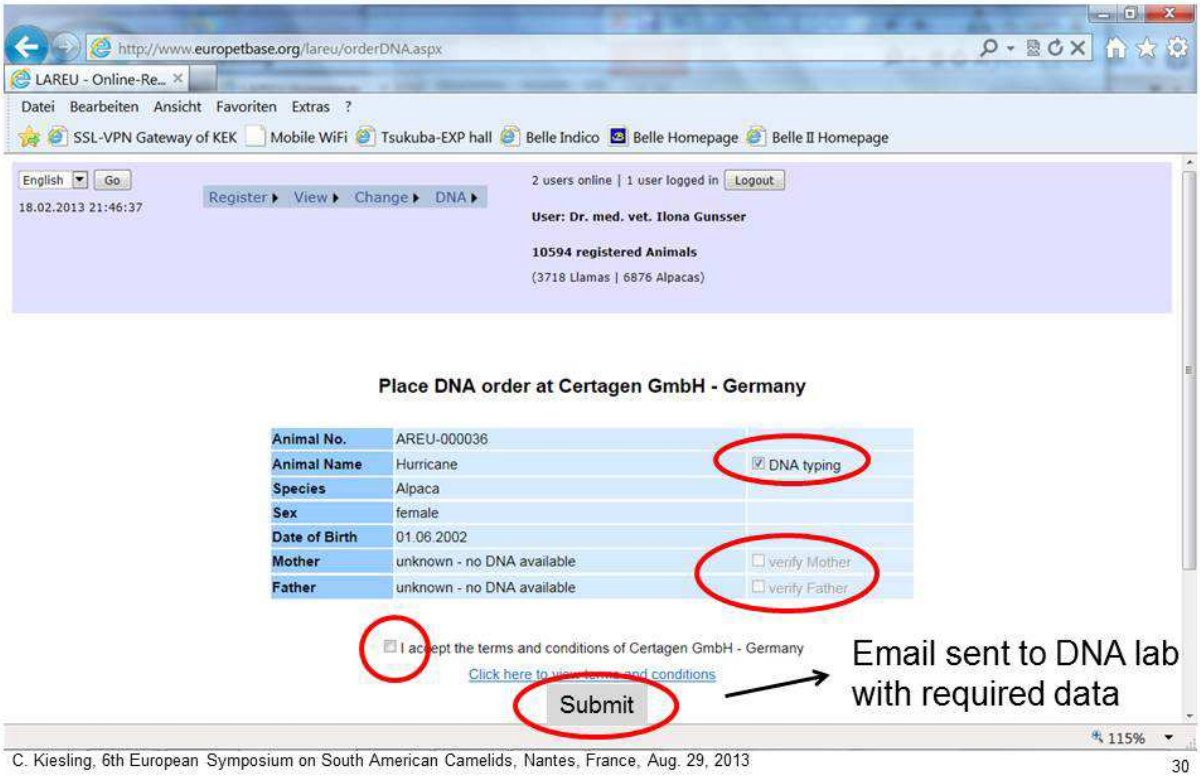
Ordering of DNA-Typing (II)



Ordering of DNA-Typing (III)



Email: Confirmation of DNA-Order for Animal-No. AREU-009999

Hello Dr. med. vet. Ilona Gunsser,

you placed a DNA-order at Certagen GmbH for your animal GKSanchez:
- DNA-typing: yes
- verify mother: no
- verify father: no

Your DNA-order-no. is: 509. To process you order, please take the DNA-sample of your animal according to the instructions, sign the attached PDF and send it to Certagen GmbH together with the DNA-Sample. The second PDF contains a barcode-label. Please cut it out and attach it to the DNA-sample for proper identification at Certagen GmbH. When the DNA-data is uploaded by Certagen GmbH, you will be informed automatically via email.

Kind regards The LAREU-Team

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Labeling of Swab

Les Lamas de Paris
Mag. Erasmus von Rotterdam
Place de l'Etoile 23
80802 Paris
France
Telephone 1: +33 (89) 34 72 72
Telephone 2: +33 (89) 32354-258
Mobile phone: +33 (171) 720 6428
FAX: +33 (89) 39 18 02
eMail: christian.kiesling@cern.ch
HomePage: http://www.physik.uni-muenchen.de

Certagen GmbH
Abt. Lama+Alpaka
Marie-Curie-Str. 1
53359 Rheinbach
Germany
FAX: +49 (2226) 871609
eMail: alpaka@certagen.de
HomePage: www.certagen.de

Please cut out and glue onto DNA sample tube /
Bitte ausschneiden und auf das Probenröhrchen kleben

AREU-000156 D'Alpi
0 000000 005487

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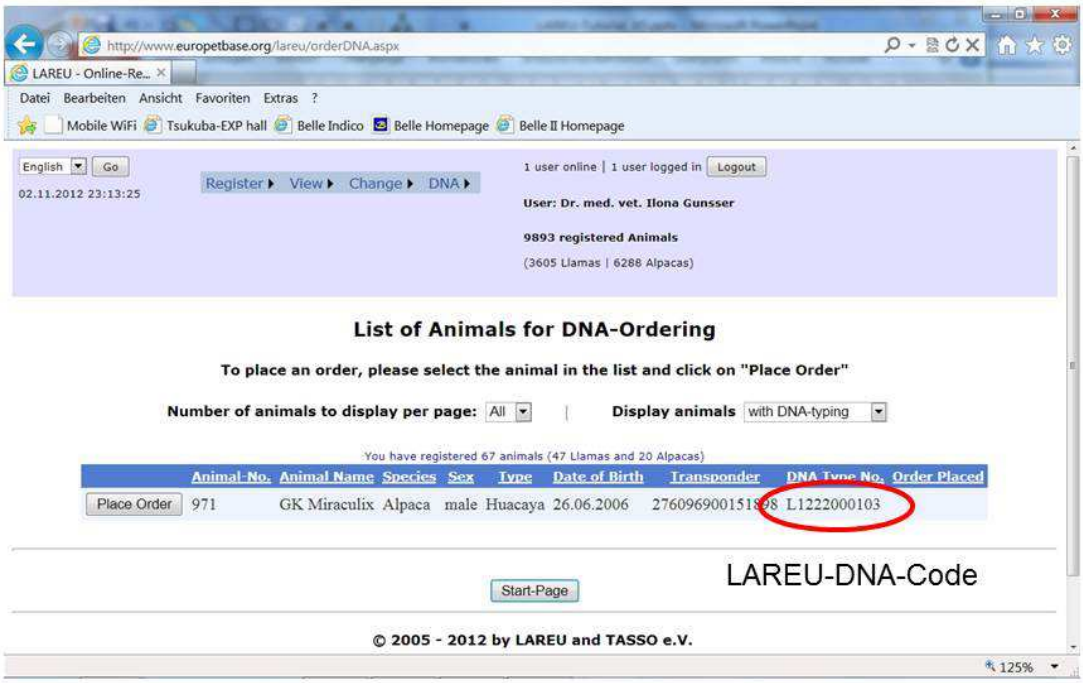
DNA-Typing done, Data uploaded



DNA-Analyis done: Confirmation Email

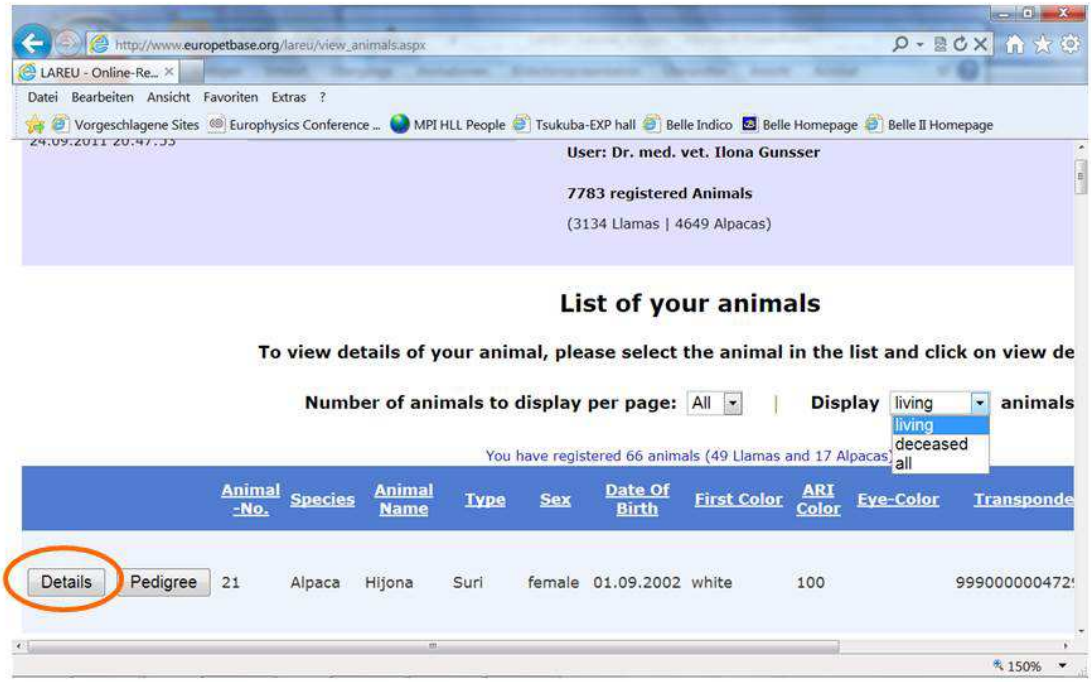
Betreff: Results of DNA set Upload

Hello Dr. med. vet. Ilona Gunsser,

the DNA data for your animal GK Skadori with the number LREU-009366 have been uploaded.
You can now inspect the marker set under the menu 'View'.

Kind regards
Certagen GmbH

Presentation of the DNA-Data (I)



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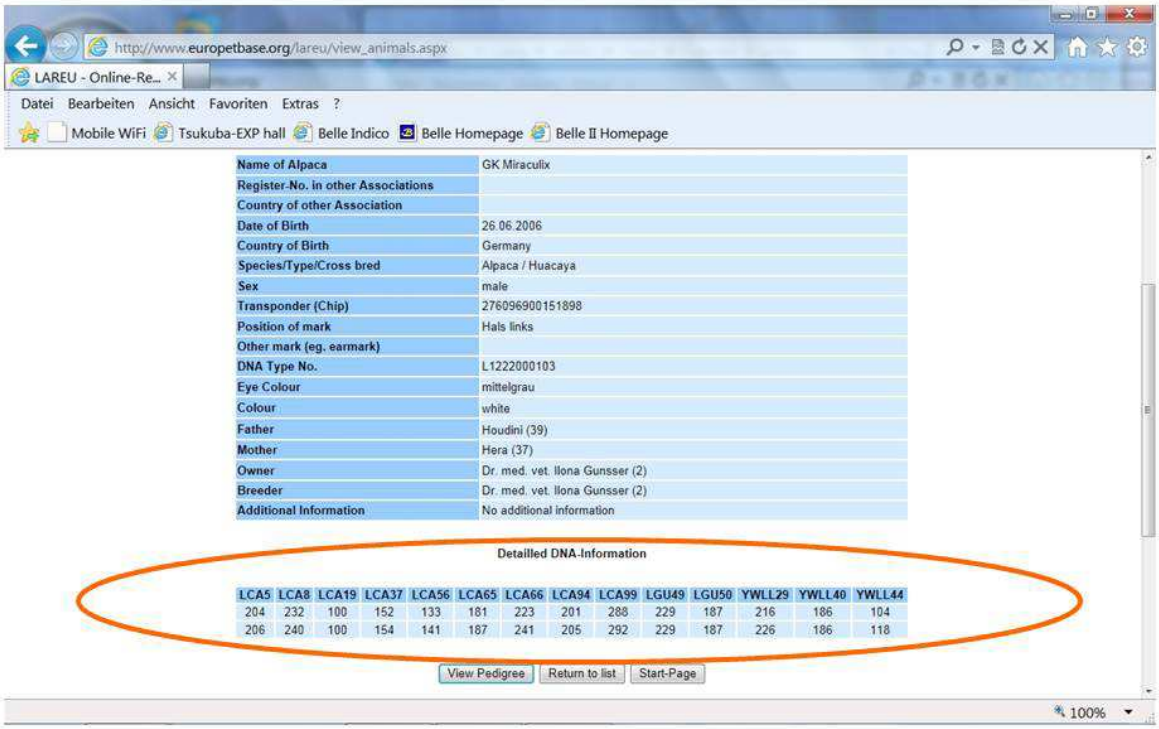
Presentation of the DNA-Data (II)



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Contacted DNA labs

Six laboratories have been contacted (by email + letter):

France:	Genindexe
Finland:	Genoscoper
Germany:	Generatio, Certagen, (Lauk-Breitling)
Netherlands:	Van Haeringen Labs
Switzerland:	Swiss Quality Test Services SQTS

We got 5 positive responses

Exception: Generatio; they are afraid to give out the allele length („may be manipulated“)

- After initial interest, the Finnish lab did not pursue the next steps,
- SQTS, very helpful in the initial phases of the project, does not (yet) see the commercial benefit implementing the LAREU system.

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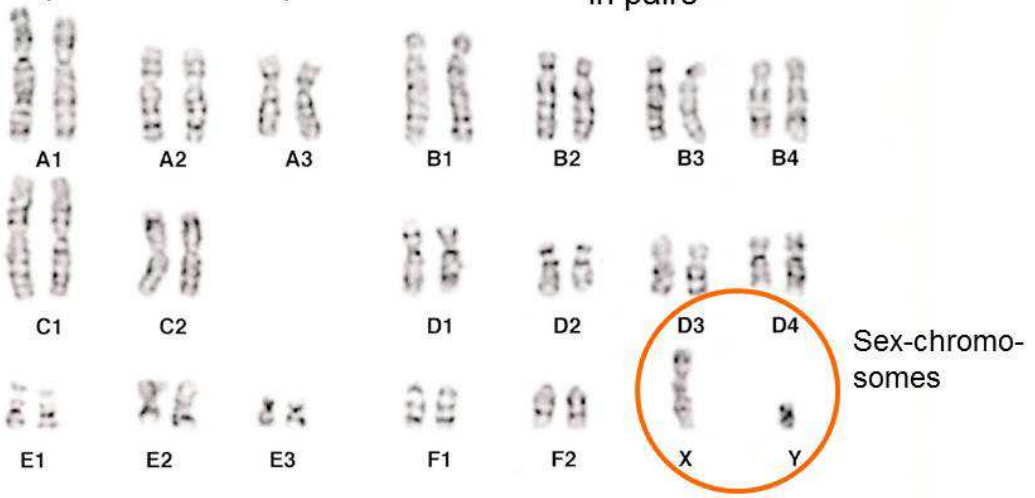
Basics of Parentage Testing

Set of chromosomes in each cell of an organism (here: mammals)

Chromosomes come always in pairs



Cat

Cat: 38 chromosomes, alpaca/llama: 74, human: 46
(one chromosome in pair from the father, the other from the mother)

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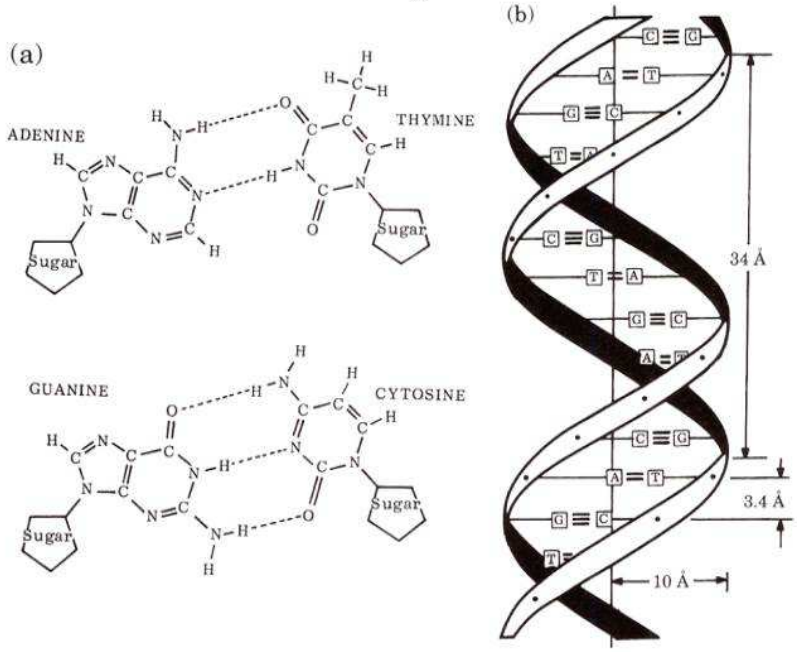
Genetic Information on the Double Helix

Chromosomes built from long strands of DNA



only 4 amino acids define the genetic information („DNA“):

Adenin (A)
Tymin (T)
Guanin (G)
Cytosin (C)

variability generated **only by the sequence** of the 4 amino complexes

Certain sections on the helix (specific sequence) = „Marker“

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Example (how it works)

- Extract single-stranded DNA material (pairs chromosomes, one from the father, one from the mother),
- search for “start” and “end” in each
- count “simple” repeat sequences (here: CA base pairs, length = 11: CA_{11})

Repeat sequences

Start (“Forward”)

GCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCAAGTGTATGT
GCATACACGTGCACACACACACACACACACACAGAGGGTGTGCACATGTG
CATGCACACTCCAAGAGACAGTGCCTAGTAAAGTGTCTCAGCACCATCTGC
AGCAAACAGGTTCTGCAAAAACCAATCCCAACTGA
TGTTCCCACAGTGACACTGT

End (“Reverse”)

For each marker pair one gets 2 numbers of repeats (one from the mother, one from the father)

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Parentage Verification

Detailled DNA-Information

Cria

LCA5	LCA8	LCA19	LCA37	LCA56	LCA65	LCA66	LCA94	LCA99	LGU49	LGU50	YWLL29	YWLL40	YWLL44
188	249	90	142	139	173	224	193	284	221	193	216	182	102
188	249	102	146	139	173	240	193	286	239	193	220	186	104

Detailled DNA-Information

Mother

LCA5	LCA8	LCA19	LCA37	LCA56	LCA65	LCA66	LCA94	LCA99	LGU49	LGU50	YWLL29	YWLL40	YWLL44
188	243	86	146	139	173	224	193	282	239	193	220	182	104
190	249	102	146	141	173	228	193	286	239	193	224	186	104

Detailled DNA-Information

Father

LCA5	LCA8	LCA19	LCA37	LCA56	LCA65	LCA66	LCA94	LCA99	LGU49	LGU50	YWLL29	YWLL40	YWLL44
188	249	90	142	139	173	224	193	284	221	193	216	182	102
188	249	102	146	139	173	240	193	286	239	193	220	186	104

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ISAG does NOT endorse commercial products or practices.

ISAG will NOT become involved in the accreditation of service genotyping laboratories or institutions.

ISAG does NOT regard participation in its Comparison Tests as indicating that a lab is internationally accredited.