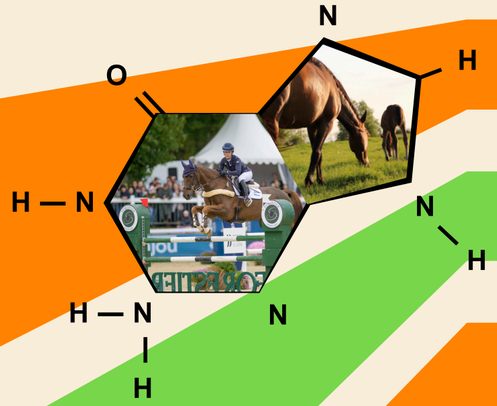
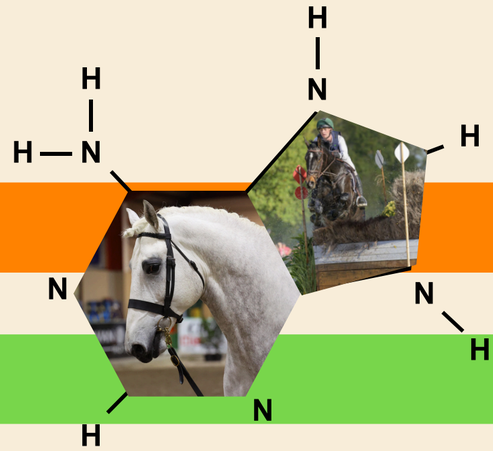
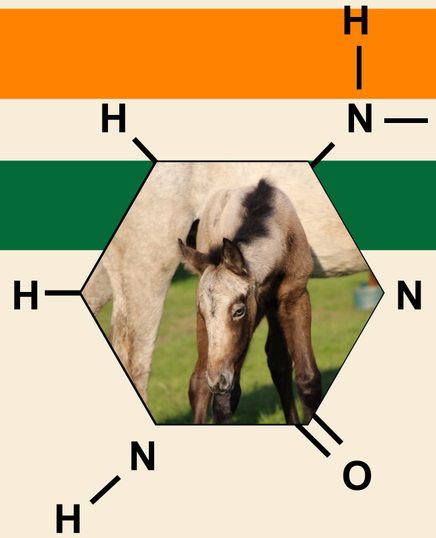
WBFSH DNA TESTING WEBINAR

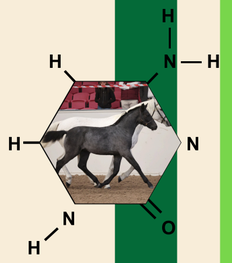


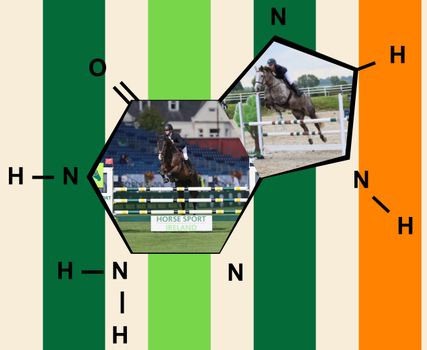




SONJA EGAN, PHD
HEAD OF BREEDING, INNOVATION AND DEVELOPMENT

HORSE SPORT IRELAND





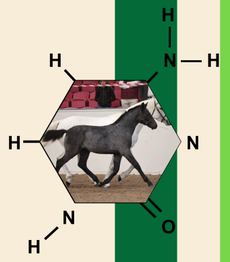
WELCOME!

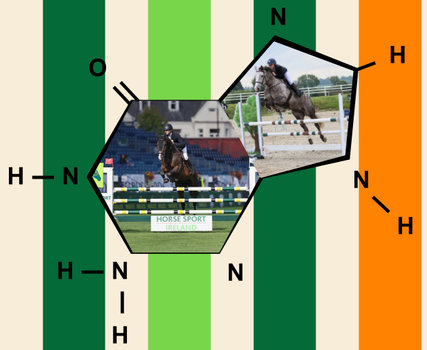
WHO AM I?

Head of Breeding Innovation and Development at Horse Sport Ireland, recently led the transition from equine DNA testing from MS - SNP in Ireland with grant support from the Irish State. Represent the ISH studbook at WBFSH. I am also on the WBFSH Scientific Advisory Committee and the WBFSH Audit Committee.

PURPOSE

Discuss the motion for mandatory DNA testing for equine parentage verification in the World Breeding Federation for Sport Horse (WBFSH) member studbooks, outlining the importance of DNA pedigree verification, and detail on options and pros and cons of the various methods.





Welcome!

What will we cover?

- What is DNA?
- Why should studbooks DNA test to record pedigrees?
- What methods are available and typically used to test equine DNA
  - What are the pros and cons of each method.
- How to identify a laboratory partner
- What is ISAG and how does their accreditation system work?
- The current WBFSH timeline
- Sample Transition protocol
- Existing research in the area of SNPs
- Q&A

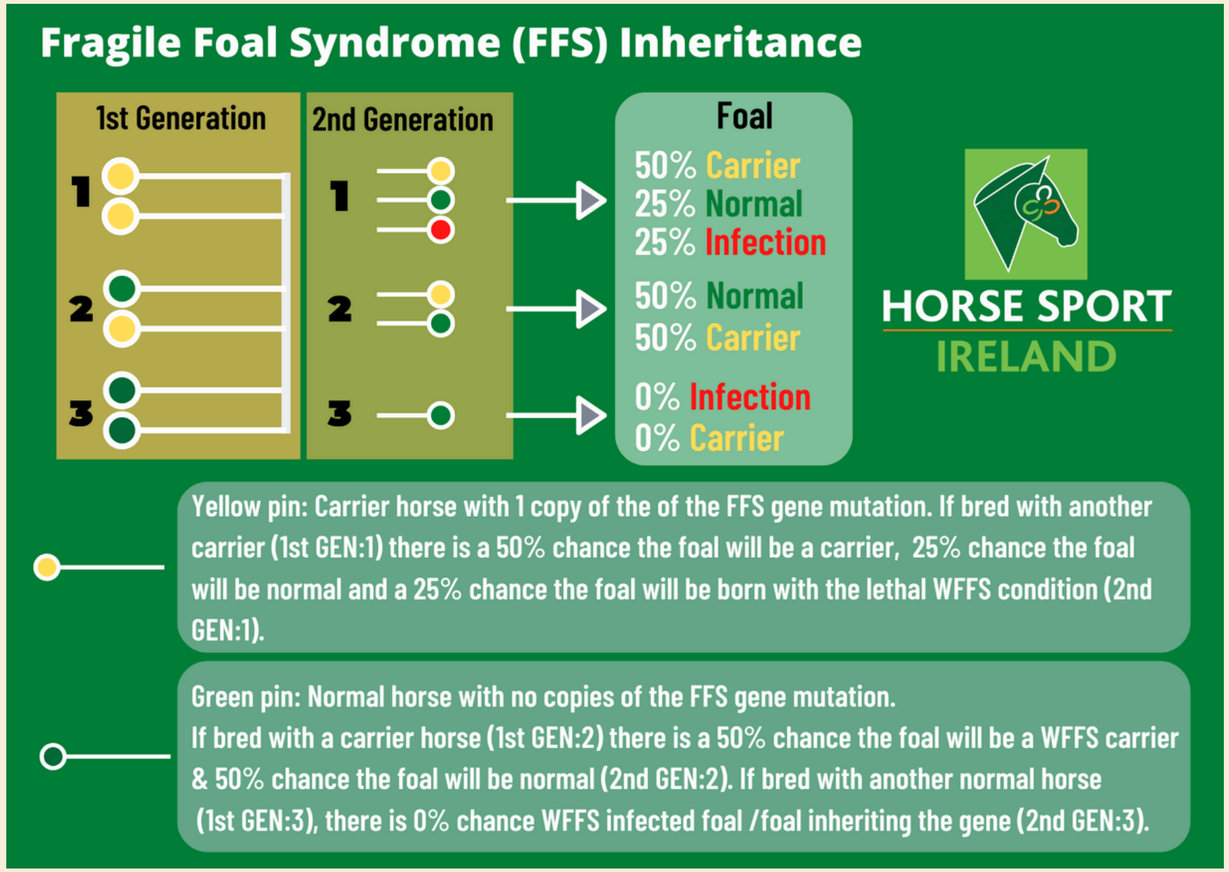
WHAT IS DNA?

- DNA, or deoxyribonucleic acid, is an essential molecule found in the nucleus of living organisms' cells.
- Like a book of genetic instructions, determining how an organism develops and functions.
- DNA has personal and unique signatures.
- DNA is the molecule that forms genes, and multiple genes are grouped into chromosomes.
- Chromosomes are present in the nucleus of every cell and contain all the genetic information of an organism.

DNA plays a central and definitive role in equine parentage verification by scientifically confirming whether a foal’s listed sire and dam are it’s true biological parents.

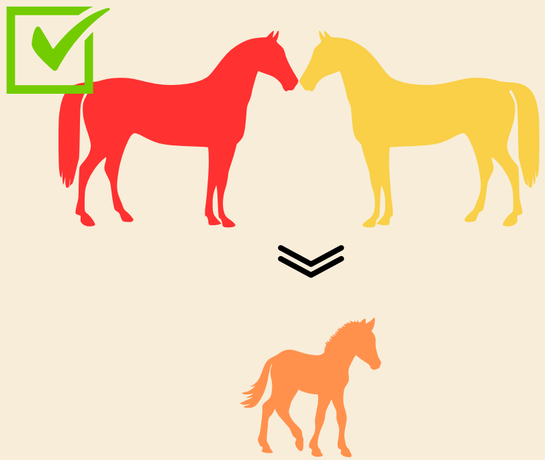
GENETIC INHERITANCE

- **Inheritance involves the transmission of genetic information from parents to offspring, determining characteristics in the progeny.**
- **Through inheritance, biological traits are continued and diversified in all species on Earth.**
- **Inheritance is essential for the continuity and**
- **adaptation of species over generations.**



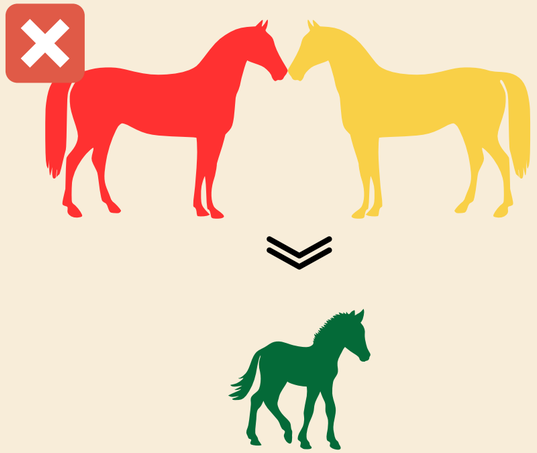
WHY DNA TEST?

DNA VERIFIED



- Scientifically verified
- Valid, accurate and repeatable
- Studbook Quality Assurance
- Breed preservation and conservation
- if SNP, supports genomic testing (inbreeding, breeding values, trait ID, etc)

‘BELIEVED TO BE BY’



- Is not definitive
- Self reported
- Open to fraudulent claims
- Can lead to incorrect pedigrees
- No validated genetic details
- Errors can undermine trust and breed integrity

COMMON ERRORS PREVENTED BY DNA TESTING

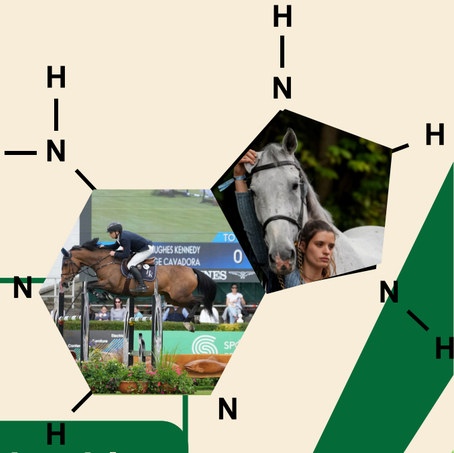
Sire/dam exclusions identified

- **Wrong sire/dam submitted on application**
- **Wrong semen issued to breeder**
- **Cross covers**

BROADER BENEFIT OF SNP DNA TESTING

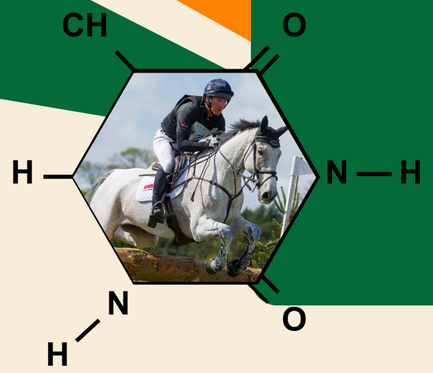
- **Accurate pedigree recording and trust in the integrity studbook registry**
- **Increased market value of the verified horse**
- **Genomic indices**

What methods are used to test equine DNA?



Microsatellites (MS/STR)

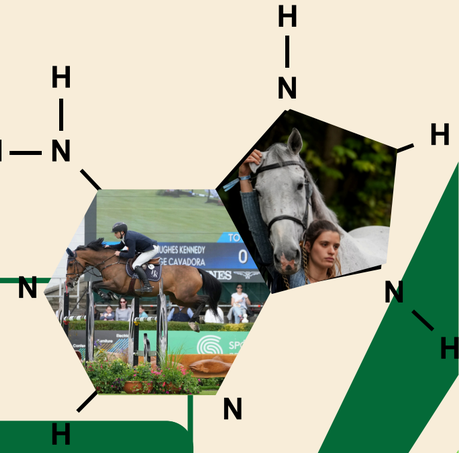
- Around since the 1970s
- Uses approx 14 - 17 markers
- Used for parentage verification only
- Widely used for parentage verification
  - Most Sport Horse studbooks
  - Thoroughbred studbooks (General Studbook)
- For example in Ireland, equines were the only species using this technology



Single Nucleotide polymorphism (SNP)

- Used in the cattle sector for a considerable time (Ireland 2009)
- Provides up to 80,000 DNA markers
  - Used for parentage verification
  - Used to identify genes/traits for interest
    - Fragile foal Syndrome
    - Hoof Wall Separation Disease
    - Coat colour
    - etc, etc
  - Used for genomic inbreeding
  - Breed composition makeup
  - Traceability
  - ETC.
- Used by Irish Studbooks, KWPN, Some German studbooks

How do they work?



Microsatellites (MS/STR)

Looks for repeating patterns in the DNA sequence, totalling 14 - 17 markers

CGTGGATAGATAGATAGATACAG

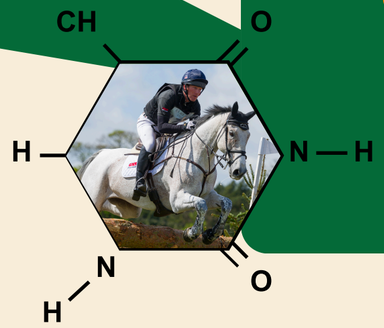
ATGTGGATAGATAGATAGATATTC

Single Nucleotide polymorphism (SNP)

Looks for individual base pair changes in the DNA, pulling 80,000 indicators

AGAGCAGTACGT

AGAGTAGTACGT



SNP Case study: Breed integrity ISH - TIH

The ISH – TIH is a sub category of Irish Sport Horse which only contains Irish component breeds and has no unknown pedigree after 1982.

- **In 2024 ISH studbook receive ISH-TIH foal application**
- **The foal excluded from nominated sire during the SNP DNA verification process**
- **Studbook requested another sample**
- **The breeder submitted a new sample but of a 2023 TIH foal – Helical system identified as immediate match to the previous 2023 foal**
- **The 2024 foal was by a Warmblood stallion and not eligible for ISH – TIH**

Feature	MS/STR	SNPs
Parentage accuracy	✅ High (proven across time and well used)	✅ Very high (greater number of markers included for parentage verification)
Standardisation	✅ ISAG Verified	✅ ISAG verified markers (SNPs) to be included in the official panel in 2024.
Data Compatibility	✅ Matches historical studbook data	⚠️ Not back compatible with STR ✅ Are SNP - MS and SNP - SNP imputation options
Cost	✅ Approximately the same (€27 - €35)	✅ Approximately the same (€27 - €35)
Other uses	❌ Parentage verification only	✅ Parentage verification genomic inbreeding, traits, etc
Mutation rate	⚠️ Slightly higher (mismatches can occur in a very small number of cases)	✅ Lower mutation rate, more stable markers
Future relevance	⚠️ In many studbooks its being phased out in favour of SNP	✅ Longer term standard for breeding and traceability

Laboratory 101

Some guidelines to look for in a lab partner

- An existing animal testing lab (Livestock i.e. cattle, pigs, sheep; pedigree dogs, TBs etc)
  - Reach out to another breed society or studbook in your region, of the same or different species!
  - The cattle sector in most countries is more advanced in terms of knowledge of animal genetics and genomics for animal breeding.
- ISO/IEC 17025 rating
  - An international standard for testing and calibration laboratories. A lab with an accreditation to this standard indicates competence in providing reliable results
- ISAG certificate

International Society for Animal Genetics (ISAG)

ISAG Panels

There is an existing ISAG panel for MS testing.

The ISAG SNP panel was approved and decided in 2024, this includes a back up panel

Rankings?

ISAG do not rank laboratories. However labs can elect to complete a ‘Comparison test’ which indicates the MS/STR testing quality of the participant i.e. labs. SNP version in development.

Therefore, it is the policy of ISAG that:

- 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.

International Society for animal genetics (ISAG)

ISAG CT test

- The International Studbook Committee (ISBC) for thoroughbreds recommend that studbooks partner with (1) ranked labs
- The ISAG SNP CT was under review at their July meeting.
- Cattle and dogs have STR/MS and SNP ISAG CT tests.

ISAG

Certificate of Participation

This is to certify that ISAG Institutional Member

[redacted]

has participated in the

2022-2023 International Horse (*Equus caballus*) STR DNA Typing Comparison Test

with the following result:

Absolute genotyping accuracy rank: 1

Total number of participating labs: **100**

Absolute genotyping Accuracy	
Rank	% Labs
1: 100% – 98%	86
2: 97.9% – 95%	5
3: 94.9% – 90%	5
4: 89.9% – 80%	2
5: Below 80%	2

THE SCORING SYSTEM:
Based on the twelve (**12**) ISAG recommended Equine (*Equus caballus*) STR DNA Markers (AHT4, AHT5,ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, VHL20)

Absolute genotyping accuracy (Aga): *(Nga - Gea) / Nga* (as percentage) considers the total number of discrepancies, that is genotyping errors and "blanks" (no genotype reported)

Nga: total number of expected genotypes (reference samples not included)
Gea: total number of genotype errors, including blanks

President: Dr. Clare Gill

Secretary: Dr. Sofia Mikko

ISAG is a scientific society that provides a forum for the exchange of information, methods and materials between members and for standardization of genotyping nomenclature. ISAG is not involved in the accreditation of service genotyping laboratories or institutions and ISAG does not regard participation in its Comparison Tests as indicating that a laboratory is internationally accredited. This certificate reflects the laboratory performance in the specific comparison test.

International Society for animal genetics (ISAG)

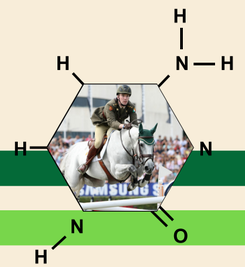
ISAG CT test

- ISAG do not provide a list of the ranked laboratories; it is kept confidential.
- Tested laboratories do receive a certificate with their ranking. If one wants to use a laboratory, ask them to provide their ISAG Comparison Test certificate in their tender/quotation for services
- ISAG recommend where there is a studbook starting DNA parentage verification (PV) that they to start with SNPs instead of STRs/MS,

WBFSH TIMELINE

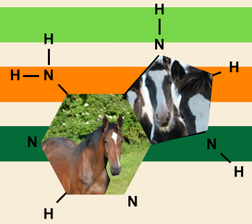
Oct 2024

Motion passed at the WBFSH General Assembly - all member stubooks to complete DNA verification in the studbook pedigree recording process



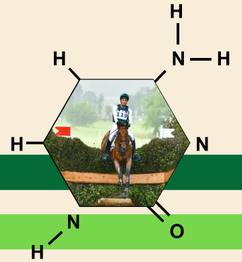
July 2025

WBFSH Host Webinar on the purpose, importance, methods and timeline for DNA verification in member studbooks.



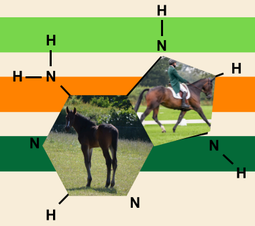
Oct 2025

WBFSH General assembly, motion and protocols to be shared and voted.



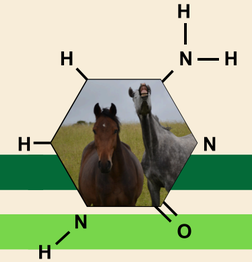
2025-2027

Member studbook transition phase where they do not already DNA test.



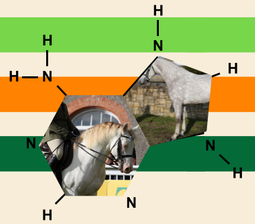
2027

DNA testing for all stallion, mare and foals mandated by the WBFSH starting in 2027.



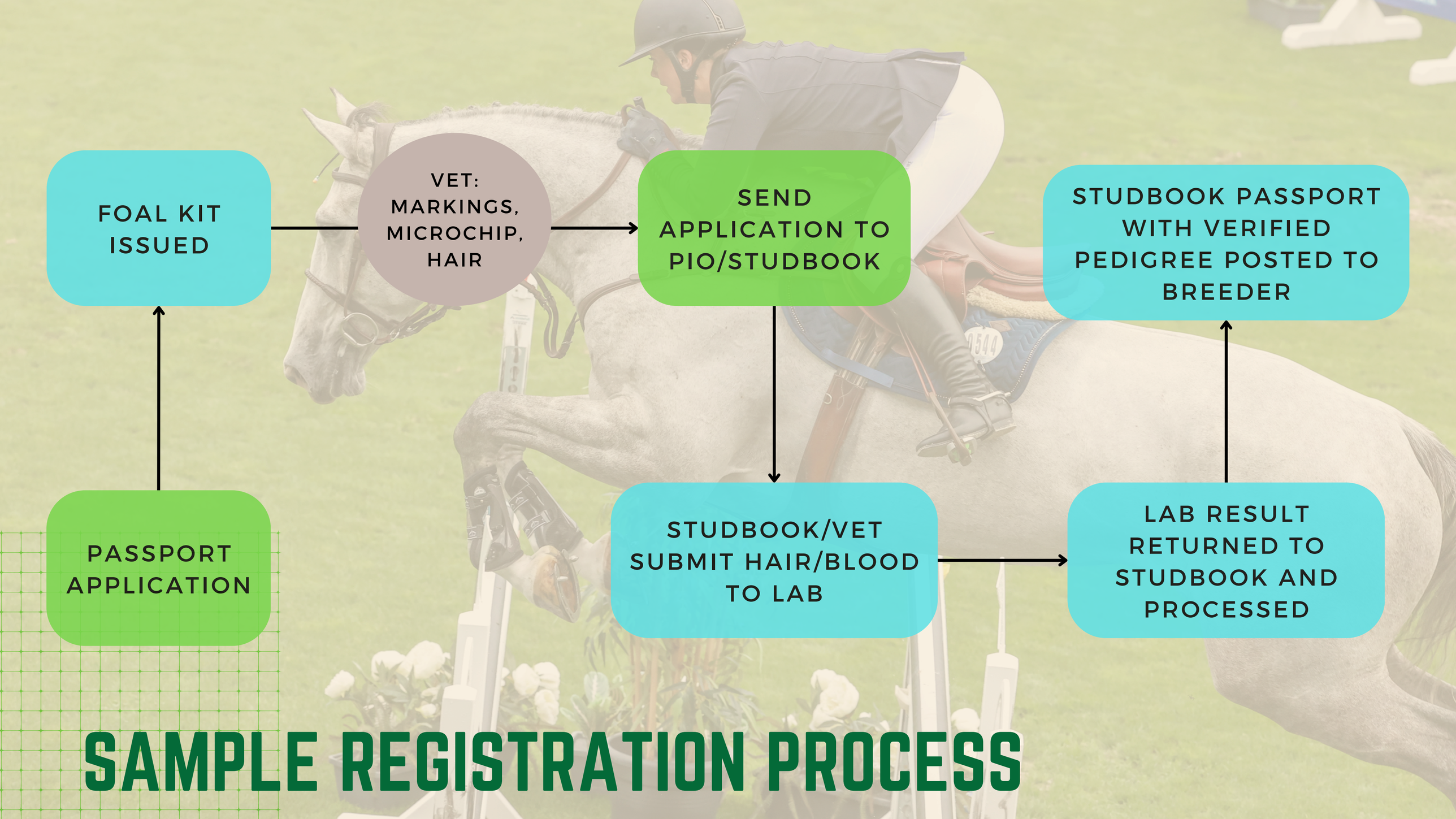
2029

From 2029 non-compliant members may face exclusion.



SAMPLE PROTOCOL

SAMPLE TIMELINE FOR DNA TESTING WITH SNP OR MS		
START DATE	**REGSITRATION**	**INSPECTIONS/CLASSIFICATIONS**
From Jan 2026 (on SNP)	All 2025 filly foals and adult mares must have hair submitted for genotyping at studbook registration. A vet should take this sample and validate it against the animal's microchip.	All animals brought forward for studbook classification/inspection must have a hair sample taken by a vet on the day (validate it against the animal's microchip). These samples should be genotyped to allow their future foals to be validated against their sires/dams.
June 2026 - Jan 2027 (on SNP)	Studbooks review most active Studbook Stallions and Mares, and most active stallions in the cross breeding programme (if relevant) and seek to test hair, share or obtain a genotype/marker from the stallion/mare owner OR animals studbook of origin for the purposes of parentage verification.	
From Jan 2027 (on SNP)	All 2026 foals and new adult registrations must be validated to their sires and dams on their passport (genotype with parentage verification)	As above – however samples will only be required for animals who are not yet genotyped.



SAMPLE REGISTRATION PROCESS

Some Existing SNP research..

Home › Journal of Applied Genetics › Article

Replacement of microsatellite markers by imputed medium-density SNP arrays for parentage control in German warmblood horses

Animal Genetics · Original Paper | Open access | Published: 29 September 2022
Volume 63, pages 783–792, (2022) Cite this article

Journal of Applied Genetics

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RESEARCH ARTICLE

SERPINB11 Frameshift Variant Associated with Novel Hoof Specific Phenotype in Connemara Ponies

Carrie J. Finno, Carlynn Stevens, Amy Young, Verena Affolter, Nikhil A. Joshi, Sheila Ramsay, Danika L. Bannasch

Published: April 13, 2015 • https://doi.org/10.1371/journal.pgen.1005122

Gmel *et al. Genetics Selection Evolution* (2024)
https://doi.org/10.1186/s12711-024-00922-6

Two Cases of Chromosome 27 Trisomy in Horses Detected Using Illumina BeadChip Genotyping

by Cliona A. Ryan [1,*], Donagh P. Berry [1], Monika Bugno-Poniewierska [2], Mary-Kate Burke [3], Terje Raudsepp [4], Sonja Egan [5] and Jennifer L. Doyle [5]

RESEARCH ARTICLE Open Access

Using high-density SNP data to unravel the origin of the Franches-Montagnes horse breed

Annik Imogen Gmel[1,2], Sofia Mikko[3], Anne Ricard[4], Brandon D. Velie[5], Vinzenz Gerber[6], Natasha Anne Hamilton[7] and Markus Neuditschko[1*]

ELSEVIER

animal

Volume 18, Issue 9, September 2024, 101278

Imputation of single nucleotide polymorphism genotypes in ungenotyped sport horses from the genotypes of their progeny

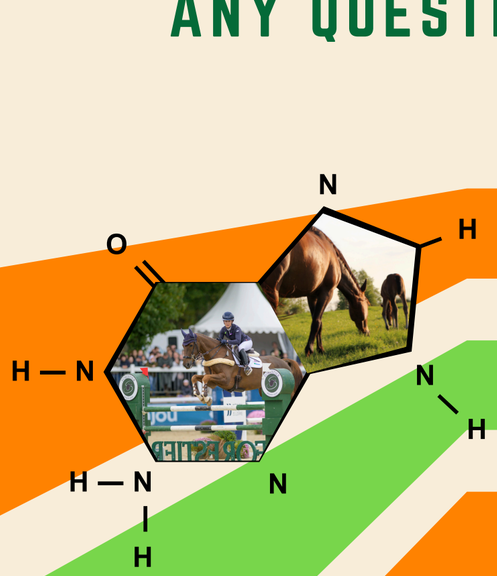
J.L. Doyle [a], S. Egan [a], D.P. Berry [b]

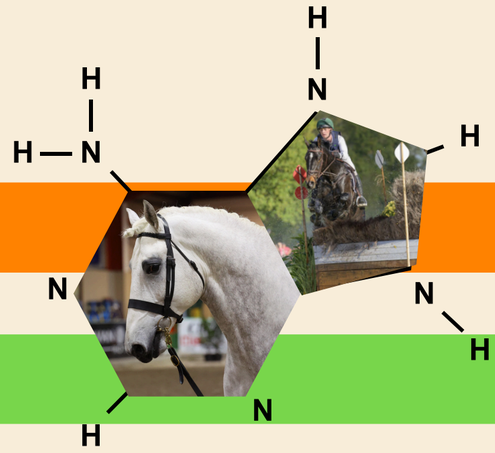
Summary

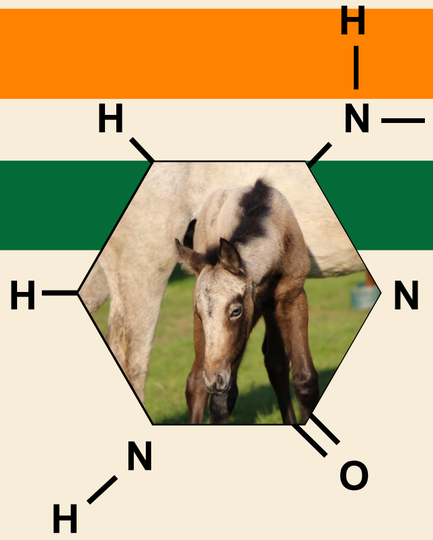
- DNA testing (blood/hair) provides a validated method of equine parentage verification
- Studbooks who complete DNA testing should consider sharing relevant markers for the purposes of parentage verification, where available.
- DNA testing will be a requirement for all WBFSH member studbook registrations by 2027.
- Parentage verification is the minimum requirement, studbooks can decide which method.
- ISAG CT tested labs are recommended - talk to local and international colleagues!
- WBFSH motion and protocol to be shared with WBFSH members to enable discussion where needed ahead of the General Assembly (GA)
    - Facilitate studbook review where necessary before the GA, the representatives of the studbooks will then vote at the GA.

THANK YOU!

ANY QUESTIONS?







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