

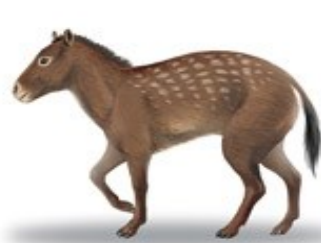
Gene Doping

SIMON COOPER
VICE-CHAIRMAN,
INTERNATIONAL STUDY BOOK
COMMITTEE

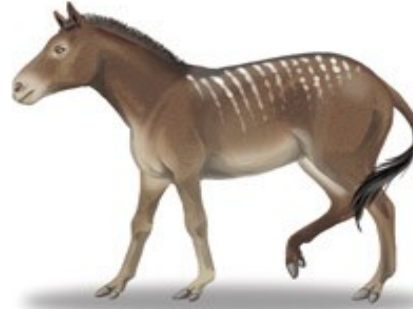
What is the future of our equine breeds?



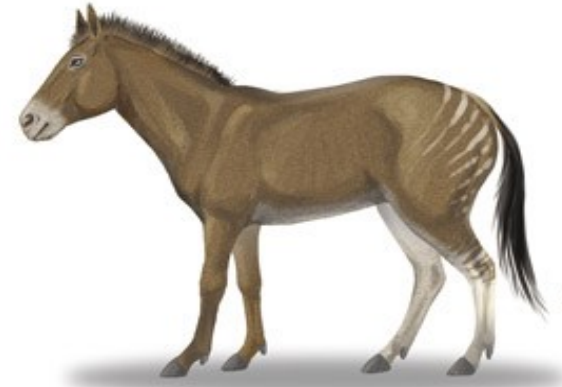
Eohippus to Equus



Eohippus
(50 million years ago)



Mesohippus
(35 million years ago)



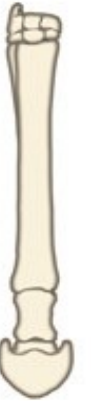
Merychippus
(10 million years ago)



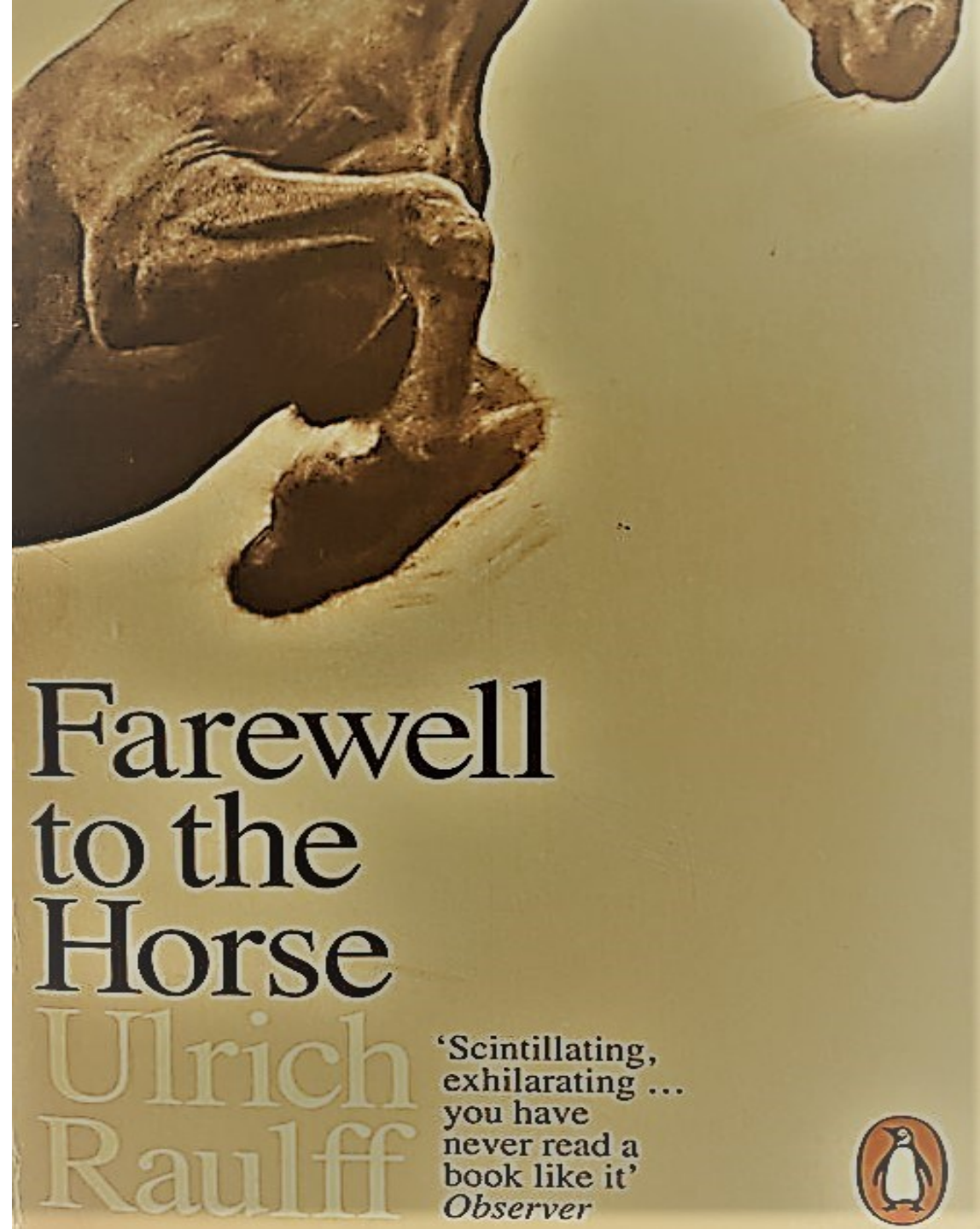
Pliohippus
(5 million years ago)



Equus
(At Present)



What is the
future of our
equines?



Farewell
to the
Horse

Ulrich
Raulff

'Scintillating,
exhilarating ...
you have
never read a
book like it'
Observer



DIY Bacterial Gene Engineering CRISPR Kit



\$159.00

Shipping: Calculated at checkout



★★★★★ 7 product reviews

Quantity:

1

Add To Cart

Add to Wishlist



‘Gene editing is no longer a rumour’

1. Best CRISPR Kit on the market ★★★★★

Posted by Camille Lienau on 5th Apr 2018

I am a high school student who purchased this kit because I had a passion to learn more about biohacking at home, and be involved with genetic engineering independently. This kit was the perfect stepping stool to give me first hand info on CRISPR and the whole process is so easy to understand, everything you need is right there in the kit, just be careful NOT to freeze your E. coli like I did :(

2. Excellent Introduction to technical details of CRISPR ★★★★★

Posted by Arthur on 14th Nov 2017

Ran three trials so far and every one was successful.

3. Works, but Protocol is Confusing ★★★★★☆

Posted by Sarah on 9th Nov 2017

Shipping took a while, but the kit included all materials including two stickers with the ODIN logo. The experiment was a success - we got a lot of growth on the plates. The only frustration was the protocol was confusing and wordy. I ended up rewriting it so it was more straightforward without the protocols and walkthroughs in the middle of the handout. Otherwise, great kit! Ordered the refill to introduce to the class next term.

'Race to sign up first genetically engineered super horse'

Daily Telegraph 27 December 2017



Race to sign up first genetically engineered super horse

Argentinian scientists say lab-made clones will be able to run faster and jump better after breakthrough

By Sarah Knapton SCIENCE EDITOR

GENETICALLY engineered horses designed to be faster, stronger and better jumpers will be born in 2019 after a breakthrough by a laboratory that already clones polo ponies.

Scientists in Argentina have successfully used a powerful DNA editing technique called Crispr to rewrite the genomes of cloned horses.

Healthy embryos were produced after the procedure, which the researchers plan to implant into a surrogate mother within two years.

The team focused on boosting the myostatin gene sequence, which is crucial to muscle development, endurance and speed. Theoretically, animals designed in such a way should be able to run faster for longer, and jump higher more easily.

Traditionally the same traits would be achieved by breeding animals that already exhibited desirable features. But it can take many generations to develop a beneficial trait.

Daniel Sammartino, the founder of Kheiron Biotech, a cloning specialist based in Buenos Aires, said: "This technology brings additional progress in horse breeding. It could be possible to achieve better horses in less time.

"Our next big challenge is not only to export our technology, but fundamentally develop these scientific advances in other animals for multiple purposes."

The first cloned horse was created in 2003 by US company ViaGen and cloned polo ponies are now widespread.

Last year, Adolfo Cambiasso rode six horses cloned from

the same mare, to help his team win the Argentinian open.

Though cloning itself cannot improve a line, some believe it offers advantages over the original donor horse, as breeders can make sure the environment and training is a perfect fit, meaning that it is likely the "daughter" clone will be better than the original.

In 2013, the The International Federation for Equestrian Sports (FEI) lifted the ban on cloned horses competing internationally after a review found they were unlikely to have any advantage over horses bred traditionally. But none has so far competed at the Olympic Games.

The FEI said there were no rules yet in place to stop genetically engineered horses competing.

Shannon Gibbons, an FEI spokesman, said: "The performance of a cloned horse is unlikely to match that of the original horse for a number of reasons, including the maternal uterine environment, nutrition, training and the understanding that clones are not exactly the same as the original.

"Additionally, as progeny of cloned horses will be produced by conventional reproductive methods, such as natural covering or artificial insemination, maintaining fair play is protected.

"The FEI will therefore not forbid participation of clones or their progenies in FEI competitions. However, we will continue to monitor further scientific research."

British equestrian bodies also said there was nothing preventing genetically edited animals from competing.

The results of the research will be published early next year in the journal *Cloning and Stem Cells*.

The first horses genetically engineered using the Crispr DNA-editing technique are expected to be born in 2019



CHARLOTTE GRAHAM FOR THE TELEGRAPH

Hunting for a v huntsmen took hunts off, peop Meanwhile, po

Hive of v

By Camilla Turner

RIVAL beekeepers h for a rise in hive thef ity of the hobby creat mand for the best qu

Hundreds of thou been taken from ap England and Wales s cash in on an incr beekeeping market. tire hives are being reported stolen over

Martin Smith, pub at the British Beeke said it was a "sad fa



X-GENE HORSES?

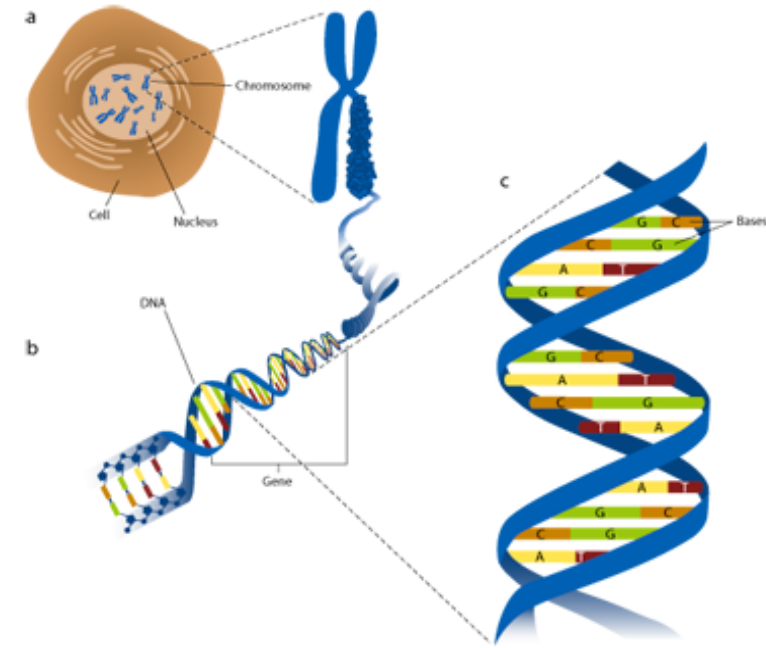
Posted 15 October 2023

Official Veterinarians have confirmed a horse has tested positive to gene doping at the recent Ocala Show.

“We have contacted the relevant connections and will not be giving out any more information at this time” the Chief Veterinarian announced yesterday.

The announcement will be a bitter blow for the equine sector and the breeding industry as it attempts to regain the trust of the public after a series of scandals.

1. **DNA** (**D**eoxyribo**N**ucleic **A**cid)
2. All DNA in an animal is called the **genome**
3. The genome is strands called **chromosomes**
4. Horses have 32 pairs of chromosomes - 31 non-sex & 1 sex (X & Y)
5. There is a small amount called **mitochondrial DNA** only inherited from the dam



What is DNA?

1. The administration of normal genes into an animal's cells **to treat or cure a disease** (transgenesis)
2. There are no genetic therapies approved by veterinary pharmaceutical regulators for use in the horse



What is Gene Therapy?

Oncept canine melanoma vaccine (prescribed for horses) is a DNA vaccine that treats melanoma:
Generates an anti-tumour immune response

<https://www.sporthorsevets.com/equine-melanoma/>

Contributes to health, not performance

Should this be banned?

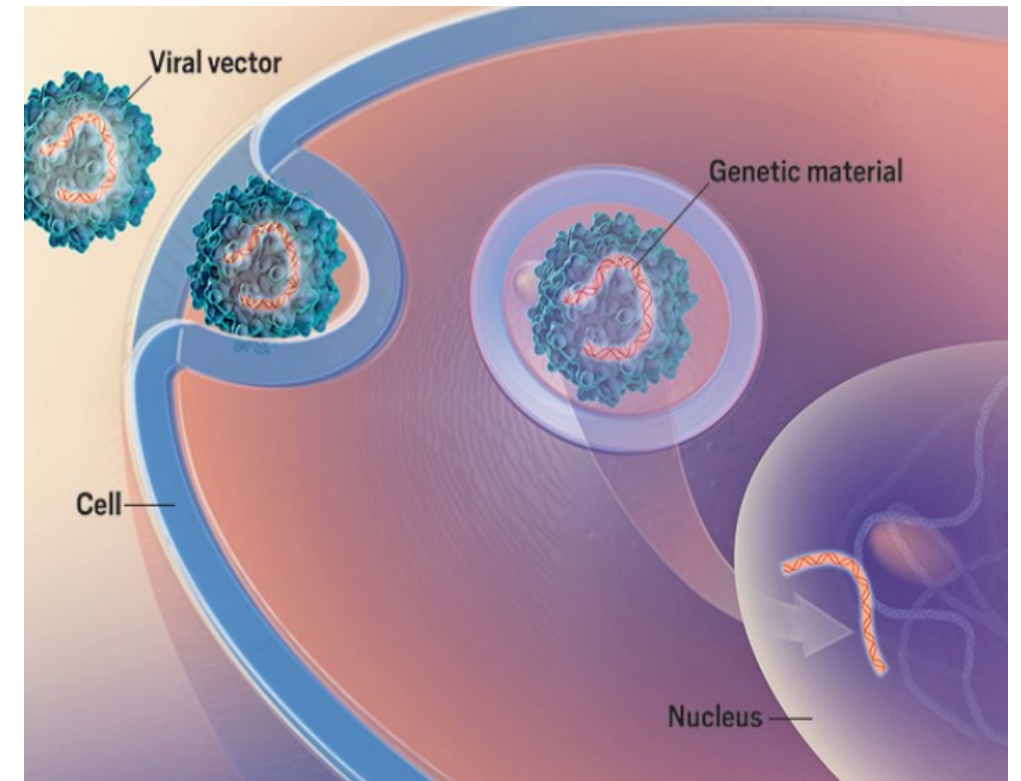
The Conundrum

1. A genetic therapy technique that allows **targeted modification** of the DNA sequence within a cell
 2. Gene editing can delete, insert or substitute the original sequence with a desired sequence (the order of the 4 chemical building blocks, called 'bases' of the DNA molecule – **A**denine & **T**hymine and **C**ytosine & **G**uanine)
-



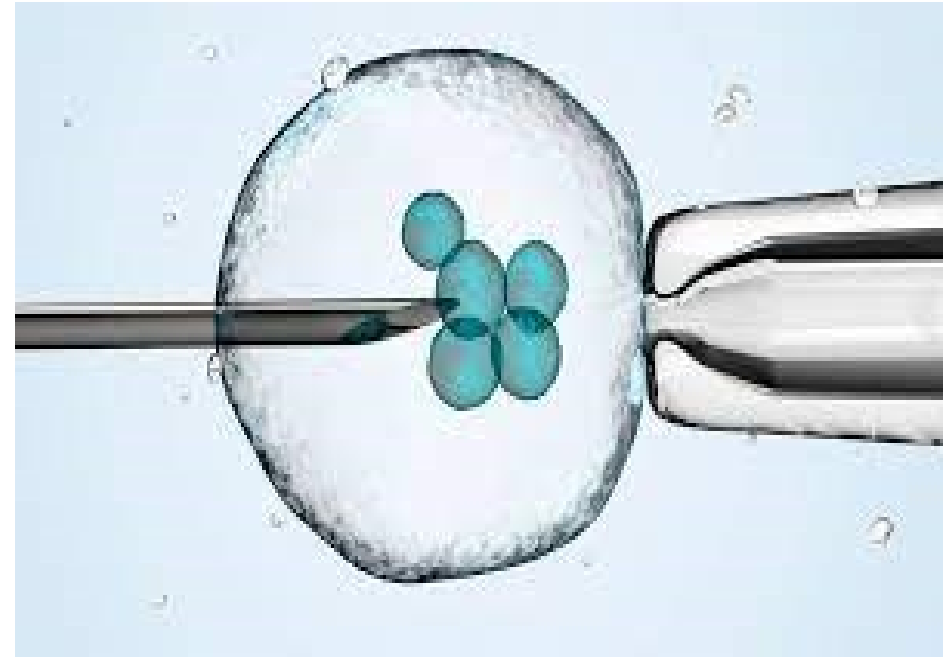
What is Gene/Genome Editing?

1. The administration of normal genes into an animal's cells to **enhance performance** instead of treating a disease
2. It is simple to order synthetic gene sequences online



What is Gene Doping?

1. Genetically modified eggs or sperm
 2. Pregnancy / embryo
 3. Foal
 4. Yearling
 5. In training/competition
 6. Post competition / before entering breeding
-



When can gene doping be carried out?

Article 6B

2. Prohibition on Gene Editing and Genome Editing

c. The use on, or administration or application to, any horse of Gene Editing or Genome Editing is prohibited at all times.

The International Agreement
(IABRW)

Article 6B

1. Prohibition on use or administration of Genetic Therapy

c. Exempted Genetic Therapy

A Genetic Therapy may be used or administered to a specific horse with the express prior approval of a Racing Authority if that Genetic Therapy is used to treat an injury or disorder formally diagnosed by a veterinarian, and:

- a. **is not capable of modifying a horse's heritable genome;***
 - b. does not pose a threat to the welfare of horse;*
 - c. does not pose a threat to the integrity of racing, either by having the potential to enhance or harm the performance of a horse in a race.*
-

Gene Therapy - IABRW

British Government Questionnaire Q1 2023

1. What breeding techniques/technologies do you currently use in your sport?
2. Is there currently any interest in using **PRECISION BREEDING** (PB) technologies in your equine sector?
3. What are the pros and cons of using PB technologies in the equine sector?
4. If you have concerns, what are they?
5. Is there likely to be any interest in using these technologies in future? If so, when?
6. Do you set standards or rules relating to breeding practices for equines?
7. Do you foresee an interest for imported precision bred equines or germinal products?

The Genetic Technology (Precision Breeding) Bill

Department of Environment, Food & Rural Affairs [April 2023]:

Precision breeding (PB) describes a range of technologies, such as gene editing, that enables DNA to be edited much more efficiently and precisely than current breeding techniques. This is different to Genetic Modification (GM)...

...Scientific advice is that there is no greater risk to the environment than organisms developed through traditional breeding.'

Precision Breeding

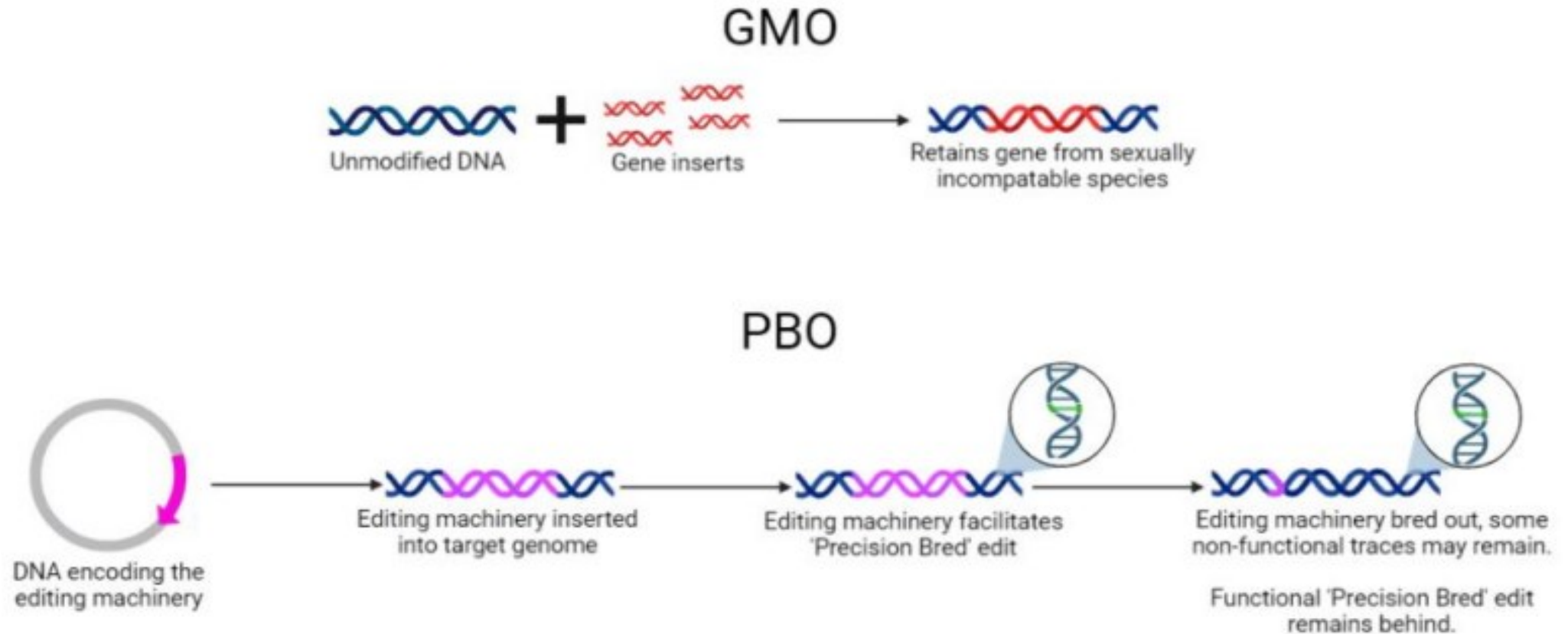
Animal welfare 'safeguards' in the Act

“The system will require a developer to seek a marketing authorisation from Defra [Government] before any precision bred animals can be marketed in England.”

“When applying, the developer must confirm that the health and welfare of the animal (and its qualifying progeny) is not expected to be adversely affected by any trait resulting from precision breeding. This will be in the form of an animal welfare declaration and accompanying evidence.”

Genetic Modification & Precision Breeding

“...Precision Bred Organisms (PBOs) are not genetically modified?”



1. Molecular biology technique called **PCR**
2. PCR targets a specific DNA sequence and makes millions of copies of it, which can then be examined by sequence, length or melting temperature
3. This method has also been upscaled to detect up to twelve genes in one reaction with a specialised instrument



How can you detect
transgenesis?

1. Comparison of the DNA sequence - **Whole Genome Re-sequencing (WGR)**
2. The DNA sequence of the foal is compared to the DNA sequence of its parents to identify artificially introduced differences in the sequence
3. Alternative - sequencing only regions of the genome likely to be targeted for gene doping



How can you detect gene editing?

**Normal
Genome**

CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGA TTGTTTCATGCTGGTATGCCT TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGA TTGTTTATGCTGGTATGCCT TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA

**Edited
Genome**

CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGGTAT **ag** TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGG **---** CTTGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGG **--ca---** TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGGTAT **gg** TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCT **-----** TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGGTA **-----** TGGCATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAAT **-----aaaata-----** TTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA
CTGACAAGACGAAATATTTTGTAGAAAAATGACAAGATTGTTTATGCTGGT **-----** ATAGTTTGTCAAGGTACGACAGGCAACATTCTTCGAGGA

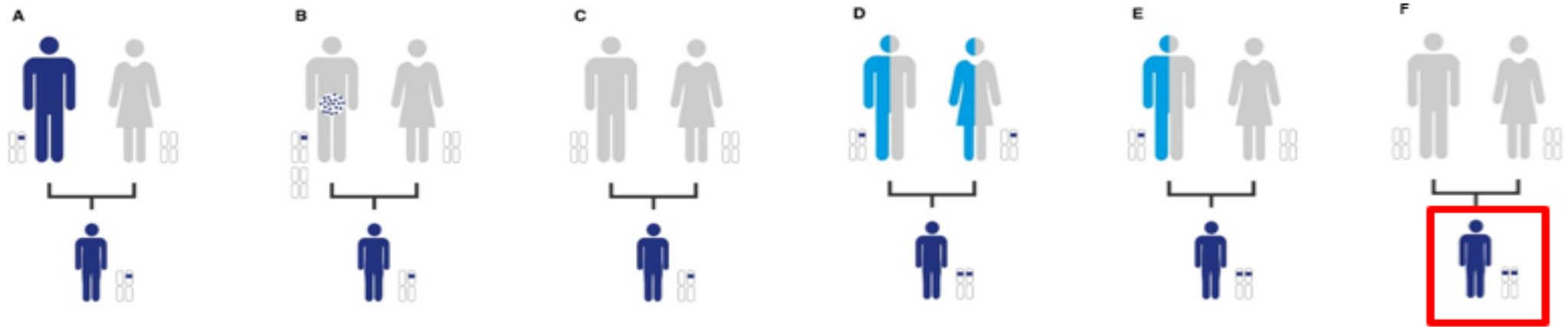
What is detected by the tests?

Gene-editing [as opposed to transgenesis - easier to detect] would have to be proved – i.e. that the mutation is not natural/spontaneous.

Information from the parents is required to show that at least one copy was not inherited.

Questions, regulations and
prosecution framework

Methods of inheritance and novel mutations



- A) Heterozygous: inherited from one parent
- B) Heterozygous: inherited from mosaic parent
- C) Heterozygous: *de novo* mutation in offspring
- D) Homozygous: inherited from each parent
- E) Homozygous: inherited from one parent plus *de novo*
- F) **Homozygous: two *de novo* mutations at the same point**

De Novo Mutations: ‘a genetic alteration that is present for the first time in one family member as a result of a variant (or mutation) in a germ cell (egg or sperm) of one of the parents, or a variant that arises in the fertilized egg itself during early embryogenesis’

Q1. Should tests be conducted only on foals and/or first-season stallions/mares, horses in training/competition?

Q2. Should retrospective testing be conducted on parents and potential siblings to assess the reach of abuse and how far should this go back?

Retrospective testing for
gene doping?

- Q1. Who owns the sample? Who owns the data?
- Q2. Chain of custody of samples (Hair or Blood)
Anonymisation of samples
Storing of samples
- Q3. Do the Stud Book's rules allow for additional samples from the parents?
-

Samples and data

Q1. Should the gene editing tests be charged to owners and breeders?

Q2. Should testing be restricted to accredited laboratories?

Costs and testing
laboratories

Q1. 'Accreditation' or 'Registration' of breeders?

Q2. Could you prove exactly when the gene-editing had occurred?

Q3. Who would you prosecute?

Prosecution

A question for you – ‘May’ or ‘Shall’

*“Given that gene editing or genome editing of an embryo can modify the heritable genome, any horse discovered to have been the subject of such practice [**may / shall**] be excluded or removed from the relevant stud book.”*

What can you do – First Steps

1. Inform and discuss gene doping threats and your Stud Book Rules with your stakeholders (Art. 6B of IABRW)
2. Include a ban on gene doping in your Stud Book Rules
3. Start collecting samples now for future testing - knowing that samples collected are a deterrent



Phosphoenolpyruvate Carboxykinase (**PEPCK**)



This is
only the
beginning but
awareness is
deterrence
