

# Project Overview

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## Science fact not fiction: Detecting gene edited racehorses

July 2019 – May 2021

### Objectives

This project will develop a cheaper and simpler method to detect gene editing in horses. It will give Australian and International Stud Books an efficient method to identify illegally edited horses, helping the industry uphold integrity and protect the welfare of its most important participants. This will be achieved by creating a custom kit (test) that identifies and isolates the DNA sequences from the genes most

likely to be targeted for gene editing. The genomic regions identified will be DNA sequenced using next generation sequencing. The sequences derived will be compared to the horse's parents, and to a library of normal horse genome variation. This will allow analysts to identify variation that has been artificially introduced to the horse with illegal gene editing.

### Background

The horseracing industry relies on the principle of fair play in competition. This is important for both for the welfare of its horses and consumer confidence in wagering, a major source of income to the industry. Antidoping control is one way the industry can protect itself from breeches to integrity. Gene doping is the non-therapeutic transfer of genes or genetically modified cells to artificially enhance athletic performance in humans and animals. The World Anti-Doping Agency (WADA) and horseracing authorities have been aware of the threat gene doping poses for the industry for around 15 years. In 2018, our team announced that we had developed a world first test to detect gene doping with traditional gene therapy methods in horses.

Technology has continued to evolve and there is a new focus on gene editing, a more refined way of performing gene therapy. Gene editing is most successful when performed on an embryo and is now attracting the attention of the thoroughbred industry. This technology presents a new challenge for the industry particularly given all forms of artificial reproductive technologies are banned in the breeding of thoroughbred horses. Currently, the only way to identify whether a horse has been gene edited is to compare its DNA sequence to that of its parents via whole genome sequencing (technology that maps the horse's entire

blueprint of DNA). Differences in a foal's sequence compared to its parents could indicate an artificially created mutation – the result of gene editing. While effective, whole genome sequencing is expensive, requires specialist knowledge interpret the analysis, and creates a large amount of data that must be securely stored.



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## Research overview

This project will identify the genes most likely to impact a horses' athletic performance and in turn be of interest to someone wanting to gene edit a horse. As a first step the team will examine and review the existing literature available. Using the team's findings, a list of potential target genes will be used to develop a custom test that will isolate the identified sequences from within a whole genome DNA sample.

We will then optimise the laboratory procedures to ensure we can derive the largest amount of useful sequence at the most affordable price. The data generated in this pilot part of the study will also be used to develop a simple step-by-step analysis method that can be used by analysts without any further specialist training.

The final part of this project will be validation of the initial findings by screening parent horses and their foals. This will give us the opportunity to properly test and optimise the kit and analysis pipeline. It will also generate new data illustrating what level of variation we should expect to observe between parents and offspring. It is normal to see some differences in these DNA sequences, because when cells replicate to create sperm or eggs, it is inevitable that errors in the DNA will occur. It is the number and types of these changes that is of interest to us. This will allow us to identify artificially introduced mutations in the DNA.

## Outcomes and implications

Australia is a world leader in the development of tests to detect gene doping using normal gene therapy methods. Using gene editing to gene dope a horse is a more serious threat to the industry's integrity because gene editing is most useful when performed on an embryo. Any sort of embryonic manipulation of a Thoroughbred will lead to that horse being excluded from the Stud Book.

This project will develop a kit to detect gene editing in horses. This method will be cheaper and easier to use than currently available whole genome sequencing techniques.

It will provide the thoroughbred horses industry and its authorities, Australian and International Stud Books, a relatively cheap and easy method to identify illegally edited horses. This will help the industry uphold integrity and protect the welfare of its most important participants.

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## Contact

### **Dr Natasha Hamilton**

Director, Equine Genetics Research Centre Racing Australia  
Laboratory 2, Hunter Valley Equine Research Centre,  
2 Randwick Way Scone 2337

02 8072 1914

Natasha.Hamilton@racingaustralia.horse

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