

## **“Development of a synthetic reference material for a multiplex test panel for anabolic gene doping”**

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### **Project Overview**

Gene doping is believed to become a new threat to sport and the anti-doping community has been focusing efforts on developing a test for its detection. Methodology to detect doping genes in athletes' blood already exists and work is underway to validate an erythropoietin gene doping test for implementation in testing laboratories.

Recently, we expanded our detection capability to include four additional candidate genes with potential to increase muscle size and strength. Using commercial PCR assays, we developed a multiplex 'anabolic gene doping detection panel'. The panel allows simultaneous detection of several 'sport-specific' genes in one sample, reducing the test's cost and turn-around-time.

Acceptance of this method for routine testing requires suitable reference materials (RM) as controls to ensure the method performs as intended. During method development, cDNA-based controls for each gene are commonly used, but these are unsuitable for routine testing, because inadvertent sample contamination with such control would lead to a false-positive test result.

We propose to develop a single synthetic DNA-RM for testing doping with four 'anabolic' genes that will overcome this problem. The RM will incorporate four modified sequences detectable by the 'anabolic gene doping detection panel' with similar specificity and sensitivity as each doping gene. However, the products from the RM and the doping genes will differ, allowing discrimination between true-positive and false-positive test results. We will characterise the RM for purity, quantity, homogeneity and stability using digital PCR and other molecular techniques. Furthermore, using this RM we will validate the multiplex 'gene doping detection panel' using a model in vitro system.

This research is crucial in the development of a reliable routine method for detection of gene doping with several genes that could be potentially used in 'power' sports.

### **Result and Conclusion:**

Gene doping is believed to be a new threat to sport and our laboratory has been focusing efforts on developing tests for its detection. Through these efforts, the first test for erythropoietin gene doping was developed and is

currently being implemented in testing laboratories. To expand our detection capability to other genes, we recently developed an 'anabolic' gene doping detection panel which targets doping genes that have potential to increase muscle size and strength. The panel consists of commercial PCR assays that were optimised and validated to work equally well when each assay is used on its own or in combination with one or two other assays to allow doping genes to be analysed in a sample simultaneously.

To use the panel of PCR assays in routine testing, suitable reference material(s) (RM) are required for use in method's quality controls. In this project, we generated a single synthetic DNA RM that can be used in testing doping with any of the four 'anabolic' genes using their specific PCR assays performed separately. A characteristic property of this RM is that, due to its unique design, it does not generate a false-positive test result in a routine laboratory setting if an athlete's sample is inadvertently contaminated with the RM.

The current study expands the arsenal of gene doping detection targets and has made significant contribution to progress the developed method towards a reliable, reproducible and robust routine test for detecting doping with any of four 'anabolic' genes. This test could be included in the fight against doping in sport in the near future.