Project Overview

"Gene doping detection by next generation sequencing"

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Gene doping represents a threat to the integrity of sport and the suitable for publication on WADA's website health of athletes. The anti-doping community has been focusing efforts on developing a test for its detection. The current methodology to detect doping genes in an athletes' blood uses the polymerase chain reaction (PCR) that targets unique sequences in a doping gene, which correspond to exon-exon junctions in the intronless transgene. These so-called real-time PCR assays detect unique sequences in the complementary DNA (cDNA) for human erythropoietin (EPO) and other doping genes such as insulin-like growth factor-1, growth hormone, growth hormone releasing hormone and follistatin.

As the sequences of cDNA of Epo and other doping genes are known, it is relatively easy to aggravate these tests, which will then result in a false-negative result. Recently, we developed a new gene doping detection assay that will overcome this problem. The test is based on targeted sequencing of doping genes with potential to detect any doping gene in any context with a very high sensitivity. Using an in-house designed next generation sequencing assay, we developed a gene doping detection assay for cDNA of EPO which targets all potential exon-exon junctions of all possible EPO-transcripts.

We propose to evaluate and further develop a multiplex 'gene doping detection panel' which targets genes for, among others, insulin-like growth factor-1, growth hormone, growth hormone releasing hormone and follistatin. The panel allows simultaneous detection of several 'sport-specific' genes in one sample, reducing the test's cost and turn-around-time. This research is crucial in the development of a reliable routine method for detection of gene doping that may be potentially used in all sports.

Results and Conclusions:

The main aim of the project was to evaluate and further develop a next generation sequencing-based multiplex 'gene doping detection panel'. First we developed probes for the detection by sequencing of Erythropoietin, Insulin-like growth factor and Growth Hormone. Second, we evaluated the developed probes in the multiplex sequencing of these genes. Finally, we optimized and fine-tuned the developed method and determined the sensitivity of the developed method.

Our results show that, using the developed probes for next generation sequencing, we were able to simultaneously detect plasmid-derived cDNA copies of Erythropoietin, Insulin-like growth factor and Growth Hormone in a

background of genomic DNA with 100% specificity. We were able to detect EPO GH1, GH2, IGF1 and IGF2 cDNA in concentrations below 0.01 percent gDNA at all exon-exon boundaries. For quantification of the amount of cDNA we spiked a GFP plasmid into the samples and found stable numbers of GFP across samples, enabling quantification of gene-doping cDNA levels.

Publications:

- De Boer EN et al., A next-generation sequencing method for gene doping detection that distinguishes low levels of plasmid DNA against a background of genomic DNA. Eddy N. de Boer, Gene Ther. (2019) Gene Therapy (published on-line),
  https://rdcu.be/bJMVv
  https://doi.org/10.1038/s41434-019-0091-6