AptaTrich

D-PhD12-2

Responsible Partner: Anses

Contributing partners: Bfr, McGill University

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GENERAL INFORMATION

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Development of an aptamer-based test for *Trichinella* detection

1. PhD12-FBZSH9-AptaTrich

1.2. **Deliverable 2 :** D-PhD12-2 'Aptamers set on whole larvae are selected'

Selection of aptamers against fixed *T. spiralis* muscle larvae

Following the isolation and fixation of *T. spiralis* muscle larvae (ML) and New Born Larvae (NBL), several round of aptamer selection by SELEX were conducted in order to produce aptamers specific for the target organism, i.e. *T. spiralis* ML. First, 10 ul of a 100uM stock ssDNA library, corresponding to 1nmol of ssDNA, was heated and subsequently cooled to yield three dimentional sequence structures capable of binding to the target of interest. These sequences were incubated with a defined number of *T. spiralis* ML for a specific period of time before washing and removing non-specific sequences. Larvae-bound sequences were retrieved by heating in water at 95C for 10mins. There sequences were subjected to symmetric and asymmetric PCR amplification to favor the amplification of the sequences of interest. These products were quantified using a nanodrop to measure absorbance at 260nm. Finally, the PCR products were used in the following round of selection. The two first SELEX rounds were only positive selections but the three next were successively positive and negative selections.

For each round of selection, the number of PCR cycles had to be optimized to reduce the formation of non-specific byproducts which could potentially interfere with later selection. Furthermore, the quantity of larvae, the duration of incubation, and the number of post-incubation washes were modified as selection progressed. This functions to increase selection stringency with the goal of eliminating sequences with less affinity and specificity for the target.

Currently, 5 rounds of selection have been successfully accomplished. By subjecting the samples to gel electrophoresis, the expected products of 80bp are visible. With further rounds of selection however, the products on the gel become increasingly more faint and less defined. We believe that the issue may be in our method of ssDNA isolation following PCR amplification, which yields a mixture of dsDNA and ssDNA. Therefore, much work is being done to optimize a better protocol for the extraction of ssDNA aptamers following PCR amplification. Nevertheless, the aptamers were bound to *T. spiralis* ML.