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METSCAN and Novel nuclear barcode regions for the identification of flatfish species

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In the context of the European Union(EU) Regulation on Genetically Modified Organisms (GMOs), validation of Polymerase Chain Reaction (PCR-)based detection methods is a fundamental task of the European Union Reference Laboratory for Geneticallly Modified Food and Feed (EU-RL GMFF) that requires to integrate the combination of both the experimental approach and of bioinformatics analyses for sequence similarity searches. In addition, the EU-RL GMFF may be requested by Commission Services to evaluate the specificity level of methods developed to detect GMOs other than those submitted under the â€˜Food and Feedâ€™ regulations, such as GMOs intended to be released in the environment or not approved GMOs for which emergency measures are issued.
Within this framework, here we show the implementation of METSCAN, a tool that allows the performance of complex bioinformatics analyses on the specificity of PCR-based detection methods. METSCAN relies on the power of a High Performance Computing cluster which runs HTCondor scheduler, through a simple user interface. METSCAN has the objective to make in silico predictions on the detection methodsâ€™ specificity, to direct recommendations on the need or not for experimental testing of method specificity and, in case, to define what source of DNA, e.g. vector, plant species or other GMOs can cross-react with each detection method.

Monitoring of the food chain to fight fraud and protect consumer health relies on the availability of methods to correctly identify the species present in samples, for which DNA barcoding is a promising candidate. The nuclear genome is a rich potential source of barcode targets, but has been relatively unexploited until now. We have developed a CPU high demanding bioinformatic pipeline that processes available genome sequences to automatically screen large numbers of input candidates, identifies novel nuclear barcode targets and designs associated primer pairs, according to a specific set of requirements. By using the High Performance Computing cluster which runs HTCondor scheduler, we have implemented the fast execution of this complex task to tackle specifically fish fraud. The obtained results have been analysed in silico and tested in laboratory to efficiently identify flatfishes of the Pleuronectidae family. In addition, by using in silico methods, a dataset of fish barcode reference sequences from the ever-growing wealth of publicly available sequence information has been generated, to facilitate and speedup, labour-intensive laboratory work. The short lengths of these new barcodes target regions render their analysis ideally suited to next-generation sequencing techniques, allowing characterisation of multiple fish species in mixed and processed samples. Their location in the nucleus also improves currently used methods by allowing the identification of hybrid individuals.

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