Designing an Edna Assay for River Otter (*Lontra canadensis*) Detection in Streams

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Environmental DNA (eDNA) is a highly promising field of survey science that hasn't been fully explored on mammalian species. The river otter species (*Lontra canadensis*), is a prime candidate for testing eDNA's uses and limitations with mammals. An eDNA primer was therefore designed that fully amplifies river otter DNA, but does not amplify any other species, including closely related ones . Candidate primer sets were generated using the computer programs BioEdit 7.2.5 , Mega6 , eprimer3 , and Life Technologies. Once primer set possibilities were identified, those with the most base pair differences in non-target species were selected and purchased. Then, using qPCR techniques, two primer sets (OTTER_2 and OTTER_3) were tested against thirteen target DNA samples and seventeen varying non-targets. The primers were effective in amplifying all target species, but also amplified many non-target species in later PCR cycles. Hence, an internal probe was additionally designed to add specificity for the OTTER_2 primer set. The probe was not as effective as decreasing non-target amplification as hypothesized, which lead to the prediction that the samples themselves may have been contaminated with river otter DNA. To test this, the non-targets that amplified despite the additional probe were re-extracted and run through qPCR with the OTTER_2 primer set. Any amplified results will be sequenced. If sequenced results produces otter DNA, the samples are contaminated and not indicative of assay specificity, if it produces non-target DNA, then the assay must be redesigned. It is the eventual goal to use this assay in management scenarios.