Internal sensitivity and reproducibility study of UrsaPlex13 kit for identification of American black bears

By: Katelyn Caric and Hannah Noel
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Abstract

The increasing number of bear attacks on humans each year poses a threat not only to human life but also to bear conservation efforts (Bombieri 2019). To identify individual bears related to wildlife forensic cases, methods need to be validated using SWGDAM guidelines to ensure they uphold legal stringency. This internal validation study will test the sensitivity and reproducibility of the UrsaPlex13 kit developed by Erin Meredith at California Department of Fish and Wildlife to identify 13 different discriminating STR profiles of Ursus americanus, the American black bear (Meredith et al. 2019). The samples used in this study underwent a 1:5 dilution series to test the sensitivity of the assay for different DNA concentrations. A PCR multiplex consisting of ten somatic and three sex markers was used to produce STR profiles of the different sample dilutions. Profiles were assessed in GeneMapper-IDX™ software to see how well the assay performed for different concentrations of DNA. The process described above was replicated four times to verify that the results are reproducible between different runs and analysts. It is expected for the UrsaPlex assay to reproduce the same results after each trial run and have the capability of handling evidentiary samples of varying quantities of genetic material. These results will help further validation of the UrsaPlex kit in Western Carolina University's Forensic Science laboratory so that it may be used in future wildlife forensic cases involving American black bears. On a global scale, validation studies of such kits can help build comprehensive DNA databases of varying species other than humans which can be used in both legal and conservation efforts.

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The increasing number of bear attacks on humans each year poses a threat not only to human life but also to bear conservation efforts (Bombieri 2019). To identify individual bears related to wildlife forensic cases, methods need to be validated using SWGDAM guidelines to ensure they uphold legal stringency. This internal validation study will test the sensitivity and reproducibility of the UrsaPlex13 kit developed by Erin Meredith at California Department of Fish and Wildlife to identify 13 different discriminating STR profiles of Ursus americanus, the American black bear (Meredith et al. 2019). The samples used in this study underwent a 1:5 dilution series to test the sensitivity of the assay for different DNA concentrations. A PCR multiplex consisting of ten somatic and three sex markers was used to produce STR profiles of the different sample dilutions. Profiles were assessed in GeneMapper-IDX™ software to see how well the assay performed for different concentrations of DNA. The process described above was replicated four times to verify that the results are reproducible between different runs and analysts. It is expected for the UrsaPlex assay to reproduce the same results after each trial run and have the capability of handling evidentiary samples of varying quantities of genetic material. These results will help further validation of the UrsaPlex kit in Western Carolina University's Forensic Science laboratory so that it may be used in future wildlife forensic cases involving American black bears. On a global scale, validation studies of such kits can help build comprehensive DNA databases of varying species other than humans which can be used in both legal and conservation efforts.

Introduction

Efforts to create discriminating DNA profiles of bears involved in attacks and related incidents within national parks have been made in the past decade. Research on differentiation non-invasive bear samples by sex have given insight on 3 bear specific markers: one X- specific Bear ZFX, and two Y-specific Bear SMCY and 318.2 (Bidon et. al 2013). The UrsaPlex13 STR multiplex assay has been developed to include 11 discriminating STR markers and 3 bear-specific sex-determining markers to help identify the bears involved (Meredith et. al 2020).

The American Black Bear populates most forests in the US and Canada, leading to many interactions between bears populations and humans, whether it be attacks, property damage, or illegal poaching (Meredith et. al, 2020). The Great Smoky Mountain National Park is no exception, resulting in numerous incidents where black bears have been in close

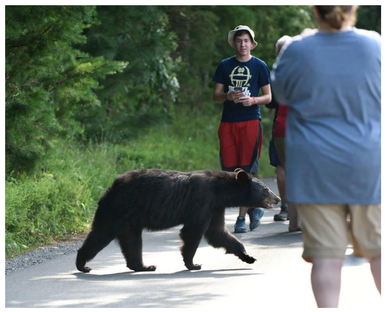

Figure 1. A bear in close proximity of humans in Cades Cove, Great Smoky Mountain National Park. Such habituation of bears to human presence can lead to nuisance issues and potential bear attacks.

Introduction Cont.

contact with campsites. In these circumstances, it would be advantageous for the park to have a reliable method of identifying which bears perpetrated these incidents to prevent euthanization of bears that were uninvolved.

For evidentiary samples from the Great Smoky Mountain National Park to be tested using UrsaPlex13, the assay must be internally validated. As per the Scientific Working Group on DNA Analysis Methods guidelines, internal validation should include the following studies: known and non-probative samples, sensitivity, and stochastic studies, precision and accuracy studies, mixture studies, and a contamination assessment (Martins et. al, 2018). This study is focused on sensitivity as well as the precision and accuracy of the UrsaPlex13 Assay. Sensitivity was tested using serial dilutions of the DNA samples while precision and accuracy were shown through repeated tests by two analysts to determine the reproducibility of the results across those different trials.

Methodology

DNA Extraction	• DNA was extracted from tissue and blood samples provided by the Great Smoky Mountain National Park
Quantitation	• Each sample was quantified using the Qubit fluorometer
Sample Dilution	• Each sample underwent a 1:5 dilution series, allowing for sensitivity testing at different DNA concentrations. • Reference Table 1 for concentrations.
PCR	• Polymerase Chain Reaction with UrsaPlex13 primers was used to produce STR profiles for each sample and their dilutions.
Analysis	• GeneMapper™ ID-X software was used to compare the assay's performance for different concentrations of DNA
Repetition	• This process was replicated four times to verify reproducibility between runs and analysts.

Results

Reproducibility of UrsePlex13 Assay

		Obtained Alleles: Analyst 1		Obtained Alleles: Analyst 2	
Sample	Concentration (ng/ul)	1st Repetition	2nd Repetition	1st Repetition	2nd Repetition
621 D1	11.4	0	18	19	18
621 D3	0.458	19	19	19	17
621 D4	0.0915	15	11	13	16
621 D5	0.0183	6	1	2	2

Reproducibility of UrsePlex13 Assay Cont.

		Obtained Alleles: Analyst 1		Obtained Alleles: Analyst 2	
Sample	Concentration (ng/ul)	1st Repetition	2nd Repetition	1st Repetition	2nd Repetition
1004 D1	7.64	19	19	19	19
1004 D3	0.306	18	14	18	17
1004 D4	0.0611	0	1	7	3
1004 D5	0.0122	0	0	1	0
1073 D1	2.5	19	20	20	20
1073 D3	0.1	0	0	0	0
1073 D4	0.02	2	3	7	1
1073 D5	0.004	0	0	0	0
1088 D1	3.68	18	18	18	18
1088 D3	0.147	0	0	0	0
1088 D4	0.0294	5	4	7	2
1088 D5	0.00589	1	0	0	0

Table 1. Concentration of each sample dilution with obtained alleles for each analyst and repetition

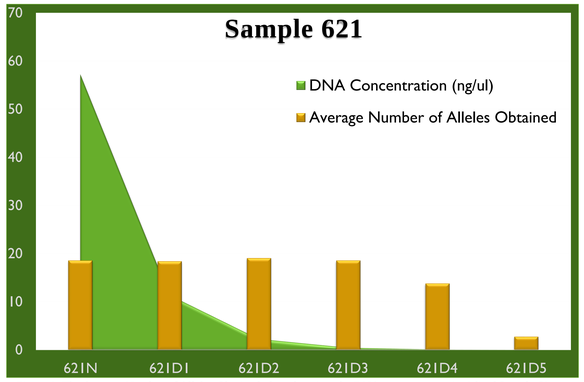

Figure 2. The Average Number of Alleles Obtained plotted against the DNA concentration (ng/ul) of Sample 621

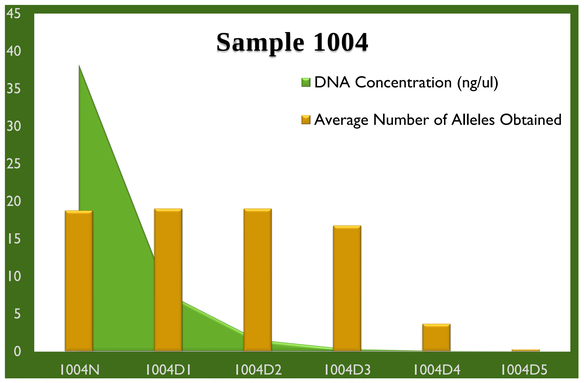

Figure 3. The Average Number of Alleles Obtained plotted against the DNA concentration (ng/ul) of Sample 1004

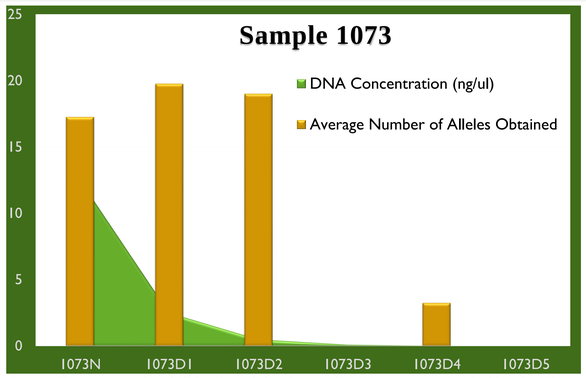

Figure 4. The Average Number of Alleles Obtained plotted against the DNA concentration (ng/ul) of Sample 1073

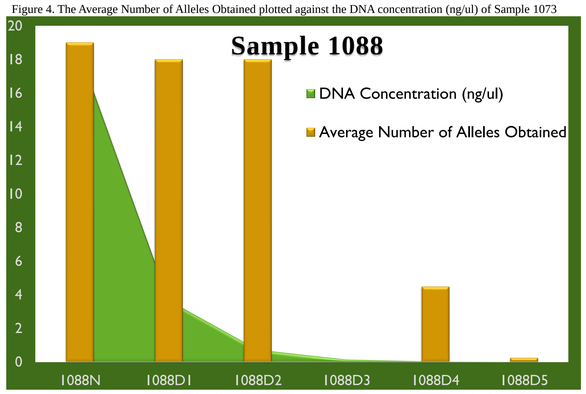

Figure 5. The Average Number of Alleles Obtained plotted against the DNA concentration (ng/ul) of Sample 1088

Conclusion

Four samples were chosen to represent the results of the internal validation of the sensitivity and reproducibility of the UrsaPlex assay. For reproducibility, the same or a similar number of alleles were obtained for each sample by different analysts when the concentration of the sample was ≥0.5 ng/ul as depicted in Table 1. This trend occurs in the sensitivity study as well, in which the number of alleles obtained reduces when the concentration of the sample is less than 0.5 ng/ul as seen in Figures 2-5. This suggests that the UrsaPlex assay can provide consistent allele calls for non-degraded samples that have ≥0.5 ng/ul of DNA. Samples 1088 and 1073 showed failure to yield allele calls in their third serial dilutions (Table 1). Since this remained consistent across all four runs, this could be due to contamination disrupting the PCR or an issue with that specific dilution in the dilution series. Testing should be performed to check for contamination in those samples and for machine failure. The future direction of this project would be to continue the internal validation with non-probative DNA samples as well as a study on contamination to see whether the assay cross-amplifies human DNA.

References

Bidon, T., et al. (2013). A sensitive and specific multiplex PCR approach for sex identification of ursine and tremarctine bears suitable for non-invasive samples. Molecular Ecology Resources., 13, 362-368.
Bombieri, G., et al. (2019) Brown bear attacks on humans: a worldwide perspective. Scientific Reports, 9, 8573.
Martins, C., et al. (2018). Internal validation of two new retrotransposons-based kits (InnoQuant® HY and InnoTyper® 21) at a forensic lab. Forensic Science International, 283, 1-8.
Meredith, E. P., et al. (2020). UrsaPlex: An STR multiplex for forensic identification of North American black bear (Ursus americanus). Forensic Science International: Genetics, 102161.

Acknowledgements

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