
















pooledpeaks: Open-Source Fragment Scoring and Analysis Workflow for Population Genetic Analyses of Microsatellite Markers in Pooled Samples

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


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Summary

Microsatellite markers are short, highly variable, multi-repeat DNA sequences (aka short tandem repeats) that appear throughout the genome and can be used to estimate population genetic metrics ([Silva et al., 2006](#); [Vieira et al., 2016](#)). These markers are frequently evaluated using fragment analysis which is based on Sanger sequencing. The pooledpeaks R package provides tools to analyze fragment analysis results (.fsa files). It provides functions that fall in three subcategories: 1) peak scoring, 2) data manipulation, and 3) genetic analysis. The package was designed for the use of microsatellite markers on pooled parasite samples, but the peak scoring functions are applicable to any fragment analysis. The peak scoring functions were partially adapted from Fragman, a package designed to score microsatellite markers in cranberries ([Covarrubias-Pazaran et al., 2016](#)). Although Fragman works for the older file version, newer versions cannot be read. In addition to revising this outdated function, we also added features including expanded scoring parameter options and exporting resulting scoring plots as a pdf file for review. The data manipulation functions were created to clean and format the data from the called peaks and transform them into allele frequencies. These frequencies can then be input into the genetic analysis functions for calculation of diversity and differentiation measures adapted from a range of papers ([Chao et al., 2008](#); [Foulley & Ollivier, 2006](#); [Jost, 2008](#); [Long et al., 2022](#); [Nei, 1973](#)). An in-depth walk-through of how to use the analysis pipeline can be found in the vignette.

Statement of Need

While a plethora of methods exist for downstream statistical analysis of allele frequencies, processing raw fragment data is limited by available software. Of the limited software that can read the .fsa binary raw data file format, nearly all require purchase or registration, are primarily built for Windows, are inefficient for analyzing large batches of files, and are highly dependent on individual researcher experience. Additionally, a previous R package allowing for

the analysis of .fsa files is incompatible with the updated file version. When using fragment analysis for microsatellite markers on pooled samples, once the raw data is extracted and scored, it must be cleaned and transformed into allele frequencies using a second software, such as Excel, which is limited in its capacity for automation and version control. Another platform shift is often required to analyze the resulting allele frequencies. These factors highlight the need for a comprehensive scoring and analysis pipeline that is open-source, offline, reproducible, consistent between researchers, and that does not require platform switching between steps.

Ongoing Research Projects

This package is currently being used to analyze genetic clustering of *Schistosoma mansoni* pooled egg samples from four Brazilian communities, as well as the relatedness of *Schistosoma haematobium* populations around Lac de Guiers in Senegal and from Gabon.

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