Nanopore Multiplex Amplicon Sequencing (NMAS-Seq) Method to Investigate Hybridization in *Schistosoma haematobium*

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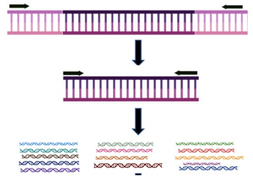
Introduction

Reports of ongoing hybridization between the human (*S. haematobium*) and bovine (*S. bovis*) parasite in many African countries [1] is thought to be associated with the sharing of water bodies by both livestock and humans. The suggestion that a species complex exists within the *S. haematobium* group of schistosomes may influence transmission potential [2]. Rapid, cost-effective, field-deployable genotyping methodologies are needed to detect the genotypes of circulating species in endemic sites. Therefore, we developed a Nanopore multiplex amplicon sequencing (NMAS-Seq) platform using 12 genetic markers of varying phylogenetic strength to generate high-resolution data for investigating the occurrence of *Schistosoma* hybrids in Nigeria. Our approach identified that all 95 schistosome isolates from 4 sampling sites resolved as hybrids.

Methods

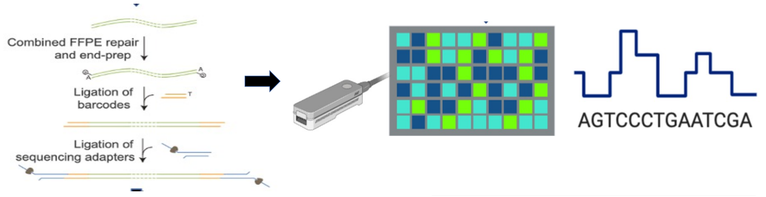
Field Survey

Nested PCR

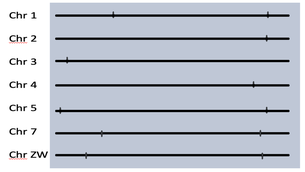


N (Amplicons) = 1344

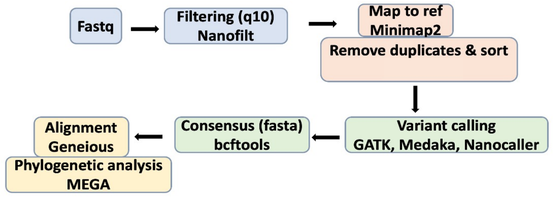
Library prep and Multiplex amplicon sequencing



Chromosomal location of markers



Bioinformatic analysis



Sequencing stats

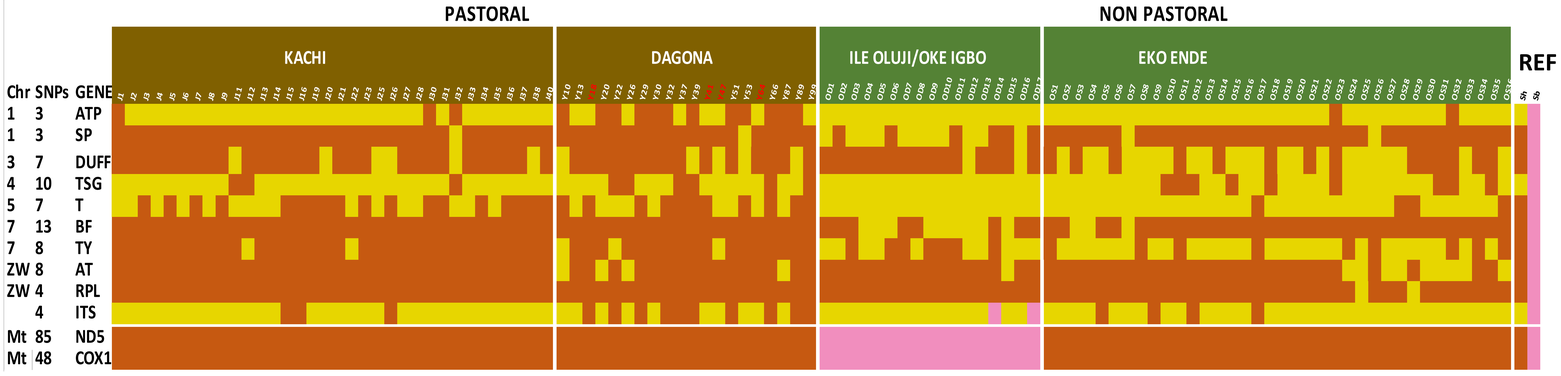
Number of reads	
Total	362071
Pass (qscore ≥ 10)	187295
Reads mapped to ref	122453
Coverage	
Mean Depth	22
Average coverage	54X

Discussion

- We demonstrated the applicability of a low-cost, adaptable and field-deployable minION sequencing technique for genotyping schistosomes
- Based on the mixed genetic profiles of ten nuclear markers, the sampled populations are *S. haematobium* with different levels of admixed ancestry from *S. bovis* [3]
- In all but one sampling sites, the mitochondrial profile were *S. haematobium* (Fig.1). In Ile Oluji/Oke Igbo), an agrarian community with no cultural practice of human and animal sharing water bodies, the mitochondrial profile was *S. bovis*
- Our data suggest that admixed *S. haematobium* is widespread in Nigeria irrespective of human/cattle contact in waterbodies
- The NMAS-Seq technique was 70% less expensive than Sanger sequencing
- We propose this multilocus typing scheme as a cost-effective, high fidelity alternative to study genetic diversity between *S. haematobium*, *S. bovis*, and hybrids

Results

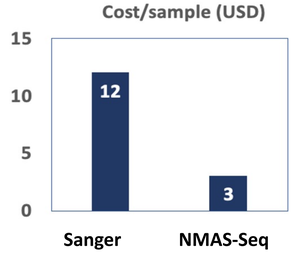
Figure 1: NMAS-Seq identified varying levels of genetic admixture across the study sites



n= 119. Dagona (Yobe) = 25; Kachi (Jigawa) = 40; Ile Oluji/Okeigbo (Ondo) =17; Eko Ende (Osun) =37

S. haematobium *S. bovis* *S. haematobium x S. bovis admixture*

Figure 2: Cost comparison between NMAS-Seq and Sanger



Literature cited

1. Rey et al (2021). https://doi.org/10.1016/j.meegid.2021.104727
2. Borlase et al (2021) https://doi.org/10.1073/pnas.2110711118
3. Kincaid-Smith et al (2021). https://doi.org/10.1371/journal.pntd.0010062

Acknowledgements and Funding

Funding: International Foundation of Science (IFS) grant I3-B-6522-1 and the Intramural Research Program at the NIH

Further information

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