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SNP marker panels for parentage assignment and traceability in the Florida bass (*Micropterus floridanus*)

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ABSTRACT

The Florida bass (*Micropterus floridanus*) is a species endemic to peninsular Florida that is held in high esteem by bass anglers for its tendency to attain a larger maximum size and aggressiveness relative to that of its sister taxon, the Northern largemouth bass, *Micropterus salmoides*. Hatchery rearing and stocking of Florida bass outside of their native range are commonplace, particularly in the southern United States. In many cases, however, there has been minimal assessment of the persistence and success of these fish. Genetic markers are an important tool for tagging and tracing the contributions of particular lines and crosses of fish. Single nucleotide polymorphism (SNP) markers, in particular, can provide rapid and affordable genotyping of large numbers of fish. In the present study, we generated 58,450 genome-wide SNPs and population-level genotypes for Florida bass using a cost-effective genotyping-by-sequencing method. A total of 58 SNPs were shown to assign parents to offspring with 100% accuracy, irrespective of sex and with the presence of full-sib relationships. Depending on the population, sex information, and genetic relationships between parents, we also demonstrated that smaller SNP subsets may be sufficient for parentage assignment. The accuracy and assignment power of the SNP panels were found to compare favorably to those of 10 microsatellites genotyped on the same parents and progeny. This study demonstrated the utility of simple and low-cost GBS techniques for SNP discovery and the relatively small number of variable SNPs needed for accurate parentage assignment in Florida bass. The SNP resources created in this study should facilitate parentage-based research and breeding, genetic tagging, and conservation of Florida bass.

1. Introduction

The artificial propagation of aquatic species and subsequent release into natural environments, also known as stock enhancement, has been a widely utilized and frequently criticized approach in conservation and supplementation efforts (Allendorf and Ryman, 1987; Bert et al., 2007; Maceina and Murphy, 1992; Sekino et al., 2005; Waples and Drake, 2004). Additional forms of stocking involve introducing non-native species (e.g. Florida bass *Micropterus floridanus*) to enhance specific fisheries attributes (e.g. growth; Buynak and Mitchell, 1999; Buynak et al., 1999b). One of the challenges in stock enhancement is to maintain pedigree information for hatchery brood individuals. Reliable pedigree information allows fisheries managers to track ecological or life-history characteristics of released fish, to estimate genetic parameters and breeding values, and to minimize inbreeding in broodstocks (Bert et al., 2007; Liu et al., 2016). The predominant approaches for pedigree development and hatchery stocks evaluation in aquaculture involve the use of physical tags to determine the origin and age of recaptured fish (Bergman et al., 1992; Steele et al., 2013). However, there are known drawbacks to these traditional tagging techniques including tissue damage, decreased swimming capacity, premature tag loss, and risk related to juvenile handling vulnerability (Jepsen et al., 2015). Therefore, alternative tagging techniques are needed. One emerging technology is the use of parentage-based tagging (PBT), a genetic-based tagging method, to create a database of parental genotypes from hatcheries and later assign each progeny back to their parents, thereby

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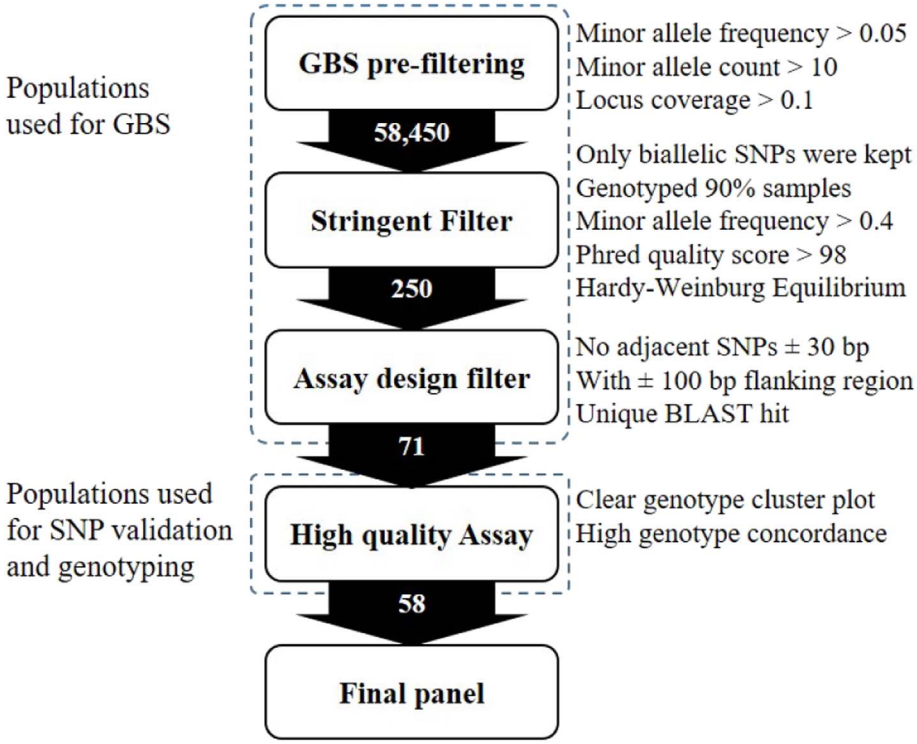

Fig. 1. Workflow outlining the steps used in marker identification and selection of SNP panels for parentage. The remaining number of SNPs after each step are listed between the boxes. Details of filtering and populations used for data mining are given adjacent to each box.

reconstructing the pedigree and identifying the origin and brood year for each sampled offspring (Steele et al., 2013). The implementation of large-scale PBT project in steelhead (*Oncorhynchus mykiss*) has demonstrated the feasibility of this method in building parent-offspring relationship with a number of advantages such as low cost and higher tagging rates compared with traditional tagging methods (Steele et al., 2013).

Advances in molecular technologies have allowed scientists to develop DNA markers that are polymorphic and robust for parentage analysis. In aquaculture, parentage assignment studies came of age in the 1990s with the advent of microsatellite markers (Estoup et al., 1998; Herbinger et al., 1995; Vandeputte and Haffray, 2014). However, this parentage assignment approach has frequently encountered issues associated with genotyping error, null alleles, and mutations that limit its resolving power (Ball et al., 2010; Kalinowski et al., 2007). Single nucleotide polymorphisms (SNPs) are codominant, biallelic molecular markers valued for their genome-wide distribution, abundance, ease of multiplexing and low genotyping error rate for high-throughput analyses (Pritchard et al., 2012; Slate et al., 2009). SNPs are rapidly replacing microsatellites in parentage studies as the development of SNPs is more efficient and less expensive for nonmodel aquatic species (Hauser et al., 2011; Hess et al., 2015; Jin et al., 2014; Liu et al., 2016; Steele et al., 2013). Additionally, with advances in SNP genotyping approaches, SNPs are expected to become one of the major marker systems for routine parentage analysis in a variety of aquatic species (Yue and Xia, 2014). Given that reference genomes are currently available for only a limited number of taxa, genotyping-by-sequencing (GBS) data is proposed as one of the best options for cost-effective SNP discovery and subsequent parentage studies (Kaiser et al., 2016). GBS is a simple, reproducible, highly multiplexed approach that was originally developed for SNP identification and genotyping in crop genomes and populations (Elshire et al., 2011). GBS has been increasingly used for genetic and genomic research in nonmodel organisms, such as linkage map construction (Bielenberg et al., 2015; Uncu et al., 2016), marker-assisted selection (MAS) (Kim et al., 2016), trait mapping (Liu et al., 2014), and estimating genetic diversity (Peterson et al., 2014).

The Florida bass (*Micropterus floridanus*) is a highly prized sportfish native to peninsular Florida that attains larger maximum sizes relative to its sister taxon, the Northern largemouth bass, *Micropterus salmoides* (Barthel et al., 2010). The distribution of Florida bass overlaps with that of the Northern largemouth bass forming a natural hybrid zone in the southeastern US; however, the scope of this introgression has been expanded dramatically via stocking efforts (Barthel et al., 2010; Johnson and Fulton, 1999; Li et al., 2015; Philipp et al., 1983). Although Florida bass have been extensively studied from a stocking and management perspective, genetic efforts have mainly focused on the development of markers capable of assessing population structure and/or Florida/Northern ancestry (Li et al., 2015; Maceina et al., 1988; Philipp et al., 1983; Seyoum et al., 2013) and extralimital introduction (Hargrove et al., 2017). Relatively little attention has been paid to developing tagging methods for pedigree development in this species. Recently, microsatellites have been used for parentage assignment in *M. floridanus* to investigate the mating patterns in hatchery environments (Austin et al., 2012; Hargrove and Austin, 2017). However, the assignment power and minimum number of microsatellite markers needed to accurately conduct parentage analysis in Florida bass groups were not estimated. Thus, the goal of the present study was to develop SNP markers suitable for Florida bass parentage analysis through GBS followed by validation and panel creation using Agena MassARRAY technology. Further, we evaluated the accuracy of SNP markers in parentage analysis relative to previously utilized microsatellite markers. And lastly, we compared the performance of multiple parentage programs (Cervus and SNPPIT; Kalinowski et al., 2007; Anderson, 2010). Our comprehensive assessment should provide valuable information for investigators developing SNPs via GBS for nonmodel organisms, and the SNP resources reported here should be of high utility in pedigree tracing of hatchery-reared Florida bass used for stock enhancement or a genetic selection program.

2. Material and methods

The workflow outlining the steps used in marker identification and selection of SNP panels for parentage is summarized in Fig. 1.