**Black Bass Strain ID via Single Nucleotide Polymorphism (SNP) Genetic Sampling**

**Scope:**

To provide SNP Strain ID analysis services to the Louisiana Department of Wildlife and Fisheries (LDWF) on fin tissue samples of *Micropterus salmoides/nigricans* to include DNA extraction from tissue samples, low density genotyping, and “strain” ID analysis (% Florida vs Northern).

**Requirements:**

* Samples shall be screened for bi-allelic variation across an established panel of 35 diagnostic SNP loci for *Micropterus salmoides/nigricans* according to established protocol (see below).
* Each shipment of samples will require a report of the results. The report, when complete, can be submitted to the District Biologist Manager who collected the data, but must also be sent to our state Black Bass Coordinator, Zane Leblanc, zleblanc@wlf.la.gov, and must at a minimum, include the following:
  + Report date
  + Report number
  + Sample receipt date
  + Number of samples received
  + Methods
  + Brief description of results
  + Structure Bar plot describing results for each population submitted in the sample
  + A digital Excel spreadsheet with detailed “strain” assignment results. The Excel spreadsheet columns should include sample number or ID, M.salmoides/nigricans percentages, “strain” assignment, number of SNPs genotyped, population, and any notes.
* LDWF will supply a schedule annually each October (see below) with batch sizes and expected delivery dates. Any changes to the schedule will be brought to the attention of the vendor point of contact as soon as possible for scheduling.
* Samples will be submitted as preserved tissues in 70% ETOH in either sample collection tubes or 96-well plates.
* Shipping will be paid separately per batch by LDWF. LDWF staff will notify vendor with each batch if they wish to have the samples returned or not at LDWF’s expense.
* Invoices should accompany reports for each batch of samples.
* LDWF will retain ownership of all samples, raw data and testing reports.
* The number of samples will vary annually but will likely be approximately 1,800 samples.
* Pricing for testing should be per sample for approximately 1,800 samples per year.
* Sample batches should be completed and invoiced within 120 calendar days of receipt.

**SNP Testing Criteria**

The reference samples must include an adequate number of specimens (at least 30 individuals from each subspecies) that originated from appropriate waterbodies within the subspecies ranges.

* A minimum of 30 SNPs genotyped in the test individual.
* Bayesian analysis utilized by the program STRUCTURE is required to produce the Florida largemouth bass ancestry assignment.

Analysis criteria are below:

* -Note that individual fish to have at least 98.5% Florida largemouth bass alleles/ancestry in order to be considered a pure Florida largemouth bass.

|  |  |  |
| --- | --- | --- |
|  |  |  |
| **Reference Populations (previously genotyped)** | **Qty** | **Unit** |
| NLMB | 30 | Sample |
| SFLB | 30 | Sample |
| SFLB | 30 | Sample |
|  |  |  |
| **Number of SNPs Genotyped** | 35 | Each |
| **Minimum allowed for assignment analyses** | 30 | Each |
|  |  |  |
|  |  |  |
| **Length of Burn-in Period** | 300000 | Iterations |
| **Number of MCMC Reps after Burn-in:** | 200000 | Replicates |
| **Inital ALPHA** | 1 | N/A |
| **ALPHAMAX** | 10 | N/A |
| **ALPHAPROPSD** | 0.025 | N/A |
| **Set K** | W | N/A |
| **Number of Iterations** | 3 | Each |
|  |  |  |
|  |  |  |
| **Assignment to SFLB** | The sample average (3 iterations) to SFLB > 0.9845 | Q-value |

2025 Louisiana Department of Wildlife & Fisheries Largemouth Bass

**Genetic Sampling Plan/Schedule**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | |  |  |
|  | |  |  |  |
| District | Waterbody | | Number | Season |
| 1 | Lake Bistineau | | 120 | Fall |
| 2 | Poverty Point Reservoir | | 120 | Spring |
| 2 | Bussey Brake | | 120 | Fall |
| 3 | Black River/Cocodrie | | 120 | Spring |
| 5 | Vernon Lake | | 120 | Spring |
| 6 | Crooked Creek | | 120 | Spring |
| 8 | Bayou Bienvenue | | 120 | Spring |
| 9 | Atchafalaya River Basin | | 120 | Fall |
| 10 | Grand Bayou Reservoir | | 60 | Spring |
| 10 | Sibley Lake | | 120 | Spring |
|  | |  |  |  |
|  | |  |  |  |
|  | |  |  |  |
|  | |  |  |  |
| **Spring** | | **780** |  |  |
| **Fall** | | **360** |  |  |
| **Total** | | **1,140** |  |  |
|  | |  |  |  |

Notes:

1. Plan subject to changes as other needs arise.
2. Plan does not include sampling conducted by LDWF and submitted to testing lab by LDWF, but paid for by other entities.