

Procedures for Collecting Blood Samples from Wild Horses and Burros for Genetic Analysis

During the next four to five years all HMA's will be gathered and data will be collected on Wild Horse and Burro populations to facilitate preparation of Population Management Plans (PMP's). A portion of this process involves the collection of blood samples for genetic baseline data. This attachment outlines procedures to be used for collecting blood samples for genetic analysis.

Blood samples for establishing baseline genetic data should be collected from horses and burros in each HMA as gathers are accomplished. These samples should be collected from release horses/burros except where gate cut gathers are used. They will be collected by breeding population. If the breeding populations are not clearly defined, then samples from adjacent herds should be kept separate for initial analysis. The geneticist can answer the question of interbreeding between the populations. This testing should be accomplished and results compared prior to making decisions on combining HMA's for management purposes. Mixing samples from non-interbreeding herds can give misleading estimates of genetic variation.

Minimum sample size is 25 per cent of AML or a minimum of 25 samples and not more than 100 per population. A sample is defined as the collective blood for an individual animal. (i.e., two tubes for horses-one tube for burros). Blood should be drawn from both mares and studs in a ratio similar to the sex ratio released, or in the case of partial gathers the estimated sex ratio. Animals of any age class may be sampled.

The test will consist of looking at 29 systems (17 typing and 12 DNA). For burros, only the DNA will be analyzed because some of the typing tests are species specific to horses. The data will be compared to similar data from both domestic and other wild horse/burro populations. The primary value of this initial data is a baseline against which future samples can be compared to identify genetic drift and any narrowing of diversity through inbreeding. In the short term diversity can be determined, herds may be separated or combined for management based on the data, rare alleles identified and a determination of founders (historical origin of herd). A sample of DNA will be preserved (frozen) for each horse tested. A report on the analysis will be provided by Dr. Gus Cothran of the University of Kentucky.

Procedures:

Two tubes of blood need to be collected from each horse (One red top and one yellow top vacutainer of blood). One sample needs to be collected from each burro (One yellow top vacutainer of blood-only DNA is analyzed for burros). The red is the clot sample and the yellow contains an anticoagulant and needs to be inverted several times to mix the anticoagulant with the blood. The sample needs to be drawn directly into the vacutainer. Training will be provided at the Compliance/Animal Health Course (4700-05) or any veterinarian can draw the sample.

Sometimes an animal is not cooperative and a half tube or less may be obtained. If the tube is much less than ½ full then another sample should be collected or find a more cooperative animal. After the samples have been collected both tubes (in the case of burros-one yellow top tube) should be placed in a zip lock bag along with a data sheet describing as a minimum: 1. Date Collected, 2. HMA Name, 3. Animal Age., Sex, and Color. Forms will be provided with collection supplies.

The bag should be rolled up and placed in the refrigerator pending shipment. DO NOT FREEZE THE SAMPLES. In the field the samples need to be kept cool but not frozen. If it is warm at the collection site, a small cooler with ice or re-freezable blue ice should be used to cool the samples. Be sure and place newspaper between the ice and samples so a sample does not freeze. After collection put the samples in a refrigerator pending shipment. The orientation of the tubes in storage is not critical (i.e. do not have to stand them up). Some individuals prefer to identify the samples by grease marking the tubes then providing a master list. That is also ok. However, be sure the tubes are in plastic bags so that if one breaks it does not obliterate the markings on other tubes.

Samples should be packed in Styrofoam coolers with blue ice and appropriate packaging material for shipment (the coolers vaccine comes in work great). The samples should be shipped overnight mail to arrive during working hours Monday/Friday. Do not ship so they arrive on the weekend.

Ship Samples To:

CSU Diagnostic Laboratory
BLM Research Project
300 West Drake Road
Fort Collins, CO 80526

ATTN: Dr. Elizabeth Mumford
Tel# 970-491-1850

You should call Dr. Mumford and notify her that you are sending samples from _____ HMA, approximate number of samples to be mailed and the anticipated date of arrival. Collection plans should provide for shipment and receipt of samples by Dr. Mumford within three weeks of collection or earlier. Dr. Mumford will bank some of the serum for future studies and will then forward the samples to Dr. Gus Cothran of the University of Kentucky for typing and genetic analysis. ALL SAMPLES COLLECTED FOR GENETICS WILL BE HANDLED THIS WAY. A report will be generated by Dr. Cothran that will detail procedures, findings and recommendations.

The Washington Office Operations Staff for the Wild Horse and Burro Program in Reno, Nevada has supplies on hand and will provide them on request. Contact Ron Hall at 775-861-6623.

Attachment 2-2

Samples will be collected on the first gather of an HMA after October 1, 2001, unless an area has been adequately sampled (25 per cent of AML-no less than 25 samples) within the last 10 years. If an HMA has been adequately sampled over 5 years ago a 10% sample size should be collected and compared to earlier data.

Data will be collected during scheduled gathers so budget impacts will be limited to collection and analysis costs. The cost for the analysis, handling and the report is \$30.00 per sample. The cost will be covered by WO-260.