Supplemental Information

Fecal DNA Extraction Method

My DNA extraction method is a variant of a previously published method (Wehausen et al. 2004). That method used only carefully scraped material from the very outer layer of dry fecal pellets in a Qiagen QIAamp DNA Stool Mini Kit DNA extraction. I have since found that the following adaptation of the AquaGenomic Stool and Soil Protocol (MultiTarget Pharmaceuticals, Colorado Springs, CO, USA) yields notably better DNA, as evidenced by downstream PCR results. It uses 30 mg of fecal scrapings in a 2 ml screwtop microcentrifuge tube to which about 2–3 mm of 1 mm zirconia/silica beads (Biospec Products, Bartlesville, OK, USA) are added followed by 375 uL of AquaGenomic Solution. This is lightly vortexed to get all fecal material into solution and the tube is then incubated at 70C for 5 min followed by intense vortexing in a beadbeater (Biospec Products, Bartlesville, OK, USA) at 4200 rpm for 10 sec. It is then lightly centrifuged to re-establish the liquid solution, followed by light vortexing to release any pelleted fecal material into solution, after which it is incubated another 20 min at 70C. Next it is vortexed for 60 sec followed by centrifuging for seven minutes at ≥ 12,000 xg. Fecal samples provide generally low concentrations of DNA from the target species. I attempt to maximize the amount of DNA isolated by drawing all of the supernatant from the centrifuged sample and transferring it to a new 1.5 ml microcentrifuge tube, which also is centrifuged for seven minutes at ≥ 12,000 xg and the supernatant again transferred to a new 1.5 ml microcentrifuge tube. I have found the amount of supernatant to vary greatly among samples (180–260 uL). This extra centrifugation step removes yet more insoluble fecal material after which the supernatant volume can be measured relative to the next step. A volume of AquaPrecipi Solution (MultiTarget Pharmaceuticals) equal to half the total supernatant volume is added to the transferred supernatant, along with an equal volume of ethanol. The rest of the AquaGenomic Stool and Soil Protocol is then followed. Pigment in the final eluted DNA is not uncommon for this extraction method and, in contrast with the Qiagen QIAamp DNA stool extraction method, I have not found such pigment to be correlated with failed PCRs. The instructions for the AquaGenomic Stool and Soil Protocol suggest adding Proteinase K prior to the initial incubation if mtDNA is sought. While the method for distinguishing mule deer and bighorn sheep fecal samples is based on mtDNA, I have not found the addition of Proteinase K necessary in the above DNA isolation procedure.

Literature Cited

- Wehausen, J. D., R. R. Ramey II, and C. W. Epps. 2004. Experiments in DNA extraction and PCR amplification from bighorn sheep feces: the importance of DNA extraction method. Journal of Heredity 95:503–509.

GenBank Accession Numbers of Dloop Sequences Used for Primer Development

<u>Mule Deer</u>

64 *Odocoileus heminionus hemionus*

Montana: FJ189159, FJ18916, FJ189161, FJ189162, FJ189164, FJ189167, FJ189168, FJ189169, FJ189170, FJ189155

Idaho: FJ189124, FJ189126, FJ189127, FJ189129, FJ189130, FJ189134, FJ189136, FJ189140, FJ189141

Wyoming: FJ189326, FJ189327, FJ189328, FJ189329, FJ189330, FJ189331

Nevada: FJ189198, FJ189201

Utah: FJ189297

New Mexico: KM061096, KM061101

Colorado: FJ189109, FJ189111, FJ189113, FJ189114, FJ189116, FJ189117

British Columbia, Canada: FJ189003, FJ188996, FJ188998

Alberta, Canada: FJ188901, FJ188902, FJ188908, FJ188909, FJ188915, FJ188912, FJ188904, FJ188921, FJ188913, FJ188918, FJ188914, FJ188920, FJ188906, FJ188903

North Dakota: FJ189171, FJ189172, FJ189174

South Dakota: FJ189255

Oregon: FJ189219.1, FJ189223

Washington: FJ189299, FJ189298, FJ189300, FJ189302, FJ189317

22 *O. h. californicus*

California: FJ189062, FJ189052, FJ189026, FJ189054, FJ189047, FJ189077, FJ189059, FJ189030, FJ189081, FJ189079, FJ189075, FJ189080, FJ189078, FJ189024, FJ189076, FJ189049, FJ189048, FJ189027, FJ189022, FJ189046, FJ189028, FJ189023

7 *O. h. inyoensis*

California: FJ189038, FJ189039, FJ189034, FJ189032, FJ189040, FJ189041, FJ189036

21 *O. h. crooki*

Texas: FJ189283, FJ189285

Sonora, Mexico: FJ189270, FJ189266, FJ189269, FJ189268

Chihuahua, Mexico: FJ189094, FJ189090, FJ189095

Coahuila: FJ189122

Arizona: FJ188935, FJ188936, FJ188941, FJ188930, FJ188925, FJ188933, FJ188943, FJ188939, FJ188945, FJ188938, FJ188940

11 *O. h. eremicus*

Arizona: FJ188959, FJ188961, FJ188962, FJ189278

Sonora, Mexico: FJ189278, FJ189271, FJ189274, FJ189275, FJ189276, FJ189280, FJ189281

4 *O. h. peninsulae*

Baja California, Mexico: FJ188975, FJ188978, FJ188973, FJ188973

3 *O. h. fuliginatus*

California: FJ189065, FJ189068, FJ188967

Bighorn Sheep

Desert Bighorn: AY903993, AY903995, AY903996, AY903998, AY903999, AY904001, AY904002, AY904003, AY904004, AY904006, AY904009, AY904011, AY904013, AY904014, AY904016, AY904017, AY116623, AF076913, AF076911, AF076915, AF076912, AF076916, AF076914, KU363655, KU363675, KU363674, KU363639, KU363679, KU363678, KU363673, KU363671, KU363664, KU363646

Sierra Nevada Clade: KU363690, KU363653

Rocky Mountains and Oregon: MK381324, MK381329, MK381326, MK381325, MK381344, MK381336, MK381323, MK381341, MK381338, MK381345, MK381330, MK381328, MK381319, MK381355, MK381353, MK381346, MK381339, MK381334, MK381333, MK381327, KU363685, KU363680, KU363689, KU363688, KU363687, KU363686, AY091486, MH094035, NC015889, JX484768, JX484769, JX484771