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Microsatellite Development Work Order

Please read through the details for each option carefully, fill out the work order form on pages 3 and 4 and mail it with your samples.

Sample specifications:

Option 1 - 4) You need to enclose two samples of high molecular weight DNA (≥ 150 uL of ≥ 50 ng/uL of > 10 kb DNA per sample). Please either include a picture of an aliquot run on a 1% agarose minigel or nanodrop measurements. If you run a gel please provide information about the amount of DNA loaded and the ladder/size standard used.

Option 4) ≥ 24 additional DNA samples suitable for PCR (≥ 150 uL of 20 ng/uL). Please include a picture of aliquots run on a 1% agarose minigel or nanodrop measurements. We screen at least 8 samples across up to 48 loci so we do use a lot of DNA. It is really important to send enough DNA.

Summary of work & limitations:

Option 1) We will digest the DNA, ligate the DNA with linkers, enrich with biotinylated probes, and recover the enriched DNA via PCR. We will send you: a) DNA eluted after enrichment, b) PCR amplicons from the eluted DNA (i.e., recovered DNA), and c) primer so you can recreate fresh amplicons. Generally, at least 2 of the libraries we develop will yield $> 50\%$ of clones with microsatellite repeats. We guarantee that at least one of the 3 libraries will have $> 25\%$ of sequences with microsatellite repeats.

Option 2) In addition to option 1, we will sequence the library using 454 massively parallel sequencing. The 454 sequencing will occur at the University of Georgia's Genomics Facility. The resulting sequences will be analyzed and aligned with bioinformatics tools and then we will identify microsatellite repeats using MsatCommander, and design primers using MsatCommander and Primer3. We guarantee that we will generate ≥ 750 non-redundant sequences and that primers can be designed for ≥ 36 loci. We can not guarantee what proportion of primer pairs designed will be amplifiable in your lab, what proportion will yield scorable results from the genomic DNA of your critters, or what proportion are polymorphic, etc.

Option 3) In addition to option 2, we will order primers for 36 loci. We will attempt PCR optimization for all loci, and screen the successfully optimized & polymorphic loci across 24 individuals (some DNA samples do not amplify at all loci, but we will attempt to amplify all 24). If at least 8 loci are polymorphic we will also co-author (with you) a primer note describing these results including basic statistics (observed and expected heterozygosities, number of alleles at each locus, polymorphic information content) and tests for deviations from Hardy-Weinberg and linkage disequilibrium. We will do our best to identify 8 polymorphic loci, but we cannot guarantee that because some critters simply don't have "normal" levels of polymorphism.

Microsatellite Development Pricing for One or More Species*

Costs are in US dollars and are for each step

| Option | 1 species | 2 at once | 3 at once | 4 at once | 5 at once |
|---|-----------|-----------|-----------|-----------|-----------|
| 1. Enrichment | 1,750 | 3,150 | 4,450 | 5,500 | 6,400 |
| 2. Sequence via 454 and design \geq 36 primer pairs | 3,250 | 5,850 | 8,300 | 10,250 | 11,800 |
| 3. Test 36 primer pairs | 4,250 | 7,650 | 10,800 | 13,400 | 15,500 |
| Total for all 4 options | 9,250 | 16,650 | 23,550 | 29,150 | 33,700 |

*in order to receive the discount for multiple species we must receive the DNA and work order forms for the additional species within one month of receiving DNA from the first species as doing extra species is only a cost savings for us if we do them around the same time.

Work Order Details

How many species would you like us to enrich? _____

Species name(s): _____

Common name(s): _____

How many DNA samples are you sending? _____

Please describe anything in particular (e.g. inbred, highly endangered, not diploid...) about your species we should know?

Please put a check next to which option you would like us to perform.

_____ Option 1: Produce doubly enriched library.

_____ Option 2: Option 1 plus sequence, & design \geq 36 primer pairs

_____ Option 3: Option 2 plus order \geq 36 pairs of primers, test 8 individuals across these loci, and screen an additional 16 individuals across scorable, polymorphic loci.

Based on the option you have selected and how many species you are sending, please refer to the pricing table, determine the total cost, and enter it here:

Projected cost for work: \$ _____

Contact for DNA samples & microsatellite development:

Name: _____

Shipping address: _____

Email: _____

Phone: _____

Fax: _____

Contact for payment issues:

Name: _____

Shipping address: _____

Email: _____

Phone: _____

Fax: _____

Prior to shipping any DNA, please send an email to msatdevo@gmail.com.

US mail & International mail:

Dr. Stacey Lance
Savannah River Ecology Laboratory
PO Drawer E
Aiken, SC 29802

FedEx or UPS (within U.S. only):

Dr. Stacey Lance
Savannah River Ecology Laboratory
Savannah River Site, Building 737-A
Aiken, SC 29808

I _____ [print name & institution] understand and agree to the above terms and conditions, as well as those outlined on the web pages.

_____ [sign & date]