

**PCR primer cocktails for DNAHouse samples\***

Cocktail/Primer name	Primer sequence 5'-3'	Product/primer position (with reference to mouse mitochondrion)	References
<b>C_VF1LFt1-C_VR1LRt1 (COI-2)</b>			
LepF1_t1	TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG	6472-7129	
VF1_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG	6446-6471	1
VF1d_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG	6446-6471	2
VF1i_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCAIAIGAIATIGG	6446-6471	2
LepRI_t1	CAGGAAACAGCTATGACTAAACTCTGGATGTCCAAAAATCA	7155-7130	1
VR1d_t1	CAGGAAACAGCTATGACTAGACTCTGGTGGCCRAARAAYCA	7155-7130	2
VR1_t1	CAGGAAACAGCTATGACTAGACTCTGGTGGCCAAAGAACATCA	7155-7130	3
VR1i_t1	CAGGAAACAGCTATGACTAGACTCTGGTGICCIAAIAICA	7155-7130	2
<b>C_FishF1t1A C_FishR1t1 (COI-3)</b>		6475-7126	
VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	6448-6474	3
FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	6448-6474	3
FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAACATGAGAA	7152-7127	3
FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	7152-7127	4
<b>M13 sequencing primers</b>			
M13F (-21)	TGTAAAACGACGGCCAGT	5	
M13R (-27)	CAGGAAACAGCTATGAC	5	
<b>Folmer primers</b>			
LCO1490	GGTCAACAAATCATAAAGATATTGG	6	
HC02198	TAAACTTCAGGGTGACCAAAAAATCA	6	

\*DNA extraction, PCR amplification, and sequencing were performed at Center for Conservation Genetics, American Museum of Natural History

\*Table adapted from Ivanova et al. 2007

**References**

- 1 Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences, USA, 101, 14812–1481
- 2 Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, 6, 998–1002.
- 3 Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA
- 4 Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544-548.
- 5 Messing J (1983) New M13 vectors for cloning. Methods in Enzymology, 101, 20–78.
- 6 Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Marine Biol Biotechnology 3: 294:299.