

Amplicon Templates Used in Positive Control		
Target Species	Target Gene	Primer Pair
METABARCODING TARGETS (Modified Positive)		
ALL Ranid Frogs	16s	100 + 60
All Salamanders	16s	101 + 61
ALL Bony Fish	16S	63 + 64
All Chondrostei	16S	116 + 117
Phytophthora	CytB	92 + 93
<i>Batrachochytrium dendrobatidis</i>	ITS	94 + 95
ALL Crayfish	16S	77 + 78
Universal Mussel	16S	200+201
MUSSELS		
<i>Obovaria unicolor, Obovaria jacksoniana and Lampsilis ornata</i>	COI	203+204
<i>Obovaria unicolor</i>	ND2	205+206
<i>Obovaria jacksoniana</i>	ND2	207+208
<i>Lampsilis ornata</i>	ND2	209+210
<i>Elliptio arca and Elliptio arctata</i>	COI	211+212
<i>Elliptio arca and Elliptio arctata</i>	ND1	213+214
<i>Pleurobema decisum</i>	COI	215+216
<i>Pleurobema decisum and P. perovatum</i>	ND1	217+218
<i>Plerobema perovatum</i>	COI	219+220
<i>Medionidus acutissimus</i>	ND1	221+222
<i>Medionidus acutissimus</i>	COI	223+224
<i>Hamiota perovalis</i>	COI	225+226
<i>Ligumia recta and Ligumia subrostrata</i>	ND1	227+228
<i>Ligumia recta</i>	COI	229+230
<i>Quadrula asperata and Quadrula verrucosa</i>	ND1	231+232
<i>Fusconaia cerina</i>	COI	233+234
<i>Fusconaia cerina</i>	ND1	235+236
<i>Megalonaias nervosa</i>	ND1	237+238
<i>Obliquaria reflexa</i>	ND1	239+240
FISH		
<i>Cyprinella venusta</i>	COI	241+242
<i>Cyprinella venusta</i>	CytB	243+244
<i>Lepomis cyanellus, Lepomis gulosus, Lepomis macrochirus</i>	COI	245+246+ 247
<i>Ameiurus natalis and Ictalurus punctatus</i> (all three primers run together)	CytB	253+254+255
<i>Noturus munitus</i>	CytB	256+257
<i>Noturus munitus</i>	ND5	258+259
<i>Micropterus salmoides and M. punctulatus</i>	CytB	260+261
<i>Micropterus salmoides and M. punctulatus</i>	COI	262+263
<i>Percina nigrofasciata</i>	CytB	264+265
<i>Percina breviceauda</i>	ND2	268+269
<i>Percina breviceauda</i>	COI	270+271
<i>Cottus carolinae</i>	CytB	273+274
<i>Etheostoma caeruleum</i>	CytB	281+282
<i>Pomoxis annularis</i>	CytB	285+286
<i>Crystallaria asprella</i>	ND2	287+288
<i>Crystallaria asprella</i>	COI	289+290
TURTLES		
<i>Macrochelys temminckii</i>	CytB	291+292
<i>Macrochelys temminckii</i>	COI	293+294
**SE Graptemys and Trachymys Turtles: nigrinoda, gibbonsi, concinna, and scripta elegans	CytB	304+305
<i>Graptemys nigrinoda and G. gibbonsi</i>	COI	295+296
<i>Pseudemys concinna</i>	CytB	299+300
<i>Sternotherus odoratus and S. carinatus</i>	CytB	306+307