

## Supplementary Materials

**Table S1. GenBank (NCBI, Bethesda, MD) samples used for primer-probe development and *in silico* testing of the qPCR assay. For each individual, the quantity of mismatches are provided, for both forward (F) and reverse (R) primers, as well as the number of probe mismatches.**

<i>Primer-probe Development</i>					
Species	GenBank No.	Locality (State)	No. F Primer Mismatches	No. R Primer Mismatches	No. Probe Mismatches
<i>Acris blanchardi</i>	EF988109	Illinois	0	0	0
<i>Acris blanchardi</i>	EF988127	Missouri	0	1	0
<i>Acris blanchardi</i>	EF988141	Oklahoma	0	0	0
<i>Acris blanchardi</i>	EF988144	Mississippi	0	1	0
<i>Anaxyrus americanus</i>	EU938446	Kansas	4	2	2
<i>Lithobates catesbeianus</i>	AY083293	Ohio	6	0	0
<i>Lithobates clamitans</i>	AY083282	Missouri	5	1	1
<i>Pseudacris crucifer</i>	EF988160	Minnesota	4	3	1
<i>Primer-probe Specificity Confirmation</i>					
Species	GenBank No.	Locality (State)	No. F Primer Mismatches	No. R Primer Mismatches	No. Probe Mismatches
<i>Acris blanchardi</i>	EF988097	Iowa	0	0	0
<i>Acris blanchardi</i>	EF988098	Iowa	0	0	0
<i>Acris blanchardi</i>	EF988099	Iowa	0	0	0
<i>Acris blanchardi</i>	EF988100	Iowa	0	0	0
<i>Acris blanchardi</i>	EF988101	Arkansas	0	1	0
<i>Acris blanchardi</i>	EF988102	Arkansas	0	1	0
<i>Acris blanchardi</i>	EF988103	Arkansas	0	1	0
<i>Acris blanchardi</i>	EF988104	Arkansas	0	0	0
<i>Acris blanchardi</i>	EF988105	Arkansas	0	1	0
<i>Acris blanchardi</i>	EF988106	Arkansas	0	0	0
<i>Acris blanchardi</i>	EF988107	Arkansas	0	1	0
<i>Acris blanchardi</i>	EF988108	Illinois	0	0	0
<i>Acris blanchardi</i>	EF988114	Kansas	0	0	0
<i>Acris blanchardi</i>	EF988115	Wisconsin	0	0	1
<i>Acris blanchardi</i>	EF988116	Wisconsin	0	0	0
<i>Acris blanchardi</i>	EF988117	Louisiana	0	0	0
<i>Acris blanchardi</i>	EF988119	Louisiana	0	0	0
<i>Acris blanchardi</i>	EF988120	Texas	0	0	0
<i>Acris blanchardi</i>	EF988121	Texas	0	0	0
<i>Acris blanchardi</i>	EF988122	Texas	0	0	0
<i>Acris blanchardi</i>	EF988133	Kentucky	0	0	0
<i>Acris blanchardi</i>	EF988137	Ohio	0	0	0
<i>Acris blanchardi</i>	EF988138	Minnesota	0	0	0
<i>Acris blanchardi</i>	EF988139	Minnesota	0	0	0
<i>Acris blanchardi</i>	EF988140	Oklahoma	0	0	0
<i>Acris blanchardi</i>	EF988142	Mississippi	0	0	0
<i>Acris blanchardi</i>	EF988143	Mississippi	0	0	0
<i>Acris blanchardi</i>	EF988145	Mississippi	0	0	0
<i>Acris blanchardi</i>	EU835890	---	0	1	0
<i>Acris blanchardi</i>	EU835891	---	0	1	0
<i>Acris blanchardi</i>	EU835892	---	0	1	0
<i>Acris blanchardi</i>	EU835893	---	0	2	0
<i>Acris blanchardi</i>	EU835888	---	0	0	0
<i>Acris blanchardi</i>	EU835889	---	0	1	0
<i>BLAST Confirmation of Primers against Non-target Anurans</i>					
Species	GenBank No.	Locality (State)	No. F Primer Mismatches	No. R Primer Mismatches	No. Probe Mismatches
<i>Hyla cinerea</i>	FJ226874	Georgia	3	3	4
<i>Hyla cinerea</i>	KJ536188	Florida	3	3	4
<i>Lithobates palustris</i>	KX269353	New York	4	3	4
<i>Lithobates sylvaticus</i>	EU203528	---	4	2	3
<i>Lithobates sylvaticus</i>	EU203529	---	4	2	3
<i>Lithobates sylvaticus</i>	EU203530	---	4	1	3
<i>Lithobates sylvaticus</i>	EU203531	---	4	1	3
<i>Lithobates sylvaticus</i>	EU203532	---	3	1	3
<i>Lithobates sylvaticus</i>	EU203533	---	4	1	3

Table S1. Continued.

<i>Lithobates sylvaticus</i>	EU203534	---	5	1	3
<i>Lithobates sylvaticus</i>	EU203535	---	4	1	3
<i>Lithobates sylvaticus</i>	EU203536	---	4	1	3
<i>Lithobates sylvaticus</i>	EU203537	---	4	1	3
<i>Lithobates sylvaticus</i>	EU203538	---	4	1	3
<i>Pseudacris clarkii</i>	KJ536214	Texas	3	1	2
<i>Pseudacris clarkii</i>	KJ536216	Kansas	3	1	2
<i>Pseudacris fouquettei</i>	KJ536226	Louisiana	3	2	2
<i>Pseudacris fouquettei</i>	KJ536227	Arkansas	2	2	2
<i>Pseudacris maculata</i>	EF988161	Minnesota	3	2	2
<i>Pseudacris maculata</i>	KJ536215	Illinois	3	1	2
<i>Pseudacris maculata</i>	KJ536217	Kansas	3	1	2
<i>Pseudacris regilla</i>	AY363181	---	3	0	5
<i>Pseudacris regilla</i>	AY363182	---	3	0	5
<i>Pseudacris regilla</i>	AY363183	---	3	0	6
<i>Pseudacris regilla</i>	AY363184	---	3	0	5
<i>Pseudacris regilla</i>	AY363185	---	3	0	5
<i>Pseudacris regilla</i>	AY363186	---	3	0	5
<i>Pseudacris regilla</i>	AY363187	---	3	0	5
<i>Pseudacris regilla</i>	AY363188	---	3	0	5
<i>Pseudacris regilla</i>	AY363190	---	3	0	5
<i>Pseudacris regilla</i>	AY363191	---	3	0	5
<i>Pseudacris regilla</i>	AY363192	---	3	0	5
<i>Pseudacris regilla</i>	AY363193	---	3	0	5
<i>Pseudacris regilla</i>	AY363194	---	3	0	5
<i>Pseudacris regilla</i>	AY363195	---	3	0	5
<i>Pseudacris regilla</i>	AY363196	---	3	0	5
<i>Pseudacris regilla</i>	AY363197	---	3	0	5
<i>Pseudacris regilla</i>	AY363198	---	3	0	5
<i>Pseudacris regilla</i>	AY363199	---	3	0	5
<i>Pseudacris regilla</i>	AY363200	---	3	0	5
<i>Pseudacris regilla</i>	AY363201	---	3	0	5
<i>Pseudacris regilla</i>	AY363206	---	3	0	4
<i>Pseudacris regilla</i>	AY363207	---	3	0	5
<i>Pseudacris regilla</i>	AY363208	---	3	0	5
<i>Pseudacris regilla</i>	AY363209	---	3	0	5
<i>Pseudacris regilla</i>	AY363210	---	3	0	5
<i>Pseudacris regilla</i>	AY363211	---	3	0	5
<i>Pseudacris regilla</i>	AY363212	---	3	0	5
<i>Pseudacris regilla</i>	AY363213	---	3	0	5
<i>Pseudacris regilla</i>	AY363214	---	3	0	5
<i>Pseudacris regilla</i>	AY363215	---	3	0	5
<i>Pseudacris regilla</i>	AY363216	---	3	0	5
<i>Pseudacris regilla</i>	AY363217	---	3	0	5
<i>Pseudacris regilla</i>	AY363219	---	3	0	5
<i>Pseudacris regilla</i>	DQ195206	California	3	1	3
<i>Pseudacris regilla</i>	KJ536195	Oregon	3	0	5
<i>Pseudacris regilla</i>	KJ536196	Oregon	3	0	5