

Development and validation of environmental DNA assays for the detection of endangered and threatened freshwater mussels

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Online Resource 1: DNA Sequences of Freshwater Mussel Sequences Aligned for Designing TaqMan Assays. GenBank accession, species, type of source sequence, and if the sequence was used in the design of the TaqMan assay (Y=Yes).

Accession	Species	Type	TaqMan Assay		
			Avar12s	ERan16s	ETriCytb
NC_029210.1	<i>Aculamprotula tientsinensis</i>	Genome	Y	Y	Y
NC_037431.1	<i>Alasmidonta heterodon</i>	Genome	Y		
OP497997.1	<i>Alasmidonta varicosa</i>	Isolate	Y		
MG938673.1	<i>Alasmidonta varicosa</i>	Genome	Y		
NC_022803.1	<i>Anodonta anatina</i>	Genome	Y	Y	Y
NC_026674.1	<i>Anodonta arcuiformis</i>	Genome	Y	Y	Y
NC_026792.1	<i>Anodonta euscaphys</i>	Genome	Y	Y	Y
NC_026673.1	<i>Anodonta lucida</i>	Genome	Y	Y	Y
NC_023955.1	<i>Arconaia lanceolata</i>	Genome	Y	Y	Y
NC_012716.1	<i>Cristaria plicata</i>	Genome	Y	Y	
NC_026306.1	<i>Cuneopsis pisciculus</i>	Genome	Y	Y	Y
DQ208545.1	<i>Epioblasma brevidens</i>	Isolate		Y	
DQ208589.1	<i>Epioblasma brevidens</i>	Isolate			Y
OP473572.1	<i>Epioblasma rangiana</i>	Isolate		Y	
DQ208583.1	<i>Epioblasma torulosa rangiana</i>	Isolate			Y
DQ208546.1	<i>Epioblasma triquetra</i>	Isolate		Y	
OP485442.1	<i>Epioblasma triquetra</i>	Isolate			Y
DQ208590	<i>Epioblasma triquetra</i>	Isolate			Y
NC_023806.1	<i>Lamprotula gottschei</i>	Genome	Y	Y	Y
NC_023346.1	<i>Lamprotula leai</i>	Genome	Y	Y	
NC_021404.1	<i>Lamprotula tortuosa</i>	Genome	Y	Y	Y
NC_005335.1	<i>Lampsilis ornata</i>	Genome	Y	Y	Y
NC_026686.1	<i>Lanceolaria grayana</i>	Genome	Y	Y	Y
NC_015481.1	<i>Lasmigona compressa</i>	Genome	Y	Y	Y
NC_029491.1	<i>Lepidodesma languilati</i>	Genome	Y	Y	Y
NC_028522.1	<i>Leptodea leptodon</i>	Genome	Y	Y	Y
NC_013661.1	<i>Pyganodon grandis</i>	Genome	Y	Y	Y
NC_013658.1	<i>Quadrula quadrula</i>	Genome	Y	Y	Y
NC_024943.1	<i>Sinanodonta woodiana</i>	Genome	Y	Y	Y
NC_015483.1	<i>Toxolasma parvus</i>	Genome	Y	Y	Y
NC_033976.1	<i>Unio crassus</i>	Genome	Y	Y	Y
NC_033854.1	<i>Unio delphinus</i>	Genome	Y	Y	Y
NC_026111.1	<i>Unio douglasiae</i>	Genome	Y	Y	Y
NC_015310.1	<i>Unio pictorum</i>	Genome	Y	Y	Y
NC_015479.1	<i>Utterbackia imbecillis</i>	Genome	Y	Y	Y

Online Resource 2: Non-target unionid species present in field validation streams. Species inventories obtained from the Pennsylvania Natural Heritage Program database (<https://www.naturalheritage.state.pa.us/>, accessed 2017) and Selckmann et al. 2018.

French Creek (at Carlton), Pennsylvania, USA <i>Alasmidonta marginata</i> <i>Amblema plicata</i> <i>Anodontoides ferussacianus</i> <i>Cincinnatia integra</i> <i>Cyclonaias tuberculata</i> <i>Epioblasma torulosa rangiana</i> <i>Epioblasma triquetra</i> <i>Fusconaia subrotunda</i> <i>Lampsilis fasciola</i> <i>Lasmigona complanata</i> <i>Lasmigona compressa</i> <i>Leptodea fragilis</i> <i>Ligumia nasuta</i> <i>Pleurobema clava</i> <i>Pleurobema sintoxia</i> <i>Quadrula cylindrica cylindrica</i> <i>Simpsonaias ambigua</i> <i>Toxolasma parvum</i> <i>Villosa fabalis</i> <i>Villosa iris</i>	Pine and Marsh Creeks, Pennsylvania, USA <i>Alasmidonta varicosa</i> <i>Alasmidonta undulata</i> <i>Alasmidonta marginata</i> <i>Elliptio complanata</i> <i>Strophitus undulatus</i> <i>Lasmigona subviridis</i>
Shenango River, Pennsylvania, USA <i>Alasmidonta marginata</i> <i>Amblema plicata</i> <i>Anodontoides ferussacianus</i> <i>Epioblasma torulosa rangiana</i> <i>Epioblasma triquetra</i> <i>Fusconaia subrotunda</i> <i>Lampsilis fasciola</i> <i>Lampsilis radiata</i> <i>Lasmigona compressa</i> <i>Ligumia nasuta</i> <i>Obovaria subrotunda</i> <i>Pleurobema clava</i> <i>Pleurobema cordatum</i> <i>Pleurobema sintoxia</i> <i>Quadrula cylindrica cylindrica</i> <i>Quadrula verrucosa</i> <i>Villosa fabalis</i> <i>Villosa iris</i>	Flat Brook, New Jersey, USA <i>Alasmidonta heterodon</i> <i>Alasmidonta varicosa</i> <i>Alasmidonta undulata</i> <i>Anodonta implicata</i> <i>Elliptio complanata</i> <i>Pyganodon cataraeta</i> <i>Strophitus undulatus</i>
	Cherry Creek, Pennsylvania, USA <i>Elliptio complanata</i> <i>Alasmidonta undulata</i>
	Allegheny River (Hunters Station), Pennsylvania, USA <i>Alasmidonta marginata</i> <i>Amblema plicata</i> <i>Cyclonaias tuberculata</i> <i>Epioblasma torulosa rangiana</i> <i>Fusconaia flava</i> <i>Fusconaia subrotunda</i> <i>Lampsilis fasciola</i> <i>Lasmigona complanata</i> <i>Lasmigona compressa</i> <i>Leptodea fragilis</i> <i>Plethobasus cyphus</i> <i>Pleurobema clava</i> <i>Pleurobema sintoxia</i> <i>Quadrula cylindrica cylindrica</i> <i>Somatogyrus integra</i> <i>Villosa fabalis</i> <i>Villosa iris</i>
Aquashicola Creek and McMichaels Creek, Pennsylvania, USA <i>Elliptio complanata</i>	Potomic River, West Virginia, USA <i>Elliptio complanata</i> <i>Lampsilis sp.</i> <i>Alasmidonta varicosa</i> <i>Strophitus undulates</i>

Online Resource 3: Species in the order Unionida detected by the a.) EcomCOI and b.) Avar12s TaqMan Assays. Both assays were tested for the potential of cross amplification with non-target species in silico using Primer Blast (Ye et al. 2012) against the NCBI database (5/15/2025). Primer Blast specificity stringency included mismatches within the last four base-pairs of the 3' end of a primer or five or more base-pair mismatches overall to unintended targets.

a.)

Species		
<i>Cyclonaias nodulata</i>	<i>Elliptio jayensis</i>	<i>Fusconaia escambia</i>
<i>Elliptio arca</i>	<i>Elliptio mcmichaeli</i>	<i>Fusconaia iheringi</i>
<i>Elliptio arctata</i>	<i>Elliptio nasutilus</i>	<i>Fusconaia lananensis</i>
<i>Elliptio complanata</i>	<i>Elliptio producta</i>	<i>Fusconaia masoni</i>
<i>Elliptio congaraea</i>	<i>Elliptio pullata</i>	<i>Fusconaia mitchelli</i>
<i>Elliptio crassidens</i>	<i>Elliptio purpurella</i>	<i>Fusconaia ozarkensis</i>
<i>Elliptio dariensis</i>	<i>Elliptio roanokensis</i>	<i>Fusconaia subrotunda</i>
<i>Elliptio downiei</i>	<i>Elliptio waccamawensis</i>	<i>Hemistena lata</i>
<i>Elliptio fisheriana</i>	<i>Eurynia dilatata</i>	<i>Megalonaias nervosa</i>
<i>Elliptio fumata</i>	<i>Fusconaia askewi</i>	<i>Plethobasus cooperianus</i>
<i>Elliptio hopetonensis</i>	<i>Fusconaia burkei</i>	<i>Pleurobema cordatum</i>
<i>Elliptio icterina</i>	<i>Fusconaia chunii</i>	<i>Pleurobema barnesiana</i>

b.)

Species (Range)
<i>Acuticosta chinensis</i> (Asia)
<i>Unio elongatulus</i> (Europe)
<i>Unio delphinus</i> (Africa, Europe)
<i>Unio pictorum</i> (Asia, Europe)
<i>Unio terminalis</i> (Europe)

References

Pennsylvania Natural Heritage Program (2017) Species lists. Retrieved November 17, 2017.

<https://www.naturalheritage.state.pa.us/Lists.aspx>

Selckmann GM, Smith Z, Cummins J, Griggs A, Buchanan, C (2018) Biological Surveys of Three Potomac River Mainstem Reaches (2012-2014) with Considerations for Large River Sampling. ICPRB Report 18-2. Interstate Commission on the Potomac River Basin. https://www.potomacriver.org/wp-content/uploads/2018/12/LargeRiver_2012_2014-FInal.pdf

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T (2012) Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 13:134. <https://doi.org/10.1186/1471-2105-13-134>