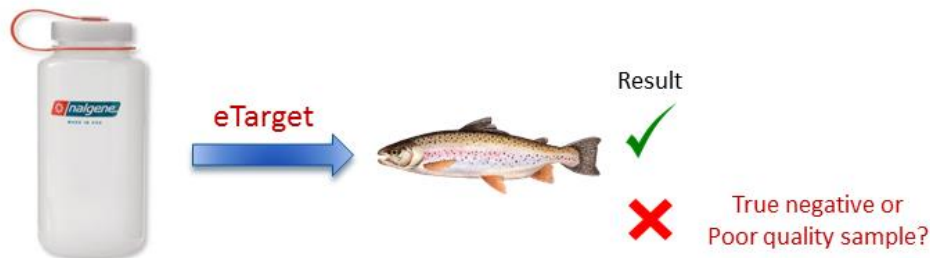


## Environmental DNA (eDNA) testing:

- Cells from the target species are released into the environment
- DNA from these cells can be collected from a water sample and tested for the presence of a target species of interest
- Completely non-invasive

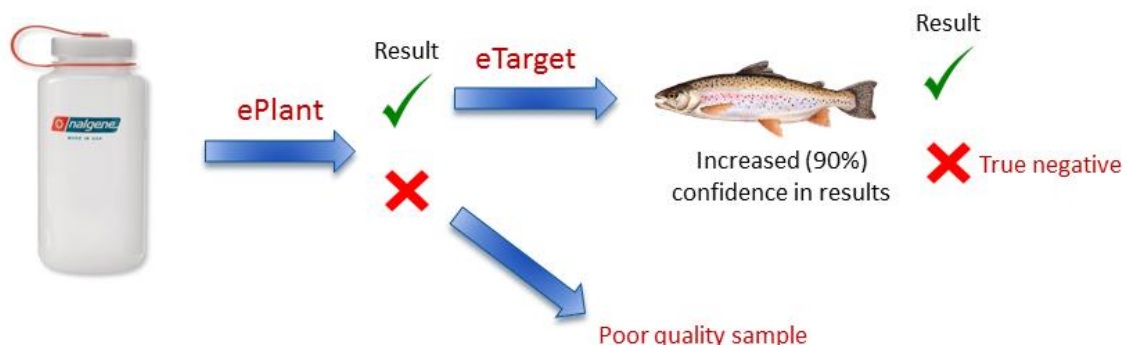
### Problem:

- The eDNA test is completely dependent upon a technique called qPCR
- Test reliability is dependent on appropriate design, validation, and execution
- Current eDNA tests cannot distinguish between a poor quality DNA sample and the actual absence of the target animal species (eTarget)



### Solution:

- We introduced several innovations to eDNA tests to improve reliability
  - Veldhoen et al (2016) Implementation of Novel Design Features for qPCR-Based eDNA Assessment. PLoS ONE 11(11): e0164907. doi:10.1371/journal.pone.0164907
- We created a direct test for DNA quality through an internal DNA amplification control (ePlant) that is always present in a water sample
- A positive ePlant result means a viable sample that can be tested with eTarget
- A negative ePlant result means a poor quality sample



## Available Validated eDNA Tests

Species	Common Name
<i>Ambystoma mavortium</i> *	Western tiger salamander
<i>Ambystoma tigrinum</i> *	Eastern tiger salamander
<i>Anaxyrus (Bufo) boreas</i>	Western toad
<i>Ascaphus montanus</i>	Rocky mountain tailed frog
<i>Ascaphus truei</i>	Pacific (Coastal) tailed frog
<i>Lithobates (Rana) catesbeiana</i>	North American bullfrog
<i>Lithobates (Rana) pipiens</i>	Northern leopard frog
<i>Oncorhynchus clarkii</i>	Cutthroat trout
<i>Oncorhynchus kisutch</i>	Coho salmon
<i>Oncorhynchus mykiss</i>	Rainbow trout
<i>Oncorhynchus nerka</i>	Sockeye salmon
<i>Oncorhynchus tshawytscha</i>	Chinook salmon
<i>Rana aurora</i>	Northern red-legged frog
<i>Rana cascadae</i>	Cascades frog
<i>Rana luteiventris</i>	Columbia spotted frog
<i>Rana pretiosa</i>	Oregon spotted frog
<i>Sorex bendirii</i>	Pacific water shrew
<i>Thymallus arcticus</i>	Arctic grayling
<i>Spea intermontana</i>	Great Basin spadefoot
eFish	Generic fish
eFrog	Generic frog
eMammal	Generic mammal not human

\*Species are indistinguishable in current test

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## **Aquatic Animal Health Monitoring with Minimal Impact**

- Innovations in genomics technology allow us to obtain a snapshot of fish or frog health
- RNA molecules are isolated from animal tissues that indicate which genes are being used
- Some genes are important in normal growth and development and can serve as markers for disease
- Some genes can be activated by stress or pollutants
- A small tissue sample from a fin is enough to evaluate stress or exposure to pollutants

### **Enhanced reliability with the PROAQUA advantage**

- We have developed tests based upon the detection of gene activity important in development, reproduction, and growth
- Test reliability is dependent on appropriate design, validation, and execution and is appropriate for the species of interest
- The tests are very sensitive and can reveal sublethal deleterious effects
- They are more informative than typical toxicity tests that test for mortality or morbidity

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