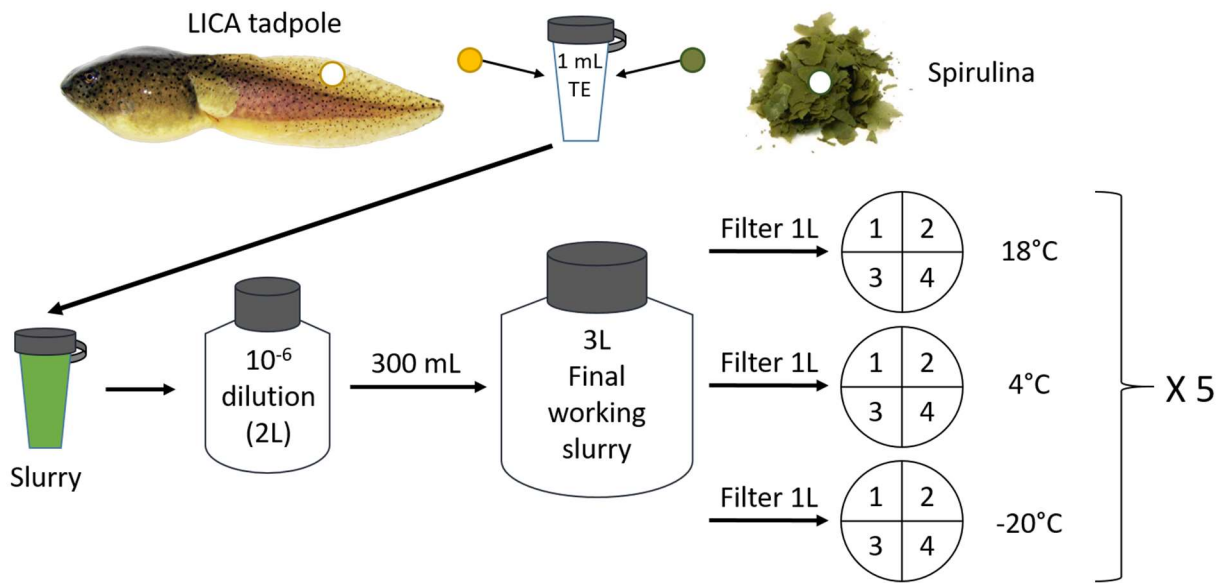
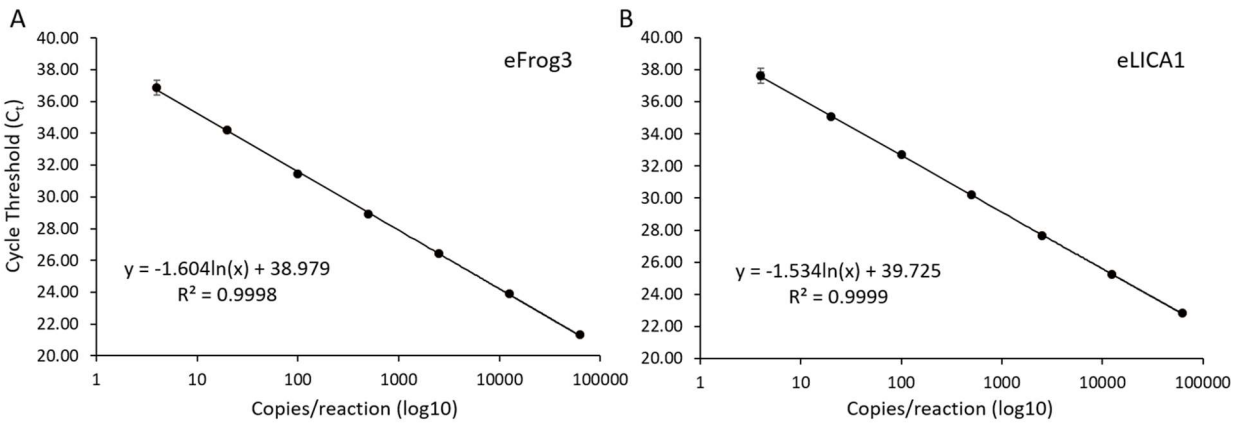
Supplementary Information



Supplementary Figure 1. Schematic of the method for creating a standard DNA slurry. Four millimeter-diameter biopsies of American Bullfrog (LICA) tail fin and a Spirulina flake were added to 1 mL TE buffer pH 8, then homogenized into a slurry. A 10^{-6} dilution using recirculated fresh water from the Aquatics Facility was made which was further diluted 10-fold to the final working slurry. This dilution was determined to be optimal as it was the most dilute slurry to still obtain 100% detections for the target species. One liter final working slurry was filtered and the filter was stored at the indicated temperatures (n=5 per temperature). A quarter of each filter was processed at each of 4 times (1-4) at 1 week, 1 month, 5 months, and 12 months.



Supplementary Figure 2. Standard curves of gBlocks synthetic DNA serial dilution curves for (A) eFrog3 and (B) eLICA1 eDNA assays used to calculate copy number in Figure 3. There is a very strong linear relationship between cycle threshold (C_t) and copies/reaction.