Amphibia-Reptilia

Detection of elusive fire salamander larvae (Salamandra salamandra) in streams via environmental DNA

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Supplementary material

Calculation for creating the standard curve

We followed the manual by Applied Biosystems (http://www6.appliedbiosystems.com/support/tutorials/pdf/quant_pcr.pdf) for creating a standard curve with plasmid DNA templates.

Please note that this formulae is not appropriate for this experimental setup as it does not take the nuclear DNA present in the DNA extracts into consideration. This calculation can be used for samples containing plasmid DNA templates only. Therefore, the concentrations in our results are not comparable to other studies but comparable within the study.

Firstly, calculate the mass of a single plasmid molecule by inserting the fragment size into the following formulae.

(1) $(mass\ of\ plasmid\ plasmid\ (g)) = (fragment\ size\ (bp)) \times (1{,}096 \times 10^{-21})$

Then multiply the resulting mass with the copy number of the target fragment you wish to have in the solution (30-300,000). This gives the mass of plasmid DNA that is needed.

(2) $(mass\ of\ plasmid\ DNA\ needed\ (g)) = (copy\ number\ of\ interest) \times (mass\ of\ single\ plasmid(g))$

To calculate the final concentration of plasmid DNA that is needed to achieve the copy number of interest, divide the mass of plasmid DNA needed by the volume to be pipetted in each reaction.

(3) $(Final\ concentration\ of\ plamsid\ DNA\ \left(\frac{g}{\mu l}\right)) =$

$\left(mass\ of\ plasmid\ DNA\ needed\ (g)\right) \div (DNA\ volume\ in\ qPCR\ (\mu l))$

In the following example, we take the 800bp fragment length of the D-loop and 5µl DNA volume that is used for the qPCR reaction into account:

(1) $(8.768\ \times\ 10^{-19}\text{g}) = (800\ \text{bp}) \times (1.096 \times 10^{-21}\ \frac{\text{g}}{\text{bp}})$

(2) $(2.6\ \times\ 10^{-13}\text{g}) = (300{,}000\ \text{copies})\ \times (8.768\ \times\ 10^{-19}\text{g})$

(3) $\left(5.26 \times\ 10^{-14}\ \frac{\text{g}}{\mu\text{l}}\right) = (2.6\ \times\ 10^{-13}\text{g})\ \div (5\mu\text{l})$

The final concentration of plasmid DNA that contains 300,000 copies of the 800 bp D-loop is $5.26 \times\ 10^{-14}\ \frac{\text{g}}{\mu\text{l}}$.

Table S1. RFU values in percent for primer combinations F7/R13, F7/R2 and F6/R3 with combination F7/R13 as reference (100%).

DNA concentration in DNA copies	RFU value in % with F7/R13 as reference values		
	primers F7/R13	primers F7/R2	primers F6/R3
300,000	100	66.87	42.69
3,000	100	66.55	60.79
30	100	78.67	126.76

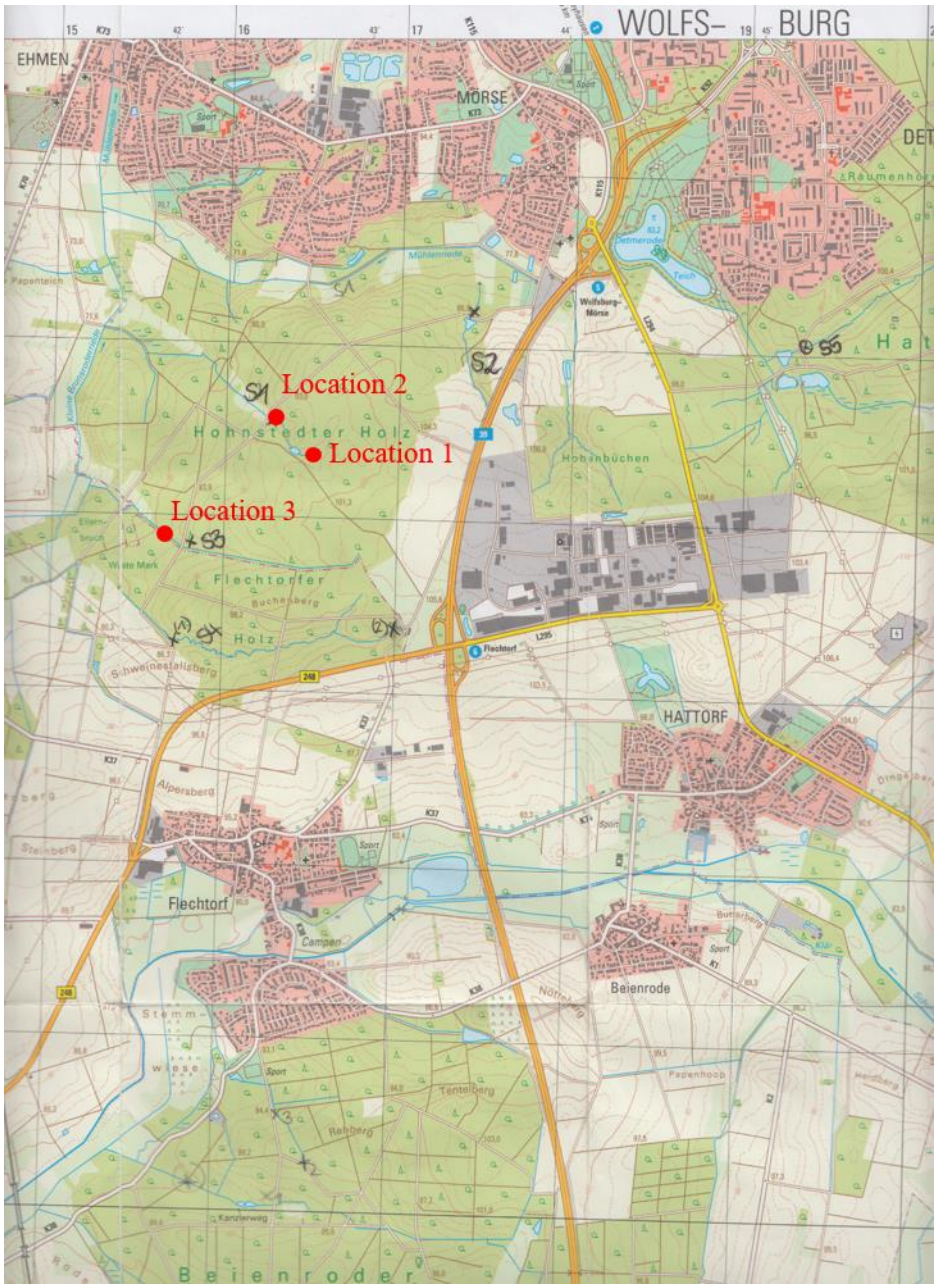

Figure S1. Sampling locations (location 1-3, marked with a red dot) near Wolfsburg.

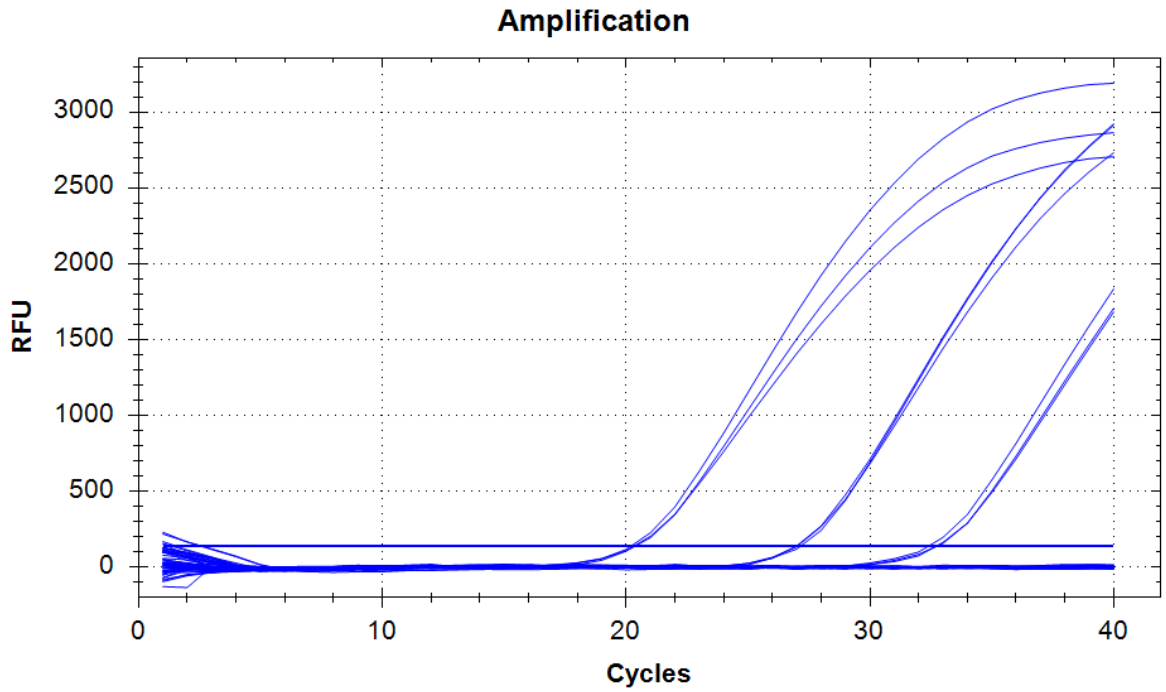

Figure S2. Graphical display of the RT-qPCR for the cross-amplification with primer F7/R13. Only the three replicates of the dilution series (300,000, 3,000 and 30 eDNA copies) of *S. salamandra* were amplified. The PCR replicates of *I. alpestris*, *L. helveticus*, *L. vulgaris* and *T. cristatus* showed no amplification