Genome size estimation in amphibians through Real-Time PCR
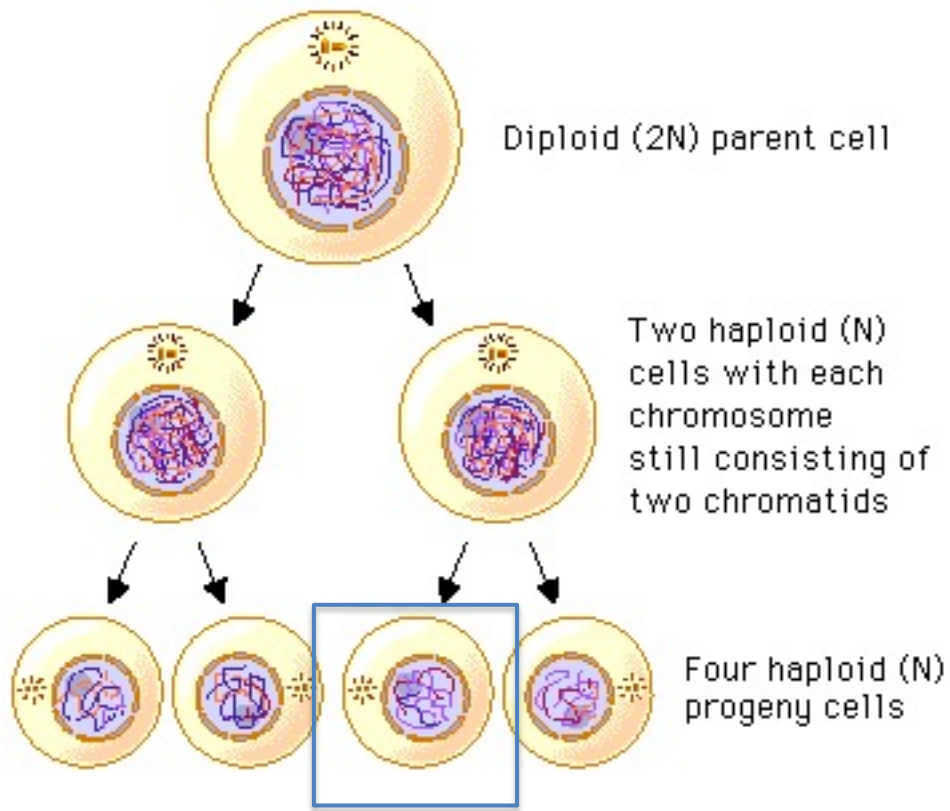
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SEVINOMICS Spring Meeting

Estación Biológica Doñana CSIC

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The Basis of Genome Size

Diploid (2N) parent cell

Two haploid (N) cells with each chromosome still consisting of two chromatids

Four haploid (N) progeny cells

Genome size is the total amount of DNA contained within one copy of a single genome

C-value: mass (in picograms) of DNA per haploid nucleus

Why should anyone care about genome size?

Genome size is a major consideration when choosing targets for complete genome sequencing projects
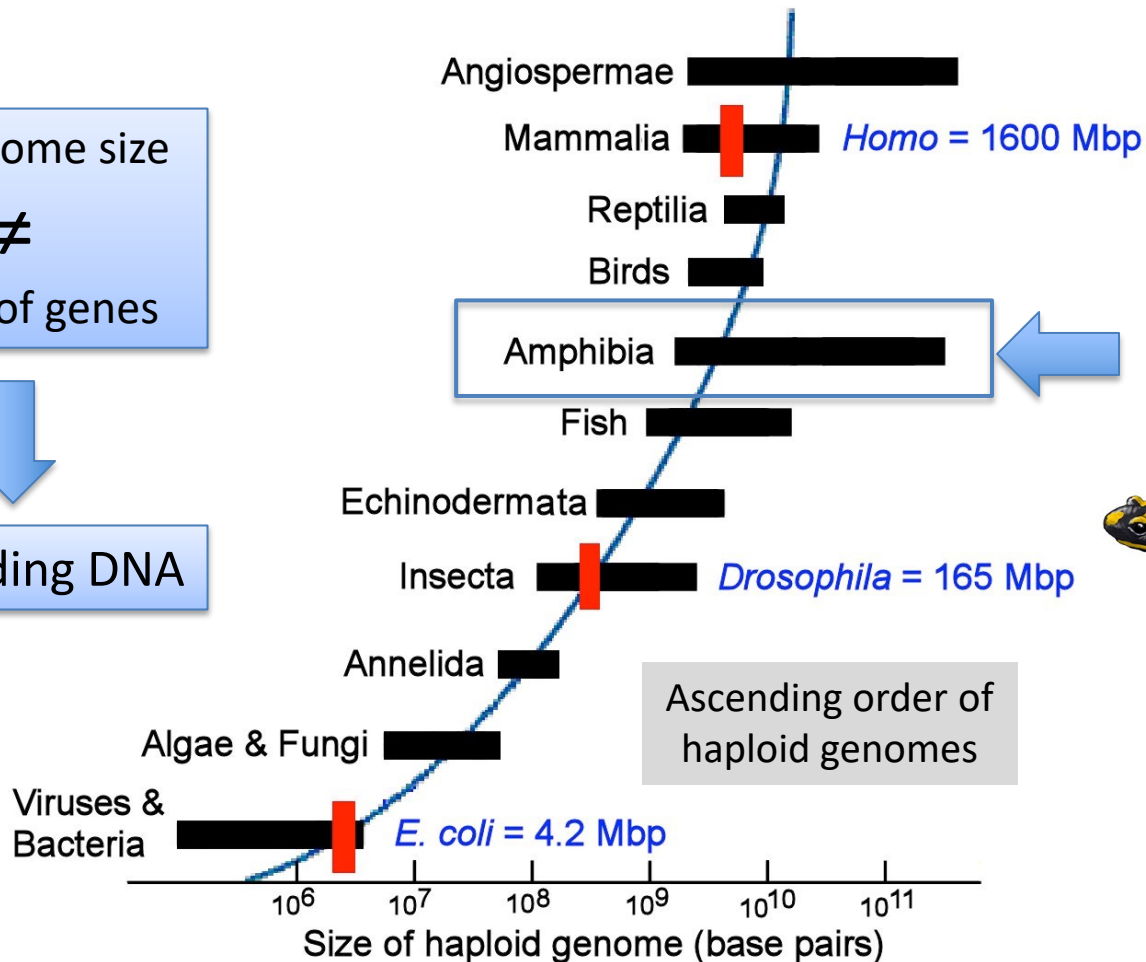

- Mapping
- Construction libraries
- Reading the sequence of nucleotides
- Reassembling the individual sequenced fragments

The C-value Enigma

(+) Genome size

≠

(+) Nº of genes

Noncoding DNA

Angiospermae

Mammalia   *Homo* = 1600 Mbp

Reptilia

Birds

Amphibia   **WHY?**

Fish

Echinodermata

Insecta   *Drosophila* = 165 Mbp

Annelida

Ascending order of haploid genomes

Algae & Fungi

Viruses & Bacteria   *E. coli* = 4.2 Mbp

10^6 10^7 10^8 10^9 10^{10} 10^{11}
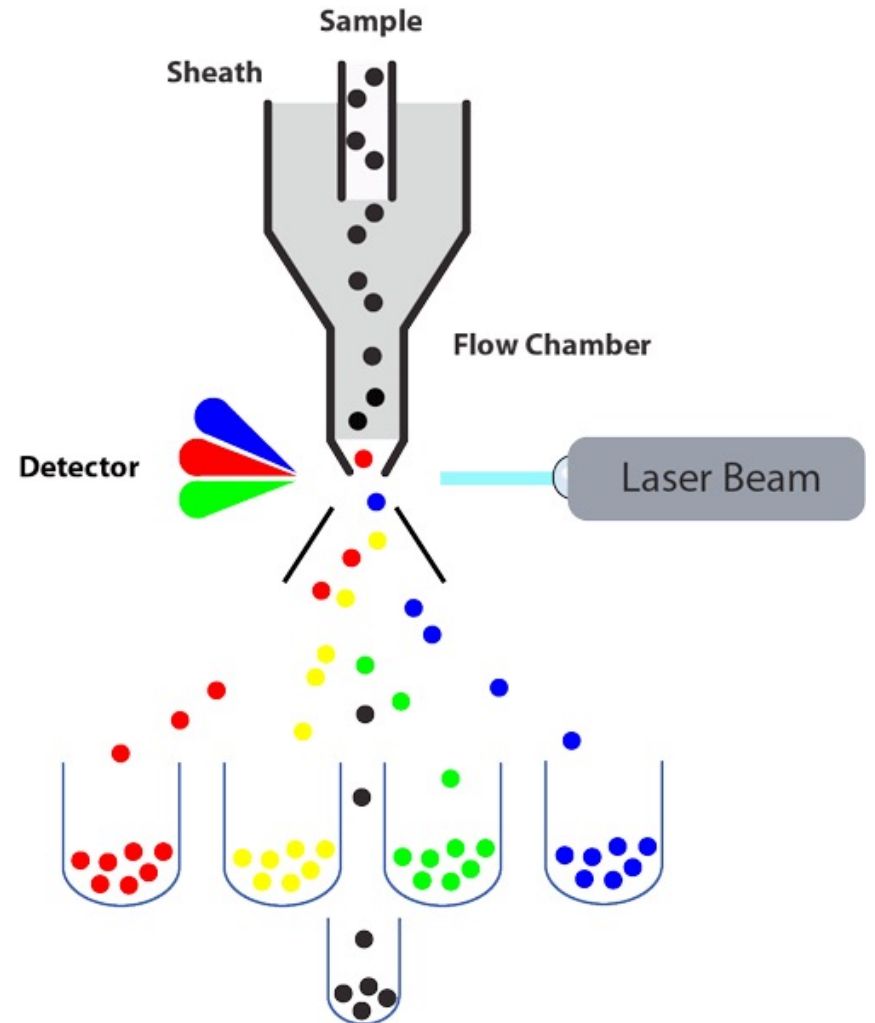
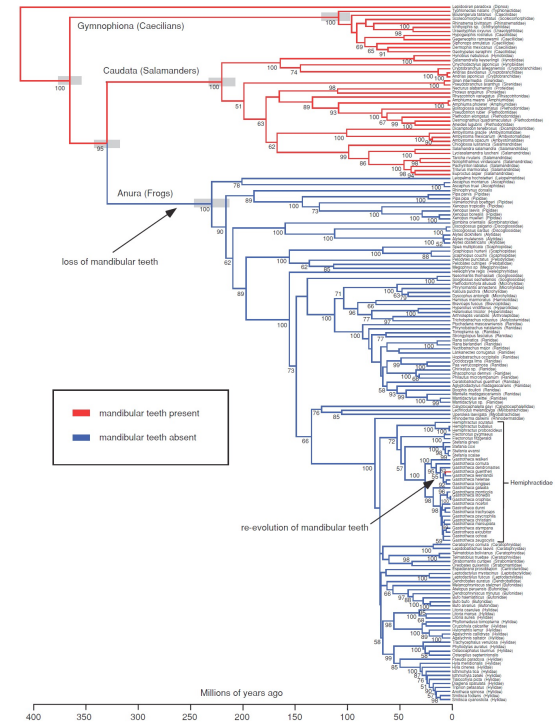Size of haploid genome (base pairs)

Classical Methods for Estimating the C-value

- Measuring the phosphate content

- Reassociation kinetics

- Flow cytometry

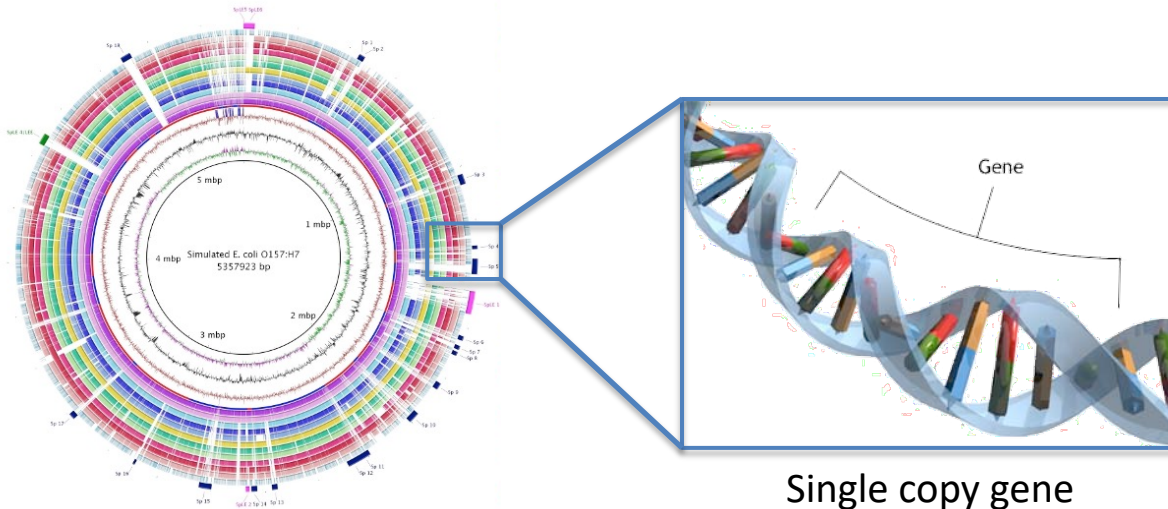- Image analysis

- Absorption cytometry

Issues and Challenges

Classical methods require the use of fresh non-processed tissues.
This means genome size **can't be estimated** on museum samples

GOAL: to develop a method to estimate genome
size <u>across all amphibians</u> (frogs, newts, salamanders and caecilians)

The Idea Behind

Single copy gene

Absolute quantification of a single copy gene in a genomic DNA sample by real-time PCR
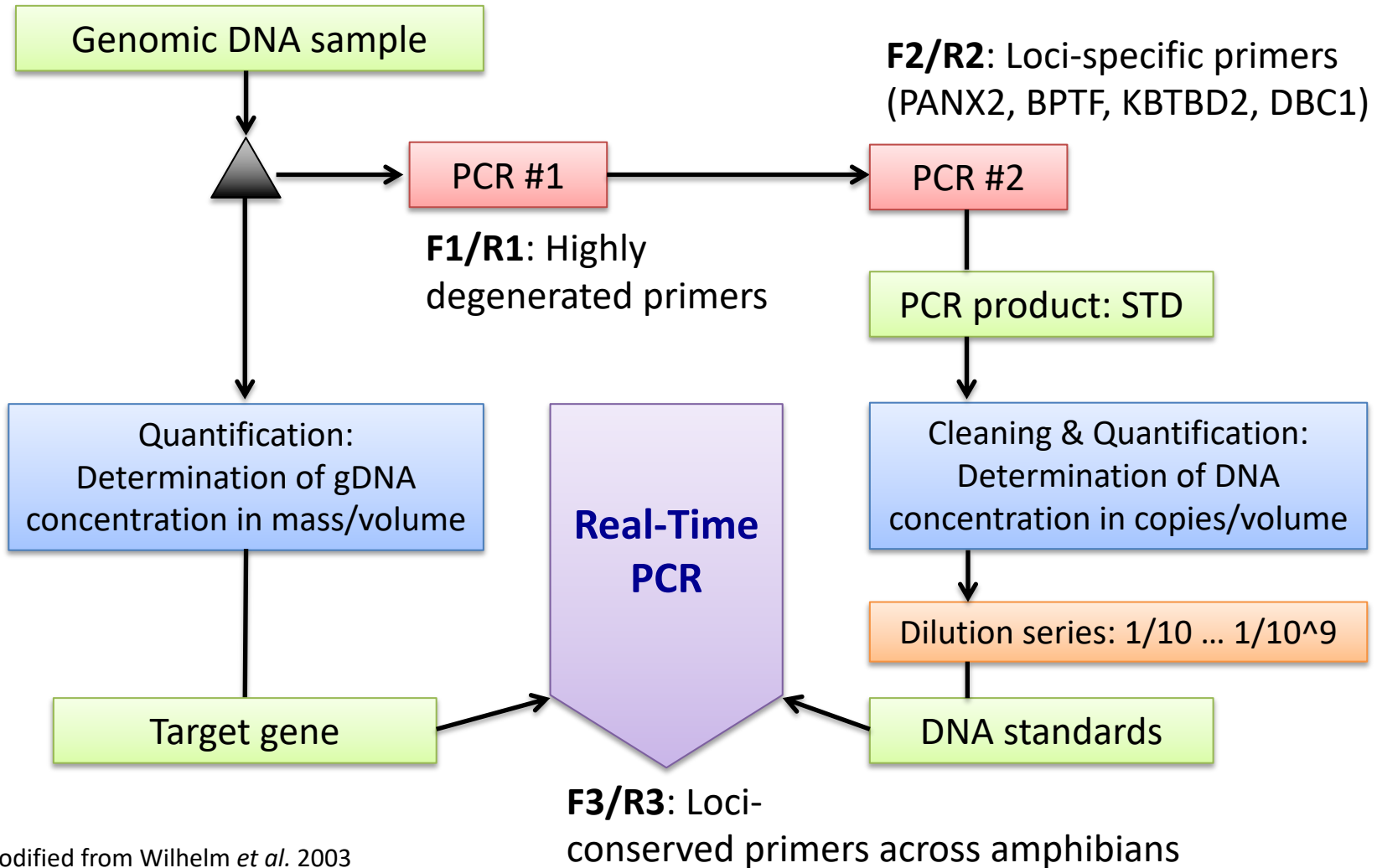
$$C = \frac{m}{N}$$

C = Mass of DNA per haploid nucleus (ng)

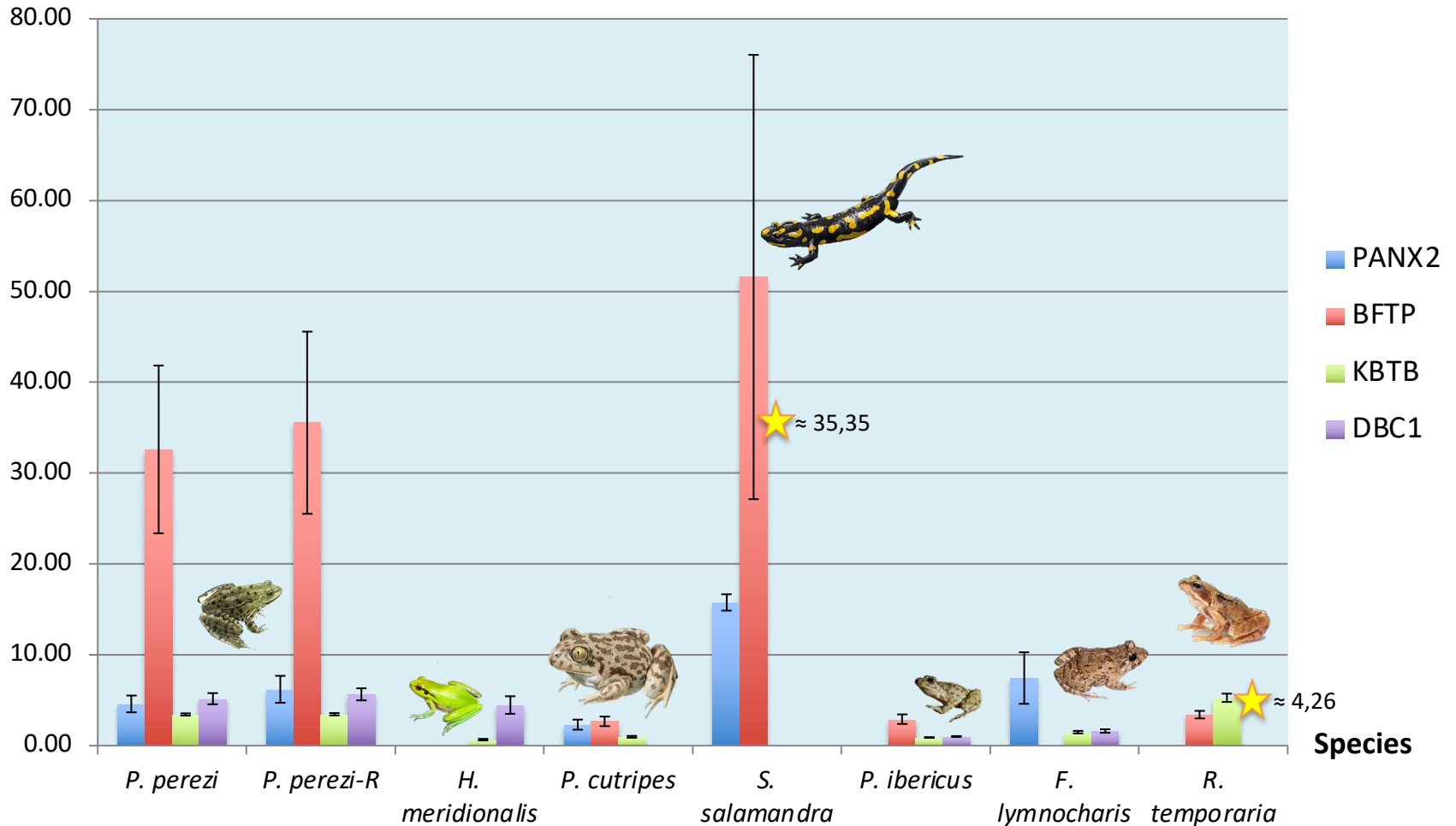m = Total mass of template DNA (ng / uL)

N = Copy number of the target sequence (copies / uL)

The approach

Genomic DNA sample

F2/R2: Loci-specific primers
(PANX2, BPTF, KBTBD2, DBC1)

PCR #1 → PCR #2

F1/R1: Highly degenerated primers

PCR product: STD

Quantification: Determination of gDNA concentration in mass/volume

Real-Time PCR

Cleaning & Quantification: Determination of DNA concentration in copies/volume

Dilution series: 1/10 … 1/10^9

Target gene

DNA standards

F3/R3: Loci-conserved primers across amphibians

Modified from Wilhelm *et al.* 2003

Results to date

C-value (pg)

Species

Legend: PANX2, BFTP, KBTB, DBC1

≈ 35,35

≈ 4,26

Conclusions and Future Perspectives

- **Positive part**: Estimations seem accordant with published data

- **Negative part**: High variation within technical replicates

Validation of the method

- ✧ To use more loci / markers

- ✧ To use biological replicates

- ✧ To use different DNA extraction methodologies

Rana temporaria   C-value ≈ 4,26

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