Optimizing flow cytometry for genome size estimation in salamanders: a call for nondestructive methods in amphibian genomics

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We present a non-destructive method of estimating genome size in salamanders with flow cytometry, using propidium iodide-stained nuclei isolated from blood cells.

Image 1: The Olm (*Proteus anguinus*)

References:

1. Decena-Segarra, L. P., *et al.* Genome Size, and Biological Size in a Diverse Clade of Salamanders. (2020)
2. Galbraith, D.W. *et al.* Rapid Flow Cytometric Analysis of the Cell Cycle in Intact Plant Tissues. (1983)
3. Doležel, J. *et al.* Estimation of nuclear DNA content in plants using flow cytometry. (2007)

BACKGROUND

Salamaders (Urodela) possess some of the largest genomes among vertebrates, ranging from 9.3–120 gigabases (Gb). Genome size has been shown to strongly influence embryonic developmental time as well physical body and cell size and may serve as an important tool in research pertaining to evolution, phylogenetics and speciation of salamanders. Additionally, genome size is highly variable even among closely related taxons of salamanders - intraspecific genome size variance may thus help to elucidate taxonomic relationships among different subpopulations of species such as the endangered *Proteus anguinus*. Despite its great potential, research in the field of salamander genome size estimation has often been hampered by inconsistent, laborious and inappropriate methods. Sorely needed is a reliable protocol that provides consistent, comparable and accurate results. In our work, we present an efficient and simple method for estimating genome size in salamanders using flow cytometry.

METHODS

- The Iberian ribbed newt (*Pleurodeles waltl*), the Axolotl (*Ambystoma mexicanum*) and The Olm (*Proteus anguinus*) were included in our study.
- Animals were anesthetized with 0,03-0,5% tricaine methanesulfonate (MS-222), buffered with sodium bicarbonate to a pH of 7.0-7.5.
- Blood was collected from the ventricle using a heparinized 26 G needle and a 1.0 mL syringe
- Nuclei of blood cells were extracted in Galbraith's lysis buffer for 30 minutes on ice and then stained with 100 µg/ml propidium iodide for an additional 30 minutes on ice and in the dark.
- Fluorescence was measured using a BD FACSMelody Cell Sorter.
- Genome size measurements were cross-checked using Feulgen densitometry
- Morphology of the stained nuclei was examined by fluorescence microscopy to verify the success of cell lysis.
- Various cell fixatives and parameters of sample preparation were assessed.

Image 2: The Iberian ribbed newt (*Pleurodeles waltl*)

Image 3: The Axolotl (*Ambystoma mexicanum*)

RESULTS, ASSAYED PARAMETERS

All specimes survived the blood taking; full recovery varied between individuals in the time frame of 10-40 minutes.

Comparison between DAPI and propidium iodide. Samples stained with 100 µg/mL of propidium iodide were compared with samples stained with a DAPI concentration of 4 µg/mL and 8 µg/mL. Compared to propidium iodide staining, DAPI staining resulted in poor separation of *P. waltl* and *A. mexicanum* peaks.

Comparison of different lysis treatments: Galbraith buffer was compared with a modified lysis buffer of 0,1% sodium citrate in 0,1% Triton X-100i. The modified lysis buffer did not prove to be a suitable substitute as it resulted in large cell nuclei aggregates.

Comparison of four cell fixation methods. Blood samples were fixed using four different treatments (70% EtOH, 3:1 methanol acetic acid, frozen at -20 °C in PBS with 1mM EDTA, 10% formalin). Samples were analyzed 7 and 14 days after fixation. This experiment is still in progress.

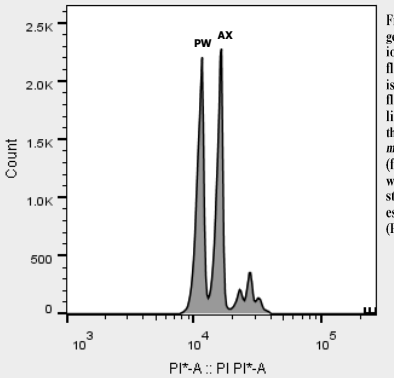

Figure 1: Estimation of genome size in propidium iodide-stained nuclei using flow cytometry. The x-axis is the intensity of the fluorescence and is linearly correlated with the genome size. Here, *A. mexicanum* (AX) peak (fluorescence intensity) was used as internal standard for the estimation of *P. waltl* (PW) genome size.

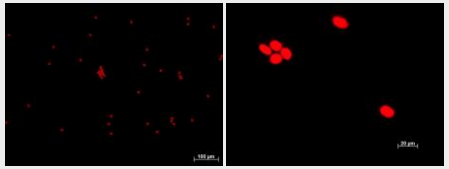

Image 4: *P. waltl* nuclei stained with propidium iodide (fluorescent microscopy)