MOL231: Bioinformatical and experimental analysis of IL-2 proteins from Atlantic Salmon

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***Equal contribution**

SCAN ME

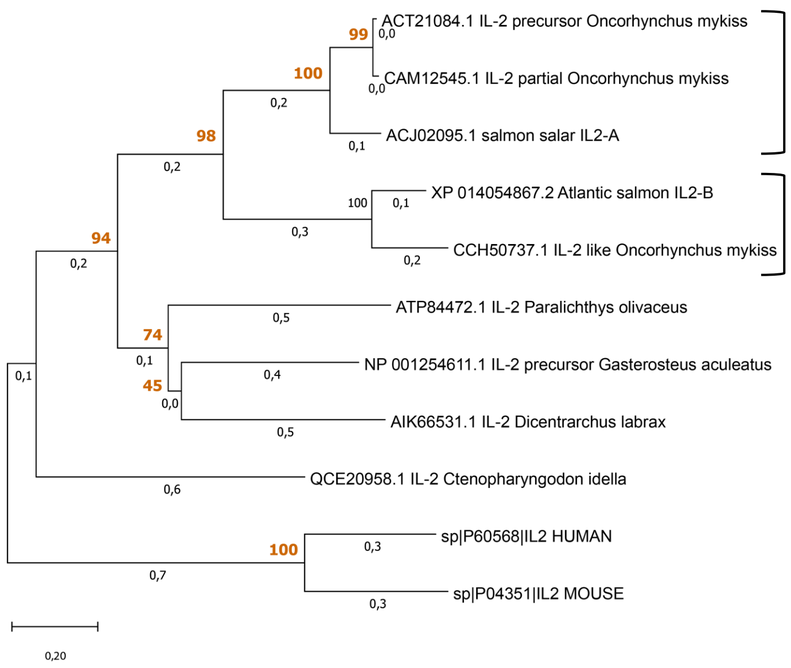
Aim:Applying methods of molecular biology to analyze, clone, and express recombinant salmon IL-2 proteins to determining their solubility.

Backround and Summary: Interleukin-2 (IL-2) is a protein produced by T-cells (CD4+ and CD8+). It is required for regulation of immune response and can act as a growth factor for T- and B-cells[1]. This makes them interesting targets for research of immune response, and immune cell differentiation and proliferation[2]. Atlantic salmon (*Salmo salar*) has two paralogs, IL-2A and IL-2B, which were examined in this project. These variants contain minor differences between their sequences and structures, which might affect their function[3].

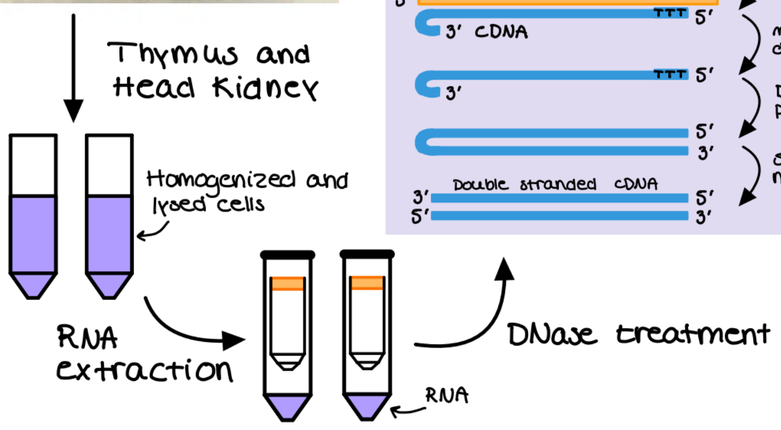
1. Multiple sequence alignment of IL-2 relatives

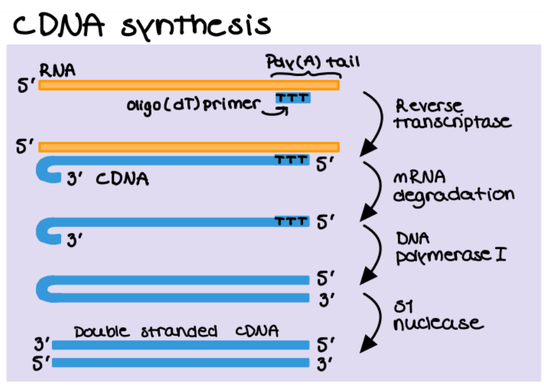
IL-2 signature

2. Phylogenetic tree

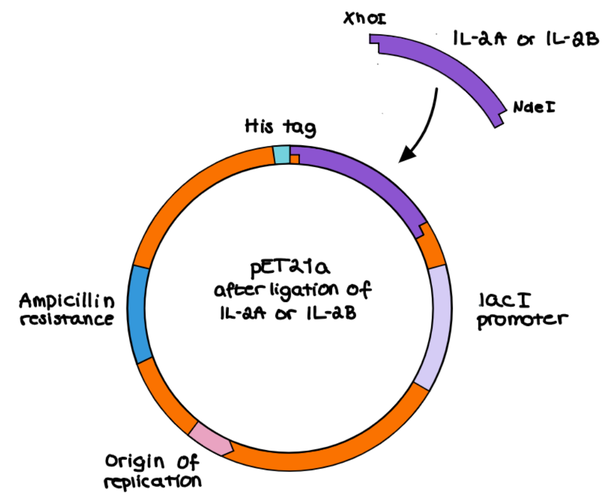


3. RNA Extraction and cDNA Synthesis



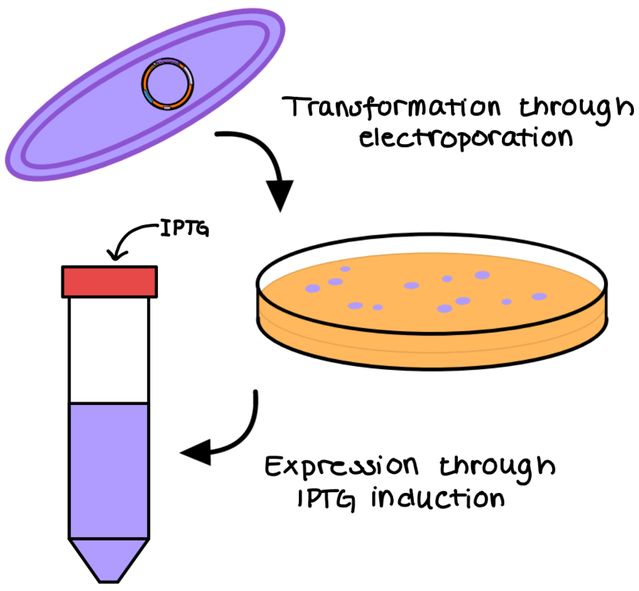


4. Cloning

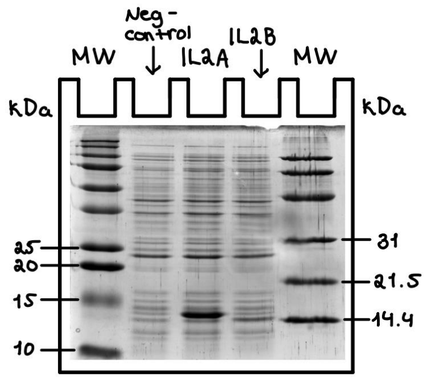


Restriction digestion with XhoI and NdeI. The His-tag is within the reading frame and used later for expression in Western Blot.

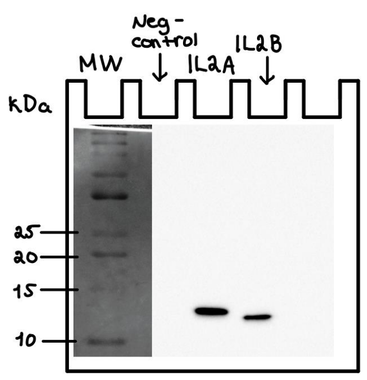
5. Transformation and Expression



6. SDS-page and Western Blot

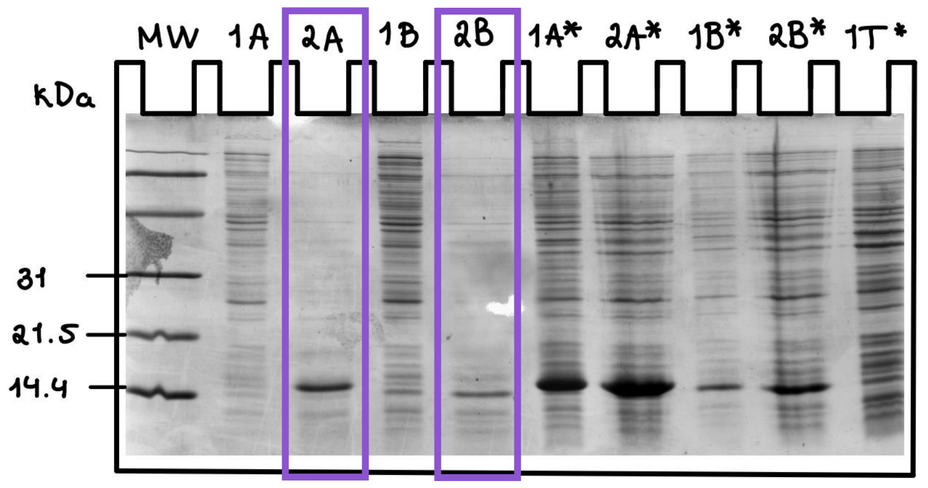


SDS-page colored with coomassie



Western Blot expressed with anti-his antibodies

7. Autoinduction and Solubility



1A and 1B: CE (TE-buffer)
2A and 2B: CE (buffer A)
1A* and 1B*: Sonicated cells (TE-buffer)
2A* and 2B*: Sonicated cells (buffer A)
1T*: neg. control (pET21a TE-buffer)
Both IL-2A (2A) and IL-2B (2B) were shown to be soluble in buffer A (50mM Tris-HCl, 0.5M NaCl, 20% glycerol, 10mM Imidazole).

Conclusion: Both IL-2A and IL-2B were soluble in buffer A. Further research could focus on isolating the proteins and subjecting T-cells to them to measure the response.

References:
[1] "IL2 Protein Expression Summary - the Human Protein Atlas." *Www.proteinatlas.org*, www.proteinatlas.org/ENSG00000109471-IL2#gene_information. Accessed 2 May 2022.
[2] "OMIM Entry - * 147680 - INTERLEUKIN 2; IL2." *Www.omim.org*, www.omim.org/entry/147680?search=IL2&highlight=il2. Accessed 2 May 2022.
[3] Wang, Tiehui, et al. "Interleukin (IL)-2 Is a Key Regulator of T Helper 1 and T Helper 2 Cytokine Expression in Fish: Functional Characterization of Two Divergent IL2 Paralogs in Salmonids." *Frontiers in Immunology*, vol. 9, 26 July 2018, 10.3389/fimmu.2018.01683. Accessed 30 Nov. 2020.