MOL231

Multicistronic mRNA in salmon CHSE cells

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Background and aim

The currently available conventional vaccines for salmon are not efficient enough. A promising solution is the development of mRNA vaccines due to their advantages: rapid development, adaptability, large scale production and no integration of the host cells genome. The next promising idea with mRNA vaccines is to make a multicistronic in vitro transcripted (IVT) mRNA which can lead to expression of several proteins, enabling vaccination against several diseases using only one vaccine.

Aim: Make a multicistronic IVT mRNA for CHSE salmon cells expressing green, red and blue fluorescent protein (GFP, RFP, BFP).

Methods

- Growing CHSE cells
- Transfection
- IVT
- Cloning
- Transformation of *E.coli*
- Plasmid isolation
- Agarose gel electrophoresis
- Flow cytometry
- Fluorescence microscopy
- SDS-PAGE and Western Blot

Results

The 5′ Cap of a mRNA is important for efficient translation. Different 5′ caps were tested and protein expression was measured with flow cytometry, showing that ARCA, ARCA 2′O-Me and FCE 2′O-Me gave the highest percentage of positive cells (Fig. 1A,B). The ARCA caps were preferred as they gave stronger fluorescent intensity than FCE caps (Fig. 1C). Thereafter, a multicistronic template for IVT was made (Fig. 2A). Successful cloning was confirmed with restriction enzyme cutting with HindIII (Fig. 2B). Following IVT, mRNA was transfected in CHSE cells and expression of GFP, RFP and BFP was monitored with fluorescent microscopy (Fig. 3), as well as SDS-PAGE and Western blot (Fig. 4A,B).

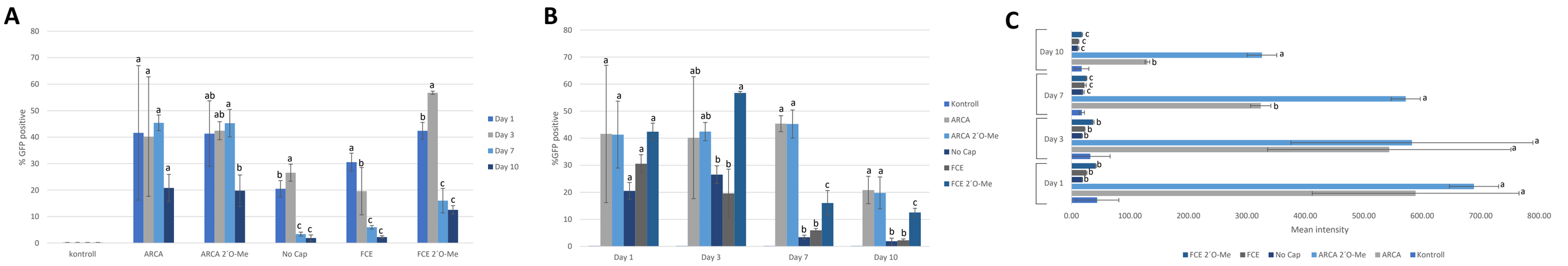

Figure 1: Effect of different 5′Caps. *A, B. GFP expression in CHSE cells from mRNA with different 5′ Cap structures, measured at four separate days post-transfection. C) Mean intensity of the positive GFP-transfected CHSE cells with five different mRNAs at four separate days post transfection. One-way ANOVA was used to determine if the results were statistically significant.*

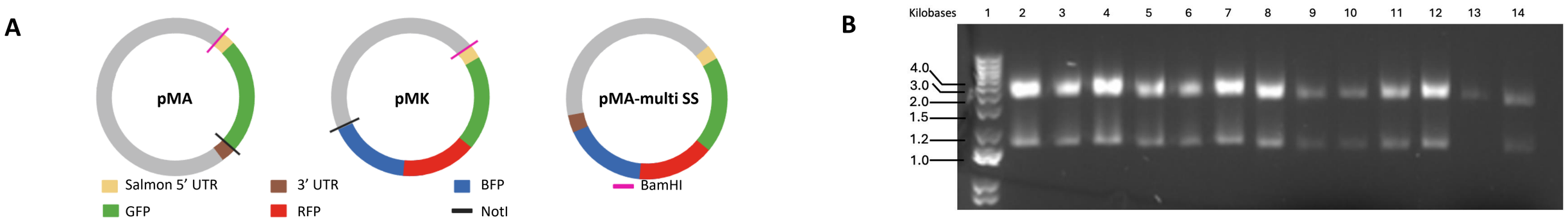

Figure 2: Overview of the cloning procedure to make the template for the multicistronic mRNA *. A. The sequence encoding GFP, RFP and BFP were cut from the pMK vector and inserted into pMA replacing GFP. The restriction enzymes, BamHI and NotI were used for cloning. B. HindIII was used to screen for positive clones (lane 2-14), visualized in a 1% agarose gel. Lane 1: 1Kb+ DNA ladder.*

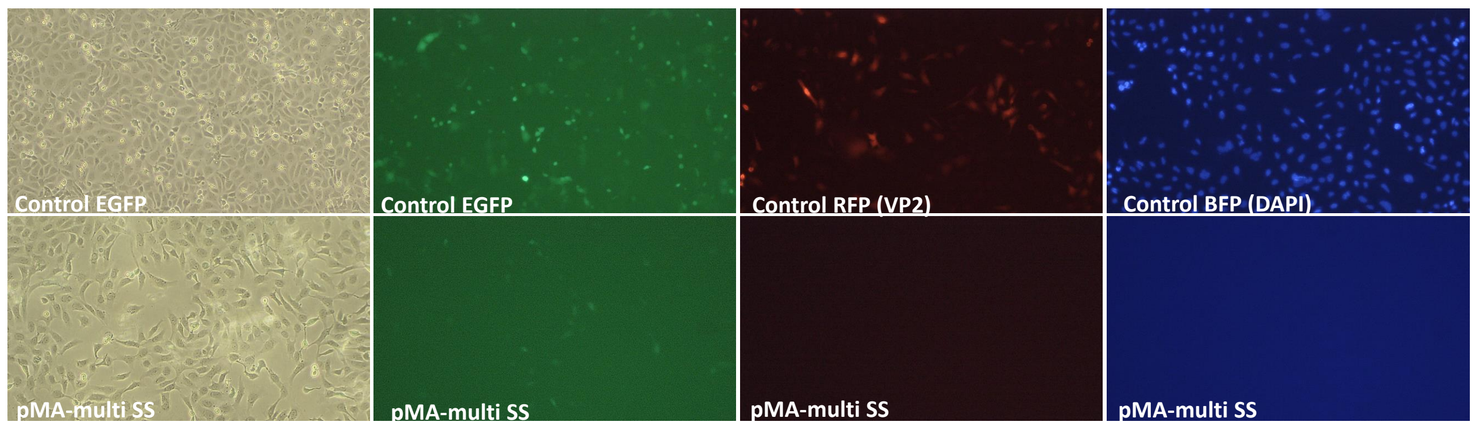

Figure 3: Microscopy images of the control samples: EGFP, RFP and BFP, and the CHSE cells transfected with pMA-multi SS mRNA shown at 10 X magnification. *pMA-multi SS transfected cells showed positive GFP expression, but no visible expression of RFP and BFP.*

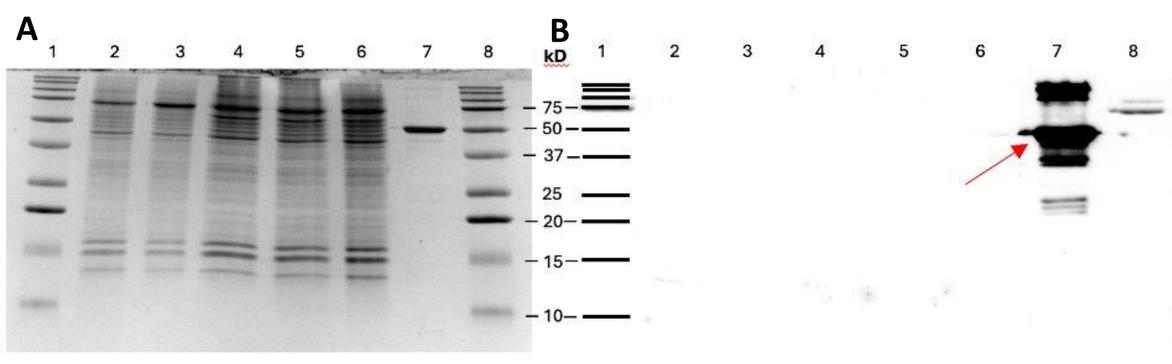

Figure 4: A. SDS-PAGE gel stained with Coomassie for visualization. B. Western Blot image showing positive HisTag control. *Sample distribution in A. and B. 1: Precision Plus Protein standard, 2: pMA-multi SS (harvested with Trypsin, colony 10), 3: pMA-multi SS (harvested with Trypsin, colony 6), 4: pMA-multi SS (harvested with RIPA, colony 10), 5: pMA-multi SS (harvested with RIPA, colony 6), 6: transfection control, 7: positive HisTag control and 8: Precision Plus Protein standard. B) No visible bands indicating no pMA-multi SS protein, possibly due to too low expression and intensity.*

Discussion and conclusion

- ARCA and ARCA 2′O-Me were determined as the best functioning 5′ Cap structures (Fig. 1). ARCA was further utilized.
- Cloning of pMA-multi SS was successful and verified by gel electrophoresis (Fig. 2B).
- By IVT, the pMA-multi SS mRNA was transcribed. Fluorescence microscopy confirmed the functional mRNA (Fig. 3).
- No bands was visible on Western Blot (Fig. 4B), except the positive HisTag control.
- Further work will include optimalization to ensure efficient protein expression and eventually replace the fluorescent proteins with antigens.

References

LEE, K., KIM, S. Y., SEO, Y., KWON, H., KWON, Y. J. & LEE, H. 2019. Multicistronic IVT mRNA for simultaneous expression of multiple fluorescent proteins. *Journal of Industrial and Engineering Chemistry,* 80**,** 770-777.

MARUGGI, G., ZHANG, C., LI, J., ULMER, J. B. & YU, D. 2019. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. *Mol Ther,* 27**,** 757-772.

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