



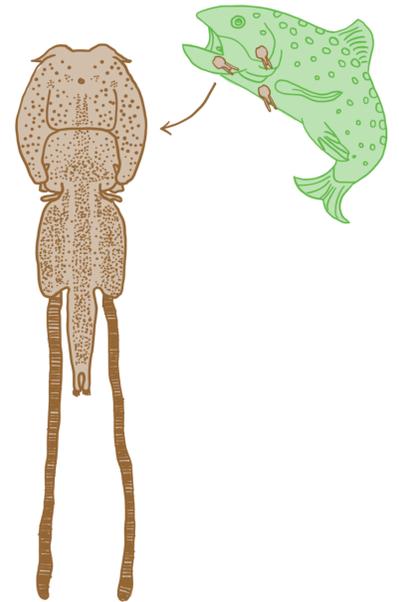
# MOL231: Heterologous expression of salmon louse proteins in yeast *Pichia pastoris*

Hanne Arstein, Virginie Comorge

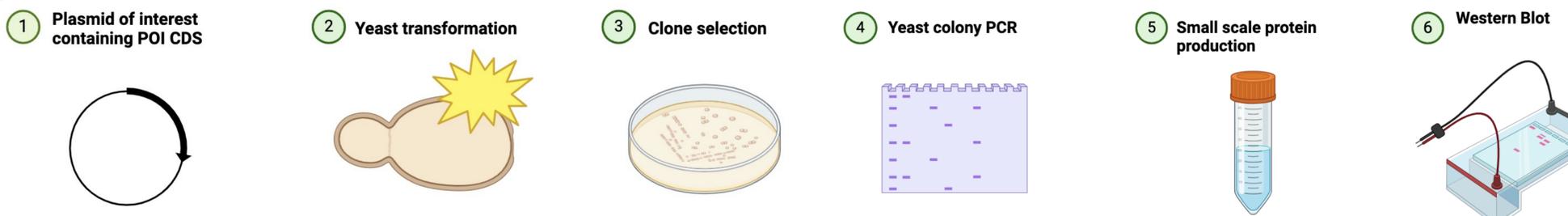
Department of Biological Science, University of Bergen, Bergen, Norway

## Background

One of the most deleterious threats for salmon is the salmon louse<sup>1</sup>. The development of vaccines can protect farmed salmon and prevent the spreading of lice larvae, but we still lack knowledge about potent salmon louse antigens that can be targeted. Antigens might be secreted by labial glands, as the parasite feeds directly from the skin and blood of the host<sup>2</sup>. Five genes coding for labial secreted proteins have been identified and some of them have been shown to modulate host immune response<sup>3</sup>. The genes coding for Labial Gland Protein 1 (LGP1) and Labial Gland Protein 1 – like (LGP1L) are possibly key regulators for labial gland secretion, but we do not know their function. This project aims to set up a new system to produce LGP1 and LGP1L using the yeast *Pichia pastoris*.



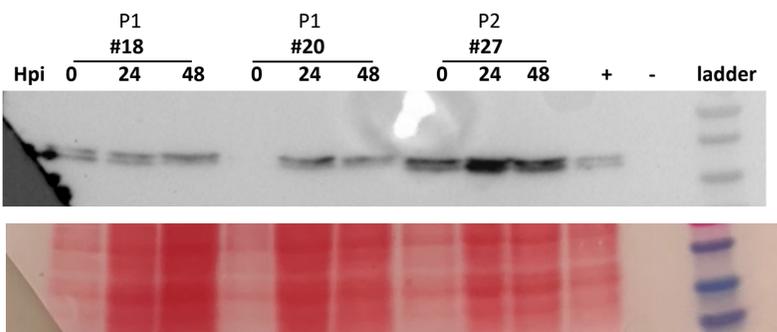
## Methods



We will test if the system allows production of LGP1 in *P. pastoris* strains mutated in the gene coding for Protease 1 (P1) and strains mutated in the gene coding for Protease 2 (P2). The system should allow protein production in medium because the proteins should be secreted.

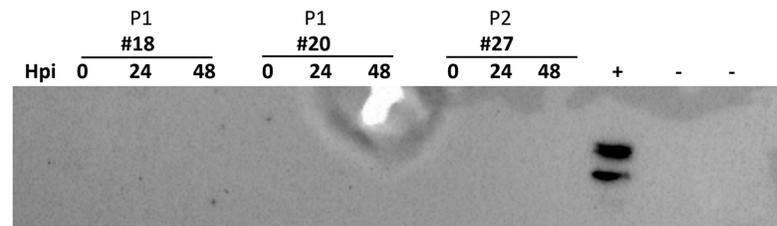
## Results and discussion

### LGP1 is produced in *P. pastoris* pellets



**Figure 1: Western blot on *Pichia pastoris* pellets.** Samples from lysate *Pichia pastoris*, 0, 24 and 48 hours post induction.

### No protein expression of LGP1 in *P. pastoris* medium



**Figure 2: Western blot on *P.pastoris* medium samples.** Samples from *Pichia pastoris* medium, 0, 24 and 48 hours post induction.

We tested different strains of *P. pastoris* to see if LGP1 production happened. We successfully obtained protein production in pellets after optimizing some parameters. Despite the use of two strains mutated in genes coding for proteases: *prb1* (Protease 1) or *pep4* (Protease 2), no expression of LGP1 has been detected in medium. This could indicate that protein secretion did not happen, or that they have been degraded anyway. We tried to select clones that have a production only after 24 hpi and 48 hpi to prevent the use of strains that had a leakage in the protein production. The ponceau staining shows there is less protein overall at 0 hpi because the yeast grew over 48 hpi. An OD measurement of each culture could have made the amounts of cells in each sample more equal.

## Conclusion

LGP1 were produced in *Pichia pastoris*, but was not secreted to the medium.

## Future work

See if *Pichia pastoris* double mutant *prb1 pep4* could secrete LGP1 in medium. If not, find a way to lysate *P. pastoris* in large scale production.

## References

1. Thorstad, E. B. & Finstad, B. Impacts of salmon lice emanating from salmon farms on wild Atlantic salmon and sea trout. vol. 1449 <https://brage.nina.no/nina-xmlui/handle/11250/2475746> (2018).
2. Braden, L. M., Barker, D. E., Koop, B. F. & Jones, S. R. M. Comparative defense-associated responses in salmon skin elicited by the ectoparasite *Lepeophtheirus salmonis*. *Comp Biochem Physiol Part D Genomics Proteomics* 7, 100–109 (2012).
3. Øvergård, A.-C. et al. Small, charged proteins in salmon louse (*Lepeophtheirus salmonis*) secretions modulate Atlantic salmon (*Salmo salar*) immune responses and coagulation. *Sci Rep* 12, 1–16 (2022).

