Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: **CA17103**

Grantee name: **Maria Francisca Coutinho**

Details of the STSM

Title: **A simple and effective F0 knockout method to generate zebrafish knock-out models for MPS III**

Start and end date: 03/10/2022 to 07/10/2022

Description of the work carried out during the STSM

The main purpose of this STSM was to allow the grantee to become familiar with the Cas9/guide RNA (gRNA) ribonucleoprotein (RNP) microinjection protocol in zebrafish to promote F0 knockout for a single gene. This is a crucial step for the overall success of a CRISPR-Cas9-based targeted gene knockout in this animal.

The whole protocol may be summarized as follows: crRNA selection and resuspension; crRNA/tracrRNA annealing; gRNA/Cas9 assembly; RNP pooling and, finally, injection. While simple, the overall success of the knockout approach depends most on the reliability of the injections. For instance, if some eggs were missed during injections, the F0 population would include a proportion of wild-type animals, which would reduce the effect size between the control and the experimental group and make the phenotype less likely to be detected. That is why it is of upmost relevance to get any researcher who will be attempting this sort of injection protocol for disease modelling, to try and perform this protocol in a lab that already has it all optimized.

During this whole week, the grantee had not only the opportunity to see it performed by experienced users, but also, and most importantly, to attempt it by herself. Briefly, the candidate learnt how to select single-stage zebrafish embryos and to inject them in the yolk at the single-cell stage before the cell inflates. She first attempted the protocol with a soluble dye and, later during the week, replicated it with her target-specific RNPs. She also learned how to prepare the whole experimental setting in a time-effective manner, as timing is crucial for the protocol's success. Finally, she realized how she could identify the successful injections (Figure 1) and verify the mutagenic potential of gRNAs before undertaking an F0 phenotypic screen in the microinjected zebrafish embryos, through a rapid sequencing-free method called headloop PCR.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

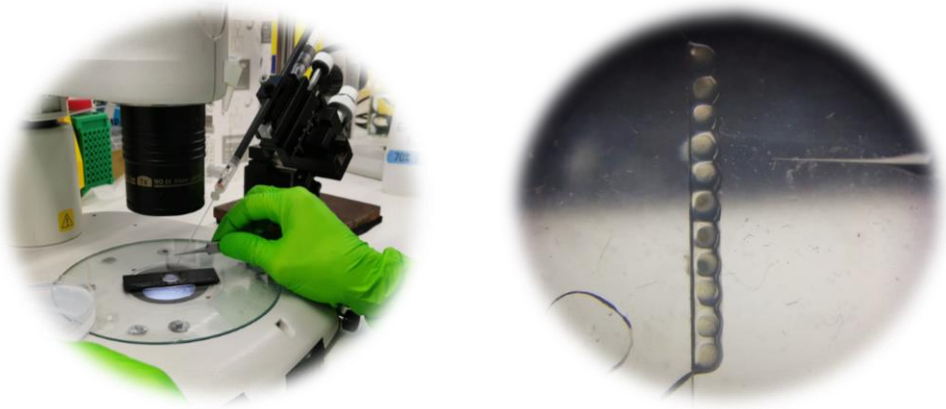

Figure 1: a) Microinjection setup; b) one-cell stage zebrafish embryos as seen under the lens, just a few seconds after microinjection (one can confirm RNPs have entered the yolk by observing a slight and transient colour change: the yolk gets darker (*). Then, as the RNPs diffuse, the grey colour disappears).

Description of the STSM main achievements and planned follow-up activities

During this STSM, it was possible for the grantee to achieve all proposed goals, and carry on all the envisaged activities, namely the microinjection protocol.

That method is particularly relevant within the scope of an ongoing project the grantee is currently running entitled "*Neurological disease modelling for Sanfilippo: a key step towards understanding and treating a rare genetic disorder*" (EXPL/BTM-SAL/0659). With this project, she aims to create knockout zebrafish models for Sanfilippo to be used in the research for new RNA-based therapies. Thus, it was of upmost importance for the grantee to learn and perform this technology at Prof. Steve Wilson's lab (Cell and Developmental Biology, UCL, London, UK), which was the one who generated the original protocol to model inborn errors of metabolism in zebrafish (https://doi.org/10.7554/eLife.59683). Now, she will bring that knowledge to other colleagues from Sandra Alves' lab on Lysosomal Storage Disorders (Research and Development Unit, Human Genetics Department from the National Institute of Health Dr. Ricardo Jorge, in Portugal) and implement the method in Portugal, at CIIMAR (*Centro Interdisciplinar de Investigação Marinha e Ambiental*).

Overall, their method is an econocal and scalable pipeline of upmost utility for both pathophysiological studies and drug screening protocols. By learning the whole setup, in a lab where the protocol is well-optimized, the chances for the applicant to latter implement that same method with success will greatly improve.