MAST PreASSEMBLE report

Yacine Badis

Background and Summary: To pilot Cas9-mediated genome editing on the brown algal model *Ectocarpus siliculosus (E.si),* the MASTS Aquaculture Forum awarded £2240 for my previous TRAFALGAR (Transgene-free Algal Genome Remodelling) project in collaboration with the Biological Station of Roscoff. While TRAFALGAR allowed the development and in-vitro validation of sgRNA**s** targeting a gene of interest in *E.si.,* another major outcome enabled by MASTS support has been the successful submission of a EMBRC-ASSEMBLE+ grant (H2020-INFRAIA; 218k€ to SAMS) including 12 month of contract for me to further TRAFALGAR in collaboration with SAMS and Roscoff. The overarching objective of this project is to share the TRAFALGAR tools developed at SAMS and test them in-vivo thanks to Roscoff's expertise in functional genomics and lipid based transfection of *Ectocarpus* gametes. Finally, Another consequence of the interest sparked by the MASTS-enabled TRAFALGAR project has been appointment in early 2017 of a 1 year fixed-term research technician in Roscoff, to work collaboratively with me on the development of those genome editing tools. While the 1-year research technician appointed in Roscoff was already operational, the start date of the TRAFALGAR-ASSEMBLE+ funding would only support my visits starting **on the 1st of October** 2017. Hence, and to fully potentiate this collaborative research effort despite this 4-month gap, MASTS supported my cost of living for a visit to Roscoff between the 1st and 15th of June 2017, with the following objectives:

(i) Transfer the sgRNA in-vitro synthesis tools developed during TRAFALGAR

(ii) Assist the appointed technician with Cas9-sgRNA In-vitro validation and preparation of biological material

(iii) Layout an appropriate 4-month scientific strategy to fully synchronize Roscoff research efforts with the upcoming ASSEMBLE+-TRAFALGAR funding

I am glad to say that this visit was a real success with respect to the defined objective, as I was able to repeatedly train the appointed technician Celine Caillard in the realisation of the sgRNA in-vitro synthesis tools and the in-vitro validation of our defined targets. A direct consequence of this visit was also the redefinition of an optimal strategy (Summarized on Fig-1) to achieve this genome editing by (i) the targeting of two possible genes, one allowing a chemical screen for mutants, and one developmental gene allowing for visual selection of genome editing events (ii) the parallel testing of two entry mechanisms for the Cas9 complexes by lipofection and electroporation (iii) the development of PCR based methods for the detection of rare events to assist the screen. The ASSEMBLE+ Project has now officially started, and as of December 2017 I stayed in Roscoff to further those efforts. We now have defined 16 validated sgRNA targets to achieve the strategy explained above, the summary of those validations are displayed on Fig-2. We are currently exploring the electroporation based strategy, to ensure that all possible alternatives are guaranteed for the success of this project. Finally, because of those continuous efforts and supports from MASTS, we submitted another application for an additional two-year funding to work on this topic of high priority for algal blue-sky research. I could not be more thankful to MASTS for this continuous support.

Fig-1 Updated topics and strategies following the MASTS-enabled TRAFALGAR project

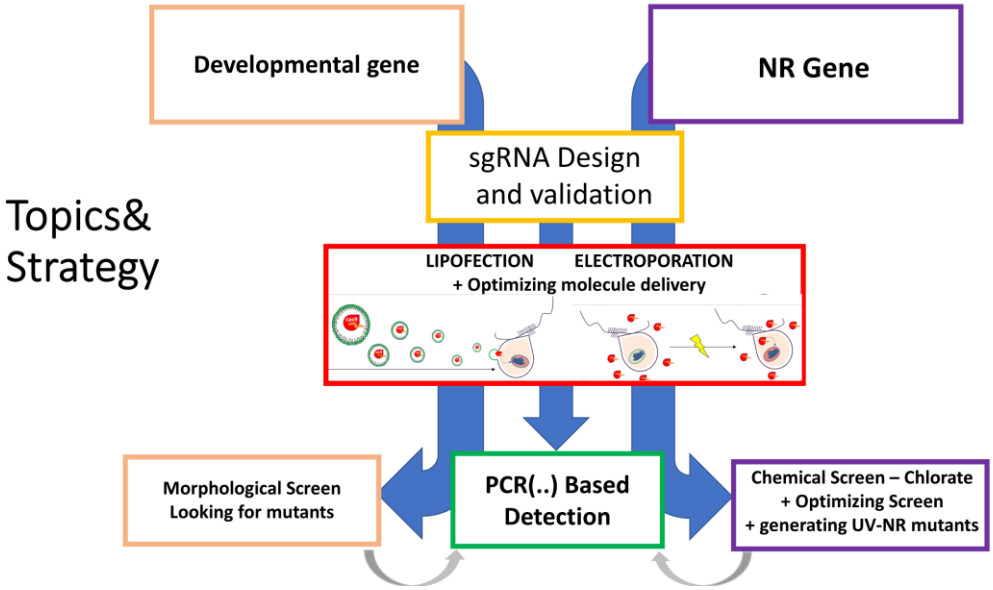

Fig-2 Test and Cas9 in-vitro validation of sgRNAs designed in the frame of this supported short term scientific mission