# MAST TRAFALGAR report

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**Summary:** This Small Grant allocated by MAST allowed the development of a **sgRNA efficiently cutting a target gene of E.siliculosus in-vitro**. Based on the initial MAST project, microinjection of untreated filaments of E.si was also piloted, but hampered by cell wall resistance (a). Attempts at protoplast microinjection (b) were not successful due to protoplast fragility. To overcome this limitation, alternative strategies were defined with the Roscoff team relying on lipid-assisted delivery of functional Cas9-sgRNA complexes (Supplementary workflow at the end of this report). Based on this successful in-vitro validation of the Cas9 tool, the only constraint is now to deliver functional complexes in E.si cells. This grant allowed me to establish pilot results and further my collaboration with the Aberdeen and Roscoff laboratories. **Amongst all, a major outcome of this project has been the submission of a EMBRC grant including 12 month of contract dedicated to furthering Trafalgar in collaboration with SAMS and Roscoff.** I am grateful for this real kick-start help. MAST will of course be acknowledged in the outcome of this work and I really look forward furthering my collaborations with MASTS.

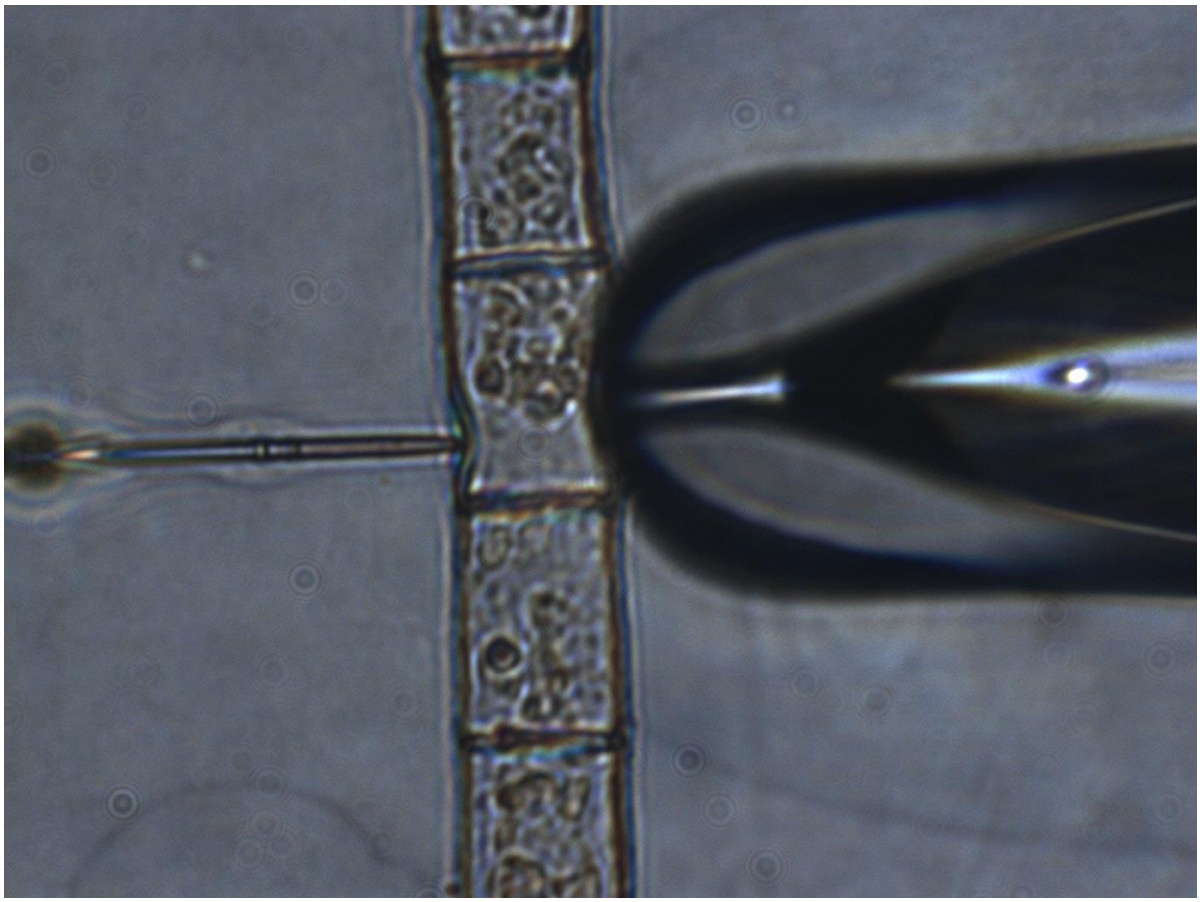
**Summary of comsummables bought with the help of the Trafalgar MASTS grant (all proof available upon request) :**

| Product | Use |
|---|---|
| GoTaq, Miniprep50, GelStain | Cas9 vector amplification |
| Alginate lyase, cellulase | Protoplast generation |
| Megascript synthesis + purification | sgRNA amplification/purification |
| T4 ligase + BsaI enzyme | Cloning of Cas9 vector and sequencing amplicons |
| sgRNAtarget build primers | Primers for sgRNA template amplification |
| Cas9 protein PNABio | In-vitro testing of sgRNA |
| Cas9 protein PNABio Roscoff | In-vitro testing of sgRNA |
| sgRNAtarget build primers 2 | Primers for sgRNA template amplification |
| Lipofectamine Reagen ts Roscoff | Piloting lipid mediated delivery of proteins |
| Lipofection reagents OZBioscience | Piloting lipid mediated delivery of proteins |
| Q5 HF PCR kit 50 reactions | Pcr detection of editing events |
| smaI Restriction enzyme | sgRNA in-vitro testing |
| 100 bp DNA Ladder | sgRNA in-vitro testing |

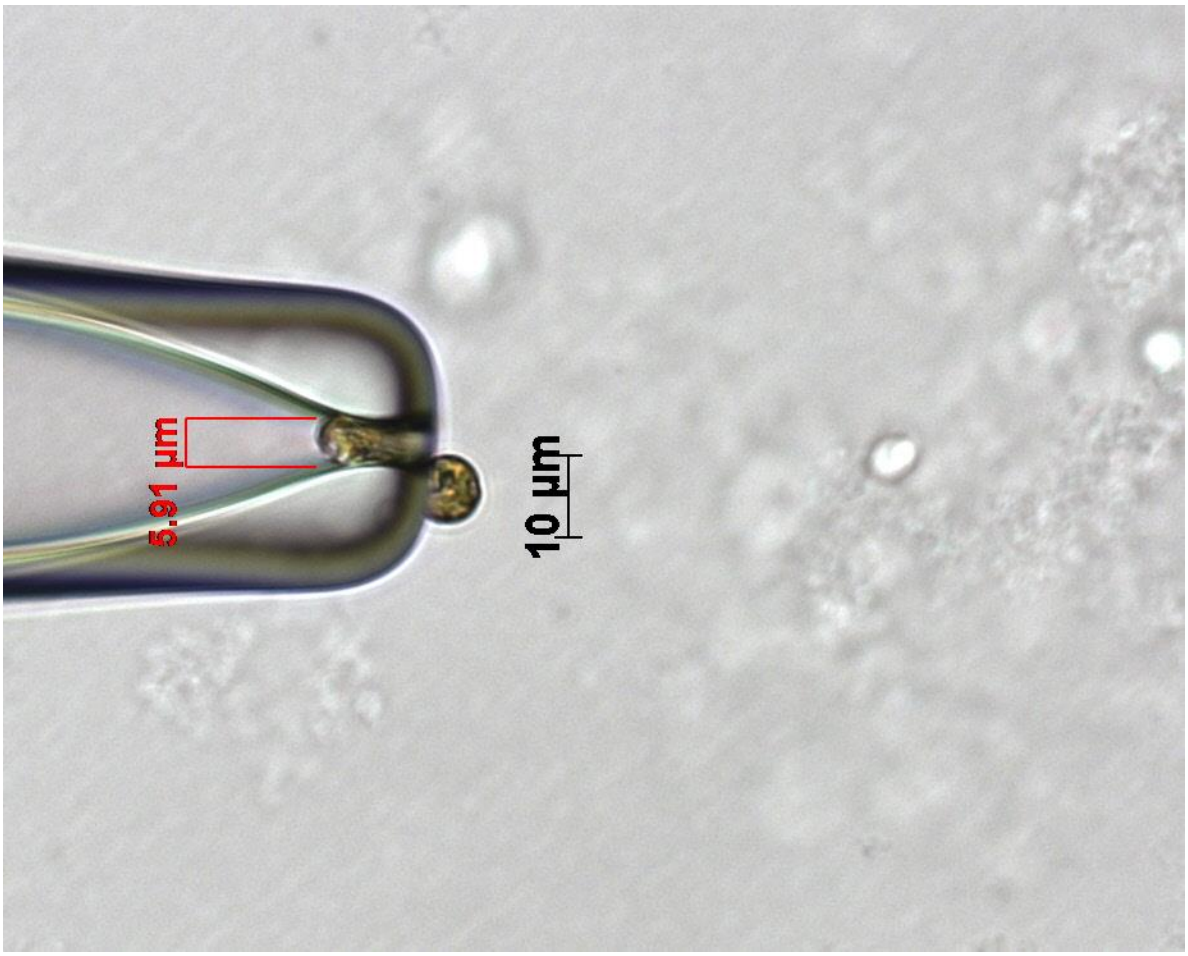
**Main Successful outcome of Trafalgar - Synthesis of sgRNAs and in-vitro validation of target cleavage**


Following Bioinformatic design of small guide RNA targeting gene A (Details on procedure upon request but omitted here for brevity), Template-free PCR primers were designed to amplify the DNA version of the sgRNA. Amplification and purification of DNA templates for in Vitro transcription of sgRNAs using turbo Pfu HF DNA Polymerase, and RNAse Free DEPC water. For each sgRNA, 100uL template-free PCR were prepared following the directions of Kistler et al., 2015. Purified PCR products were subsequently used as template for the In-vitro synthesis of 2 sgRNAs. In vitro validation of target cleavage was performed on SmaI digested plasmid incubated with Cas9 protein and with or whithout sgRNAs, thus showing a clear and efficient in-vitro cleavage of target sequence with sgRNA 551f. **The molecular tools required to pilot genome editing of E.siliculosus are now ready and validated.**

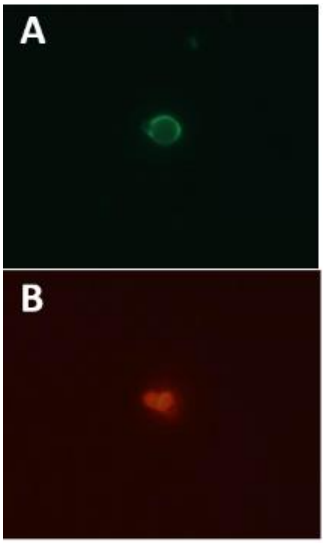
**(a)**



**(b)**



**Piloting in vivo protein delivery in Ectocarpus gametes using lipofection**




Three reagents (PDI, RMF, and HiP) were tested in pilot experiments on female gamete releases of two *Ectocarpus* strains. Briefly, a diluted fluorescent protein (Igg-FITC) is incubated with lipofection reagents for 15 minutes and further diluted before addition to equal volume of gamete suspension. Gametes are observed after overnight incubation using fluorescence microscopy to detect potential delivery of Igg-FITC in gametes. Those preliminary experiments allowed us to exclude one reagent (RMF) as precipitation occurred after seawater addition. Although very preliminary, the use of the HiP reagent resulted in the observation of few fluorescent gametes (A) that still displayed chloroplast integrity (B). Further efforts (maximising the contact of fluorescent vesicles and gametes, varying reagents, gamete concentration and gamete strains) are needed to thoroughly assess the efficiency of this strategy. This work, as well as the exploration of protein electroporation, will be continued in Oban thanks to the material kindly shared by Dr Mark Cock.