

Justification for use of the negotiated procedure

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We have recently received the Symbiosis model systems award #9382 titled ‘**Cryotomographic visualization of symbiosis initiation in the *Euprymna scolopes-Vibrio fischeri* association**’ (PI: A Briegel; Co-PIs: McFall-Ngai, Ruby and Septer) from the Moore foundation. For this research, we got an allocated budget to acquire equipment to establish a workflow in in order to perform large volume processing of samples for cryoEM. More specifically, to set up the entire workflow in our institute, we need three pieces of equipment:

- high pressure freezer (HPF)
- cryo-ultramicrotome (cryoMT)
- light microscope with cryostage (cryoLM)

Furthermore, in order to establish this workflow at the Leiden Institute, the biological sample needs to be processed, in sequence, by the different pieces of equipment and ultimately transferred to the instruments available at NeCEN for further processing.

In short, the sample has to be high-pressure frozen in the HPF, then pre-trimmed with the cryoMT and transferred to the cryoLM for target determination. From here, the workflow will continue with the instrumentation of the NeCEN facility: first, the Thermo Fischer Scientific (TFS) Aquilos cryoFIB instrument for further thinning of the sample and finally the sample will be transferred to the TFS TITAN KRIOS instruments for tomography data collection. This workflow is not routine. On the contrary, large volume sample preparation for cryoEM is very much in the developmental stage. In order to succeed, the following requirements are essential:

- (1) **integrated workflow to ensure safe sample transfer between instruments**
- (2) **optimal integration of the instrumentation in the existing equipment park**
- (3) **fast and expert support from the vendor**

***Leica Microsystems* is the only suitable choice as a vendor to provide the necessary equipment (HPF, cryoMT, cryoLM).** It is the only vendor that can meet the requirements outlined above:

- (1) This is the only vendor on the market that can provide an integrated solution for the entire workflow, offering all three pieces of equipment and expert for development of the workflow. Because of this integrated solution, their workflow greatly minimizes sample contamination between instruments and the need to design special attachments for switching between instruments from different vendors.
- (2) Furthermore, *Leica Microsystems* is closely collaborating with *TFS* to integrate their instruments into one common workflow. Therefore, their instruments are optimally set up for the completion of the full large volume workflow at NeCEN, since *TFS* is the vendor for all instruments present at the NeCEN center (Aquilos FIB instrument, Talos cryoEM screening instrument, TITAN Krios cryoEM for high resolution data collection). The *Leica Microsystems* instruments will ensure optimal integration and enable the complete cryoEM workflow.

Additionally, *Leica Microsystems* and *TFS* are continuing their integrative developments for the expansive workflow to ensure minimal sample contamination and maximum throughput. This will be essential for the success of these challenging experiments.

- (3) Furthermore, as mentioned above, the workflow that will be established with this equipment is not standard, but still largely in development. This fact makes a close partnership with the vendors absolutely essential. Co-ordination between three different companies will be highly challenging and would ultimately highly likely result in substantial delays. This can only be avoided by purchasing the instrumentation from the same vendor, in the case *Leica Microsystems*. Additionally, the Leica experts for all parts of this workflow are in close proximity to Leiden University to help with troubleshooting and instrument support.

As outlined above, only Leica can offer the solution that is necessary to fulfill the requirements to perform the research funded by the Moore grant. They are the only company that can meet all set requirements: They can provide the necessary **(1) integrated workflow to ensure safe sample transfer between instruments**, the **(2) optimal integration of the instrumentation in the existing equipment park**, and guarantee **(3) fast and expert support** through their local experts.