High Quality *De Novo* Genome Sequencing of the Common Ice Plant (*Mesembryanthemum crystallinum* L.) - a Functional Genomics Resource for Crassulacean Acid Metabolism (CAM) Biodesign

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Project Goals: Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that exploits a temporal CO_2 pump with nocturnal CO_2 uptake to improve the water-use efficiency (WUE) and the adaptability of plants to hotter, drier climates. The long-term goal of the CAM Biodesign project is to introduce CAM into C_3 photosynthesis plants, such as *Arabidopsis* and *Populus,* and thereby enhance WUE and photosynthetic performance. Major project goals include: 1) defining the genetic basis of key CAM modules in both eudicot and monocot CAM species; 2) characterizing the regulation of 'carboxylation', 'decarboxylation', and 'inverse stomatal control' gene modules of CAM using a wide variety of functional genomic approaches including loss-of-function studies and transcriptome profiling in model CAM species; 3) deploying advanced genome engineering technologies to enable transfer of fully functional CAM modules into C_3 species; and 4) analyzing the effects of these transgenic modules on 'stomatal control', CO_2 assimilation and transpiration rates, biomass yield, and WUE in *Arabidopsis* and *Populus*. Successful transfer of CAM into target C3 photosynthesis species might allow expansion of biofuel feedstock product into water-limited, semi-arid or seasonally dry environments.

The common ice plant (*Mesembryanthemum crystallinum* L., Azoaceae, Caryophyllales) is an important halophytic and facultative crassulacean acid metabolism (CAM) model in which CAM can be induced by salinity or water-deficit stress (1, 2). To improve our understanding of the molecular genetic basis of CAM, water-use efficiency, and salinity tolerance in plants, a high-quality, annotated genome assembly was generated for ice plant using two different genome sequencing platform, Illumina and PacBio, resulting in 95,569,388,632 and 33,865,183,009 bases, with read depths of coverage of 254 X and 90 X, respectively. We assembled the reads in two steps, first with ABySS, SparseAssembler, String Graph Assembler, and SOAPdenovo2 for Illumina reads and SMRTanalysis for PacBio reads, independently. The resulting assemblies were compared with each assembler output with the Illumina mate-pair reads used for scaffolding. The scaffold from the PacBio was combined with the Illumina assembly resulted in the longest scaffolds of 11.6 Mb, N50 & NG50 of 3.2 Mb, 957 scaffolds, and a total size is 382.6 Mb, which is close to the ice plant genome size estimate of 390 Mb from flow cytometry analysis. This accuracy and completeness of the genome assembly was improved further using BioNano Genomics Irys® single-molecule next-generation mapping (NGM) with an increase in scaffold N50 from 3.2 Mb to 4.0 Mb. A total of 44,208 open reading frames were annotated using this genome assembly. This high-quality draft genome assembly will enable a comprehensive identification of genes required for the operation of CAM and for the adaptation

of this halophytic species to salinity stress conditions. This high-quality ice plant genome sequence will also serve as a reference for comparative CAM genomics, tracing the evolution of CAM, and CAM Biodesign efforts aimed at introducing the improved WUE of CAM into bioenergy crops.

References

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