Detecting Darwinian Evolution via Nanopore Sequencing

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Introduction: One definition of life is a "self-sustaining system capable of Darwinian evolution". Thus, the ability to measure evolution is highly relevant to the search for life beyond Earth (Neveu et al. 2018). We previously discussed potential space experiments to measure evolution using nanopore sequencing in a satellite, on/in the International Space Station (ISS), or for life detection (Saboda et al. 2019).

Nanopore Sequencing as an Evolution Metric: We used nanopore sequencing to detect mutations in *Bacillus subtilis* 168 (DSM 402), a model organism and veteran space traveler, that had been exposed to Mars-like solar radiation (broad spectrum UV, 200-400 nm) daily for 69 days, or approximately 700 generations. The UV-evolved strain MW01 was found to be 3-times more UV-resistant than the ancestral strain (Wassmann et al., 2010, *Fig. 1A*). BreSeq analysis (Deatherage and Barrick, 2014) revealed mutations in genes related to metabolism and iron-containing proteins, both hallmarks of the SOS response consistent with the oxidative stress of radiation exposure. Strain MW01 and its dark-evolved counterpart (DE69) were compared to their ancestor and a 3-times faster substitution rate was observed under UV irradiation than in the dark.

Comparison of Nanopore and Illumina Detection of Evolution: Here, we also compare long read nanopore sequencing (NS) data to more accurate, short read Illumina sequencing (IS) data for the ancestral (A), dark evolved (D), and UV-evolved (MW01) isolates, evaluating the potential for nanopore sequencing to detect evolution (*Fig. 1B*).

Mutations in MW01 found with IS were comprised of 81% base substitutions and 19% small indels. NS produced the opposite skew, with 9% base substitutions and 91% small indels, most likely false positives due to this common failure mode of NS.

NS correctly called 52.4% of all 288 mutations found with IS of isolate MW01 (*Fig. 1C*). Of these, it called 67.2% of the 61 synonymous, and 56.9% of the 123 nonsynonymous mutations detected with IS. NS found that mutated genes were primarily enriched in functions related to metabolic pathways, Flavin mononucleotide (FMN), and flavoprotein, respectively. Those found with IS were primarily enriched in functions related to FMN, Phosphopantetheine, and flavoprotein, respectively.

The two sequencing methods were in agreement about two of the top three primary functions associated with mutated genes from the UV-evolved isolate—flavoprotein and FMN. Flavoproteins contain a flavin cofactor, either FMN or flavin adenine dinucleotide (FAD). These are photosensitizers, which absorb UV radiation and, in turn, generate singlet oxygen and other reactive oxygen species (ROS). The ROS cause cellular damage; therefore, mutations affecting Flavin-containing proteins could minimize radiation damage.

Is evolution repeatable? We address this question in a forthcoming paper that probes the effects of prolonged solar radiation exposure on four parallel populations, from the *Bacillus subtilis* UV radiation experiment, in contrast with a population grown in the dark.

Conclusions: With future improvements in nanopore sequencing and analysis methods, including nanopore-specific optimization of BreSeq, it will be feasible to measure evolution within the constraints of future robotic and human space exploration.

References: Deatherage and Barrick (2014) *Methods in Molecular Biology* 1151, 165-88. Neveu et al. (2018) *Astrobiology* ast.2017.1773. Saboda et al. (2019) IEEE Aerospace Paper 2077 (In press). Wassmann et al. (2010) *Astrobiology* 10(6), 605-15.

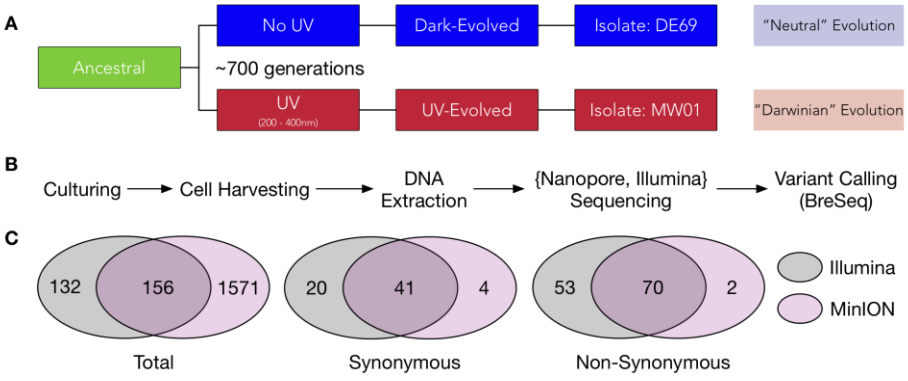

Figure 1. A) *Bacillus subtilis* evolution experiment performed by Wassmann et al. (2010) resulted in isolation of dark evolved strain DE69 and UV-evolved strain MW01. B) Methods used in the present study. C) Variants in isolate MW01 compared to the ancestral strain as a function of sequencing technology.