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Development of a pipeline for precision cloning and effective storage of large synthetic or natural human DNA fragments.

Proposer and Contact information:

Principle Investigator: Bogumil Karas, Ph.D.

Dr. Karas is an Assistant Professor in the Department of Biochemistry with a focus on Synthetic Biology at Western University, London, Ontario, Canada. Concurrently, Dr. Karas is the CEO/Founder of Designer Microbes Inc., whose offices reside in London, Ontario, Canada. Dr. Karas gained his expertise at the prestigious J. Craig Venter Institute (JCVI), under careful guidance and mentorship from Nobel Laureate Dr. Hamilton Smith, where he directed the development of multiple technologies to enable and accelerate genome engineering initiatives. The proposed applicant's efforts at JCVI culminated in three patent applications in DNA transfer technologies, the creation of a first-in-world synthetic organism driven by a minimal genome (Hutchison et al. 2016), and 18 peer-reviewed publications in top-tier world-renowned journals (Nature, Science, ACS Synthetic Biology).

Proposed Location:

Research for the proposed project would be performed at Designer Microbes Inc. (700 Collip Circle, London, ON, Canada, N6G4X8) and Western University (1151 Richmond Street, Department of Biochemistry, London, Ontario, Canada, N6A5C1).

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Background:

In 2001, the sequence of the first human genome was published. The project lasted over a decade and incurred costs approaching 2.7 billion US dollars. Today, an individual human genome can be sequenced within a 24-hour period at a fraction of the cost, approaching \$1000 USD. In 2016, the *Genome Project – Write* [GP-Write] was initiated as an extension of the Human Genome Project whose effort is to synthesize the human genome. One of the main objectives of GP-Write is to develop economical methods to synthesize and store large DNA fragments. In addition, the medical community is facing a similar problem with capturing large human DNA fragments/genes that would be used to identify specific genetic disorders (haplotype analysis) or as a platform for the development of novel medicines (using human genes in mouse model experiments). As the cost of creating synthetic DNA continues to decline, we will require the appropriate host strains and efficient tools to store large DNA fragments that are currently not available. Dr. Karas has spent five years at the J. Craig Venter Institute

optimizing techniques for capturing DNA of various sizes and with variable G+C contents. In one case, Dr. Karas demonstrated that a near-complete 1.5 Mb genome of the bacterium *Acholeplasma laidlawii* could be cloned in *S. cerevisiae* after the identification and removal of a single toxic gene (Karas et al. 2012). In other instance, whole eukaryotic “unclonable” chromosomes could be cloned in *S. cerevisiae* simply with the addition of few extra yeast origins of replication (Karas, Molparia, et al. 2013). Finally, whole 1.8 Mb bacterial chromosomes could be cloned in *S. cerevisiae* by using a novel method where DNA is directly transferred from the donor bacterial cell to yeast by cell fusion (Karas, Jablanovic, et al. 2013). Based on these interesting findings, it is evident that yeast is an excellent host for cloning and storing DNA fragments up to few mega base pairs. However, the process requires further optimization as it is still very labor-intensive and requires personnel with significant experience. To meet the ambitious HGP-Write goals, we need build on our progress to establish methods for cloning even larger DNA fragments for capturing and storing of natural or synthetic chromosomes to facilitate the challenging endeavours enacted by HGP-write.

Technical Idea:

Our research proposal is focused on the development of more effective tools and techniques that will permit precise and efficient capture of human DNA and storage in a stable and benign host vehicle strain. We would begin by designing novel cloning vectors for DNA capture that would combine and improve upon all existing knowledge to enable this feat. Our vector would contain novel combinations of positive and negative selection markers for capturing large DNA fragments. We would also determine the ideal length and composition of vectors that would result in decreased background colonies to improve selection capabilities (i.e. improve the signal-to-noise ratio in the selection process). We will work with bioinformatics teams in an iterative process to predict which DNA fragments can be cloned directly in yeast and which fragments require extra origins of replication to improve stability in the host strain. In addition, we endeavor to incorporate genome-editing technologies, such as CRISPR/Cas9, which has been shown to improve the capture of DNA fragments by pre-processing the targeted regions. These new findings would allow us to establish rules for cloning maximum sizes of DNA with varying complexities. Upon completion of this pilot experiment, the whole process could be automated to enable faster cloning procedures and the stable storage of large, precise DNA fragments.

Utility:

Once our tools are developed, they could then be used to:

- 1) Capture large DNA fragments/genes from an individual to investigate any genetic disease with ease.
- 2) Provide DNA templates to enable synthesis of designer DNA fragments.
- 3) Build a first-in-world library of all human genes cloned with their native regulatory elements (promoters, introns, terminators) in yeast.

Fit for GP-Write:

Based on the experiments described above, we would establish a platform for storing large DNA fragments to be used in the Human Genome Project-Write. We hope to establish collaborations with GP-Write partners who are developing improved DNA synthesis technologies. In addition, since Dr. Karas is located in Canada, it would help to attract the critical mass of Canadian scientists working on the goals of GP-Write.