DNA assembly via optimized and evolved cellular homologous recombination

(DRAFT v1)

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Draft purpose: The author would like to seek feedback before further developing this proposal. Also, the author will not be engaged in the direct laboratory execution of this project, and whether this proposal can find a home in GP-write remains to be addressed. Author can contribute some funding, but probably not all required funding.

Background: Two of the primary bottlenecks for genome engineering are DNA synthesis and DNA assembly. There are many interesting ideas for next-generation DNA synthesis. However, DNA assembly continues to be problematic. Often, the task of DNA fragment assembly into larger fragments is automated using individual reactions to join two or more molecules together, but usually not all of the molecules. To assemble a 1 billion bp DNA molecule, there might be many tens of thousands of different reactions.

Cell-based homologous recombination is an extremely popular technique in molecular biology. Often it's some kind of yeast technique.

Venter/JCVI of course did some work with homologous recombination of large fragments in cells.

Technical idea: Fundamentally, a cell strain can be evolved or designed that is specifically skilled at assembling large quantities of DNA fragments into single DNA molecules. Cells are already used for this purpose in the laboratory, although the limits of this behavior and the limits of how far it can be optimized have not yet been thoroughly explored. Using directed evolution, a strain of cells could be developed such that the cells act as "puzzle solvers". The puzzle is the collection of DNA fragments they are given. The puzzle solution is the correctly assembled DNA molecule. The initial selection rounds will be related to homologous recombination of short DNA fragments into relatively small, but larger, DNA molecules. Over time, the inputs will increase in size and/or quantity, and larger outputs will be selected.

Utility: Speculatively, this technique could be used to develop a "one pot" genome assembly method. Millions of small DNA fragments could be correctly assembled into large DNA constructs.

GP-write fitness: This proposal is directly related to a major bottleneck in large-scale, low-cost genome engineering. The GP-write community is also exceptionally skilled at the techniques required to execute this project.