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for ENGINEERING BIOLOGY

Pilot Project Proposal
(Not to exceed two pages)

Name of Project: Synthesizing a Prototrophic Human Genome

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Background:
Chronic malnutrition is a global health challenge that lead to a variety of systemic diseases, mortality and negative life outcomes, afflicting especially children and adults in underdeveloped and developing countries. In contrast to bacterial cells, which can produce all necessary metabolites needed for growth and maintenance from simple sugars and elemental building blocks, humans are metabolically incapable of biosynthesis of 9 out of the 20 amino acids that are needed for life. Instead, these 9 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are derived from diet. In addition to amino acids, a variety of essential vitamins needed to be consumed as well since they cannot be produced by the body, including vitamin A, B1 (thiamine), B2 (riboflavin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folate), B12 (cobalamin), E and K. While certain vitamins can be produced by humans, they are often not generated at sufficient levels and often require significant dietary supplementation (e.g. vitamins B3 (niacin), C and D). Amino acid and vitamin deficiencies as a result of chronic malnutrition and food shortage can potentially be addressed using synthetic biology approaches. Here, we suggest the possibility of introducing the missing biosynthetic pathways necessary for production of these otherwise essential metabolites in humans from simple sugars from diet.

Technical Idea:
In general, the simpler amino acids are easily derived from core intermediates of metabolism (e.g. alanine and aspartate can be derived from a single step from pyruvate and oxaloacetate). In comparison, more complex amino acids, which require up to 15 enzymatic steps, are the ones that humans are unable to biosynthesize. Due to unknown evolutionary pressures or historically contingent evolutionary trajectories, humans (or related mammals) have yet to evolve the capabilities to synthesize these essential compounds. The biosynthesis of essential amino acids and vitamins are generally well-characterized and the genes and pathways responsible for them are known. We propose

to heterologously introduce these biosynthetic pathways for amino acids and vitamins (as well as the regulatory factors) into the human genome as to enable human cells to be less dependent on (or completely independent of) exogenous supplementation of these metabolites. It is anticipated that the first proof-of-concept studies will be done in the context of cell lines including stem cells, which can be grown in defined media. In the future, one could imagine extending other pathways such as photosynthesis machinery from algae or plants to generate autotrophic human cells.

Utility:
In addition to the eventual goal of generating a prototrophic human cells to combat mal-nutritional conditions, one could imagine this study to be of utility for understanding the biochemical milieu needed for mammalian organismic development, cellular differentiation, and nutritionally-associated aging processes. Since, current mammalian production cell lines (e.g. CHO cells) require growth media that are expensive to scale up, one could imagine using these prototrophic cells with cheaper grow media formulations at a larger bio-production scale, which could lead to more economical biosynthesis of various drugs and compounds that require mammalian cell lines.

"Fit" For GP-write:
The proposed 1% projects fits well with the general and tactical aims of synthesizing a subset of genes and pathways *de novo* that are human-optimized and cannot be generated in any other fashion. It is likely that a variety of pathway configurations need to be explored to optimize biosynthetic levels, which is suited for a DNA synthesis project of this size. The chromosomal integration sites will need to be stable. Thus this effort will likely leverage technical resources, advances, and activities generated by other HGSP projects (e.g. building additional chromosomes or discovering safe chromosomal insertion sites). In order to generate all essential amino acids, vitamins, and other metabolite pathways, we anticipate that >200 kb of DNA will need to be synthesized, possibly more to test various expression/pathway configurations.