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Pilot Project Proposal
(Not to exceed two pages)

Name of Project: Empirically designing genomically-recoded human cell lines

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Background:
The UltraSafe Cell Line pilot project proposes to develop a platform for engineering a biocontained human cell line that exhibits resistance to viruses and produces proteins using an expanded amino acid repertoire. Such cell lines would have immediate biomedical applications in the production of biological therapeutics. Furthermore, the construction of these cell lines will reveal crucial design rules for human genomes, thereby improving our ability to predict mutations that will be tolerated in cell culture.

Technical Idea:
Genomically recoded human cell lines exhibiting noncanonical genetic codes could be produced by replacing all instances of target codons with synonymous codons and then deleting the translation factors responsible for decoding these target codons. Since protein-coding sequences occupy roughly 1% of the entire human genome, any number of codons could be changed while synthesizing merely 1% (3.3×10^7 bp) of the human genome. However, given our tenuous understanding of how to design genomes, it remains difficult to predict which mutations will be tolerated—even for synonymous codon replacements. Fortunately, it is likely that only a subset of codons need to be reassigned in order to achieve virus resistance, biocontainment, and expanded amino acid repertoires. For instance, the UAG (canonically translated as stop) and AUA (canonically translated as isoleucine) codons only account for 1% of the total number of translated codons in human cells and could be functionally reassigned with 1.5×10^5 changes.

This project demands an unprecedented number of changes to the human genome, requiring the knowledge to design viable changes and the technology to implement these changes. A similar challenge has been addressed in Escherichia coli by using an in vivo genome engineering strategy capable of simultaneously evaluating large numbers of desired mutations across heterogenic populations of cells. By multiplexing small numbers

of changes across many parallel populations, it was possible to rapidly assess the fitness impacts of individual codon replacements. This strategy was implemented to completely reassign the UAG codon, to evaluate 12 additional codons for genome-wide reassignment, and to derive genome design rules that are now being implemented to synthesize a genomically recoded Escherichia coli with 7 codons reassigned. A similar genome engineering strategy will be implemented in human cell culture to rapidly assess fitness changes associated with replacing each instance of a target codon. The results will indicate tolerated mutations that could be implemented in a synthetic human genome and illuminate design rules that could benefit future redesigns of the human genome. To accomplish these goals, the GP-write team will need to develop an optimized pipeline capable of rapidly prototyping desired mutations in human cell culture:

1. Develop a method to rapidly introduce targeted genome modifications in multiplex (e.g., reduce CRISPR toxicity and/or develop alternative genome engineering methods; optimize the number of alleles that can be modified in each transfection)
2. Develop a rapid and robust selection for living cells in 96-well plates (e.g., only living cells remain adherent)
3. Develop a strategy to rapidly evaluate heterogenic cell populations (e.g., MIP sequencing)
4. Develop software capable of designing the necessary synthetic DNA and analyzing viable codon replacements