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Pilot Project Proposal
(Not to exceed two pages)

Name of Project: Stable Haploid Human Pluripotent Stem Cells

Proposer and Contact Information:

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Background:

In yeast, and more recently in mammalian cells, haploid cells have provided an effective tool to evaluate gene function. Haploid cells carry a single copy of each chromosome and gene. Therefore, the phenotype of haploid cells provides a functional readout of the single allele present in the cell. In contrast, diploid cells usually carry two copies of each gene, and the change or deletion of one copy often has no functional consequences because of allelic complementation. Methods to modify the genome of a diploid cell commonly result in heterozygosity. In contrast, the modification of a haploid cell will always result in a hemizygous cell. Haploid human pluripotent stem cells have two key advantages relevant to HPG write: they are capable of differentiation into any cell type, and they are human, providing a tool to functionally interrogate the human genome. However, current haploid cell cultures have a significant disadvantage: they diploidize and require repeated sorting for DNA content.

Technical Idea:

We will determine the mechanism of diploidization in haploid human pluripotent stem cells. Determining the mechanisms will inform us regarding the manipulations required to stabilize haploid cell cultures. Our approach is based on the observations that cell cultures continually progress from haploid to diploid, and from diploid to tetraploid. However, while tetraploid cells do not take over diploid cultures, diploid cultures overtake haploid ones. Characterizing the differences between haploid, diploid and tetraploid cells should point us to the mechanism that we can use to stabilize haploid cultures. We will use live-cell imaging of cells marked by H2B-GFP and centromere-

cherry to directly observe diploidization. We anticipate that we will be able to design strategies that stabilize haploid cells.

Utility:

Stable haploid human pluripotent stem cells will be exceedingly useful for HGP-write, as they reduce the workload by half. For a 3 billion base pair genome, this is a significant reduction. Each sequence introduced into haploid cells will provide a direct readout of the functionality of a synthetic sequence. The current rate of diploidization of haploid human pluripotent stem cell cultures requires sorting every 5-6 passages, or about once a month. In our preliminary and currently unpublished results, we show that despite ongoing diploidization, gene editing can be performed while maintaining a haploid culture. However, for more extensive studies, including multiple and sequential genetic gene substitution, the number of sorting rounds required would be astronomical, and potentially result in irreversible damage to the genome, as it uses Hoechst staining and UV illumination.

“Fit” For GP-write

This is a project that will directly support the aims of a human GP write project. Insight gained in this proposal will also be useful for mouse haploid human stem cells. There is significant interest within the HPG community in using haploid human pluripotent stem cells for this purpose.

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