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Pilot Project Proposal
(Not to exceed two pages)

Name of Project: Safety and Containment; Chromatin and Chromosome Structure

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Background:
In reducing the grand vision to practical and achievable goals, I have settled on two issues as an initial foci. 1.) Safety and containment: Can we build reliable tools that would prevent a synthetic genome from ever emerging following hybridization with a natural genome? 2) What can we learn about the contribution of DNA sequence to chromatin and chromosome structure at a more macro way than has been previously possible?

The synthetic yeast genome can play a vital role. There will likely be substantial pushback on the goals of GP-write. But demonstration of a class of questions that can only be answered by use of a synthetic genome will cause the community to better embrace the goals and the perception (and perhaps reality) of the opportunity costs that GP-write could bring.

Technical Idea:
Safety and Containment
If synthetic genomes are to play a role in agriculture, then we will face a daunting scale issue given that millions acres are under cultivation for the few major crops that have been engineered (corn, soybean, cotton, and canola in the US). While the probability of an adverse event is low, the number of opportunities could be enormous. The common occurrence of hybrids in nature makes me think that any synthetic organism could find a way to hybridize with a natural organism at some frequency. So, I think methods should be developed that would ensure that no recombinants from such a hybrid would survive. The classic Drosophila balancer chromosomes provide one type inspiration.

The synthetic yeast genome gives us the chance to see how much containment can be achieved in a well-understood context. The project would consist of using the genome

scrambling capability being put into the Sc2.0 to first scramble one chromosome and then multiple chromosomes to learn the efficiencies by which scrambled balancers could be prevented from surviving meiosis, and to analyze the failure modes that would let a recombinant gamete survive. Ancillary activities would include the development of toxin-antidote combinations, such as restriction-modification gene pairs that could be glued into chromosomes at centromeres such that, if the pair were ever separated, the cell would die.

Chromatin and Chromosome Structure

We have been relatively blind to a middle range of chromatin structures that are bigger than nucleosomes, and beyond the limits of light microscopy. The various Chromosome Conformation Capture technologies, especially Hi-C are making a contribution, but to date they have been largely data collecting exercises rather than experiments. The Sc2.0 and scrambled derivatives provide the logical opportunity to determine what shapes the contact maps that these chromosomes provide.

The Drosophila polytene chromosomes are natures own magnifier of chromatin structure and provided the classic proof that chromatin structure was determined by the underlying DNA sequence. If we could introduce substantial synthetic sequences into Drosophila, we could use polytene chromosomes to uncover the principles behind how particular sequence creates particular structure.