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Pilot Project Proposal
(Not to exceed two pages)

Name of Project: High-throughput HAC Design to Test Connections Between Gene Expression, Location and Conformation

Proposer and Contact Information:
Jeffrey Way, Harvard Wyss Institute
Pamela Silver, Harvard University

Background:
We propose to build two regions of the human genome. Both are about 1 megabase. We would also develop technology for moving these from complete assemblies in yeast into human cells. These would be used in characterizing long-range gene expression mechanisms, and also in engineering humans for an optimized antiviral immune response.

Technical Idea:
The ultimate goal is to enable controlled expression of adjacent genes under different promoters while using insulators to separate these genes and minimize the undesired effects of proximal regulators. We will construct a combinatorial library of ~1 MB segments with different promoters and insulators to assay for optimal gene expression regulation that will be used in the above experiments to generate a complete 4DN picture. We will then apply the designs to current gene expression tools in development in the Silver lab:

(i) **Mammalian interferon promoters**: The Type I interferon promoters and genes exist in a cluster, possibly providing interactive use of one another's regulatory components. This cluster is adjacent to p16/INK4a and the polyamine synthesis salvage enzyme methylthioadenosine phosphorylase (MTAP) in a segment of ~900 kb. This entire segment is often deleted in tumor cells, driven by the tumor cells' need to eliminate p16 and possibly also to eliminate the interferons. Loss of MTAP is probably deleterious, and may be the basis for candidate cancer drugs inhibiting polyamine synthesis. Tumor cell lines have a variety of different deletions of this region, but systematic comparison is not possible because the lines vary in many other ways. We will put about 1 Mb including these genes into the HAC, with adjacent reporters and combinatorial permutations in which segments are deleted as in natural tumors. We will characterize domain organization by Chromosome Conformation Capture (3C), intranuclear

movement, and expression of nearby genes in response to IFN inducers such as poly dI/dC.

The p16-MTAP-Type I IFN gene cluster on chr. 9p. Deletions from p16 extending through this region are common in tumors. IFN genes are highly inducible in most cells with essentially no basal expression.

(ii) **Mammalian estrogen-responsive regions**: We previously characterized numerous estrogen receptor binding sites at significant distances from the genes that they regulate. We will place a ~1 mb segment including a gene such as NRIP-1, which has ER binding sites ~100 kb away from the regulated transcription start, into the synthetic segments in various wild-type and deleted forms. We will characterize domain organization by 3C, intranuclear movement, and expression of nearby genes in response to estrogen.