

# NIH Grant Application

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## Title: **Testing the effects of genetic variations using MINIME technology.**

### Principal Investigators:

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### SUMMARY:

As a result of advances in the ability to routinely sequence human genomes, a large number of genetic variations have been discovered that are hypothesized to be involved in the predisposition to common human diseases. As most of these diseases have polygenic origins, however, it has been difficult to determine the importance of individual genetic variants in an unambiguous manner. This difficulty has drastically limited the value of genetic screening and has hindered the development of more effective methods to manage such predisposed patients and their families.

We here propose to investigate the effects of such variants through experimental rather than epidemiological methods. In essence, we intend to create model organisms retaining most human physiognomic properties but devoid of the essential cognitive characteristics that distinguish humans from more classically used experimental organisms. Although rodents and primates have in certain cases been useful for investigating these issues, the tens of millions of differences between the genomes of humans and all other organisms has precluded use of the latter for the investigation of most variants of interest. The new organisms, called MINIMEs (for miniature manlike entities), should be useful for a variety of purposes. In particular, isogenic MINIMEs, differing only in the genetic variants to be assessed, will be generated and tested in a prospective fashion for the development of particular diseases.

The generation of MINIMEs will involve standard stem-cell engineering principles and will incorporate several novel features, as follows:

1. The *Emx1*, *Emx2*, and *Nkx-2.1* homeobox genes will be deleted in homozygous manner from the neurogenic cells developing in human embryonic stem-cell (ES) explants. These disruptions will ensure that the corpus callosum and telencephalic regions of the brain will be absent from the organisms, rendering them completely devoid of any higher cognitive or emotional functions. ES embryos will be cultivated on artificial amnions previously developed in our laboratory (see, for example, PubMed VVV48306). Fetuses at the 32-somite stage will be transferred to post-amniotic environments modified as described below.

2. Photosynthetic and prototrophic genes from *Arabidopsis thaliana* will be inserted into the germ line of the ES cells under the control of the keratin 14 promoter so that they will be expressed exclusively in the skin. These *Arabidopsis* genes have been shown to confer photosynthetic properties to the skin of rodents, and we anticipate that analogous results will be obtained in the MINIMEs. When exposed to visible light and provided with minerals and nitrogen sources through surgically implanted

time-release capsules, MINIMEs should be fully capable of supporting their own growth. Deletions of appropriate growth hormone-related genes will ensure that the MINIMEs are no larger than 5 kg in size, thus further minimizing the cost and space required for their maintenance. All experiments on MINIMEs will be submitted for approval to our University's CCALF (Committee for the Care of Alternative Life Forms) before their initiation.

3. Many of the most important diseases to be investigated do not develop until adulthood. To speed up development of MINIMEs, the RecQ helicase genes *WRN* and *PRG*, responsible for the Werner and Hutchinson-Gilford forms of progeria, respectively, will be inactivated through targeted homologous recombination. Mutations in these genes lead to premature aging in both humans and mice, and the combination of mutations is expected to lead to adult characteristics within 3 to 5 years, thereby making a variety of investigations possible within the tenure of the grant.

4. Although the first experiments to be performed will be designed simply to assess the role of individual genetic variations in specific diseases, they should eventually provide unique opportunities to investigate disease mechanisms. For example, through the use of transposon-based technologies, variants can be created in specific tissues at specific times of development. Experiments on the resultant MINIMEs should define the specific cells (for example, immune cell or parenchymal cell subtype) responsible for observed phenotypes and should allow determination of whether the disease is reversible upon elimination or replacement of the variation.

In summary, this technology has the capacity to resolve much of the confusion concerning the causal nature of the numerous genetic variations that have been discovered in the last 60 years. Funding of this application should lead to novel strategies for diagnosing, preventing, and treating the large number of human diseases with a genetic component.

The authors are a father-son team whose members are at various stages of their scientific careers. Bert has a lab. Jacob seeks employment in a lab. And Joshua is frequently used as a subject in someone else's lab. Joshua T. Vogelstein, Washington University, St. Louis, MO 63105, USA. E-mail: jtv1@cec.wustl.edu. Jacob V. Vogelstein, Brown University, Providence, RI 02912, USA. E-mail: vitas@brown.edu. Bert Vogelstein, Howard Hughes Medical Institute at Johns Hopkins University, Baltimore, MD 21231, USA. E-mail: vogelbe@welchlink.welch.jhu.edu

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