

*"Correction of Albinism Mutations by Targeted Genomic Rearrangements" led by Dr. Murray Brilliant at the University of Arizona - This is a request for additional funding to continue an existing project for which we provided funding.*

*This project is investigating a novel gene therapy approach to direct genomic rearrangements in living organisms, which would ultimately lead to increased pigmentation in individuals with albinism. They have already demonstrated an ability to restore pigment production in cultured cells and are now looking to perform this same transformation in the retinal cells of animals and, ultimately, people with albinism.*

The requested funding was to continue to test our reagents to measure and optimize delivery systems (lipid aided transfection and viral vectors) and homologous recombination (of the  $p^{un}$  allele and compound heterozygotes). We also proposed to measure the effect of cell-cycle on homologous recombination. These were issues that the NIH reviewers wanted clarified.

We have made significant progress in culturing RPE cells. However, these cells are difficult to grow, require expensive reagents and labor-intense efforts. We have recently developed dermal melanocyte cell lines from  $p^{un}$  mice. While these cells are different from the RPE, they are more readily accessible and because the functional readout is the same (pigmentation) we can use these to pretest the various reagents before we use them on the harder to produce RPE cultures. Several of these lines have been developed and testing with a fluorescent protein marker (GFP) has demonstrated much better transfection efficiency using Lipofectamine 2000 than with Fugene6, FugeneHD, or Turbofect. Additionally, initial testing with a lentiviral vector (GFP readout) showed relatively poor transfection efficiency. These studies are continuing.

Most genetic disorders (including oculocutaneous albinism) are recessive and most people with these disorders are compound heterozygotes. We plan to test the efficacy of homologous recombination to convert one DNA homologue to a wild-type sequence (and the other homologue to harbor two mutations). To obtain a compound heterozygous mouse model for OCA2, we are collaborating with Dr. Yoichi Gondo, Team Leader of the Population and Quantitative Genomics unit of the RIKEN Genomic Science Center. Dr. Gondo was trained in the Brilliant laboratory and at the time was key in the identification of the mouse  $p$  gene and the elucidation of the structure of the  $p^{un}$  mutation. We have developed a multiplex of  $p$  gene exons in *Mus musculus* so that RIKEN Genomics Science Center can screen through their library of mutants using the TGCE screening method. Primer pairs for all 24 exons have been designed and tested. Twenty of the twenty four amplicons have already been optimized. RIKEN will next identify the appropriate point mutations in the mouse OCA2 gene. It may take up to 6 to 8 months to derive, ship, and establish the mouse strains in our animal care facility at the University of Arizona.

Unlike, RPE cells, dermal melanocytes can be easily grown under dividing and non-dividing conditions to test the effects of cell-cycle on homologous recombination. In non-dividing cells, double stranded DNA breaks are resolved by end-joining. We can use these cells to measure the relative frequency of homologous recombination and end-joining as well as the frequency of errors in these processes.

Based on these results, we are currently preparing a new submission for NIH funding. We thank the VFT Foundation for their generous support for this project.