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An arrayed human genomic library constructed in the PAC shuttle vector pJCPAC-Mam2 for genome-wide association studies and gene therapy

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Abstract

The Human Genome Project has ushered in the era of big science. The International HapMap Project's original goal was to catalog the millions of single nucleotide polymorphisms (SNPs) present in the population into many haplotypes in an attempt to establish links between certain variants and specific diseases. All of this research has uncovered over 150 risk loci for more than 60 common diseases and traits. Genome-wide association studies (GWAS) are underway to identify candidate genes for various complex traits such as autism, bipolar disorder, diabetes, obesity and schizophrenia. We constructed an arrayed 115,000-member human genomic library in the PAC shuttle vector pJCPAC-Mam2 that can be propagated in both bacterial and human cells. Microgram quantities of a given PAC clone can be recovered in *E. coli* and then transfected into any human cell line of interest. Transient transfection of a p53-containing PAC clone into the p53-null Saos-2 human osteosarcoma cells demonstrated that both p53 mRNA and protein were produced. Additionally, expression of the p53 protein triggered apoptosis in a subset of the Saos-2 cells as evidenced by an Annexin V assay. When a p53-GFP PAC clone was transfected into Saos-2 cells, immunofluorescence studies demonstrated that the fusion protein was localized to the nucleus and that a subset of cells exhibited the hallmark blebbing that is associated with apoptosis. This library should serve as a valuable resource to validate potential disease genes identified by GWAS in both human cell lines and in animal models such as rats.

Biography

Jonathon Coren obtained his Ph.D. in Genetics from Cornell University in 1991. He did a postdoc at Thomas Jefferson University from 1991-1993. Jon next pursued a postdoc in Nat Sternberg's lab at DuPont Merck Pharmaceutical Company where he modified the P1 bacteriophage cloning system into a P1 Artificial Chromosome (PAC) system. He started as a tenure-track Assistant Professor at Southwestern Oklahoma State University in Weatherford, Oklahoma in 1999. Jon received a $100, 000 NIH R15 AREA grants HG002216-01A1 in September of 2001. He started teaching at Elizabethtown College in 2002 and was tenured and promoted to Associate Professor in 2006.