Molecular Methods (for Health ABC AS01-21, Ferrell project)

Because there is limited amount of DNA on each participant, we initially "bulked up" the genomic DNA via whole genome amplification, using the Genomiphi Kits (product #25660002) from Amersham Biosciences, 800 Centennial Ave, Piscataway, NJ 08855).

The majority of the polymorphisms of interest are SNPs. SNP sites were genotyped by the fluorescence polarization (FP) protocol of Chen et al. (1999; http://psy-sur1.bsd.uchicago.edu/geno/snp/snp.html). About 5% of sites that we have tested do not give acceptable results by FP. For any site that fails by FP, we will use standard RFLP, oligonucleotide ligation assay (Nickerson et al. 1990) or the ABI TaqMan system (Applied Biosystems, Inc.).

Some of the variations of interest are length polymorphisms which may be functional (the AR intragenic CAG repeat, etc.) or may simply mark the gene of interest (IGFI (CA)n, ERA 3'-(A)n, etc). Repeat polymorphisms will be genotyped by amplification using a fluorescently labeled forward primer and resolution of the amplified products on the ABI 3700 sequencer and analysis using the Genotyper software package.