



Announcement

Affymetrix GeneChip *P. falciparum* 10K SNP Genome Array Analysis

This application-based service project is jointly funded by the National Institute of Allergy and Infectious Diseases (NIAID), MR4, and the Burroughs Wellcome Fund. Samples will be processed by Stacy Ricklefs at NIAID's Rocky Mountain Laboratories (RML) Research Technologies Section, headed by Stephen Porcella.

MR4 is accepting applications for submission and analysis of a limited number of *Plasmodium falciparum* DNA samples using a new *P. falciparum* 10K SNP Affymetrix array designed by Xin-zhuan Su at NIAID, Robert Stephens at NCI-Frederick, and colleagues. Application instructions are at the end of this announcement.

Technical Description, Sample Processing, and Requirements

Upon approval, investigators will submit *P. falciparum* DNA samples to the RML Research Technologies Section for processing. DNA samples will be processed with the malaria custom-targeted genotyping panel, which makes use of Molecular Inversion Probe (MIP) technology (Affymetrix), along with universal tags enabling the samples to be hybridized to GeneChip Universal Tag Arrays. MIP technology allows for very dense, multiplexed reactions. Oligo probes are designed with 5' and 3' sequences that are complementary to flanking sequences surrounding the target polymorphism. Internal to the probe are universal PCR primers, cleavage sites, and probe-specific sequences complementary to the oligo tags on the microarrays. The assay involves annealing and ligation of the probe to genomic DNA, thus forming a circular oligo. A cleavage step results in an inverted linear probe with the universal PCR primers exposed on the ends. The SNP-specific amplicons are amplified, labeled, and hybridized to the microarray.

Panel validation has been completed between Affymetrix and RML. During validation at Affymetrix, ~2000 SNPs were filtered from the panel due to the inability to be genotyped. The filtered panel consists of 8079 SNPs. RML ran 24 DNAs which were clustered against the 77 DNAs that Affymetrix ran. 8045 of the 8079 SNPs passed QC. SNPs with less than 80% of the samples being successfully genotyped will be marked as failures. This threshold for passing assays can be adjusted if desired by the investigator. The genotypes for triplicate 3D7 samples were in 99.8% concordance.

For accepted projects, submission requirements for analysis at RML are 300ng of DNA from each sample isolated from cultured parasites at a concentration of 10ng/uL on dry ice. This DNA requirement allows RML to compensate for variation seen in DNA quantitation. If your project samples are unable to meet this minimum requirement (e.g., whole genome amplified field samples less than 300 ng in total), alternative arrangements may be possible but we cannot guarantee the outcome of sample

processing. DNA samples may be consolidated from multiple investigators during processing, as 24 samples are processed in each run.

The data output from the 10K MIP array analysis can be provided in Excel format and does not require proprietary software. Data can be evaluated by end users according to their needs.

Application Instructions

Investigators must be registered with MR4 prior to consideration. MR4 registrants must complete an application form, stating their project title and background for the research materials and/or study. Suggested sample size requests are between 1 and 24 samples. Inordinately large sample sets will not be considered because of the expense and limited number of available Affymetrix chips that have been produced for this project.

Investigators who are accepted for participation will be given instructions on sample submission for processing. *Do not send samples to MR4 when submitting the application. Affymetrix chips will NOT be sent to investigators under this program.* All DNA samples will be processed by NIAID's RML Research Technologies Section.

The application deadline has been extended to June 30, 2010. The number of participants and number of samples accepted under this program are limited. Not all requests will be accommodated. Investigators will be notified within one to two months of application submission whether their application/study samples have been approved for inclusion.