Use of European Collection of Cell Cultures (ECACC) DNA Control Panels (HRC-1) in the Quality Control of High Throughput SNP Studies at MRC geneservice.

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MRC geneservice is a not-for-profit contract research organization and molecular biological resource centre supported by the Medical Research Council (MRC). It is a division of the UK Human Genome Mapping Project Resource Centre (UK HGMP RC). Developing innovative functional genomic products and technology services making them available to academic and commercial research communities worldwide.

A Single Nucleotide Polymorphism (SNP) arises when a nucleotide within a genome can exist as one of two variants. SNPs arise every 500–1000 bases in the human genome. A SNP is of greatest value for genetic mapping studies when both alleles have a frequency greater than ~20%. The special virtues of SNPs for genetic analysis are their ease of discovery and assay. Their high density in the human genome and their effects, in some cases, on transcription and translation make SNPs valuable for association studies.

One assay utilised at MRC geneservice is the 5' nuclease assay (also known as the TaqMan assay). The allelic discrimination is based on the characteristic 5' to 3' exonuclease activity of Taq DNA polymerase. PCR using flanking primers is performed including fluorescent oligonucleotide probes in a homogeneous assay. The probes consist of a 5' reporter dye and a 3' quencher dye, and are specific to the region containing the base change in the region to be amplified. The 5' nuclease activity cleaves the probe if hybridisation occurs, releasing the reporter from the quencher. Two different probes with different fluorogenic reporters are added to the reaction for allele discrimination, one specific to each of the variant alleles to be typed. If there is a mismatch between the probe and target DNA sequence, the hybridisation is significantly reduced, therefore stopping the cleavage of reporter from quencher, and release of fluorescent signal (see figure 1). The amounts of each signal released indicate which allele(s) of the target region is present. The probes have been improved by the introduction of two moieties: nonfluorescent quenchers to reduce background, and minor groove binder to increase the sensitivity of the assay.

The homogeneous nature of the assay with standard reaction conditions means implementation and automation is made simpler, with flexibility as no multiplexing is involved. Reactions are set up using Matrix *Plate* Mate Plus liquid handling robots, before cycling in a KBiosystems Super Duncan thermal cycler. The data is collected using the ABI 7900HT Sequence Detection System with automated 384 well plate loading, to generate up to 100,000 genotypes per day.

In high throughput applications several quality control (QC) steps are required to reduce wastage of customer DNA and reagents that may arise if DNA is of insufficient purity or if the SNP is intractable. A key component of our QC is the use of DNA of uniform concentration and consistently high quality provided by ECACC in its Human Random Control (HRC-1) DNA Panels.

Initially all our newly designed SNP assays are tested in duplicate using 48 ECACC DNA samples, to check that the TaqMan primers and probes function as required with 100% concordance between the duplicate assays. This provides adequate evidence that an assay is reliable.

Despite having a very robust assay, DNA of insufficient purity can still have serious implications on the results that can be obtained, increasing the scatter seen for each genotype, and leading to an increase in the number of samples which cannot be scored. In extreme cases, problems with DNA quality - whether it be very seriously degraded or contains impurities or inhibitors – can prevent accrual of any data.

Our second quality control step compares data from an assay using the ECACC DNA to data using the validated assay with a subset of the customer's DNAs. This enables us to estimate the success rate for the customer's DNAs, and to highlight any DNA related problems prior to starting a large-scale project.

In conclusion, quality control steps are essential at the early stages of high throughput studies to stop the wastage of valuable reagents and DNA, however for these QC steps to be reliable, a source of high quality reference control DNA is required. MRC geneservice has found the ECACC HRC-1 DNA panel to be excellent for this purpose, and so is an essential component of our high throughput SNP genotyping service.

Used by MRC geneservice these New Disease Panels and the Random Control Panels are now available through Sigma. The DNA samples are highly purified and dispensed to give a uniform concentration necessary for controlling validating assays. Each set is delivered in a 96 well format from which the appropriate samples may be selected.

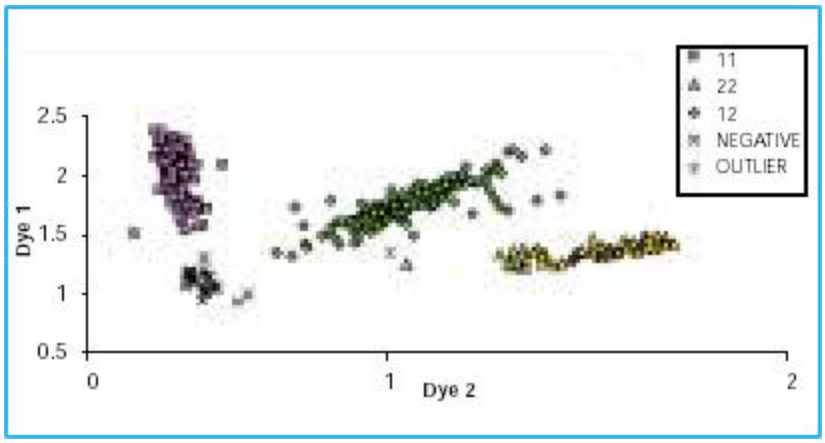
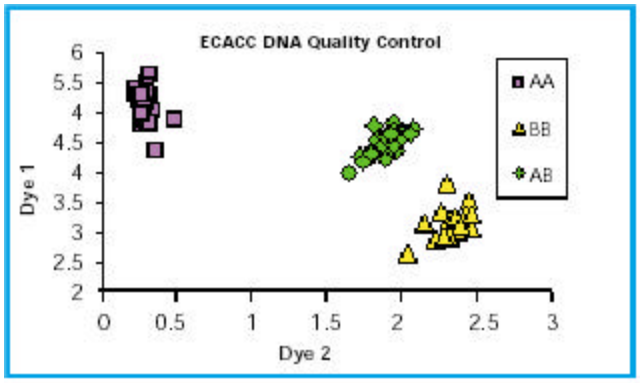


Figure 1: Scatter plot of standard results from 5' nuclease assay reactions analysed on an ABI PRISM 7900HT Sequence detection System using customer DNA. The clusters of output of the fluorescent data are seen: only FAM signal, homozygous allele 1; only VIC signal, homozygous allele 2; increase in both FAM and VIC signal, heterozygous, both alleles present. The negative control is also shown.



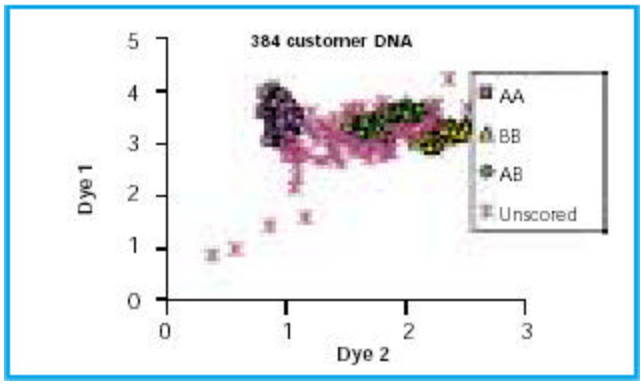


Figure 2: Comparison of 96 ECACC control DNA's and 384 Customer DNA's using the same 5' nuclease assay as in Fig 1. a) The reduced scatter and tighter clustering using the ECACC control DNA shows that the assay is functional. b) Using customer DNA it has resulted in increased scatter and poor grouping, decreasing the effectiveness of the assay and the reliability of the data.