Taqman® Assay Methods

The Taqman® Assay Principles

Taqman SNP analysis utilizes the 5' exonuclease activity of Taq DNA polymerase and the quenching effects of specific fluorescent dyes to determine the presence or absence of each allele within an individual genome. Primers are designed against a conserved region of the genome flanking the locus of interest. Two probes are designed across the locus of interest, one for each allele. Each probe is labeled with a different reporter dye as well as a quencher molecule. Proximity to the quencher dye inhibits the fluorescence of the reporter molecule. During thermocycling, the probe anneals to the locus of interest in an allele-specific manner. As the Taq DNA polymerase extends the primers, it also degrades the annealed probe, allowing the fluorescent dye to come out of the sphere of influence of the quencher and thus become detectable.

Reaction Set up

20 ng of gDNA is dried in the well of a 384 well plate. Five microliters of a master mix consisting of 1X Taqman® Genotyping Master Mix plus 1X appropriate Taqman Assay for Allelic Discrimination is added. PCT is performed according to the recommendations for the assay.

Data capture and Delivery

The GRC utilizes an ABI 7900HT instrument with the capability of running SNPs in either 96 or 384 well format. Following PCR, the fluorescent signal is read for each reporter dye for each well on the plate. A sample yielding signal in only one wavelength is homozygous for the appropriate allele. A sample resulting in equal fluorescence from each reporter is determined to be heterozygous. Results are returned in a text file containing sample ID, (if provided, plate location if not) and SNP genotyping call and are available through a web interface.

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