CDC Methods for Genotype Determination for the Following Genes: Insulin, HLA DRB1, DQA1, DQB1, 3 Microsatellites (TH01, TPOX and CSFPO1) and Sex Specific Marker Amelogenin

Gene: Insulin
Technique: Allelic Discrimination
Description: The genotyping method for the insulin gene is an allelic discrimination reporter assay developed at CDC on the ABI 7700. The polymorphism of interest in the insulin gene involves a single nucleotide change at the –23 position. This method involves two reporter oligonucleotide probes that are specific to the polymorphism. Each probe is bound with a unique fluorescent molecule. If an individual is homozygous, only one of the two unique reporter molecules will be detected. If an individual is heterozygous, both of the unique reporter molecules will be detected.

Gene: HLA DRB1
Technique: PCR followed by Sequence Based Typing (SBT)
Description: The genotyping method for DRB1 involves a two-step process. First, the second exon of DRB1 is PCR amplified from an individual. The second step involves automated DNA florescent sequence analysis of the PCR product using a combination of both generic and sequence specific primers (SSP). The sequence of the DRB1 gene is then analyzed to determine a high resolution genotype. Currently there are 304 possible alleles. This method is commercially available from Forensic Analytical.

Gene: HLA DQA1
Technique: SSP-PCR followed by SBT
Description: The genotyping method for DQA1 was developed in our laboratory at CDC and involves a SBT of exons 2 and 3. Analysis of exon 2 requires an SSP-PCR to divide the 22 possible alleles into two low resolution subsets. The exon 3 PCR amplifies all possible alleles in generic reaction. All three of these PCR reactions are then sequenced using automated DNA florescent sequence analysis. The sequences of the DQA1 gene are then analyzed to determine a high resolution genotype.

Gene: HLA DQB1
Technique: PCR followed SBT and Allelic Discrimination
Description: The genotyping method for DQB1 was developed in our laboratory at CDC and involves a SBT of exon 2 with additional allelic discrimation assays in exon 3. Analysis of exon 2 requires a PCR that amplifies all possible 49 alleles in generic reaction. The PCR reaction is then sequenced using automated DNA florescent sequence analysis. The sequences of the DQB1 gene are then analyzed to determine a high resolution genotype. In rare cases when specific heterozygous genotypes cannot be resolved, SSP-PCR followed by SBT is performed. Additionally, there are several cases where the characteristic polymorphisms are located in exon 3. The polymorphisms located in exon 3 are analyzed using florescent allelic discrimination assays of the characteristic sites to provide the highest resolution genotyping.

Gene:	Three Short Tandem Repeats (STR) Microsatellites (TH01, TPOX, CSF1PO) and Amelogenin (sex specific loci)
Technique:	PCR followed by Florescent Fragment Analysis of STRs
Description:	Three microsatellites, TH01, TPOX, and CSF1PO and the sex specific Amelogenin loci are co-amplified with florescent labeled primers. The number of repeats that an individual has at each loci are determined using the Applied Biosystems Fragment Analyzers with the GeneScan software. The number of repeats for each of the three microsatellites is determined within the trio families to verify family relationships and detect sample mix-ups. Additionally the Amelogenin loci provides sex verification. This method is a commercially available from Applied Biosystems.