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Development of an autosomal SNP multiplex containing 20 SNP loci plus Amelogenin

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Abstract. A single nucleotide polymorphism (SNP) multiplex, comprising 20 autosomal SNPs plus Amelogenin, was developed to analyse highly degraded and low copy number (LCN) DNA template, i.e. <100 pg, for scenarios including mass disaster identification. © 2005 Published by Elsevier B.V.

Keywords: SNP; Biallelic marker; Casework; Validation

1. Introduction

A biallelic SNP multiplex was developed, with primers designed to be close to the single polymorphic base. Analysis of short fragment lengths increases the likelihood of obtaining a result when STRs fail, for example, with degraded samples or low copy number templates [1,2]. To achieve a large stable multiplex we have used the amplification refractory mutation system (ARMS) [3] combined with universal reporter primers (URP) in a two-phase PCR reaction, to amplify DNA fragments ranging from 57 to 146 bp in length. As the multiplex was intended for use with samples too degraded for conventional profiling, a computer program was specifically developed to aid interpretation. The discrimination power of the system was estimated to be 1 in c. 4.5 million, from a white Caucasian population database. Comparisons using casework samples profiled with both the SNP multiplex and low copy number (LCN) AMP*Fl*STR® SGM plus™ (Applied Biosystems) [4] demonstrated a greater likelihood of obtaining a profile using SNPs for certain sample types. Kinship analysis [5] and discrimination power calculations [6] were

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used to assess the usefulness of the system compared to standard DNA profiling techniques.

2. Materials and methods

DNA was extracted from a set of samples using Qiagen™ QiaAmp Mini-Kits (Cat. No. 51306) according to the manufacturer's protocol. SNP multiplex PCR and capillary electrophoresis was carried out on a range of samples, including a dilution series of control DNA extracts, as described by Dixon et al. [1]. All data were analysed using an in-house computer program, Celestial™ (Fig. 1).

3. Results and discussion

Peak height data, collated from dilution series experiments, were used to define the interpretation criteria for use in Celestial™. A series of thresholds determined by experimentation is used to decide whether to designate an allele. A series of heterozygous loci was analysed with decreasing quantities until one of the alleles dropped-out. The peak height/areas of the remaining allele was recorded. This parameter gives the homozygote threshold (*Ht*). If a single allele is observed and its height is less than *Ht* then it is given an 'F' designation [4] to indicate the possibility of allele dropout and the locus may be genotypically heterozygous.

Heterozygous balance (*Hb*) was defined as p_A/p_B where A is the peak height of the smallest allele and B is the peak height of the largest allele [1]. If a calculated *Hb* parameter for a given locus was less than expected (from experimentation), then this indicated potential contamination or presence of a mixture.

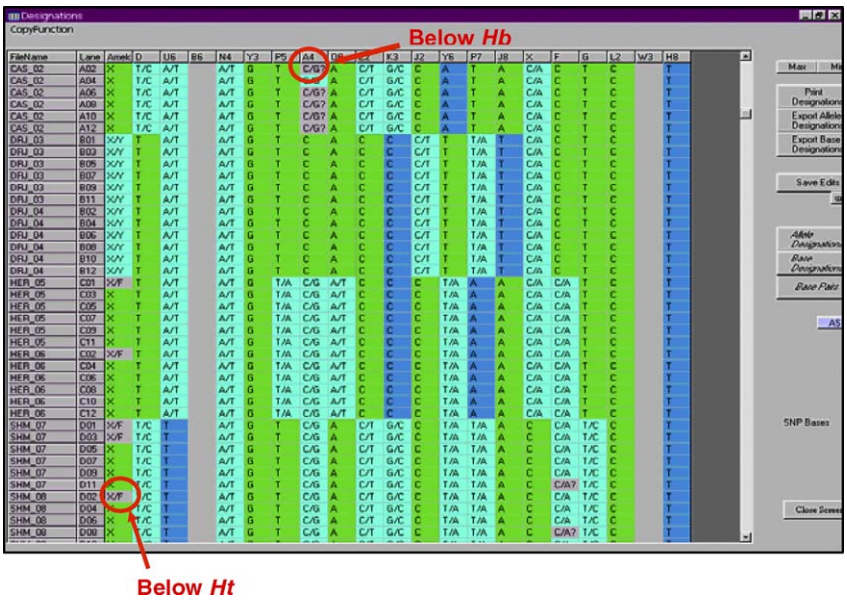

Fig. 1. Output screen from Celestial™ automated SNP genotyper program. The software highlights loci where the *Hb* and *Ht* parameters are below threshold.

All samples, including a further dilution series of controls, were analysed by Celestial™ using *Hb* and *Ht* parameters to designate loci. A total of 42 control samples were all successfully genotyped. An important feature of the experimental design was that *Hb* and *Ht* were individually assessed for each locus. By linking together the multiplex characteristics with a computer analytical program, this simplifies the construction of new batches of multiplexes. When large numbers of loci are multiplexed it is almost impossible to ensure complete reproducibility of inter-loci balance, hence the parameters used by Celestial™ will be prepared specifically to a given batch of multiplex.

In the validation described by Dixon et al. [1], a set of casework samples was amplified using LCN SGM+ and the SNP multiplex. Three out of eight samples gave an increased percentage profile when amplified using the SNP multiplex. Out of these, one sample obtained from muscle gave a higher discrimination power with SNPs than with LCN SGM+ (1 in 1400 compared to 1 in 25 for SNPs and SGM+, respectively).

4. Conclusions

Interpretation guidelines were based on dilution series data identifying the threshold limits for both *Hb* and *Ht*, allowing both optimal (0.5–1.0 ng) and sub-125 pg DNA template to be amplified and genotyped with confidence in the result. These parameters were encoded into Celestial™ software in order to automate the process of interpretation. Further studies on casework samples highlighted the usefulness of SNPs to obtain a result when STRs fail or give a partial profile. The use of a partial SGM+ profile and a partial SNP profile is more discriminating than either profiling techniques individually.

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