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# A validation study of a multiplex INDEL assay for forensic use in four Chinese populations

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ABSTRACT

Insertion/deletion (INDELs) marker sets can serve as a useful supplementary tool for human identification. A commercial kit, the Qiagen DIPplex® Investigator kit, multiplexes 30 biallelic autosomal INDELs and Amelogenin for forensic use. We performed a validation study based on the DIPplex® kit in four Chinese populations: Han, Tibetan, Uyghur, and Kazakh. There were no significant departures from Hardy–Weinberg equilibrium or significant linkage disequilibrium (pair-wised $r^2 < 0.2$) between the 30 INDELs. The random match probabilities were in the range of $3.84 \times 10^{-11}$ to $1.20 \times 10^{-12}$, and the power of exclusion was >0.99. The multiplex PCR was optimized for a 5-μL volume, full profiles were obtained with 0.062 ng/μL of template DNA, and excellent performance was obtained with degraded casework samples. This study demonstrates that the multiplex INDEL assay can be used as a supplementary method for degraded DNA detection in the studied Chinese populations.


## 1. Introduction

Multiplex short tandem repeats (STRs) profiling is a robust, reliable, and sensitive method for individual identification and paternity testing, which is widely used in worldwide forensic DNA laboratories [1–3]. However, STR profiling has several potential limitations. First, the long amplicon sizes of STR loci, generally ranging from 100 to 400 bp, make it difficult to analyze degraded DNA samples, which are usually shorter than 200 bp in length. The STR loci of longer amplicons are also difficult to type. Second, the high mutation rate of STRs (*c.* $10^{-3}$) can confound the kinship results with lower discrimination power.

Single nucleotide polymorphism (SNP) analysis is a complementary strategy to overcome the limitations of STR profiling [4]. The amplicons can be <100 bp, which makes them detectable in highly degraded DNA samples. Further, the relatively low mutation rate of SNPs is attractive for kinship analysis. Indeed, one often requires SNP typing to confirm or overturn STR results [5–7]. In spite of the above advantages, SNP genotyping platforms require complex chemistry and operation procedures, and thus, SNP typing is not yet routinely used in forensic practice.

Insertion–deletion markers (INDELs) are biallelic length polymorphisms caused by the insertion or deletion of several bases. INDELs show many of the advantages of SNPs and are also more convenient for use in multiplex panels compared to SNPs [8]. The multiplex design can be easily implemented as STR kits and detected by capillary electrophoresis [9], and the amplicon sizes can be <200 even 100 bp, which is suitable for analyzing degraded DNA [10]. Commercial INDEL kits have recently been made available, but like STRs kits, population indices should be assured before their widespread use in new populations [11–13]. In this study, we validated a panel of 30 INDELs and Amelogenin (Qiagen DIPplex® Investigator kit) with four Chinese subpopulations. We also conducted sensitivity testing and degraded samples analyses.

## 2. Materials and methods

### 2.1. Sample collection and DNA preparation

Unrelated training samples from four major Chinese population groups included: 97 Han Chinese (35 male and 62 female) recruited from Beijing, 94 Uyghurs (49 male and 45 female) from Urumqi, 96 Kazakhs (48 male and 48 female) from Ili, and 94 Tibetans (48 male and 46 female) from Lhasa. Whole blood samples (2 mL) were obtained from unrelated healthy volunteers with written informed consent. Each participant completed a brief

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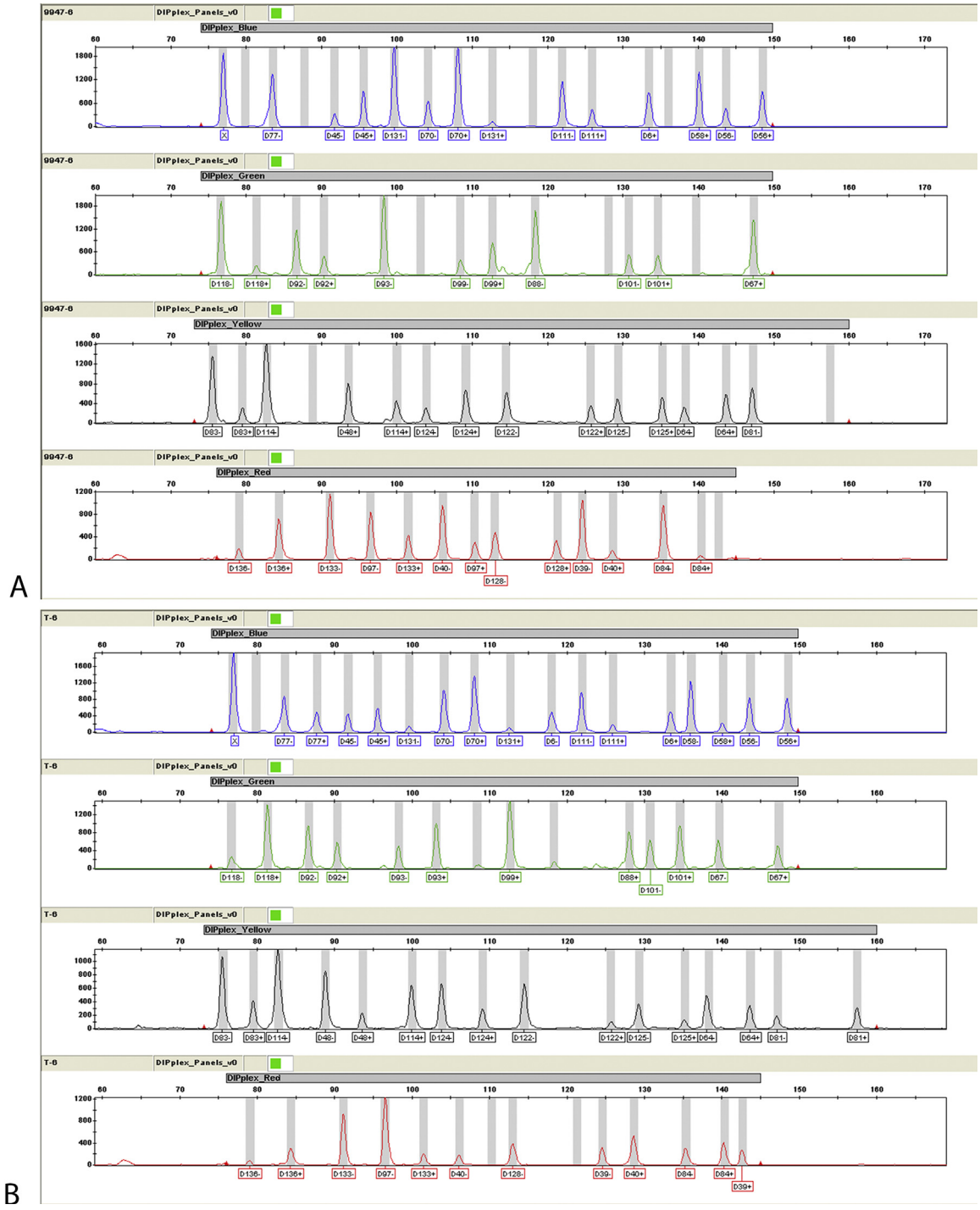


**Fig. 1.** Full profiles obtained from 9947A (A) and T150 (B) at a concentration of 0.062 ng/μL.

questionnaire of general information, including sex, age, population group, etc. This study was approved by the Review Board of the Institute of Forensic Sciences, Ministry of Public Security of China. In addition, four partially degraded DNA samples were collected from crime cases of our laboratory for INDELs typing. The sensitivity study of the DIPplex® kit (Qiagen, Hilden, Germany) was assessed by analyses of dilution series of genomic DNA (0.016, 0.031, 0.062, 0.125, 0.25, 0.5, and 1.0 ng/μL) from a standard DNA 9947A (Promega, Madison, WI, USA) and a training sample (T150).

DNA from whole blood samples was extracted with a QIAamp® DNA blood midi kit (Qiagen). Four degraded samples were extracted from teeth and bone fragments using the skeletal extraction method [14]. DNA quantification was performed by real-time PCR using a Quantifiler™ Human DNA Quantification kit (Life Technologies, Carlsbad, CA, USA) on an AB 7500 (Life Technologies) according to the manufacturer's recommendations. All DNA samples were diluted into 0.5–1.0 ng/μL for amplification.

### 2.2. INDEL typing

We used a commercially available INDEL kit: Investigator DIPplex® kit (Qiagen). According to the manufacturer's protocol, we optimized the PCR volume to 5 μL, containing 4 μL PCR mix and 1 μL DNA templates. The PCR mix contained 1 μL reaction mix