STR-Polymorphisms in non-human Primates

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Introduction

PCR STR systems have proven to be powerful tools in forensic DNA typing. Here we report on the STR systems HumD21S11, HumVWA and HumTH01 in which VNTR polymorphisms can also be detected in many non-human primates, and are therefore useful for DNA typing purposes such as paternity testing or choosing breeding partners in monkeys and apes. There are some structural differences compared to human alleles, which are associated with the evolutionary distances between the different primate groups. As only few data exist for some species these results should be regarded as preliminary.

Materials and methods

The primate species and numbers of individuals investigated were as follows:

Group	Anthr.Apes	Old World Monkeys	New World Monkeys	Prosimians
Spec	Chimpanzee (14) Gorilla(4) Gibbon(5)	Rhesus(23) Crab-eating Macaque(11) Green Monkey(1)	Capuzine(2) Tamarine(1)	Whitehead Lemur(1) Catta(1)

DNA was isolated from single and pooled hair roots in 50 µl reaction volumes containing 5 µl 10X PCR buffer (Promega,USA), 10 µl proteinase K (2mg/ml), 3 µl 0.1M DTT.
The samples were incubated at 37°C overnight or at 56°C for 3 hours. Blood samples were collected on cotton fabric and extracted with Chelex 100 according to Wiegand et al.(1993). Aliquots of 1-10 µl were used for PCR.
PCR amplification was performed using published conditions and primers.
HumTH01 : (Edwards et al. 1992)
HumVWA : (Kimpton et al. 1992)
HumD21S11: (Sharma and Litt 1992) with the following modifications:
temperatures (29 cycles):
94°C - 15s
62°C - 30s
72°C - 75s

10x PCR-buffer and Tth-polymerase (ITC,FRG)

Thermocyclers: HumTH01 and D21S11: Triothermoblock (Biometra,FRG)
HumVWA: TC9600 (PerkinElmer/Cetus,USA)

The electrophoretical separation of amplified HumTH01 and HumD21S11 fragments was carried out in polyacrylamide gels (6% T, 3% C; thickness 750 µm) and denaturing polyacrylamide gels (6% T, 3% C, 10 M urea) for HumVWA fragments with piperazine diacrylamide as cross-linker (Budowle et al. 1991) using a discontinuous buffer system (Allen et al. 1989). The separation distance was 20 cm (15 cm for HumD21S11). Bands were visualized by silver staining according to Budowle et al. (1991).

Results and discussion

STR loci could be detected in a variety of non-human primates. In HumD21S11 a high degree of polymorphism could be observed as exemplified for Chimpanzees and Crab-eating Macaques (Fig.1).

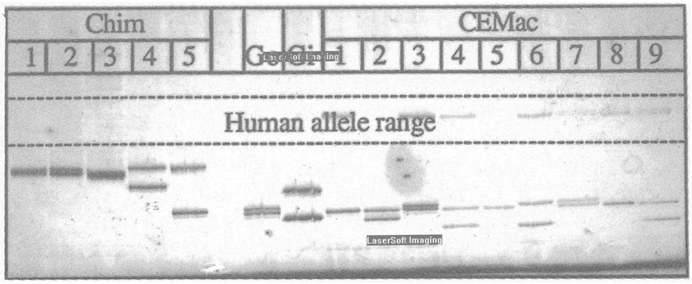

Fig.1: Polymorphism in D21S11. The range of human fragments is marked by broken lines.
Chim: Chimpanzee
Go: Gorilla
Gi: Gibbon
CEMac: Crab-eating Macaque

Concerning the allele sizes, it is evident that shorter alleles are more frequent in non-human primates. Sequence data indicate that this is due to less repeat units (data not shown). Phylogenetically older species such as Crab-eating Macaques show alleles relatively far outside the human range, while in Chimpanzees the fragments are intermediate in size and nearly reach the lower allele range of humans.

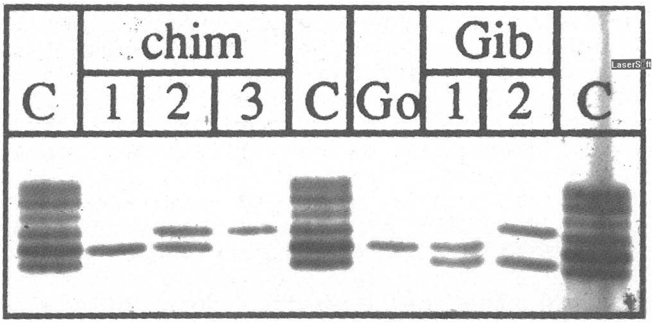

Fig.2: Polymorphic patterns in HumTH01. The fragments of the apes are located within the human range.
C: Human allele cocktail
chim: Chimpanzee
Go: Gorilla
Gib: Gibbon

Polymorphism in HumTH01 could only be detected in anthropomorphous apes (Fig.2). In this system the alleles of the apes are located within the human range and the alleles 5-7 are most common. Old world monkeys show no polymorphism, all species investigated so far are monomorphic having only one fragment with 3 repeats. The Capuzine monkey (a New world species) also shows allele 3 and in the less evolved Tamarine an even shorter allele could be found. Polymorphism in the HumVWA system seems to be phylogenetically older than in HumTH01 and can already be observed at the level of the prosimians. The bands of anthropomorphous apes are partly within the human range and partly outside (Fig.3). Again one can find the tendency of decreasing allele sizes associated with the distances in the evolutionary tree. For an overview of the family tree of primates see Washburn (1985).

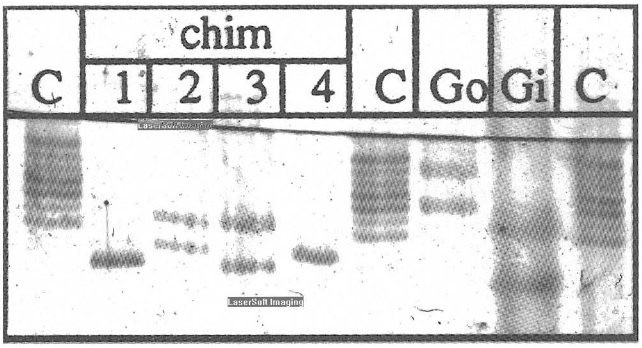

Fig.3 The HumVWA system in apes. Bands can be observed within and without the human range
C: Hum. allele cocktail
chim: Chimpanzee
Go: Gorilla; Gi: Gibbon

Codominant inheritance of STR alleles in a mendelian fashion could be observed in all species investigated so far (8 meioses in Chimpanzees, 3 in Gibbons, 6 in Crab-eating Macaques, 4 in Rhesus monkeys). This fact and the high degree of polymorphism found in most primate species demonstrates that PCR STR systems that have been established in forensic DNA profiling are also informative DNA markers for non-human primates.

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