# ANOMALOUS ELECTROPHORETIC BEHAVIOUR OF HUMACTBP2 (SE33)

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## INTRODUCTION

The microsatellite tetrameric tandem repeat sequence in the 5'flanking region of the human ACTBP2 (SE33) locus (Polymeropoulos et al. 1982) was one of the first STRs evaluated for forensic applications. Because it is higly polimorphic, population studies in caucasians and forensic validation studies have recently been carried out (Polymeropoulos et àl. 1992, Weigand et al. 1993).
The sequence variability of this tetranucleotide repeat has recently been reported (Urquhart et al. In Press) and it has been found that it is an AT rich sequence, with at least 30 alleles, some of which may vary by as litle as 1 base.
In the course of the last EDNAP exercise (Gill et al. submitted for publication) anomalous electrophoretic mobility was observed in some samples which showed different SE33 patterns in agarose and polyacrylamide. Because of this, a study of the electrophoretic mobility in SE33 fragments was carried out using agarose and polyacrylamide at various T and C values- -in some of which denaturing conditions were employed.

## MATERIAL AND METHODS

The primers and amplification conditions for HUMACTBP2 were made as described by Polymeropoulos et al. (1982). The sizes of the ACTBP2 control alleles, determined by sequencing, ranged from 234 to 318 bp.
The electrophoretic conditions are shown in table I. As for PAGE, various T and C values were used including the additions of different concentrations of denaturing agents.
Horizontal discontinous polyacrylamide electrophoresis was performed using 33mM TRIS-Sulfate pH 9.0 as a gel buffer and 0.52M TRIS-Borate pH 8.5 as an electrode buffer in 2% agarose strips. The gels were subsequently silver stained.
The agarose gel consisted of 4.5% Metaphor in TAE, and were run in TAE at 80 V for 5 hours. ACTBP2 fragments were made visible using EtBr.
Msp I digest of pBR322 and a conventional 123 bp were used as ladders.
Polyacrylamide and agarose gel pore sizes were calculated from Ferguson plots of the ladders as described by Holmes et al.(1991).

## RESULTS AND DISCUSSION

Fig.1 shows the different mobilities of PBR322/MspI and the ACTBP2 heterozygote (16-19) used as the control. This ACTBP2 sample was a heterozygote in agarose (Fig.2) and T:5 C:10 gels

(Fig.3) and a pseudohomozy gote in T:10 C:3.3 gels (Fig.4) even when different denaturing conditions were used (Fig.5).
The pBR 322/MspI digest ladder shows different patterns at different T and C values. and has an electrophoretic behaviour in polyacrylamide at high C values similar to that observed in agarose gels. The use of denaturing conditions has only a minimal influence on the mobility of the pBR 322/MspI ladder. Using as reference the normal electrophoretic behaviour of the 123 bp repeat ladder, at least 5 regions of the pBR 322/MspI digest ladder are responsible for that electrophoretic behaviour.
As much as for SE33 fragments as for the pBR322/MspI ladder, different mobilities can be observed for the same DNA fragment in gels with different compositions having the same effective median pore radious. Therefore absolute mobility does not correlate with gel pore size unless the physical properties and composition of the gel are held constant.
From the forensic point of view the more interesting finding was the anomalous migration in the SE33 fragments which is probab ly due to differences in DNA gel interations which are affected by variation in gel compositions and by a DNA bending phenomenum (to which AT rich sequences are more suceptibles). Anomalous migration in AT rich DNA sequences has been reported for AT rich VNTRs and other DNA sequences (Marini et al. 1982, Ulanovsky et al. 1986).
These results are in complete agreement with the last EDNAP results and support the conclusions of the EDNAP report.
We believed that the use of AT rich STRs showing anomalous electrophoretic behaviour should be avoided, but if they are to be used, common electrophoretic systems should be employed.
Each STR should be tested in order to investigate the possible electrophoretic problems before validation.

REFERENCES

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Ulanovsky L, Bodner M, Trifonov EN, Choder M (1986) Proc. Natl. Acad. Sci. USA 83, 862-866.
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Weigand P, Budowle B, Rand S, Brinkmann B (1993) Int. J. Leg. Med. 105, 315-320.

Table 1.- Electrophoretic conditions.

PAGE.-

* TRIS-Sulfate pH 9.0 (gel),
* TRIS-Borate pH 8.5 + AGE 2% (strips)
* 600 V, 15°C, 3 hours. Silver staining.

| T/C | Pore radii | Denaturing conditions |
|---|---|---|
| T:5 C:3.3 | 99 nm | T:5 C:3.3 + UREA 9M |
| T:8 C:3.3 | 80 nm | T:10 C:3.3 + UREA 9M + FORMAMIDE 5% |
| T:10 C:3.3 | 70 nm | T:10 C:3.3 + UREA 6M + FORMAMIDE 15% |
| T:5 C:10 | 27 nm | T:10 C:3.3 + UREA 6M + FORMAMIDE 15% |
| | | T:10 C:3.3 + UREA 9M + FORMAMIDE 15% |

AGE.-

* TAE pH 7.8/ 80 V, 5 hours./ EtBr staining
* METAPHOR 4.5%. Pore radii.- 62 nm.

Fig.1.- Different mobilities of pBR322/MspI (—— and the ACTBP2 heterozygote 16-19 (---)

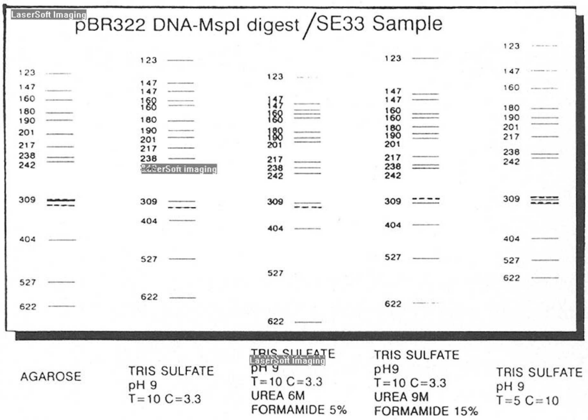
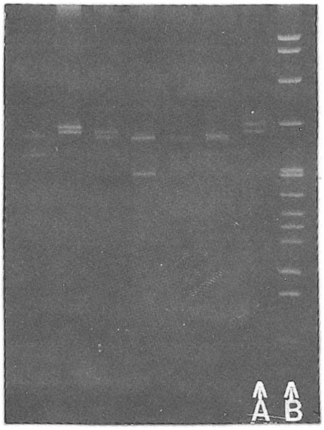



Fig.2.-
Metaphor 4.5%
A.- ACTBP2 control 16-19
B.- pBR322/MspI digest



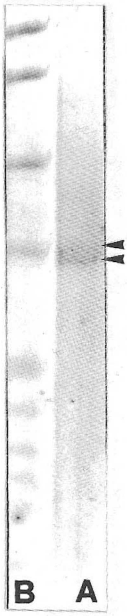




Fig.3

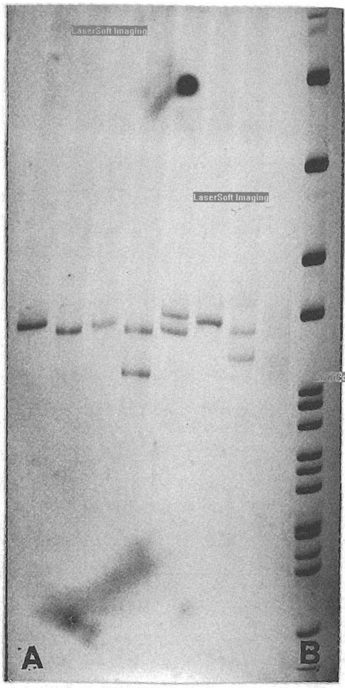



Fig.4

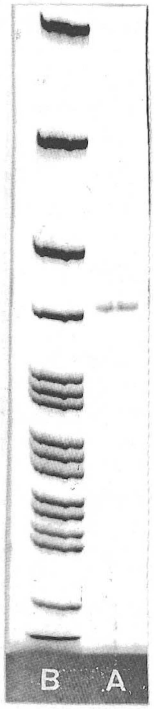



Fig.5

Fig.3.- PAGE T:5 C:10. A.- ACTBP2 control 16-19. B.- pBR322/MspI
Fig.4.- PAGE T:10 C:3.3. A.- ACTBP2 control 16-19. B.- pBR322/MspI
Fig.5.- PAGE T:10 C:3.3+Urea 6M+Formamide 5%. A.- ACTBP2 control 16-19. B.- pBR322/MspI