SPANISH POPULATION DATA FOR THE SHORT TANDEM REPEAT ACTBP2 (SE33)

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INTRODUCTION

Short tandem repeat (STR) loci can exhibit a high degree of polymorphism. One of these STR loci, called ACTBP2 (or SE33) is highly polymorphic and has been reported to be rather robust for forensic analyses [1].

For ACTBP2 to be useful for identity testing, some population data are desirable. This paper describes frequency data for ACTBP2 alleles from a Spanish sample population.

MATERIALS AND METHODS

Population: 120 unrelated Spanish Caucasians from Andalucía.

DNA isolation: organic extraction [2]

DNA quantitation: slot-blot + probe D17Z1 [3]

Primer sequences: 5′-AATCTGGGCGACAAGAGTGA-3′
5′-ACATCTCCCCTACCGCTATA-3′

ANALYTICAL CONDITIONS

1. PCR reaction (final volume: 50 µl)
5 ng DNA
1 µl dNTPs each (0.2 µM concentration each)
5 µl 10X Taq polymerase buffer
1 µl Primer A (from 12.5 µM stock)
1 µl Primer B (from 12.5 µM stock)
0.5 µl Taq polymerase (2.5 IU)
Qs H_2O to 50 µl

2. Thermal Cycler (Perkin Elmer 9600)

Two different sets of cycle programs were linked for a total of 26 cycles as follows:

# 1: 10 cycles.	# 2: 16 cycles.
94 C x 10 sec	90 C x 10 sec

60 C x 10 sec	+	64 C x 10 sec
72 C x 60 sec		72 C x 60 sec

3. Gel Electrophoresis Conditions: according to Lorente, et al [4].

5. Silver Staining: according to Budowle, et al [5].

6. Allele numbers were assigned after comparison with an allelic ladder.

RESULTS & DISCUSSION

SE33 was highly polymorphic in our Spanish population sample (Fig. 1). We have observed 22 different allele categories, with frequencies ranging from 0.013 (alleles 2, 12 and 22) to 0.125 (allele 7); all the other alleles have frequencies less than 0.096. The observed heterozygosity was 89.2%

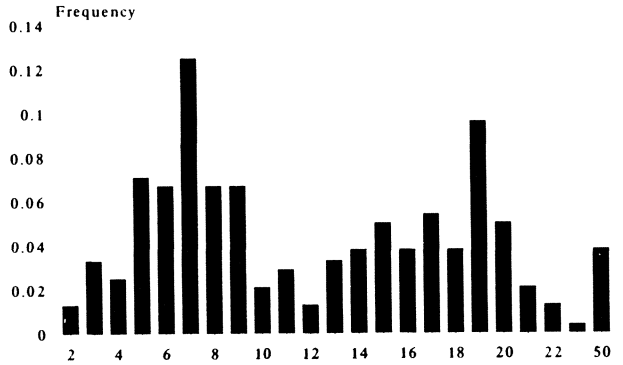

Figure 1. Histogram of ACTBP2 allele/bin frequency distribution for 120 Spanish Caucasians.

As a convention for naming alleles, the ladder bands were given an even number designation (i.e., 2-22). If an allele migrated the same distance as a band in the ladder, the allele was designated with a corresponding even number. If the allele fell between two bands of the ladder, it was given the appropriate odd number designation.

A test for independence for ACTBP2 can be problematic because of the allele designation strategy used. The even numbered alleles generally represent discrete data, while the odd numbered allele frequencies are derived from binned data. This is complicated further by the limited resolution and measurement error of the electrophoretic system. Regardless, the exact test [6] was used for a test of allelic independence. The data suggest that .....

ACTBP2 is a very polymorphic locus. The degree of polymorphism is such that ACTBP2 can be a very informative tool for laboratories interested in human identification. However, the complexity of ACTBP2 necessitates careful designation of alleles, so interlaboratory comparisons can be meaningful. Further work should be performed in order to classify alleles in this system before it is adopted for forensic identification. The potential of a two base pair difference in size between two alleles requires that a laboratory show that it has achieved two base pair resolution; otherwise a binning strategy for allelic data may be necessary. We have amplified ACTBP2 alleles with different sets of primers such that the amplified products for the same allele differ in length by two bases. When such amplified alleles are co-electrophoresed in the same lane, they can be resolved (data not shown).

Future developments for ACTBP2 should focus on producing a common allelic ladder which can be used in a variety of laboratories. Additionally, comparison of different electrophoretic systems may have to be compared for typing efficacy of ACTBP2.

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