

# HIGH RESOLUTION HLA-B\*27 TYPING BY PCR-SSP AND ALLELE FREQUENCIES IN 'WELSH' BLOOD DONORS

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## Introduction

The HLA-B27 gene is a major histocompatibility gene with one of the strongest associations with a human pathological condition. 95% of ankylosing spondylitis (AS) patients carry HLA-B27 and there is also a strong association, although less so, with reactive arthritis and other diseases termed the spondyloarthropathies in most ethnic groups.

There are currently 24 allelic variants of the HLA-B27 gene. Not all B27 alleles have been shown to have a positive association with AS, for example B\*2706 and B\*2709 have both been shown to have either no association or a negative association.

Typing for the presence of HLA-B27 is routinely used as an aid for AS diagnosis. Serological based methods were historically employed but flow cytometry or DNA based methods are now preferred.

HLA typing to the allelic level is important in bone marrow transplant matching and population genetics studies as well as for studies of HLA and disease association.

## Methods

We designed a PCR-SSP typing set comprised of 24 mixtures employing 29 primers to differentiate the 24 alleles of the B\*27 family. The typing set works under the same conditions as our low resolution class I HLA PCR-SSP typing.

The typing set was able to differentiate the following B\*27 alleles:

B\*2701, B\*2702, B\*2703, B\*2705, B\*2707, B\*2708, B\*2709, B\*2710, B\*2711, B\*2712, B\*2713, B\*2714, B\*2716, B\*2717, B\*2718, B\*2719, B\*2720, B\*2723, B\*2724. B\*2704 was not able to be differentiated from B\*2715 or B\*2725 and B\*2706 was not able to be differentiated from B\*2721 (based on IMGT/HLA database v1.14).

These included all B\*27 alleles (4 digit) listed in the 2002 HLA Nomenclature.

B\*2704, B\*2715, B\*2725, B\*2706 and B\*2721 are thought to occur at a low frequency in the largely north-western European Caucasoid local population.

As part of the validation process the set was used to type 'reference' DNA samples possessing: B\*2701, B\*2702, B\*2704, B\*2705, B\*2706, B\*2707, B\*2708, B\*2709, and B\*2723. These samples had been sequenced for HLA-B, often by several laboratories, and included material from UK National External Quality Assurance Scheme for Histocompatibility and Immunogenetics; the University of California, Los Angeles, Tissue Typing Laboratory exchanges and the European Federation for Immunogenetics DNA Quality Control Exercises.

Helmberg's SCORE software was used to analyse the PCR-SSP mixtures using version 1.14 of the IMGT/HLA allele database. This confirmed the amplification reactivity of each mixture and evaluated the set for allele combination ambiguities.

Typing with 41 further mixtures differentiated the B\*27 alleles from all other HLA-B alleles except that B\*2704/15/25 could not be distinguished from B\*2720 in the presence of B\*0814.

A random contiguous group of 353 B\*27 blood donors from a total population of 4020 was typed. This represents a B\*27 carriage frequency, in the total population of 8.78%, and a gene frequency, by direct counting, of 0.04428.

## Results

A total of 7 different B\*27 alleles were identified in the group of 353 donors. These are shown, with the number of subjects each allele was found in, the percentage proportion of the B27 group and the carriage and gene frequencies in the whole population, in Table 1.

**Table 1. B\*27 alleles found in 'Welsh' blood donors**

Allele	No. Subjects	% in B*27 group	Carriage % in population	Gene freq. in population
B*2701	2	0.57	0.050	0.00025
B*2702	22	6.23	0.547	0.00274
B*2703	1	0.28	0.025	0.00012
B*2705	324	91.79	8.060	0.04055
B*2708	3	0.85	0.075	0.00037
B*2709	1	0.28	0.025	0.00012
B*2717	1	0.28	0.025	0.00012

Two donors were apparent B\*2705 homozygotes and one was heterozygous for B\*27: B\*2705, B2702.

## Comments

The B\*2705 and B\*2702 frequencies were similar to those described in other European Caucasoid populations. The presence of B\*2703 and B\*2709 suggests a minority of non-northern western European Caucasoid haplotypes in our local blood donor population.

PCR-SSP provides an accurate and simple technique for high resolution typing of HLA-B\*27 individuals. The typing set can be easily updated in order to include the detection and differentiation of newly discovered B\*27 alleles.