# TECHNICAL NOTE

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# Genetic Typing of the DQA1*4 Alleles by Restriction Enzyme Digestion of the PCR Product Obtained with the DQ Alpha Amplitype™ Kit

**REFERENCE:** Salazar, M., Williamson, J., and Bing, D. H., **"Genetic Typing of the DQA1*4 Alleles by Restriction Enzyme Digestion of the PCR Product Obtained with the DQ Alpha Amplitype™ Kit,"** *Journal of Forensic Sciences*, JFSCA, Vol. 39, No. 2, March 1994, pp. 518–525.

**ABSTRACT:** The three DQ Alpha 4 alleles, 4.1 (0501), 4.2 (0401), and 4.3 (0601) cannot be distinguished with the reverse dot blot DQ Alpha Amplitype™ Kit. A previous report (Yunis, I. et al., Tissue Antigens, Vol. 39, 1992, pp. 182–186) indicated that the typing of these three alleles can be accomplished by endonuclease digestion of the PCR product that is produced following amplification with the DQ Alpha primers with Fok I and Rsa I. We report here the use of this method to type the DQA1*0401, 0501 and 0601 alleles in the PCR product obtained with the DQ Alpha-Amplitype™ Kit. We have analyzed the PCR product obtained in over 200 forensic samples. We have found that in all of these cases, it is possible to assign a type to those samples that type as DQ Alpha 4 with the Amplitype Kit. Furthermore, we have found the technique to be useful in some cases where it has not been previously possible to distinguish individuals or samples that have the DQ Alpha 4 allele and type identically with respect to all other DQ Alpha alleles.

**KEYWORDS:** pathology and biology, polymerase chain reaction, DQ alpha, restriction enzymes

DNA analysis of forensic samples with polymerase chain reaction (PCR) methods has allowed for detection of genetic markers in samples containing as little as 2 ng of DNA. The DQ Alpha Amplitype Kit™ (Perkin Elmer, Norwalk, CT) is one of the most widely used PCR based diagnostic testing kits used for analysis of such samples, and has become almost a standard methodology in those forensic laboratories that are performing DNA based analyses of forensic materials. One of the drawbacks of this kit is that it only types six out of the eight possible DQA alleles. More specifically, the immobilized probes that are part of the reverse dot blot format detect all DQA1*4 alleles (DQA1*0401, 0501

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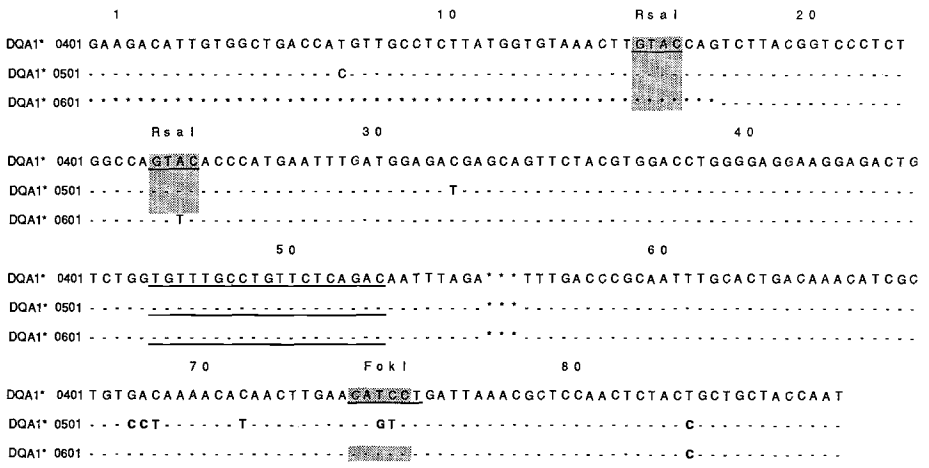


FIG. 1—*Nucleotide sequences of the DQA1*4 alleles 0401, 0501 and 0601. The presence of the restriction sites are shaded. The numbers refer to the amino acid position. The solid bar is the sequence of the probe used in the DQ Alpha Amplitype™ Kit.*

and 0601) with a single probe that recognizes a common nucleotide sequence [*1*]. The differences in the DQA1*4 alleles can be recognized by two restriction enzymes, more specifically at position 25 by Rsa I [A and T] and at position 76 by Fok I [C and T] (see Fig. 1). A restriction map of all of the DQA1 alleles is presented in Fig. 2. Thus, if the other DQA1 alleles are known, it is possible to assign the correct DQA1*4 allele with just two restriction enzymes. Studies by others [*2–4*] have demonstrated that restriction analysis of the PCR product of the second exon of the DQA1 region can be used to type all DQA1 alleles. We report here a specific application of this technique to the PCR product obtained by use of the DQ Alpha Amplitype Kit obtained from Perkin

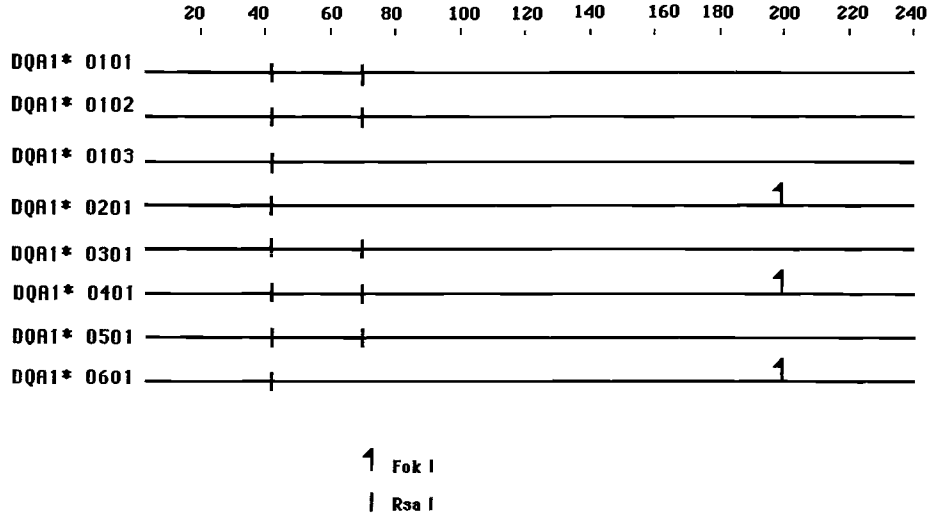


FIG. 2—*Restriction map of the DQA1 alleles with Fok I and Rsa I. The numbers refer to the nucleotide sequence.*

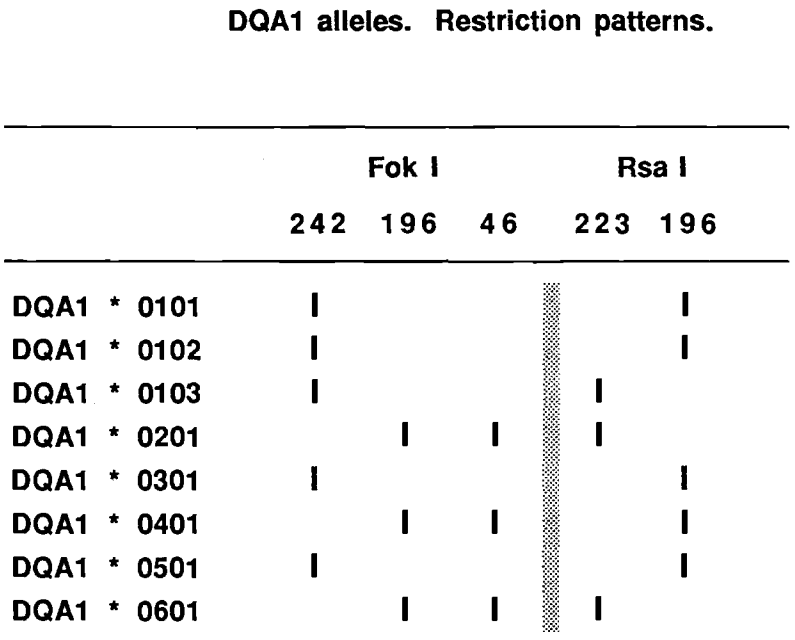


FIG. 3—*Expected patterns following restriction with Fok I and Rsa I of the PCR product produced by amplification of DQA1 alleles with primers included in the DQ Alpha Amplitype Kit. The size of the band in base pairs is included at the top. The presence of the band is noted by the vertical line.*

Elmer and developed by Cetus. The technique has been evaluated with over 200 forensic samples obtained from about 30 cases.

## Materials and Methods

### *Preparation of DNA Samples*

DNA was extracted from all reference blood samples and from stains by standard methods involving digestion with Proteinase K in the presence of Sodium Dodecyl Sulfate (SDS), extraction with chloroform-phenol-isoamyl alcohol, washing with *n*-butanol and concentration, and washing with TE buffer in Centricon 100 concentrators (Amicon, Corp. New Bedford, MA) [5]. Mixed stains containing cells derived from the victim and spermatozoa derived from assailants were separated in a two-step method into epithelial cell lysate and male lysate fractions by a method which first extracts DNA from epithelial cells and then extracts DNA from the sperm [6]. All genomic DNA samples were stored at 4°C until used. The concentration of DNA was estimated following submarine electrophoresis in TAN buffer of 5 μL of each sample in a 0.8% Agarose gel (SeaChem, FMC Corp, Rockland, ME) [7].

### *DQ Alpha Amplification and Typing*

All samples were amplified and typed in the reverse dot blot format with the DQ Alpha Amplitype kit according to the method provided by the manufacturer.

Following assignment of the DQA1 alleles, the DQA1*0401, 0501 and 0601 alleles were typed according to the method described by Yunis, et al., [3]. Briefly, 20 μL of each sample was digested separately with 5 units of each enzyme with buffers supplied by the manufacturer in a final volume of 25 μL for 3 h at 37°C in a covered 96-well microliter plate. The enzymes, Fok I and Rsa I used in this study, were obtained from New England Biolabs, Beverly, MA. The entire sample was loaded into a 4% agarose gel containing a final concentration of 2% NuSieve agarose (FMC Bioproducts, Rockland, ME), 2% LE Agarose (BRL, Gaithersburg, MD), and 0.5 μg/mL Ethidium Bromide (Sigma, St. Louis, MO) in 0.5X TBE buffer. Electrophoresis was done in submarine electrophoresis for 3 h. The progress of electrophoresis was followed with a visual marker consisting of the 123 base pair ladder obtained from BRL mixed with loading buffer containing bromphenol blue [3]. The gel was photographed under UV light irradiation.

*Study Design*

All DQA1 typings were done with the DQ Alpha Amplitype Kit prior to the analysis with the restriction enzymes. The results of the DQA typings were blinded and the results obtained by the two methods were reconciled only after both analyses were completed. In all cases, controls included reagent blanks (where available) prepared during the isolation of DNA samples, a known positive genomic DNA sample isolated from whole blood in parallel with the reference samples and evidence, the positive control (DQA1 = 0101/0501) and the DNA sample.

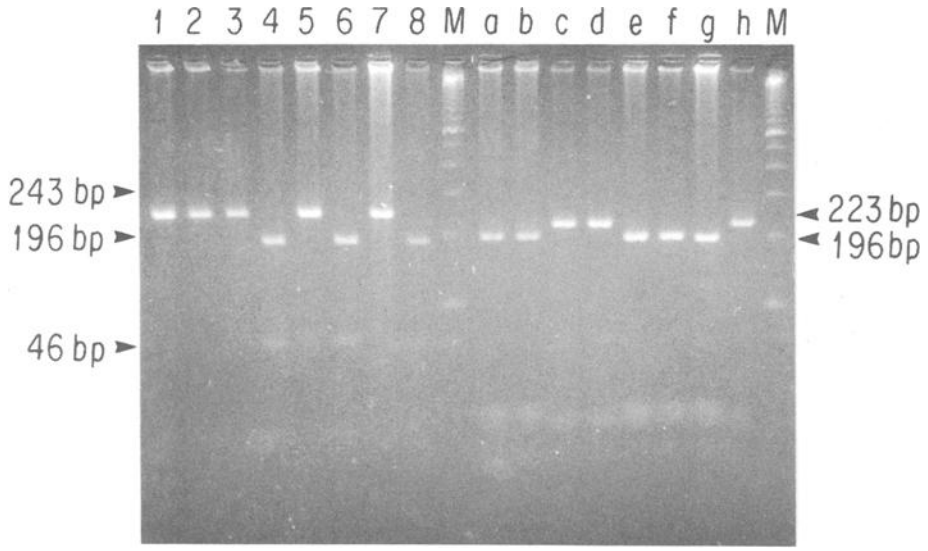


FIG. 4—*The band pattern of DNA isolated from known homozygous cell lines, amplified with the DQ Alpha Amplitype kit and restricted with Fok I and Rsa I. Lanes 1 through 8 are samples restricted with Fok I, lane M is a 100 bp ladder, and lanes a through h are samples restricted with Rsa I. 1, a is DQA1*0101, 2, b is DQA1*0102, 3, c is DQA1*0103, 4, d is DQA1*0201, 5, e is DQA1*0301, 6, f is DQA1*0401, 7, g is DQA1*0501, and 8, h is DQA1*0601.*

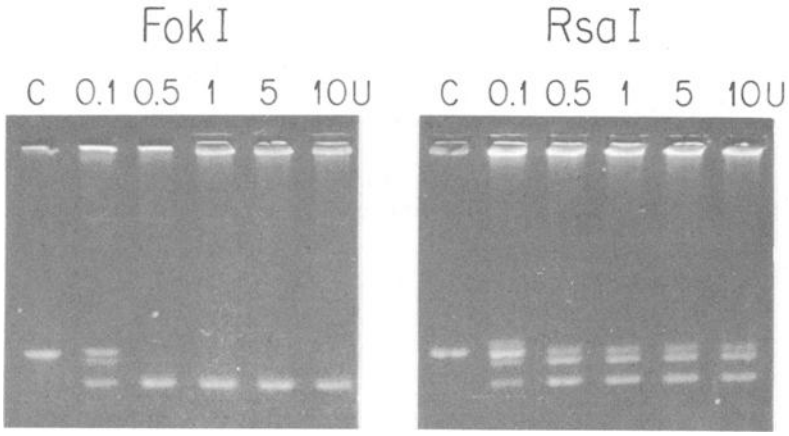


FIG. 5—*Optimization of enzyme concentrations for restriction of the PCR product obtained with the DQ Alpha Amplitype Kit. U is units of enzyme. C is the undigested control. With Fok I, 0.1 U gave partial digestion of the amplified product. The optimal amount of each enzyme was established to be 5 U.*

## Results

### *Restriction Patterns of DQA*1 Following Fok I and Rsa I Digestion*

The pattern of restriction digestion with Fok I and Rsa I of the PCR product obtained following amplification of the second exon of the DQA1 gene with the DQ Alpha Amplitype Kit is presented in Fig. 3. Figure 4 shows the patterns obtained following digestion of homozygous cell lines with these two enzymes. The results show the following: Fok I produces two fragments of 183 and 59 bp for 0401 and 0601 and one fragment of 242 bp for 0501. The 0401 and 0601 are distinguished by Rsa I, which produces a 194 bp fragment for 0401 and 221 bp fragment for 0601. The 0201 allele will give the same pattern as 0401 and 0601 with Fok I. Thus, it is necessary to have previously determined whether this allele is present with the DQ Alpha Amplitype Kit.

### *Concentration of Fok I and Rsa I*

The concentration of the restriction enzymes to be used was determined as follows: For Fok I, DNA obtained from the 11th Histocompatibility Workshop [*8*] that typed as DQA1*0401/0401 was used, and for Rsa I, the DNA typed as DQA1*0103/0501 by the

TABLE 1—*Rape and assault case.*

| | DQA Type | DQA 4 Type |
|---|---|---|
| 1. Victim | 3/4 | 0501 (4.1) |
| 2. Suspect 1 | 3/4 | 0501 (4.1) |
| 3. Suspect 2 | 3/4 | 0501 (4.1) |
| 4. Vaginal swab #1 epithelial cell lysate | 3/4 (weak 2) | 0501 (weak 0201) |
| 5. Vaginal swab #1 male cell lysate | 3/4 | 0401 (4.2) |
| 6. Vaginal swab #2 epithelial cell lysate | 3/4 | 0501 (4.1) |
| 7. Vaginal swab #1 male cell lysate | 3/4 | 0401 (4.2) |

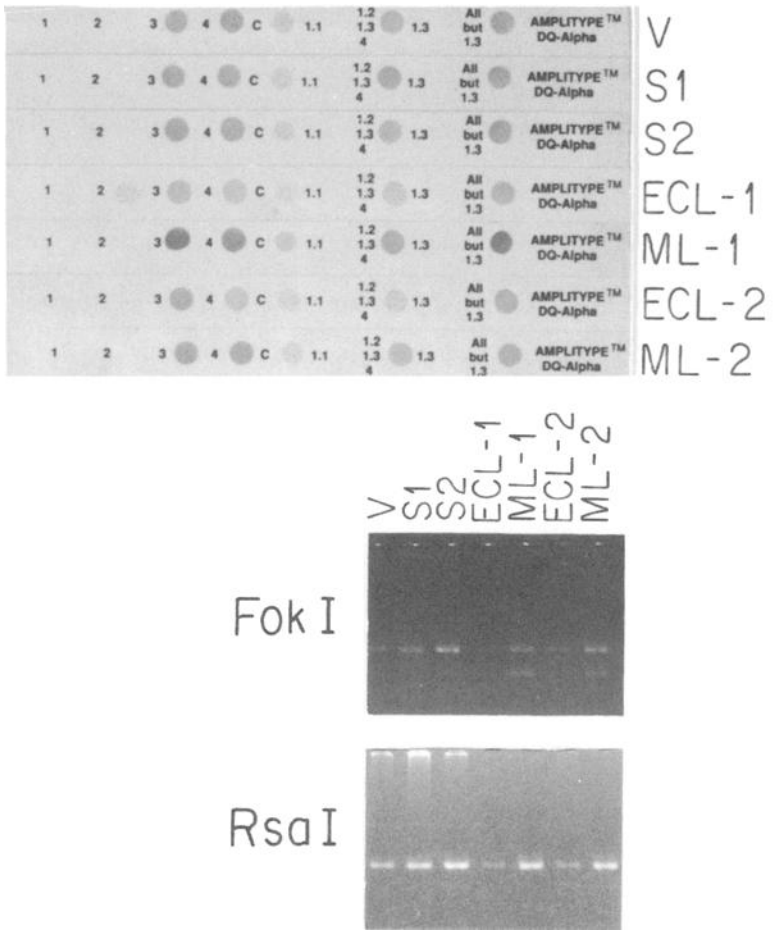


FIG. 6—*Analysis of rape and assault case with DQ Alpha Amplitype Kit followed by analysis of PCR product with restriction enzymes Fok I and Rsa I.* A. *DQ Alpha Amplitype Kit Results. V, victim, DQA1 type 3,4; S1 Suspect 1, DQA1 type 3,4; S2, Suspect 2 DQA1 type 3,4; ECL-1, epithelial cell lysate 1, DQA1 type 3,4, weak 2; ML-1, male lysate 1, DQA1 type 3,4; ECL-2, epithelial cell lysate 2, DQA1 type 3,4; ML-2, male lysate 2, DQA1 type 3,4.* B *DQA1 typing of 4 alleles. V, victim, 0501; S1 Suspect 1, 0501; S2, Suspect 2 0501; ECL-1, epithelial cell lysate, 1 0501 weak 0201; ML-1, male lysate 1, 0401; ECL-2, epithelial cell lysate 2, 0501; ML-2, male lysate 2, 0401.*

direct dot blot method that uses radiolabeled sequence specific oligonucleotides [*8*]. These samples type as 4 and 1.3/4, respectively with the DQ Alpha Amplitype Kit. These results are presented in Fig. 5. It was determined that as little as 0.5 units of each enzyme could be employed, but for routine use we chose to use 5 units to insure complete digestion of the amplified product. Ten units of the enzyme did not lead to over digestion of the PCR product (Fig. 5).

*Typing of Forensic Samples*

The utility of the method is illustrated in the case summarized in Table 1 and Fig. 6. In this case, the samples consisted of three reference samples (one victim and two sus-

pects) and two vaginal swabs. All of the samples typed as DQ Alpha 3/4 with the DQ Alpha Amplitype Kit (Fig. 6*A*). Analysis of the banding pattern following endonuclease digestion with the two enzymes indicated that the victim and the two suspects typed as DQA1*0301/0501 (Fig. 6*B*). The epithelial cell lysate typed as DQA1*0301/0501, consistent with the typing found for the victim. The male lysate typed as an 0301/0401, indicating that none of the donors of the reference samples could be the contributor of the DNA extracted, amplified and typed with the DQ Alpha Amplitype Kit.

This technique has been used to analyze 30 cases where the victim and suspect shared a DQA1*4 allele. The types observed were as follows: All three alleles have been observed; in 24 cases, DQA1*0501 was observed, in 6 cases, DQA1*0401 was observed, and in 6 cases, DQA1*0601 was observed. This includes analysis of over 200 samples which typed homozygous for DQA1*4 as well as heterozygous for DQA1*4 with all other DQA1 alleles. To date, in only one case have the typing of the four alleles proved to be informative in terms of distinguishing possible donors of DNA extracted from samples collected at crime scenes. In no case was a different type observed than was found in the reverse dot blot format. That is, there were no discrepancies in terms of detection of the other DQA1 alleles.

## Discussion

We undertook this study to show that the DQA1*401, 0501 and 0601 (DQA4 alleles) can be distinguished by restriction analysis of the PCR product detected with the DQ Alpha Amplitype Kit. The advantages of this kit are that it can detect all other DQA1 alleles, it has been optimized to type 2 ng or less of genomic DNA, and it has been developed with both positive and negative controls that allow for accurate genetic typing of forensic samples. The purpose of this study was not to suggest an alternative to the DQ Alpha Amplitype test. Rather, we suggest that the method we describe is a simple and rapid way to determine if typing the DQA1*4 allele can lead to informative results when comparison of DQA1 typing results on the reference sample does not allow for unequivocal discrimination of possible donors to DNA extracted from the physical evidence. This would be the case for example when both the victim and the suspect have the identical DQA1 type and/or where one or both alleles detected is DQA1*4.

An advantage of the procedure described in this report is that it is done on the amplified DNA obtained using the DQ Alpha Amplitype Kit and requires no further processing or consumption of the evidence. The test is done with equipment that is already required for use of the DQ Alpha Amplitype Kit and only requires the purchase of two enzymes and agarose. Thus, in laboratories that do not use radioisotopes for detection of Class II Antigens, this is an easy technique to perform, as it only adds about six hours to the total analysis. One requirement, however, is to be certain that the amplified DNA can be kept separated from other areas of the laboratory during the steps used to prepare the endonuclease digests.

It can be estimated how often a different four allele will be seen based on the genotyping results of the Class II antigens presented at the 11th Histocompatibility Workshop in 1991 [*8*]. Briefly, the most common DQA1*4 allele in Caucasians is 0501, where the estimated frequency is 20.7% (Table 2). Likewise, in both South African and North American Blacks, the predominant DQA1*4 allele is 0501, but 0401 is elevated in between 13.5% and 19.2% as compared to 3% in North American Caucasians (Table 2). In other ethnic groups, it is different. Oriental populations have increased levels of DQA1*4 allele 0601 ranging from 9.6% in Chinese to 7.3% in Koreans and to 1.9% in Japanese. Similarly, studies from our laboratory on South American Indians indicate the 0401 allele is the predominant DQA1*4 allele, with an estimated allele frequency of 10% to 20% for DQA1*0401, 10% to 20% for DQA1*0501, and none found for

DQA1*0601 (J. Yunis et al., Manuscript in preparation). Based on these results, it can be predicted that the DQ1*4 allele that will most often be found will be 0501. However, as noted in the case work analyzed for this report when different DQA1*4 alleles are found, it can provide important information. Thus, the advantage of the technique described in this report is that it allows for complete analysis of all eight DQA1 alleles and extends the utility of the DQ Alpha Amplitype Kit.

## References

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Editor

## Erratum

In the Technical Note "Genetic Typing of the DQA1*4 Alleles by Restriction Enzyme Digestion of the PCR Product Obtained with the DQ Alpha Amplitype Kit," by M. Salazar, J. Williamson and D. H. Bing, J. Forensic Sci. 39, No. 2, March 1994, pp. 518–525, Table 2 was inadvertently omitted. This table is reprinted here for the information of readers.

TABLE 2—*Allele frequencies of the HLA-DQA 4 group*

| HLA-DQA1* N= | N.A.N. 129 | S.A.N. 88 | N.A.C. 224 | S.Ch. 71 | Korean 100 | Japanese 334 |
|---|---|---|---|---|---|---|
| *0401 | 13.5 | 19.2 | 3 | 1.6 | 1 | 2.5 |
| *0501 | 21.2 | 20.8 | 20.7 | 20.8 | 10.7 | 10.3 |
| *0601 | 1.4 | 0 | 2.7 | 9.6 | 7.3 | 1.9 |

N.A.N. North American Negroids
S.A.N. South African Negroids
N.A.C. North American Caucasoids
S.Ch. Singapore Chinese