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Analysis of linkage and linkage disequilibrium for eight X-STR markers

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ABSTRACT

X-chromosomal short tandem repeats (X-STR) have proven to be informative and useful in complex relationship testing. The main feature of X-STR markers, compared to autosomal forensic markers, is that all loci are located on the same chromosome. Thus, linkage and linkage disequilibrium may occur. The aim of this work was to study population genetic parameters of eight X-STR markers, located in four linkage groups. We present haplotype frequencies, based on 718 Swedish males, for the four linkage groups included in the Argus X-8 kit. Forensic efficiency parameters have been calculated as well as the allelic association between the tested markers for detection of linkage disequilibrium. To study the occurrences of recombination between the loci, both Swedish and Somali families were typed. A mathematical model for the estimation of recombination frequencies is presented and applied on the family samples. Our study showed that the tested markers all have highly informative forensic values and that there is a significant degree of linkage disequilibrium between the STR markers within the four linkage groups. Furthermore, based on the tested families, we also demonstrated that two of the linkage groups are partially linked. A consequence of these findings is that both linkage and linkage disequilibrium should be accounted for when producing likelihood ratios in relationship testing with X-STR markers.

1. Introduction

DNA markers on the X-chromosome have been shown to be powerful tools for solving complex relationship cases [1,2]. Paternity trio cases can most easily be solved with autosomal short tandem repeat (STR) markers alone, while test of paternity duos involving a daughter or more complex family relations could gain from X-chromosomal testing. The main application of X-STR markers is however in deficient paternity cases, especially the DNA-analysis of multiple females under the hypothesis that they share the same father (e.g. Fig. 1) [3].

Males carry one X-chromosome, so their haplotype is transferred to their biological daughters. Females carry two X-chromosomes. The two female X-chromosomes are prone to recombination during meiosis, which necessitate a consideration of two features that can have an impact on the interpretation and calculation of probabilities in relationship testing. These features include physical dependency between loci (later on referred to as linkage) and the allelic dependency between alleles at different loci (later on referred to as linkage disequilibrium, LD) [4]. Linkage is the co-segregation of closely located loci in a pedigree, while linkage disequilibrium measures the allele co-segregation at a population level. For a given set of markers, linkage can be measured by obtaining recombination frequencies from family samples and linkage disequilibrium can be estimated from allele- and haplotype frequencies.

The main aim of the present study was to investigate eight X-STR markers based on a Swedish population sample. Evaluations of statistical parameters of interest for both forensic- and relationship-tests were considered. The study of the characteristics of linkage and linkage disequilibrium has been of special interest. Over the past years many articles have been published on X-STR markers with the description of typing details and population data [5–10]. However, there has not been the same activity concerning studies of how linkage and linkage disequilibrium might impact probability calculation in relationship testing. Previously, Krawczak [11]

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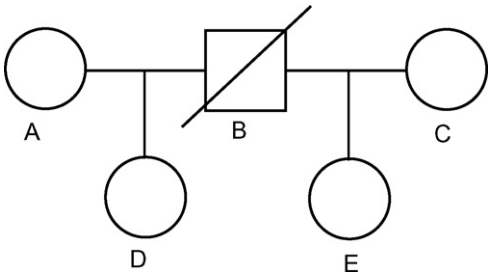

Fig. 1. Pedigree representing a deficiency case where the question is whether D and E have the same father or not.

showed in a theoretical example how the likelihood ratio was affected taking arbitrary values of linkage and linkage disequilibrium into account. Here we have made an effort to study the magnitude of linkage and linkage disequilibrium based on real population data. For the purposes described, we have analyzed the samples with the X-chromosomal kit Argus X-8.

2. Material and methods

2.1. Sample and typing

718 unrelated males and 106 unrelated females from a Swedish population were typed for the frequency estimation as well as for the evaluation of forensic parameters. The samples come from blood donors and have previously been shown to be good representatives of the Swedish population [12]. In addition, 16 Swedish and 16 Somali families were typed for the estimation of recombination fractions and mutation analysis. In these families two to nine children were accompanied by a genetically confirmed mother and father.

Multiplex PCR-amplification (Mentype® Argus X-8, Biotype) was performed on 1–10 ng of DNA extracted from blood according to the manufacturer's protocol, or directly from buccal swabs using FTA-cards. Both sample types were amplified in 10 μl reactions modified from the manufacturer's instructions. The kit includes PCR-primers of eight STR-loci located in four linkage groups, DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101 and DXS10134-DXS7423 (described in [13]). PCR-amplicons were analyzed in an ABI Prism 3100 capillary electrophoresis sequencer (Applied Biosystems) using the filter set recommended by the manufacturer, and the sequenced ladder included in the kit as reference. ROX-550 was used as size standard. Results were typed automatically using GeneMapper ID ver. 3.2 with the binset provided by the manufacturer (www.biotype.de).

2.2. Statistical methods

Haplotype frequencies for loci within the four linkage groups were calculated by simple counting based on the 718 typed males. Additionally, population parameters were computed based on haplotype frequencies as follows: mean exclusion chance (MEC) [2,14,15], power of discrimination (PD) and *P*-values for Hardy–Weinberg equilibrium (HWE). Associations between alleles from different loci were tested using the Genetic Data Analysis software program by Lewis and Zaykin [16]. In this test a comparison is done between pairs of loci. *P*-values were obtained using Fisher's exact test with 10,000 permutations. The Swedish haplotype frequencies were compared with frequencies from African, Asian and European population samples [13,17] by applying the Exact test of population differentiation [18]. *P*-values were obtained using a Markov chain of 100,000 steps. Only a comparison with a limited number of populations could be performed due to lack of matching marker sets. Mutations were studied based on the family data.

A mathematical model was set up in order to obtain estimates of recombination frequencies between two loci. This was done as follows: For N families we have X-chromosomal data from a mother and k confirmed children. We restricted ourselves to mothers heterozygous at both loci. Since the haplotype status of the mother is not known, we have to consider two possibilities. Assuming one of the possibilities, for s of the children an overcrossing (or an even number) has occurred, whereas for the remaining k-s children no overcrossings have occurred. If we assume the other state of the haplotype of the mother, k-s overcrossings have instead occurred.

Let r be the probability for an odd number of overcrossings to occur between the two tested loci. We would like to estimate r from the family data. We know, a priori, that r is somewhere in the interval between 0 and 0.5 and use a prior distribution for r that is constant on this interval.

If our data is one family as above, with k children, we get the likelihood

$$p(data|r) = 0.5 \cdot r^s(1-r)^{k-s} + 0.5 \cdot r^{k-s}(1-r)^s$$

Thus the posterior becomes

$$p(r|data) \propto p(data|r) \cdot p(r) \propto r^s(1-r)^{k-s} + r^{k-s}(1-r)^s$$

for $0 \leq r \leq 0.5$ and 0 otherwise.

Let $k_1,\ldots k_N$ and $s_1,\ldots s_N$ be the observed values for N families. Then repeated Bayesian updating gives the posterior

$$p(r|data) \propto \prod_{i=1}^{N}[r^{s_i}(1-r)^{k_i-s_i} + r^{k_i-s_i}(1-r)^{s_i}]$$

For numerical calculations, we can define $x_0,\ldots x_n$ by $x_j = j/2n$ for a suitably large n, and compute the logarithm of the posterior at each x_i as

$$y_j = \log(\, p(r = x_j|data)) = \sum_{i=1}^{N} \log[r^{s_i}(1-r)^{k_i-s_i} + r^{k_i-s_i}(1-r)^{s_i}] + C$$

for some constant C. For specificity, we compute with $C = 0$, and the mean $\bar{y}$ is subtracted from all y_j values. As an estimate for r, we use the posterior mode, which can be approximated as the x_j, where y_j is maximal. The credibility interval (95%) can be defined as the $[x_l, x_s]$, so that values y_l to y_s are larger than all y_j outside this interval, and so that the values from $\exp(y_l)$ to $\exp(y_s)$ sum to 95% of the sum $\exp(y_j)$. The obtained recombination frequencies were compared with recombination frequencies (r_h) converted from physical distances using Haldane's mapping function [4]:

$$r_h = \frac{1}{2}(1 - e^{-2d})$$

where d is the physical distance between loci.

Finally, an example is given illustrating the effect of using markers that is in LD on forensic calculations, i.e., the change in paternity index (PI) using the expected haplotype frequencies (assuming linkage equilibrium) instead of observed haplotype frequencies. Let $I_{LD} = PI_{Exp}/PI_{Obs}$ be the ratio of the different paternity indexes, where PI_{Exp} and PI_{Obs} are calculated with expected haplotype frequencies and observed frequencies, respectively. Consider a deficiency case as in Fig. 1. Assume that X-chromosomal DNA data is available for individuals A, C, D and E and that the question is whether B is the father of both D and E. The alternative is that D and E are unrelated. For simplicity we assume that the case is non-ambiguous, i.e. the paternal haplotype is deducible. The paternity index, given the pedigree in Fig. 1, for each of the four linkage groups is then $1/p_{pat}$, where p_{pat} is the frequency of the paternal haplotype (assuming absence of recombination

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Table 1
Population genetic data of forensic efficiency parameters for four pairs of linked X-STR markers in a Swedish population.

	DXS10135–DXS8378	DXS7132–DXS10074	HPRTB–DXS10101	DXS10134–DXS7423
PDF	0.9991	0.9967	0.9983	0.9975
PDM	0.9780	0.9584	0.9704	0.9637
MEC Krüger	0.9516	0.9122	0.9332	0.9088
MEC Desmarais Trio	0.9775	0.9568	0.9696	0.9625
MEC Desmarais Duo	0.9566	0.9190	0.9420	0.9292

PDF, PDM: Power of discrimination for females and males, respectively; the probability to discriminate two unrelated persons.
MEC: Mean exclusion chance; the probability to exclude a non-true relationship (trio or duo).
Note that MEC Desmarais Trio is the same as polymorphism informative content (PIC) and that PDM coincides with expected heterozygosity (HET).

Table 2
P-values from population comparison of haplotype frequencies using the Exact test for population differentiation.

Population	DXS10135–DXS8378	DXS7132–DXS10074	HPRTB–DXS10101	DXS10134–DXS7423
Germany [13]	0.020	0.301	0.002	0.346
Japan [13]	0.006	0.000	0.000	0.010
Ghana [13]	0.000	0.000	0.000	0.000
Hungary [17]	0.362	0.180	0.157	0.960

between the loci within each linkage group). We calculated I_{LD} for all observed haplotypes weighted against the number of times each haplotype was observed in the Swedish population, thus giving a representative sample. The distributions of the obtained I_{LD} for each of the four linkage groups are presented in a box plot.

3. Results

Swedish haplotype frequencies for the four linkage groups are presented in Supplementary Table 1. The calculated population and forensic efficiency parameters based on haplotype frequencies can be seen in Table 1. Hardy–Weinberg equilibrium was not tested on haplotypes but on single loci, since the haplotype status of the typed female was not known. Marker DXS7423 departed from HWE, using a 5% cut-off for statistical significance. Here we encountered the "multi-testing problem" caused by a combined total of eight tests of HWE. We addressed this problem using the method described by Zaykin et al. [19]. Applying the method for correction for multiple testing no "overall" departure from HWE could be seen ($P > 0.05$).

In order to analyze frequency differences between populations we compared the Swedish haplotype frequencies with information from four other population samples. As expected the Swedish haplotype frequencies showed more similarities with the European populations than with the non-European populations (Table 2).

The observed haplotype frequencies were also used to study the allelic association for the eight loci. Using Fisher's exact test to compare observed and expected haplotype frequencies we showed that the loci within each of the four linkage groups were in LD ($P < 0.0018$, after adjustment for multiple testing), while loci located in different linkage groups could not be shown to be in LD after adjustment for multiple testing (Table 3).

Calculated case specific paternity indexes will typically differ using expected haplotype frequencies rather than observed frequencies when loci are in LD. An illustration of such divergence is shown in Fig. 2. The box plot shows how the paternity ratio differs using expected haplotype frequencies based on allele frequencies instead of observed haplotype frequencies. Even though the medians of I_{LD} are close to one, the variations of I_{LD} are considerable. This means that for some haplotype combinations a paternity index would be significantly influenced if the observed LD is not taken into account.

Recombination fractions were estimated using both Swedish and Somali family samples (Table 4). Two recombinations were confirmed within two different linkage groups, one in a Swedish family (in linkage group DXS7132-DXS10074), and the other in a Somali family (DXS10134-DXS7423). Overall, the recombination fractions between loci located in different linkage groups were estimated to be less than 50% in two cases. In particular between linkage group 3 and 4 the observed recombination fraction was estimated to be around 25% (95% credibility interval 17%-35%). When the estimated recombination frequencies were compared with frequencies based on Haldane's mapping, only

Table 3
P-values from test for linkage disequilibrium.

Marker pair	Linkage groups	*P*-value
DXS10135/DXS8378	1/1	**0.000000**
DXS10135/DXS7132	1/2	0.124300
DXS10135/DXS10074	1/2	0.071500
DXS10135/HPRTB	1/3	0.175300
DXS10135/DXS10101	1/3	0.008700
DXS10135/DXS10134	1/4	0.141800
DXS10135/DXS7423	1/4	0.013800
DXS8378/DXS7132	1/2	0.005100
DXS8378/DXS10074	1/2	0.246600
DXS8378/HPRTB	1/3	0.242100
DXS8378/DXS10101	1/3	0.681100
DXS8378/DXS10134	1/4	0.928100
DXS8378/DXS7423	1/4	0.180700
DXS7132/DXS10074	2/2	**0.001300**
DXS7132/HPRTB	2/3	0.029300
DXS7132/DXS10101	2/3	0.142800
DXS7132/DXS10134	2/4	0.834700
DXS7132/DXS7423	2/4	0.049200
DXS10074/HPRTB	2/3	0.346400
DXS10074/DXS10101	2/3	0.011300
DXS10074/DXS10134	2/4	0.068600
DXS10074/DXS7423	2/4	0.133600
HPRTB/DXS10101	3/3	**0.000000**
HPRTB/DXS10134	3/4	0.413500
HPRTB/DXS7423	3/4	0.709000
DXS10101/DXS10134	3/4	0.024600
DXS10101/DXS7423	3/4	0.219600
DXS10134/DXS7423	4/4	**0.000000**

Abbreviations for loci in the linkage groups are as follows: 1, DXS10135 and DXS8378; 2, DXS7132 and DXS10074; 3, HPRTB and DXS10101; 4, DXS10134 and DXS7423.
Significant *P*-values after Bonferroni correction ($P < 0.0018$) are marked as bold.

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Table 4
Recombination frequencies (95% credibility interval) from Swedish and Somali families.

X-STR loci	Sweden	Somalia	Sweden and Somalia combined	Haldane's mapping function [4]
DXS10135–DXS8378	0.00 (0.00–0.052)	0.00 (0.00–0.05)	0.00 (0.00–0.03)	0.002
DXS8378–DXS7132	0.39 (0.24–0.50)	0.50 (0.29–0.50)	0.45 (0.31–0.50)	0.336
DXS7132–DXS10074	0.03 (0.001–0.11)	0.00 (0.00–0.05)	0.01 (0.001–0.05)	0.018
DXS10074–HPRTB	0.50 (0.24–0.50)	0.50 (0.32–0.50)	0.50 (0.34–0.50)	0.368
HPRTB–DXS10101	0.00 (0.00–0.07)	0.00 (0.00–0.06)	0.00 (0.00–0.03)	0.0002
DXS10101–DXS10134	0.29 (0.19–0.495)	0.22 (0.13–0.35)	0.25 (0.17–0.35)	0.136
DXS10134–DXS7423	0.00 (0.00–0.06)	0.02 (0.001–0.09)	0.01 (0.001–0.05)	0.002

The number of informative meiosis for the different pairs of loci was for the Swedish sample between 33 and 55, for the Somali sample between 45 and 69 and for the combined sample between 84 and 116.

the recombination frequency between linkage group 3 and 4 differed, thus suggesting that this might be a recombination hotspot.

Mutations occur naturally in repeated sequences at a certain frequency, this is also true for X-chromosomal markers. Two mutations were found investigating 142 meioses from Swedish families and 158 meioses from Somali families. The two mutations were both found in Somali families, one between a mother and son in marker DXS10135 (allele 29 to 28), and the other between a mother and son in marker DXS10074 (17 to 16).

4. Discussion

Over the last decade a number of publications have described the typing of X-chromosomal markers (SNPs and STRs) and also the application of these markers in forensic casework, i.e. solving complex family relationships. STR markers on the X-chromosome are valuable sources of information for a wide spectrum of family constellations [2].

Eight X-chromosomal markers included in the X-STR-kit produced by Biotype have been widely studied regarding allele frequencies for a large number of populations. Genetically and statistically interesting features of X-STR markers, such as linkage and linkage disequilibrium, have not been analyzed to the same extent. In this study we analyzed a large population sample in order to get information about how X-chromosomal markers behave both within a family and at a population level. Regarding frequencies, parameters describing the tested population, and forensic efficiency of the tested loci, the Swedish population sample was found not to deviate from other studied populations. However, one notable observation concerned the population comparison where Swedish X-STR frequencies tend to be more population specific than for autosomal markers [20].

Since DNA-results from several X-STR markers are used simultaneously in relationship testing, there is a significant risk of observing both linkage and linkage disequilibrium. Linkage disequilibrium, meaning allelic dependency, was seen for all loci within each of the four linkage groups. We also showed that the frequency of recombination between loci in different linkage groups was not always 50%, thus the assumption of independence does not hold.

Using DNA in relationship testing requires some calculation of an impartiality weight of evidence given by the DNA-result. Likelihood calculations are most often used to provide such a weight regarding the hypotheses tested. The model such likelihood calculations should be based on depends among other things on the DNA-markers analyzed. The widely used assumption of independence between DNA-markers and corresponding alleles does not hold for X-chromosomal markers, thus making interpretation and likelihood calculations more complex.

If alleles from different loci are in linkage disequilibrium there will typically be a discrepancy in the paternity index if the haplotype frequencies are inferred from allele frequencies rather than from haplotypes (Fig. 2).

All together, the X-chromosome markers included in the Mentype Argus X-8 kit offer the possibility to solve complex kinship cases where autosomal STR markers do not provide the information needed. Our study has, however, shown that both linkage and linkage disequilibrium have to be taken into consideration when making interpretation in relationship testing based on the tested X-STR markers.

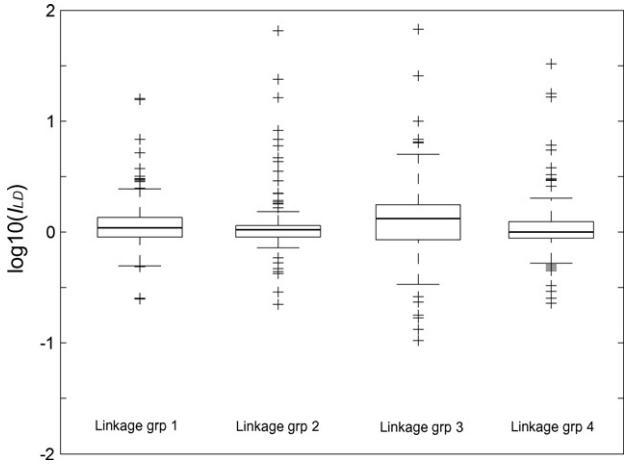

Fig. 2. Comparison of paternity indexes calculated with observed and expected haplotype frequencies for four pairs of closely linked X-chromosomal STRs. The distribution of the ratios of I_{LD} for all possible haplotypes is presented in the box plot. Derivation of I_{LD} can be found in the material and methods section. The box plot contains medians, 2.5%, 25%, 75% and 97.5% percentiles and "outliers" (indicated by +). The loci studied were as follows, starting from the left: DXS10135-DXS8378 ("Linkage grp 1"), DXS7132-DXS10074 ("Linkage grp 2"), HPRTB-DXS10101 ("Linkage grp 3") and DXS10134-DXS7423 ("Linkage grp 4").

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2008.09.006.

References

[1] R. Szibor, S. Hering, E. Kuhlisch, I. Plate, S. Demberger, M. Krawczak, J. Edelmann, Haplotyping of STR cluster DXS6801-DXS6809-DXS6789 on Xq21 provides a powerful tool for kinship testing, Int. J. Legal Med. 119 (2005) 363–369.
[2] R. Szibor, M. Krawczak, S. Hering, J. Edelmann, E. Kuhlisch, D. Krause, Use of X-linked markers for forensic purposes, Int. J. Legal Med. 117 (2003) 67–74.

[3] R. Szibor, X-chromosomal markers: past, present and future, Forensic Sci. Int. Genet. 1 (2007) 93–99.
[4] J. Ott, Analysis of Human Genetic Linkage, 3rd. ed., The Johns Hopkins University Press, Baltimore, 1999.
[5] M.T. Zarrabeitia, T. Amigo, C. Sanudo, A. Zarrabeitia, D. Gonzalez-Lamuno, J.A. Riancho, A new pentaplex system to study short tandem repeat markers of forensic interest on X chromosome, Forensic Sci. Int. 129 (2002) 85–89.
[6] M.T. Zarrabeitia, T. Amigo, C. Sanudo, M.M. de Pancorbo, J.A. Riancho, Sequence structure and population data of two X-linked markers: DXS7423 and DXS8377, Int. J. Legal Med. 116 (2002) 368–371.
[7] H. Asamura, H. Sakai, K. Kobayashi, M. Ota, H. Fukushima, MiniX-STR multiplex system population study in Japan and application to degraded DNA analysis, Int. J. Legal Med. 120 (2006) 174–181.
[8] H.Y. Lee, M.J. Park, C.K. Jeong, S.Y. Lee, J.E. Yoo, U. Chung, J.H. Choi, C.Y. Kim, K.J. Shin, Genetic characteristics and population study of 4 X-chromosomal STRs in Koreans: evidence for a null allele at DXS9898, Int. J. Legal Med. 118 (2004) 355–360.
[9] C. Tomàs, J.J. Sanchez, J.A. Castro, C. Børsting, N. Morling, Utility of X-chromosome SNPs in relationship testing, Forensic Sci. Int. Genet. Supplement Series 1 (2008) 528–530.
[10] T. Fracasso, M. Schurenkamp, B. Brinkmann, C. Hohoff, An X-STR meiosis study in Kurds and Germans: allele frequencies and mutation rates, Int. J. Legal Med. 122 (2008) 353–356.
[11] M. Krawczak, Kinship testing with X-chromosomal markers: mathematical and statistical issues, Forensic Sci. Int. Genet. 1 (2007) 111–114.
[12] A.O. Karlsson, T. Wallerstrom, A. Gotherstrom, G. Holmlund, Y-chromosome diversity in Sweden—a long-time perspective, Eur. J. Hum. Genet. 14 (2006) 963–970.
[13] D. Becker, H. Rodig, C. Augustin, J. Edelmann, F. Götz, S. Hering, R. Szibor, W. Brabetz, Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit, Forensic Sci. Int. Genet. 2 (2008) 69–74.
[14] J. Krüger, W. Fuhrmann, K.H. Lichte, C. Steffens, On the utilization of erythrocyte acid phosphatase polymorphism in paternity evaluation, Dtsch. Z. Gesamte Gerichtl. Med. 64 (1968) 127–146.
[15] D. Desmarais, Y. Zhong, R. Chakraborty, C. Perreault, L. Busque, Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA), J. Forensic Sci. 43 (1998) 1046–1049.
[16] P.O. Lewis, D. Zaykin, Genetic Data Analysis: Computer Program for the Analysis of Allelic Data, 2001, 1.1.
[17] A. Zalan, A. Volgyi, W. Brabetz, D. Schleinitz, H. Pamjav, Hungarian population data of eight X-linked markers in four linkage groups, Forensic Sci. Int. 175 (2008) 73–78.
[18] Excoffier, L.G. Laval, S. Schneider, Arlequin ver 3.0: an integrated software package for population genetics data analysis, Evolutionary Bioinformatics Online 1, 2005, pp. 47–50.
[19] D.V. Zaykin, L.A. Zhivotovsky, P.H. Westfall, B.S. Weir, Truncated product method for combining *P*-values, Genet. Epidemiol. 22 (2002) 170–185.
[20] K. Montelius, A.O. Karlsson, G. Holmlund, STR data for the AmpF*ℓ*STR Identifiler loci from Swedish population in comparison to European, as well as with non-European population, Forensic Sci. Int. Genet. 2 (2008) e49–e52.

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