Molecular Ecology Notes (2006) **6**, 985–988 doi: 10.1111/j.1471-8286.2006.01413.x

TECHNICAL NOTE

Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach

C. MIQUEL,* E. BELLEMAIN,*† C. POILLOT,* J. BESSIÈRE,* A. DURAND* and P. TABERLET*
**Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France,* †*Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Box 5003, N-1432 Ås, Norway*

Abstract

In noninvasive studies, the intersample variance in DNA quality and quantity is large, and produces multilocus genotypes of highly variable quality. Here we propose a standardized method for testing the reliability of the genotyping procedure when using the multiple-tube approach. The quality indexes generated will allow reliable comparisons among samples, loci, studies, and field and/or laboratory protocols. These indexes represent a powerful tool for the quality management of noninvasive studies.

Keywords: genotyping errors, microsatellites, noninvasive samples, quality index

Received 14 November 2005; revision received 14 February 2006; accepted 4 April 2006

Noninvasive sampling is increasingly used in conservation genetics and forensic science. Nevertheless, the application of this approach remains challenging (Taberlet *et al*. 1996; Taberlet *et al*. 1999) and can lead to genotyping errors (Paetkau 2003; Bonin *et al*. 2004). The difficulties of obtaining reliable genotypes can be partially overcome by a multiple-tube approach, i.e. by replicating, for each locus, several times the amplification from the same DNA extract (Navidi *et al*. 1992; Taberlet *et al*. 1996). In large scale noninvasive studies (e.g. Bellemain *et al*. 2005), the variance in DNA quality and quantity might be huge among samples, leading to the production of multilocus genotypes of highly variable quality, from reliable multilocus genotypes to no results at all. Due to the possibility of genotyping errors, medium-quality multilocus genotypes are usually not taken into account, although they might contain valuable information.

Here we propose a method to assess the quality of the multilocus genotypes, in order to select the most reliable results for subsequent analyses. This method also permits comparisons among genotypes, samples, loci, studies and protocols. Our goal was to provide simple and usable quality indexes that could represent the standard metrics for estimating the quality of the results when using the multiple-tube approach.

We assume the analysis of n samples for m loci. The quality index of the ith sample for the jth locus is estimated using the following steps (see Fig. 1 and Table 1):

1 Estimation of the consensus genotype after simultaneous observation of electropherograms corresponding to different repeats of jth locus for the ith sample. An allele is only taken into account, if, as in other studies, its intensity is well above the background signal, and if it is present at least twice among the different repeats.
2 Assignment of the score for each repeat. If the genotype at one repeat is identical to the consensus genotype, the score '1' is assigned, otherwise the score '0' is assigned whatever the differences (no amplification, allelic dropout, false allele, contamination, etc.). In case of heterozygous genotype, if the smaller allele is less than 20% in intensity compared to the highest allele, we suggest to assign a score of '0'.
3 Calculation of the quality index for the jth locus of the ith sample (sample/locus quality index). The scores assigned to each repeat are summed and divided by the number of repeats.

Correspondence: P. Taberlet, Fax: +33 (0)476514279; E-mail: pierre.taberlet@ujf-grenoble.fr

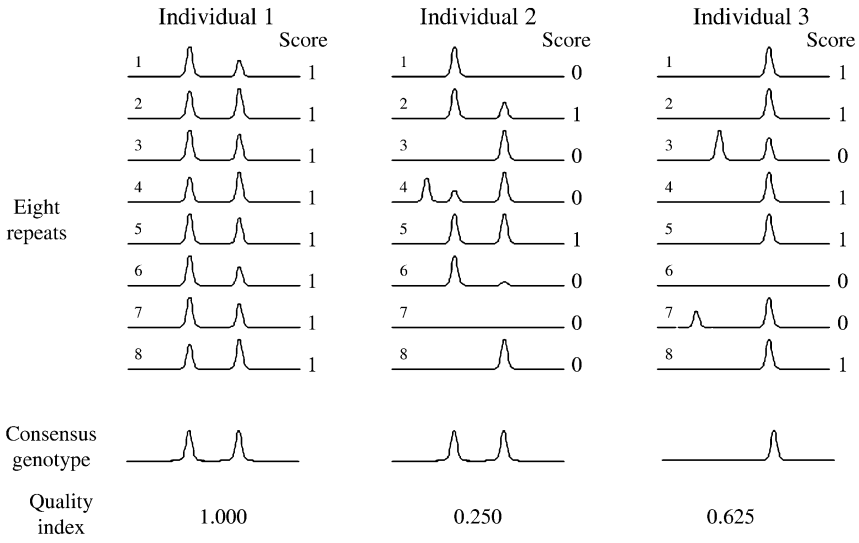

Fig. 1 Schematic illustrations of the different steps to estimate the quality index. Stutter bands are not shown on those simplified profiles. For individual 1, the profiles of all repeats clearly show two alleles (with varying intensities). For individual 2, the repeats no. 1, 3, 6 and 8 show an allelic dropout at one of the allele, repeat no. 4 shows two false alleles and repeat no. 7 shows non amplification. All those repeats are consequently scored as '0'. Individual 3 has a homozygous consensus genotype and repeats no. 3 and 7 show false alleles.

Table 1 Example showing the calculation of the sample, locus, and global quality indexes based on the estimates of the sample/locus quality indexes

	Locus 1	Locus 2	Locus *j*	Locus *m*	Quality index per sample
Sample 1	$QI_{1,1}$	$QI_{1,2}$	$QI_{1,j}$	$QI_{1,m}$	$\frac{\sum_{j=1}^{m} QI_{1,j}}{m}$
Sample 2	$QI_{2,1}$	$QI_{2,2}$	$QI_{2,j}$	$QI_{2,m}$	$\frac{\sum_{j=1}^{m} QI_{2,j}}{m}$
Sample *i*	$QI_{i,1}$	$QI_{i,2}$	$QI_{i,j}$	$QI_{i,m}$	$\frac{\sum_{j=1}^{m} QI_{i,j}}{m}$
Sample *n*	$QI_{n,1}$	$QI_{n,2}$	$QI_{n,j}$	$QI_{n,m}$	$\frac{\sum_{j=1}^{m} QI_{n,j}}{m}$
Quality index per locus	$\frac{\sum_{i=1}^{n} QI_{i,1}}{n}$	$\frac{\sum_{i=1}^{n} QI_{i,2}}{n}$	$\frac{\sum_{i=1}^{n} QI_{i,j}}{n}$	$\frac{\sum_{i=1}^{n} QI_{i,m}}{n}$	**Global quality index** $\frac{\sum_{i=1}^{n}\sum_{j=1}^{m} QI_{i,j}}{n \cdot m}$

The quality index of the *i*th sample corresponds to the mean quality index of its *m* loci. The quality index of the *j*th locus corresponds to the mean quality index of *n* samples for this locus. The global quality index corresponds to the mean quality index of *n* samples or of *m* loci. These quality indexes vary from zero to one. In some cases, the estimation of the consensus genotype might be problematic. If more than two putative alleles are present more than twice, we suggest a conservative choice by not assigning any genotype (quality index = 0). The number of repeats must be chosen beforehand using the results of a pilot study (Taberlet *et al*. 1999). In practice, we carried out the pilot study with eight repeats per locus and per sample. Then we decided if it was possible to decrease the number of repeats without compromising the reliability of the consensus genotypes. The quality indexes can be estimated from only two repeats per locus and per sample if the error rate is very low, i.e. if the pilot study demonstrated that reliable consensus genotypes can be obtained with only two repeats. An Excel Macro estimating consensus genotypes and quality indexes, and drawing the figures is available on request.

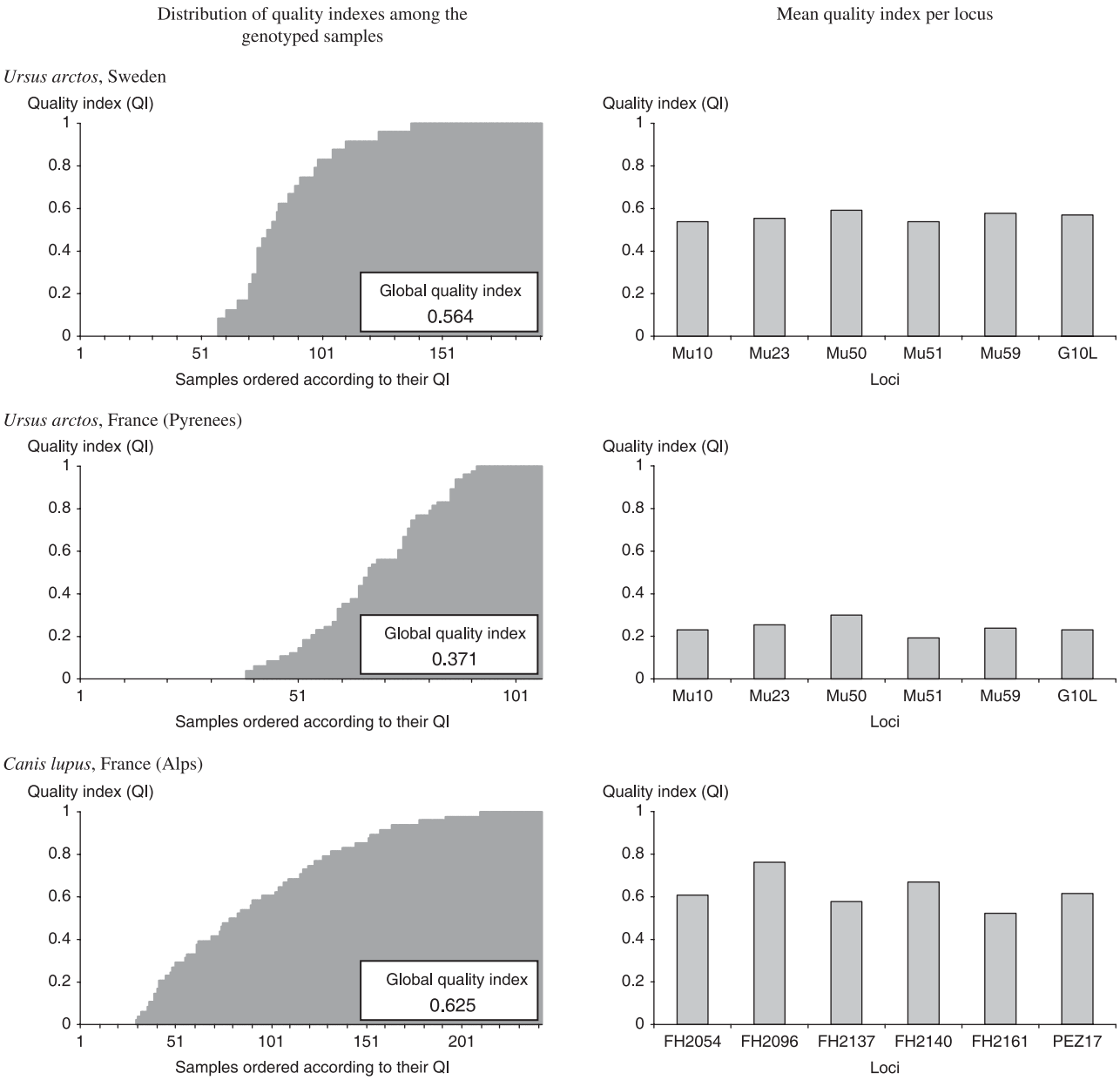

Fig. 2 Example of quality indexes obtained for three different noninvasive studies: brown bear faeces from Sweden (four repeats per locus per sample); brown bear faeces from France, Pyrenees (eight repeats); wolf faeces from the French Alps (eight repeats). The distributions of quality indexes among samples for bears from Sweden and from France are clearly different despite the use of the same experimental protocol, demonstrating that the sample quality is higher in Sweden than in France. It is interesting to note that, for comparable global quality indexes, the quality of wolf samples are more evenly distributed than those of bears from Sweden.

To illustrate the application of such quality indexes (Fig. 2), we considered three empirical data sets from faecal samples: two from brown bear (*Ursus arctos*) collected in central Sweden (Bellemain *et al*. 2005) or in France (Pyrenees; 87 samples), and one from wolves (*Canis lupus*) collected in the French Alps (242 samples). Figure 2 shows the distribution of the quality indexes for the samples and loci, as well as the global quality index for the three studies.

Estimating such quality indexes in noninvasive studies has many advantages. First, it allows performing reliable comparisons among samples, loci, studies, and field and/or laboratory protocols. In some studies, some samples are not genotyped on the basis of a preliminary assay indicating difficulties to amplify nuclear DNA. For comparisons among studies, such samples should be taken into account and assigned a quality index of zero to avoid biasing the global quality index. Second, it allows to detect problematic

loci or problematic samples, and to be cautious when considering in the subsequent analysis a multilocus genotype exhibiting a relatively low quality index. The quality index threshold, above which the sample should be considered, depends on the objectives of the study and cannot be a priori defined. In our extensive study on brown bear in Sweden (Fig. 2; Bellemain *et al*. 2005), the complete multilocus genotypes that we considered for the subsequent analysis had quality indexes above 0.625. The reliability of these results has been confirmed by consistency with field data. In such studies, it might also be interesting to compare the mean quality index of multilocus genotypes found several times with the mean quality index of unique multilocus genotypes. A lower mean quality index for unique multilocus genotypes might indicate the presence of genotyping errors. Third, by considering the quality indexes per sample (Fig. 2) and with an identified threshold, the proportion of samples that lead to a reliable multilocus genotype can be deduced. According to this proportion, it is possible to estimate the number of samples required for a future study. For example, if 300 multilocus genotypes are necessary to answer the biological question, and if only 40% of the samples can be considered according to their quality index, then 750 samples should be collected in the field. Finally, associating a quality index to each multilocus genotype can greatly facilitate the communication between field scientists and geneticists, and integrate both genetics and field data in a more reliable way. To conclude, we believe that the quality indexes will be useful in many ways and in various situations. However, we would like to emphasize that a reliable experience in genotyping is necessary to deduce the right consensus genotype and consequently to estimate correctly the quality index. Recent studies pointed out the need of standardizing the estimation of error rates in genotype data sets (Broquet & Petit 2004; Pompanon *et al*. 2005). We hope that the quality indexes we proposed here will also contribute to the quality management of noninvasive studies.

Acknowledgements

We thank Eve Zeyl for her contribution in the development of this index, Ludovic Gielly and Delphine Rioux for their help in the laboratory work. We are grateful to the Scandinavian Brown Bear Research Project and to the Office National de la Chasse et de la Faune Sauvage for providing faecal samples.

References

Bellemain E, Swenson J, Tallmon D, Brunberg S, Taberlet P (2005) Estimating population size of elusive animals using DNA from hunter-collected feces: comparing four methods for brown bears. *Conservation Biology*, **19**, 150–161.

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, **13**, 3261–3273.

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology*, **13**, 3601–3608.

Navidi W, Arnheim N, Waterman MS (1992) A multiple-tube approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *American Journal of Human Genetics*, **50**, 347–359.

Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, **12**, 1375–1387.

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics*, **6**, 847–859.

Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **26**, 3189–3194.

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution*, **14**, 321–325.