A simple genotyping method for CD247 3′-untranslated region polymorphism rs1052231 and characterization of a reference cell panel

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CD247 (or CD3-ζ chain) is an essential adaptor and signal-transducing molecule of the T-cell antigen receptor (TCR) complex, and it also couples to NK-cell activating receptors such as NKp46, NKp30 and CD16A (FcγRIII). Noncoding sequence polymorphisms and variations in CD247 expression, a tightly regulated process, have been related with an altered immune response in multiple health conditions. A single nucleotide polymorphism (T > A) at nucleotide 844 of the CD247 3′-untranslated region, rs1052231, has been related with lower CD247 gene expression and it has been investigated as a potential biomarker of autoimmune disease. We present here a simple, accurate, reliable, time-efficient, and cost-effective method for CD247-rs1052231 genotyping. Using this method, based on polymerase chain reaction with confronting two-pair primers (PCR-CTPP), we have also characterized the CD247-rs1052231 genotypes in a panel of worldwide available cell lines, which should facilitate study of the role of this polymorphism in immunity and human health.

KEYWORDS
alleles, CD247, CD3-ζ, genetic polymorphism, genotyping, human genetics, T-cell receptor, untranslated region

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location. Its long intracellular domain contains three immunoreceptor tyrosine-based activation motifs (ITAMs), unlike CD3-γ, -δ, and -ε chains bearing a single ITAM; and it assembles in disulfide-bond homodimers within the TCR/CD3-complex. It is noteworthy that CD247 is present, not only in T-lymphocytes, but also in NK cells, where it forms complexes with activating NKP46, NKP30, and CD16 (FcγRIII) receptors, enabling signal transduction and the ensuing effector functions. In NK cells, loss of the adaptor FcγR1γ-chain (a late maturation event) favors subsequent CD247 engaging to CD247, hence enhancing antibody-dependent cellular cytotoxicity. Conversely, replacement of CD247 by the FcγR1γ-chain in T-cell subsets has been observed in pathological conditions. Thus, changes in CD247 expression may modulate both innate and adaptive immunity and be potentially pathogenic.

The CD247 (CD3Z) gene is located on chromosome 1 and spans ~88 kbp. Whereas its coding sequence is virtually invariant, multiple noncoding single nucleotide polymorphisms (SNPs), alternative splicing, and post-transcriptional regulation can affect gene expression. CD247 genetic and functional variability has been related to the clinical course or susceptibility to autoimmune disease in humans and rodents, including systemic lupus erythematosus (SLE), systemic sclerosis, rheumatoid arthritis, and diabetes. The minor allele of rs1052231 T > A, affecting nucleotide 844 of the CD247 3′-untranslated region (UTR), has been associated with lower gene and protein expression, which could also be conferred by other CD247 polymorphisms. Because a genetically determined decrease in CD247 expression could further enhance the protein down-regulation observed in T cells in chronic inflammation, rs1052231 has been investigated as a susceptibility factor in autoimmune disease (i.e., SLE and rheumatoid arthritis), for which there is conflicting evidence.

To facilitate rs1052231 analysis, we have designed a genotyping assay based on polymerase chain reaction with confronting two-pair primers (PCR-CTPP). With this method, the two SNP alleles are detected simultaneously in a single, standard PCR, allowing fast and simple genotype assessment of large collections of DNA samples. For PCR-CTPP, four primers are used, which, in combination, amplify three overlapping gene regions in rs1052231 heterozygotes. External primers FP1 and RP2 (Table 1) define the area including the SNP and should produce, in all samples, the longest amplicon. The latter is thus used as an internal positive control that ensures integrity of each reaction (Figure 1). Each of the other two primers (FP2 and RP1) is specific for one of the rs1052231 alleles and, in combination with its confronting external primer (RP2 and FP1, respectively), generates an allele-specific amplicon of unique size, only in DNAs carrying that allele. External primers are chosen at different distance from the internal ones so that the three amplicons migrate as three distinct bands in agarose-gel electrophoresis. That way, depending on the sample genotype, two or three different PCR products are generated, allowing a straightforward allele assignment (Figure ). The primer sequences and PCR-CTPP conditions are shown in Table 1.

For optimization and validation of this method, we studied 52 samples, obtained after informed consent, whose genotypes we had previously assigned by means of Sanger direct sequencing (details available upon request). Correlation between the results obtained by both methods was complete, thereby demonstrating reliability and robustness of the presented PCR-CTPP genotyping assay.

To facilitate the local adjustment and validation of our method in other laboratories, we further used it to
characterize a panel of worldwide available reference cell lines. This panel comprises 50 cell lines representing all three possible rs1052231 genotypes (T/T, A/T, and A/A), thus allowing access to control DNAs (Table 2).

The method presented here enables genotype assignment of dozens to hundreds of samples in one or a small number of assays of only a few hours, using DNA amounts of, approximately, 100 ng, conventional thermocyclers, and appropriate electrophoresis chambers. The small size of all PCR products generated in this assay (less than 300 bp) should facilitate analysis of partially degraded or poor-quality DNAs. However, in severely degraded samples, the greater internal positive control band might disappear, circumstance in which the result should be disregarded because of the risk of missing the intermediate band that marks one of the alleles.

In terms of risk of contaminating reagents or samples with PCR products, CTPP is inferior to qPCR, in which reaction tubes need not be opened after amplification. In turn, PCR-CTPP, like PCR with sequence-specific primers (SSP), requires only an electrophoretic analysis after amplification; this is in contrast with methods like PCR-restriction fragment length polymorphism (RFLP) or DNA sequencing, which require further enzymatic reactions before electrophoresis. Several advantages of the method described here are shared with real-time PCR assays, used by other authors for rs1052231 analysis, but reagents and equipment costs are notably lower for PCR-CTPP. Costs are also lower in comparison with more time-consuming methods like Sanger sequencing and PCR-RFLP.

To sum up, we present an accurate, time-efficient, and cost-effective PCR-CTPP method for assessment of the CD247-rs1052231 T > A SNP, which should facilitate the study of its relevance in immunity and human health. In particular, the controverted behavior of this SNP minor allele as biomarker of autoimmune disease underlines the importance of the availability of simple and robust methods for genotype assignment.

![Diagram](image.png)

**FIGURE 1** PCR-CTPP for CD247 rs1052231-T/A genotyping. (A) General design—primers and amplicon sizes (not drawn to scale; modified from Vilches et al.28). (B) Results obtained using six DNA samples with different genotypes

![Image with bands](image2.png)

**TABLE 2** CD247 rs1052231 genotypes in a reference cell line panel

| Cell lines | Genotypes |
|------------|-----------|
| NKL        | TT        |
| BH         | 1052231-T |
| COX        | 1052231-A |
| HOR        | TA        |
| LUY        | TA        |
| PAR        | TA        |
| RML        | AA        |
| WT51       | AA        |
| JURKAT     | TA        |
| NDSWW      | TA        |
| W0049      | AA        |
| DEU        | AA        |
| NKL        | TT        |
| BH         | 1052231-T |
| COX        | 1052231-A |
| HOR        | TA        |
| LUY        | TA        |
| PAR        | TA        |
| RML        | AA        |
| WT51       | AA        |
| JURKAT     | TA        |
| NDSWW      | TA        |
| W0049      | AA        |
| DEU        | AA        |
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