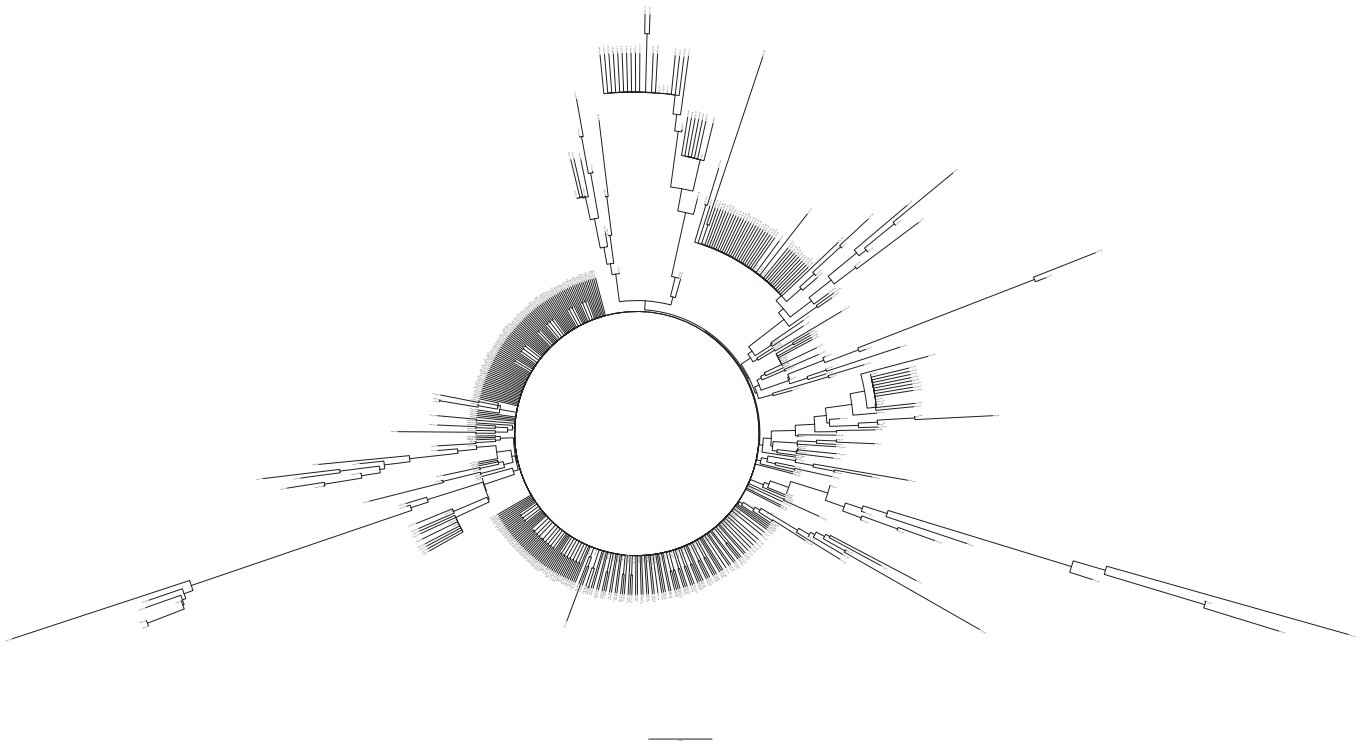
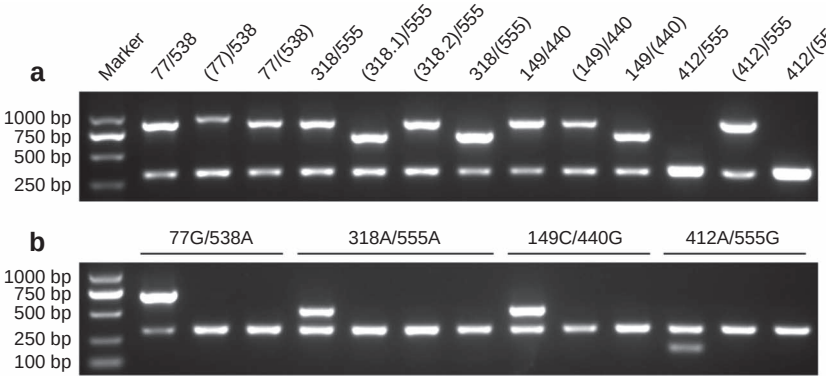


Supplemental Figure 1 A phylogenetic tree of HLA-A*02 alleles. Nucleotide sequences of exons 2 and 3 of 540 HLA-A*02 alleles were aligned with software ClustalX (version 2.1). Phylogenetic tree was generated with the program package PHYLIP (version 3.69) employing the neighbor-joining algorithm. The tree was viewed using the software FigTree (version 1.4.0). Zoom in for detail.



Supplemental Figure 2 Validation of specificities of primer sets for Main-screening2 and sub-screening on substitution templates. (**a**) Electrophoresis analysis of substitution templates generated according to Supplementary Table 1. Samples are named according to the cases listed in Supplementary Table 1. (**b**) The specificities of primer sets (77G-FW, 538A-RV), (318A-FW, 555A-RV), (149C-FW, 440G-RV) and (412A-FW, 555G-RV) were analyzed on corresponding substitution templates. PCR products were analyzed on a 1% agarose gel stained with ethidium bromide. The reactions (detailed in Table 3) applied are indicated on the top of each lane, and the templates used for each case are in the same order as analyzed in **a**. Representative results of three independent experiments.

Protocol S1. A one-tube procedure for genomic DNA preparation from PBMCs for genotyping.

1 Materials

PBMCs: 1.25×10^6~2.5×10^7 cells/mL suspended in PBS or culture medium.
Tris-Cl: 10 mM, pH 8.0, sterile.
Proteinase K stock: 20 mg/mL proteinase K (Merck, Germany) in 50% glycerol solution, stored at −20 °C.

2 Procedures

1) Transfer 2.5×10^5~5×10^5 PBMCs to a 0.2-mL PCR tube, centrifuge at 500× *g* for 5 min in a microcentrifuge. *For good performances of the cell lysates in downstream applications, it is critical to remove erythrocytes from the starter PBMCs completely.*
2) Prepare lysis buffer by 1/200 dilution of the proteinase K stock with Tris-Cl.
3) After centrifugation, aspirate the supernatant with care from the opposite side of the cell pellet. Loosen the cell pellet by flicking the bottom of the tube.
4) Add 50~100 μL lysis buffer to the tube **without any pipetting or mixing**. *A final concentration of* 5×10^3 *cells/μL is recommended. However, cell lysates of concentrations ranging between* 6.25×10^2 *and* 1.0×10^4 *cells/μL worked well in HLA-A*02 genotyping applications (see examples below).*
5) Heat at 56 °C for 1 h followed by 95 °C for 10 min on a thermocycler.
6) Centrifuge 1 min at 13 000× *g*. Store for up to one month at 4 °C or for long-term at −20 °C.

3 Examples

PBMCs from an HLA-A*02 heterozygous individual were transferred into five 0.2-mL PCR tubes at 6.25×10^4, 1.25×10^5, 2.5×10^5, 5.0×10^5 and 1.0×10^6 cells/tube respectively. The cells in each tube were then resuspended in 100 μL lysis buffer and cell lysates were prepared according to the procedures listed above. The samples were served as templates in reactions 78C/I3 and 78T/I3 (**Table 3**). In two independent experiments, cell lysates at all concentrations worked well and specific reaction patterns obtained in all cases (**Figure S3** below).

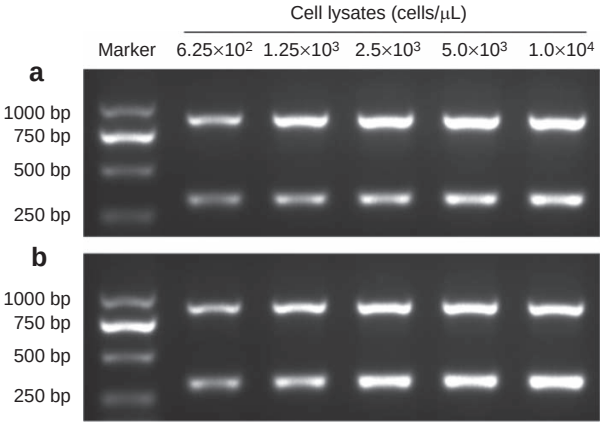


Figure S3 Examples for validating the genomic DNA preparation procedure presented above. Cell lysates of different concentrations (as indicated on the top of each lane in panel A) were prepared with PBMCs from an HLA-A*02 heterozygous individual. The quality of these samples was assessed by HLA-A*02 Main-screening1 reactions 78C/I3 **(a)** and 78T/I3 **(b)** established in this study (**Table 3**). PCR products were analyzed on a 1% agarose gel stained with ethidium bromide. Representative results of two independent experiments.

Supplemental Table 1 Generation of substitution templates for validation of specificities of primer sets for Main-screening2 and sub-screening

Primer set[a]	*Case*	*Alleles to be discriminated*	*Priming site sequences*[b]	*Substitution template*[c]	*Validation amplicon*[d]
77G-FW, 538A-RV	77/538	A*02:236	TCCTCGTCCCCAGGCTG GTGCTTGGTGGTCTGAGCT	IHW09064:77G-L-FW/A-I3-RV (883 bp)	688 bp
	(77)/538	Most alike non-A*02:236 alleles at position 77 among 538A group	TCCTCGTCCCCAGGCTc GTGCTTGGTGGTCTGAGCT	IHW09064:A-I1-FW/A-I3-RV (973 bp)	—
	77/(538)	Most alike non-A*02:236 alleles at reverse priming site among 77G group	TCCTCGTCCCCAGGCTG GcGCTTGGTGaTCTGAGCc	IHW09112:77G-L-FW/A-I3-RV (883 bp)	—
318A-FW, 555A-RV	318/555	All SA2 alleles	GACGGGGAGACACGGAAA GCCGCCTCCCACTTGT	IHW09377:78T-FW/A-I3-RV (882 bp)	510 bp
	(318.1)/555	Most alike NA2 alleles at forward priming site among GNA2/555A group	GACGaGGAGACAgGGAAA GCCGCCTCCCACTTGT	IHW09064:318A-NA2-FW/A-I3-RV (691 bp)	—
	(318.2)/555	Most alike NA2 alleles at position 318 among GNA2/555A group	GACGGGGAGACACGGAAt GCCGCCTCCCACTTGT	IHW09213:78T-FW/A-I3-RV (882 bp)	—
	318/(555)	Most alike NA2 alleles at position 555 among GNA2/318A group	GACGGGGAGACACGGAAA GCCGCCTCCCACTTGc	IHW09220:78T-FW/555G-L-RV (702 bp)	—
149C-FW, 440G-RV	149/440	Partial SNA2 alleles	CCCCGCTTCATCGCC CGGAGGAAGCGCCC	IHW09377:78C-FW/A-I3-RV (881 or 882 bp)	516 bp
	(149)/440	Most alike A2 alleles at position 149 among GA2/440G group	CCCCGCTTCATCGCa CGGAGGAAGCGCCC	IHW09220:78C-FW/A-I3-RV (881 or 882 bp)	—
	149/(440)	Most alike A2 alleles at position 440 among GA2/149C group	CCCCGCTTCATCGCC CGGAGGAAGCGCCa	IHW09211:78C-FW/555A-RV (700 or 701 bp)	—
412A-FW, 555G-RV	412/555	Another partial SNA2 alleles	GTTCTCACACCATCCAGATA GCCGCCTCCCACTTGC	IHW09112:412A-L-FW/A-I3-RV (359 bp)	178 bp
	(412)/555	Most alike A2 alleles at position 412 among GA2/555G group	GTTCTCACACCATCCAGATg GCCGCCTCCCACTTGC	IHW09377:78C-FW/A-I3-RV (881 or 882 bp)	—
	412/(555)	Most alike A2 alleles at position 555 among GA2/412A group	GTTCTCACACCATCCAGATA GCCGCCTCCCACTTGt	IHW09064:412A-L-FW/A-I3-RV (359 bp)	—

[a] Primer information is listed in Table 2.

[b] Within each case, top row for priming site of forward primer and bottom row the reverse. Mismatched nucleotides are shown in lower case.

[c] Expressed as (Parental template):(Forward primer)/(Reverse primer). Additional primers used: 77G-L-FW (5′-TCCTCGTCCCCAGGCTGTCACTCC-3′), 318A-NA2-FW (5′-GACGAGGAGACAGGGAAAGTGAAGGC-3′), 555G-L-RV (5′-GGCCGCCTCCCACTTGCGCTTGGTG-3′) and 412A-L-FW (5′-GTTCTCACACCATCCAGATAATGTTTGGCTG-3′). The control reaction (Table 3) was included in each reaction. Cycling protocol used: CP1(1 min). Results of electrophoresis analysis are shown in Supplementary Figure 2a.

[d] The control reaction was included in each reaction. Cycling protocol used: CP2(1 min) for 77G-FW/538A-RV primer set and CP2(30 s) for other sets. Results of electrophoresis analysis of these amplicons are shown in Supplementary Figure 2b.