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A high-throughput SNP typing system for genome-wide association studies

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Abstract One of the most difficult issues to be solved in genome-wide association studies is to reduce the amount of genomic DNA required for genotyping. Currently available technologies require too large a quantity of genomic DNA to genotype with hundreds or thousands of single-nucleotide polymorphisms (SNPs). To overcome this problem, we combined the Invader assay with multiplex polymerase chain reaction (PCR), carried out in the presence of antibody to Taq polymerase, as well as using a novel 384-well card system that can reduce the required reaction volume. We amplified 100 genomic DNA fragments, each containing one SNP, in a single tube, and analyzed each SNP with the Invader assay. This procedure correctly genotyped 98 of the 100 SNP loci examined in PCR-amplified samples from ten individuals; the genotypes were confirmed by direct sequencing. The reproducibility and universality of the method were confirmed with two additional sets of 100 SNPs. Because we used 40 ng of genomic DNA as a template for multiplex PCR, the amount needed to assay one SNP was only 0.4 ng; therefore, theoretically, more than 200,000 SNPs could be genotyped at once when 100 μg of genomic DNA is available. Our results indicate the feasibility of undertaking genome-wide association studies using blood samples of only 5–10 ml.

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Key words Single nucleotide polymorphism (SNP) · Multiplex PCR · Invader assay · Genotyping · Association study · Taq antibody

Introduction

The pathogenesis of common diseases, such as hypertension and diabetes mellitus, is thought to be caused, in some part, by common genetic variations such as single-nucleotide polymorphisms (SNPs) (Lander and Schork 1994; Lander 1996; Risch and Merikangas 1996; Collins et al. 1997; Kruglyak 1999). Genome-wide association studies undertaken on the basis of large-scale genotyping of SNPs in thousands of DNA samples will become one of the most potent methods for identifying genes related to complex diseases.

The Invader (Third Wave Technologies, Madison, WI, USA) assay, a method for typing SNPs, combines structure-specific cleavage enzymes with universal fluorescent probes. This assay can be applied to high-throughput genotyping (Lyamichev et al. 1999; Mein et al. 2000), but, although it does not require polymerase chain reaction (PCR) amplification of genomic DNA (Ryan et al. 1999), it does require 100 ng of genomic DNA to assay a single SNP. Therefore, only 1000 SNPs can be genotyped from 100 μg of genomic DNA (equivalent to a 5- to 10-ml sample of whole blood). Hence, this assay is not applicable for SNP genotyping on the very large scale needed for genome-wide association studies, for which 50,000–300,000 SNPs should be typed in each individual's DNA.

To reduce the amount of DNA required to less than 1 ng for the assay of a single SNP and to make genome-wide association studies feasible, we combined a multiplex PCR method with the Invader assay.

Materials and methods

Preparation of genomic DNA. Blood samples were collected from ten unrelated individuals after written informed

consent was obtained from all participants. Genomic DNAs were prepared from white blood cells by standard methods.

Primer design. The sequences of the primers used in this study were the same as those developed for PCR amplification in our earlier SNP-discovery project. Information on that project, including the sequences of primers and PCR conditions, can be found on the World Wide Web at http://snp.u-tokyo.ac.jp.

Amplification of samples. Multiplexed amplification was performed with 40 ng of genomic DNA. Each total reaction volume of 50 μl contained 50 pmol of each primer, 10 units of Ex-Taq DNA polymerase (TaKaRa Shuzo, Otsu, Japan), and 0.55 μg of TaqStart (Clontech Laboratories, Palo Alto, CA, USA). Samples were amplified in the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Initial denaturation was at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 45 s, and extension at 72°C for 3 min.

Genotyping. The Invader assay combines structure-specific cleavage enzymes and a universal fluorescent resonance energy transfer (FRET) system. Allele-specific oligonucleotide pairs and invasive probes were designed and supplied by Third Wave Technologies. FRET probes were labeled with FAM or VIC that corresponded to alleles. Signal intensity was indicated as the ratio of FAM or VIC to ROX, an internal reference. Each total reaction volume of 10 μl contained 0.5 μl of signal buffer, 0.5 μl of FRET probes, 0.5 μl of structure-specific cleavage enzyme, 1 μl of allele-specific probe mix, and 2 μl of PCR product diluted 1:10. Samples were incubated at 95°C for 5 min, and then at 63°C for 15 min, in an ABI7700 (Applied Biosystems). In this system, when correspondent alleles exist, sigmoid or quadratic signals are detected during incubation. On the other hand, when correspondent alleles do not exist, the signal is linear.

Results

First, we randomly selected 100 genomic DNA fragments in which we had discovered at least one SNP (the data are available through our SNP database, http://snp.ims.u-tokyo.ac.jp) and examined whether the DNA could be amplified sufficiently in a single tube for the Invader assay (Table 1). The 100 pairs of primers for DNA amplification were the same as those used for SNP screening. Simply mixing the 100 primer pairs with 40 ng of genomic DNA failed to amplify some fragments sufficiently to be validated by the Invader assay, partly owing to dimerization of primer sequences (Edwards and Gibbs 1994). To overcome this problem, we added to the reaction solution anti-Taq polymerase antibody (TaqStart; Clontech Laboratories) (Kellogg et al. 1994), which is known to reduce dimerization and to increase the efficiency of PCR amplification of target amplicons. Some results are shown in Fig. 1.

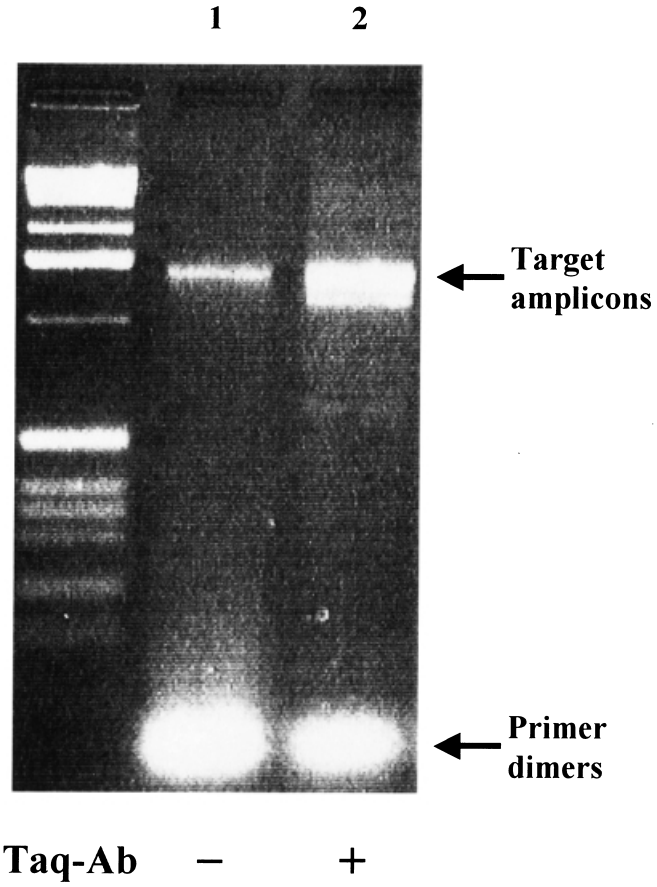

Fig. 1. Multiplex-polymerase chain reaction (PCR) products without (*lane 1*) and with (*lane 2*) anti-Taq polymerase antibody (*Taq-Ab*)

To evaluate the feasibility of amplifying multiple DNA fragments prior to assay by the Invader method, we attempted to genotype genomic DNAs from the ten unrelated individuals this way. After amplification was performed, using 40 ng of genomic DNA as template, the Invader assay was performed with unique probes corresponding to each SNP allele. Figure 2a–c shows examples of real-time scanning of fluorescent signals, and indicates that 15-min incubation at 63°C can discriminate sufficiently between alleles. To simplify the genotyping procedure, in the interests of developing a high-throughput automated scoring system, we investigated the possibility of measuring fluorescent-signal intensities in two dimensions at the end of the incubation. As shown in Fig. 2d, alleles could be discriminated at a glance. By examining 100 independent SNPs, each of which was present in one of 100 amplicons, we successfully obtained correct genotypes for 98 SNPs in the ten individuals. We examined the reproducibility of the method by repeating the experiment twice, independently, for the same 100 SNPs, and obtained identical results.

To confirm the accuracy of our method, we performed direct sequencing of 24 SNP loci in all ten samples of genomic DNA (a total of 240 fragments sequenced). The SNPs were randomly selected from among the 98 that had

Table 1. Sequences of primers

Primer names	Forward primers	Reverse primers
AC000353.27_20000214_5_FR	CCAGCAGGACTTGGTGACAG	GCAAGAAGCAGCCAGATCAAG
AC000388.1_19970529_9_FR	CAGCCACCCACTCAGTCTTG	AGGTCCTGGCTCTGCGTAAC
AC001643.1_19970529_3_FR	GCTTGAGACTCACCCTCTGATG	GTCCCGACTTGAAGGTCCAC
AC002237.1_19970606_1_FR	TCTGCCAAGCAGAAACCTAGAG	GGCACCTTGAGAGGAATGC
AC002319.1_19980203_3_FR	CTTTCCGACAACGAGAGCG	CACCTGGACTCTGCATCCTG
AC002364.1_19981204_2_FR	AGGCCGTGAGGGAATGATG	GGGTGTCTAGCATGGTGCTG
AC003005.1_19971022_1_FR	TTCAGCATAGCTCCAGAAGGC	TTTGGACCCTTGTCCTAACAAC
AC003005.1_19971022_3_FR	TGGCTCACTAAATGCACTACCAC	ACCTGGAGGTGAAGCGAGAC
AC003689.1_19981121_2_FR	ACCACAGGCTCCAGGAAGTG	TGCGTTTGCACTGGTAGGC
AF066064.1_19980603_1_FR	CACTCCCACCACCATCACTG	GCTCACGGAACTCGAAGACG
AF157101.1_1990624_1_FR	CGTTGGAAGCCTGTACTCCTTAG	AGGAGAGCTCACCCGAAGTG
AF196968.1_1991109_1_FR	GGCACCTCTCCAGGATTGTG	GAAGCCAGGGCAAGTCATTG
AJ009610.1_1990104_4_FR	CCCAAGGCCCACTGTGTTAC	CCTGGTGCCAAGTGGTCAAG
AJ011772.1_19981005_2_FR	GAGCATTGCCCTCCTCACTG	GTGCCACAATTGATATGACCAG
AJ011931.1_19981110_5_FR	GCCTCTACCTTTACCGTCCG	GCCACCTCCCTGTCTTCATC
AJ229043.1_19990122_1_FR	CAGCTTCAGGCCAAATGTATG	TCACACCTCCTCCTCCATTG
AL008633.1_19971029_1_FR	TCCACCCTGATCAAGTCCAG	GCATGGGTGCACTGTTGAC
AL008634.1_19981109_13_FR	AAATTAAGGCACAGGCAGTGAG	GTCCTCTGCTTTGCTCAGGC
AL008634.1_19981109_14_FR	TCTATGTGGGTAGGATCTCCAGAC	TCGAAACAGAAGATGTGGCTG
AL008638.1_19981123_4_FR	CAGCAGCAACAACAACCGTC	CCCAAGTGTGGTAGGTTTACAATG
AL008730.1_19980204_2_FR	GAAATGCCTCCCTGGAACAG	CTCTGCCAAGCCCATCTTG
AL008733.10_19991225_1_FR	TCCCTGAGCCCAGGTAAGTC	TGTTCCCTGATCCTCATCCAG
AL008734.10_19990610_1_FR	CATCCTCGTCACTGACTAATAGCG	TCAACAGCGAACTCCACCTG
AL021917.1_19980721_19_FR	GCCAGGGACTGAAGCTGAAC	AAAGCATCAGTGGGCAGAATC
AL023279.1_19990305_3_FR	CACTTTGAGCACTCTCAGGAGAAC	GCTTTGAGCAAGGCTTCCAG
AL049557.19_19990728_8_FR	GAGAACGGGCTGAGGACAAG	TGCCAGAGAAAGGGTGACTG
AL049569.13_19990803_29_FR	CCCTGAGTCTAGCTCAAATCTCTC	CCTGCTCCTTGAGCTTGTCAC
AL049569.13_19990803_5_FR	CTGAGGGTCCCTTCACCAAG	GCAACAGCCTGAATGTACACAG
AL049569.13_19990803_12_FR	CCACTGTCCTGGCTCAGATG	GAGGATGTCACGGTTCCAGTC
AL049569.13_19990803_13_FR	GTGACCTTCCTCTGTCCTATTACG	TTTCAGCAGGGACAGAGTCG
AL049575.7_19990701_2_FR	ATCCACTGGCCATTCTGCTG	GCTCAAGGCAGACTGGTGTC
AL049611.24_19991123_9_FR	AGGCAGACAAATCGCCACTC	TGCATGGGCTTCAGTAGAGC
AL049612.11_19991123_4_FR	TGTGGGCTGCTCTGAGGTAG	CCCACCCTCCTTTGGTAATG
AL049649.4_19990701_3_FR	GGGAAGACCCAGCCATAATC	GAGTTGGTGGGCACTAAGGTG
AL049650.8_19990706_9_FR	CTGGGCCTGTGTCTTCACTG	GGCAAAGGTCTTGGTGTCAAC
AL049691.17_19991123_5_FR	GCAGCCCTCTGACTATATGAGTTG	AGAACGCAGCAAGGAAGCAC
AL049694.9_19990730_1_FR	GATTAGCGTTTCTTTCAGCCATC	TCTGAATTCCCATTCTTCATGC
AL049698.3_19991123_2_FR	GGCCAAAGGTTCCAGGAGAG	CGATGCAGAGACTGTCCAGAG
AL049758.11_19991214_7_FR	CCTCCTCAGTTTCTCCAGCG	TGGGCATCTGAATGGAAGC
AL049758.11_19991214_11_FR	ACCAATCCAAGGGCTAGGTG	CAGGTCCAGCAGTGATCCATAC
AL049759.10_19991212_11_FR	GTTACAAACCTGACTTGTGGCTC	GGCTATGAGTTCCCGCTCAG
AL049795.20_19991123_31_FR	GCCAAACAATCCCTCATGATAC	ATGCTTCCTCTACCATGGCG
AL049829.2_19990617_16_FR	TCCCAACTCATTTCAGCATCTC	TGTCTGCCTCCCTGACTCTG
AL049829.2_19990617_23_FR	TTAACTGGCCCTGTCTGGTG	GTGCACACAGAGGTGTAGCG
AL049843.18_20000215_4_FR	GGGCTTCTTCTGCATGTGTG	TGCTTCCCACTGTTCTCAGC
AL096766.12_20000107_2_FR	AAACCTCACTGTCTGCTTCCTG	CAGGTGAGATCGGCACACTC
AP000168.1_19990702_5_FR	TTTGGCCTTGTTTGCCTCTG	AAGGCCACAGTTTGAGAACG
AP000171.1_19990702_8_FR	GAGTGTGGTCCATAAACTTGGC	ACCACGTCTCTAGCCAGTCG
AP000347.1_19990616_10_FR	GCTGTGTGACGTTAGGCCAG	AGATACTGGGTTCCATCCGC
AC004224.1_19980226_8_FR	GGCCATTCCCTTTATTGCG	CCACAGGCCGTCTAGCTTATG
AP000350.1_19990616_1_FR	GCTTATTCCTGCAAGGCGTC	AATGGAAGCCAAAGGCACAG
AP000350.1_19990616_4_FR	AACCCTGAGCCTGTCACCTG	TGAGCCCTGAATGCGAGTAG
AP000352.1_19990616_11_FR	TGTGACCTTCCTGGCTCTTC	AGCCTCACTGACATGCCTTG
AP000353.1_19990616_16_FR	CAACTGTGAGTGACCGTGGAG	AGTGAGGTATTGGAATCTGAGGC
AC004448.1_19980702_1_FR	CCTGCTCCCACCATGAAGTC	ATGGACTCCTACACGATGCG
AP000493.1_19990928_9_FR	CATAAGCCGAGTGGTACAGAGC	TCCAAAGGCCATAGTTTACCAAG
AP000495.1_19990928_7_FR	TGGCTTGAGGTTCTGGCTTC	TGTGACGGGTAAGGCAGATG
AP000500.1_19990928_16_FR	CCTATGCTCAGCCAAGGTCAG	AGAACCACCTGGGCTGCTAC
AP000500.1_19990928_18_FR	CTGTGATGGGCTGCAGAATG	GGAGAGCCTCCAGTTCAAGC
AP000500.1_19990928_3_FR	CACCCAGTGCAGCCTTATAGC	ACCTCCCTCTCTGCCTTCTG
AP000501.1_19990928_18_FR	ACCACGGAGTCTGGCATCAC	CGGTCAGAACAAAGAGAGTGGAAC
AP000501.1_19990928_10_FR	TTTGTCCTTGGGCTTGGTAG	CAGGGAGAGGTATACGATGGTG
AP001041.1_20000114_2_FR	CCCATCCCGTTAAAGCACTTAG	AGGATGGGCTTCCCACTCAG
AP001054.1_20000114_8_FR	GTGTGCTTTGTTTGGTTTGCATAG	CTGGGAATGTGCCAGCAAG
AC004465.1_19980711_2_FR	GGAGAGGAGACAAACAGAGATGC	TGGGTACCAGAAACTGCTGC
AC004551.1_19980711_12_FR	TTGGATATGTGTGTGGCAGC	GTGATGTCTGCCATGGATGC
D50561.1_19990210_1_FR	CAAACAGGTCACATTTGCTGAAG	TGGCCCACACAGACTAATAAGC
NT_002717.1_19991111_7_FR	GGAGGCCTGACAGCCATATC	GCCATATGTGGAACAAGCAGC
U07563.1_19960131_4_FR	TTCTTTCTGCCATCAAGTTGC	GCTTTGCCAGGAGCCTAGTG
X56832.1_19970625_2_FR	TGTGATCTTCCAATTCCTCCTG	TATGGCAGGGAAGGAAGCAC

Table 1. Continued

Primer names	Forward primers	Reverse primers
X74107.1_19970625_17_FR	TGCACCAGACAGGGTAGCTG	CCATCCAGCCAAGTCCTTGTAG
X78901.1_19970612_1_FR	CCAGACGGCTTAGAGCACTG	CGAGAGCATCCACCAGAGTG
X87344.1_19980924_41_FR	GGACGGATGAAGATGAACGC	ATCCCAGCAGCCCTCTTAGC
X91863.1_19961120_2_FR	TTGCCTTGAACTTGCTCTGC	CACACCTGCCCTTTATTGGTC
Y08378.1_19970804_3_FR	TCTCCAGCAGGTACAGGCAC	GCCTTCATCTCTGCAAGCTC
Y12852.1_19971110_2_FR	CCTCATGTCCTGGTGCATTG	AGCTGCGAAGAGAGCCAGAG
Y16792.1_19981106_4_FR	GATCACAGGTCGTAGGCAGATG	CCATCCAGACTGGGTCAGTG
Z54246.1_19960313_1_FR	CTCCATGTGGGAATCGGTG	TCAAGGCACATCACATGGTC
Z93241.11_19990512_20_FR	CCCTGAAGCTAAACATCACCAG	TCTGTCCCTGTGTGACCTGC
Z93241.11_19990512_21_FR	TCATGGTGAAGGGAGTTCTGAG	CGGATATGTCAGGTGGAGGC
Z96074.4_19990621_3_FR	CAAGGTGGTCAGTCTGGCTC	TGGGAATCATTAAGAGATGCCTG
Z97192.1_19980821_7_FR	TGTCCAGGAGCCTGCCTATC	GCCTCCAGTTCTGCCAACTC
Z97632.1_19990423_15_FR	TCCCTCCCTTCTTGCCACTC	TGCAACTAAGGGTGAGTGTTCG
Z97876.1_19971031_3_FR	CCATGATGAAAGGGAAATAGAGC	TGCTACGTAAGTGGTTGACGC
Z98200.8_19991123_11_FR	TCTGTCCAGGTTTCTGTCATCTC	CCAAACTTGGAGGTGGTGTC
Z98257.1_19980311_8_FR	TGTCCAAGGCAGAGGAATCTC	CAAGAGAGCTACAGGGTGCTTG
Z98258.1_19971009_3_FR	ACACCAAGCCTTTGAGGCAC	AGCAGCCCAAGAGCTCAGAC
Z98259.1_19970804_3_FR	CGCAGGTGACTTCTTAGGAGAAC	GCAGCTGGACGTTGTCATTC
AC000024.1_19981031_7_FR	CTGGGACTCTGGGACAGATG	TGGTGCAGCTGGACTCTATTG
AC000029.17_19990924_21_FR	AACCAGACCTCCAGCCACTC	CCTACAAGGTTTGCCCTACACTAC
AC000063.1_19961114_14_FR	ACCAGATGCATAGCAGTGGC	CCCATCCCAGCATCCTAGAG
AC002106.1_19970902_2_FR	CAATTCTCCTTTGCGAGGTG	TGCCACTGTCGTTAAGCCTG
AC002297.1_19970610_2_FR	AGTCCCTTCCCGGTCTTCTG	TGCTATGAGCAGTGGTCACG
AC002297.1_19970610_3_FR	TCATCCTTTCTGGGATCCTG	CCAGCTGGCTGCATCTATTC
AC002400.1_19991123_15_FR	ACCTTGGGTGCTGTAGGTGC	CCTCCAAGCTGGAGTTCCTC
AC004556.1_19981211_2_FR	TGCCTTGGCCTAAGTGTGTG	CCATGCAGTAGCTGAGGCTG
AC002467.1_20000216_2_FR	TCTCAATGTGCAAATGTGGC	TCCCTCCCTCCTTGTATCCG
AC005088.2_19990505_1_FR	GGAGGGAATAGCCAGCACTC	GACCCTTTGAAGGTTGCTGC
AC005137.1_19980930_1_FR	CTGGTCCCTGTCACCAGAAG	AGGTGGCTGTGAAACTGGTG
AC003026.1_19991123_10_FR	TGTTGATGCTGGGTGGATTC	GCCCTCTATAATCAAACACGAGC

been genotyped successfully. The sequencing results were 100% identical to the genotyping data obtained by the Invader assays coupled with multiplex PCR amplification of the 100 genomic fragments. To confirm whether this method could be applied for any combination of SNPs, we evaluated it using two different sets of 100 SNPs selected from our database. The same experimental procedures again yielded high success rates; we obtained reliable signal intensities for 96 and 98 SNPs, respectively, in the two additional sets of 100 SNPs examined. We believe, therefore, that this method should be applicable to any combination of SNPs.

Furthermore, to achieve a higher throughput, we developed our original 384-well reaction card (Fig. 3a), in which only 1 μl of reaction mixture is required for one SNP assay. After samples are spotted in the well, the card is sealed tightly with an overlaid cover plate that is welded on ultrasonically. As shown in Fig. 3b, to avoid reaction mixtures contaminating each other, grooves were prepared around the reaction wells. Multiple Invader assays, using this card, provided sufficiently high fluorescent signals.

Discussion

The greatest advantage of the system described here is the significant reduction in the amount of genomic DNA required for the genotyping of a single SNP — to 0.4 ng. Even if we were to carry out a genome-wide association study involving 100,000 SNPs, only 40 μg of genomic DNA would be required from each person tested. Although 50–60 μg may be necessary for repeated experiments if genotyping fails for some SNPs, 5- to 10-ml samples of blood from any participant are likely to be sufficient. Our procedure also reduces the cost of the PCR to much less than $0.01 per SNP. The improvements described here should make genome-wide SNP genotyping more realistic and should have a great impact on studies designed to identify genes that confer susceptibility to various common diseases.

Another advantage of our system is a reduction in the time required for the Invader assay. The flow-chart in Fig. 3c illustrates the mechanics of our high-throughput genotyping system. Although a multiplex PCR process is required prior to the Invader assay, the PCR time for all SNPs is considered to total only a few minutes, because 100 fragments are amplified together. And because of the high copy-number of each target DNA fragment amplified by the PCR, 20 min of incubation and 5 min for scanning and allele discrimination are sufficient; the original protocol took 4 h. Furthermore, using our cards, we can incubate many samples simultaneously in a water bath at 63°C, and, thus, there is no need to occupy scanning machines for incubation. By our estimation, we can carry out the genotyping of 300,000–400,000 SNPs in 1 day with our high-throughput system, and this is equivalent to genotyping about 100 million SNPs over the course of a year.

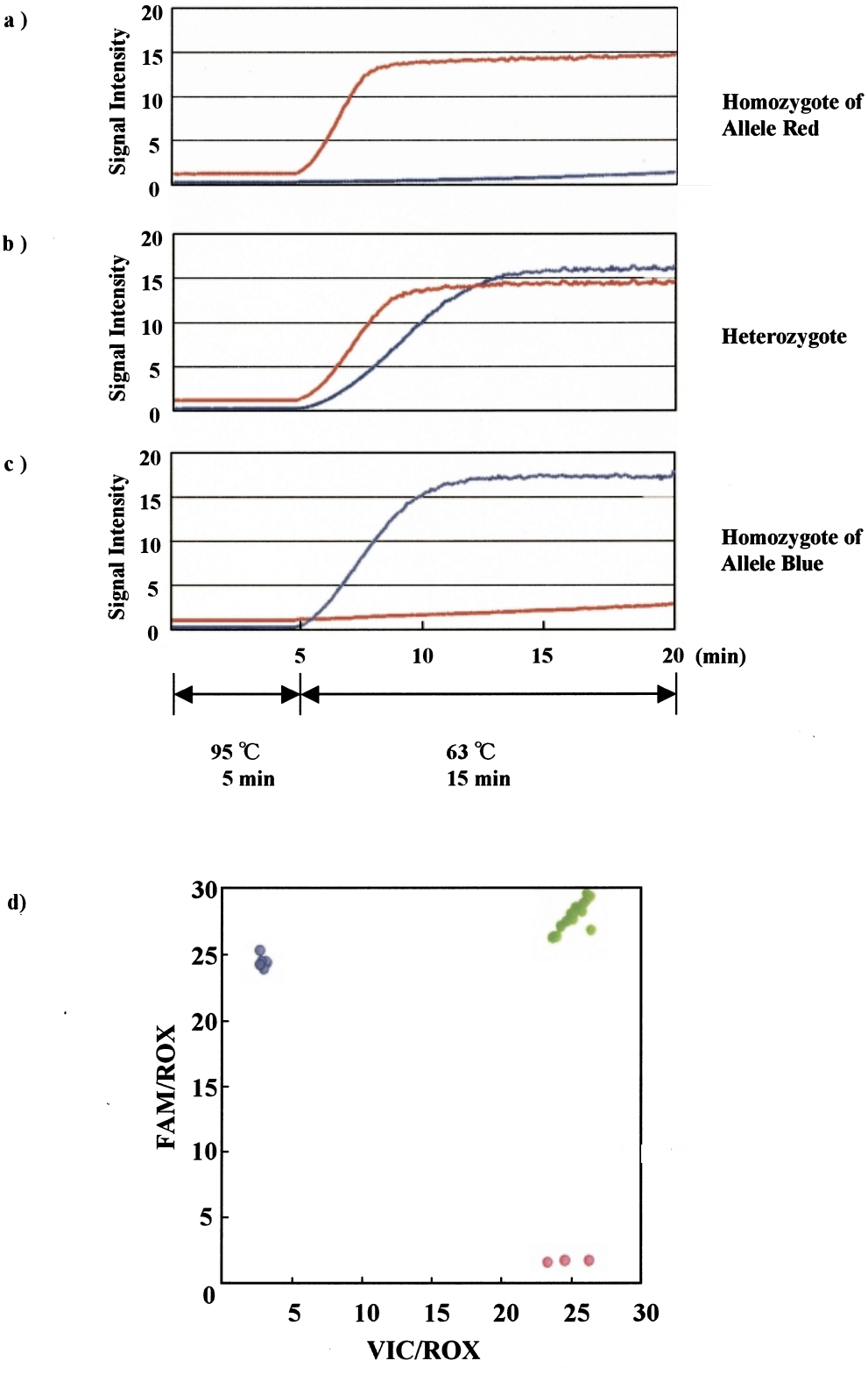

Fig. 2a–d. Representative experimental results for an Invader assay after simultaneous PCR amplification of 100 genomic fragments. Signal intensities were recorded continuously during incubation in the ABI7700 (Applied Biosystems, Foster City, CA). *Red curves* indicate the intensity of the VIC signal divided by that of ROX, an internal-control fluorescent dye. *Blue curves* indicate relative FAM signals calculated in the same way. The respective fluorescent signals were detectable in homozygous individuals (**a** and **c**) and both signals were detected in a heterozygous sample (**b**). Note that all signals reached a plateau within 15 min. **d** Allelic discrimination of 30 samples (triplicate experiments with 10 samples). The *horizontal axis* shows the signal intensity of VIC/ROX; the *vertical axis* shows the signal intensity of FAM/ROX. The cluster of *red spots* indicates a strong VIC/ROX signal and a weak FAM/ROX signal; the samples in this cluster were judged to be homozygous for allele 1. Similarly, samples indicated by *green spots* are heterozygous, and samples yielding *blue spots* are homozygous for allele 2

At present, more than 90% of the human genomic sequence has been determined, and the next decade will be an era of "functional genome analyses", including the identification of genes related to diseases with complex traits. Genome-wide association studies should play an important role in finding genes related to diseases that do not show Mendelian inheritance. Because such studies will require the assay of hundreds of millions of SNPs, genotyping methods with very high throughput will be indispensable. Moreover, one of the most important issues in making genome-wide association studies feasible is the amount of genomic DNA required from each human participant:

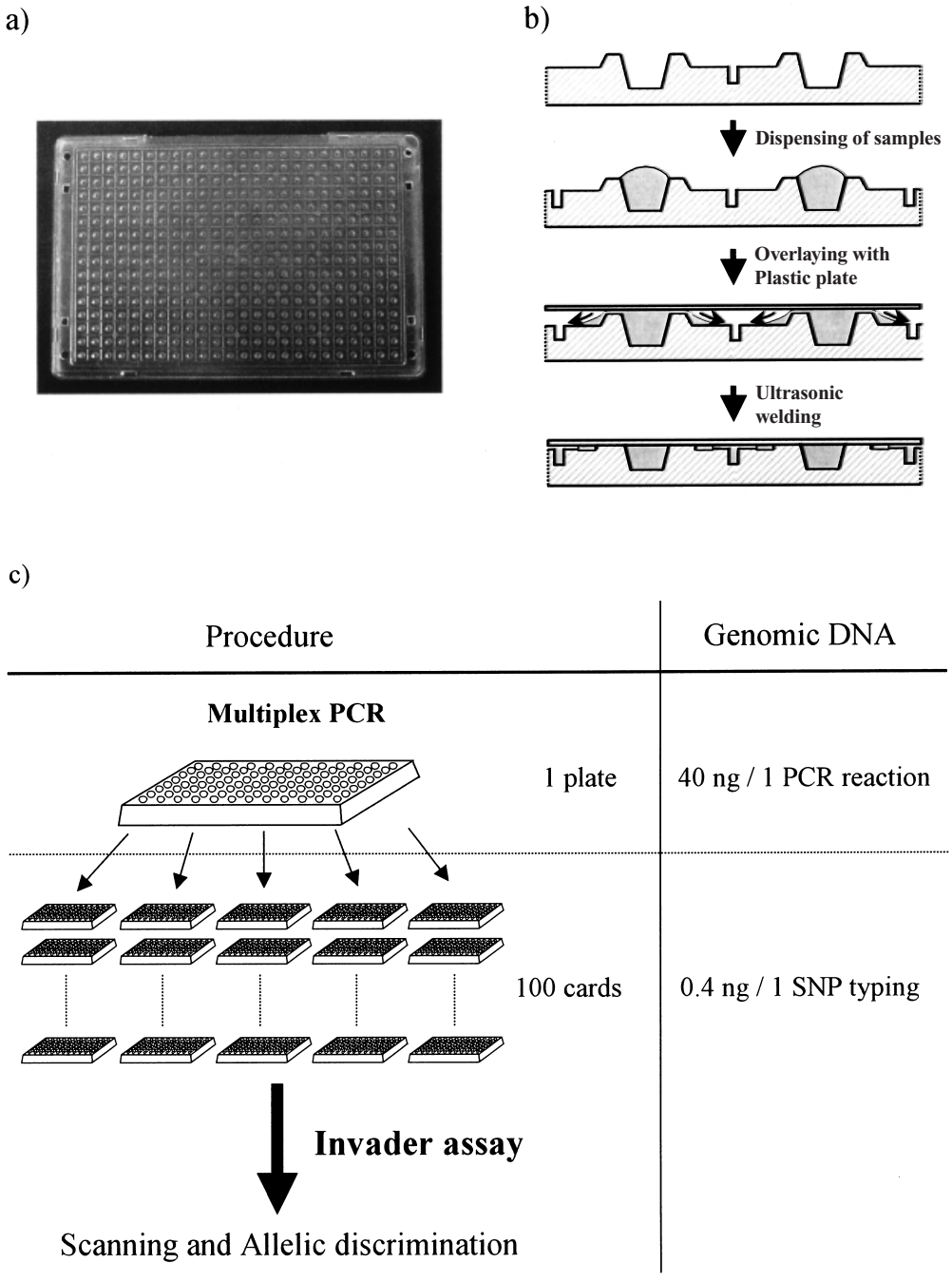

Fig. 3a–c. High-throughput genotyping system. **a** Overview of the 384-well reaction card. **b** Method of sealing up the samples. After the wells are filled with reaction solution, each well is covered with a plastic plate. At this point, some portion of the sample overflows from the well, to keep residual air bubbles to a minimum, and the overflow is trapped by the grooves surrounding the wells. **c** Experimental flow-chart for Invader assay procedure, after the simultaneous PCR amplification of 100 genomic fragments, also showing the calculated amount of DNA required in each step. *SNP*, Single-nucleotide polymorphism

the source is limited and cannot be increased by simply spending more money. Although some promising new approaches have been reported, until now, none has successfully reduced to a practicable level the amount of genomic DNA required from an individual blood sample.

Here, however, we have reported a high-throughput and highly efficient tool for genotyping SNPs. Although it merely combines easily available methods, our modification provides a way to solve the most serious practical problems in genome-wide association studies. Using the automated sample-flow system, along with our newly developed 384-well cards, we have established a protocol for genotyping up to 100 million SNPs per year.

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