Electronic Supplementary Material (ESI) for Analyst.

SUPPLEMENTARY MATERIAL

Digital versatile discs as platforms for multiplexed genotyping based on selective ligation and universal microarray detection

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1) Probe design

The developed method (DVD-array-MLPA) employs the specificity of ligase enzymes to join oligonucleotide probes to achieve allelic discrimination. The ligation set is composed of two probes, called discrimination ligation probes (DLP), and a common ligation probe (CLP).

DLPs comprise two regions: amplification barcode for universal PCR (5'-end) and specific upstream sequences (3'-end), as described in Figure SI.1. DLPs are allele-specific and bind to the template at the studied site. Their 3'-ends match the polymorphism or mutation sites because ligases are more sensitive to mismatches at this end.

CLPs are composed of three regions: a sequence complementary to the specific downstream region (5'-end), a barcode for the universal array and an amplification tail for universal PCR (3'-end), as Figure SI.1 describes. CLPs bind to the template next to the allele-specific probe. Thus, ligation products are formed depending on the nucleotide in the polymorphism or mutation position. If a DLP binds to a template (single-stranded DNA) at the SNP site with perfect complementarity, the DNA ligase joins it with the adjacent CLP.

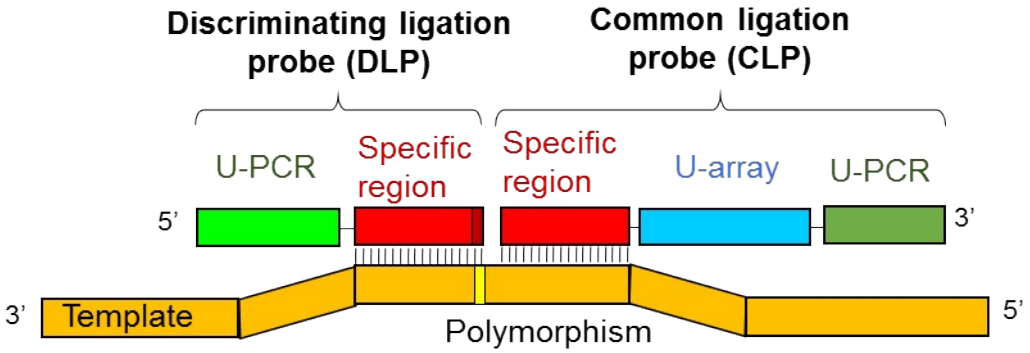

Figure SI.1. *Design of ligation probes.* U-PCR: Universal PCR. U-array: Universal array.

Each reaction mixture contains a specific DLP and the CLP. There are several general restrictions to be considered to design oligonucleotide sets.

- The sequences of amplification barcodes are the same for all DLPs and CLPs, which differ from one another. Thus ligation products can be simultaneously amplified

using two common primers. Specifically, the forward primer has the same sequence as the 5'-tail of DLPs and the reverse primer is complementary to the tail of CLPs.

- The sequences of hybridization barcodes differ for each polymorphism. Thus amplification products hybridize individually to the perfect-match probes attached to the analytical surface.

- Assay selectivity strongly depends on the combination of these oligonucleotide sequences. A careful selection is required to choose the probe sets to satisfactorily ligate/amplify/detect the given template region.

Specific design constraints were incorporated to achieve success in the simultaneous detection of several targets.

- The key discrimination stage is the annealing of DLPs and CLPs to the DNA template before the allele-specific activity of the ligase. Thus a key design constraint is the melting temperatures of the candidate specific sequences.

- Hybridization barcodes must be short oligonucleotides with no homology to any human genome sequence to thus avoid false signals due to non specific hybridizations. Efficient selection leads to improved hybridization conditions. Nevertheless, two processes can interfere: the formation of stable secondary structures or partial hybridizations with templates.

- The potential formation of hairpins must be checked because the length of both ligation probes can lead to stable secondary structures.

The nucleotide sequences for the studied polymorphisms were retrieved from the SNP database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). A program, in Visual Basic language, was used to support the selection of oligonucleotides. The input data were the FASTA sequence of the gene regions around the targeted SNP and the design constraints. Alleles were indicated by IUPAC codes (G/C: S, A/T: W, G/A: R, T/C: Y, G/T: K, A/C: M). The thresholds, expressed as melting temperatures, were > 58 ºC and <44 ºC respectively for the perfect-match regions and for all the plausible double-strand molecules. Another restriction was that the variation of Gibbs free energy of the DNA complex between the probe and template was high (ΔG° > 24 kcal mol^{-1}). Barcode length was 22 nucleotides (melting temperature 65.0 ± 1.3 ºC). Despite the presence of barcodes and universal tails, the estimated stability of the undesirable hairpins and other secondary structures should be low in all cases ($\Delta G^{\circ} \approx$ -3 kcal mol^{-1}).

The selected sets are listed in Supplementary Material (Excel file).

2) Setup of the ligation method

The human genomic DNA extracted from blood (about 5 mL) and buccal swabs (2 cotton swabs per person) was used. Samples were collected and immediately frozen (-20 ºC). The DNA extraction protocols are described in previous studies[1] and were performed in less than one month after sampling. The extracts were stored at - 20 ºC, during a maximum of one year. No evidences of contamination or degradation were observed according to the measurements by colorimetric method and gel electrophoresis.

Experiments focused on studying the ligation step under different experimental conditions, including annealing buffer composition, the amount of ligase, incubation time and temperature. For optimization purposes, polymorphism rs4680, located in the catechol-o-methyltransferase gene (*COMT*), was selected as the model (Assembly GRCh38.p2, chromosome 22, position 19963748). The positive ligation mixture contained perfect-match probes for the targeted region. Five negative controls were simultaneously analyzed (Table SI.1). Controls 1 and 2 were incomplete reaction mixtures (lack of a probe). Controls 3 and 4 corresponded to the *COMT*-selective probes, but no template or non human DNA (bacterial genomic DNA) was employed. Control 6 included no adjacent probes to target the sequence, complementarily to the *DRD3* gene (DLP) and the *COMT* gene (CLP). The studied variables were a composition of hybridization buffer (buffer 1: 200 mM Tris-HCl (pH 8.3), 250 mM KCl, 100 mM $MgCl_2$, 5 mM NAD, and 0.1% Triton® X-100, buffer 2: NAD, Tris-HCl pH 8.5, $MgCl_2$, non ionic detergents), a probe annealing time (5-960 min), enzyme concentration (1×) and ligation temperature (54-70 ºC). Later products were amplified using amplification mixtures with specific primers (5'-GAGTCGAGGTCATATCGT-3' and 5'-GACTCACTATAGGCAGAC-3'). The formation of the amplified products was determined by fluorescence.

Table SI.1. *The reaction mixture for ligation optimization.*

Type	Ligation probes (5'-3' sequence)	Probe	Template
Positive	GAGTCGAGGTCATATCGTG-ATGGTGGATTTCGCTGGCG	DLP	Human DNA
	[P]-TGAAGGACAAGGTGTGCATG-GTCTGCCTATAGTGAGTC	CLP	
Control 1	GAGTCGAGGTCATATCGTG-ATGGTGGATTTCGCTGGCG	DLP	Human DNA
Control 2	[P]-TGAAGGACAAGGTGTGCATG-GTCTGCCTATAGTGAGTC	CLP	Human DNA
Control 3	GAGTCGAGGTCATATCGTG-ATGGTGGATTTCGCTGGCG	DLP	-
	[P]-TGAAGGACAAGGTGTGCATG-GTCTGCCTATAGTGAGTC	CLP	
Control 4	GAGTCGAGGTCATATCGTG-ATGGTGGATTTCGCTGGCG	DLP	*Salmonella* strain
	[P]-TGAAGGACAAGGTGTGCATG-GTCTGCCTATAGTGAGTC	CLP	
Control 5	GAGTCGAGGTCATATCGTG-ACACCATGCTCTGCTGTATCAGGG	DLP'	Human DNA
	[P]-TGAAGGACAAGGTGTGCATG-GTCTGCCTATAGTGAGTC	CLP	

DLP: discrimination ligation probe for the COMT gene, CLP: common ligation probe for the COMT gene, DLP': discrimination ligation probe for the DRD3 gene

Positive responses were obtained in the reaction mixtures, including the perfect-match probes to the DNA template (Figure SI.2). Two oligonucleotide probes, which bind adjacently on a target sequence, were ligated only in the presence of their complementary target DNA. If there were any mismatched probes, no ligation product was generated and the associated signal was comparable to the background (mixtures

[1] Tortajada-Genaro LA, Puchades R, Maquieira A. J Pharm Biomed Anal 2017;136:14-21.

without template). For the ligation yields, the results were similar under compared conditions, and even drastically cut the ligation time.

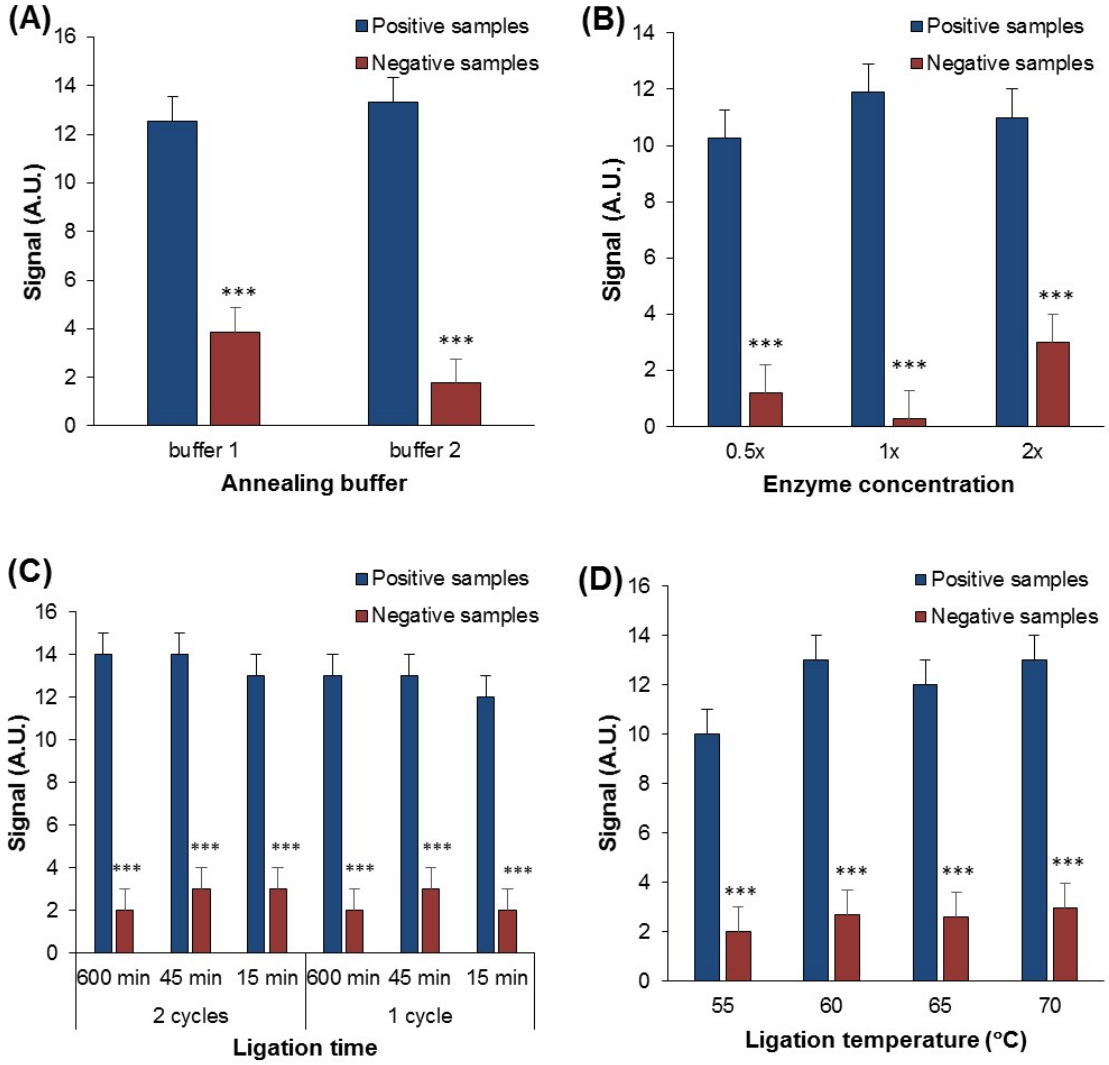

Figure SI.2. *Optimization of the probe annealing and ligation steps by array-MLPA. The mean spot intensities obtained for the perfect-match probe-template (positive samples) and the mismatch probe-template (negative samples). SNPs: rs1799971 (OPRM1), rs1800544 (ADRA2A), rs5569 (SL6CA2). (a) Type of annealing buffer, (b) Enzyme concentration, (c) Ligation cycles (number and duration), and (d) Ligation temperature.* ***: test t, p-value<0.001

The following experiments focused on gene selectivity. Ten human genome regions were PCR-amplified in a single format using the primers listed in Table SI.2. These synthetic templates (157-205 bp) were diluted (10-1000 copies) and individually incubated with the ligation mixture for the model gene (*COMT* gene), as previously described. The fluorescence measurements corresponded precisely with the expected results. Positive responses were recorded for both the wild-type and mutant mixtures in the presence of the DNA product from the *COMT* gene (Table SI.2). Low fluorescence signals (comparable to the negative controls) indicated that no ligation was produced for the unmatched templates (t-test, p-value < 0.05). Therefore, the results confirmed the absence of any significant cross-reactivity during the ligation reaction.

Table SI.2. *Results in the experiments for checking ligation selectivity using the ligation reagents for genotyping SNP rs4680 (the COMT gene).*

Gene	Synthetic ligation template Primers (5'-sequence-3') [1]	length (bp)[2]	Relative fluorescence[3] WT-ligation	M-ligation
COMT	GGGCCTACTGTGGCTACTCA CCCTTTTTCCAGGTCTGACA	176	100	100
ADRA2A	TCCCTTTTCTCCCAAGATCC GGCGGGTACCTTGAGCTAGA	164	5 ± 3	6 ± 3
HTR1B	CAGCTGATAACCGACTCCCC CCTAGCGGCCATGAGTTTCT	162	8 ± 4	6 ± 3
LPHN3	GGGTGATTTTCCCTTCCAAA GGCCACACAATTCTTTTCTTG	178	6 ± 3	5 ± 3
OPRM1	CCCCCACGAACGCCAGCAAT AGGCTGTCTCTCCCGCCCAG	157	6 ± 3	7 ± 4
SLC6A2	GACCCTAATTCCTGCACCCC ATGCAGAACAGGGCGAGAAG	163	6 ± 4	7 ± 4
ABCB1	GTCCCAGGAGCCCATCCT CCCAGGCTGTTTATTTGAAGAG	172	8 ± 3	5 ± 3
SLCO1B1	CTTACCTTTTCCCACTATCTCA GTGAAAATATTCAGTAGATAAGCA	180	5 ± 3	6 ± 3
GRIK4	AAGAAGTGGACTGGTTTGAGAA GCAGAGCATCTCAAATTTAGG	205	7 ± 3	8 ± 4
ACTB	AATCTGGCACCACACCTTCTAC ATAGCACAGCCTGGATAGCAAC	170	9 ± 4	8 ± 4

[1] The PCR primers used to generate ligation templates

[2] Length of the ligation templates

[3] Relative fluorescence measured after amplifying the ligation products formed in the reaction mixture that contained the wild-type (WT) or mutant (M) ligation probe.

Ligation selectivity was evaluated from a two-factorial experiment. The factors were template (wild-type or mutant homozygous) and DLP in the reaction mixture. The last nucleotides were guanine and adenine for the wild-type and mutant probes, respectively. Positive responses were recorded only for the perfect-match complexes.

3) Setup of universal amplification-barcode hybridization

This research focused on the selectivity of the detection of allele-specific products, and demonstrated that amplification was independent of the specific barcode used in the CLP.

Experiments were run using ligation reagents to genotype SNP rs2235048 (*ABCB1* gene). Each mixture contained the same DLP (GAGTCGAGGTCATATCGTG-TGCTAATTTCTCTTCACTTCTGGGAG) and different CLP oligonucleotides. The barcode sequence between the specific targeted region (ACCAGCCCCTTATAAATCAAACTA) and the reverse universal primer (GTCTGCCTATAGTGAGTC) was changed (Table SI.3). After amplification with the universal primers, fluorescent signals were measured. The results were comparable to those of the amplification with no barcode in all cases (tail-1) and independently of the specific barcode sequence that was introduced (ANOVA test, $p = 0.62$).

Table SI.3. *List of the tested barcodes for the universal amplification-hybridization experiments. Target polymorphism: rs2235048 (the ABCB1 gene).*

	Barcode sequence (5'-3')	Barcode length	Relative fluorescence
Tail-1	-	0	100 ± 4
Tail-2	AGGCGATAGGCTGTACGAATCG	22	98 ± 4
Tail-3	GCTCGAAGAGGCGCTACAGATC	22	95 ± 6
Tail-4	CTTTTCCCGTCCGTCATCGCTC	22	94 ± 4
Tail-5	CTCGGTGGTGCTGACGGTGCAA	22	91 ± 5
Tail-6	CGACTCCCTGTTTGTGATGGAC	22	97 ± 4

The immobilization of the probes on the top DVD layer was studied using a doubled-labelled oligonucleotide. The 5'-end biotin modification enabled the indirect passive immobilization via disc coating with streptavidin. The 3'-end Cy5-modification enabled the fluorescence quantification of the attached probes as function of the printed molecules. The immobilization yield were measured, estimating a surface density of about 0.5 pmol/cm^2. These experiments confirmed that a blocking stage was unnecessary because background signals were comparable to those obtained on raw material (t-test, p-value < 0.05). The interpretation was the hydrophobic nature of the polycarbonate surface (contact angle 90º) and, consequently, unspecific adsorption on disc surface was minimized.

The specific barcode probes were immobilized on the DVD surface in array format (spot diameter 500 ± 20 μm). The amplification products were hybridized because the probe sequence was the same as the barcode. The analyzed data were the signals corresponding to the mean spot intensities associated with a specific probe, after incubating the product formed from a specific ligation probe (specific barcode tail). High signals were recorded when the product and probe presented a perfect match, while lower signals corresponded to no complementary sequences (Figure SI.3). Thus barcode hybridization was successful and each product hybridized specifically on its corresponding probe.

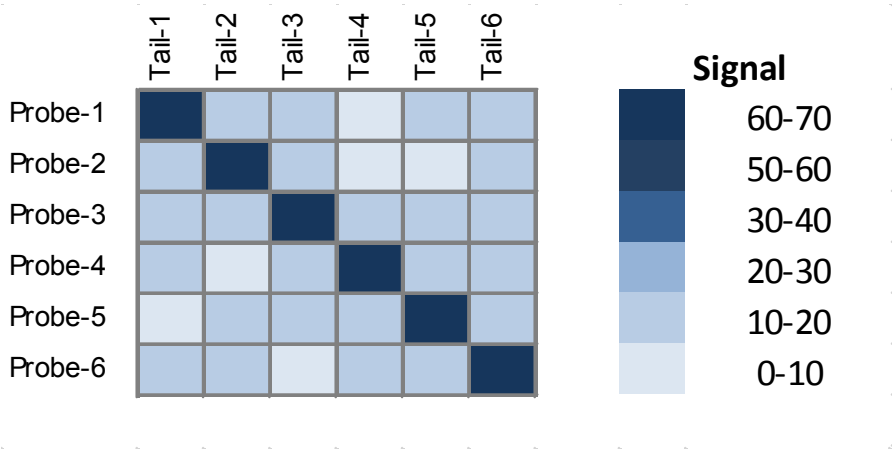

Figure SI.3. *Study of cross-reactivity depending on the barcode sequence. Signal: normalized spot intensity. Target polymorphism: rs2235048.*

4) Experiments to test multiplexing capability

Polymorphism rs2319398, located in the glycogen synthase kinase 3 beta gene (*GSK3B*), was selected as the model (Assembly GRCh38.p7, chromosome 3, position 119894095). The DNA template from a homozygote patient (wild-type CC) was chosen. The tested formats were taken from an assay run only for the genotyping of SNP rs2319398 (1-plex) to an assay for 28 polymorphisms (28-plex). The ligation reaction mixture contained between two and fifty-six oligonucleotides, respectively. The microarray layout varied from 1 to 28 kinds of probes (4 or 6 replicates per probe), respectively. The control probes were also immobilized. Table SI.4 indicates the oligonucleotide sets selected in each case. The optimized assay (ligation, universal amplification, barcode hybridization and optical reading) was applied.

Figure SI.4 shows the images captured using a DVD reader for the tested multiplexing formats. Excellent assay selectivity results were obtained. First, all the sets of ligation oligonucleotides were ligated in the same reaction along several genes with no interferences encountered in other kinds of discrimination reactions (i.e. allele-specific amplifications). Second, the specific amplification/hybridization of the multiplexed products was correctly achieved by the barcode addresses included in the ligation probes.

Table SI.4. *List of the tested SNPs in the multiplexing experiments. Patients' variants are included.*

	Gene	Variant	1-plex	3-plex	5-plex	9-plex	15-plex	22-plex	28-plex	Genotype
1	*GSK3B*	rs2319398	Yes	Yes	Yes	Yes	Yes	Yes	Yes	CC
2	*CYP1A2*	rs762551		Yes	Yes	Yes	Yes	Yes	Yes	CA
3	*HTR1A*	rs10042486		Yes	Yes	Yes	Yes	Yes	Yes	CC
4	*ABCB1*	rs2235048			Yes	Yes	Yes	Yes	Yes	AA
5	*RGS4*	rs2661319			Yes	Yes	Yes	Yes	Yes	CC
6	*COMT*	rs4680				Yes	Yes	Yes	Yes	GG
7	*DRD3*	rs963468				Yes	Yes	Yes	Yes	GG
8	*HTR2A*	rs6313				Yes	Yes	Yes	Yes	AG
9	*LPHN3*	rs6551665				Yes	Yes	Yes	Yes	AA
10	*CACNG2*	rs2284017					Yes	Yes	Yes	CT
11	*CYP2C19*3*	rs4986893					Yes	Yes	Yes	GG
12	*CYP2C9*2*	rs1799853					Yes	Yes	Yes	CT
13	*GRIA3*	rs4825476					Yes	Yes	Yes	AA
14	*NR3C1*	rs10482633					Yes	Yes	Yes	TT
15	*ANKK1*	rs1800497					Yes	Yes	Yes	GG
16	*DRD2*	rs6277						Yes	Yes	AA
17	*CYP2C19*17*	rs12248560						Yes	Yes	CC
18	*CYP2C9*3*	rs1057910						Yes	Yes	AA
19	*CYP2D6*	rs16947						Yes	Yes	AG
20	*CYP2D6*	rs1135840						Yes	Yes	GG
21	*CYP2D6*41/69*	rs28371725						Yes	Yes	CC
22	*DRD4*	rs11246226						Yes	Yes	AC
23	*GNB3*	rs5443							Yes	CT
24	*GRIK2*	rs2518224							Yes	AC
25	*GSK3B*	rs13321783							Yes	CC
26	*LOC729622*	rs4675690							Yes	CT
27	*LPHN3*	rs2345039							Yes	GG
28	*NR3C1*	rs852977							Yes	AA

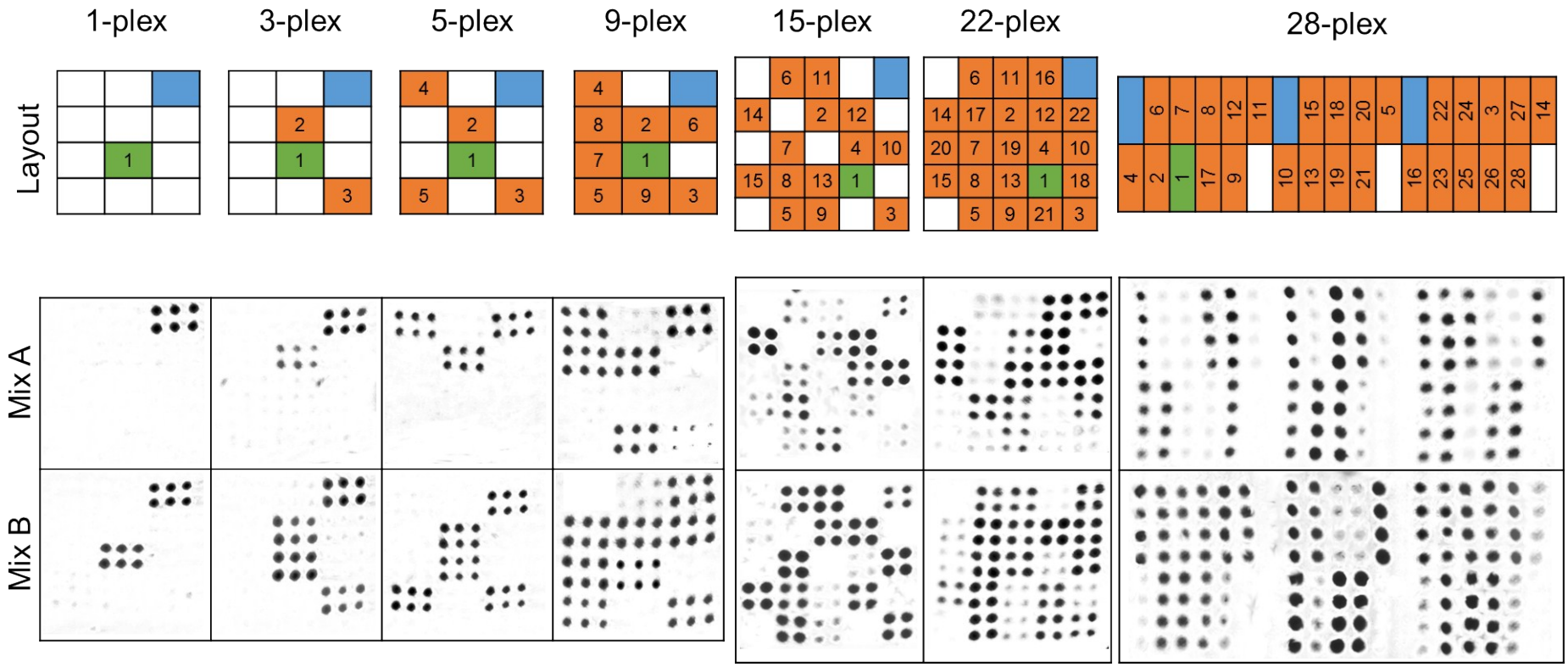

Figure SI.4. *Images obtained for the simultaneous genotyping of 1, 3, 5, 10, 15, 22 or 28 SNPs. Numbers indicate the probe set selected for the multiplexed experiments. The colors used in the probe array layout were; blue: positive controls, white: negative controls, green: probes for rs2319398 (located in the GSK3B gene), orange: probes for the other tested SNPs.*

The identification of a specific SNP (rs2319398) and other target SNPs was studied in different reaction media. For this purpose, three reaction mixtures for the 10-plex assays were used, as Table SI.5 indicates. After the ligation reaction, each solution was amplified using the universal primers and was hybridized in the array for 28 variants. The results are summarized in Figure SI.5.

Table SI.5. *List of the tested SNPs used. Patients' variants are included.*

Mix 1			**Mix 2**			**Mix 3**		
Gene	Variant		Gene	Variant		Gene	Variant	
GSK3B	rs13321783	CT	GSK3B	rs2319398	CT	GSK3B	rs2319398	CT
ABCB1	rs2235048	CC	LPHN3	rs6551665	AG	DRD2	rs6277	TT
COMT	rs4680	AG	CACNG2	rs2284017	CT	DRD4	rs11246226	AC
CYP1A2	rs762551	AC	CYP2C9*3	rs1057910	AC	GNB3	rs5443	CC
CYP2C19*17	rs12248560	CC	CYP2D6	rs16947	AG	GRIK2	rs2518224	AC
CYP2C19*3	rs4986893	GG	CYP2D6	rs1135840	CG	HTR1A	rs10042486	TT
CYP2C9*2	rs1799853	CC	CYP2D6*41/69	rs28371725	CC	LOC729622	rs4675690	CT
DRD3	rs963468	AG	DRD2/ANKK1	rs1800497	CC	LPHN3	rs2345039	CG
GSK3B	rs2319398	GT	GRIA3	rs4825476	AG	NR3C1	rs852977	AA
HTR2A	rs6313	AG	RGS4	rs2661319	AG	NR3C1	rs10482633	AA

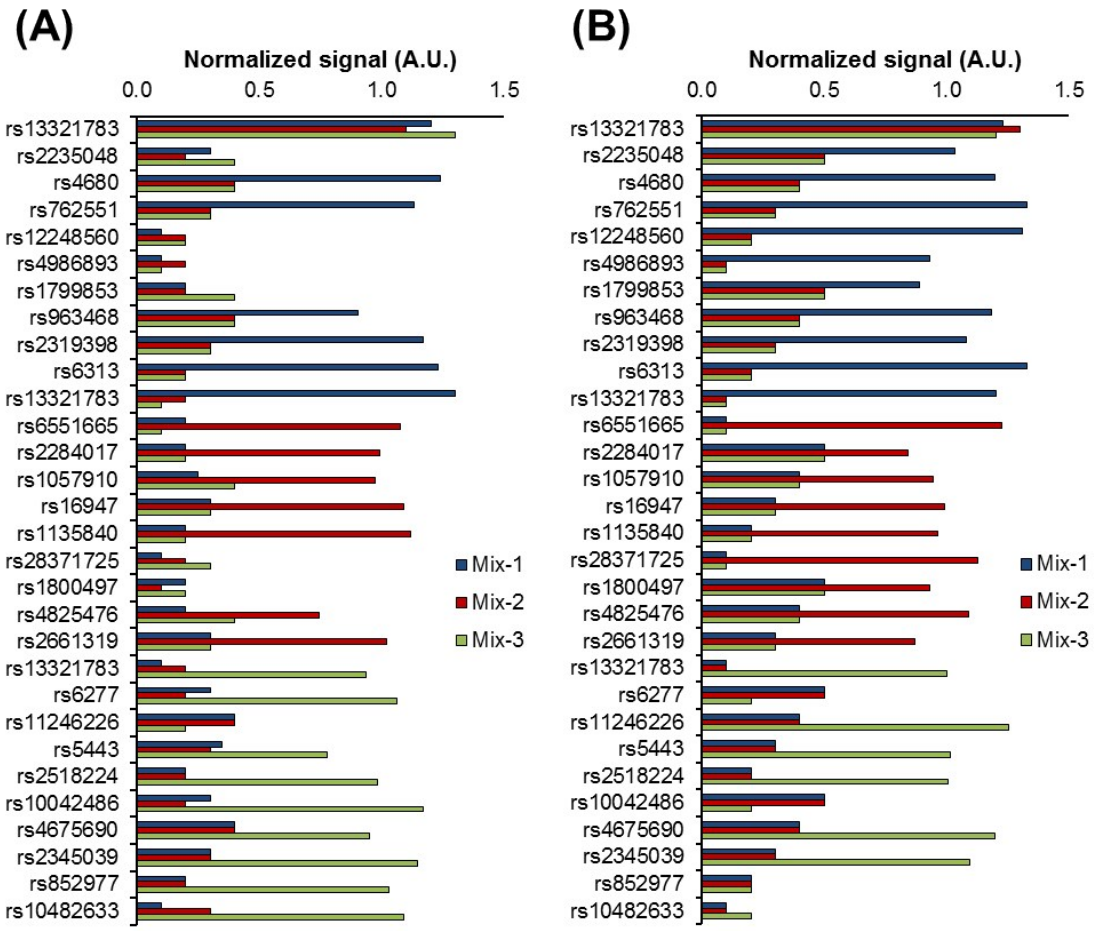

Figure SI.5. *The mean spot intensities for each SNP using different ligation mixtures in a 10-plex format: (A) signals of allele A and (B) signals of allele B.*

5) Analyzing patients

The assay performances were evaluated by applying the SNP genotyping method of biological human samples.

DNA extraction was performed using the PureLink Genomic DNA Mini Kit (Invitrogen), according to the manufacturer's instructions. The DNA concentration was determined using NanoDrop 2000/2000c (Thermo Scientific). The reference genotyping method was the GoldenGate assay with VeraCode Technology (Illumina).

Buccal cells were collected by rolling a swab (Catch-All sample collection swab, Epicenter) on the inside of a cheek. DNA extraction was performed using the PureLink Genomic DNA Mini Kit (Invitrogen). Briefly, the swab was incubated with 500 µL of PBS (1 X), 20 µL of Proteinase K and 20 µL of RNAse at room temperature for 2 min. Lysate buffer was added and incubated for 20 minutes at 55 ºC. Purification was performed by a spin column-based centrifugation procedure.

Figure SI.6 shows the scheme of the protocol stages and the array layout performed on the DVD surface.

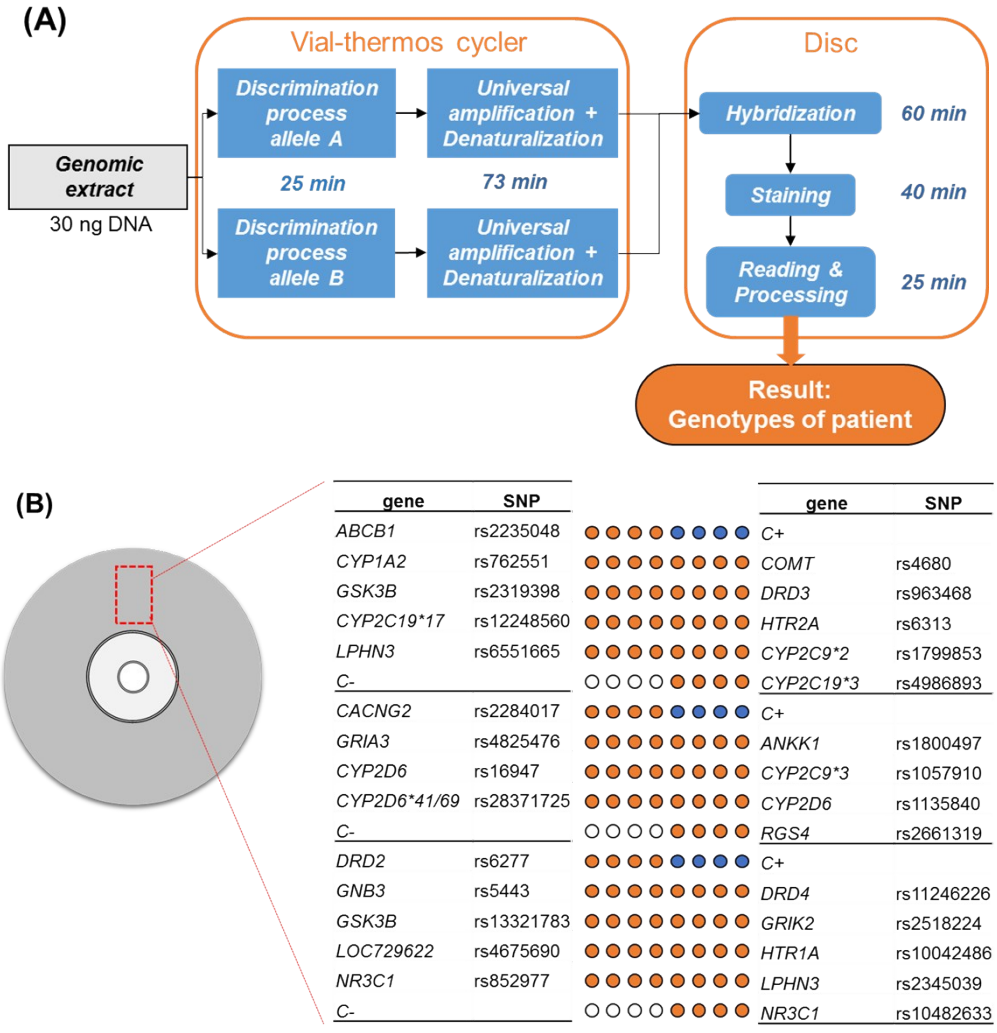

Figure SI.6. *(A) Analysis workflow for patient genotyping, including the analysis time. (B) Array layout for the analysis of patients with 28 genetic variants and controls. Spot replicates: 4 per probe. Patient/disc: 8.*

From the spot intensities of the wild-type and mutant probes (X_{iA} and X_{iB}) from the human samples of a training set, a multiple regression model was adjusted (function: $D_i = c_{i0} + c_{iA} X_{iA} + c_{iB} X_{iB}$). Table SI.6 shows the regression coefficients of the discriminant functions (c_i) for each studied polymorphism. The discriminant function number was *np-1*, and np was the number of genotype populations. For instance, the populations identified for SNP rs2235048 were CC, CT or TT. Thus two discriminant functions were required to differentiate the three populations.

Table SI.6. *Coefficients of the discrimination analysis models.*

			Function 1			Function 2		
			c_0	c_A	c_B	c_0	c_A	c_B
1	*ABCB1*	rs2235048	-0.09	0.09	-0.09	-2.29	0.05	0.04
2	*ANKK1*	rs1800497	-1.41	0.17	-0.03			
3	*CACNG2*	rs2284017	-0.28	0.11	-0.07	-3.07	0.04	0.06
4	*COMT*	rs4680	-0.42	0.13	-0.15	-2.08	0.04	0.09
5	*CYP1A2*	rs762551	0.23	0.08	-0.11	-2.11	0.06	0.03
6	*CYP2C19*17*	rs12248560	-0.27	0.12	-0.07			
7	*CYP2C19*3*	rs4986893						
8	*CYP2C9*2*	rs1799853	0.50	0.18	-0.06			
9	*CYP2C9*3*	rs1057910						
10	*CYP2D6*	rs16947	0.75	0.11	-0.14	-3.27	0.08	0.04
11	*CYP2D6*	rs1135840	-0.86	0.15	-0.07	-5.09	0.01	0.09
12	*CYP2D6*41/69*	rs28371725	0.01	0.14	-0.06			
13	*DRD2*	rs6277	-0.29	0.07	-0.12	-2.06	0.04	0.04
14	*DRD3*	rs963468	0.99	0.13	-0.16	-1.87	0.07	0.04
15	*DRD4*	rs11246226	-0.15	0.09	-0.09	-2.05	0.05	0.05
16	*GNB3*	rs5443	1.24	0.07	-0.13	-2.05	0.05	0.03
17	*GRIA3*	rs4825476	0.74	0.07	-0.13	-2.67	0.06	0.09
18	*GRIK2*	rs2518224	-0.70	0.08	-0.11	-1.95	0.03	0.06
19	*GSK3B*	rs2319398	0.77	0.07	-0.13			
20	*GSK3B*	rs13321783	-0.31	0.11	-0.17			
21	*HTR1A*	rs10042486	0.67	0.10	-0.09	-3.41	0.02	0.06
22	*HTR2A*	rs6313	0.60	0.09	-0.12	-1.94	0.07	0.02
23	*LOC729622*	rs4675690	0.54	0.08	-0.09	-2.27	0.06	0.05
24	*LPHN3*	rs6551665	0.13	0.07	-0.05	-3.29	0.04	0.05
25	*LPHN3*	rs2345039	0.11	0.06	-0.08	-1.80	0.03	0.04
26	*NR3C1*	rs852977	-0.02	0.11	-0.17	-1.56	0.06	0.03
27	*NR3C1*	rs10482633	0.08	0.08	-0.18	-2.36	0.06	0.03
28	*RGS4*	rs2661319	-0.02	0.10	-0.08	-2.59	0.05	0.05

By substituting data for a new sample (X_{iA} and X_{iB}), a score can be calculated and a patient can be classified in a population group. Table SI.7 depicts the genotypes obtained by analyzing the human samples of the validation sets by the DVD-array-MLPA method.

Table SI.7. *Genotyping results in the patients' samples. Red results indicate a discrepant assignation versus the value reported by the GoldGate assay (Illumina).*

	ABCB1	ANKK1	CACNG2	COMT	CYP1A2	CYP2C19*17	CYP2C19*3	CYP2C9*2	CYP2C9*3	CYP2D6	CYP2D6	CYP2D6*41/69	DRD2	DRD3
	rs2235048	rs1800497	rs2284017	rs4680	rs762551	rs12248560	rs4986893	rs1799853	rs1057910	rs16947	rs1135840	rs28371725	rs6277	rs963468
1	GG	AG	CT	AG	CA	CC	GG	CC	AA	GG	CC	CG	CC	AA
2	GG	GG	CT	AG	CA	CC	GG	CC	CA	AG	CG	CG	TT	AG
3	AG	GG	CC	AA	CA	CC	GG	CC	AA	AG	CG	AG	TT	AA
4	AG	GG	CC	AG	CA	CT	GG	CC	AA	AG	CG	CG	TT	AA
5	AA	GG	CC	AG	AA	CC	GG	CC	AA	AG	CG	CG	CT	AG
6	AA	AG	CC	AG	AA	CC	GG	CC	AA	GG	CC	CG	CT	GG
7	AG	GG	TT	AG	AA	CT	GG	CC	AA	GG	CG	CG	CC	GG
8	AA	GG	CT	GG	CA	CC	GG	CT	AA	AG	GG	CG	TT	GG
9	GG	AG	TT	GG	CA	CT	GG	CT	AA	AG	CG	CG	CC	GG
10	AG	GG	CT	AG	AA	CT	GG	CC	AA	GG	GG	CG	TT	GG
11	GG	GG	CT	AG	CA	CC	GG	CC	AA	AG	CG	GG	CT	GG
12	AA	GG	CC	GG	CA	CC	GG	CT	AA	AG	CG	CG	TT	AG
13	AG	GG	TT	AG	AA	CC	GG	CT	AA	GG	CG	CG	TT	AA
14	GG	AG	CT	AA	AA	CC	GG	CC	AA	AG	CG	CG	CT	AA
15	AG	GG	CT	AA	AA	CC	GG	CC	AA	AG	CG	CG	TT	AG
16	AG	GG	CC	AG	CA	CC	GG	CC	AA	AG	CG	AG	CT	AG
17	AA	GG	TT	GG	CA	CC	GG	CC	AA	AG	CG	CG	CT	GG
18	AA	GG	CC	AG	CC	CT	GG	CC	AA	GG	GG	CG	TT	GG
19	AA	GG	CC	AG	CA	CC	GG	CT	AA	AA	GG	CG	CT	AA
20	GG	AG	CT	GG	CA	CC	GG	CC	AA	AG	CG	CG	CT	AA
21	AA	AG	CT	AA	CC	CC	GG	CC	AA	GG	GG	CG	TT	AG
22	AG	AG	TT	AA	CC	CC	GG	CC	AA	AG	CG	CG	CT	AA
23	GG	AA	CT	AA	AA	CT	GG	CC	AA	AG	CG	CG	CT	AG
24	AG	GG	CC	AA	AA	CC	GG	CC	AA	AG	CG	CG	TT	AG
25	AG	GG	CT	GG	CA	CT	GG	CC	AA	GG	CC	CG	TT	AG
26	AA	AG	CT	AA	AA	CC	GG	CC	AA	GG	CG	CG	CT	GG
27	AG	AG	CC	AA	AA	CT	GG	CC	AA	GG	CC	CG	CT	AG
28	AA	GG	TT	GG	AA	CC	GG	CT	AA	AG	GG	CG	CT	AG
29	AG	GG	CT	GG	CA	CC	GG	CC	AA	GG	GG	CG	TT	GG
30	AG	GG	CC	AG	AA	CT	GG	CC	AA	GG	CC	CG	TT	GG
31	AA	GG	CC	AA	CA	CC	GG	CC	AA	GG	CC	CG	TT	AG
32	AA	GG	CT	AG	AA	CC	GG	CC	AA	AG	CG	CG	CT	AG
33	AG	GG	CT	AA	CA	CC	GG	CC	AA	AG	CG	AG	CC	AG
34	AG	GG	CC	AG	CA	CC	GG	CT	AA	AG	CG	AG	CT	AG
35	GG	GG	CT	AG	CA	CT	GG	CC	AA	GG	GG	CG	TT	GG
errors	0	0	2	0	2	1	0	0	0	0	1	0	1	1

(Continuation)

	DRD4	GNB3	GRIA3	GRIK2	GSK3B	GSK3B	HTR1A	HTR2A	LOC729622	LPHN3	LPHN3	NR3C1	NR3C1	RGS4
	rs11246226	rs5443	rs4825476	rs2518224	rs2319398	rs13321783	rs10042486	rs6313	rs4675690	rs6551665	rs2345039	rs10482633	rs852977	rs2661319
1	AC	TT	AA	AA	GG	TT	CC	GG	CC	AG	CG	AA	AA	CT
2	AC	CC	AG	AC	GT	TT	TT	AG	CT	AG	CG	AA	AA	CT
3	AC	CT	AG	AC	TT	CT	CT	GG	CC	AG	CG	AA	AA	CC
4	CC	CT	AA	AA	TT	CC	CT	AG	CT	AA	CG	GG	AA	CC
5	AA	CC	AA	AA	GG	CT	CT	GG	CT	GG	CC	AA	AA	CC
6	AC	CC	AA	AA	TT	CC	CC	AG	CT	AA	CG	AG	AA	TT
7	AA	CT	AA	AA	TT	CC	CT	AG	TT	AA	CG	AG	AC	CC
8	AC	TT	AA	AC	GT	CC	CC	AG	CT	AA	GG	AA	AA	CC
9	-	CT	AA	AA	GG	CT	CT	AA	CT	AG	GG	GG	AC	CT
10	AA	CC	AA	AA	GG	TT	TT	AG	TT	AG	CC	AA	AC	CC
11	AC	CC	AA	AA	GT	CT	CT	AA	CC	AA	GG	AG	AA	CT
12	CC	CC	GG	AA	GT	CT	CT	AG	CT	AA	GG	GG	AC	CT
13	AC	TT	AA	AC	GT	TT	CC	AG	CT	AG	CG	AG	AA	CT
14	AC	CC	AG	AA	GT	CT	TT	AA	CC	AA	GG	AG	AC	CT
15	AA	CC	AG	AA	TT	CC	CC	AG	TT	GG	CC	AG	AC	TT
16	CC	CT	AA	AA	GG	CT	CT	AG	CT	AG	GG	AA	AA	CT
17	AC	CT	AG	AA	GT	CT	TT	AA	CC	AA	GG	AA	AA	CT
18	AA	CT	GG	AA	GT	CT	CC	AG	CC	AA	GG	AG	AC	CC
19	CC	CT	AG	AA	GT	CT	CT	AA	CT	GG	CG	AA	AA	CT
20	AC	TT	AG	AA	GT	CT	CT	AG	CC	AG	GG	AA	AA	CT
21	AA	CC	AA	AA	GT	TT	CT	AG	CT	AG	CG	AA	AA	CT
22	CC	TT	GG	AC	GT	TT	CC	AG	CC	AA	CG	AA	AA	CC
23	AA	TT	GG	AA	GT	TT	CT	AG	TT	AG	CG	GG	AC	TT
24	CC	CT	AA	AC	GG	CT	TT	AG	TT	AG	CG	AA	AA	TT
25	AA	CC	GG	AA	GT	TT	CT	AG	CC	AG	CG	AA	AA	CT
26	AC	CC	AA	AA	GT	CT	CC	AG	CC	GG	CG	GG	CC	CT
27	AC	CC	AA	AA	GG	CT	CT	AG	CT	AG	CC	AA	AA	CC
28	AC	TT	AA	AA	GT	TT	TT	AG	TT	AA	GG	AA	AA	CT
29	AA	CT	AA	AA	GG	TT	TT	AA	TT	AG	CC	AG	AA	CC
30	CC	CT	AA	AC	GG	CT	CC	GG	TT	AG	CG	AA	AA	TT
31	AA	CT	AA	AA	GT	TT	CC	AA	CT	AG	CG	AG	AC	CC
32	AA	TT	AA	AA	GG	TT	CC	AA	CC	GG	CC	AA	AA	CT
33	AC	CT	AA	AA	GG	TT	TT	AG	CT	AA	GG	AA	AA	TT
34	AC	CT	AA	CC	GG	TT	TT	AG	CT	AA	GG	AA	AA	CC
35	CC	CT	AA	AA	GG	TT	TT	AA	CC	AG	CG	AG	AA	CT
errors	0	2	2	3	4	4	1	0	3	1	0	0	0	0