CE IVD

INSTRUCTION FOR USE

SPMED™ Genotyping Kit: CYP2D6

REF SGSAA1

SPMED
(주)에스피메드

CONTENTS

**** Be sure to read all the contents in the user manual before using.***

1. Intended use

This product is an in vitro diagnostic medical device that qualitatively analyzes seventeen (17) variants of the CYP2D6 gene (100C>T, 1023C>T, 1611T>A, 1707delT, 1758G>A, 1846G>A, 1887insTA, 2549delA, 2573_2574insC, 2615_2617delAAG, 2850C>T, 2988G>A, 3183G>A, 3877G>A, 4125_4133dup GTGCCCACT, deletion, duplication) from genomic DNA extracted from human whole blood by single base extension.

This kit is IVD reagents for pharmacogenetics used by a health-care professional. The test results can be used as an aid to clinicians in determining therapeutic strategy and treatment dose related to drugs metabolized by CYP2D6 gene.

2. Principles of the examination method

This kit is designed to determine the genotype by amplifying and analyzing the specific nucleotide sequence of 17 mutations of CYP2D6 to confirm the presence or absence of a CYP2D6 mutation and a specific nucleic acid sequence. After amplifying the gene with CYP2D6 specific primer, SNaPshot reaction is performed using specific primer for detecting specific SNP (Single Nucleotide Polymorphism) of CYP2D6 (100C>T, 1023C>T, 1611T>A, 1707delT, 1758G>A, 1846G>A, 1887insTA, 2549delA, 2573_2574insC, 2615_2617delAAG, 2850C>T, 2988G>A, 3183G>A, 3877G>A, 4125_4133dupGTGCCCACT, deletion, duplication). The SNP of the product obtained through the SNaPshot reaction can be confirmed as a peak, and the variation of the SNP can be confirmed by the color of each peak.

It is a qualitative analysis In vitro diagnostic medical device that uses the Single-base extension (SBE) test method, that can identify mutations in peak form by combining amplified CYP2D6 products, fluorescent substances including ddNTP, and primers prepared up to the sequence immediately before the mutation. The CYP2D6 mutation is determined based on the analysis results of Wild Type DNA included in the kit. Samples with CYP2D6 mutations show peaks with distinct color and height differences compared to Wild Type DNA, which can accurately detect CYP2D6 mutations. Identifying CYP2D6 mutations can help with the selection and dose control of drugs metabolized by CYP2D6.

PCR is carried out using one of the components, 2X PCR Amplification Mix and 3 types of primers (CYP2D6 APM1, APM2, APM3). After separating double strands of human genomic DNA at 94 ℃, using CYP2D6 specific primers at 64 ℃ Amplifies. And one of the components SNaPshot Multiplex Reagent and 100C>T, 1023C>T, 1611T>A, 1707delT, 1758G>A, 1846G>A, 1887insTA, 2549delA,

2573_2574insC, 2615_2617delAAG, 2850C>T, 2988G>A, 3183G>A, 3877G>A, 4125_4133dup GTGCCCACT, deletion, duplication specific SNaPshot primers (SPM1, SPM2) is used to perform SNaPshot reaction. By analyzing the product after the reaction, a genetic analyzer (sequencer) can be used to identify each fluorescently labeled SNP as a peak.

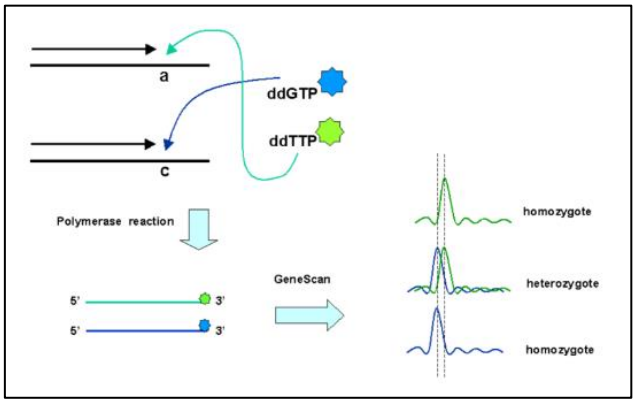
Table 1. CYP2D6 SNP detected by SPMED™ Genotyping Kit: CYP2D6

No.	CYP2D6 SNP	
	SPM1	SPM2
1	100C>T	1023C>T
2	1611T>A	1707delT
3	1758G>A	1846G>A
4	1887insTA	2549delA
5	2573_2574insC	2615_2617delAAG
6	2850C>T	3183G>A
7	2988G>A	Duplication
8	3877G>A	
9	4125_4133dupGTGCCCACT	
10	Deletion	

* Description of single-base extension

Single-base extension (SBE) is called SNaPshot and is a method used to test multiple mutations at once. The single-base extension is a method of constructing a primer up to the sequence immediately before the mutation, binding it to the complementary site of the mutation, and expanding one base to perform a genetic test with a fluorescent label or a mass label. This test method has the advantage of being less expensive to experiment and multiple tests for multiple mutations than other genetic test methods such as sequencing.

Figure 1. Single Base Extension

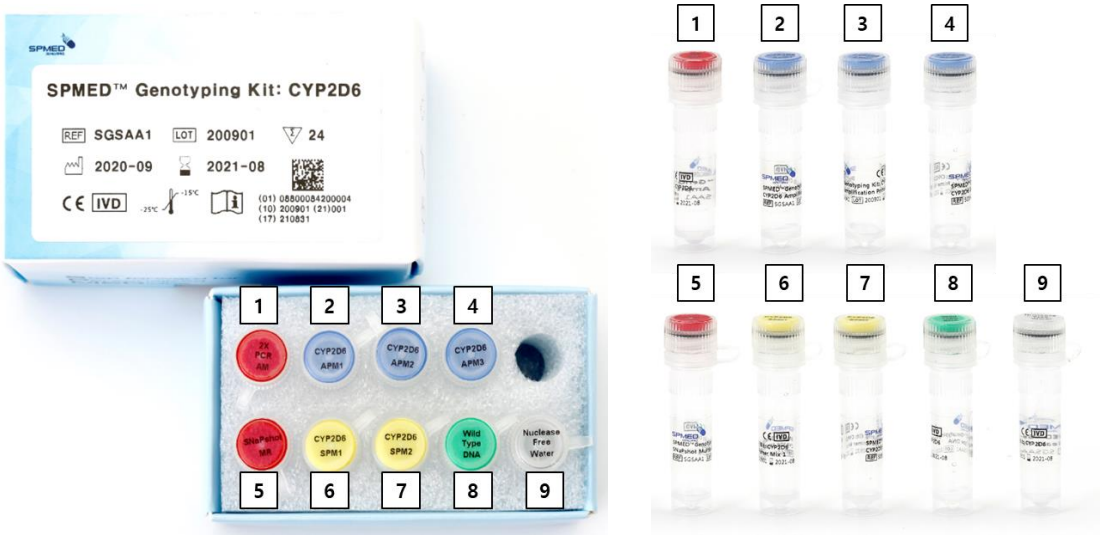


3. Metrological traceability

The standard materials used in quality control of SPMED™ Genotyping Kit: CYP2D6 were confirmed by the reference measurement procedure, Sanger Sequencing method from the third party organizations, PharmacoGenomics Research Center, INJE university, and/or Coriell Institute, for each of 17 variant types and Wild Type.

4. Components

4.1 Appearance



4.2 Detailed components

No.	Component	Purpose	Appearance features	Volume	Quantity
1	2X PCR Amplification Mix	Polymerase that can amplify DNA	Colorless, odorless liquid in	800 ㎕	1EA
2	CYP2D6 Amplification Primer Mix 1	Oligomer for amplifying CYP2D6 gene	a transparent 2 ml plastic tube (red lid)	100 ㎕	1EA
3	CYP2D6 Amplification Primer Mix 2	Oligomer for amplifying CYP2D6 gene	Colorless, odorless liquid in	100 ㎕	1EA
4	CYP2D6 Amplification Primer Mix 3	Oligomer for amplifying CYP2D6 gene	a transparent 2 ml plastic tube (blue lid)	100 ㎕	1EA
5	SNaPshot Multiplex Reagent	Polymerase for amplifying SNP of CYP2D6	Colorless, odorless liquid in	50 ㎕	1EA
6	CYP2D6 SNaPshot Primer Mix 1	Oligomer to detect CYP2D6 gene mutation	a transparent 2 ml plastic tube (blue lid)	25 ㎕	1EA
7	CYP2D6 SNaPshot Primer Mix 2	Oligomer to detect CYP2D6 gene mutation	Colorless, odorless liquid in	25 ㎕	1EA
8	Wild Type DNA	Reagents containing wild type DNA	a transparent 2 ml plastic tube (blue lid)	100 ㎕	1EA
9	Nuclease Free Water	Sterilized distilled water	Pink, odorless liquid in	1 ㎖	1EA

5. Additional required reagents and equipment

5.1 Reagent

Classification	Reagent Name	Cat.No.	Manufacturer
Gene extraction reagent	QIAamp DSP DNA Mini Kit	61304	QIAGEN
DNA Electrophoresis	Agarose	-	-
	0.5X TAE or TBE buffer	-	-
	1kb DNA ladder marker	-	-
Clean Up Enzyme	ExoSAP-IT	78201	Applied Biosystems
	Shrimp Alkaline Phosphatase	783901000UN	Applied Biosystems
SNaPshot reaction	1/2 term buffer (200mM Tris-HCl, 5Mm MgCl2, pH9.0)	-	-
Sequencing reagent	GeneScan™ 120 LIZ™ Size Standard	4324287	Applied Biosystems
	Hi-Di™ Formamide	4311320	Applied Biosystems
	Cathode Buffer Container 3500 Series each	4408256	Applied Biosystems
	Anode Buffer Container 3500 Series each	4393927	Applied Biosystems
	Conditioning Reagent 3500 Series each	4393718	Applied Biosystems
	POP-7™ Polymer 3500 series each	4393714	Applied Biosystems
	3500 Genetic Analyzer 8-Capillary Array, 50 cm	4404685	Applied Biosystems
	96-well Plate Septa	4315933	Applied Biosystems

5.2 Instrument

No.	Equipment Name	Manufacturer
1	Veriti 96-Well Thermal Cycler	Applied Biosystems
2	3500Dx Genetic Analyzer	Applied Biosystems
3	GeneMapper® Software (Version 5 recommended)	Applied Biosystems
4	Pipettes	-
5	Vortex	-
6	Centrifuge	-
7	PCR tube and cap	-
8	Gel tray and Electrophoresis equipment	-
9	Gel documentation equipment	-

6. Storage and shelf life after first opening

Before Opening	12 months at -25~ -15℃
After Opening	6 months at -25~ -15℃

1) SPMED™ Genotyping Kit: CYP2D6 must be stored frozen at temperatures between -25 ℃ and -15 ℃, avoiding UV rays or sunlight, and all components are guaranteed for stability only when stored under the storage conditions below until the expiration date.

2) The validity period of the product is 12 months from the date of manufacture before opening, and each component must be used within 6 months from the date of opening. It is recommended not to use products that have expired.

3) Do not exceed 5 times for freezing and thawing of the product. Repeated freezing and thawing of the product may cause abnormality in product quality, such as deterioration of enzyme activity. In case of frequent use, aliquot to a certain amount for storage. The tubes are tightly closed after use for further storage.

4) Avoid storage in places where chemicals or other foreign substances may be mixed.

5) If the product packaging is damaged due to transportation problems, etc., please contact your dealer to resolve the problem.

- Reagents used should be kept away from direct sunlight and stored according to the storage method.

7. Warnings and precautions

7.1 General precautions

- This product must be used for in vitro diagnostic use only and should not be used for any other purpose.
- Only an experienced expert should be familiar with the kit manual and conduct inspections to obtain accurate results.
- This product is an in vitro diagnostic medical device that assists in the selection and dose control of drugs metabolized by CYP2D6 gene through CYP2D6 genotyping, cannot be diagnosed solely by the results of this test. The clinical judgment based on the measurement results should be finalized at the judgment of a physician along with other test results.

7.2 Product warranty and responsibility

- We guarantee the quality of the products used within the validity period, which is 12 months from the date of manufacture, and each component must be used within 6 months of opening.
- The product performance and results are guaranteed only when the product is used in accordance with the instructions in this manual, should not be used for purposes other than the intended use of this product.
- If there is a problem due to the wrong use or carelessness of the experimenter, it cannot be exchanged.
- We are not responsible for any problems arising from the use of methods other than those specified in this manual. For efficient market reporting and processing, the customer must communicate the problem in detail to us within 30 days of the problem.

7.3 Safety warnings and first aid Tips

- Avoid contact with reagents by the eyes, respiratory system or skin.
- When reagents get into the eyes, the eyes should be washed with running water. If irritation persists, see a doctor.
- If the reagent contacts with the skin, the contacted area should be thoroughly washed with soap and water. If irritation persists, see a doctor.

8. Primary sample collection, handling and storage

8.1 Sample preparation

1) When the specimen is whole blood, blood is collected in a tube containing an anticoagulant (EDTA or heparin), and the blood is sufficiently mixed with the anticoagulant to prevent blood clotting.

2) The extraction of nucleic acid (DNA) is recommended using a commercially available DNA extraction kit. For extraction method, refer to the manufacturer's instructions.

No.	Reagent Name	Manufacturer
1	QIAamp DSP DNA Mini Kit	QIAGEN

3) Depending on the DNA extraction kit and extraction method, the yield of DNA may vary, which may affect the test results.

8.2 Sample storage

1) If the specimen is not used on the same day, it should be dispensed in an appropriate amount and stored in frozen (-25 ~ -15 ℃) conditions before testing.

2) However, it will not be stored beyond 12 months.

3) When the specimen needs to be freezing and thawed, it is recommended not to exceed 3 times for whole blood and 5 times for DNA, and it is recommended to use an appropriate amount by dispensing when used frequently.

- The number of repetitions of freezing and thawing of the specimen used for the test should not exceed 3 times for whole blood and 5 times for DNA.
- Specimens used in the test (whole blood, DNA) can be stored for up to 12 months under frozen conditions (-25 ~ -15 ℃).

9. Examination procedure

9.1 Gene amplification

1) Make 20μl of the mixture as follows.

PCR	Sample		Control	
	Composition	Quantity	Composition	Quantity
CYP2D6 Amplification Primer Mix 1	Genomic DNA	100ng	Wild Type DNA	4 ㎕
	2X PCR Amplification Mix	10 ㎕	2X PCR Amplification Mix	10 ㎕
	CYP2D6 APM1	4 ㎕	CYP2D6 APM1	4 ㎕
	Nuclease Free Water	Up to 20 ㎕	Nuclease Free Water	2 ㎕
	Total amount	20 ㎕	Total amount	20 ㎕
CYP2D6 Amplification Primer Mix 2	Genomic DNA	100ng	Wild Type DNA	4 ㎕
	2X PCR Amplification Mix	10 ㎕	2X PCR Amplification Mix	10 ㎕
	CYP2D6 APM2	4 ㎕	CYP2D6 APM2	4 ㎕
	Nuclease Free Water	Up to 20 ㎕	Nuclease Free Water	2 ㎕
	Total amount	20 ㎕	Total amount	20 ㎕
CYP2D6 Amplification Primer Mix 3	Genomic DNA	100ng	Wild Type DNA	4 ㎕
	2X PCR Amplification Mix	10 ㎕	2X PCR Amplification Mix	10 ㎕
	CYP2D6 APM3	4 ㎕	CYP2D6 APM3	4 ㎕
	Nuclease Free Water	Up to 20 ㎕	Nuclease Free Water	2 ㎕
	Total amount	20 ㎕	Total amount	20 ㎕

2) The mixed solution is spin down for 1-2 seconds, then vortexed for 2-3 seconds, and then spin down for 5 seconds.

3) PCR is performed under the following temperature conditions.

PCR condition	
(1) Initial denaturation: 94℃, 5min (2) Denaturation: 98℃, 20sec (3) Annealing: 64℃, 30sec (4) Extension: 72℃, 3min (5) Final extension: 72℃, 5min	The process (2)~(4) is repeated 35 cycles.

4) PCR products should be confirmed by electrophoresis immediately, or stored at 2 ~ 8 ℃ for less than a week and -25 ~ -15 ℃ for more than a week until electrophoresis is performed.

9.2 Electrophoresis

1) PCR products of CYP2D6 Amplification Primer 1-3 are electrophoresed on a 1% agarose gel. Electrophoresis is according to the device manufacturer's recommendations.
2) Stain agarose gel with the final concentration of Ethidium Bromide (EtBr) at 0.5 ㎍/㎖.
3) Electrophoresis buffer solution is used in the same way as agarose gel buffer solution.
4) Each PCR product is mixed with an appropriate amount of 6x loading dye and loaded to agarose gel.
5) Electrophoresis is performed until the lower loading Dye (Two Dye System) is located on the lower 2/3 of the entire agarose gel.
6) When electrophoresis is completed, confirm by comparing with PCR product amplified with positive control DNA with UV transilluminator. Each band should be the same size as the PCR product amplified with wild type DNA and have a thickness similar to 90% or more.

9.3 PCR Clean Up

1) Prepare 7.5μl of the mixture as follows.

Mix type	Composition	Quantity
PCR products of CYP2D6 APM1, APM2 (for CYP2D6 SPM 1)	Exo-SAP IT	2.5 ㎕
	PCR products of CYP2D6 APM1	2 ㎕
	PCR products of CYP2D6 APM2	3 ㎕
	Total amount	7.5 ㎕
PCR products of CYP2D6 APM1, APM3 (for CYP2D6 SPM 2)	Exo-SAP IT	2.5 ㎕
	PCR products of CYP2D6 APM1	2 ㎕
	PCR products of CYP2D6 APM3	3 ㎕
	Total amount	7.5 ㎕

2) The mixed solution is spin down for 1-2 seconds, then vortexed for 5 seconds or more, and then spin down for 5 seconds.

3) Perform PCR clean-up process under the following conditions.

PCR Clean up condition
(1) 37℃, 35min (2) 80℃, 15min

9.4 SNaPshot reaction

1) Prepare a mixed solution as follows.

Primer type	Composition	Quantity
CYP2D6 SNaPshot Primer Mix 1 (for CYP2D6 SPM 1)	1/2 term buffer	4 ㎕
	SNaPshot Mutiplex Reagent	1 ㎕
	Product of PCR clean up	7.5 ㎕
	CYP2D6 SNaPshot Primer Mix 1	1 ㎕
	Total amount	13.5 ㎕
CYP2D6 SNaPshot Primer Mix 2 (for CYP2D6 SPM 1)	1/2 term buffer	4 ㎕
	SNaPshot Mutiplex Reagent	1 ㎕
	Product of PCR clean up	7.5 ㎕
	CYP2D6 SNaPshot Primer Mix 2	1 ㎕
	Total amount	13.5 ㎕

2) SNaPshot reaction is performed under the following conditions.

SNaPShot condition	
(1) 96℃, 10sec (2) 50℃, 5sec (3) 60℃, 30sec	All processes are repeated 40 cycles.

9.5 SNaPshot Clean up

1) Make 15μl of mixed solution as follows.

Composition	Quantity
SAP	1.5 ㎕
SNaPshot product	13.5 ㎕
Total amount	15 ㎕

2) The mixed solution is spin down for 1-2 seconds, then vortexed for 5 seconds or more, and then spin down for 5 seconds.

3) SNaPshot clean-up is performed under the following conditions.

SNaPShot Clean up condition
(1) 37℃, 60min (2) 60℃, 15min

4) The completed SNaPshot clean-up product is immediately prepared for sequencing electrophoresis using a genetic analyzer. Samples should be stored at 2-8 ℃ if stored for less than a week and -25 ~ -15 ℃ if stored for less than a week until sequencing electrophoresis using a genetic analyzer.

9.6 Preparation of electrophoresis using genetic analyzer

1) Make a mixed solution as follows.

Composition	Quantity
SNaPshot Clean Up product	1 ㎕
Hi-Di™ Formamide	8.5 ㎕
GeneScan™-120LIZ size standard	0.5 ㎕
Total amount	10 ㎕

2) Heat denaturation is performed under the following conditions.

Heat denaturation
95℃, 5min

3) After the heat denaturation process, immediately after being sufficiently cooled in an ice state, electrophoresis is performed using a genetic analyzer. The operation of the genetic analyzer follows the

4) recommendations of the device manufacturer.

- Reagents for testing are sensitive to moisture as well as heat, so open immediately before use, and use immediately after opening.

	- Unopened products are stable for up to 12 months under frozen conditions (-25 ~ -15 ℃), and open products are stable for up to 6 months under frozen conditions (-25 ~ -15 ℃), so do not exceed them. - The number of repetitions of freezing and thawing of the product should not exceed 5 times. If necessary, dispense reagents and store them. - The expiration date of the product is indicated on the outside of the package, and the product whose expiration date has passed shall not be used. - Do not use if the package is damaged or the seal is peeled off. - Reagents are used according to the method of use described in this manual, and the reliability of the measured value cannot be guaranteed for use other than the method of use and the purpose of use.

10. Control procedure

1) This product contains Wild Type DNA, a control material. For quality control, it is recommended to test with the control material at each test.

2) The PCR result of the specimen should be compared with the wild type DNA PCR result. After confirming that the size between the results and the thickness of the band is more than 90% similar, the test of the next step is performed.

3) All test results should not detect bands and peaks higher than 100 in the negative control (No Template Control, NTC).

4) Verify that each mutant peak appears visually and in the expected color. If other colored peaks appear, retest the sample.

5) If bands and peaks are not detected in the control group indicated by wild type DNA, the test results are invalidated and the specimens are retested.

6) Verify that the Wild Type DNA test results have been properly analyzed.

7) If more frequent control experiments are required due to the quality control standards of the laboratory in which this test is conducted, the tests should be conducted based on the criteria set in the laboratory.

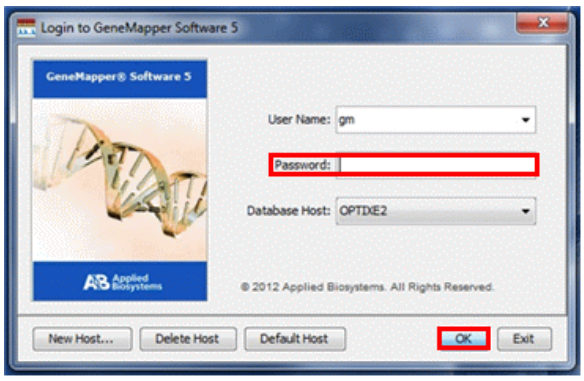
11. Analysis of examination results

✓ This is a method of analyzing the results of using this product using the test equipment software (GeneMapper® Software, Version 5). For more detailed information, refer to the manual of the software manufacturer (Applied Biosystems).

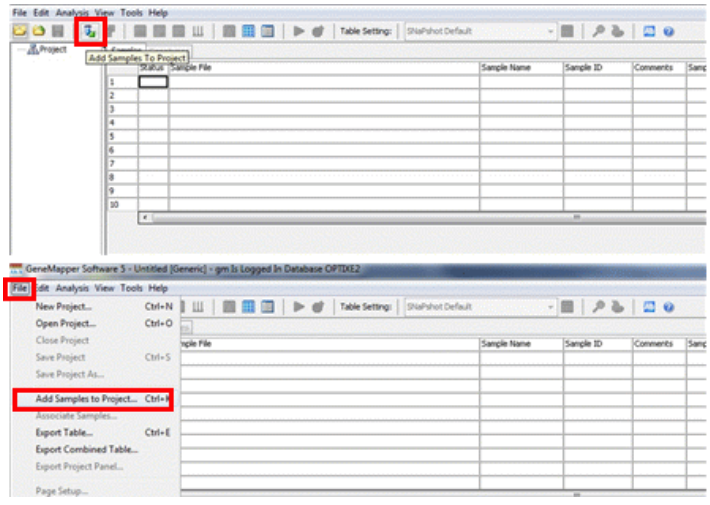
1.1 Analysis of results using GeneMapper® Software Version 5

1) Double-click the GeneMapper 5 icon to activate it.

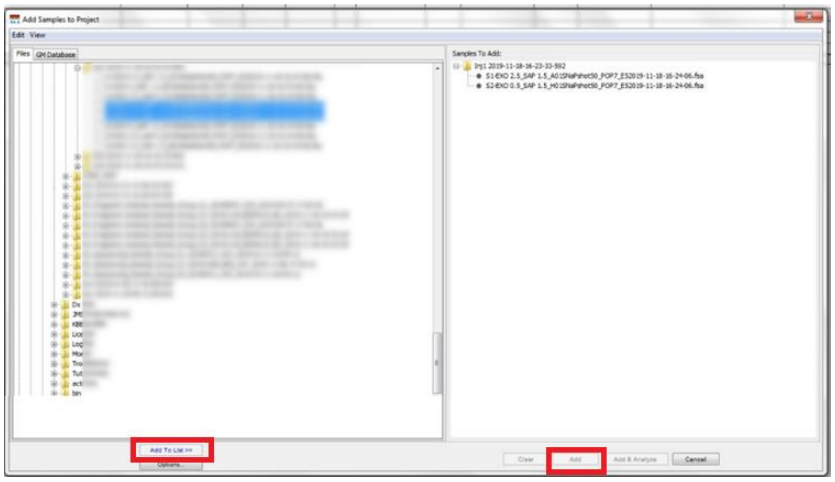
2) When activated, the following password window appears. After entering the password, click 'OK' on the bottom right.



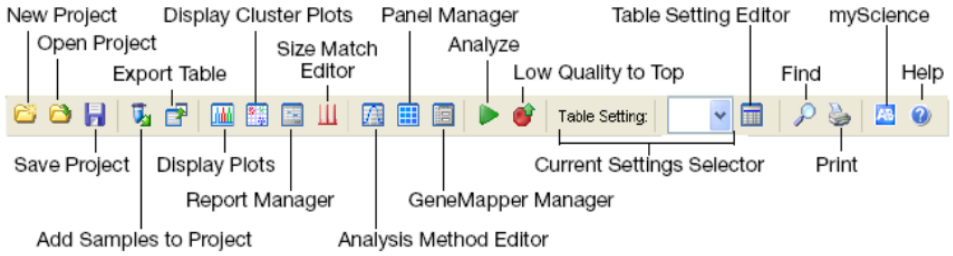
3) Click 'File' at the top of the menu, then click 'Add Samples to Project'. Clicking the icon performs the same function.



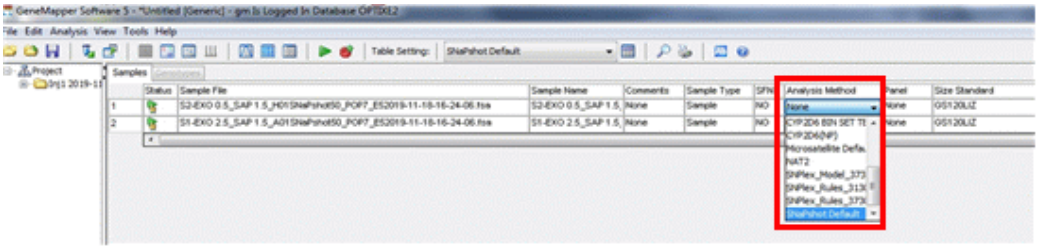
4) The screen to specify the path to load the saved data appears as shown below. Find and click the raw data in the personal folder, click 'Add to List' at the bottom left, and check that the raw data list has been added to the right screen. After checking, click 'Add' at the bottom right.



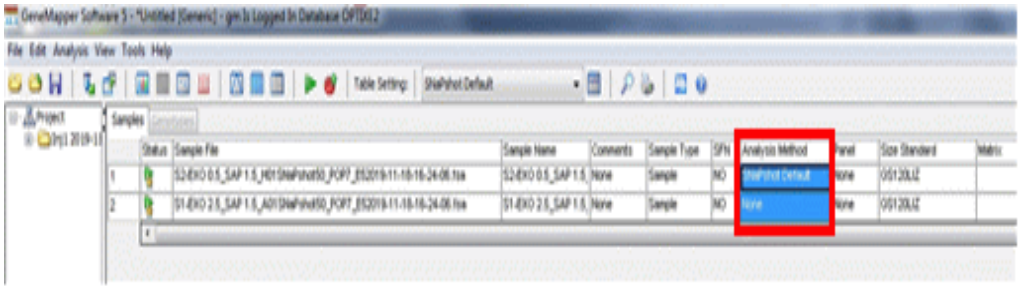
5) Each icon at the top of the menu has the following meaning.



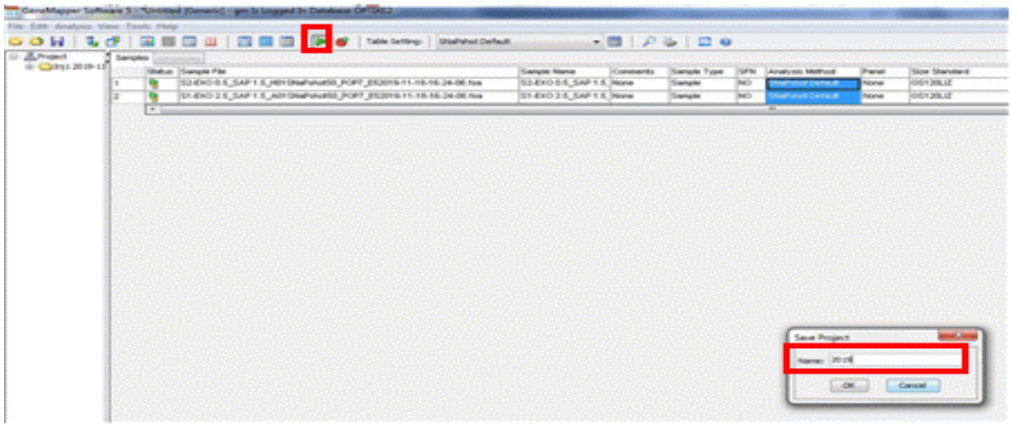
6) Check if the imported raw data has been added, click 'Analysis Method' and select 'SNaPshot Default'.



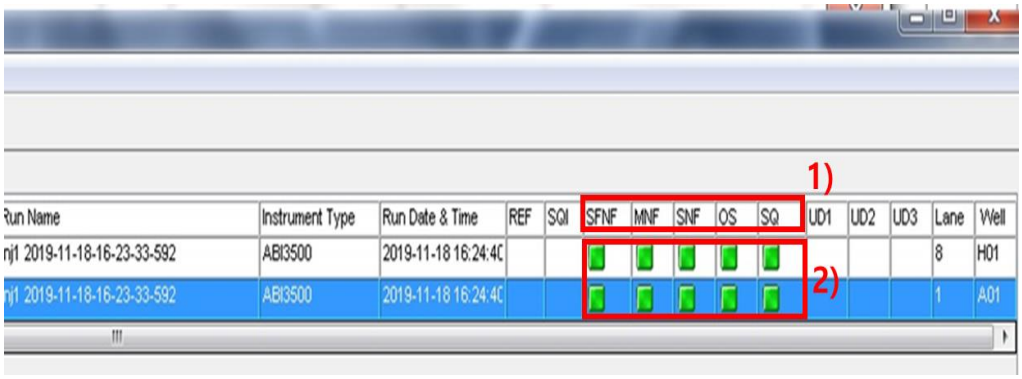
7) Select the entire column using the mouse and Ctrl button for the 'Analysis method', and then press Ctrl + D to apply it to all files.



8) If you click the ' Analyze' icon at the top of the menu, a warning window appears asking you to save the project as shown below. Enter an appropriate name and click OK.



9) When the analysis is completed, the following screen is displayed, and the QC result (Genotype Quality) is displayed.



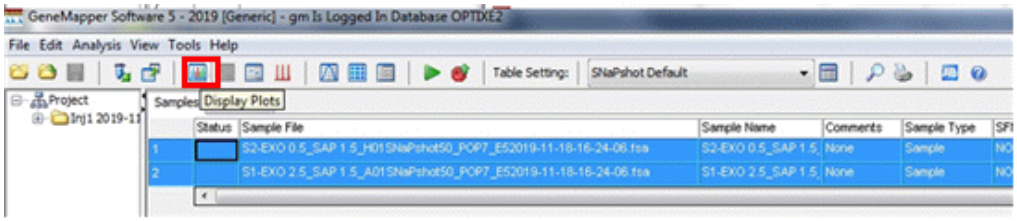
(1) QC result display column

SFNF (Sample file not found)	It is a case where a sample file is not found.
MNF (matrix not found)	It can be safely ignored because the matrix has not been set for the analysis.
SNF (size standard not found)	This is the case when the size standard is not detected.
OS (Off Scale)	When the signal of each peak appears outside the size standard.
SQ (Sizing Quality)	It is a case where sizing is not performed properly.

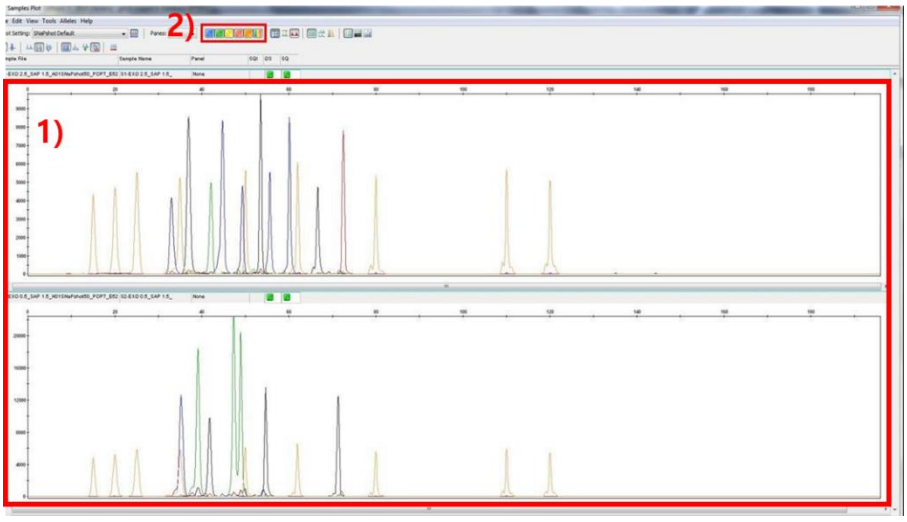
(2) Peak Quality

	The analysis is normally completed
	The analysis is completed, but there is a lack of quality.
	The case where the analysis failed.

10) Select a file to analyze using the mouse and click  'Display Plots' icon at the top of the menu.



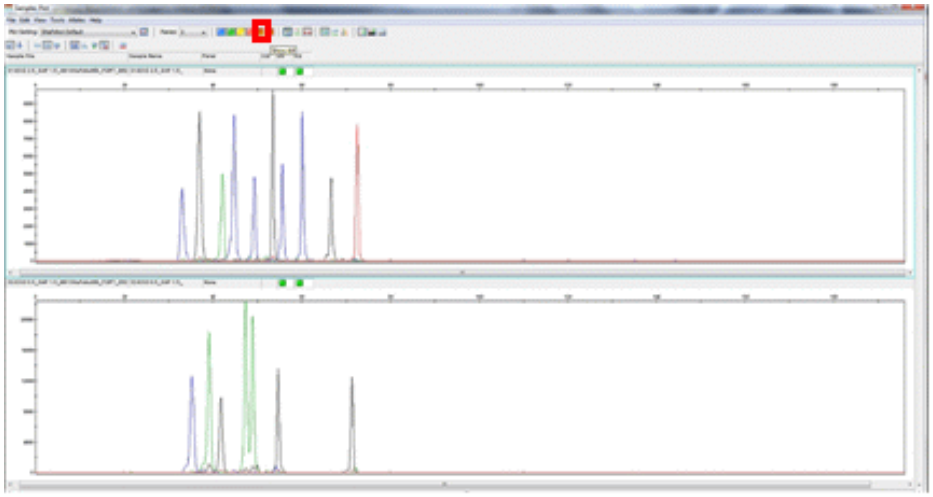
11) Click 'Display plots' and the following screen will appear.



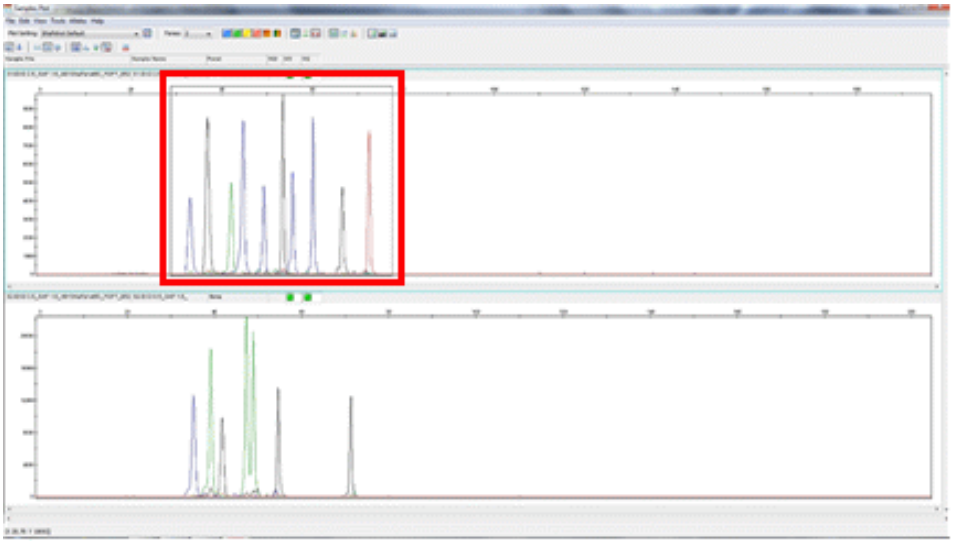
The meaning of the peak color and peak display/non-display icon displayed on the screen is as follows.

	1) peak color	2) peak display/non-display icon
A peak	**Green**	
T peak	**Red**	
G peak	**Blue**	
C peak	**Black**	
GS120LIZ (size standard)	**Orange**	
All		

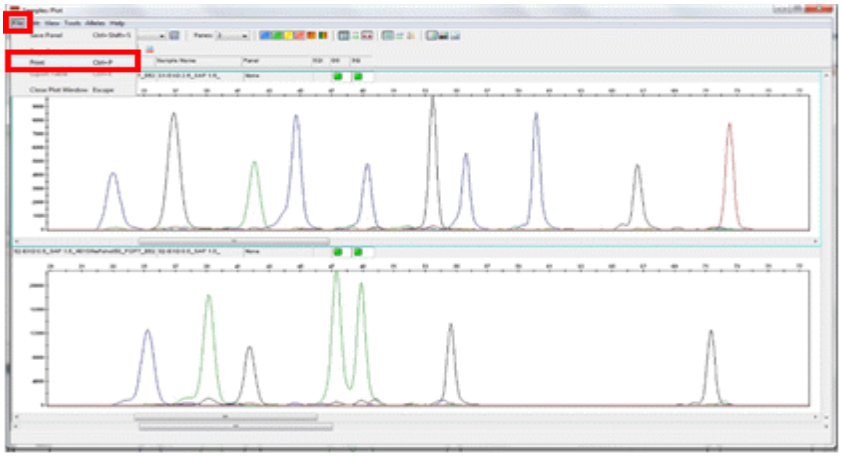
12) Click on the orange GS120LIZ( )'to disable the size standard peak.



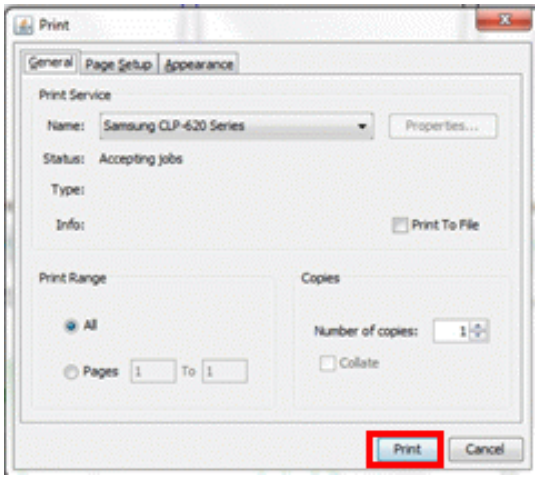
13) For convenience of analysis, use a mouse to enlarge as needed.



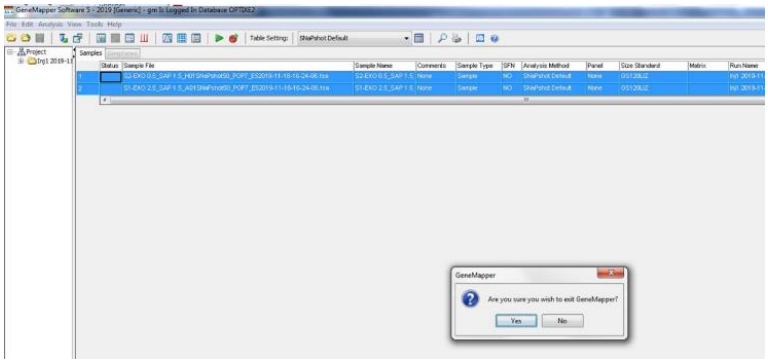
14) To print each confirmed peak, click 'Print' in 'File' on the top menu.



15) When you click Print, the following screen appears. After checking the printer connected to the computer, click 'print'.



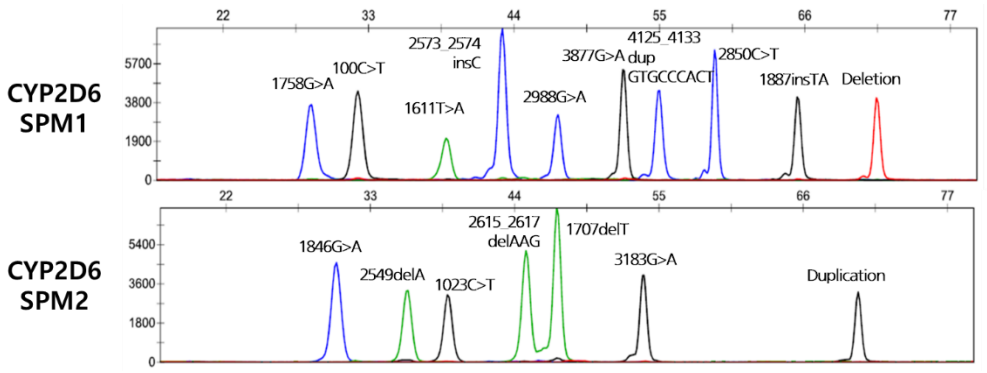
16) When all the processes are completed, click 'X' at the top right to finish. If you click 'X', the following warning window appears. Click 'Yes' to exit the software.



12. Interpretation of results

12.1 SNP analysis

1) When '11. Analysis of result' is completed, the peak as shown in the figure below is printed. The peak result is analyzed by dividing into SPM1 (10 SNPs) and SPM2 (7 SNPs), and the CYP2D6 SNP corresponding to each SPM is shown in the figure below.



2) The variation result according to the SNP peak color is shown in the table below. The test result of the analysis sample must be derived by comparing it with the wild type DNA test result, and the wild type DNA result is displayed in wild type for all mutations below.

SNaPshot Set	SNP	Type	Peak color	Genotype
SPM1	1758G>A	Wild type	Blue	G/G
		Heterozygous mutant type	Blue/Green	G/A
		Homozygous mutant type	Green	A/A
	100C>T	Wild type	Black	C/C
		Heterozygous mutant type	Black/Red	C/T
		Homozygous mutant type	Red	T/T
	1611T>A	Wild type	Green	T/T
		Heterozygous mutant type	Green/Red	T/A
		Homozygous mutant type	Red	A/A
	2573_2574insC	Wild type	Blue	-/-
		Heterozygous mutant type	Blue/Black	-/C

		Homozygous mutant type	Black	C/C
	2988G>A	Wild type	Blue	G/G
		Heterozygous mutant type	Blue/Green	G/A
		Homozygous mutant type	Green	A/A
	3877G>A	Wild type	Black	G/G
		Heterozygous mutant type	Black/Red	G/A
		Homozygous mutant type	Red	A/A
	4125_4133 dupGTGCCCACT	Wild type	Blue	-/-
		Heterozygous mutant type	Blue/Red	-/GTGCCCACT
		Homozygous mutant type	Red	GTGCCCACT /GTGCCCACT
	2850C>T	Wild type	Blue	C/C
		Heterozygous mutant type	Blue/Green	C/T
		Homozygous mutant type	Green	T/T
	1887insTA	Wild type	Black	-/-
		Heterozygous mutant type	Black/Red	-/TA
		Homozygous mutant type	Red	TA/TA
	Deletion	Wild type	Red	-/-
		Heterozygous mutant type	Red/Black	-/DEL
		Homozygous mutant type	Black	DEL/DEL
SPM2	1846G>A	Wild type	Blue	G/G
		Heterozygous mutant type	Blue/Green	G/A
		Homozygous mutant type	Green	A/A
	2549delA	Wild type	Green	A/A
		Heterozygous mutant type	Green/Blue	-/A
		Homozygous mutant type	Blue	-/-
	1023C>T	Wild type	Black	C/C
		Heterozygous mutant type	Black/Red	C/T
		Homozygous mutant type	Red	T/T
	2615_2617delAAG	Wild type	Green	AAG/AAG
		Heterozygous mutant type	Green/Blue	-/AAG

		Homozygous mutant type	Blue	-/-
	1707delT	Wild type	Green	T/T
		Heterozygous mutant type	Green/Black	-/T
		Homozygous mutant type	Black	-/-
	3183G>A	Wild type	Black	G/G
		Heterozygous mutant type	Black/Red	G/A
		Homozygous mutant type	Red	A/A
	Duplication	Wild type	Black	-/-
		Heterozygous mutant type	Black/Red	-/DUP
		Homozygous mutant type	Red	DUP/DUP

12.2 Genotype analysis

1) Determination of CYP2D6 genotype

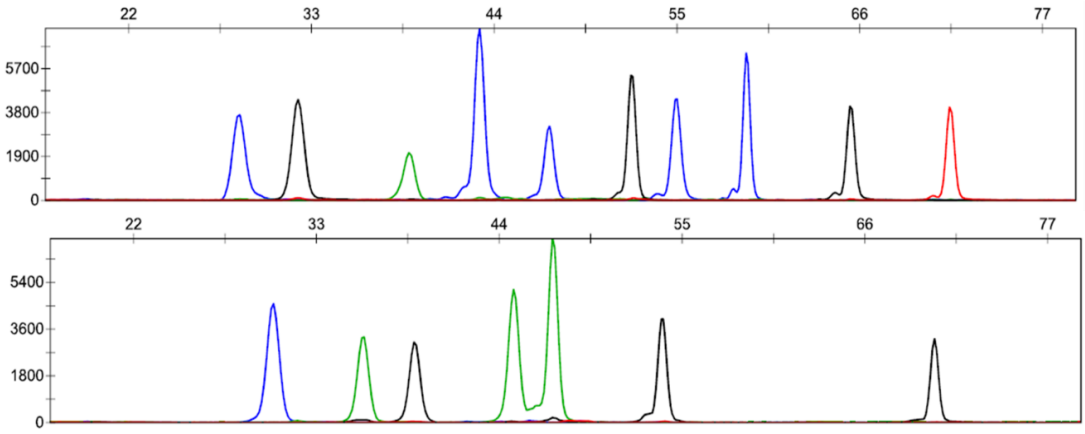
The results of 17 mutations in each CYP2D6 are identified to determine the genotype of CYP2D6 based on the following criteria. Determination of the genotype follows 'CYP2D6 Allele Nomenclature'.

Haplotype	Allele	*14	*10B	*49	*21	*41	*52	*18	*2	*60	*3	*4	*6	*9	*17	*29	*5	*XN
	A.A change	G169R	P34S	F120I	267 frameshift	Splicing defect	E418K	468_470dup VPT	R296C	S183X	259 frameshift	Splicing defect	118 frameshift	K281del	T107I	V338M	-	-
	N.T change	1758G>A	100C>T	1611T>A	2573 _2574insC	2988G>A	3877G>A	4125_4133 dupGTGCCC ACT	2850C>T	1887insTA	2549delA	1846G>A	1707delT	2615_2617 delAAG	1023C>T	3183G>A	Deletion	Duplication
	Wild type	G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
	Mutant type	A	T	A	C	A	A	T	T	A	G	A	G	G	T	A	G	A
*1		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*1XN		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*2		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*2XN		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	A
*3		G	C	T	G	G	G	G	C	G	G	G	T	A	C	G	A	G
*4		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	G
*4X2		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	A
*5																	G	
*6		G	C	T	G	G	G	G	C	G	A	G	G	A	C	G	A	G
*9		G	C	T	G	G	G	G	C	G	A	G	T	G	C	G	A	G
*10B		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*10BX2		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*14B		A	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*17		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	G
*17XN		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	A
*18		G	C	T	G	G	G	T	C	G	A	G	T	A	C	G	A	G
*21B		G	C	T	C	G	G	G	T	G	A	G	T	A	C	G	A	G
*29		G	C	T	G	G	G	G	T	G	A	G	T	A	C	A	A	G
*41		G	C	T	G	A	G	G	T	G	A	G	T	A	C	G	A	G
*49		G	T	A	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*52		G	T	T	G	G	A	G	C	G	A	G	T	A	C	G	A	G
*60		G	C	T	G	G	G	G	C	A	A	G	T	A	C	G	A	G

A.A change: Amino acid change, N.T change: Nucleotide change

2) Example of results: CYP2D6 Wild Type DNA (Control material)

(1) Peak display



(2) SNP analysis

SNaPshot Set	SNP	Type	Peak color	Genotype
SPM1	1758G>A	Wild type	Blue	G/G
	100C>T	Wild type	Black	C/C
	1611T>A	Wild type	Green	T/T
	2573_2574insC	Wild type	Blue	-/-
	2988G>A	Wild type	Blue	G/G
	3877G>A	Wild type	Black	G/G
	4125_4133 dupGTGCCCACT	Wild type	Blue	-/-
	2850C>T	Wild type	Blue	C/C
	1887insTA	Wild type	Black	-/-
	Deletion	Wild type	Red	-/-
SPM2	1846G>A	Wild type	Blue	G/G
	2549delA	Wild type	Green	A/A
	1023C>T	Wild type	Black	C/C
	2615_2617delAAG	Wild type	Green	AAG/AAG
	1707delT	Wild type	Green	T/T
	3183G>A	Wild type	Black	G/G
	Duplication	Wild type	Black	-/-

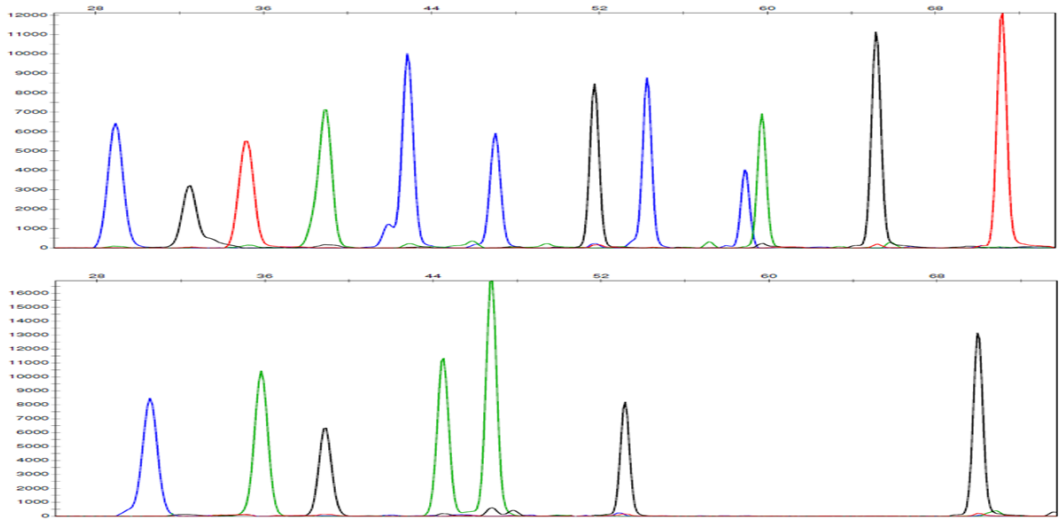
(3) Genotyping results: CYP2D6 *1/*1

Haplotype	Allele	*14	*10B	*49	*21	*41	*52	*18	*2	*60	*3	*4	*6	*9	*17	*29	*5	*XN
	A.A change	G169R	P34S	F120I	267 frameshift	Splicing defect	E418K	468_470dup VPT	R296C	S183X	259 frameshift	Splicing defect	118 frameshift	K281del	T107I	V338M	-	-
	N.T change	1758G>A	100C>T	1611T>A	2573 _2574insC	2988G>A	3877G>A	4125_4133 dupGTGCCC ACT	2850C>T	1887insTA	2549delA	1846G>A	1707delT	2615_2617 delAAG	1023C>T	3183G>A	Deletion	Duplication
	Wild type	G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
	Mutant type	A	T	A	C	A	A	T	T	A	G	A	G	G	T	A	G	A
*1		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*1XN		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*2		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*2XN		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	A
*3		G	C	T	G	G	G	G	C	G	G	G	T	A	C	G	A	G
*4		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	G
*4X2		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	A
*5																	G	
*6		G	C	T	G	G	G	G	C	G	A	G	G	A	C	G	A	G
*9		G	C	T	G	G	G	G	C	G	A	G	T	G	C	G	A	G
*10B		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*10BX2		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*14B		A	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*17		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	G
*17XN		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	A
*18		G	C	T	G	G	G	T	C	G	A	G	T	A	C	G	A	G
*21B		G	C	T	C	G	G	G	T	G	A	G	T	A	C	G	A	G
*29		G	C	T	G	G	G	G	T	G	A	G	T	A	C	A	A	G
*41		G	C	T	G	A	G	G	T	G	A	G	T	A	C	G	A	G
*49		G	T	A	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*52		G	T	T	G	G	A	G	C	G	A	G	T	A	C	G	A	G
*60		G	C	T	G	G	G	G	C	A	A	G	T	A	C	G	A	G

A.A change: Amino acid change, N.T change: Nucleotide change

3) Example of results: Sample with CYP2D6 mutation

(1) Peak display



(2) SNP analysis

SNaPshot Set	SNP	Type	Peak color	Genotype
SPM1	1758G>A	Wild type	Blue	G/G
	100C>T	Heterozygous mutant type	Black/Red	C/T
	1611T>A	Wild type	Green	T/T
	2573_2574insC	Wild type	Blue	-/-
	2988G>A	Wild type	Blue	G/G
	3877G>A	Wild type	Black	G/G
	4125_4133 dupGTGCCCACT	Wild type	Blue	-/-
	2850C>T	Heterozygous mutant type	Blue/Green	C/T
	1887insTA	Wild type	Black	-/-
	Deletion	Wild type	Red	-/-
SPM2	1846G>A	Wild type	Blue	G/G
	2549delA	Wild type	Green	A/A
	1023C>T	Wild type	Black	C/C
	2615_2617delAAG	Wild type	Green	AAG/AAG
	1707delT	Wild type	Green	T/T
	3183G>A	Wild type	Black	G/G
	Duplication	Wild type	Black	-/-

(3) Genotyping results: CYP2D6 *2/*10B

Haplotype	Allele	*14	*10B	*49	*21	*41	*52	*18	*2	*60	*3	*4	*6	*9	*17	*29	*5	*XN
	A.A change	G169R	P34S	F120I	267 frameshift	Splicing defect	E418K	468_470dup VPT	R296C	S183X	259 frameshift	Splicing defect	118 frameshift	K281del	T107I	V338M	-	-
	N.T change	1758G>A	100C>T	1611T>A	2573 _2574insC	2988G>A	3877G>A	4125_4133 dupGTGCCC ACT	2850C>T	1887insTA	2549delA	1846G>A	1707delT	2615_2617 delAAG	1023C>T	3183G>A	Deletion	Duplication
	Wild type	G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
	Mutant type	A	T	A	C	A	A	T	T	A	G	A	G	G	T	A	G	A
*1		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*1XN		G	C	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*2		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*2XN		G	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	A
*3		G	C	T	G	G	G	G	C	G	G	G	T	A	C	G	A	G
*4		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	G
*4X2		G	T	T	G	G	G	G	C	G	A	A	T	A	C	G	A	A
*5																	G	
*6		G	C	T	G	G	G	G	C	G	A	G	G	A	C	G	A	G
*9		G	C	T	G	G	G	G	C	G	A	G	T	G	C	G	A	G
*10B		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*10BX2		G	T	T	G	G	G	G	C	G	A	G	T	A	C	G	A	A
*14B		A	C	T	G	G	G	G	T	G	A	G	T	A	C	G	A	G
*17		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	G
*17XN		G	C	T	G	G	G	G	T	G	A	G	T	A	T	G	A	A
*18		G	C	T	G	G	G	T	C	G	A	G	T	A	C	G	A	G
*21B		G	C	T	C	G	G	G	T	G	A	G	T	A	C	G	A	G
*29		G	C	T	G	G	G	G	T	G	A	G	T	A	C	A	A	G
*41		G	C	T	G	A	G	G	T	G	A	G	T	A	C	G	A	G
*49		G	T	A	G	G	G	G	C	G	A	G	T	A	C	G	A	G
*52		G	T	T	G	G	A	G	C	G	A	G	T	A	C	G	A	G
*60		G	C	T	G	G	G	G	C	A	A	G	T	A	C	G	A	G

A.A change: Amino acid change, N.T change: Nucleotide change

13. Performance characteristics

13.1 Analytical performance characteristics

No.	Classification	Test method and results
1	Analytical sensitivity (limit of detection)	The Limit of Detection of SPMED™ Genotyping Kit: CYP2D6, was measured by standard substances with 5-fold dilution. The CYP2D6 mutation was repeatedly tested more than 20 times to confirm the sample amount capable of determining 95% or more as positive. As a result, the limit of detection of SPMED™ Genotyping Kit: CYP2D6 was confirmed to be 100 ng / reaction.
2	Analytical sensitivity (Cut-off Value)	To measure the cut-off value of SPMED™ Genotyping Kit: CYP2D6, 17 standard substances were diluted in 5 steps for each concentration, and 17 types of CYP2D6 mutants were repeatedly tested 276 times. The results of 17 types of CYP2D6 mutants at 100 ng / reaction identified as the limit of detection (LoD) are consistent with the pre-determined peak range, peak color, and peak height criteria, and the analysis result for each CYP2D6 SNP is 100% consistent. Therefore, it was confirmed that the criterion was appropriate.
3	Accuracy (Concordance)	To measure the accuracy of SPMED™ Genotyping Kit: CYP2D6, the results of the SPMED™ Genotyping Kit: CYP2D6 test using 17 reference materials containing 17 variants of CYP2D6 were compared with the results of the test using the gold standard sequencing method as a reference method. As a result of comparing the reference method and the results of genotyping, 100% accuracy (concordance) of SPMED™ Genotyping Kit: CYP2D6 was confirmed.
4	Analytical specificity (Cross-reactivity)	The specificity of the SPMED™ Genotyping Kit: CYP2D6 (cross-reactivity) was confirmed using CYP2D6 wild type standards and cross-linked substances such as 11 pathogenic bacteria and viral nucleic acids. As a result, SPMED™ Genotyping Kit: CYP2D6 was confirmed to have no influence of cross-substance.
5	Analytical specificity	The interference reaction of SPMED™ Genotyping Kit: CYP2D6 was confirmed by using two standard substances whose

	(Interference)	genotypes were confirmed by sequencing and six types of endogenous / exogenous interference substances. As a result, SPMED™ Genotyping Kit: CYP2D6 was confirmed to be free from interference.
6	Precision (Repeatability)	To confirm the precision (repeatability) of the SPMED™ Genotyping Kit: CYP2D6, CYP2D6 wild type standard substances diluted with three concentrations were repeatedly measured under defined conditions. As a result, it was confirmed that the precision of SPMED™ Genotyping Kit: CYP2D6 was 100%.
7	Precision (Reproducibility)	To confirm the precision (reproducibility) of the SPMED™ Genotyping Kit: CYP2D6, the precision between two laboratories was compared using the CYP2D6 wild type standard substances, and the precision between two testers in the laboratory was measured. As a result of testing under the specified conditions, the reproducibility of SPMED™ Genotyping Kit: CYP2D6 was 100%.

13.2 Diagnostic performance characteristics

No.	Classification	Test method and results
1	Clinical sensitivity/ specificity	The diagnostic performance (sensitivity, specificity) of SPMED™ Genotyping Kit: CYP2D6 was evaluated with 276 samples delivered from the Human Resource Bank. 276 CYP2D6 wild, heterozygote, and homozygote mutant types were tested for clinical efficacy using SPMED™ Genotyping Kit: CYP2D6. Clinical sensitivity and specificity were measured in comparison with gold standard sequencing, allele specific PCR (ASP) for confirming CYP2D6 deletion, restriction fragment length polymorphism (RFLP) for confirming CYP2D6 duplication. As a result of the measurement, the clinical sensitivity (positive agreement rate) and specificity (negative agreement rate) of SPMED™ Genotyping Kit: CYP2D6 were 100%.

14. Limitations of the examination procedure

1) This product contains seventeen (17) mutations of the CYP2D6 gene known as major functional mutations (100C>T, 1023C>T, 1611T>A, 1707delT, 1758G>A, 1846G>A, 1887insTA, 2549delA, 2573_2574insC, 2615_2617delAAG, 2850C> T, 2988G>A, 3183G>A, 3877G>A, 4125_4133dup GTGCCCACT, Deletion, Duplication) can only be tested, and other CYP2D6 genetic mutations cannot be detected.

2) Depending on the concentration of the sample, the degree of band and peak is determined. In other words, if the concentration of the specimen is low, bands and peaks may be faint, so in the case of a test that must be performed, two or more persons read it or confirm it by another test method.

3) This product is a kit for analyzing the CYP2D6 genotype, and the final diagnosis should not be based solely on the genotype results, but should be made by expert judgment based on other test methods and clinical findings.

4) Since this product applies the SNaPshot method for CYP2D6 genotyping analysis, genotypes such as *1/*2+dup, *1/*10B+dup, *2/*10B+dup, *2/*41+dup, etc. Is unable to distinguish which of the two strands of the CYP2D6 gene DNA has a duplication. Additional experiments are required to determine the duplication strand.

15. Literature references

1) Sequence-based CYP2D6 genotyping in the Korean population. Lee SY[1], Sohn KM, Ryu JY, Yoon YR, Shin JG, Kim JW. Ther Drug Monit. 2006 Jun;28(3):382-7.

2) Genetic polymorphism of hepatocyte nuclear factor-4alpha influences human cytochrome P450 2D6 activity. Lee SS, Cha EY, Jung HJ, Shon JH, Kim EY, Yeo CW, Shin JG. Hepatology. 2008 Aug;48(2):635-45.

3) Visualization of CYP2D6 genotype to phenotype correlation in Korean. Kim EY, Jung HJ, Lee SS, Shon JH, Shin SG, Shin JG. AMIA Annu Symp Proc. 2007 Oct 11:1009-10.

4) Auto-interpreter for CYP2D6 SNaPshot genotyping. Ong S, Jeong HE, Lee SS, Shon JH, Shin JG, Kim EY. AMIA Annu Symp Proc. 2008 Nov 6:1006.

5) Discovery of novel functional variants and extensive evaluation of CYP2D6 genetic polymorphisms in Koreans. Lee SJ, Lee SS, Jung HJ, Kim HS, Park SJ, Yeo CW, Shin JG. Drug Metab Dispos. 2009 Jul;37(7):1464-70.

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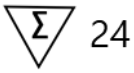
16. Symbol information

Symbol	Meaning
	Storage at -25 to -15℃
	Validity
	Batch code (Lot number)
	Catalog number
	Contains sufficient for <24> tests
	Caution

Symbol	Meaning
	Refer to user manual
	Date of manufacture
	Manufacturer
	Authorized representative in the European Community
	In vitro diagnostic medical device
	CE Mark

SPMED Co., Ltd.

611, 612 Ho, 111, Hyoyeol-ro,

Buk-gu, Busan, 46508, Republic of Korea

Tel : + 82 51 362 1101

Fax : + 82 51 361 1102

Website: www.spmed.kr

E-mail : help@spmed.kr

EC REP **QARAD EC-REP BV**

Pas 257 2440 Geel, Belgium