

Mutector™

Mutation Detection Kit

CYP 2D6

Genotyping Reagents

User Manual V1.3

Cat No. GP11



www.trimgen.com

CONTENTS

Introduction	4
Overview of Mutector™ Assay	5
Materials Provided	6
Materials Required	7
Equipment Required	7
DNA Sample Preparation	8
Sequencer Setup	8
Thermal Cycling Programs	9
Mutector™ Assay Protocol	10
A. PCR Amplification	10
B. PCR Product Clean Up	13
C. STA (Genotyping) Reaction	14
D. Sample Loading	16
E. Data Analysis	16
F. Troubleshooting	17

Storage

Upon receipt of the kit, store at -20°C until use. At this temperature the reagents are stable for 6 months.

After first use, store all of reagents at $2-8^{\circ}\text{C}$ and keep them protected from direct light. At this condition the reagents are stable for 1 month.

Notice to Purchaser

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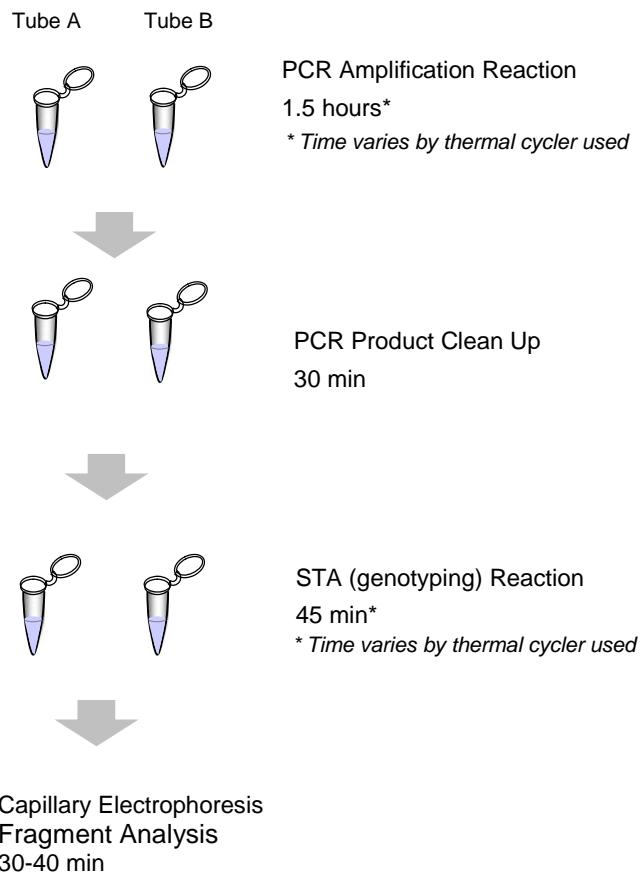
Introduction

CYP2D6 Mutector™ Kit contains reagents to identify 10 variations in CYP 2D6 gene. The assay is two tube test, the SNPs identified by the assay are listed in the table below:

Test tube	SNPs
Tube A	<i>4180G>C</i> <i>2850C>T</i> <i>2549delA</i> <i>1846G>A</i> <i>2988G>A</i>
Tube B	<i>1707delT</i> <i>2915-17delAAG</i> <i>100C>T</i> <i>1023C>T</i>

The allele information and correspondent enzyme activity are not provided in this manual.

Overview of CYP2D6 Genotyping Assay



Tube -A for alleles *2, *3, *4, *41

Tube-B for alleles *6, *9, *10, and *17

Materials Provided:

The Mutector™ CYP2D6 Genotyping contains reagents for 32 tests.

Materials	GP11	Description
Master Mix	2 x 600 ul	PCR Reagent Mix
C-UP 1	65 µl	PCR Product Clean UP Enzyme 1
C-UP 2	65 µl	PCR Product Clean UP Enzyme 2
C-UP Buffer	780 µl	Buffer for Clean UP Enzymes
ST-F*	780 µl	Reagent Mix for Allele Detection
Loading Buffer*	2 x 650 ul	Sample Loading Buffer with Size Standards
PCR-P A	70 µl	PCR Primers for *2, *3, *4, *41, 4180G>C
PCR-P B	70 µl	PCR Primers for *6, *9, *10, *17
DP- A	70 µl	Detection Primers for *2, *3, *4, *41, 4180G>C
DP- B	70 µl	Detection Primers for *6, *9, *10, *17
CTL- A	50 µl	Control DNA for *2, *3, *4, *41, 4180G>C
CTL- B	50 µl	Control DNA for *6, *9, *10, *17

*** Light sensitive:** Keep these reagents protected from direct light.

CYP 2D6 V1.3

Materials required:

0.2 ml PCR tubes (8-well strip tube)

DS-32 Matrix Standard Kit for calibration of sequencer
(Applied Biosystems Pat No. 4345831)

Equipment required:

Thermal Cycler:

Any type of thermal cycler with a 0.2 ml tube block is acceptable for performing the assay.

Sequencer:

Applied Biosystems Genetic Analyzer

Instrument	Data Collection	Data Analysis
Genetic analyzer 3100	Data Collection Software v3.0 or v3.1	GeneMapper® Software v4.0 or v4.1
Genetic analyzer 3700		
Genetic analyzer 3130		
Genetic analyzer 3500	3500 Data Collection Software v1.0	GeneMapper® Software v4.1

CYP 2D6 V.1.3

DNA Sample Preparation:

Reagents for DNA preparation are not provided with the kit.

Any commercially available DNA extraction kit is acceptable.

DNA concentration adjustment:

When using a column or bead DNA extraction method, adjust the final concentration of extracted DNA to **100 – 200 ng/–l**.

Sequencer Setup:

First time users should set up the analysis program for the ABI sequencer (one time setup). After setup, the program can apply to all Mutector™ tests for data analysis.

Step I. GeneMapper® Setup

www.trimgen.com/docs/PartI-GeneMapper-Setup.pdf

Step II. Data Collection® Software Setup

www.trimgen.com/docs/PartII-Data-Collection-Setup.pdf

Step III. Data Analysis Using GeneMapper®

www.trimgen.com/docs/PartIII-Data-Analysis-GeneMapper.pdf

CYP 2D6 V.1.3

Important

Spectral calibration is required before running the test

The sequencer needs to be calibrated with the DS-32 calibration kit (Applied Biosystems cat No. 4345831). **This is a one-time calibration** to set up spectral channels to collect the test results. Refer to the DS-32 Matrix standards kit to prepare the DS-32 matrix standards. Run a Matrix Standard Set DS-32 (5FAM, JOE, NED, ROX) to perform a spectral calibration.

Thermal Cycling Programs:

Program 1 (PCR)	
1 cycle	94°C 2 min
35 cycles	94°C 20 sec
	53°C 30 sec
	72°C 30 sec
1 cycle	72°C 1 min
Hold at 4°C	

Program 2 (PCR Product Clean-up)	
	37°C 25 min
	95°C 5 min
Hold at 4°C	

Program 3 (STA Reaction)	
1 cycle	94°C 1 min
20 cycles	94°C 20 sec
	50°C 45 sec
	72°C 10 sec
Hold at 4°C	

CYP 2D6 V1.3

Mutector™ Assay Protocol:

A. PCR Amplification

Thaw all reagents and keep on ice. Spin down the reagents before use.

Negative and positive controls are recommended for each run.

§§ Tube A (alleles *2, *3, *4, *41 and 4180G>C)

A.1. Prepare PCR Reaction Mix A:

Calculate the amount of Master Mix and PCR primers using following formula.

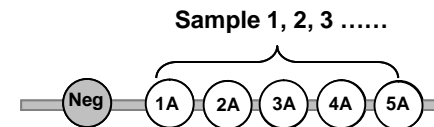
$$\text{Master Mix} = 18 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of Samples/Controls}} \right) \times 1.1^* = \text{_____} \text{ -l}$$

$$\text{PCR-P A} = 2 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of Samples/Controls}} \right) \times 1.1^{**} = \text{_____} \text{ -l}$$

* Adjustment for pipetting error.

Label a tube with "A" and transfer the above reagents to the "A" tube and gently mix the contents (avoid bubble). This is the PCR Reaction Mix A.

A.2. Collect 0.2 ml PCR tubes, 1 tube per sample and label the tubes as shown below:



Neg: Negative control

A.3. Transfer 20 -l from the tube "A" into each of the tubes.

CYP 2D6 V1.3

- A.4. Add 2 -l of nuclease free water to the "Neg" tube.
- A.5. Add 1-2 -l of sample DNA (50-200 ng/μl) to each sample tube.

§§ Tube-B (Alleles *6, *9, *10, and *17)

A.6. Prepare PCR Reaction Mix B:

Calculate the amount of Master Mix and PCR primers using following formula.

Master Mix = $18 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of Samples/Controls}} \right) \times 1.1^* = \text{_____} \text{ -l}$

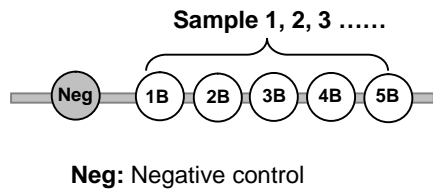
PCR-P B = $2 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of Samples/Controls}} \right) \times 1.1^{**} = \text{_____} \text{ -l}$

* Adjustment for pipetting error.

CYP 2D6 V.1.3

Label a tube with "B" and transfer the above reagents to the "B" tube, gently mix the contents (avoid bubble). This is the PCR Reaction Mix B.

- A.7. Collect 0.2 ml PCR tubes, 1 tube per sample and label the tubes as shown below:



- A.8. Transfer 20 -l each from the tube "B" into all of the tubes.
- A.9. Add 2 -l of nuclease free water to the "Neg" tube.
- A.10. Add 1-2 -l of sample DNA (50-200 ng/μl) to each sample

tube.

- A.11. Cap the tubes and mix the contents. After spin, place the PCR tubes in a thermal cycler and run **Program 1**.

Program 1

1 cycle	94°C 2 min
35 cycles	94°C 20 sec
	53°C 30 sec
	72°C 30 sec
1 cycle	72°C 1 min
	Hold at 4°C

CYP 2D6 V.1.3



The procedure can be temporarily stopped after **Program 1**. The PCR products can be stored at 2-8°C for next day test.

B. PCR Products Clean Up

B.1. Prepare PCR Clean-Up (C-UP) Mix:

Calculate the amount of C-UP Buffer, C-UP1 and C-UP2 using following formula:

$$\text{C-UP Buffer} = 9 \mu\text{l} \times \left(\frac{\quad}{\# \text{ of PCR tubes}} \right) \times 1.2^* = \quad \mu\text{l}$$

$$\text{C-UP1} = 1 \mu\text{l} \times \left(\frac{\quad}{\# \text{ of PCR tubes}} \right) = \quad \mu\text{l}$$

$$\text{C-UP2} = 1 \mu\text{l} \times \left(\frac{\quad}{\# \text{ of PCR samples}} \right) = \quad \mu\text{l}$$

* Adjustment for pipetting error.

CYP 2D6 V1.3

Label a tube with "C-UP" and transfer the above reagents to the **C-UP** tube and gently mix the contents (avoid bubble). This is the PCR Clean-Up Mix.

- B.2.** Collect 0.2 ml strip tubes, one tube for each PCR reaction. Label the tubes the same as the PCR tubes.
- B.3.** Transfer 11 μl **C-Up mix** to new tube.
- B.4.** After PCR reaction finished, spin the PCR tube and transfer 5 μl of **PCR product** to each C-Up tube (the remaining PCR products can be stored at -20°C for re-test).
- B.5.** Gently mix the content and spin the tubes.
- B.6.** Incubate the tubes in a thermal cycler using **Program 2**.

Program 2
37°C for 25 min
95°C for 5 min
Hold at 4°C

STOP The procedure can be temporarily stopped after **Program 2**. The C-UP products can be stored at 2-8°C for next day test.

C. STA Reaction (Genotyping)

§§ Tube Set-A (Alleles *2, *3, *4, *41, 4180G>C)

C.1. Prepare STA Mix A (SA):

Label a tube as "SA".

Prepare "SA" mix as following:

$$\text{ST-F Mix} = 11 \mu\text{l} \times \left(\frac{\quad}{\# \text{ of C-UP tubes}} + 1^* \right) \times 1.1^{**} = \quad \mu\text{l}$$

$$\text{DP-A} = 2 \mu\text{l} \times \left(\frac{\quad}{\# \text{ of C-UP tubes}} + 1^* \right) \times 1.1^{**} = \quad \mu\text{l}$$

* For CTL controls

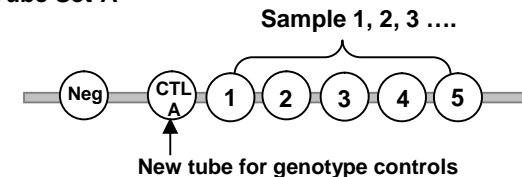
** Adjustment for pipetting error

CYP 2D6 V1.3

Transfer the above reagents to the "SA" tube and gently mix the contents (avoid bubble). This is the STA Mix A.

- C.2.** Collect 0.2 ml strip tubes, one tube for each C-UP treated sample. **Add an extra new tube for genotype controls and label the tube with CTL.**

Tube Set-A



! **The CT- A is genotype controls designed for data analysis and should be run each time.**

- C.3.** Add 13 μL of "SA" into each tube of Tube Set-B.
- C.4.** Add 5 μL of **2D6 CTL-A** into the **CTL-A** tube

- C.5. Transfer 5 µL of **C-UP treated PCR products** from the **Tube B** in C-UP section to each Set-B tube correspondingly.
- C.6. Cap the tubes, mix the contents and spin the tubes.

§§ Tube Set-B (Alleles *6, *9, *10, and *17)

C.7. Prepare STA Mix B (SB):

Label a tube as "SB".

Prepare "SB" mix as following:

ST-F Mix = $11 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of C-UP tubes}} + 1^* \right) \times 1.1^{**} = \text{_____} \mu\text{l}$

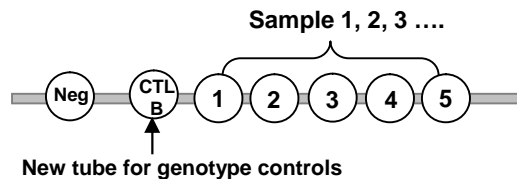
DP-B = $2 \mu\text{l} \times \left(\frac{\text{_____}}{\text{\# of C-UP tubes}} + 1^* \right) \times 1.1^{**} = \text{_____} \mu\text{l}$

* For mutation controls
 ** Adjustment for pipetting error

Transfer the above reagents to the "SB" tube and gently mix the contents (avoid bubble). This is the STA Mix B.

- C.8. Collect 0.2 ml strip tubes, one tube for each C-UP treated sample. **Add an extra new tube for genotype controls and label the tube with CTL.**

Tube Set-B



! The CTL-B is genotype controls designed for data analysis and should be run each time.

- C.9. Add 13 µL of "SB" into each tube of Tube Set-B.
- C.10. Add 5 µL of **2D6 CTL-B** into the **CTL-B tube**
- C.11. Transfer 5 µL of **C-UP treated PCR products** from the **Tube B** in C-UP section to each Set-B tube correspondingly.
- C.12. Cap the tubes, mix the contents and spin the tubes.
- C.13. Place all tubes into the thermal cycler and perform the STA reaction using **Program 3**.

Program 3	
1 cycle	94°C 1 min
20 cycles	94°C 20 sec
	50°C 45 sec
	72°C 10 sec
Hold at 4°C	



The procedure can be temporarily stopped after **Program 3**. The STA products can be stored at 2-8°C overnight.

D. Sample Loading

- D.1. Add 15 µL of the **Loading Buffer** to each well of a sequencer adapter plate.
- D.2. Transfer 5 µL of the STA reaction products into each well. Confirm and remove any bubbles in the well.
- D.3. Load the plate into the sequencer and run the pre-setup Data Collection Program.

E. Data Analysis

User can set up the allele identification method using GeneMapper (detail see GeneMapper instruction). TrimGen also provide a third party analysis software, for detail information please contact us at "inforequest@trimgen.com".