RAS Plus Mutation Detection

User Manual V1.0

Cat No. COR-D
Cat No. GP06P
Cat No. GP19P

32 reactions

TrimGen
GENETIC DIAGNOSTICS

www.trimgen.com

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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°C and keep them protected from direct light. At this condition the reagents are stable for 1 month.

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Introduction

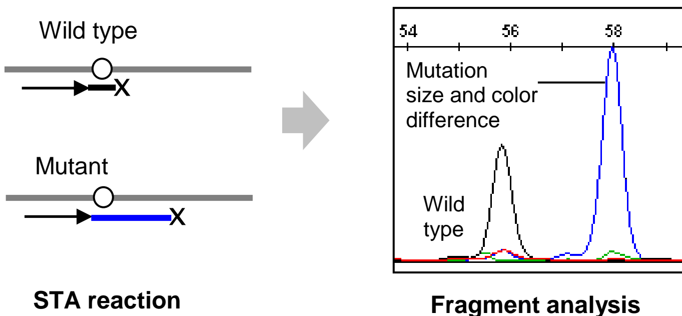
The Mutector™ RAS Plus mutation analysis reagents are designed to detect any mutation in codons 61, 117 and 146 of KRAS or NRAS gene. The following table lists most common mutations found in KRAS and NRAS exon 3 and 4:

KRAS exon 3	**KRAS exon 4**	
Codon **61**	Codon **117**	Codon **146**
Q61H (CAA >CAT) Q61L (CAA >CTA) Q61R (CAA >CGA) Q61E (CAA >GAA) Q61K (CAA >AAA) Q61H (CAA >CAC)	K117E (AAA >GAA) K117N (AAA >AAC) K117N (AAA >AAT)	A146T (GCA >ACA) A146P (GCA >CCA) A146G (GCA >GGA) A146V (GCA >GTA)
NRAS exon 3	**NRAS exon 4**	
Codon **61**	Codon **117**	Codon **146**
Q61H (CAA >CAT) Q61L (CAA >CTA) Q61R (CAA >CGA) Q61E (CAA >GAA) Q61K (CAA >AAA) Q61H (CAA >CAC)	K117A (AAG >GCG) K117N (AAG >AAC) K117N (AAG >AAT)	A146T (GCC >ACC) A146P (GCC >CCC) A146V (GCC >GTC)

The reagents have been developed based on TrimGen's proprietary Shifted Termination Assay (STA) technology.

STA (Shifted Termination Assay) technology

The STA reaction extends primers with specially modified nucleotides to increase signal strength and fragment size, generating mutation peaks that are different from wild type in both color and size.

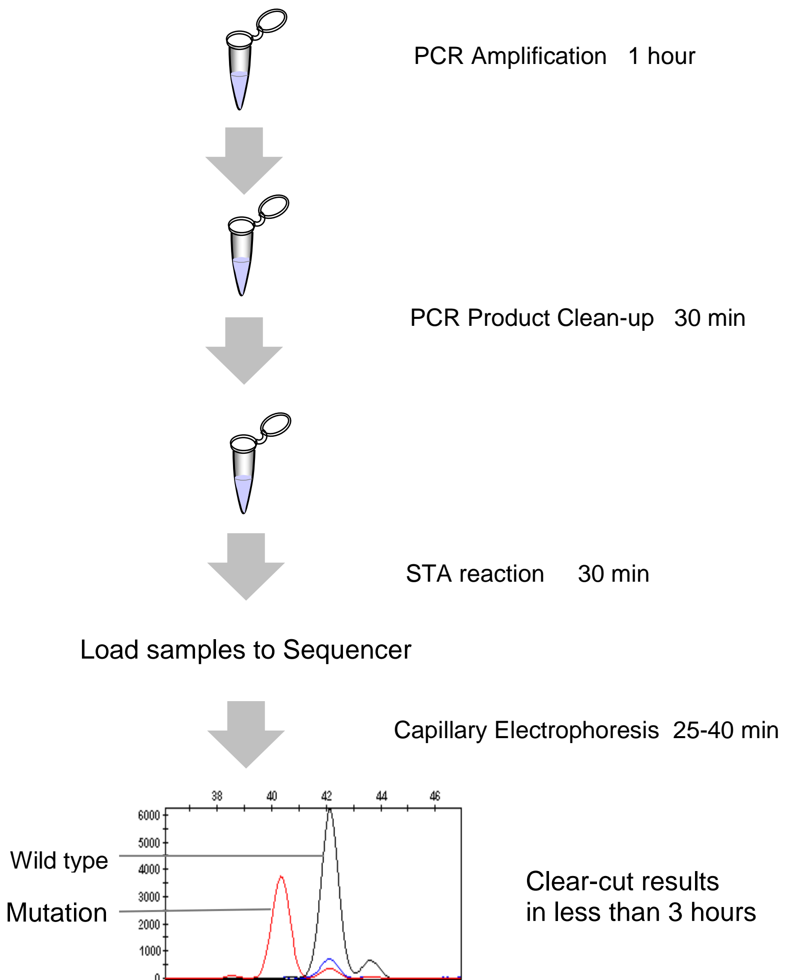


STA reaction **Fragment analysis**

STA can detect any mutations in a target codon, for example, a target codon “CAG”, the STA assay can detect base “C” change to A/G/T; base “A” change to C/G/T; base “G” change to A/C/T. STA can simultaneously detect those mutations in a single tube.

Because of its unique mutation signal enrichment, STA has much higher sensitivity than sequencing and detects mutations that are often missed by sequencing or other primer extension methods.

Overview of Mutector™ Assay



Materials Required:

Reagents:

Core Reagents *Cat.* **COR-D**

KRAS Plus Primer Set *Cat.* **GP06P**

NRAS Plus Primer Set *Cat.***GP19P**

Core Reagents are common reagents for both KRAS plus and NRAS plus mutation analysis.

Other Materials Required:

0.2 ml PCR tubes (8-well strip tube)

Applied Biosystems DS-32 Matrix Standard kit (Cat. No. 4345831). This kit will be used for a one-time calibration to set up the correct spectral channels for all STA assays.

If your sequencer has already been calibrated with the DS-32 Matrix Standard, you do not need to order the kit for re-calibration.

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

<table>
<tr><th>Instrument</th><th>Data Collection</th><th>Data Analysis</th></tr>
<tr><td>Genetic analyzer 3100</td><td rowspan="3">Data Collection Software v3.0 or v3.1</td><td rowspan="3">GeneMapper® Software v4.0 or v4.1</td></tr>
<tr><td>Genetic analyzer 3700</td></tr>
<tr><td>Genetic analyzer 3130</td></tr>
<tr><td>Genetic analyzer 3500</td><td>3500 Data Collection Software v1.0</td><td>GeneMapper® Software v4.1</td></tr>
</table>

DNA Sample Preparation

The assay is compatible with DNA samples extracted using any commercially available kit.

TrimGen provides a quick DNA extraction kit for FFPE, FNA (fine needle aspiration) and fresh or frozen tissue samples. The extract ensures a high success rate (over 99%) of PCR amplification.

Product information:
WaxFree™ DNA for 50 samples (Cat. WF-50)
WaxFree™ DNA for 100 samples (Cat. WF-100)

DNA concentration:
Adjust DNA concentration to **20-80 ng /µl** for PCR amplification.

If TrimGen's WaxFree™ kit is used for sample DNA extraction, the final extract can directly be used for PCR amplification.

Sequencer Calibration

Spectral calibration is required before running the test

A One-time spectral calibration with Applied Biosystems DS-32 Matrix Standard kit (Cat No. 4345831) is required for all STA assays. Refer to the DS-32 kit manual for performing a spectral calibration.

If your sequencer already calibrated with the DS-32 Matrix standard, you do not need to perform a re-calibration.

Setup Data Analysis Program

A one-time setup of the data analysis program is required for the first-time user of Mutector™ kit. After setup, the program can be applied for data analysis of all Mutector™ tests.

GeneMapper® 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

Thermal Cycling Programs

Program 1 (PCR)

1 cycle	94°C 2 min
35 cycles	94°C 30 sec 55°C 30 sec 72°C 30 sec
1 cycle	72°C 5 min
	Hold at 4°C

Program 2 (Clean-up)

	37°C 25 min 95°C 5 min
	Hold at 4°C

Program 3 (ST reaction)

1 cycle	94°C 2 min
20 cycles	94°C 20 sec 60°C 20 sec 70°C 20 sec
	Hold at 4°C

Mutector™ Assay Protocol:

A. PCR Amplification

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

A negative control (water) is recommended to run with samples each time. If needed, a DNA sample with a known mutation can be included as a positive control.

A.1. Prepare PCR Reaction Mix:

Master Mix = 18 x (________+ 2*) x 1.1 = _________ µl**
# of Samples

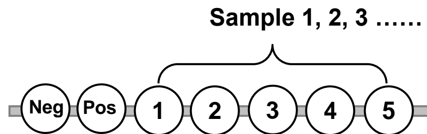
K- or N-Plus PCR-P = 1 x (_______+ 2*) x 1.1 = _______ µl**
# of Samples

* tubes for negative and positive controls.

** Adjustment for pipetting error.

Mix reagents gently and spin down

A.2. Collect 0.2 ml PCR strip tubes and label tubes as shown below:



Neg: negative (water) control
Pos: positive sample control

A.3. Transfer **19 µl** of **PCR Reaction Mix** into each tube.

A.4. Add **1 µl** of nuclease-free water to the "**Neg"** tube.

A.5. (Optional) Add **1 µl** of the DNA sample with a known mutation to the **"Pos"** tube.

A.6. Add **1 µl** of sample DNA (20-80 ng/µl) to each sample tube.

Note: If the sample DNA concentration is too low, you may add 2-3 µl of the sample DNA. Adding too much sample DNA may inhibit the PCR reaction.

A.7. Mix the contents of each tube gently and spin down.

A.8. Place all PCR tubes in a thermal cycler and run **Program 1**.

Program 1

1 cycle	94°C 2 min
35 cycles	94°C 30 sec 55°C 30 sec 72°C 30 sec
1 cycle	72°C 5 min
	Hold at 4°C

Option: The procedure can be stopped after **Program 1**. The PCR products can be stored at 4°C for 2-3 days.

During the PCR amplification process, prepare steps B1-B3.

B. PCR Product Clean Up

B.1. Prepare **C-UP Mix:**

C-UP Buffer = 9 µl x (________) x 1.1* = _________ µl
# of PCR tubes

C-UP 1 = 1.0 µl x (__________) x 1.1* = __________ µl
# of PCR tubes

C-UP 2 = 1.0 µl x (__________) x 1.1* = __________ µl
# of PCR tubes

* Adjustment for pipetting error

Mix reagents gently and spin down

B.2. Collect 0.2 ml strip tubes, one tube for each PCR reaction. Label each tube the same way as PCR tubes.

B.3. Add **11 µl** of **C-UP Mix** to each new tube.

B.4. Transfer **5 µl** of PCR products from step **A.8** to each tube (remaining PCR products can be stored at –20°C for re-testing).

B.5. Mix the contents of each tube gently and spin down.

B.6. Place all tubes in a thermal cycler and run **Program 2.**

Program 2

37°C for 25 min

95°C for 5 min

Hold at 4°C

During the clean-up incubation, prepare steps C1-C3.

C. STA Reaction (Mutation Detection)

C.1. Prepare **ST Reaction Mix**.

ST-D = 11 x (_______ + 1*) x 1.1 = ______μl**
\# of C-UP samples

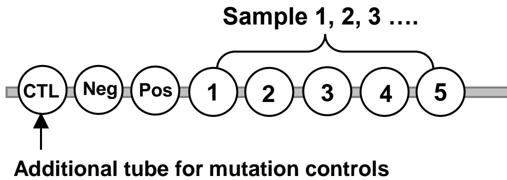
K- or N-Plus DP = 2 x (_______ + 1*) x 1.1 = _____ μl**
\# of C-UP samples

*One extra tube for mutation controls (CTL)

** Adjustment for pipetting error.

Mix reagents gently and spin down

C.2. Collect 0.2 ml strip tubes, one tube for each C-UP treated sample. **Add an additional tube** for mutation controls (Control) and label tubes as shown below:



The CTL must be run each time.

C.3. Transfer **13 μl** of **ST reaction mix** (from step C.1) into each tube.

C.4. Add **5μl** of **C-UP treated samples from step B.6** to corresponding tubes.

C.5. Add **5μl** of **K- or N-Plus CTL** to the **"CTL"** tube.

C.6. Mix the contents of each tube gently and spin down.

C.7. Place all tubes in a thermal cycler and run **Program 3**.

Program 3	
1 cycle	94°C 2 min
20 cycles	94°C 20 sec 60°C 20 sec 70°C 20 sec
	Hold at 4°C

During the STA reaction, prepare step D1 and set up sequencing running file.

D. Sample Loading

D.1. Add **15 µl** of **Loading Buffer** to each empty well of a 96-well sequencing plate.

D.2. Transfer **5 µl** of **ST reaction products from step C.7** into each well.

Note: avoid making bubbles, they may affect the capillary electrophoresis.

D.3. Load the plate onto the sequencer and run the pre-set Data Collection Program (ref. page 7).

F. Data Analysis

Data analysis is available at
http://www.trimgen.com/RAS-Plus-data-analysis