Seegene

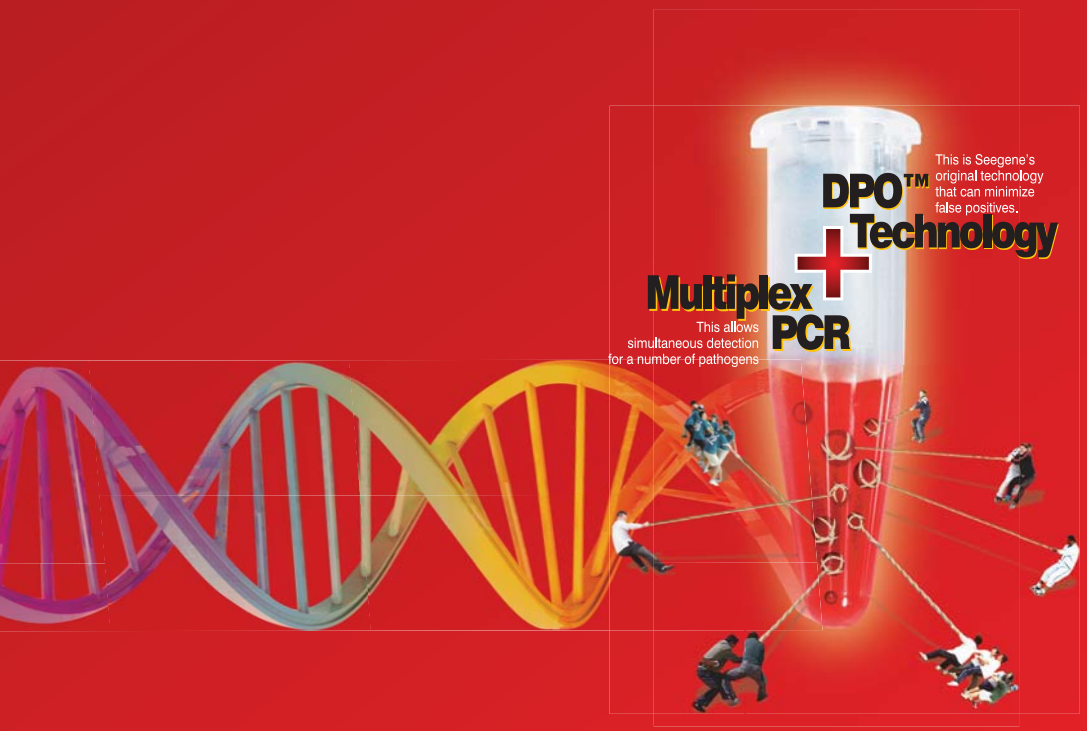
Research Use Only

Seegene's Product Information

Multi-detection at Once

Seeplex® Multi-detection System

Molecular Diagnostics
Automation
DPO™-based Multiplex PCR



Seegene, Inc.

Pioneer in Multiplex PCR for Molecular Diagnostics

New Standard for Multi-pathogen Tests

Seegene, Inc. has invested significantly in research and development, which has resulted in Seegene's own innovative technologies, methods and products in the field of molecular diagnostics. Founded in 2000 and based in Rockville, MD and Seoul, Korea, Seegene, Inc. is pioneering the field of multi-pathogen testing. Seegene specializes in oligo technologies and has two core oligo platforms named ACP™ (Annealing Control Primer) and DPO™ (Dual Priming Oligonucleotide). Seegene applies Seeplex®, its breakthrough multiplexing PCR technology platform, that enables a new standard in simultaneous multi-pathogen detection. The Seeplex® platform utilizes DPO™ and ACP™ to create multi-pathogen tests delivering maximum specificity, reproducibility and sensitivity. With over 300 citations and several patents and patents pending, Seegene has been offering technical services to over 1,000 global institutes in more than 25 countries. Seegene is actively working with both scientific and business partners.

Multi-pathogen Detection at Once

Seegene's mission is to integrate Seeplex® with disease diagnostics to provide a new platform for effectively treating patients. Seegene is poised to become the leading company for enabling unique molecular diagnostic systems in multiple pathogen detection and multiple SNP detection for human, plant, animal, and microorganisms.

Seegene is seeking strategic business partners to collaborate in the development of unique, world-class molecular diagnostic products and technologies.

Seegene, Inc. obtained ISO9001 and ISO13485.

Seegene, Inc. is ISO9001:2000 certified by Australia International Association Facilitators (IAF) in May, 2007. ISO 9001:2000 (Quality management systems-Requirements) is intended for use in any organization which designs, develops, manufactures, installs and /or services any product or provides any form of service. Additionally, the company obtained ISO 13485:2003 certification from British Standards Institution (BSI) in October, 2007.

Seegene, Inc. obtained CE markings on Seeplex.

The CE marking is a mandatory European marking for certain product groups to indicate conformity with the essential health and safety requirements set out in European Directives. So far, Seegene, Inc. has 7 different CE marked products and those are:

Seeplex® RV12 ACE Detection (catalog # RV6C00Y)
Seeplex® RV5 ACE Screening (catalog # RV6550Y)
Seeplex® RV12 Detection (catalog # RV1211)
Seeplex® RV6 Detection (catalog # RV2210)
Seeplex® RV7 Detection (catalog # RV 3210)
Seeplex® STD5 Detection (catalog # SD2510Y)
Seeplex® HPV4 ACE Screening (catalog # HP1400Y)
Seeplex® HPV6 Genotyping (catalog # HP1511Y)
Seeplex® HPV18 ASE Genotyping (catalog # HP5J10Y)

Available Soon

BRAF ACE Detection

Cat. No. **BR6300Z** Size-25 rxns

Detection of BRAF V600E mutation primarily present in papillary thyroid cancer

New Products

Seeplex® RV12 ACE Detection

Simultaneously detecting 12 major respiratory viruses

Cat. No. **RV6C00Y** Size-50 rxns

- Influenza A virus
- Influenza B virus
- RSV A
- RSV B
- Parainfluenza virus 1
- Parainfluenza virus 2
- Parainfluenza virus 3
- Coronavirus 229E/NL63
- Coronavirus OC43/HKU1
- Rhinovirus A/B
- Adenovirus
- Metapneumovirus

Seeplex® RV5 ACE Screening

Rapid screening of the most prevalent respiratory viruses

Cat. No. **RV6550Y** Size-50 rxns

- Influenza A virus
- Influenza B virus
- Human respiratory syncytial virus A/B
- Other respiratory viruses 1(AdV, PIV 1/2/3, BoV)
- Other respiratory viruses 2(MPV, HRV, CoV 229E/NL63/OC43/HKU1)

Seeplex® HPV4 ACE Screening

Simultaneously genotyping (HPVs 16 and 18) and screening for HPV high-risk and low-risk

Cat. No. **HP1400Y** Size-50 rxns

- HPV-16
- HPV-High Risk Common (11 HPV Types)
- HPV-18
- HPV-Low Risk Common (5 HPV Types)

Seeplex® HPV/STD4 ACE Screening

Simultaneously screening for STD and HPV high-risk/low-risk

Cat. No. **SH1400Y** Size-50 rxns

- *Chlamydia trichomatis*
- HPV-High Risk Common (13 HPV Types)
- *Neisseria gonorrhoeae*
- HPV-Low Risk Common (5 HPV Types)

DPO™ Technology (Super Multiplex PCR)

Novel Oligo Platform for PCR : DPO™ (Dual Priming Oligonucleotide)

DPO™ technology is a fundamental tool for blocking extension of non-specifically primed templates generating consistently high specificity. The strength and utility of this DPO™ technology can be successfully incorporated into molecular diagnostics systems such as multiplex diagnostics and SNP genotyping systems.

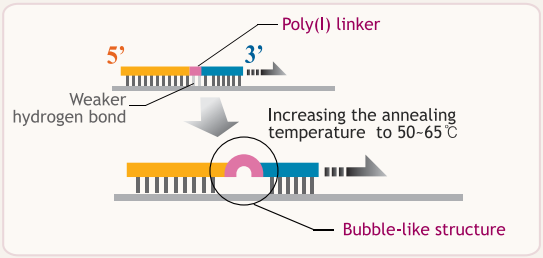
Structure of DPO™

DPO™ is structurally and fundamentally different from conventional primers. It comprises of two separate priming regions joined by a polydeoxyinosine linker. The linker forms a "bubble-like structure" which itself is not involved in priming, but rather delineates the boundary between two parts.

Principles of DPO™

DPO™ has two functional priming regions (one is longer than the other) separated by the poly (I) linker. These two unequally distributed priming regions generate dual priming reactions resulting in only target-specific products (as illustrated below).

Deoxyinosine has a relatively low melting temperature compared to the natural bases, due to weaker hydrogen bonding so that the poly (I) linker will form a bubble-like structure at a certain annealing temperature and separates a single primer into two functional regions.

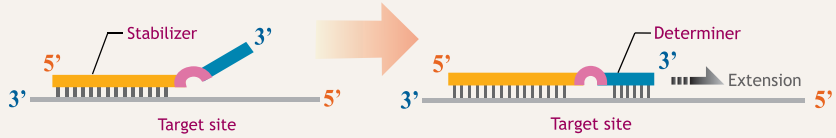


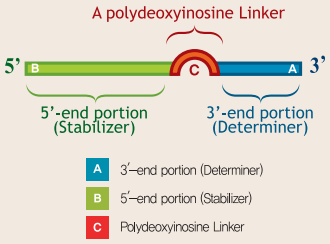
Step 1: First priming reaction

The longer 5'-segment preferentially binds to the template DNA and initiates "stable annealing"
- it acts as a "Stabilizer".

Step 2: Second priming reaction

The short 3'-segment selectively binds to a target site and determines "target-specific extension"
- it acts as a "Determiner".





Reference

Chun, et al., Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. *Nucleic Acids Research* 2007;35(6):e40

Roh et al. Comparison of the Seeplex Reverse Transcription PCR Assay with the R-mix Viral Culture and Immunofluorescence Techniques for Detection of Eight Respiratory Viruses. *Annals of Clinical & Laboraroty Science*, Vol. 38, No. 1 (2008) pp. 41-46.

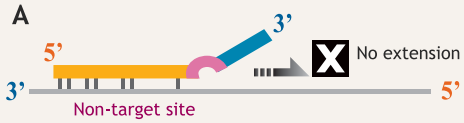
Kim et al. Direct detection of lamivudine-resistant hepatitis B virus mutants by a multiplex PCR using dual-priming oligonucleotide primers. *Journal of Virological Methods*, 149 (2008) pp. 76–84

Patents

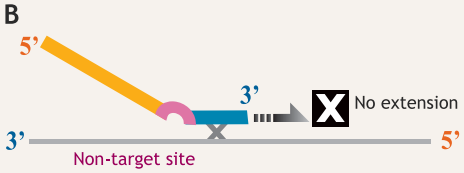
1. Process Using Dual Specificity Oligonucleotide and Dual Specificity Oligonucleotide
2. Annealing Control Primer and Its Uses

Key Features

DPO™ offers several advantages in PCR. For example, DPO™ generates unparalleled specificity by blocking the extension of non-specifically primed templates.



Although the longer 5'-segment binds a non-target site, the short segment resists non-specific extension.



The short 3'-portion alone fails to make a priming at an annealing temperature.

Key Advantages

- Unparalleled specificity
- Guaranteed reproducibility
- No primer competition and dimerization
- Multiplex assay
- Single base discrimination

Applications

Multiple-pathogen Detection

- High specificity without production of any non-specific or false-positive results
- Reliable, rapid, practical and cost-effective detection method
- Specific and simultaneous detection of multiple pathogens without any false results

Multiple-SNP Detection

- Specific and simultaneous analysis of multiple single nucleotide polymorphic sites

Multiple-genotyping

- Specific and simultaneous discrimination of multiple pathogen subtypes with similar genetic background

Magtration System 12GC® (Precision System Science Co.)

Full automation of whole process from cell lysis to nucleic acid extraction

Nucleic acid purification system optimized for Seeplex® products.

Features

Full automation from cell lysis to nucleic acid extraction
All that you need is samples in your hands.

High-speed extraction
12 samples extraction in 35 minutes

Very easy and simple operation
Start nucleic acids extraction right after sample and cartridge loading

0% carry-over contamination between samples
Introduction of disposable cartridges and tips

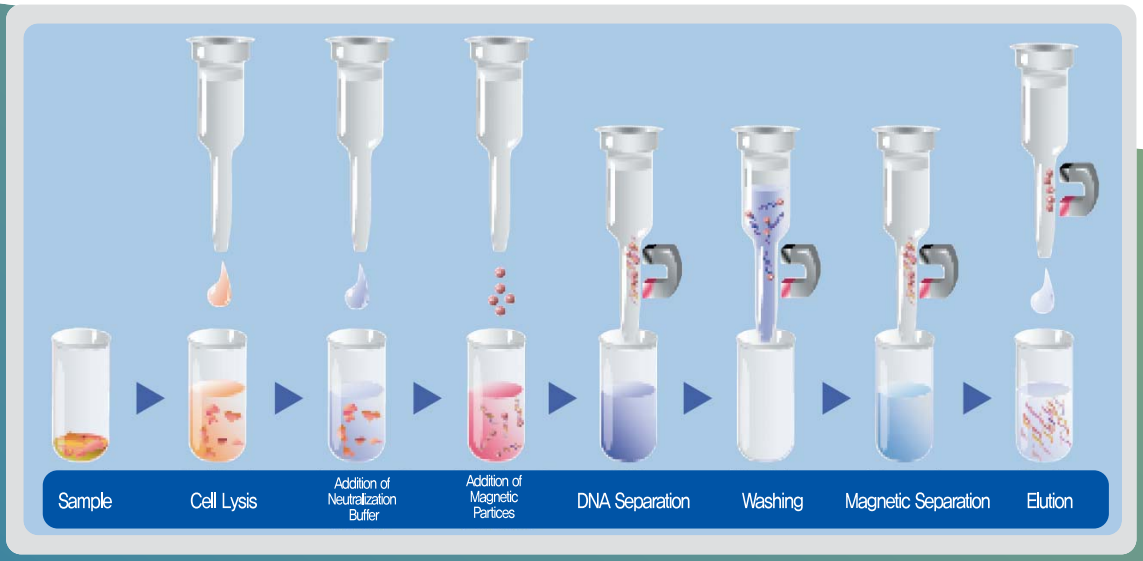
Nucleic acids extraction from various sources
Isolation of DNA or RNA from whole blood, cell, tissue, bacteria, virus etc.

Nucleic acids extraction with high-purity
Can be used for PCR or sequencing reaction right after extraction

Supply with all reagents necessary for nucleic acids extraction in forms of cartridge
Hazardous organic solvent or manual reagent preparation is not necessary.

Magtration® Technology

Magtration® (Magnetic Filtration) Technology allows extraction of nucleic acid with high purity without performing additional filtration, centrifugation and chloroform/phenol extraction steps which are generally required for usual nucleic acid extraction.



* This image was copied from PSS's catalogue.

Seeplex® Technology

Seeplex®system is the multiplex PCR system based on dual priming oligonucleotide (DPO™) technology, which greatly improves the specificity and yet keeps the sensitivity of multiplex PCR as high as that of single PCR. This is going to be a new guideline for mass screening with reasonable cost and great efficiency in the molecular diagnostic market.

Diagnostic system of next generation for ;

Simultaneous detection of multiple pathogens

Seeplex® System is an innovative diagnostic system designed for a simultaneous detection of multiple pathogens by just one-time PCR reaction using Seegene's proprietary DPO™ technology.

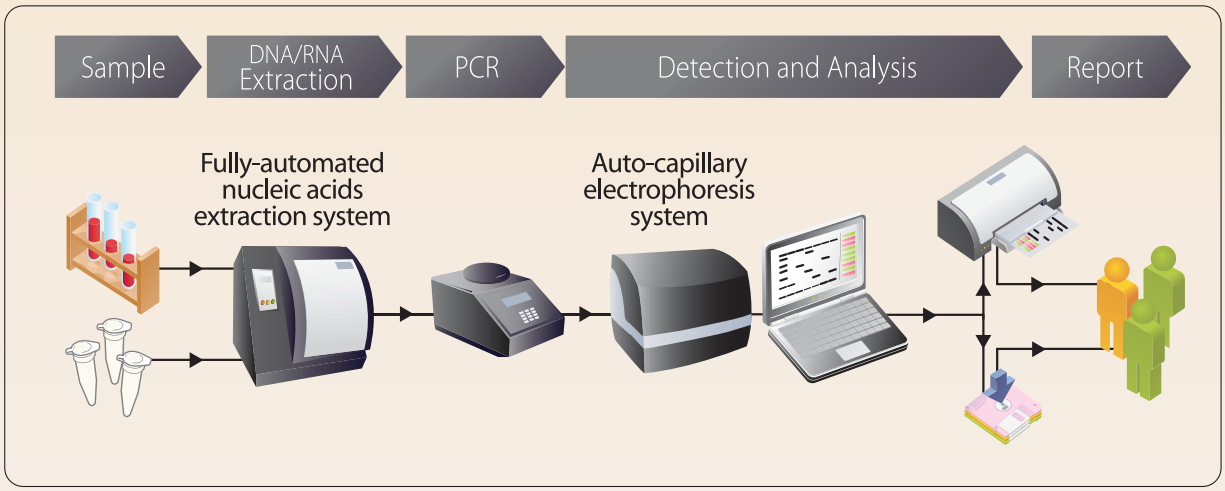
Mass screening by adapting various automatic detection systems

It is designed to work with various auto detection systems like Auto Sequencer (AS) and Auto Capillary Electrophoresis (ACE) instruments to offer computerized data, reproducibility, speed and accuracy.

Diagnostic test that can be easily performed using PCR technique

DPO™ completely eliminates artifacts that are easily found in the conventional PCR methods, hence maximizing specificity and reproducibility, which consequently allow customers to perform tests using widely-used PCR technique.

Seeplex® System for Automatic Detection



Auto-capillary electrophoresis system

LabChip® 90 System

Simultaneous detection for 96T or 384T depending on your number of tests & samples

LabChip® 90 System has various applications such as Multiplex PCR and Restriction Digest Fragment Analysis (LabChip® Kit for DNA).
(Automated detection system optimized for Seeplex® products)

Key Features

Automated electrophoresis and detection

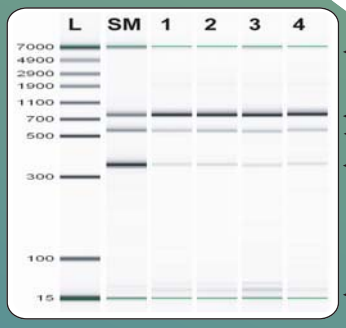
High-throughput & High speed
(Analysis of 96 samples using 96- or 384-well plate within 1 hr)

Direct analysis of samples without any further sample preparation step after PCR reaction

Cost effective

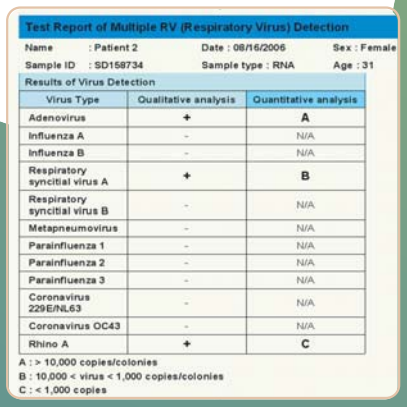
Data

The result obtained using LabChip® 90 System after PCR by Seeplex® MTHFR C677T ACE



L: Ladder | SM : Size Marker |
1~4 : Clinical Samples | N : Negative Control

Report form samples



Test Report of Multiple RV (Respiratory Virus) Detection

Name : Patient 2 Date : 08/16/2006 Sex : Female
Sample ID : SD158734 Sample type : RNA Age : 31

Results of Virus Detection

Virus Type	Qualitative analysis	Quantitative analysis
Adenovirus	+	A
Influenza A	-	N/A
Influenza B	-	N/A
Respiratory syncitial virus A	+	B
Respiratory syncitial virus B	-	N/A
Metapneumovirus	-	N/A
Parainfluenza 1	-	N/A
Parainfluenza 2	-	N/A
Parainfluenza 3	-	N/A
Coronavirus 229E/NL63	-	N/A
Coronavirus OC43	-	N/A
Rhino A	+	C

A : > 10,000 copies/colonies
B : 10,000 < virus < 1,000 copies/colonies
C : < 1,000 copies

Auto-capillary electrophoresis system

ScreenTape® System

Fully automated solution for gel electrophoresis

Simultaneous analysis up to 16 PCR samples within only 16 mins !

User-friendly software and report form which is easy to interpret (Automated detection system optimized for Seeplex® products)

Key Features

High-speed
(Automated simultaneous analysis of 16 PCR samples within 16 mins.)

Not necessary to use a whole strip at once, unused lanes can be re-used later

No requirement for EtBr, buffer and gel document system

Easy handling of data with barcode on ScreenTape

Automated analysis of PCR bands
(Sample loading, electrophoresis and analysis)

Process

ScreenTape

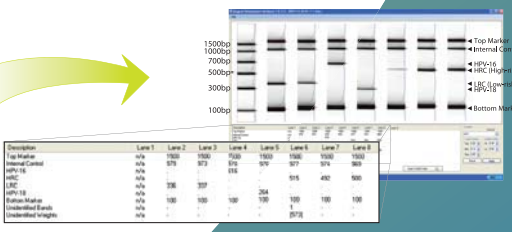
ScreenTape with the size of credit card containing multiple mini-gels does not require additional reagents or buffers.

1.TapeStation®

Load sample tubes and ScreenTape into the TapeStation™ after PCR reaction.

2. Electrophoresis & Analysis

Automatic analysis of PCR bands



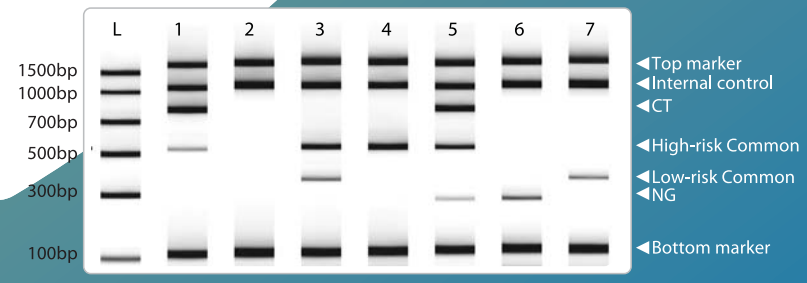
Report form

Sample ID	B2:2	C2:3	D2:4	E2:6	F2:7	G2:11	H2:13
IC	+	+	+	+	+	+	+
CT	+	-	-	-	+	-	-
HRC	+	-	+	+	+	-	-
LRC	-	-	+	-	-	-	+
NG	-	-	-	-	+	+	-
Unidentified	0	0	0	0	0	0	0

Positive Negative L : Ladder 1~7 : Clinical samples

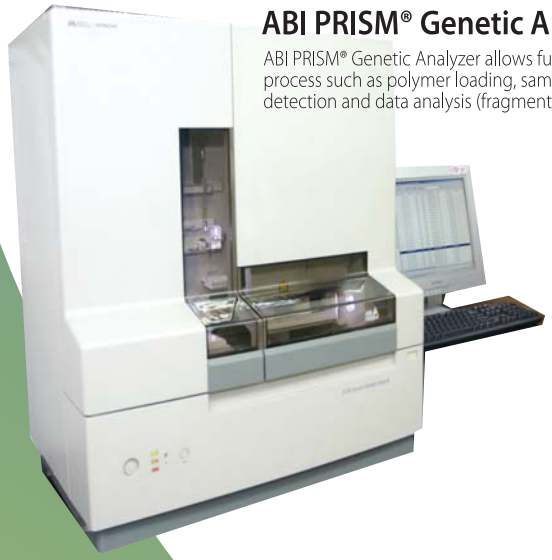
Data

The result obtained using ScreenTape® System after PCR by the Seeplex® HPV/STD4 ACE Screening



Auto-sequencer electrophoresis system

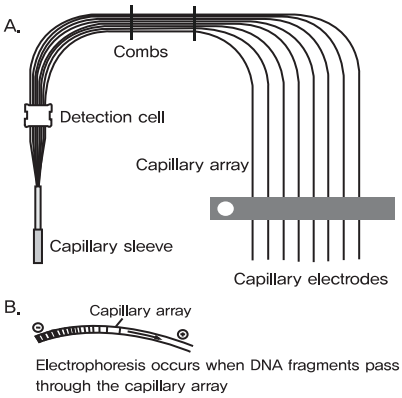
ABI Series (Applied Biosystems Inc.)
MegaBACE 1000 (Amersham Pharmacia Biotech Inc.)
Automated detection of multiplex PCR products from Seeplex® system



ABI PRISM® Genetic Analyzer

ABI PRISM® Genetic Analyzer allows full automation of whole process such as polymer loading, sample injection, separation, detection and data analysis (fragment analysis).

Principle



Multiplexed PCR products labeled with fluorescent dye are separated in gel matrix filled capillary by high voltage electrophoresis and the fluorescence with given wavelength emitted from PCR products can be detected by laser.

Applicable for Seeplex® Products

Seeplex® RV/PB18 ASE Detection
Simultaneous detection of 18 respiratory viruses and pneumonia bacteria in just one tube

Seeplex® HPV18 ASE Genotyping
Simultaneous genotyping of 18 different HPV types - 13 high-risk HPV types and 5 low-risk HPV types

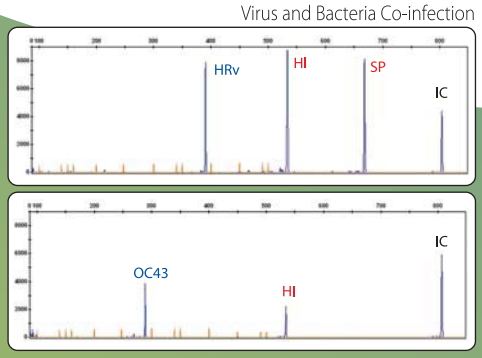
Seeplex® STD9 ASE Detection / STD7 ASE Detection
Simultaneous detection of 9 or 7 STD pathogens

Seeplex® HBV Lamivudine-resistant ASE
Simultaneous detection of 3 lamivudine resistant HBV types

Data

The result obtained using ABI PRISM® 3100-Avant Genetic Analyzer after PCR by Seeplex® RV/PB18 ASE Detection

Virus and Bacteria Co-infection



HRv, Human rhinovirus;
OC43, Human coronavirus OC43/HKU1;
SP, *Streptococcus pneumoniae*;
HI, *Haemophilus influenzae*;
IC, Internal Control

* Red peak indicates standard size marker.

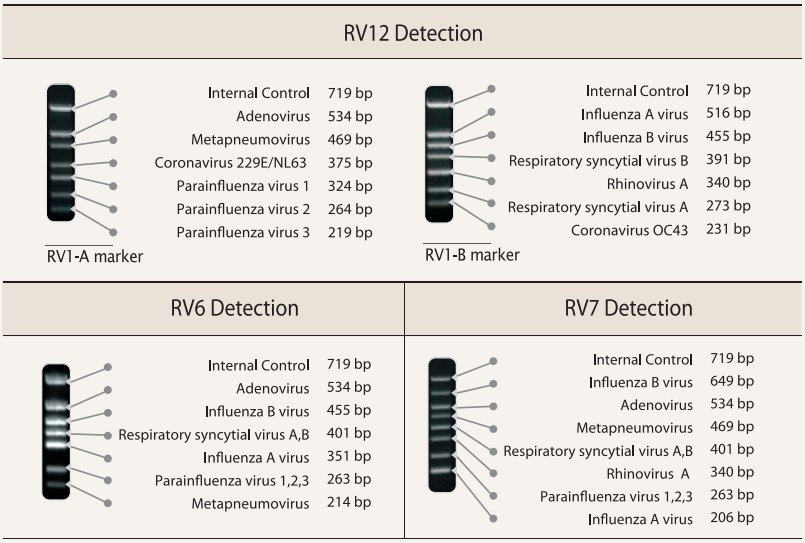
1.1 Agarose Gel-based Method

1.1.1 RV Detection

The Seeplex® RV Detection series are designed to detect the most common respiratory viruses from patients' samples including nasopharyngeal aspirates, nasopharyngeal swabs and bronchoalveolar lavage.

The conventional methods (culture and IFA, etc.) to detect respiratory viruses take several days or do not have enough sensitivity. Therefore, many of the recently developed diagnostic methods employ PCR methods. Among them, Seegene's Respiratory Virus Detection is outstanding in terms of sensitivity and specificity by applying DPO™ technology and detects the largest numbers of respiratory viruses.

Fig. 1. Band information



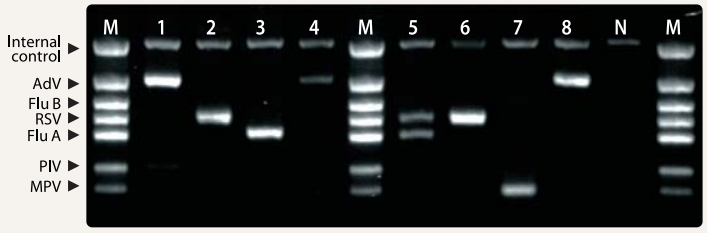
Seeplex® RV Detection

Product	Cat. No.	Size
RV12 Detection	RV1211	50 rxns
RV6 Detection	RV2210	50 rxns
RV7 Detection	RV3210	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

CE Seeplex® RV Detection series have received CE Mark.

Fig. 2. & Table 1. Example



M : RV2 Marker | 1~8 : Clinical samples | N: Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® RV6 Detection (Fig. 2) and result analysis (Table 1).

Patient	Infected Viruses	Patient	Infected Viruses
1	Adenovirus	5	Respiratory syncytial virus A, B & Influenza A virus
2	Respiratory syncytial virus A, B	6	Respiratory syncytial virus A, B
3	Influenza A virus	7	Metapneumovirus
4	Adenovirus	8	Adenovirus

***Citation:** 1. Roh *et al.*, Comparison of the Seeplex Reverse Transcription PCR Assay with the R-mix Viral Culture and Immunofluorescence Techniques for Detection of Eight Respiratory Vlruses. Annals of Clinical & Laboraroty Science, 2008, 38(1), 41-46.
2. Yoo et al., Detection of 12 Respiratory Viruses with Two-set Multiplex Reverse Transcriptase-PCR Assay Using a Dual Priming Oligonucleotide System. Korean J Lab Med, 2007, 27:420-7.
3. Chang-Seok Ki, Suk Ran Kim and Nam Yong Lee. Rapid Identification of 12 respiratory viruses with a multiplex PCR assay using dual priming oligonucleotide (DPO) system. 11th Asian Pacific Congress of Clinical Biochemistry (October 14th~19th 2007, Beijing, China).
4. M.N. Kim. Evaluation of Performance of Multiplex PCR using Dual Priming Oligonucleotide for Detecting Human Respiratory Viruses. 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) (17-20 September 2007, USA).

1.2 Auto-capillary Electrophoresis (ACE)

1.2.1 RV12 ACE Detection

The Seeplex® RV 12 ACE Detection for auto-capillary electrophoresis system is designed to detect 12 major respiratory viruses, 11 respiratory RNA viruses & 1 DNA virus, from patients' samples including nasopharyngeal aspirates, nasopharyngeal swabs and bronchoalveolar lavage.

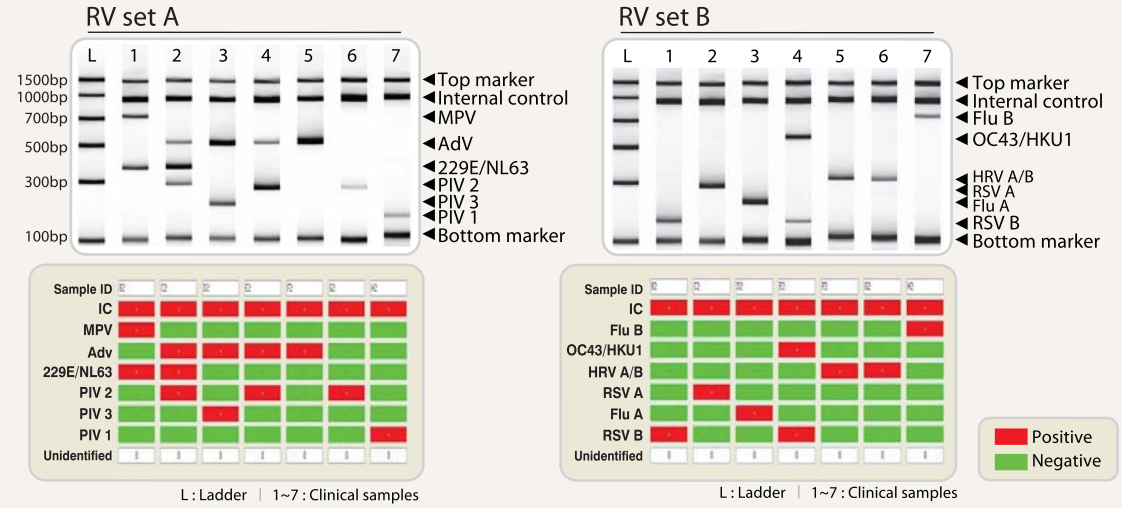
The conventional methods (culture and IFA, etc.) to detect respiratory viruses take several days or do not have enough sensitivity. Therefore, many of the recently developed diagnostic methods employ PCR methods. Among them, Seegene's Respiratory Virus Detection is outstanding in terms of sensitivity and specificity by applying DPO™ technology and detects the largest numbers of respiratory viruses.

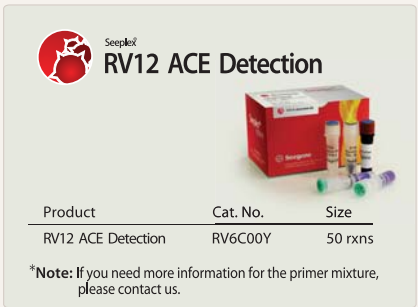
Simultaneously detecting 12 major respiratory viruses

- Influenza A virus
- Influenza B virus
- Respiratory syncytial virus A
- Respiratory syncytial virus B
- Parainfluenza virus 1
- Parainfluenza virus 2
- Parainfluenza virus 3
- Coronavirus 229E/NL63
- Coronavirus OC43/HKU1
- Rhinovirus A/B
- Adenovirus
- Metapneumovirus

CE **Seeplex® RV 12 ACE Detection has received CE Mark.**

Figure & Table. Example (ScreenTape® System)





Patient	Infected Viruses
1	MPV, CoV 229E/NL63, RSV B
2	AdV, Cov 229E/NL63, PIV2, RSV A
3	AdV, PIV3, Flu A
4	AdV, PIV2, CoV OC43/HKU1, RSV B
5	AdV, HRV A/B
6	PIV2, HRV A/B
7	PIV1, Flu B

Figure & Table. Amplified PCR products using the Seeplex® RV 12 ACE Detection. In this example, more than one respiratory virus was successfully detected in 7 patients' samples (lanes 1-7).
(Rapid & easy interpretation using ScreenTape® System)

1.2.2 RV5 ACE Screening

The Seeplex® RV5 ACE Screening for auto-capillary electrophoresis system is designed to screen the most prevalent respiratory viruses from patients' samples including nasopharyngeal aspirates, nasopharyngeal swabs and bronchoalveolar lavage.

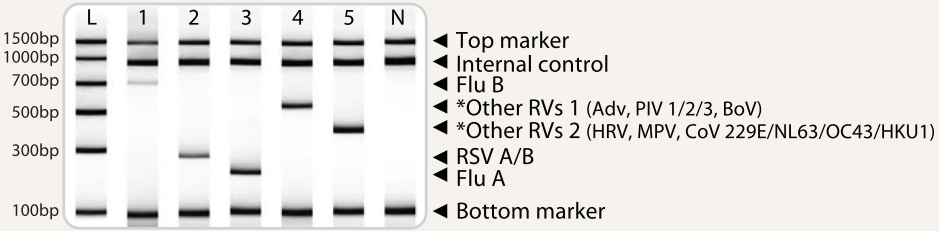
The conventional methods (culture and IFA, etc.) to detect respiratory viruses take several days or do not have enough sensitivity. Therefore, many of the recently developed diagnostic methods employ PCR methods. Among them, Seegene's Respiratory Virus Test is outstanding in terms of sensitivity and specificity by applying DPO™ technology and detects the largest numbers of respiratory viruses.

Rapid screening of the most prevalent respiratory viruses

- Flu A
- Flu B
- RSV A/B
- Other respiratory viruses 1 (AdV, PIV 1/2/3, BoV)
- Other respiratory viruses 2 (MPV, HRV, CoV 229E/NL63/OC43/HKU1)

Flu A (Influenza A virus), Flu B (Influenza B virus), RSV A/B (Respiratory syncytial virus A/B), AdV (Adenovirus), PIV 1/2/3 (Parainfluenza virus 1/2/3), BoV (Bocavirus), HRV (Rhinovirus), MPV (Metapneumovirus), CoV 229E/NL63/OC43/HKU1 (Coronavirus 229E/NL63/OC43/HKU1)

Figure & Table. Example (ScreenTape® System)



L : Ladder | 1~5 : Clinical samples | N : Negative control

Positive
Negative

Patient	Infected Viruses
1	Flu B
2	RSV A/B
3	Flu A
4	Other RVs 1 (AdV, PIV 1/2/3, BoV)
5	Other RVs 2 (HRV, MPV, CoV 229E/NL63/OC43/HKU1)

Figure & Table. Amplified PCR products using the Seeplex® RV5 ACE Screening. In this example, more than one respiratory virus was successfully detected in 5 patient samples (lanes 1-5). (Rapid & easy interpretation using ScreenTape® System)

CE Seeplex® RV5 ACE Screening has received CE Mark.

Seeplex® RV5 ACE Screening

Product	Cat. No.	Size
RV5 ACE Screening	RV6550Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

1.2.3 PneumoBacter ACE Detection

The Seeplex® PneumoBacter ACE Detection for auto-capillary electrophoresis system is designed to simultaneously detect 6 pneumonia bacteria from nasopharyngeal aspirates, nasopharyngeal swabs and bronchoalveolar lavage.

Pneumonia is usually caused by viruses and bacteria. This product is a multiplex PCR product to test bacterial pneumonia. Bacterial culture and serological testing for detection of pneumonial bacteria have low sensitivity and are time-consuming. By applying an innovative DPO™ based multiplex PCR system having optimized specificity and sensitivity, the Seeplex® PneumoBacter ACE Detection simply, rapidly, and accurately detects 6 pneumonia bacteria in respiratory specimens.

Simultaneously detecting 6 pneumonia bacteria

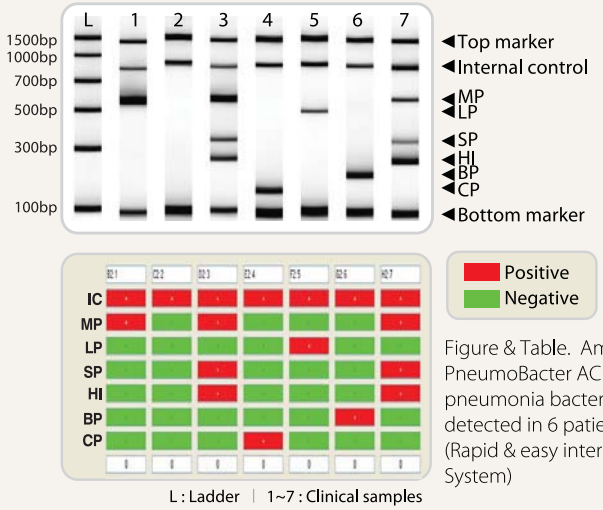
Typical pneumonia

- *Streptococcus pneumoniae* (SP)
- *Haemophilus inflenzae* (HI)

Atypical pneumonia

- *Chlamydophila pneumoniae* (CP)
- *Legionella pneumophila* (LP)
- *Bordetella pertussis* (BP)
- *Mycoplasma pneumoniae* (MP)

Figure & Table. Example (ScreenTape® System)



L : Ladder | 1~7 : Clinical samples

Figure & Table. Amplified PCR products using PneumoBacter ACE Detection. In this example, pneumonia bacteria were successfully detected in 6 patient samples (lanes 1, 3-7). (Rapid & easy interpretation using ScreenTape® System)

Patient	Infected Viruses
1	*Mycoplasma pneumoniae*
2	-
3	*Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus inflenzae*
4	*Chlamydophila pneumoniae*
5	*Legionella pneumophila*
6	*Bordetella pertussis*
7	*Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus inflenzae*

Seeplex®
PneumoBacter ACE Detection

Product	Cat. No.	Size
PneumoBacter ACE Detection	PB1610Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

1.3 Auto-sequencer Electrophoresis (ASE)

1.3.1 RV/PB18 ASE Detection

The Seeplex® RV/PB18 ASE Detection is designed to detect simultaneously 13 respiratory viruses and 5 pneumonia bacteria from patients' samples such as nasopharyngeal aspirates, nasopharyngeal swabs and bronchoalveolar lavage.
Capillary-based auto sequencers including ABI3130/3100, MegaBACE 1000 are applicable for the Seeplex® RV/PB18 ASE Detection.

13 Respiratory Viruses + 5 Pneumonia Bacteria + Internal Control

13 Respiratory Viruses:
Influenza A virus, Influenza B virus, Human respiratory syncytial virus A, Human respiratory syncytial virus B, Human rhinovirus, Human coronavirus OC43/HKU1, Human coronavirus 229E/NL63, Human adenovirus, Human parainfluenza virus 1, Human parainfluenza virus 2, Human parainfluenza virus 3, Human bocavirus, Human enterovirus

5 Pneumonia Bacteria:
Mycoplasma pneumoniae, Haemophilus influenzae, Streptococcus pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila

Key Features

- Small volume (0.2ml) of samples needed
- Multiplex PCR in just one tube
- Internal control can check PCR inhibition
- No cross-reaction
- Low cost and fast result

Product	Cat. No.	Size
RV/PB18 ASE Detection	RP5J10Y	50 rxns

Limitations of the Existing Methods

Now, the unnecessary abuse of antibiotics can be prevented.

Existing diagnostic methods have tested for some viruses and some bacteria. However, it is difficult to accurately diagnose co-infection of viruses and bacteria because until now there has been no method to detect two types using one test, and thus the proper prescription cannot be made at the appropriate time.

Consequently, the unnecessary abuse of antibiotics for common cold symptoms has been rampant under circumstances where the accurate diagnosis of pathogens, which requires the use of antibiotics, cannot be performed.
However, Seegene's products make it possible to prescribe quickly in the early stage of symptoms with just one test by determining whether a respiratory infection has been caused by viruses or bacteria or both.
If the influenza A or influenza B virus is detected using Seegene products, Oseltamivir or Zanamavir can be prescribed.
This product will help with the development of specific antiviral preparations and antibiotics in the long term, and ultimately make customized prescriptions possible.

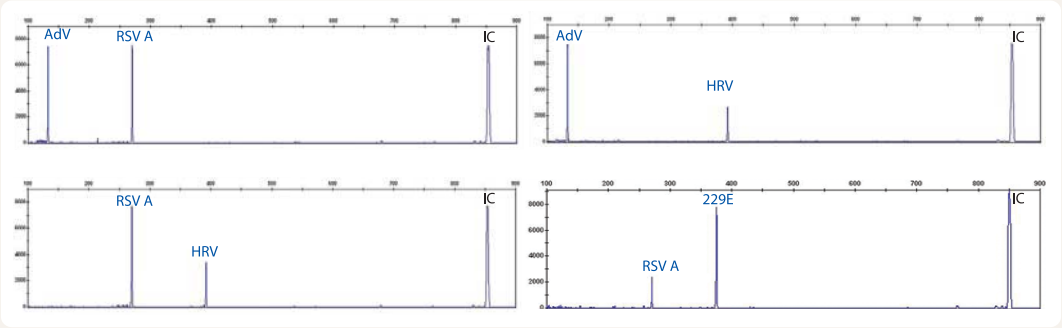
Target Pathogens

- 18 pathogens
  - 5 Bacteria
    - *Legionella pneumoniae*
    - *Streptococcus pneumoniae*
    - *Chlamydophila pneumoniae*
    - *Haemophilus influenzae*
    - *Mycoplasma pneumoniae*
  - 13 Viruses
    - 11 RNA viruses
      - Influenza A virus
      - Influenza B virus
      - Human respiratory syncytial virus A
      - Human respiratory syncytial virus B
      - Human parainfluenza virus 1
      - Human parainfluenza virus 2
      - Human parainfluenza virus 3
      - Human coronavirus 229E/NL63
      - Human coronavirus OC43/HKU1
      - Human rhinovirus
      - Human enterovirus
    - 2 DNA viruses
      - Human adenovirus
      - Human bocavirus

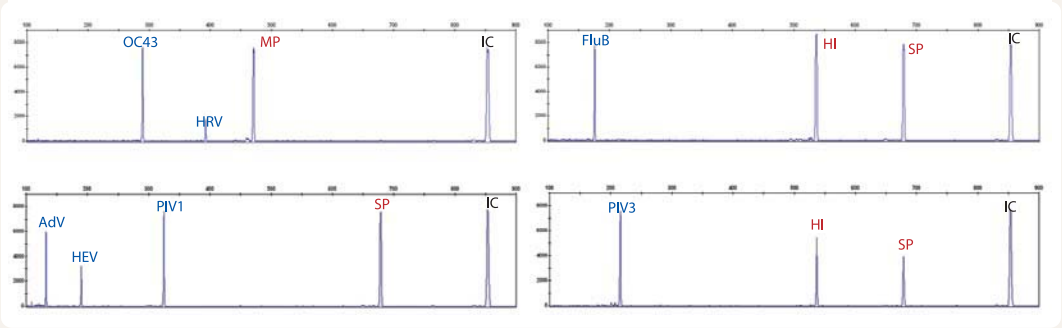
Examples

Seeplex® RV/PB18 ASE Detection with clinical samples

Virus infection



Virus and Bacteria Co-infection



IC, Internal Control;
HRV, Rhinovirus;
RSV A, Respiratory syncytial virus A;
229E, Coronavirus 229E/NL63;
AdV, Adenovirus;
HEV, Enterovirus;
FluB, Influenza B virus;
OC43, Coronavirus OC43/HKU1;
PIV 1, Parainfluenza virus 1;
PIV 3, Parainfluenza virus 3;
SP, *Streptococcus pneumoniae*;
HI, *Haemophilus influenzae*;
MP, *Mycoplasma pneumoniae;*

Press Release

"Seegene Introduces a Diagnostic Test Kit Capable of Detecting 18 Different Virus- and Bacteria-Born Respiratory Infections in a Single Tube"

Clinical Lab Products (Top story/Reading Record Hit) - http://www.clpmag.com/clprime/2007-09-19_01.asp
European Hosptial (Front page) - http://www.european-hospital.com/topics/article/2545.html
Genetic Engineering & Biotechnology News - http://www.genengnews.com/news/bnitem.aspx?name=22898704&taxid=37&taxid=14
Rapid microbiology - http://www.rapidmicrobiology.com/news/2955h0.php
LabTechnologist.com - http://www.labtechnologist.com/news/ng.asp?n=79720-affymetrix-atcc-illumina-micronic-seegene

***Others:** Investor's Business Daily, The Business Gazette, PharmaBiz.com, SelectScience, GenomeWeb News, Medical Device Daily, BioSpectrum Asia, Medical News Today, Diagnostics Focus Medical Devices, Yahoo, etc.

2.1 Agarose Gel-based Method

2.1.1 STD Detection

The Seeplex® STD Detection series are designed to detect 4, 5, or 6 different sexually transmitted disease (STD) pathogens from various patient samples (endocervical/urethral swabs).

The conventional methods (ELISA, Giemsa stain, culture, etc.) to detect STD pathogens take several days or suffer from poor sensitivity. Therefore, many of the recently developed diagnostic methods employ PCR methods. Among them, Seegene's STD Detection is outstanding in terms of sensitivity and specificity using applied DPO™ technology and currently detects the largest number of sexually transmitted diseases.

Fig. 1. Band information

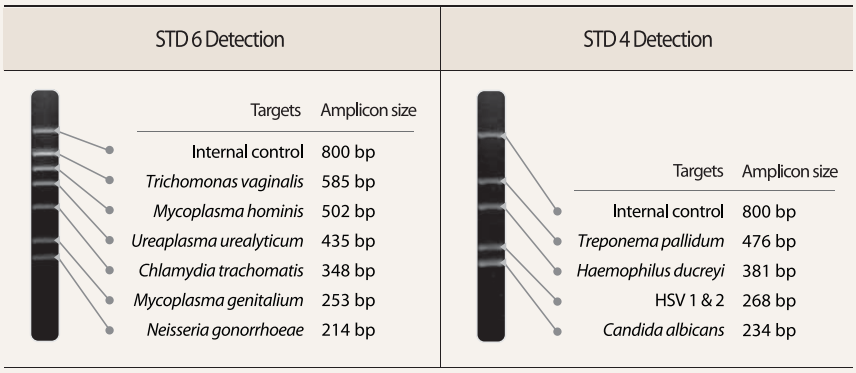
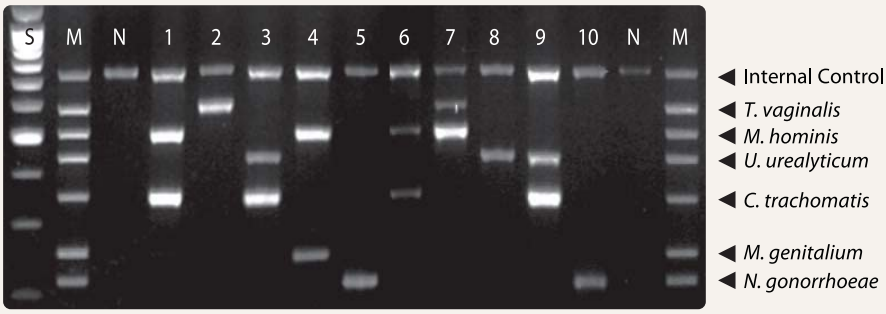


Fig. 2 & Table 1. Example



S : 100bp ladder | M : Positive marker | 1~10 : Clinical samples | N : Negative control

Patient	Infected Pathogens	Patient	Infected Pathogens
1	*M. hominis, C. trachomatis*	6	*M. hominis, C. trachomatis*
2	*T. vaginalis*	7	*T. vaginalis, M. hominis*
3	*U. urealyticum, C. trachomatis*	8	*U. urealyticum*
4	*M. hominis, M. genitalium*	9	*U. urealyticum, C. trachomatis*
5	*N. gonorrhoeae*	10	*N. gonorrhoeae*

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® STD6 Detection (Fig. 2) and result analysis (Table 1). More than one STD pathogen was successfully detected in 10 patient samples. The results also show that internal controls were all amplified, thereby confirming there were no test errors.

***Citation:** Tae-Hyoung Kim et al., Detection of Cryptic Microorganisms in Patients with Chronic Prostatitis by Multiplex Polymerase Chain Reaction, Korean J. Urol.(2007) 48:304-309.

2.2 Auto-capillary Electrophoresis (ACE)

2.2.1 STD ACE Detection

The Seeplex® STD ACE Detection series for auto-capillary electrophoresis system are designed to simultaneously detect 4 or 6 sexually transmitted disease (STD) pathogens from patients' samples (endocervical/ urethral swabs).

The conventional methods (ELISA, Giemsa stain, culture, etc.) to detect STD pathogens take several days or suffer from poor sensitivity. Therefore, many of the recently developed diagnostic methods employ PCR methods. Among them, Seegene's STD Detection is outstanding in terms of sensitivity and specificity using applied DPO™ technology and currently detects the largest number of sexually transmitted diseases.

STD4 ACE Detection

Simultaneously detecting 4 different STD pathogens

- *Treponema pallidum*
- *Haemophilus ducreyi*
- HSV 1 & 2
- *Candida albicans*

STD6 ACE Detection

Simultaneously detecting 6 different STD pathogens

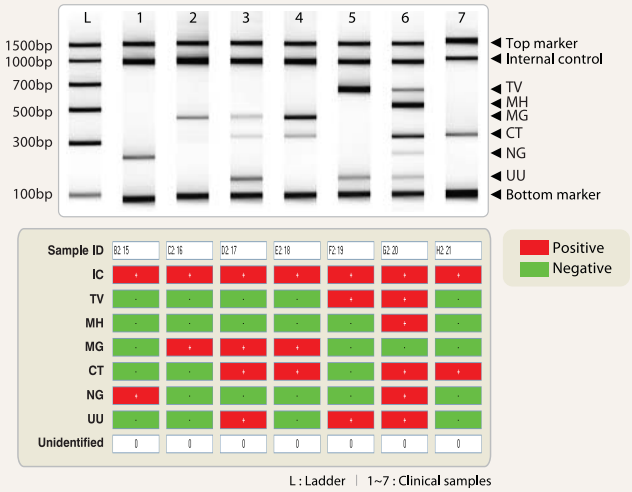
- *Trichomonas vaginalis* (TV)
- *Mycoplasma hominis* (MH)
- *Mycoplasma genitalium* (MG)
- *Chlamydia trachomatis* (CT)
- *Neisseria gonorrhoeae* (NG)
- *Ureaplasma urealyticum* (UU)

Seeplex®
STD ACE Detection

Product	Cat. No.	Size
STD4 ACE Detection	SD6400Y	50 rxns
STD6 ACE Detection	SD6600Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

Figure & Table. Example (ScreenTape® System)



Patient	Infected Viruses
1	NG
2	MG
3	MG, CT
4	MG, CT
5	TV
6	TV, MH, CT, NG, UU
7	CT

Figure & Table. Amplified PCR products using STD6 ACE Detection. In this example, STD pathogens were successfully detected in 7 patient samples (lanes 1-7). (Rapid & easy interpretation using ScreenTape® System)

2.3 Auto-sequencer Electrophoresis (ASE)

2.3.1 STD ASE Detection

The Seeplex® STD ASE Detection series are designed to detect simultaneously 9 or 7 different STD pathogens from patients' samples such as endocervical and urethral swabs. Capillary-based auto sequencers including ABI3130/3100, MegaBACE 1000 are applicable for the Seeplex® STD ASE Detection .

Seeplex® STD9 ASE Detection

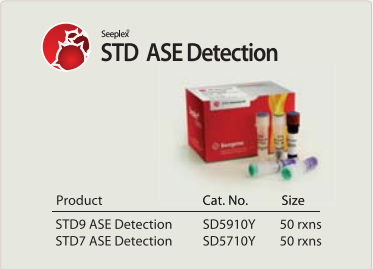
9 Pathogens: *Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Treponema pallidum, Chlamydia trachomatis*, HSV, *Neisseria gonorrhoeae, Haemophillus ducreyi, Ureaplasma urealyticum* + Internal Control

Seeplex® STD7 ASE Detection

7 Pathogens: *Trichomonas vaginalis, Gardnerella vaginalis, Mycoplasma genitalium, Chlamydia trachomatis*, HSV, *Neisseria gonorrhoeae, Ureaplasma urealyticum* + Internal Control

Key Features

- Small volume (0.2ml) of samples needed
- Simultaneous detection of 9 or 7 STD pathogens
- Multiplex PCR in just one tube
- Internal control can check PCR inhibition
- Low cost and fast result



Product	Cat. No.	Size
STD9 ASE Detection	SD5910Y	50 rxns
STD7 ASE Detection	SD5710Y	50 rxns

Overcoming Limitations of the Existing Tests

It is necessary to detect multiple STD pathogens in just one test.

Sexually transmitted diseases (STD), which are usually contracted through sexual intercourse, are common to the extent that more than 50% of all adults may be infected at least once in their lifetime. The currently used STD pathogen test products can detect only 2 types of STD pathogens (C. trachomatis and N. gonorrhoeae). However, other STD pathogens in addition to the above 2 types can also lead to serious diseases and other health problems, including adult infertility and genetic anomalies in newborn babies. Most STDs have no symptoms and about 30% of infected individuals are unaware of their infections. As such, it is important to test for as many pathogens as possible at one time for preventing the spread of pathogen and proper treatment.

Various diseases caused by STD pathogens

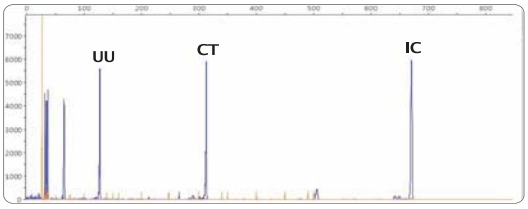
Seegene can provide the fastest and most accurate diagnostic method for the following STD pathogens with just one test, for proper diagnosis and treatment.

STD pathogens	Diseases caused by STD pathogens
T. vaginalis	Nongonococcal urethritis
M. hominis	Nongonococcal urethritis, acute endometritis, endocervicitis, pelvic inflammatory disease (PID)
U. urealyticum	Nongonococcal urethritis, meningitis, pneumonia, persistent pulmonary hypertension (PPH), chronic infection of CNS, infant and child mortality
C. trachomatis	Vaginitis, dysuria, frequent urination
M. genitalium	Nongonococcal urethritis, prostatic urethritis, puerperal fever, endometritis, endocervicitis
N. gonorrhoeae	Endocarditis, dialysis, genital mucosal infection, arthritis, bacteremia
H. ducreyi	Chancroid, lymphadenopathy, appearance of small lumps, genital ulcers with secretion
T. pallidum	Syphilis, systemic anomaly (general anomaly: heart), preterm delivery, infant and child mortality
HSV 1&2	Child mortality, brain damage, genital ulcers, conjunctivitis, keratitis, herpetic paronychia
G. vaginalis	Bacterial vaginosis or nonspecific vaginitis

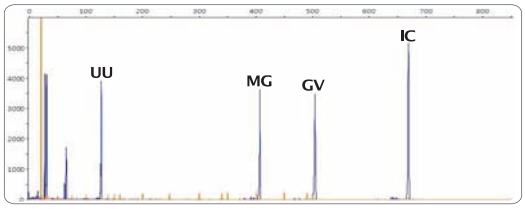
Examples

- Seeplex® STD9 ASE Detection or STD7 ASE Detection with clinical samples

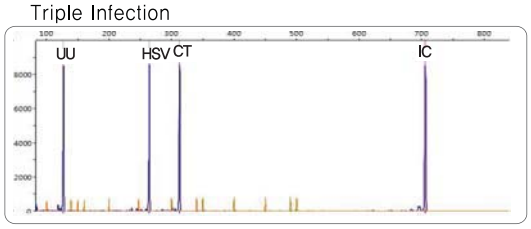
Double Infection



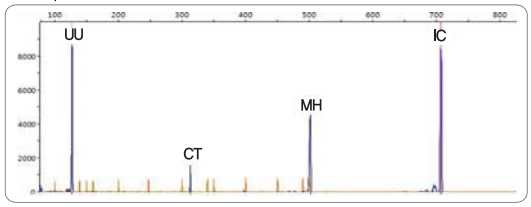
Triple Infection



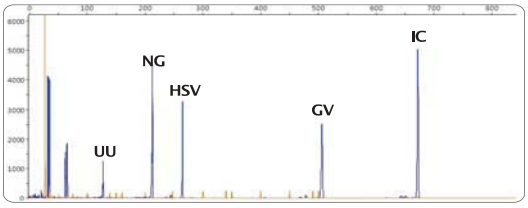
Triple Infection



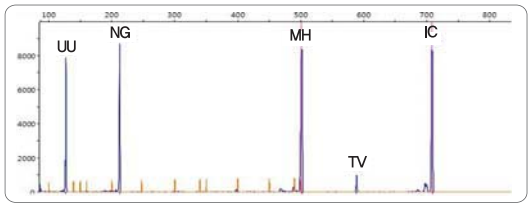
Triple Infection



Quatre Infection



Quatre Infection



* Red peak indicates standard size marker.

UU, *Ureaplasma urealyticum*; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; GV, *Gardnerella vaginalis*; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; TV, *Trichomonas vaginalis*; IC, Internal Control

3. Human Papillomavirus Detection

3.1 Agarose Gel-based Method

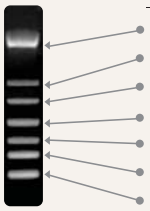
3.1.1 HPV6 Genotyping

The Seeplex® HPV6 Genotyping is designed to detect 6 HPV types from patients' cervical swabs.

Papillomaviruses are a diverse group of DNA viruses that infect the skin and mucous membranes of humans and many animals. To date, more than 100 types of HPV have been identified, of which 30 different HPVs have been found to infect the genital mucosa. It is estimated that 70~80 % women are infected by HPV at least once in their lifetime. Several studies have reported that the severity of HPV infections is influenced by genotypes of the infecting virus. For example, high risk types of HPV are the major cause of cervical cancer, whereas low risk types of HPV are frequently detected in benign lesions such as condylomata acuminata. Therefore, genotyping of HPV is important to correctly identify women who may be at risk of developing cervical cancer and to indicate appropriate therapy for women with HPV.
The Seeplex® HPV6 Genotyping with enhanced sensitivity and specificity, which utilizes DPO™ technology, allows detection of 6 types of HPV (6/11/16/18/31/45) types at one time.

Fig. 1. Band information

HPV6 Genotyping



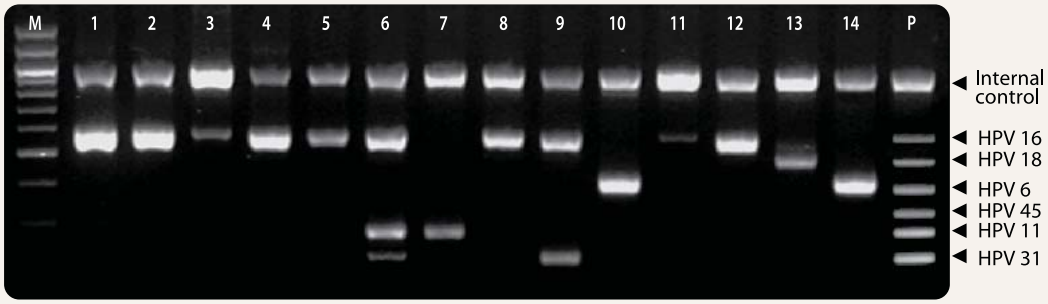
Targets	Amplicon
Internal control	1000 bp
HPV 16	588 bp
HPV 18	502 bp
HPV 6	425 bp
HPV 45	370 bp
HPV 11	293 bp
HPV 31	244 bp

Seeplex®
HPV6 Genotyping

Product	Cat. No.	Size
HPV6 Genotyping	HP1511Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

Fig. 2 & Table 1. Example



M : 100bp ladder | 1~14 : Clinical samples | P : HPV Marker

Clinical Sample	HPV type	Clinical Sample	HPV type	Clinical Sample	HPV type
1	16	6	11,16, 31	11	16
2	16	7	11	12	16
3	16	8	16	13	18
4	16	9	16, 31	14	6
5	16	10	6		

CE Seeplex® HPV6 Genotyping has received CE Mark. (Cat. No. HP1511Y)

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® HPV6 Genotyping (Fig. 2) and result analysis (Table 1). 6 genotypes of HPV were successfully detected in 14 patient samples.

3.2 Auto-capillary Electrophoresis (ACE)

3.2.1 HPV4 ACE Screening

The Seeplex® HPV4 ACE Screening is designed to simultaneously genotype (HPVs 16 and 18) and screen for HPV 11 high-risk and 5 low-risk types from patients' samples (cervical swabs).

Cervical cancer, which progresses from the precancerous stage to invasive cancer, has 7-20 years of precancerous stage; consequently early diagnosis is possible when HPV infection is suspected. HPV low-risk group, including HPVs 6 and 11, may cause genital warts. On the other hand, HPV high-risk group may lead to the development of cervical cancer; especially, HPVs 16 and 18 are associated with 70% of cervical cancer case. Seeplex® HPV4 ACE Screening can indentify HPVs 16 and 18 and also screen for HPV high and low-risks at the same time.

Simultaneously genotyping (HPVs 16 and 18) and screening

HPV 16] Genotyping
HPV 18]

11 HPV High-risk common]
5 HPV Low-risk common] Screening

* Note: **HRC (HPV High risk Common, 11 types) :**
31, 33, 35, 45, 51, 56, 58, 59, 66, 67, 70

LRC (HPV Low Risk Common, 5 types) :
6, 11, 42, 43, 44

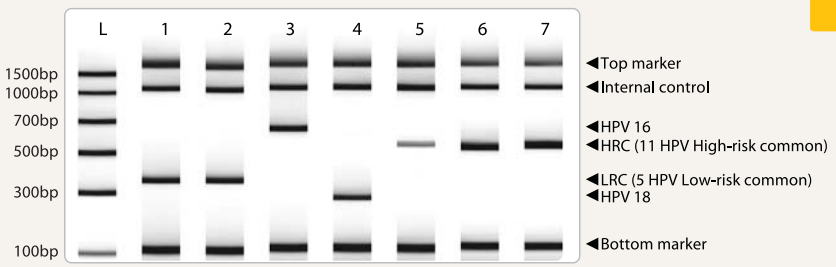
* HPV High-risk common primers might detect HPV types 39, 52 and 68 by cross-reactivity.

Seeplex®
HPV4 ACE Screening

Product	Cat. No.	Size
HPV4 ACE Screening	HP1400Y	50 rxns

***Note:** If you need more information for the primer mixture, please contact us.

CE **Seeplex®HPV4 ACE Screening has received CE Mark. (Cat. No. HP1400Y)**



L : Ladder | 1~7 : Clinical samples

Clinical Sample	Result	Clinical Sample	Result
1	LRC	5	HRC
2	LRC	6	HRC
3	HPV 16	7	HRC
4	HPV 18		

Figure & Table. Amplified PCR products using Seeplex® HPV4 ACE Screening In this example, HPVs were successfully detected in 7 patient samples (lanes 1-7).
(Rapid & easy interpretation using ScreenTape® System)

3.2.2 HPV/STD4 ACE Screening

The Seeplex® HPV/STD4 ACE Screening for auto-capillary electrophoresis system is designed to simultaneously screen for the most common STD and HPV high-risk/low-risk types from patients' samples (cervical/urethral swabs).

The Seeplex® HPV/STD4 ACE Screening detects the most common STD-causing pathogens, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and detects 13 High Risk HPVs and 5 Low Risk HPVs, separately. It is a very useful diagnostic method for prevention and timely treatment of STD and cervical cancer because two most frequently detected STD-causing pathogens and high-risk HPV causing cervical cancer can be separately detected in a single tube.

Simultaneously Screening for STD and HPV high-risk/low-risk

Chlamydia trachomatis (CT)
Neisseria gonorrhoeae (NG)] STD pathogens

13 HPV High–risk common
5 HPV Low–risk common] HPVs

* Note: **HRC (HPV High risk Common, 13 types) :**
16, 18, 31, 33, 35, 45, 51, 56, 58, 59, 66, 67, 70

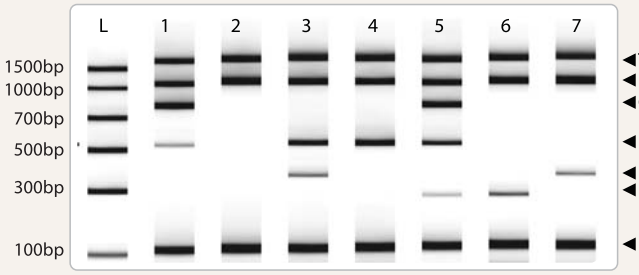
LRC (HPV Low Risk Common, 5 types) :
6, 11, 42, 43, 44

* HPV High-risk common primers might detect HPV types 39, 52 and 68 by cross-reactivity.

Seeplex®
HPV/STD4 ACE Screening

Product	Cat. No.	Size
HPV/STD4 ACE Screening	SH1400Y	50 rxns

***Note:** If you need more information for the primer mixture, please contact us.



L : Ladder | 1~7 : Clinical samples

Clinical Sample	Result	Clinical Sample	Result
1	CT, HRC	5	CT, HRC, NG
2	-	6	NG
3	HRC, LRC	7	LRC
4	HRC		

Figure & Table. Amplified PCR products using Seeplex® HPV/STD4 ACE Screening. In this example, *C. trachomatis*, *N. gonorrhoeae*, and HPVs were successfully detected in 6 patient samples (lanes 1, 3-7). (Rapid & easy interpretation using ScreenTape® System)

3.3 Auto-sequencer Electrophoresis (ASE)

3.3.1 HPV18 ASE Genotyping Available soon

The Seeplex® HPV18 ASE Genotyping is designed to detect simultaneously of 18 different HPV types from patients' samples such as cervical swabs. Capillary-based auto sequencers including ABI3130/3100, MegaBACE 1000 are applicable for the Seeplex® HPV18 ASE Genotyping.

13 High Risk Types

16, 18, 31, 33, 35, 45, 51, 56, 58, 59, 66, 67/52, 70/39/68

5 Low Risk Types

6, 11, 42, 43, 44
+ Internal Control

CE Seeplex® HPV18 ASE Genotyping has received CE Mark. (Cat. No. HP5J10Y)

Key Features

- Small volume (0.2ml) of cervical swab samples needed
- Multiplex PCR in just one tube
- Internal control can check PCR inhibition
- No cross-reaction
- Low cost and fast result

Seeplex®
HPV18 ASE Genotyping

Product	Cat. No.	Size
HPV18 ASE Genotyping	HP5J10Y	50 rxns

Cross-reaction a Major Limitation of the Existing Methods

Existing HPV DNA detection kit shows a high false positive rate.

Currently, commercially available HPV DNA detection and/or genotyping test employs probe-hybridization method which generates many false positives due to cross-reactivity between probes and amplified PCR products (de Cremoux P et al.:Am J Clin Pathol. 2003 Oct;120(4):483-4:, Castle PE et al.: Cancer Epidemiol Biomarkers Prev. 2002 Nov;11(11):1394-9., Vernon SD et al.: J Clin Microbiol. 2000 Feb;38(2):651-5 etc.).
Cross-reactivity can be solved by using PCR method with high specificity. Seeplex products completely overcome the limitations of current products by employing DPO™ primer technology with high specificity.

Significance of HPV Genotyping

HPV genotyping is very important in diagnostic tests.

The importance of genotyping, which is capable of detecting all infected genotypes beyond the presence of simple HPV infection, is on the rise along with the importance of multiplexing detection of HPV subtypes. Seegene's product can provide 18 HPV screening and genotyping simultaneously in one test. This product is necessary to identify HPV subtypes which have the potential to cause cancer.
This is because different relative risks are presented by HPV subtypes with regard to cervical cancer. As such, it is required in the efficiency testing of a vaccine, and can be used significantly in epidemiological studies.
Thus, HPV genotyping should be the ordinary early diagnosis method.

Seeplex® is very fast !

Seeplex® System

Sample → PCR → Detect

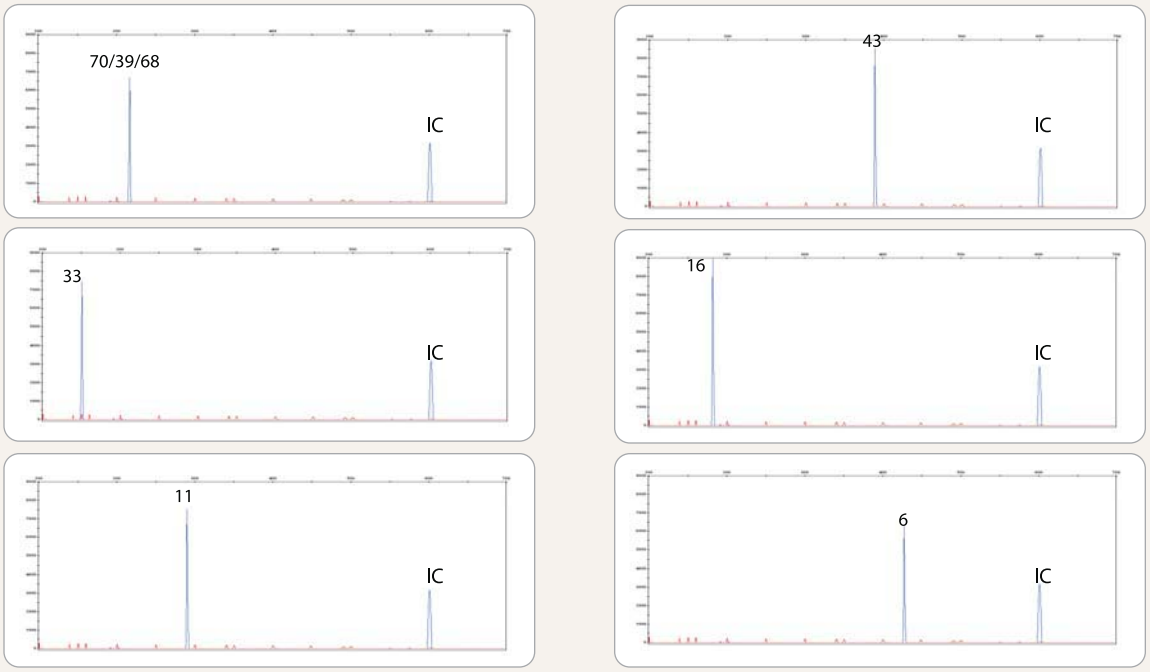
VS.

hc2 system

Sample → Denature → Hybridize → Capture → Detect → Read after

Examples

- Seeplex® HPV18 ASE Genotyping with clinical samples



* Red peak indicates standard size marker.
IC, Internal Control

Press Release

"Seegene Introduces Breakthrough DNA Test to Simultaneously Detect 18 Different Human Papillomaviruses (HPVs)"

Genetic Engineering & Biotechnology News - http://www.genengne ws.com/news/bnitem.aspx?name=20137985&taxid=14
Clinical Lab Products - http://www.clpmag.com/clprime/2007-07-18_08.asp
Bioforumnews - http://www.bioforum.it/news05.htm
Labnews.com - http://www.labnews.com/en/nav1_01/01_001.php?id=5055
Medical News Today - http://www.medicalnewstoday.com/articles/76586.php
***Others:** Marketwire, Bioscience Technology, Hospital Buyer, Medical News Today, Diagnostics Focus Medical Devices, etc.

4.1 Agarose Gel-based Method

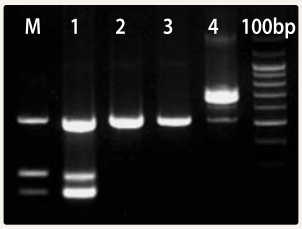
4.1.1 TB Detection 2

The Seeplex® TB Detection 2 is designed to detect M. tuberculosis complex (TBC) in various specimens such as sputum, bronchial wash, cerebrospinal fluid (CSF), body fluid and tissue .

Seeplex® TB Detection 2 uses multi-target (IS6110 and MPB64) PCR instead of a single-target PCR for specific detection of M. tuberculosis. Insertion sequence IS6110 is the most widely used target gene for detection of M. tuberculosis DNA. However, several M. tuberculosis strains that lack this insertion sequence have been isolated[1]. To prevent false-negative results caused by lack of this insertion sequence TB Detection carries out both IS6110 and MPB64 nested PCR. MPB64 has conserved in M. tuberculosis complex genome and has been a highly specific protein for the M. tuberculosis complex[2]. As simultaneously amplifying IS6110 and MPB64 DNA based on the nested PCR, Seeplex® TB Detection 2 can effectively eliminate false positive and false negative results and be a useful method for detection of TBC. This product has been validated for use with specimens including sputum, bronchial wash, cerebrospinal fluid (CSF), body fluid and tissue.

1) Juana Magdalena, et. al., J. Clin. Microbiol. 1998; 36(4); 937-943
2) Naoki Hasegawa, et. al., J. Clin. Microbiol. 2002; 40(3); 908-912

Fig. 1. Band information



M : TB2-Marker | 1 : Positive sample | 2 : Negative sample | 3 : Negative control (no-template) | 4 : Positive control PCR using TB2-control as the template | 100bp : 100 bp ladder (Seegene, Cat. No. M0100)

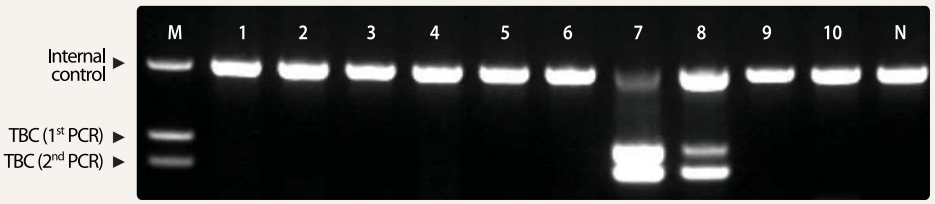
Table 1.

TB amplicon sizes for specimens		
	First PCR	Second PCR
Internal Control	520 bp	520 bp
TBC	260 bp	200 bp

Table 2.

TB amplicon sizes for positive control		
	First PCR	Second PCR
Internal control	520 bp	520 bp
Positive control	760 bp	700 bp

Fig. 2 & Table 3. Example



M : TB-2 Marker | 1~10 : Clinical samples | N : Negative control

Patient	TBC	Patient	TBC
1	ND	6	ND
2	ND	7	TBC-Positive
3	ND	8	TBC -Positive
4	ND	9	ND
5	ND	10	ND

Fig. 2 & Table 3. Amplified PCR products using the Seeplex® TB Detection 2 (Fig. 2) and result analysis (Table 3). Of ten clinical samples (sputum), TBC was positive in 2 samples (lanes 7 and 8).

ND* : Not Detected

***Note:** As shown in lane 7 of Fig. 2., the internal control may be weakened when there are strong TBC bands due to mutual competition.

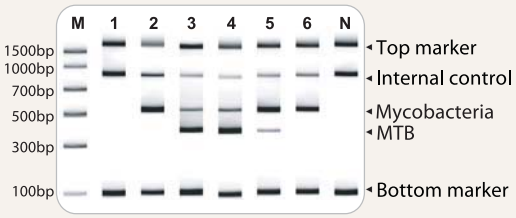
4.2 Auto-capillary Electrophoresis (ACE)

4.2.1 MTB/NTM ACE Detection

The Seeplex® MTB/NTM ACE Detection is designed for identification of *Mycobacterium tuberculosis* (MTB) dissociated from non-tuberculosis mycobacteria (NTM) grown in culture media.

Culturing is a standard method to diagnose active tuberculosis. However, culturing is very difficult to distinguish MTB from NTM since they have very similar colony morphologies. Seeplex® MTB/NTM ACE Detection is a PCR-based testing product which can accurately discriminate MTB from NTM for both liquid and solid culture media. Therefore, the kit would be useful to appropriately diagnose and treat patients with lung diseases by either MTB or NTM infection.

Figure1 & Table1. Example (ScreenTape® System)



Lane	Result	Lane	Result
1	ND*	4	MTB
2	NTM	5	MTB
3	MTB	6	NTM

ND* : Not Detected

Figure1 & Table1. Amplified PCR products using Seeplex® MTB/NTM ACE Detection.

Seeplex®
MTB/NTM ACE Detection

Product	Cat. No.	Size
MTB/NTM ACE Detection	TB1110Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

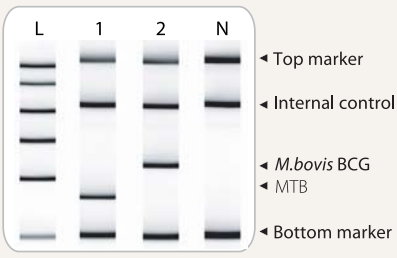
4.2.2 MTB/BCG ACE Detection

Seeplex® MTB/BCG ACE Detection is designed for the identification of *Mycobacterium tuberculosis* (MTB), and for the discrimination of M. bovis BCG (BCG) in clinical samples including sputum, body fluid, bronchial washing, urine, stool, CSF, and bonemarrow aspiration.

Seeplex® MTB/BCG ACE Detection is made not only to test for tuberculosis but also to discriminate BCG simultaneously with single PCR reaction by designing primers using Region of Difference (RD) 1 gene. RD 1 gene is present in all virulent Mycobacterium tuberculosis strains; however, attenuated Mycobacterium bovis BCG vaccine strain does not contain this gene*. By differentiating MTB from M. bovis BCG, an appropriate drug regimen is prescribed resulting in prevention of administering an unnecessary antituberculosis drug and reducing the incidence of drug resistance.

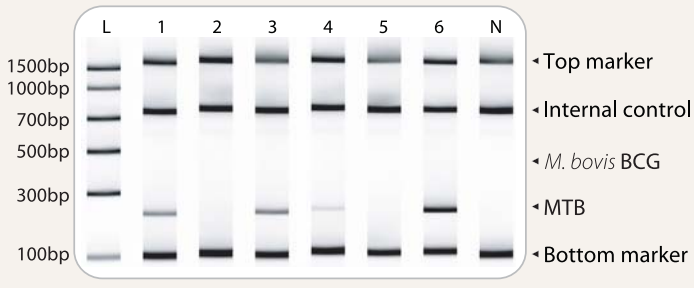
*Daugelat S. et al. Microbes Infect. 2003 Oct; 5 (12): 1082-95

Figure1. Band information (ScreenTape® System)



L: Ladder | 1: *Mycobacterium tuberculosis*
2 : *M.bovis* BCG | N : Negative control

Figure2 & Table1. Example (ScreenTape® System)



L : Ladder | 1~6 : Clinical samples | N : Negative control

Seeplex®
MTB/BCG ACE Detection

Product	Cat. No.	Size
MTB/BCG ACE Detection	TB3110Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

Lane	Result	Lane	Result
1	MTB	4	MTB
2	ND*	5	ND
3	MTB	6	MTB

ND* : Not Detected

Fig. 2 & Table 1. Amplified PCR products of six specimens using the Seeplex® MTB/BCG ACE Detection were electrophoresed using Auto Capillary Electrophoresis system. (Rapid & easy interpretation using ScreenTape® System)

5.1 Agarose Gel-based Method

5.1.1 Candida Detection

The Seeplex® Candida Detection series are designed to detect 8 Candida species.

Candida species are ubiquitous fungi found throughout the world as normal body flora. However, unfortunately, candidiasis is also the most common mycotic infection, causing a variety of diseases. Candidiasis can range from superficial disorders such as diaper rash to invasive, rapidly fatal infections in immunocompromised hosts. Candida albicans is commonly responsible for candidiasis, but other Candida species are also considered as emergent pathogens. In particular, there are species-specific differences in the susceptibility of Candida spp. to the currently used therapeutic drugs, and a PCR test which allows species identification is critical for therapeutic planning and accurate epidemiological records. Among various PCR methods, Seegene's Candida ID is outstanding in terms of sensitivity and specificity through utilization of the DPO™ technology.

Fig. 1. Band information

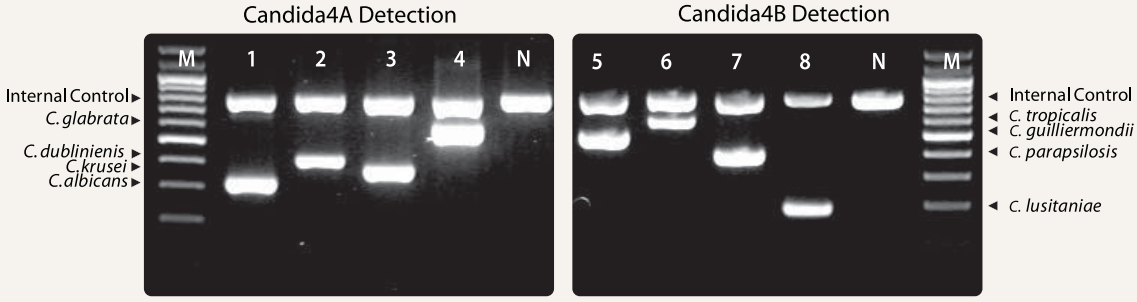
Candida4A Detection		Candida4B Detection	
Targets	**Size (bp)**	**Targets**	**Size (bp)**
Internal control	800	Internal control	800
C. glabrata	608	*C. tropicalis*	603
C. dublinienis	424	*C. guilliermondii*	507
C. krusei	379	*C. parapsilosis*	419
C. albicans	323	*C. lusitaniae*	194

Seeplex®
Candida Detection

Product	Cat. No.	Size
Candida4A Detection	CA1410Z	25 rxns
Candida4B Detection	CA1420Z	25 rxns

*Note: If you need more information for the primer mixture, please contact us.

Fig. 2 & Table 1. Example



M : 100bp ladder | 1~8 : ATCC reference strains | N : Negative control

Lane	Strain	Lane	Strain
1	*C. albicans*	5	*C. guilliermondii*
2	*C. dublinienis*	6	*C. tropicalis*
3	*C. krusei*	7	*C. parapsilosis*
4	*C. glabrata*	8	*C. lusitaniae*

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® Candida4A/4B Detection (Fig. 2) and result analysis (Table 1). 8 type strains of Candida obtained from ATCC were correctly identified by using the product.

***Citation:** Lee M. K. et al., Identification of Candida Species by Multiplex Polymerase Chain Reaction, Korean J. Clin. Microbiol. (2006) 9 (2): 119-124.

6.1 Auto-capillary Electrophoresis (ACE)

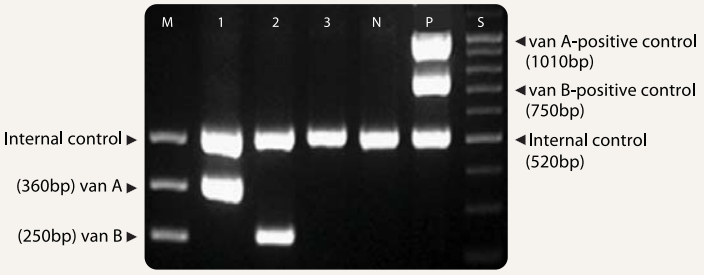
6.1.1 VRE ACE Detection

The Seeplex® VRE ACE Detection detects the vanA and vanB genes which cause vancomycin-resistance in enterococci isolated from stool culture in a single test.

Enterococci are normally indigenous bacteria found in the GI tract and female genitals, but recently they were recognized as major pathogens causing urinary tract infections and sepsis by nosocomial infection. Vancomycin, which is used in the treatment of enterococci infection, is a drug that has an antibacterial effect by forming a complex with the D-alanyl-D-alanine terminal of the peptidoglycan precursor to block transglycosylation and transpeptidation during the synthesis of the cell wall of Gram-positive cocci. However, vancomycin-resistant enterococci (VRE) tend to multiply rapidly due to the high frequency and continuous use of vancomycin.

The Seeplex® VRE ACE Detection is a PCR method that incorporates DPO™ technology with high sensitivity and specificity, and is a product that can test for the vancomycin-resistant vanA gene and the vanB gene of enterococci in one test.

Fig 1. Band information



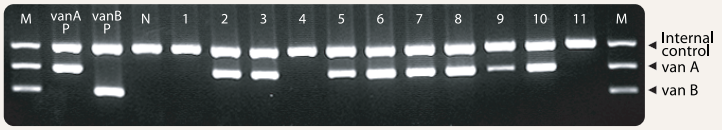
M : VRE size marker, 1: vanA positive sample,
2: vanB positive sample, 3: Negative sample,
N: Negative control, P : Positive control
S : 100 bp ladder

Seeplex®
VRE ACE Detection

Product	Cat. No.	Size
Seeplex® VRE ACE Detection	VR1210Y	50 rxns

*Note: If you need more information for the primer mixture, please contact us.

Fig. 2 & Table 1. Example



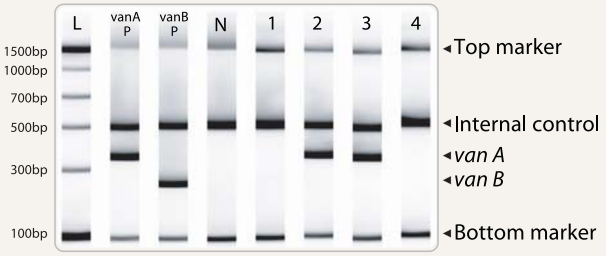
M : VRE size marker | P : Positive control | 1-11: Clinical samples | N : Negative control

Clinical Sample	Result	Clinical Sample	Result
1	-*	7	vanA
2	vanA	8	vanA
3	vanA	9	vanA
4	-	10	vanA
5	vanA	11	-
6	vanA		

*Note: "-" means that neither van A nor van B gene was detected.

Fig. 2 & Table 1. Amplified PCR products of 11 patient samples using the Seeplex® VRE ACE Detection (Fig. 2) and result analysis (Table 1).

Fig. 3 & Table 2. Example



L : Ladder | P: Positive control
N: Negative control | 1~4 : Clinical samples

Clinical Sample	Result
1	-
2	vanA
3	vanA
4	-

Fig. 3 & Table 2. Identification of the van A and van B genes using the Seeplex® VRE ACE Detection and Auto Capillary Electrophoresis

6.1.2 ClaR-H. pylori ACE Detection

The Seeplex® ClaR-H. pylori ACE Detection is designed to detect two types of point mutations (A2143G and A2142G) causing clarithromycin-resistance in *Helicobacter pylori* isolated from a gastric biopsy.

Helicobacter Pylori, a major cause of chronic gastritis, is strongly associated with the development of gastric and duodenal ulcers and has been linked with gastric adenocarcinoma and B-cell mucosa-associated lymphoid tissue lymphoma. Current treatment of *H. pylori* infection consists of a triple or quadruple regimen that includes antibiotics and a proton inhibitor, such as amoxicillin and clarithromycin. Among these, the presence of clarithromycin-resistant *H. pylori* has been found, which has presented a serious obstacle to the treatment of *H. pylori* using clarithromycin. Thus, the detection of clarithromycin-resistant H. pylori is needed to increase the efficiency of the treatment and to prescribe other antibiotics, by diagnosing the presence of clarithromycin-resistant H. pylori before treatment with antibiotics. Most clarithromycin-resistant Helicobacter pylori have point mutation at the 2142 and 2143 base sequences of the 23S rRNA gene, and exist as A2142G and A2143G, respectively.
Seeplex® ClaR-H. pylori ACE Detection has been developed to detect these two kinds of point mutations specifically using DPO™ technology.

Fig. 1. Band information

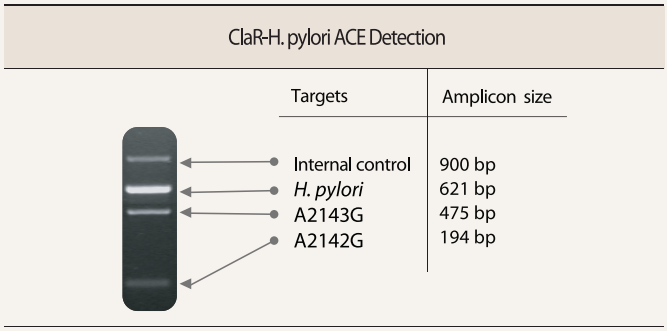
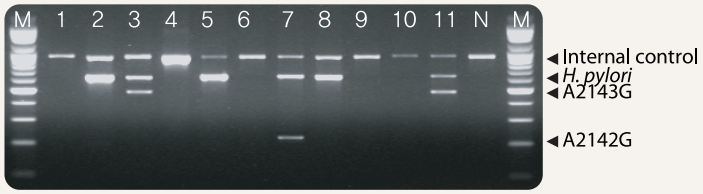


Fig. 2 & Table 1. Example



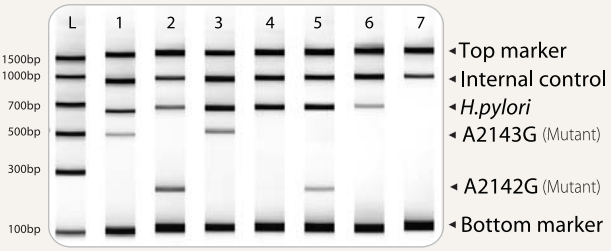
M: 100 bp ladder, 1~11: Clinical samples, N: Negative control

Fig. 2 & Table 1. Amplified PCR products of 11 clinical samples using the Seeplex® ClaR-H. pylori ACE Detection were electrophoresed on agarose gel.

Clinical Sample	Result	Clinical Sample	Result
1	ND*	7	A2142G
2	*H. pylori*	8	*H.pylori*
3	A2143G	9	ND
4	ND	10	ND
5	*H. pylori*	11	A2143G
6	ND		

ND* : Not Detected

Fig. 3 & Table 2. Example



L : Ladder | 1~7 : Clinical samples

Fig. 3 & Table 2. Amplified PCR products of 7 clinical samples using the Seeplex® ClaR-H. pylori ACE Detection were electrophoresed on Auto-capillary electrophoresis system.

Clinical Sample	Result	Clinical Sample	Result
1	A2143G	5	A2142G
2	A2142G	6	*H.pylori*
3	A2143G	7	ND*
4	*H.pylori*		

ND* : Not Detected

6.1.3 HBV Lami-resistant ACE

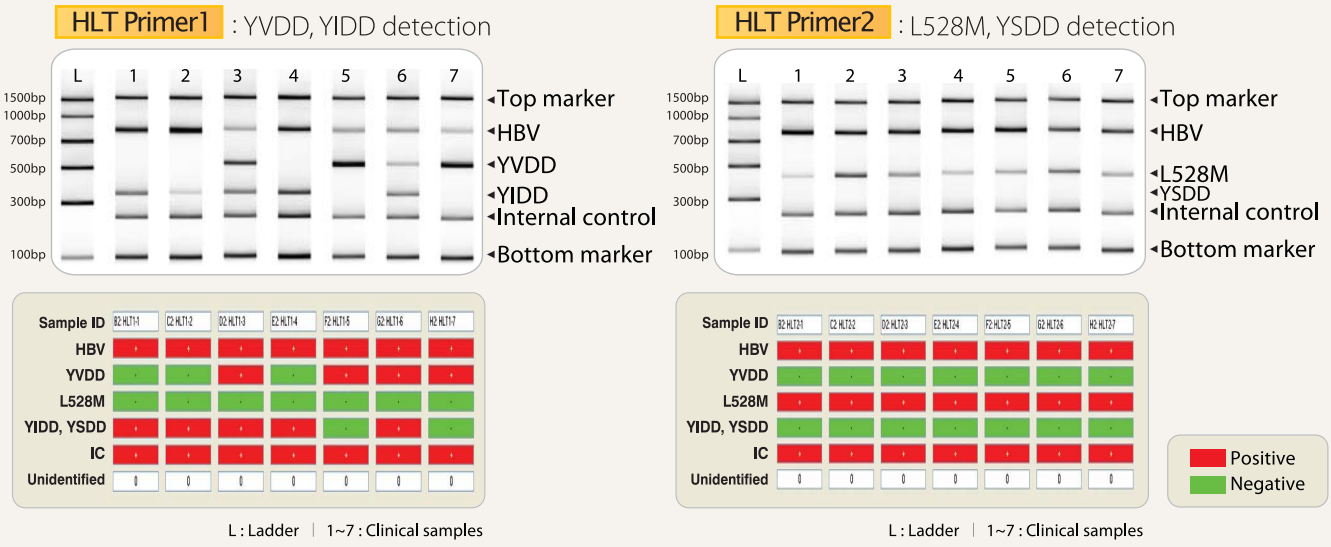
The Seeplex® HBV Lami-resistant ACE is designed to detect 4 lamivudine resistant hepatitis B viruses from patients' serum or plasma.

Lamivudine, an oral nucleoside analogue drug, powerfully inhibits the proliferation of B-type hepatitis virus by activating DNA polymerase, and as such is recognized as a safe and effective hepatitis drug. However, 10-15% of patients who take lamivudine for over two years, and over 50% who take it for three years, develop a drug-resistant virus, making treatment difficult. Thus, to effectively treat B-type hepatitis, since drug resistance development time varies according to patients, the HBV Lami-resistant should be implemented as quickly as possible in order to substitute drugs currently in use with more appropriate ones.

Note that single nucleotide mutations are responsible for drug-resistant HBV and make diagnosis difficult. The Seeplex® HBV Lami-resistant ACE detects lamivudine-resistant HBV variants with a single nucleotide mutation in the viral DNA polymerase simply and accurately using DPO™ technology without the need for additional steps such as sequencing.

Figure & Table. Example (Analysis of Seeplex® HBV Lami–resistant ACE PCR results using Auto Capillary Electrophoresis System)

* Test results were obtained using HLT Primer1 & HLT Primer2 on sample #1~7.



Lane	HBV type	Lane	HBV type
1	YIDD, L528M	5	YVDD, L528M
2	YIDD, L528M	6	YVDD, YIDD, L528M
3	YVDD, YIDD, L528M	7	YVDD, L528M
4	YIDD, L528M		

Figure & Table. Amplified PCR products using the Seeplex® HBV Lami-resistant ACE and result analysis. One or more YMDD motif mutations were detected among 7 HBV patients (lanes 1-7).
(Rapid & easy interpretation using ScreenTape® System)

Seeplex®
HBV Lami-resistant ACE

Product	Cat. No.	Size
HBV Lami-resistant ACE	BV1410Z	25 rxns

*Note: If you need more information for the primer mixture, please contact us.

6.2 Auto-sequencer Electrophoresis (ASE)

6.2.1 HBV Lami-resistant ASE

The Seeplex® HBV Lami-resistant ASE is designed to detect 3 lamivudine resistant HBV types from patients' serum and plasma. Capillary-based auto sequencers including ABI3130/3100, MegaBACE 1000 are applicable for the Seeplex® HBV Lami-resistant ASE.

3 Lamivudine–resistant HBVs (YVDD, YIDD, L528M) + Internal Control

Key Features

- Small volume (0.2ml) of samples needed
- Simultaneous detection of 3 lamivudine resistant HBVs
- Multiplex PCR in just one tube
- Internal control can check PCR inhibition
- Low cost and fast result

Seeplex®
HBV Lami-resistant ASE

Product	Cat. No.	Size
HBV Lami-resistant ASE	BV5410Z	25 rxns

Overcoming Limitations of the Existing Tests

It has overcome the low sensitivity of the existing HBV Lamivudine–resistant ASE and shortened the test time.

Sequencing method has been considered as the standard method for HBV Lamivudine-resistant testing. However, it has a limitation in that at least 20% of the HBV mutations with Lamivudine-resistance should exist among the total number of infected HBV viruses to detect HBV mutations with Lamivudine-resistance using a sequencing method in HBV infected specimens.This product can accurately detect using the Seeplex® system, which incorporates Seegene's DPO™ technology, even if the Lamivudine-resistant variation accounts for less than 2% of the total infected HBV. Therefore, it can be used for the early diagnosis of Lamivudine-resistant variation before the viral breakthrough (the regeneration of a virus lost by antiviral preparation). Also, the testing time is short (4 hours) and the experiment is easy to conduct, making it the optimal monitoring method for antiviral treatment.

Result from clinical tests using sequencing, RFMP, and Seeplex® system

Sample No.	Sequencing	RFMP	Seeplex® Products
1	YIDD/L528M	YIDD/L528M	YIDD/YVDD/L528M
2	YVDD/L528M	YVDD/L528M	YIDD/YVDD/L528M
3	YIDD/L528M	YIDD/L528M	YIDD/YVDD/L528M
4	YIDD/L528M	YIDD/L528M	YIDD/L528M
5	YIDD	YIDD/YVDD/L528M	YIDD/YVDD/L528M
6	YVDD/L528M	YVDD/L528M	YVDD/L528M
7	YIDD/YVDD/L528M	YIDD/YVDD/L528M	YIDD/YVDD/L528M
8	wild	wild	YVDD
9	YIDD	YIDD	YIDD/L528M
10	YIDD/L528M	YIDD/L528M	YIDD/L528M

Table1. Result comparison among sequencing, RFMP, and Seeplex® Products with 10 clinical samples.

#1, 2, 3, 8, and 9 : additional as well as identical mutants were detected only by the Seeplex® Products
#4, 6, 7, and 10 : identical mutants were detected by all sequencing, RFMP, and Seeplex® Products
#5: additional mutants were detected by RFMP and Seeplex® Products

The above results clearly indicate the Seeplex® Products is the most accurate and sensitive method for detection of lamivudine resistant HBV mutants.

Examples

* Red peak indicates standard size marker.

Fig.1. Representative examples of Seeplex® HBV Lami-resistant ASE for 4 samples.

The identity of peaks (PCR amplicons) generated by Seeplex® were all confirmed by individual sequencing (data not shown).

IC, Internal Control

7.1 Agarose Gel-based Method

7.1.1 ApoE Genotyping

The Seeplex® ApoE Genotyping is designed to identify the Apolipoprotein E (ApoE) genotypes from blood samples.

Apolipoprotein E (ApoE) has a major role in the metabolism of plasma lipoproteins. There are three common isoforms (E2, E3, and E4) of ApoE protein and these isoforms differ in one amino acid sequence, an Arg (CGC) or Cys (TGC) at residue 112 and 158. The three isoforms arise from three alleles (E2, E3, and E4) which are combined in six different genotypes (homozygous genotypes: E2/E2, E3/E3, E4/E4, heterozygous genotypes: E2/E3, E2/E4, E3/E4). These genotypes are known to be associated with the risk of developing cardiovascular diseases and Alzheimer diseases. The ApoE4 protein has been reported to be a major risk factor for Alzheimer's diseases and coronary artery diseases. The ApoE2 protein is known to be protective for Alzheimer's diseases, but associated with type III hyperlipoproteinnemia. Therefore, determining the genotype of ApoE is considered to be an important test for healthy people on the aspect of precautions as well as patients with the diseases.

Fig. 1. Band information

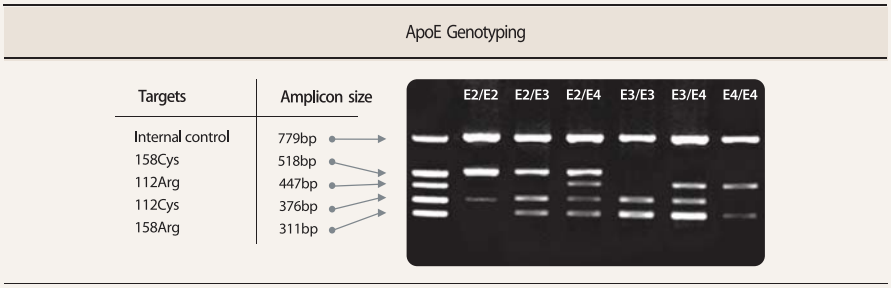
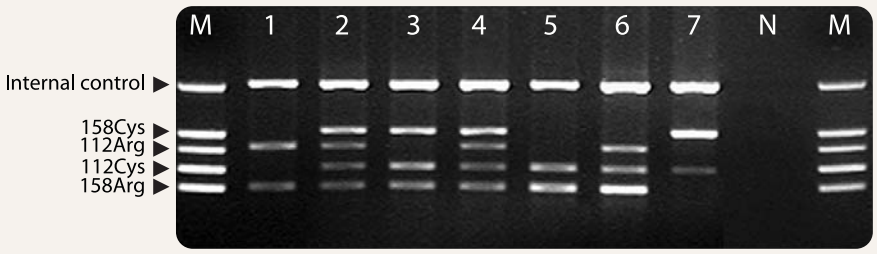


Fig 2 & Table 1. Example



M : ApoE size marker | 1~7 : Clinical samples | N : Negative control

Lane	Result	Lane	Result
1	E4/E4	5	E3/E3
2	E2/E4	6	E3/E4
3	E2/E3	7	E2/E2
4	E2/E4		

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® ApoE Genotyping (Fig. 2) and result analysis (Table 1). Seven subjects were genotyped for ApoE as shown in the Fig. 2. The genotyping results of ApoE were compared with sequencing data and the same results were obtained.

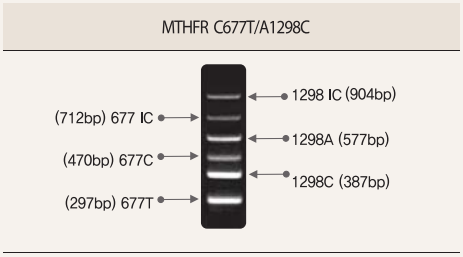
7.1.2 MTHFR C677T/A1298C

The Seeplex® MTHFR C677T/A1298C is designed to detect the MTHFR C677T and A1298C from blood samples.

MTHFR is a key enzyme in folic acid metabolism, which catalyzes the synthesis of methionine through re-methylation of homocysteine to methionine[1]. Two common SNPs in MTHFR are C677T and A1298C. The MTHFR C677T consists of a 677C->T transition and results in alanine to valine amino acid substitution at codon 222. The homozygous 677TT genotype, which occurs in approximately 10 percent to 15 percent of Caucasian and Asian populations, has been shown to have 30 percent in vitro MTHFR enzyme activity compared with the wild-type and the heterozygous 677CT genotype has been found to have 60 percent of wild-type enzyme activity[2]. Secondly, MTHFR A1298C results in amino acid change at codon 429. The homozygous 1298CC genotype, which occurs in approximately 5 percent to 10 percent of Caucasians, has been shown to have 60 percent of the wild-type MTHFR activity in vitro[2]. The genetic polymorphism of MTHFR, C677T and A1298C have been associated with increased plasma homocysteine levels, which cause hyperhomocysteinemia. The SNP of MTHFR has been reported to be an independent risk factor for cardiovascular diseases[3] and cause the risk of birth defects, especially neural tube defects, and recurrent embryo losses in early pregnancy[2),4)].

1) Jae-Hong Park, et. al., Journal of the Korean Rheumatism Association, Vol. 10, No. 3, 283-292, 2003.
2) Kim Robien, et. al., Clinical Cancer Research, Vol. 10, 7592-7598, 2004.
3) Nam-keun Kim, et. al., J Korean Neurol Assoc, Vol. 21;6, 606-613, 2003.
4) Isotalo PA, et. al., Am J Hum Genet. 2000; 67(4), 986-90.

Fig. 1. Band information



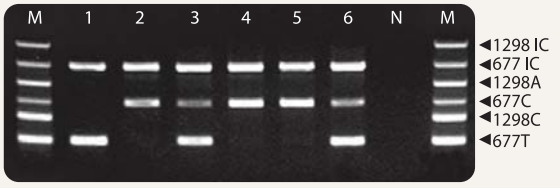
Seeplex®
MTHFR C677T/A1298C

Product	Cat. No.	Size
MTHFR C677T/A1298C	MT1210Z	25 rxns

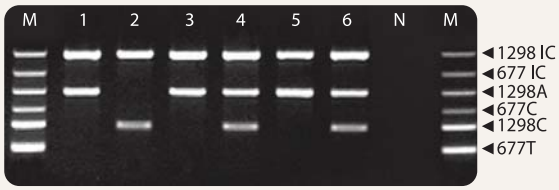
*Note: If you need more information for the primer mixture, please contact us.

Fig. 2 & Table 1. Example

Genotyping for the MTHFR C677T



Genotyping for the MTHFR A1298C



Multiplex genotyping for the MTHFR C677T and A1298C

M : MTHFR size marker, 1~6 : Clinical samples, N : Negative control

Lane	C677T Genotype	A1298C Genotype
1	TT	AA
2	CC	CC
3	CT	AA
4	CC	AC
5	CC	AA
6	CT	AC

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® MTHFR C677T/A1298C (Fig. 2) and result analysis (Table 1). The MTHFR C677T and A1298C genotypes were successfully determined in 6 human samples.

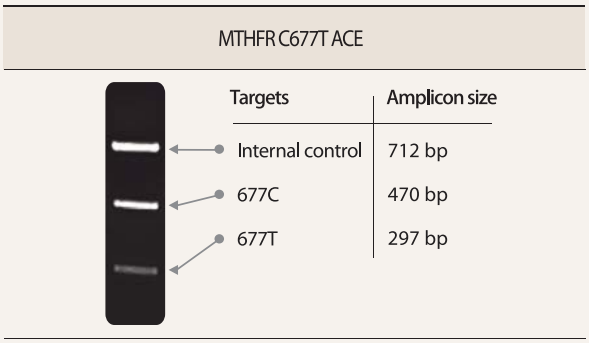
7.2 Auto-capillary Electrophoresis (ACE)

7.2.1 MTHFR C677T ACE

The Seeplex® MTHFR C677T ACE is designed to detect C677T SNP of the MTHFR gene from blood samples.

The MTHFR C677T polymorphism causes hyperhomocysteinemia which is associated with various disease conditions such as cardiovascular diseases, cerebrovascular diseases, and deep venous thrombosis. Therefore, to predict and prevent such diseases, the MTHFR C677T genotype should be tested as quickly as possible. However, such a test is difficult because it must detect single nucleotide polymorphism (SNP). The Seeplex® MTHFR C677T ACE is a unique product which provides a simple and accurate SNP detection without additional steps such as sequencing by applying DPO™ technology.

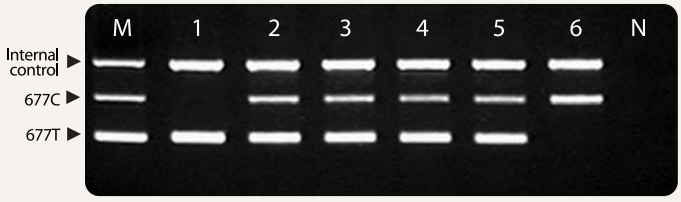
Fig. 1. Band information



Product	Cat. No.	Size
MTHFR C677T ACE	MT1110Z	25 rxns

*Note: If you need more information for the primer mixture, please contact us.

Fig.2 & Table 1. Example

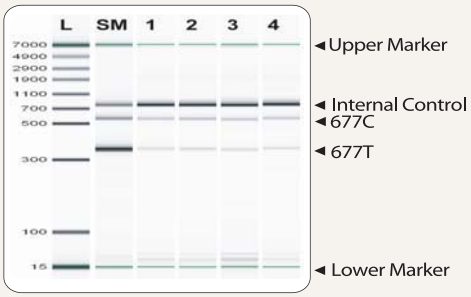


M : MTHFR size Marker | 1~6 : Clinical Samples | N : Negative Control

Lane	C677T Genotype	Lane	C677T Genotype
1	TT	4	CT
2	CT	5	CT
3	CT	6	CC

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® MTHFR C677T ACE (Fig. 2) and result analysis (Table 1). The MTHFR C677T genotypes were successfully determined in 6 human samples.

Fig. 3 & Table 2. Example



L: Ladder | SM : Size Marker |
1~4 : Clinical Samples | N : Negative Control

Lane	Type	Lane	Type
1	CT	3	CT
2	CT	4	CT

Fig. 3 & Table 2. Identification of the MTHFR C677T genotype using the Seeplex® MTHFR C677T ACE and Auto Capillary Electrophoresis (LabChip90® System).

7.2.2 CYP2C19 ACE Genotyping

The Seeplex® CYP2C19 ACE Genotyping is designed to detect the CYP2C19 *2 and *3 alleles from blood samples.

Cytochrome P450 2C19(CYP2C19) is involved in the metabolism of several therapeutic agents, such as anticonvulsants, antidepressants, proton pump inhibitors, HIV-protease inhibitors and antimalarial drugs[1]. However, if a single point mutation occurs in the CYP2C19 gene, it produces an inactive CYP2C19 enzyme; in turns, the metabolic rates of the drugs are decreased and thus undesirable side effects at standard dosages may arise.

Therefore, identification of the single nucleotide polymorphism (SNP) in the CYP2C19 gene is very important before starting drug treatments. Besides the wild-type CYP2C19 *1 allele, 20 variant CYP2C19 alleles with mutations have been identified and are named as CYP2C19 *2 to *21. Of those mutated alleles, CYP2C19 *2 and CYP2C19 *3 are the most prevalent alleles worldwide. The CYP2C19 *2 and the CYP2C19 *3 alleles are found in ~87 % and ~98% of poor metabolizers (PMs) in Caucasians and Asians, respectively[2]. By applying an innovative DPO™ based multiplex PCR system having maximized specificity and sensitivity, the Seeplex® CYP2C19 ACE Genotyping simply, rapidly, and accurately detects two SNPs (CYP2C19 *2 and CYP2C19 *3 allele) of the CYP2C19 gene without further experiments or expensive instruments.

1)Koori Nakamota et al., Pharmacogenetics and Genomics 2007, 17:103-114
2)Linder MW, ALDES R Jr. Pharmacogenetics in the practice of laboratory medicine. Mol Diagn 1999;4:365-79.

Fig. 1. Band information

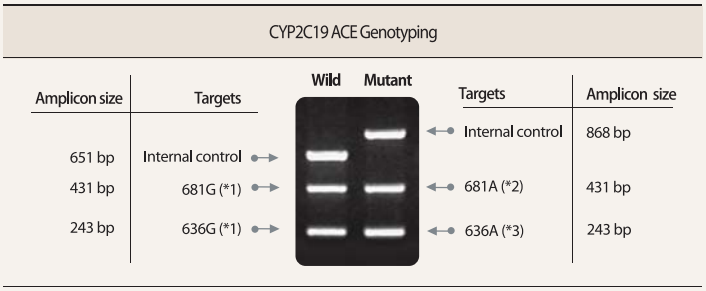
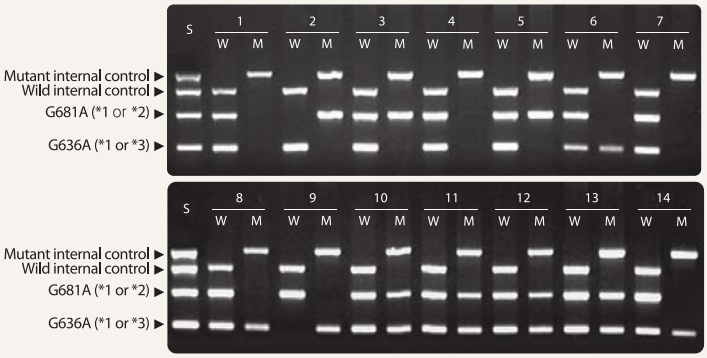


Fig. 2 & Table 1. Example

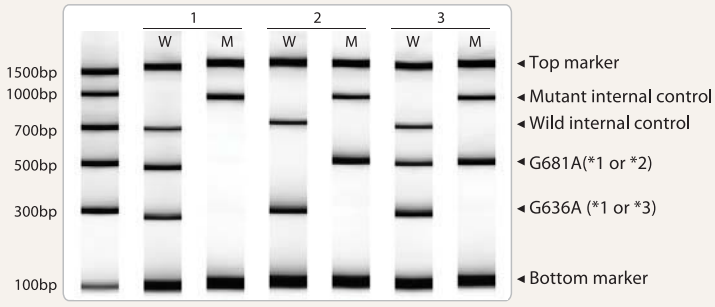


S : CYP2C19 Marker | W : PCR using wild primer mix | M : PCR using mutant primer mix
1-14: Clinical samples

No.	Genotype	No.	Genotype	No.	Genotype	No.	Genotype
1	*1 / *1	5	*1 / *2	9	*3 / *3	13	*2 / *3
2	*2 / *2	6	*1 / *3	10	*2 / *3	14	*1 / *3
3	*1 / *2	7	*1 / *1	11	*2 / *3		
4	*1 / *1	8	*1 / *3	12	*2 / *3		

Fig. 2 & Table 1. Amplified PCR products of 14 patient samples using the Seeplex® CYP2C19 ACE Genotyping (Fig. 2) and result analysis (Table1).

Figure3 & Table 2. Example (ScreenTape® System)



No.	Genotype
1	*1 / *1
2	*2 / *2
3	*1 / *2

Fig. 3 & Table 2. Amplified PCR products of three patient samples using the Seeplex® CYP2C19 Genotyping (Fig. 3) and result analysis (Table 2).

Three patient samples were genotyped for CYP2C19 *1, *2 and *3 alleles using CYP2C19 Genotyping system. The genotyping results of CYP2C19 were compared with sequencing data and the 100 % concordance confirms the accuracy of the Seeplex® CYP2C19 genotyping system.

8.1 Agarose Gel-based Method

8.1.1 FLT3 Genotyping

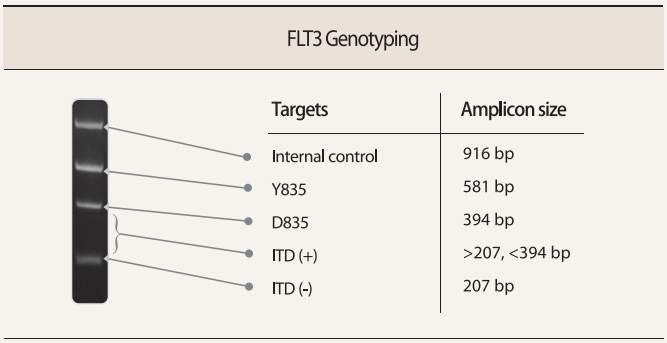
***Notice:** Not available for use or sale in USA, Japan and EU

The Seeplex® FLT3 Genotyping is designed to detect two somatic mutations of ITD (internal tandem duplication) in the JM domain and the D835Y in the tyrosine kinase domain of FMS like tyrosine kinase 3 (FLT3) in one PCR step.

The most frequent genetic drifts toward the acute myelogenous leukemia (AML) are the D835Y and ITD mutations of FLT3. The internal tandem duplication (ITD) mutation in the JM domain of FLT3 causes constitutive activation of the FLT3. The ITD mutation of FLT3 is observed in 17-26 % of AML patients[1]. The D835Y mutation of FLT3 is the substitution of tyrosine for aspartic acid at codon 835 within the activation loop of the kinase domain of FLT3. This missense point mutation is observed in 7 % of AML and also induces constitutive activation of the FLT3[1]. The ITD and D835Y mutations in FLT3 are somatic and occur independently. Ligand-induced activation of FLT3 plays a critical role in proliferation and survival of hematopoietic cells. However, the ITD and D835Y mutations in the receptor tyrosine kinase FLT3 constitutively promote proliferation and survival, thus providing the leukemic cell with a growth advantage.

1) Stem Cell , Vol. 24, No. 5, May 2006, pp. 1174-1184.

Fig. 1. Band information



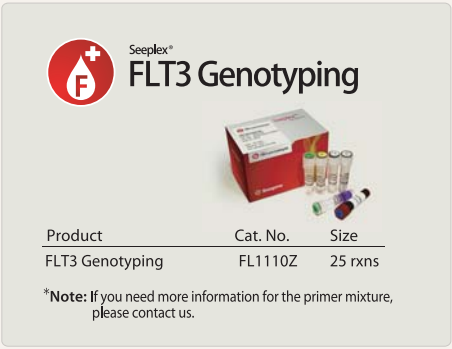
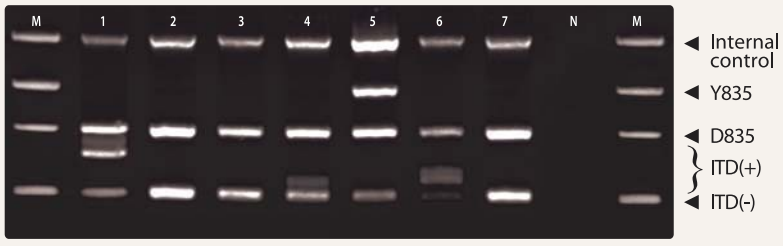


Fig. 2 & Table 1. Example



M : FLT3 Marker | 1~7 : Clinical samples | N : Negative control

Lane	Type	Lane	Type
1	ITD mutation detected	5	D835Y mutation detected
2	No mutation detected	6	ITD mutation detected
3	No mutation detected	7	No mutation detected
4	ITD mutation detected	N	Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® FLT3 Genotyping (Fig. 2) and result analysis (Table 1). The ITD and D835Y mutations of FLT3 are detected in 4 human clinical samples (lanes 1, 4, 5 and 6).

8.1.2 Leukemia BCR/ABL

The Seeplex® Leukemia BCR/ABL is designed to detect the BCR/ABL fusion gene [t(9;22)(q34;q11)] from blood and bone marrow.

The Philadelphia chromosome is formed by fusion of ABL (Ableson) proto-oncogene on chromosome 9 with the BCR (Breakpoint Cluster Region) gene on chromosome 22. The BCR/ABL fusion turns out to be positive in 90~95 % of CML (Chronic Myelogenous Leukemia) patients, 25 % of adult ALL (Acute Lymphocytic Leukemia) patients and 5 % of child ALL (Acute Lymphocytic Leukemia) sufferers. Depending on the breakpoint of the BCR gene, different types of BCR/ABL fusion genes (major type: b2a2, b3a2, minor type: e1a2, micro type: c3a2, etc.) are generated.

Chromosome tests usually have a high false negative rate as well as unexplainable mosaic forms of chromosome and require a sufficient amount of cells at division stage and require significant time and effort. Even though RT-PCR can be performed even with a limited amount of specimen, using conventional primers require additional nested PCR steps to detect BCR/ABL after initial RT-PCR.

The Seeplex® Leukemia BCR/ABL detects 8 types of the BCR/ABL fusion gene simultaneously with only one PCR reaction after cDNA synthesis using DPO™ technology, which maximizes specificity and sensitivity.

Fig. 1. Band information

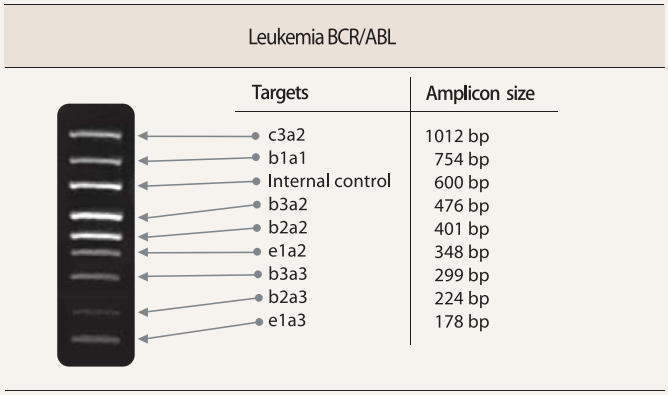
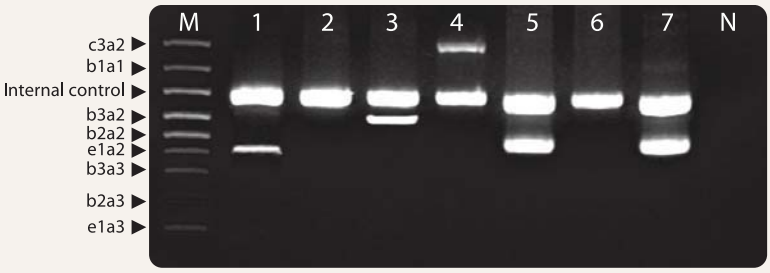


Fig. 2 & Table 1. Example



M : BCR/ABL Marker | 1~7 : Clinical samples | N : Negative control

Lane	Result	Lane	Result
1	e1a2	5	b2a2
2	No translocation	6	No translocation
3	b3a2	7	b2a2
4	c3a2	N	Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® Leukemia BCR/ABL (Fig. 2) and result analysis (Table 1). Seven patients were tested for detection of the BCR/ABL fusion gene. As shown on the agarose gel, the BCR/ABL fusion gene was observed in lanes 1, 3, 4, 5, and 7.

8.1.3 Leukemia PML/RARa

The Seeplex® Leukemia PML/RARa is designed to detect the PML/RARa fusion gene [t(15;17)(q22;q21)] from blood and bone marrow.

[t(15;17)(q22;q21)] translocation generates the formation of gene fusion between the PML gene on chromosome 15 and the retinoic acid receptor α (RAR α) gene on chromosome 17 and is a chromosomal abnormality found in most cases of acute promyelocytic leukemia (APL (M3)). It is important to discriminate APL from other leukemia disease by an accurate PCR detection system since clinical remission of APL is possible by treating with retinoic acid only. Chromosome tests usually result in high false negative rate as well as unexplainable mosaic forms of chromosome and require a sufficient amount of cells at division stage and require significant time and effort. Even though RT-PCR can be performed even with a limited amount of specimen, using conventional primers require additional nested PCR steps to detect PMA/RARa after initial RT-PCR.

The Seeplex® Leukemia PML/RARa detects bcr1, bcr2, bcr3 and their variant types of PML/RARa simultaneously with only one PCR reaction after cDNA synthesis using DPO™ technology, which maximizes specificity and sensitivity.

Detection limit: 10 copies (100 % detection), 5 copies (80 % detection)

Fig. 1. Band information

Leukemia PML/RARa

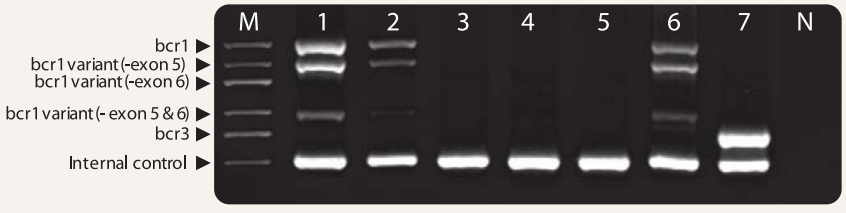
Targets	Amplicon size
bcr2 variant (*ins intron 6)	954bp
bcr1	867bp
bcr2 variant (-exon 5, ins intron 6)	809bp
bcr1 variant (-exon 5)	724bp
bcr1 variant (-exon 6)	607bp
bcr1 variant (- exon 5 & 6)	464bp
bcr3	393bp
bcr3 variant	> 312bp, < 393bp
Internal control	312bp

*ins : insertion

Product	Cat. No.	Size
Leukemia PML/RARa	PM1110Z	25 rxns

*Note: If you need more information for the primer mixture, please contact us .

Fig. 2 & Table 1. Example



M : PML/RARa Marker | 1~7 : Clinical samples | N : Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® Leukemia PML/RARa (Fig. 2) and result analysis (Table 1). Seven patients were tested for detection of the PML/RARa fusion gene. As shown on the agarose gel, the PML/RARa fusion gene was observed in the lanes 1, 2, 6, and 7.

Lane	Result	Lane	Result
1	bcr1, bcr1 variant(-exon 5), bcr1 variant (-exon 5 & 6)	5	No translocation
2	bcr1, bcr1 variant(-exon 5), bcr1 variant(-exon 5 & 6)	6	bcr1, bcr1 variant(-exon 5), bcr1 variant(-exon 5 & 6)
3	No translocation	7	bcr3
4	No translocation	N	Negative control

8.2 Auto-capillary Electrophoresis (ACE)

8.2.1 Leukemia AML1/ETO ACE

The Seeplex® Leukemia AML1/ETO ACE is designed to detect the AML1/ETO fusion gene [t(8;21)(q22;q22)] from blood and bone marrow.

t(8;21)(q22;q22)] translocation generates the formation of gene fusion between the ETO gene on chromosome 8 and the AML1 gene on chromosome 21 and turns out to be present in 20~40 % of acute myelogenous leukemia (AML (M4)) cases. If the disease recurs at a long term after bone marrow transplantation, the fusion gene will continuously be observed. Chromosome tests usually result in high false negative rate as well as unexplainable mosaic forms of chromosome and require a sufficient amount of cells at division stage and require significant time and effort. Even though RT-PCR can be performed even with a limited amount of specimen, using conventional primers require additional nested PCR steps to detect AML1/ETO after initial RT-PCR.

The Seeplex® Leukemia AML1/ETO ACE detects the AML1/ETO fusion gene with only one PCR reaction after cDNA synthesis using DPO™ technology, which maximizes specificity and sensitivity.

Detection limit: 10 copies (100 % detection), 5 copies (80 % detection)

Fig. 1. Band information

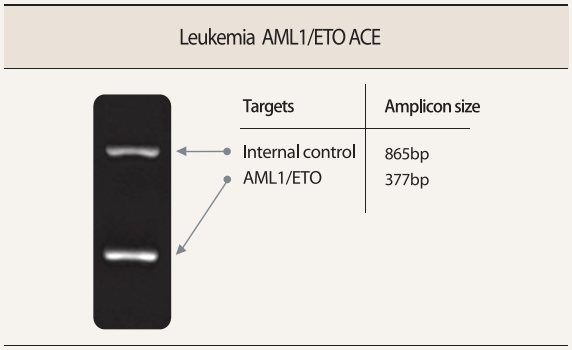
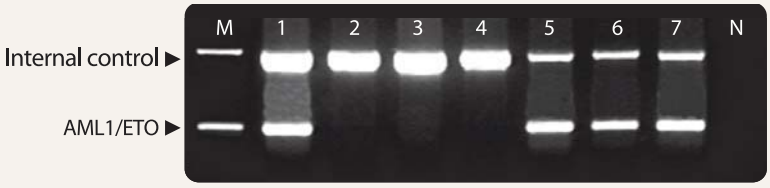


Fig. 2 & Table 1. Example

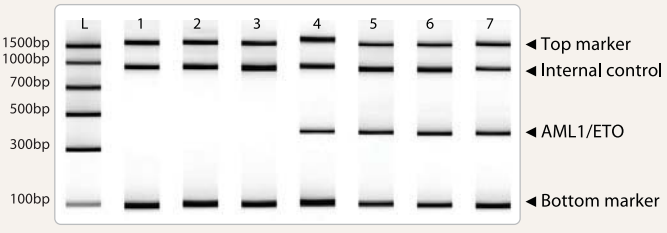


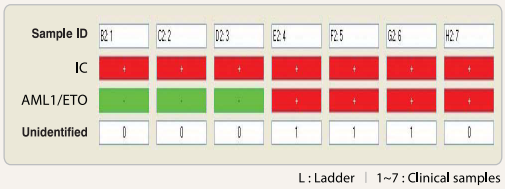
M : AML1/ETO Marker | 1~7 : Clinical samples | N : Negative control

Lane	Result	Lane	Result
1	AML1/ETO	5	AML1/ETO
2	No translocation	6	AML1/ETO
3	No translocation	7	AML1/ETO
4	No translocation	N	Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® Leukemia AML1/ETO ACE (Fig. 2) and result analysis (Table1). Seven patients were tested for detection of the AML1/ETO fusion gene. As shown on the agarose gel, the AML1/ETO fusion gene was observed in lanes 1, 5, 6, and 7.

Figure3. Example (ScreenTape® System)





L : Ladder | 1~7 : Clinical samples

Figure3. Amplified PCR products using Seeplex® Leukemia AML1/ETO ACE.
(Rapid & easy interpretation using ScreenTape® System)

8.2.2 JAK2 ACE Genotyping

***Notice:** Not available for use or sale in USA and EU

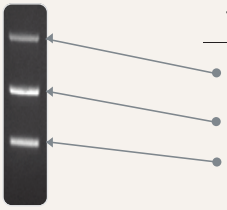
The Seeplex® JAK2 ACE Genotyping is designed to identify the JAK2 genotype at codon 617 of Janus kinase 2 (JAK2).

JAK2 plays a critical role in signal transduction by hematopoietic growth factor and cytokine receptors. The JAK2 mutation, V617F is detected to 30 in 90 % of patients with bcr/abl rearrangement negative chronic myeloproliferative disorders[1] such as polycythemia vera, essential thrombocytosis and idiopathic myelofibrosis. Especially, in polycythemia, the JAK2 mutation (V617F) contains a phenylalanine substituted for valine leading to a lack of inhibition of the JH1 domain and constitutive JAK2 kinase activity without the coupling of ligands to hematopoietic receptors inducing erythrocytosis[2][3]. The JAK2 mutation is somatic and occurs at the level of a hematopoietic stem cell. The JAK2 mutant test is useful in understanding molecular pathogenesis and clinical aspects of chronic myeloproliferative disorders and in developing more effective therapies for diseases in the future.

1) The 47th Science Council of the Korean Society for Laboratory Medicine 2006.
2) PNAS online newsboard (doi : 10.1073/pnas.0601462103) 7. April. 2006.
3) J Transl Med. (doi : 10.1186/1479-5876-4-41) 11 Oct. 2006.

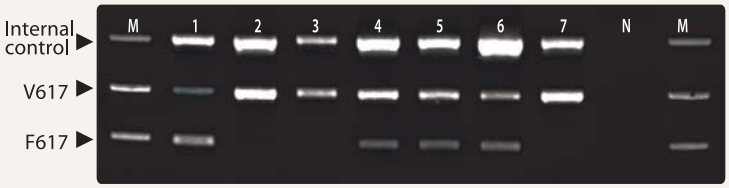
Fig. 1. Band information

JAK2 ACE Genotyping



Targets	Amplicon size
Internal control	813 bp
Wild (V617)	543 bp
Mutant (F617)	352 bp

Fig. 2 & Table 1. Example (Agarose gel)



Lane	Type	Lane	Type
1	mutant	5	mutant
2	wild	6	mutant
3	wild	7	wild
4	mutant	N	Negative control

Fig. 2 & Table 1. Amplified PCR products using the Seeplex® JAK2 ACE Genotyping (Fig. 2) and result analysis (Table 1). The JAK2 V617F mutations are observed in lanes 1, 4, 5, and 6.

Figure3. Example (ScreenTape® System)

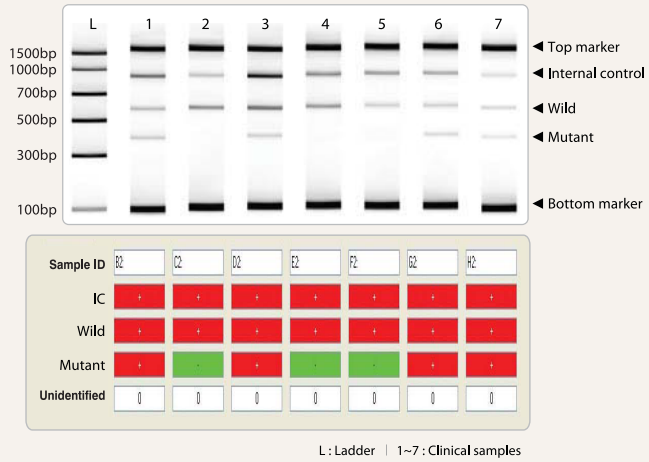


Figure3. Amplified PCR products using Seeplex® JAK2 ACE Genotyping.
(Rapid & easy interpretation using ScreenTape® System)

Ordering Information

* ASE (Auto-sequencer Electrophoresis)
* ACE (Auto-capillary Electrophoresis)

Cat. No.	Product	Size	Page	Detection Method
Seeplex® RV Detection				
RV1211	Seeplex® RV12 Detection	50 rxns	9 p	Agarose gel
RV2210	Seeplex® RV6 Detection	50 rxns	9 p	Agarose gel
RV3210	Seeplex® RV7 Detection	50 rxns	9 p	Agarose gel
RV6C00Y	Seeplex® RV12 ACE Detection	50 rxns	10 p	ACE
RV6550Y	Seeplex® RV5 ACE Screening	50 rxns	11 p	ACE
RP5J10Y	Seeplex® RV/PB18 ASE Detection	50 rxns	13 p	ASE
Seeplex® PneumoBacter Detection				
PB1610Y	Seeplex® PneumoBacter ACE Detection	50 rxns	12 p	ACE
Seeplex® STD Detection				
SD2200Y	Seeplex® STD4 Detection	50 rxns	15 p	Agarose gel
SD2510Y	Seeplex® STD5 Detection	50 rxns	15 p	Agarose gel
SD2100Y	Seeplex® STD6 Detection	50 rxns	15 p	Agarose gel
SD6400Y	Seeplex® STD4 ACE Detection	50 rxns	16 p	ACE
SD6600Y	Seeplex® STD6 ACE Detection	50 rxns	16 p	ACE
SD5710Y	Seeplex® STD7 ASE Detection	50 rxns	17 p	ASE
SD5910Y	Seeplex® STD9 ASE Detection	50 rxns	17 p	ASE
Seeplex® HPV Detection				
HP1511Y	Seeplex® HPV6 Genotyping	50 rxns	19 p	Agarose gel
HP1400Y	Seeplex® HPV4 ACE Screening	50 rxns	20 p	ACE
HP5J10Y	Seeplex® HPV18 ASE Genotyping Available soon	50 rxns	22 p	ASE
Seeplex® STD/HPV Test				
SH1400Y	Seeplex® HPV/STD4 ACE Screening	50 rxns	21 p	ACE
Seeplex® TB Detection				
TB2110Y	Seeplex® TB Detection 2	50 rxns	24 p	Agarose gel
TB1110Y	Seeplex® MTB/NTM ACE Detection	50 rxns	25 p	ACE
TB3110Y	Seeplex® MTB/BCG ACE Detection	50 rxns	25 p	ACE

Cat. No.	Product	Size	Page	Detection Method
Seeplex® Candida Detection				
CA1410Z	Seeplex® Candida4A Detection	25 rxns	26 p	Agarose gel
CA1420Z	Seeplex® Candida4B Detection	25 rxns	26 p	Agarose gel
Seeplex® VRE Detection				
VR1210Y	Seeplex® VRE ACE Detection	50 rxns	27 p	ACE
Seeplex® ClaR-H. pylori Detection				
HC1210Z	Seeplex® ClaR-H. pylori ACE Detection	25 rxns	28 p	ACE
Seeplex® HBV Lami-resistant				
BV1410Z	Seeplex® HBV Lami-resistant ACE	25 rxns	29 p	ACE
BV5410Z	Seeplex® HBV Lami-resistant ASE	25 rxns	30 p	ACE
Seeplex® ApoE Genotyping				
AP1210Z	Seeplex® ApoE Genotyping	25 rxns	32 p	Agarose gel
Seeplex® MTHFR Genotyping				
MT1210Z	Seeplex® MTHFR C677T/A1298C	25 rxns	33 p	Agarose gel
MT1110Z	Seeplex® MTHFR C677T ACE	25 rxns	34 p	ACE
Seeplex® CYP2C19 Genotyping				
CY1210Z	Seeplex® CYP2C19 ACE Genotyping	25 rxns	35 p	ACE
Seeplex® FLT3 Genotyping				
FL1110Z	Seeplex® FLT3 Genotyping	25 rxns	36 p	Agarose gel
Seeplex® Leukemia				
BC1110Z	Seeplex® Leukemia BCR/ABL	25 rxns	37 p	Agarose gel
PM1110Z	Seeplex® Leukemia PML/RARa	25 rxns	38 p	Agarose gel
AM1110Z	Seeplex® Leukemia AML1/ETO ACE	25 rxns	39 p	ACE
Seeplex® JAK2 Genotyping				
JA1110Z	Seeplex® JAK2 ACE Genotyping	25 rxns	40 p	ACE

Seegene

is a bio-company specializing in gene discovery and molecular diagnostics through Seegene's original ACP™ and/or DPO™ based PCR technologies. We guarantee customer satisfaction and look forward to working with you.

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