# *폐암의 바이오마커 탐색 및 검증을 위한 분자 유전학적 진단기술*

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2017.2.11 백범김구기념관

## *Outline*

- 폐암의 바이오마커
- 바이오마커의 탐색과 검증
- 분자유전학적 기술
- 바이오마커와 진단

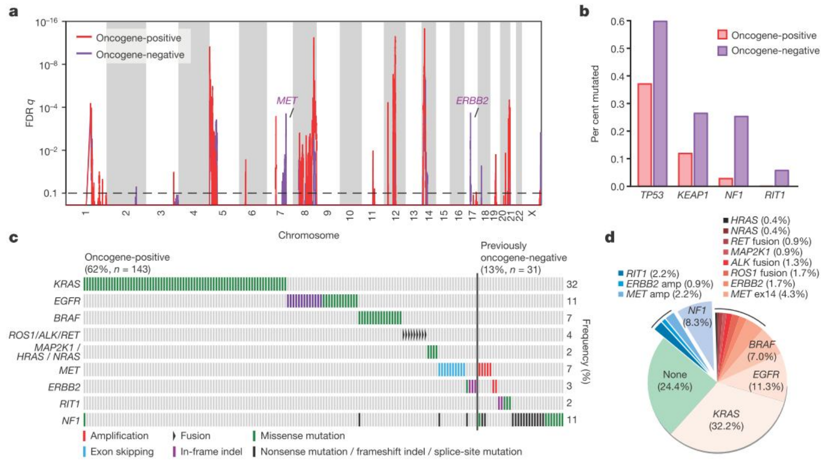
# 바이오마커

- **바이오마커 :** 객관적으로 측정할 수 있고, 정상이나 병적인 상태를 구분할 수 있거나 치료반응을 예측할 수 있는 표지자
- 바이오마커의 조건 : “objectively measured and evaluated” 이며, 상태를 나타낼 뿐 아니라 상태의 변화를 측정할 수 있어야 함.
- DNA, RNA 같은 핵산, 단백질, 지방질, 대사물질 등의 측정 양과 그 패턴의 변화 등
- 탐색된 바이오마커가 임상에서 사용되기 위해서는 임상시험을 통해 검증 된 후에 임상검사로 사용이 가능한 체외진단제품으로 개발되어야만 함.

# Comprehensive molecular profiling of lung adenocarcinoma

31 JULY 2014 | VOL 511 | NATURE | 543

The Cancer Genome Atlas Research Network*



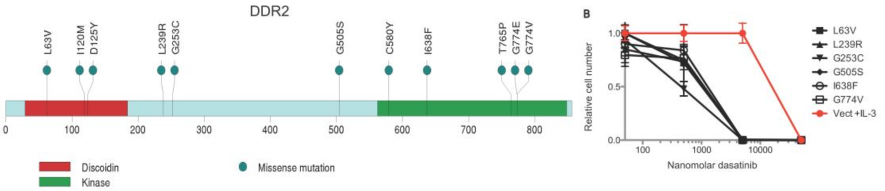
Identification of novel candidate driver genes

RESEARCH ARTICLE

Mutations in the *DDR2* Kinase Gene Identify a Novel Therapeutic Target in Squamous Cell Lung Cancer

*Cancer Discovery; 1(1); 78–89. ©2011 AACR.*

- In discovery set, 20 patients samples and matched normal DNA for 201 genes, including 90 kinase.
- In validation set, 222 samples with matched normal DNA for DDR2, except D125Y to verify the mutation as somatic.



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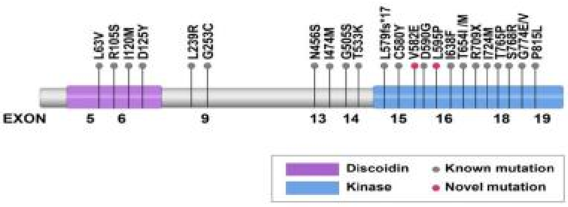
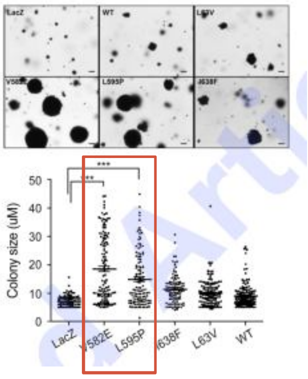
### Prevalence of Mutations in Discoidin Domain-Containing Receptor Tyrosine Kinase 2 (*DDR2*) in Squamous Cell Lung Cancers in Korean Patients

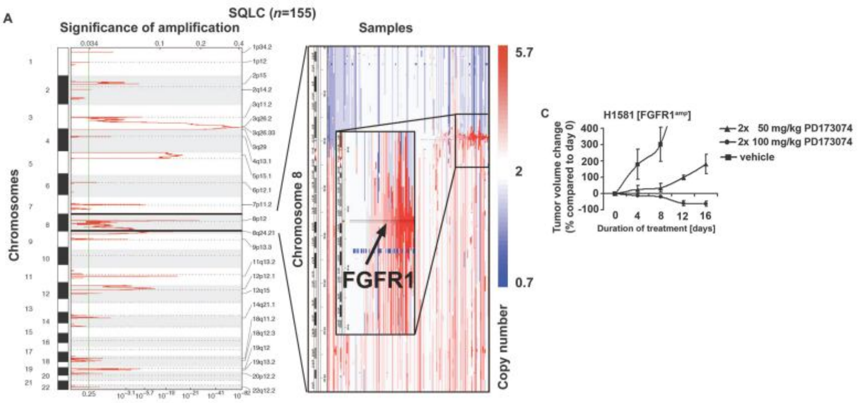
- 100 SCC patients, DDR2 Sanger sequencing on exons 6,15,16 and 18
- Two novel somatic mutations (V582E, L595P)





Lee MS, Choi YL, Cancer Res Treat, accepted

# Discovery of focal FGFR1 amplifications in up to 20% of squamous cell lung cancer



*Weiss et al.,Sci Transl Med 2010*
*Cancer Discovery 2013*

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Original Article

## Efficacy and Safety of Dovitinib in Pretreated Patients With Advanced Squamous Non-Small Cell Lung Cancer With *FGFR1* Amplification: A Single-Arm, Phase 2 Study

Sung Hee Lim, MD[1]; Jong-Mu Sun, MD, PhD[1]; Yoon-La Choi, MD, PhD[2]; Hye Ryun Kim, MD, PhD[3]; Soomin Ahn MD[2]; Ji Yun Lee, MD[1]; Se-Hoon Lee, MD, PhD[1]; Jin Seok Ahn, MD, PhD[1]; Keunchil Park, MD, PhD[1]; Joo Hang Kim, MD, PhD[3]; Byoung Chul Cho, MD, PhD[3]; and Myung-Ju Ahn, MD, PhD[1]

Cancer 2016

*FGFR1* Amplification by FISH Assay

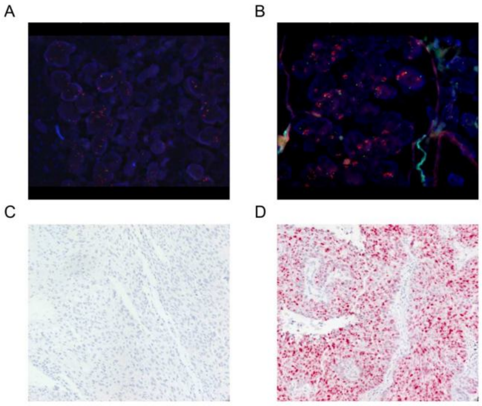
Kreatech Break-apart probe

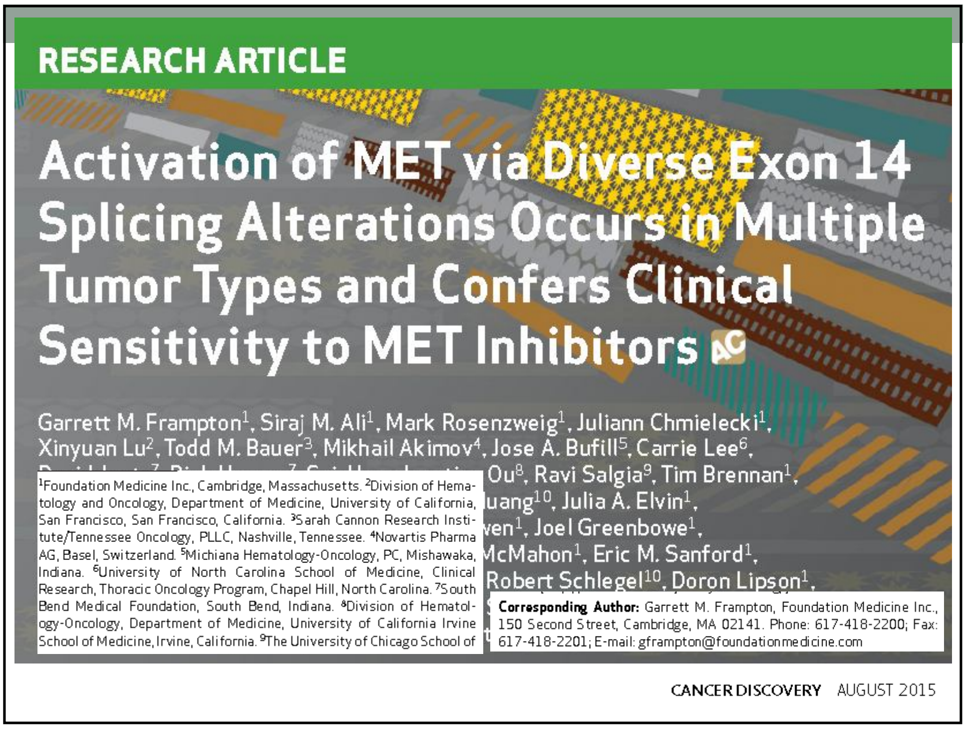
>5 FGFR1 signals and ≥ 2 FGFR1/CEN8

*FGFR1* mRNA Expression by RNA Scope Assay

Advanced Cell Diagnostics Inc

0,
1+ (1~3 dots in1%~50%),
2+ (1~3 dots in >50%),
3+ (clusters in <10% or 3~5 dots in >50% or >5 dots in <10%),
4+ (clusters in >10% or >5 dots in >10%)





RESEARCH ARTICLE

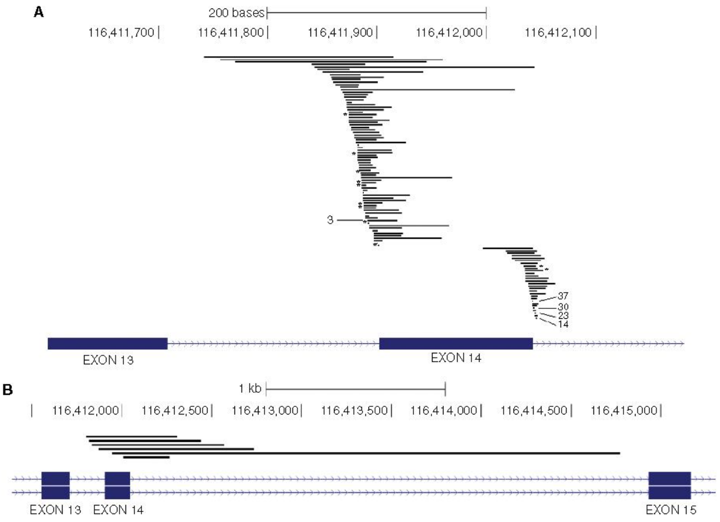
# Activation of MET via Diverse Exon 14 Splicing Alterations Occurs in Multiple Tumor Types and Confers Clinical Sensitivity to MET Inhibitors

Garrett M. Frampton[1], Siraj M. Ali[1], Mark Rosenzweig[1], Juliann Chmielecki[1],
Xinyuan Lu[2], Todd M. Bauer[3], Mikhail Akimov[4], Jose A. Bufill[5], Carrie Lee[6],
Ou[8], Ravi Salgia[9], Tim Brennan[1],
uang[10], Julia A. Elvin[1],
ven[1], Joel Greenbowe[1],
McMahon[1], Eric M. Sanford[1],
Robert Schlegel[10], Doron Lipson[1],

[1]Foundation Medicine Inc., Cambridge, Massachusetts. [2]Division of Hematology and Oncology, Department of Medicine, University of California, San Francisco, San Francisco, California. [3]Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, Tennessee. [4]Novartis Pharma AG, Basel, Switzerland. [5]Michiana Hematology-Oncology, PC, Mishawaka, Indiana. [6]University of North Carolina School of Medicine, Clinical Research, Thoracic Oncology Program, Chapel Hill, North Carolina. [7]South Bend Medical Foundation, South Bend, Indiana. [8]Division of Hematology-Oncology, Department of Medicine, University of California Irvine School of Medicine, Irvine, California. [9]The University of Chicago School of

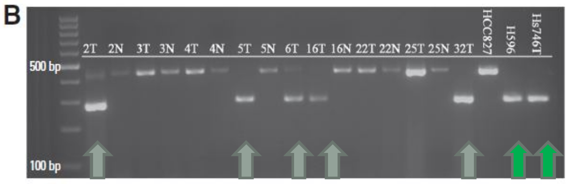
**Corresponding Author:** Garrett M. Frampton, Foundation Medicine Inc., 150 Second Street, Cambridge, MA 02141. Phone: 617-418-2200; Fax: 617-418-2201; E-mail: gframpton@foundationmedicine.com

CANCER DISCOVERY AUGUST 2015

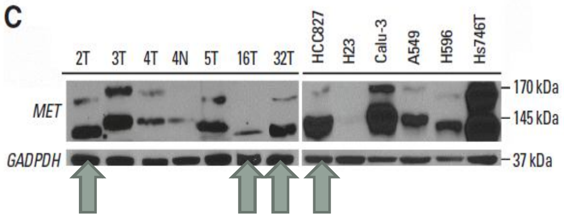


## *Identification of MET exon 14 skipping*

Real-time PCR



Western blot



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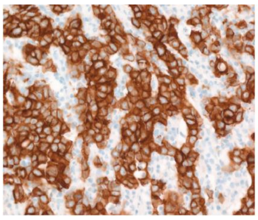
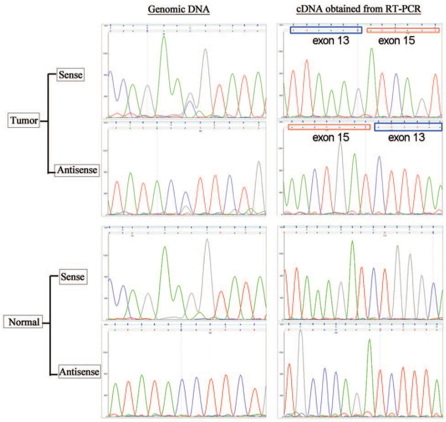
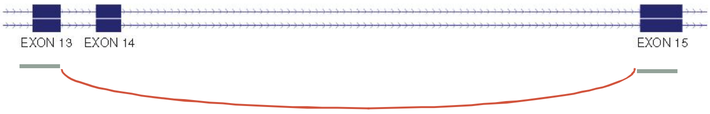
## *Identification of MET exon 14 skipping*

- *MET* exon 14 skipping in complementary DNA amplified by RT-PCR







## *MET exon 14 skipping in lung adenocarcinoma*

- 70 quintuple-negative (*EGFR/KRAS/ALK/ROS1/RET*) lung adenocarcinoma
- RT-PCR using probes spanning an exon 13-15 on 45 FFPE specimen
- Sanger sequencing

- 17 MET exon 14 skipping lung adenocarcinoma
- MET IHC : 15/15 METex14 skipping positive group,
  8/27 in negative group

Lee GD, Choi YL, JTO revision

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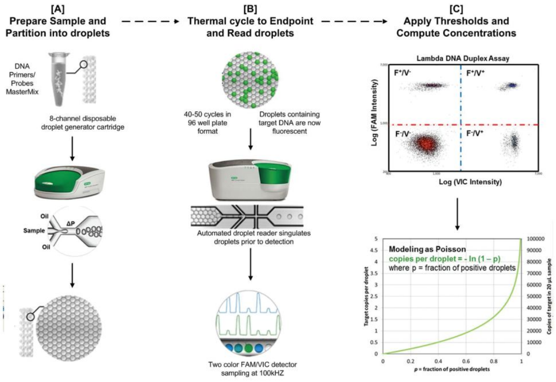
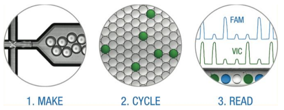
## *Issues about biomarker testing in lung cancer*

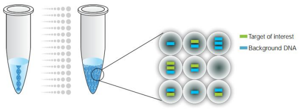
- Sample : tiny tissue, cytology specimen, ctDNA
- Gene number: single gene, multi-gene
- Target : DNA, RNA, Protein
- Technique :
  RNA : RT- PCR, digital PCR, RNA-in situ, nanostring, NGS
  DNA : Sanger, FISH, NGS, nanostring, Real-time RCR
  Protein : IHC, Multiplex-IHC
- Cut-off, QC & QA, Cost, Reimbursement

# *Ultra-sensitive detection technology Droplet digital PCR*



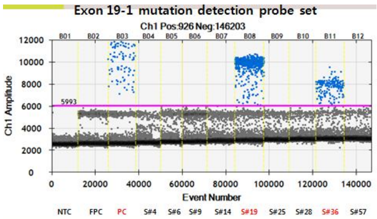
*Anal Chem. 2012 Jan 17;84(2):1003-11.*

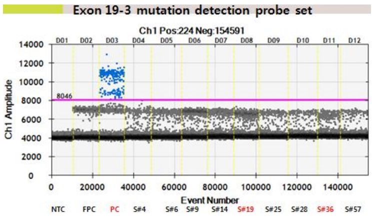


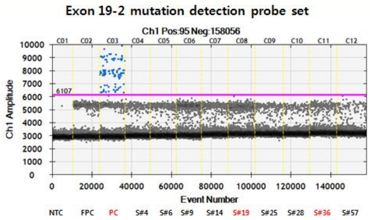


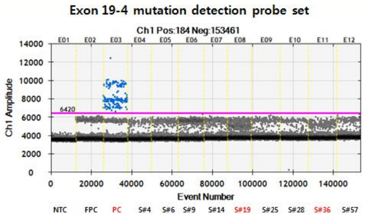
- Increased signal-to-noise
- Removal of PCR efficiency bias
- Simplified quantification
- Single template product
- Reduced reagent cost

# *Results View of ddPCR*


Exon 19-1 mutation detection probe set


Exon 19-3 mutation detection probe set


Exon 19-2 mutation detection probe set


Exon 19-4 mutation detection probe set

# RNA In situ Hybridization (RNA scope)

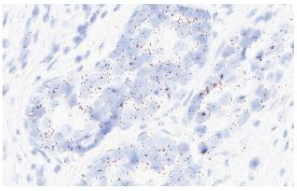


FIGURE 7. *POLR2A* detection (brown punctate dots) in colon cancer sample using RNAscope® 2.5 HD Reagent Kit•BROWN.

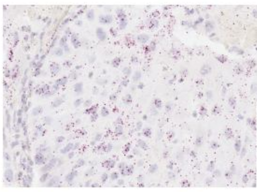
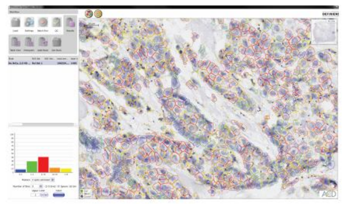


FIGURE 8. *PDL1* detection (red punctate dots) in lung cancer sampl RNAscope® 2.5 HD Reagent Kit•RED.



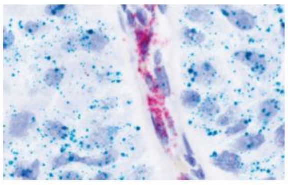


FIGURE 9. *EPCAM1* (red punctate dots) and *EGFR* (green punctate dots) expression in human breast cancer FFPE tissue using the RNAscope® 2-plex Chromogenic Kit.

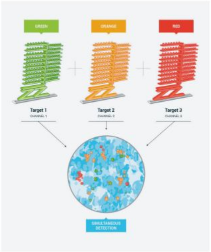
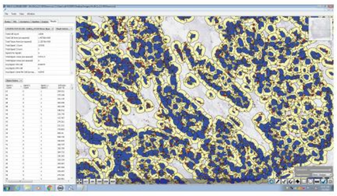
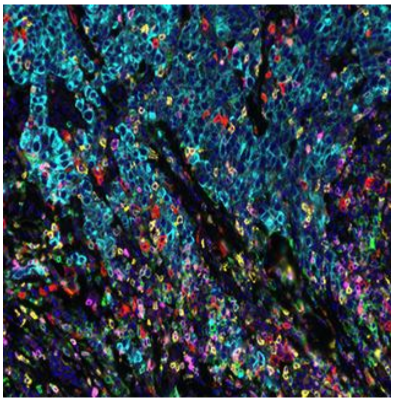


FIGURE 11. Multiplex RNA ISH analysis using RNAscope® Multiplex Fluorescent Assay.



# IHC - multiplex

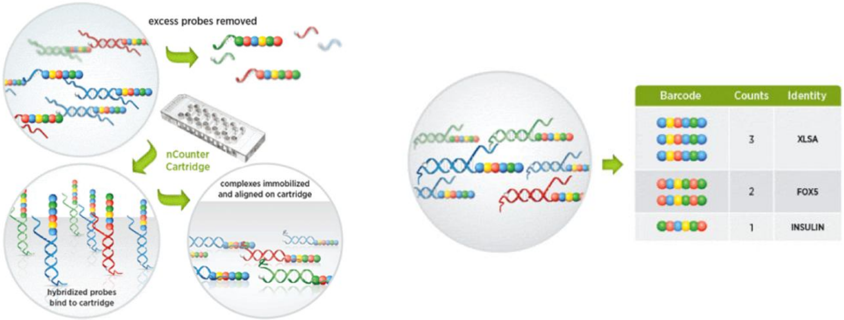
Opal Multiplex IHC Assay



# *Nanostring (nCounter)*

**Meet nCounter *SPRINT™***

A new technology is accelerating cancer research beyond the limitations of RT-qPCR and targeted RNA-Seq.
And in so doing, brings the cure to within a string's reach.



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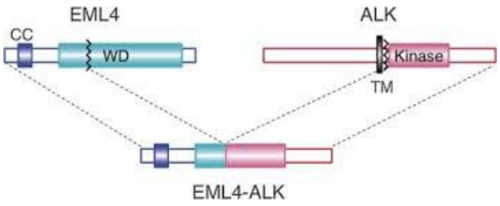
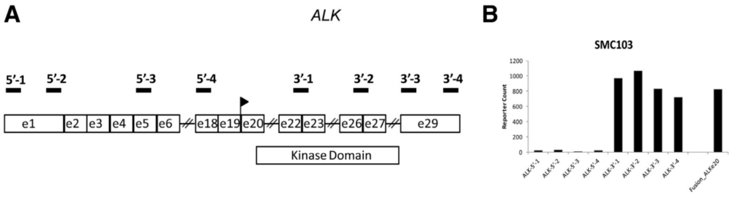
## A Single-Tube Multiplexed Assay for Detecting *ALK*, *ROS1*, and *RET* Fusions in Lung Cancer

Maruja E. Lira,* Yoon-La Choi,† Sun Min Lim,‡ Shibing Deng,* Donghui Huang,* Mark Ozeck,* Joungho Han,§ Ji Yun Jeong,¶
Hyo Sup Shim,¶ Byoung Chul Cho,‡ Jhingook Kim,|| Myung-Ju Ahn,§ and Mao Mao*

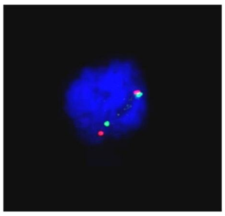
J Mol Diagn 2014, 16: 229-243





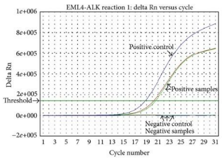
## *ALK detection Methods*

FISH



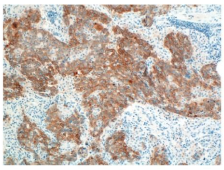
( a )

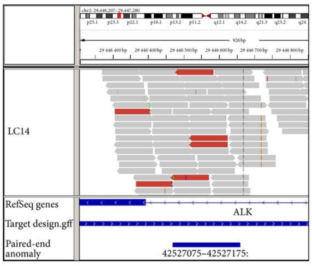
RT-PCR



( b )

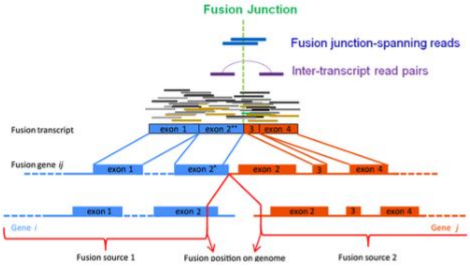
IHC





( a )

NGS with DNA or RNA



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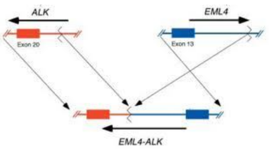
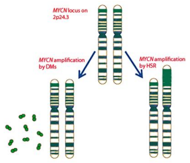
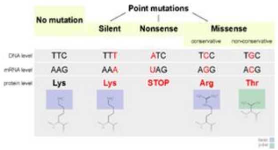
## ~~Next generation sequencing~~

## Current generation sequencing

## *Various targets for companion diagnostics*





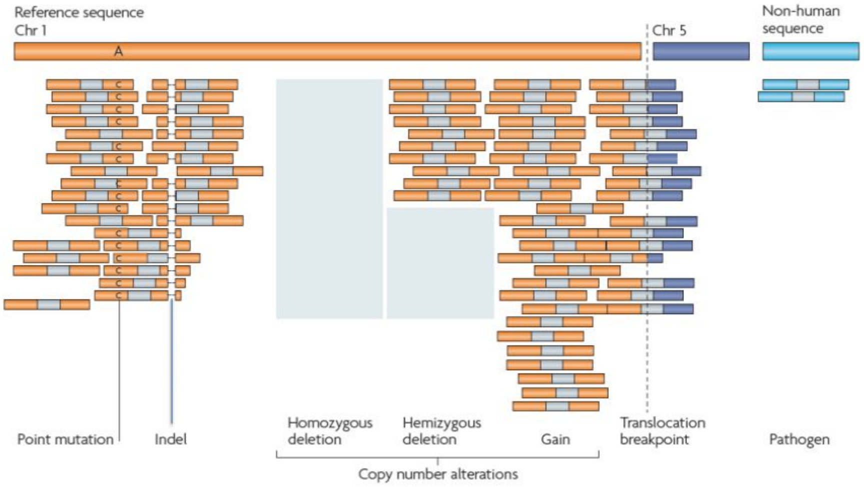


**Translocation**

**Amplification**

**Point mutation (SNV/Indel)**

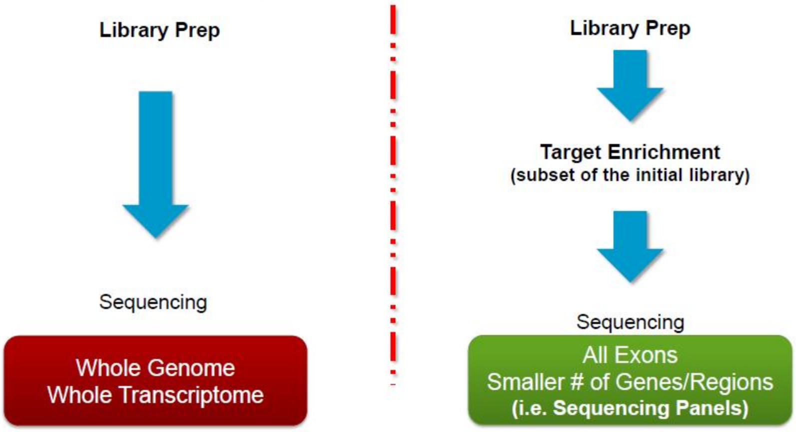
MacConaill, Cancer Discovery, 2011



Meyerson, Gabriel & Getz. Nat.Rev.Genet. (2010)

## *Whole or Targeted NGS*

Can be a 2 step or 3 step process…



**Library Prep**

Sequencing

Whole Genome
Whole Transcriptome

**Library Prep**

**Target Enrichment**
**(subset of the initial library)**

Sequencing

All Exons
Smaller # of Genes/Regions
**(i.e. Sequencing Panels)**

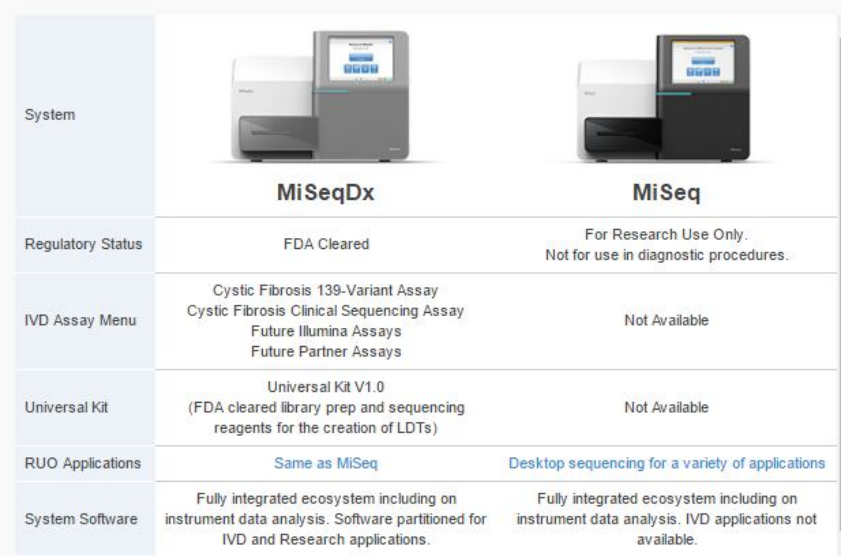
## *Lower throughput-Faster TAT*

Illumina
MiSeq Dx

Reversible Dye
Terminators

ThermoFisher
Ion PGM Dx

Monitors H+
Release

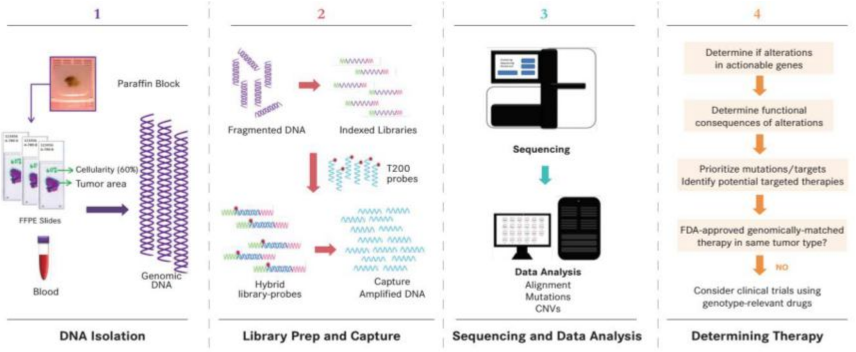


| System | MiSeqDx | MiSeq |
|---|---|---|
| Regulatory Status | FDA Cleared | For Research Use Only.<br>Not for use in diagnostic procedures. |
| IVD Assay Menu | Cystic Fibrosis 139-Variant Assay<br>Cystic Fibrosis Clinical Sequencing Assay<br>Future Illumina Assays<br>Future Partner Assays | Not Available |
| Universal Kit | Universal Kit V1.0<br>(FDA cleared library prep and sequencing reagents for the creation of LDTs) | Not Available |
| RUO Applications | Same as MiSeq | Desktop sequencing for a variety of applications |
| System Software | Fully integrated ecosystem including on instrument data analysis. Software partitioned for IVD and Research applications. | Fully integrated ecosystem including on instrument data analysis. IVD applications not available. |

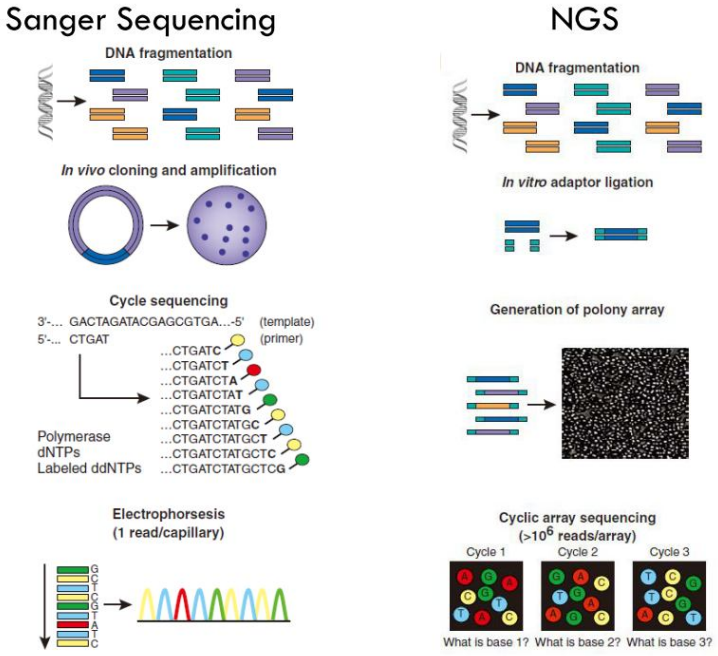
## *Nest generation sequencing*

- WGS (Whole genome sequencing)
- WES (Whole exome sequencing)
- RNA-seq
- Targeted seq (DNA, RNA)
- ChIP-seq

# *Structure of NGA Analysis*



*THE AMERICAN JOURNAL OF HEMATOLOGY/ONCOLOGY. (2015) 11(3): 17-22*



## Four main DNA sequencing methods used by NGS systems

1. Pyrosequencing

   Based on the Roche GS FLX

2. Sequencing by ligation

   Based on the Applied Biosystems SOLiD 5500xl

3. Smiconductor sequencing

   Based on the Ion PGM System, mostly used in clinical settings

4. Sequencing by synthesis

   Based on the Illumina HiSeq, currently the most popular method

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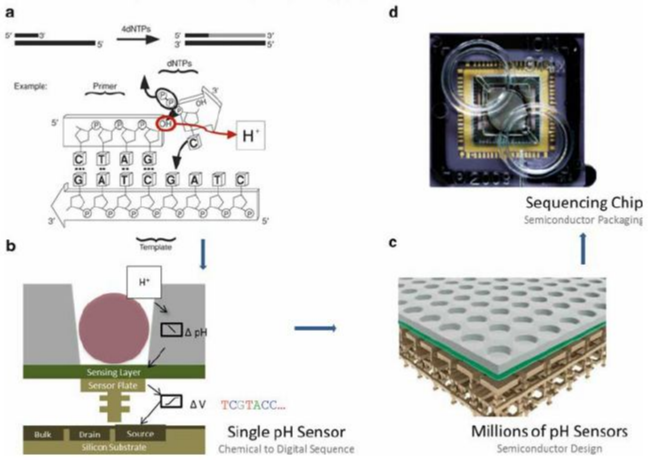
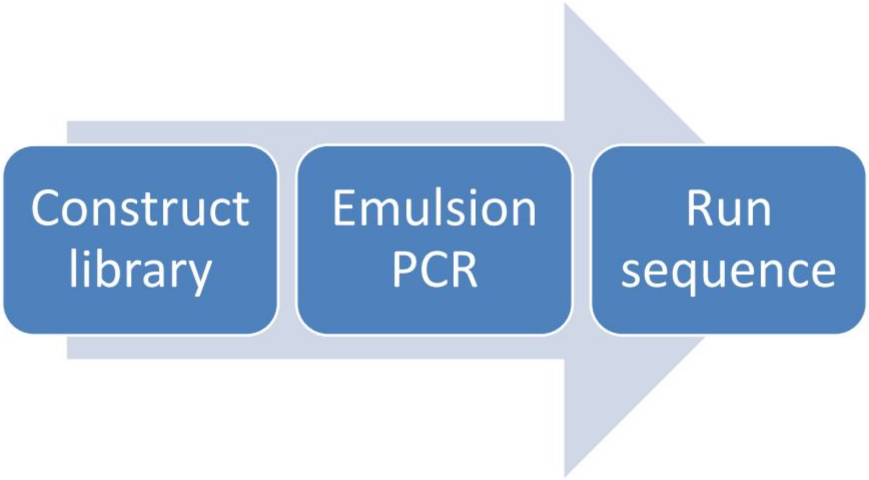
## Semiconductor sequencing : Ion Torrent

- Similar to pyrosequencing, but measures the release of H+ instead of pyrophosphate
- More cost-effective and time-efficient



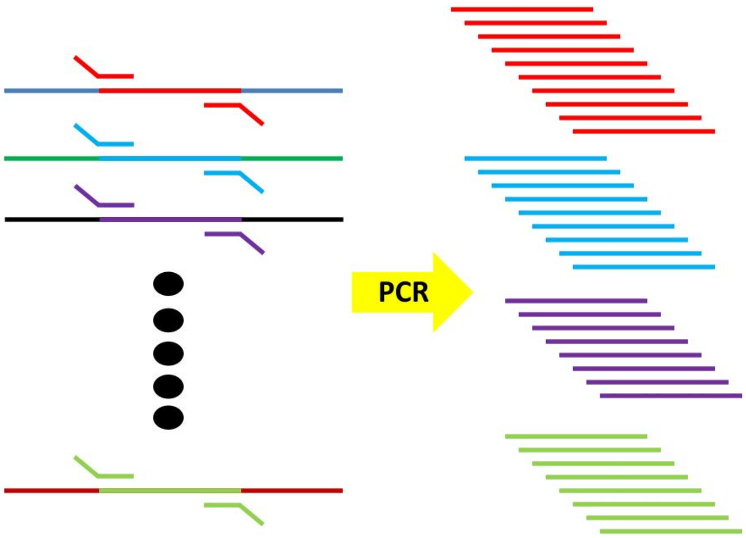
## *NGS Workflow*

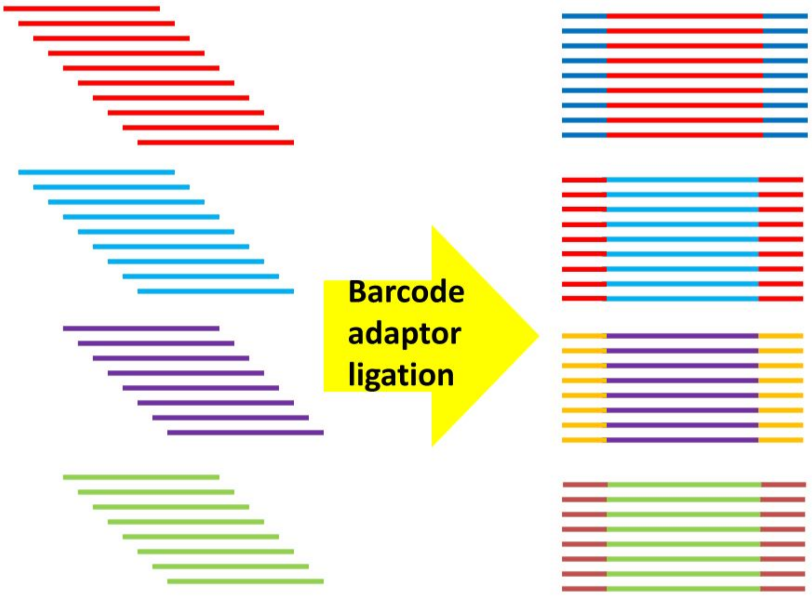


Construct library

Emulsion PCR

Run sequence





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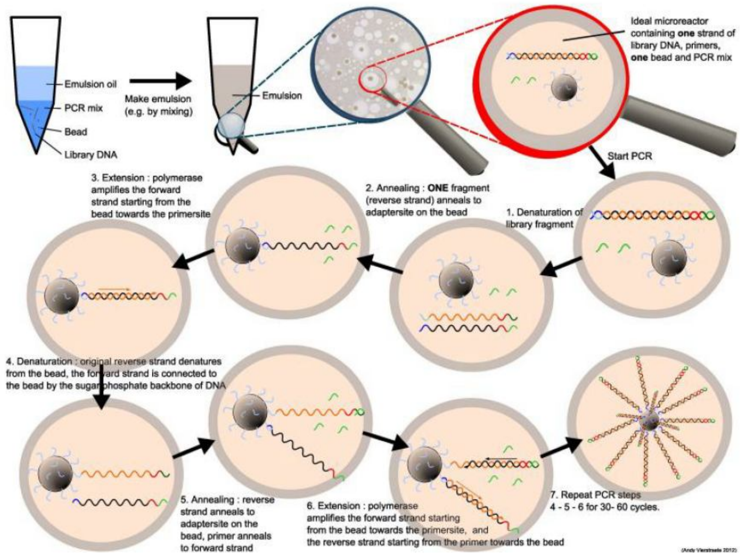
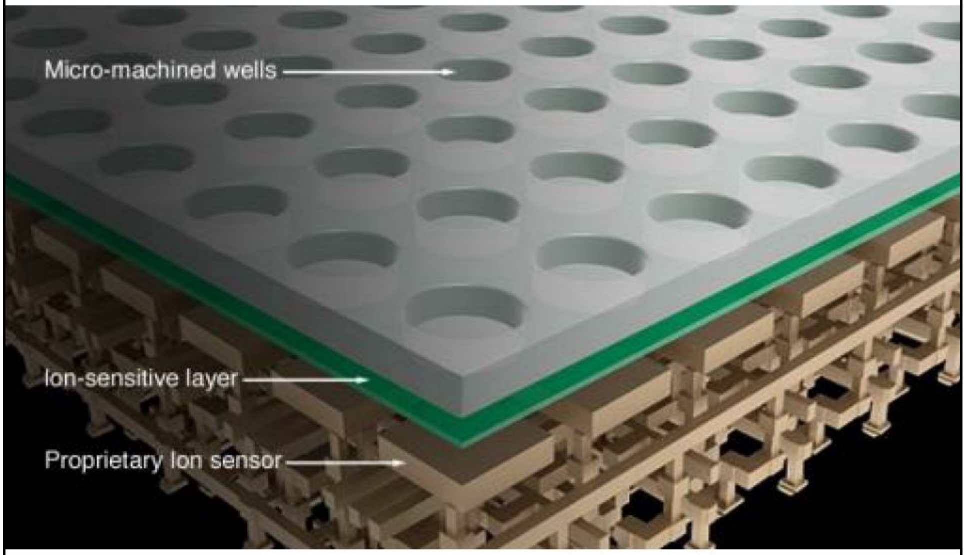
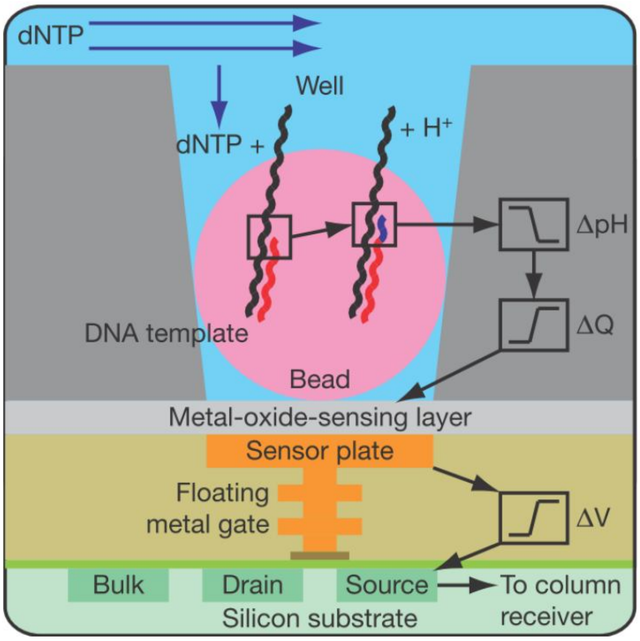
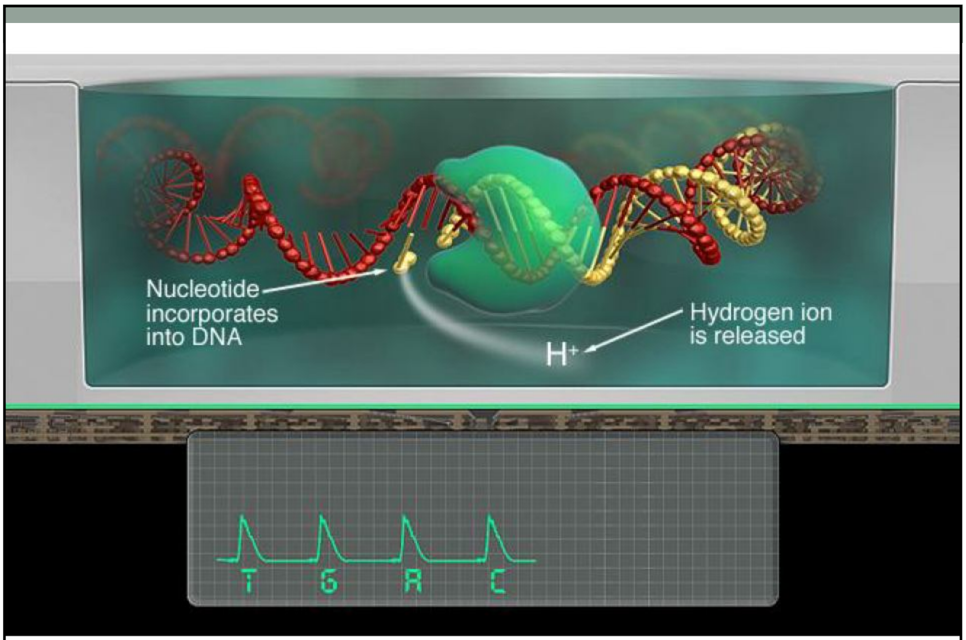
# Emulsion PCR

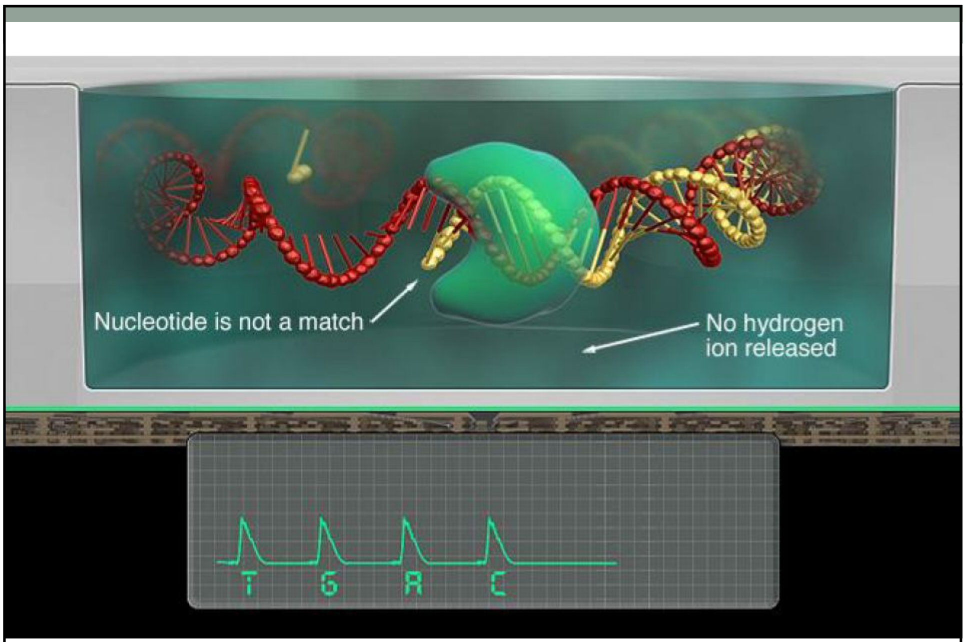



Micro-machined wells
Ion-sensitive layer
Proprietary Ion sensor


dNTP
Well
dNTP +
+ H⁺
ΔpH
ΔQ
DNA template
Bead
Metal-oxide-sensing layer
Sensor plate
Floating
metal gate
ΔV
Bulk
Drain
Source
To column
receiver
Silicon substrate

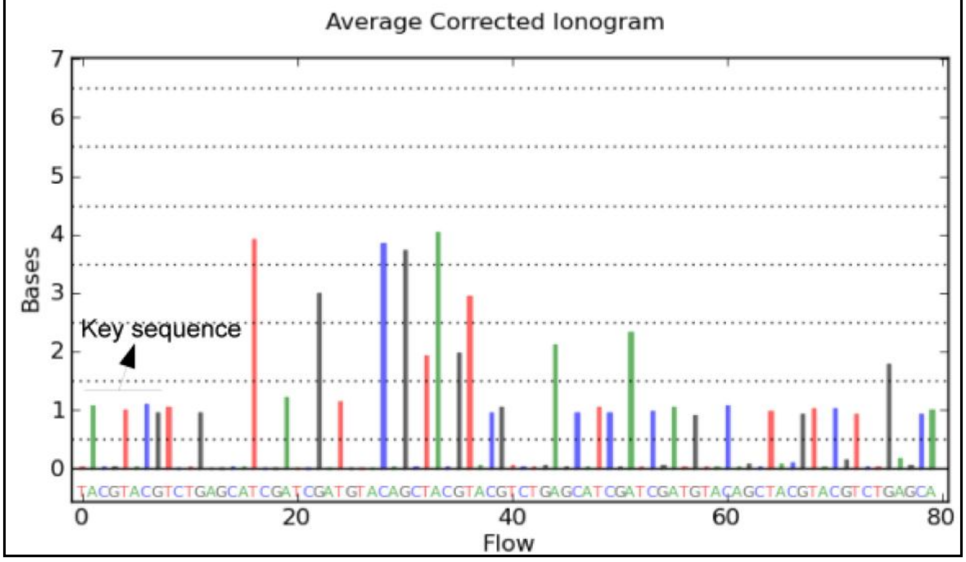


http://www.thermofisher.com



http://www.thermofisher.com

# Sequencing



## Ion AmpliSeq Cancer Hotspot Panel v2

Just one tube. Just 10ng of DNA. Just one day.

The **Ion AmpliSeq™ Cancer Hotspot Panel v2** allows translational and disease researchers to fast-track oncology research by surveying hotspot regions of 50 oncogenes and tumor suppressor genes, with wide coverage of the *KRAS*, *BRAF* and *EGFR* genes.

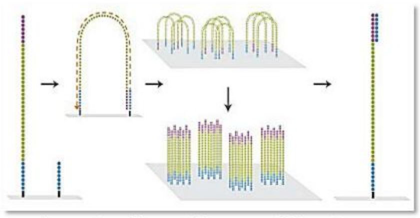
This research panel, with improved primer design, contains 207 primer pairs in a single tube and requires as little as 10ng of DNA, enabling researchers to sequence challenging samples such as formalin-fixed, paraffin-embedded (FFPE) tissue. The convenient predesigned panel allows researchers to focus on data generation and analysis, not on the labor-intensive primer design and target selection steps. While hybridization-based target selection methods require 7 to 72 hours to complete both target selection and library preparation, Ion AmpliSeq™ technology makes it possible to complete the entire process in about 3.5 hours using simple PCR reactions. Additionally, Ion AmpliSeq™ library construction steps are automated using standard 96-well plate–based protocols, further simplifying the workflow and allowing large projects to be rapidly completed without additional sample transfer steps.

"Sequencing gene panels with Ion Torrent™ technology is complete. You have the **Ion AmpliSeq™ gene selection technology**, the Ion PGM™ sequencing system and also the Ion Reporter™ analysis software.
For me, it is important to have a whole integrated solution, so researchers can advance from the tumor to results."

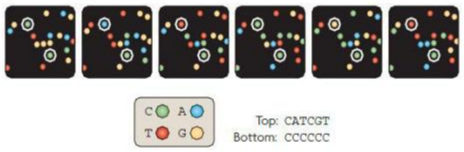
**Pierre Laurent-Puig, M.D., Ph.D.**
**Paris-Descartes University**
**Medical School, Paris, France**

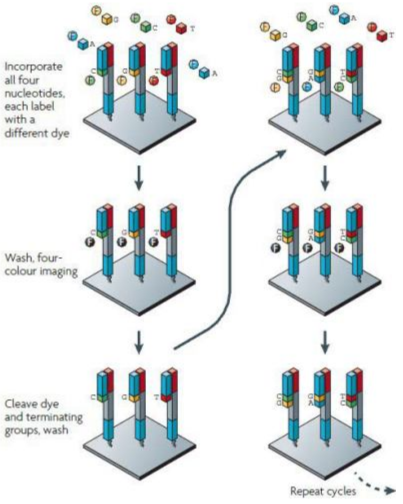
# *Sequencing by Synthesis : Illumina*

- Overcomes homopolymer issue due to terminated nucleotides
- Increased error rate with increased read lengths



**Clustering by Bridge Amplification**





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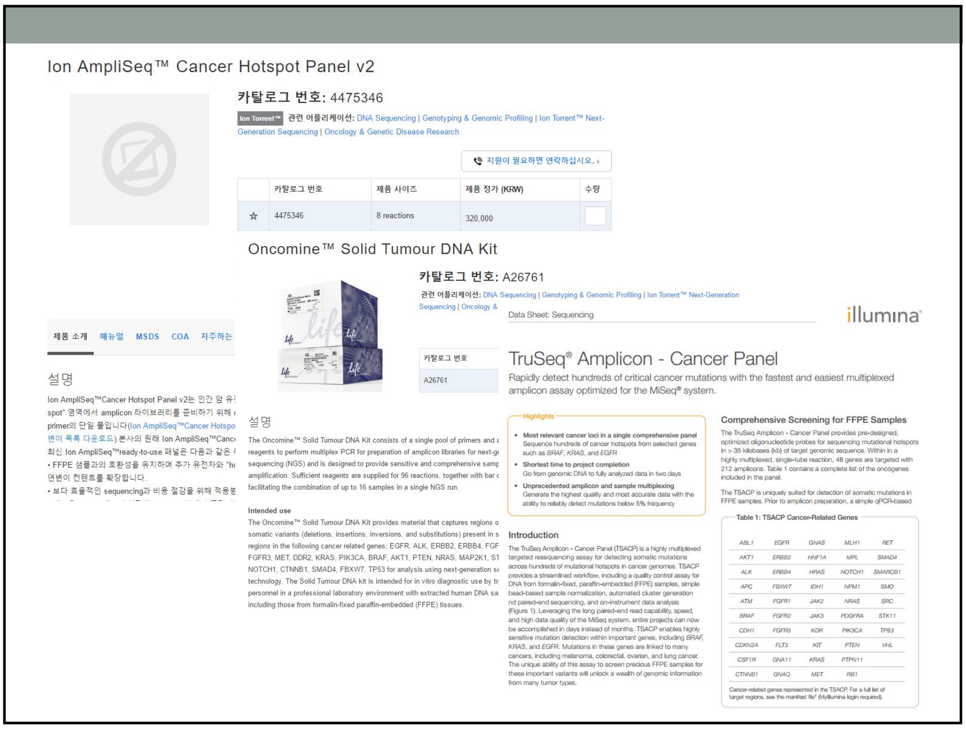


Ion AmpliSeq™ Cancer Hotspot Panel v2

**카탈로그 번호:** 4475346

Ion Torrent™ **관련 어플리케이션:** DNA Sequencing | Genotyping & Genomic Profiling | Ion Torrent™ Next-Generation Sequencing | Oncology & Genetic Disease Research

☎ 지원이 필요하면 연락하십시오. ›

| | 카탈로그 번호 | 제품 사이즈 | 제품 정가 (KRW) | 수량 |
|---|---|---|---|---|
| ☆ | 4475346 | 8 reactions | 320,000 | ☐ |

설명

Ion AmpliSeq™Cancer Hotspot Panel v2는 인간 암 유
spot" 영역에서 amplicon 라이브러리를 준비하기 위해
primer의 단일 풀입니다(Ion AmpliSeq™Cancer Hotspo
변이 목록 다운로드).본사의 원래 Ion AmpliSeq™Canc
최신 Ion AmpliSeq™ready-to-use 패널은 다음과 같은
• FFPE 샘플과의 호환성을 유지하며 추가 유전자와 "ho
연변이 컨텐트를 확장합니다.
• 보다 효율적인 sequencing과 비용 절감을 위해 적용범

Oncomine™ Solid Tumour DNA Kit

**카탈로그 번호:** A26761

**관련 어플리케이션:** DNA Sequencing | Genotyping & Genomic Profiling | Ion Torrent™ Next-Generation Sequencing | Oncology &

| 카탈로그 번호 |
|---|
| A26761 |

설명

The Oncomine™ Solid Tumour DNA Kit consists of a single pool of primers and a
reagents to perform multiplex PCR for preparation of amplicon libraries for next-ge
sequencing (NGS) and is designed to provide sensitive and comprehensive samp
amplification. Sufficient reagents are supplied for 96 reactions, together with bar c
facilitating the combination of up to 16 samples in a single NGS run.

**Intended use**

The Oncomine™ Solid Tumour DNA Kit provides material that captures regions o
somatic variants (deletions, insertions, inversions, and substitutions) present in s
regions in the following cancer related genes; EGFR, ALK, ERBB2, ERBB4, FGF
FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, ST
NOTCH1, CTNNB1, SMAD4, FBXW7, TP53 for analysis using next-generation se
technology. The Solid Tumour DNA kit is intended for in vitro diagnostic use by tr
personnel in a professional laboratory environment with extracted human DNA sa
including those from formalin-fixed paraffin-embedded (FFPE) tissues.

Data Sheet: Sequencing

illumina

## TruSeq® Amplicon - Cancer Panel

Rapidly detect hundreds of critical cancer mutations with the fastest and easiest multiplexed amplicon assay optimized for the MiSeq® system.

Highlights

- **Most relevant cancer loci in a single comprehensive panel**
  Sequence hundreds of cancer hotspots from selected genes such as *BRAF*, *KRAS*, and *EGFR*
- **Shortest time to project completion**
  Go from genomic DNA to fully analyzed data in two days
- **Unprecedented amplicon and sample multiplexing**
  Generate the highest quality and most accurate data with the ability to reliably detect mutations below 5% frequency

### Introduction

The TruSeq Amplicon - Cancer Panel (TSACP) is a highly multiplexed targeted resequencing assay for detecting somatic mutations across hundreds of mutational hotspots in cancer genomes. TSACP provides a streamlined workflow, including a quality control assay for DNA from formalin-fixed, paraffin-embedded (FFPE) samples, simple bead-based sample normalization, automated cluster generation nd paired-end sequencing, and on-instrument data analysis (Figure 1). Leveraging the long paired-end read capability, speed, and high data quality of the MiSeq system, entire projects can now be accomplished in days instead of months. TSACP enables highly sensitive mutation detection within important genes, including *BRAF*, *KRAS*, and *EGFR*. Mutations in these genes are linked to many cancers, including melanoma, colorectal, ovarian, and lung cancer. The unique ability of this assay to screen precious FFPE samples for these important variants will unlock a wealth of genomic information from many tumor types.

### Comprehensive Screening for FFPE Samples

The TruSeq Amplicon - Cancer Panel provides pre-designed, optimized oligonucleotide probes for sequencing mutational hotspots in > 35 kilobases (kb) of target genomic sequence. Within in a highly multiplexed, single-tube reaction, 48 genes are targeted with 212 amplicons. Table 1 contains a complete list of the oncogenes included in the panel.

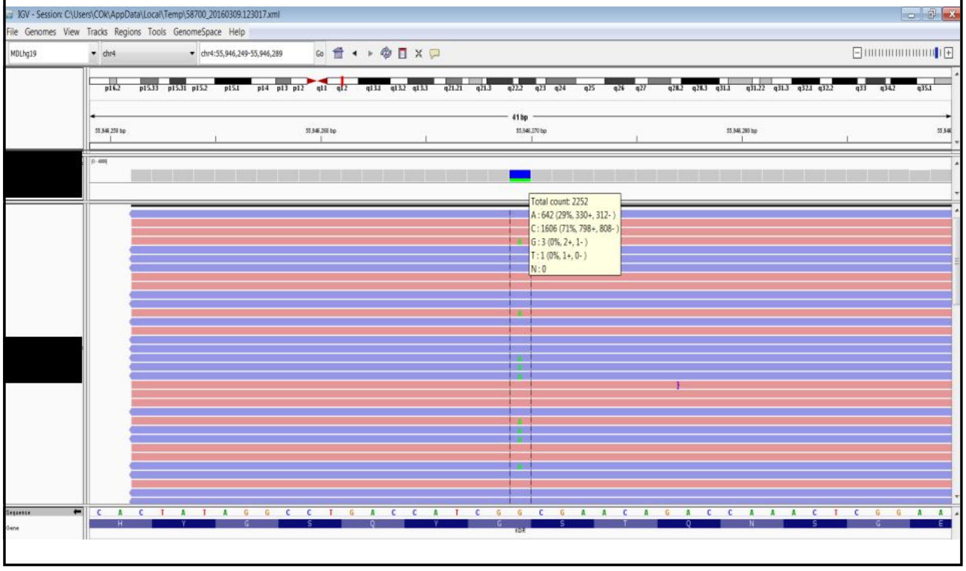
The TSACP is uniquely suited for detection of somatic mutations in FFPE samples. Prior to amplicon preparation, a simple qPCR-based

**Table 1: TSACP Cancer-Related Genes**

| | | | | |
|---|---|---|---|---|
| *ABL1* | *EGFR* | *GNAS* | *MLH1* | *RET* |
| *AKT1* | *ERBB2* | *HNF1A* | *MPL* | *SMAD4* |
| *ALK* | *ERBB4* | *HRAS* | *NOTCH1* | *SMARCB1* |
| *APC* | *FBXW7* | *IDH1* | *NPM1* | *SMO* |
| *ATM* | *FGFR1* | *JAK2* | *NRAS* | *SRC* |
| *BRAF* | *FGFR2* | *JAK3* | *PDGFRA* | *STK11* |
| *CDH1* | *FGFR3* | *KDR* | *PIK3CA* | *TP53* |
| *CDKN2A* | *FLT3* | *KIT* | *PTEN* | *VHL* |
| *CSF1R* | *GNA11* | *KRAS* | *PTPN11* | |
| *CTNNB1* | *GNAQ* | *MET* | *RB1* | |

Cancer-related genes represented in the TSACP. For a full list of target regions, see the manifest file[1] (MyIllumina login required).

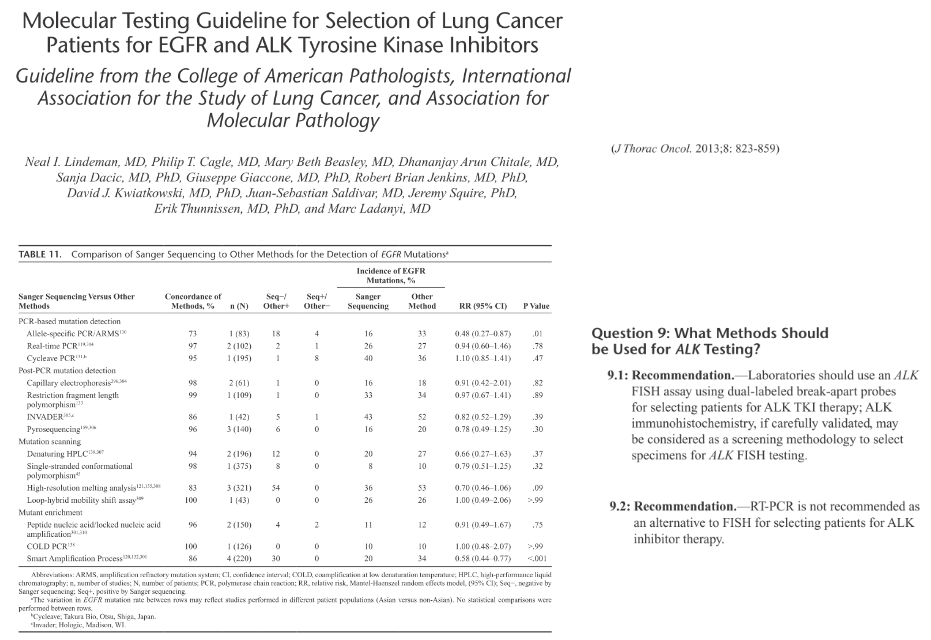
# Visualization of Mapping with IGV



[redacted]

[redacted]

Total count: 2252
A : 642 (29%, 330+, 312- )
C : 1606 (71%, 798+, 808- )
G : 3 (0%, 2+, 1- )
T : 1 (0%, 1+, 0- )
N : 0



Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

*Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology*

*Neal I. Lindeman, MD, Philip T. Cagle, MD, Mary Beth Beasley, MD, Dhananjay Arun Chitale, MD,*
*Sanja Dacic, MD, PhD, Giuseppe Giaccone, MD, PhD, Robert Brian Jenkins, MD, PhD,*
*David J. Kwiatkowski, MD, PhD, Juan-Sebastian Saldivar, MD, Jeremy Squire, PhD,*
*Erik Thunnissen, MD, PhD, and Marc Ladanyi, MD*

(*J Thorac Oncol.* 2013;8: 823-859)

**TABLE 11.** Comparison of Sanger Sequencing to Other Methods for the Detection of *EGFR* Mutations[a]

| Sanger Sequencing Versus Other Methods | Concordance of Methods, % | n (N) | Seq−/ Other+ | Seq+/ Other− | Incidence of EGFR Mutations, % |  | RR (95% CI) | P Value |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | Sanger Sequencing | Other Method |  |  |
| PCR-based mutation detection |  |  |  |  |  |  |  |  |
| Allele-specific PCR/ARMS[130] | 73 | 1 (83) | 18 | 4 | 16 | 33 | 0.48 (0.27–0.87) | .01 |
| Real-time PCR[119,304] | 97 | 2 (102) | 2 | 1 | 26 | 27 | 0.94 (0.60–1.46) | .78 |
| Cycleave PCR[131,b] | 95 | 1 (195) | 1 | 8 | 40 | 36 | 1.10 (0.85–1.41) | .47 |
| Post-PCR mutation detection |  |  |  |  |  |  |  |  |
| Capillary electrophoresis[296,304] | 98 | 2 (61) | 1 | 0 | 16 | 18 | 0.91 (0.42–2.01) | .82 |
| Restriction fragment length polymorphism[133] | 99 | 1 (109) | 1 | 0 | 33 | 34 | 0.97 (0.67–1.41) | .89 |
| INVADER[305,c] | 86 | 1 (42) | 5 | 1 | 43 | 52 | 0.82 (0.52–1.29) | .39 |
| Pyrosequencing[159,306] | 96 | 3 (140) | 6 | 0 | 16 | 20 | 0.78 (0.49–1.25) | .30 |
| Mutation scanning |  |  |  |  |  |  |  |  |
| Denaturing HPLC[139,307] | 94 | 2 (196) | 12 | 0 | 20 | 27 | 0.66 (0.27–1.63) | .37 |
| Single-stranded conformational polymorphism[45] | 98 | 1 (375) | 8 | 0 | 8 | 10 | 0.79 (0.51–1.25) | .32 |
| High-resolution melting analysis[121,135,308] | 83 | 3 (321) | 54 | 0 | 36 | 53 | 0.70 (0.46–1.06) | .09 |
| Loop-hybrid mobility shift assay[309] | 100 | 1 (43) | 0 | 0 | 26 | 26 | 1.00 (0.49–2.06) | >.99 |
| Mutant enrichment |  |  |  |  |  |  |  |  |
| Peptide nucleic acid/locked nucleic acid amplification[301,310] | 96 | 2 (150) | 4 | 2 | 11 | 12 | 0.91 (0.49–1.67) | .75 |
| COLD PCR[138] | 100 | 1 (126) | 0 | 0 | 10 | 10 | 1.00 (0.48–2.07) | >.99 |
| Smart Amplification Process[120,132,301] | 86 | 4 (220) | 30 | 0 | 20 | 34 | 0.58 (0.44–0.77) | <.001 |

Abbreviations: ARMS, amplification refractory mutation system; CI, confidence interval; COLD, coamplification at low denaturation temperature; HPLC, high-performance liquid chromatography; n, number of studies; N, number of patients; PCR, polymerase chain reaction; RR, relative risk, Mantel-Haenszel random effects model, (95% CI); Seq−, negative by Sanger sequencing; Seq+, positive by Sanger sequencing.

[a] The variation in *EGFR* mutation rate between rows may reflect studies performed in different patient populations (Asian versus non-Asian). No statistical comparisons were performed between rows.

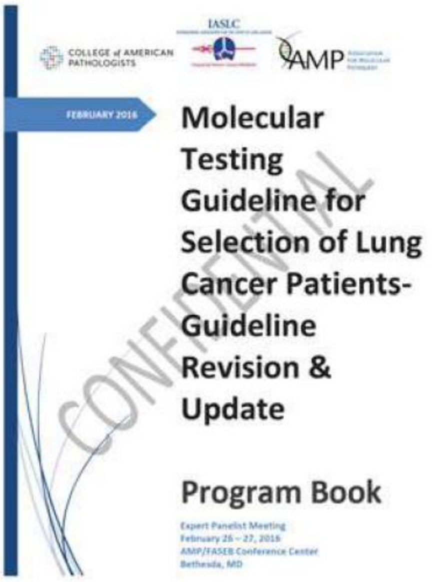
[b] Cycleave; Takura Bio, Otsu, Shiga, Japan.

[c] Invader; Hologic, Madison, WI.

**Question 9: What Methods Should be Used for *ALK* Testing?**

**9.1: Recommendation.**—Laboratories should use an *ALK* FISH assay using dual-labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for *ALK* FISH testing.

**9.2: Recommendation.**—RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.



COLLEGE of AMERICAN PATHOLOGISTS
IASLC
AMP

FEBRUARY 2016

# Molecular Testing Guideline for Selection of Lung Cancer Patients- Guideline Revision & Update

## Program Book

Expert Panelist Meeting
February 26 – 27, 2016
AMP/FASEB Conference Center
Bethesda, MD

Initiated in 2014
Writing began in 2015

This work is still in progress!

Current revisions underway!
Advisory Panel review!
Posting for public comment soon!
These are not final
Recommendations!

---

## *What other genes, previously not addressed, should be tested in lung adenocarcinoma?*

- *ROS1* : 1-2% rearrangement
- *RET* : 1-2% rearrangement
- *BRAF* : 4% half are non-V600E
- *MET* : 3% exon 14 deletion and amplification
- *ERBB2/HER2* : 2%
- *KRAS* : 30% mutation MEK inhibitors

## *Tests for ROS1 Rearrangements in NSCLC*

- Crizotinib has received FDA Breakthrough Therapy Designation Approval in ROS1 positive lung cancers.

NO GOLD STANDARD METHOD
  - FISH
  - Immunohistochemistry
  - Anchored multiplex PCR
  - Reverse transcriptase PCR
  - Next generation sequencing

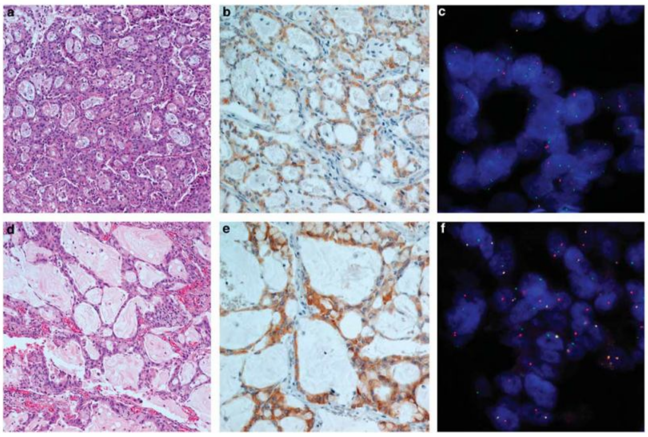
  - **In addition to histological diagnosis, important to prioritize lung adenocarcinoma biopsy tissue for *EGFR*, *ALK*, and *ROS1* testing**

---

## Comprehensive analysis of *RET* and *ROS1* rearrangement in lung adenocarcinoma

Seung Eun Lee[1], Boram Lee[1], Mineui Hong[1], Ji-Young Song[2,3], Kyungsoo Jung[2,4], Maruja E Lira[5], Mao Mao[5,7], Joungho Han[1], Jhingook Kim[6] and Yoon-La Choi[1,2,3,4]

MODERN PATHOLOGY (2015) 28, 468–479



## RET, BRAF, HER2, KRAS Considerations

- Adenocarcinomas, maybe other cell types in never smokers, etc?
- Not indicated as a routine strand-alone assay outside the context of a clinical trial?
- Can be part of larger testing panels performed either
  - Initially? or
  - when routine EGFR, ALK, and ROS1 testing are negative?

## MET in NSCLC

- MET amplification : responses to therapy with crizotinib
- MET exon14 skipping (splicing variant) : 3% of Lung Adenocarcinoma
- MET activation : response to crizotinib and cabozantinib

- MET amplification can be seen in tumors with MET exon14 deletion
- MET protein overexpression appears to correlate with MET amplification, although MET IHC is controversial
- Mutational analysis – exon 14 : Sequencing, NGS, RT-PCR
  Copy number - FISH, NGS
  IHC

## *Is IHC reliable for screening for ALK translocation?*

- ***VENTANA ALK (D5F3) CDx Assay*** is intended for the qualitative detection of the ALK protein in FFPE NSCLC tissue stained with a BenchMark XT automated staining instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI (crizotinib).
- When testing for ALK rearrangements, IHC as an equivalent alternative to FISH for adenocarcinoma

## 차세대 염기서열분석 (NGS) 임상검사실 인증제

질병의 예방·진단·치료 등을 위해 사람의 검체로 NGS 검사를 실시하는 임상검사실의 장비·시약·시설·인력·검사능력 등을 일체 평가하여 품질관리체계의 적절성과 검사결과의 정확성 및 신뢰성 등을 확보하여 해당 NGS 분석 장비 등을 임상 검사에 사용할 수 있도록 하는 제도

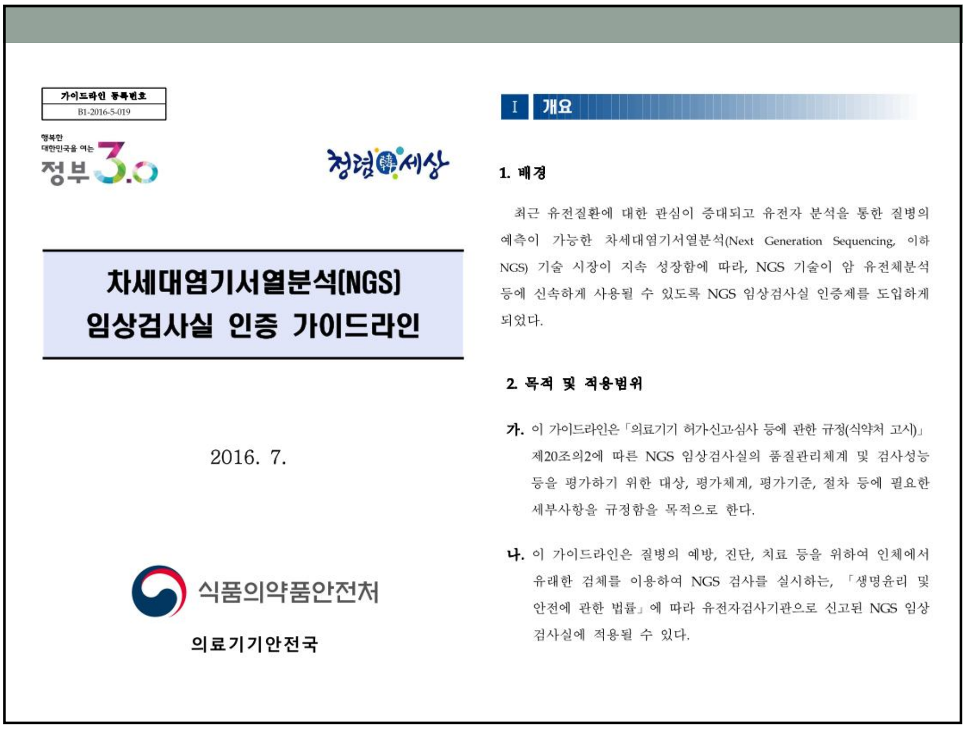
**추진상황**

NGS 유전자 검사 건강보험 급여 적용을 위한 [국민건강보험 요양급여의 기준에 관한 규칙] 개정(안) 입법예고

"임상검사실 인증서" 로 NGS 유전자 검사 요양급여 신청이 가능하도록 해당 조항 개정

인증 받은 NGS 임상검사실 검사에 사용되는 NGS 장비는 별도의 개별 허가를 면제하기 위한 [의료기기 허가 신고 심사 등에 관한 규정] (고시)개정(안) 행정예고

식약처장이 임상검사실의 품질관리체계 및 검사성능 등을 평가하여 적합하다고 인정하는 임상검사실의 차세대 염기서열분석 검사에 사용하는 유전자서열검사기는 허가 또는 인증을 받거나 신고한 것으로 본다.



| 가이드라인 등록번호 |
|---|
| B1-2016-5-019 |

행복한 대한민국을 여는 정부3.0

청렴韓세상

### 차세대염기서열분석(NGS) 임상검사실 인증 가이드라인

2016. 7.

식품의약품안전처

의료기기안전국

## I 개요

**1. 배경**

최근 유전질환에 대한 관심이 증대되고 유전자 분석을 통한 질병의 예측이 가능한 차세대염기서열분석(Next Generation Sequencing, 이하 NGS) 기술 시장이 지속 성장함에 따라, NGS 기술이 암 유전체분석 등에 신속하게 사용될 수 있도록 NGS 임상검사실 인증제를 도입하게 되었다.

**2. 목적 및 적용범위**

**가.** 이 가이드라인은 「의료기기 허가신고·심사 등에 관한 규정(식약처 고시)」 제20조의2에 따른 NGS 임상검사실의 품질관리체계 및 검사성능 등을 평가하기 위한 대상, 평가체계, 평가기준, 절차 등에 필요한 세부사항을 규정함을 목적으로 한다.

**나.** 이 가이드라인은 질병의 예방, 진단, 치료 등을 위하여 인체에서 유래한 검체를 이용하여 NGS 검사를 실시하는, 「생명윤리 및 안전에 관한 법률」에 따라 유전자검사기관으로 신고된 NGS 임상검사실에 적용될 수 있다.

---

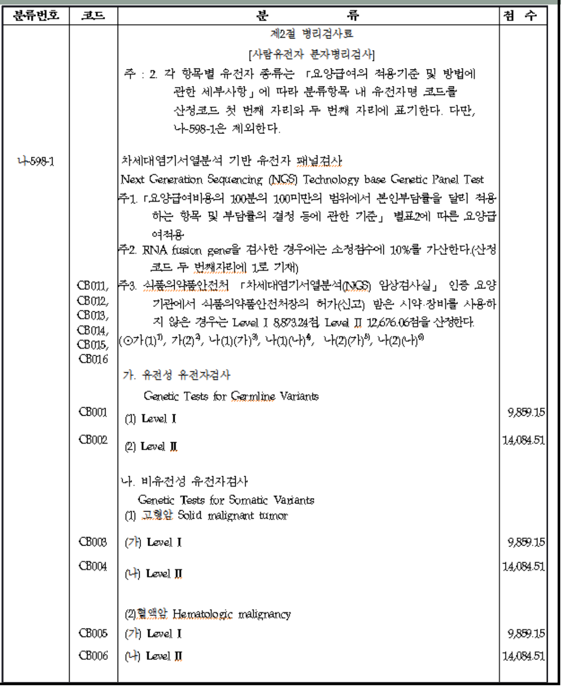
## *NGS Platforms*

- **Ion PGM Dx (with Ion OT2 Dx)**
- Ion S5 (with Ion Chef),
- Ion S5 XL (with Ion Chef)
- **MiSeqDx (with Universal Kit)**

- Ion PGM, Ion Proton
- MiniSeq, MiSeq, NextSeq 500, HiSeq 2500, HiSeq 3000, HiSeq 4000, HiSeq X Ten, HiSeq X Five

## 보건복지부 고시 제2016 – 271호

제1편 제2부 제2장 검사료 제2절 병리검사료 [사람유전자 분자병리검사]의 주 2를 일부변경, ‘나-600 염색체검사’란 앞에 ‘나-598-1 차세대염기서열분석 기반 유전자패널검사’란을 다음과 같이 신설



| 분류번호 | 코드 | 분　　　류 | 점 수 |
|---|---|---|---|
| | | 제2절 병리검사료<br>[사람유전자 분자병리검사]<br>주 : 2. 각 항목별 유전자 종류는 「요양급여의 적용기준 및 방법에 관한 세부사항」에 따라 분류항목 내 유전자명 코드를 산정코드 첫 번째 자리와 두 번째 자리에 표기한다. 다만, 나-598-1은 제외한다. | |
| 나-598-1 | | 차세대염기서열분석 기반 유전자 패널검사<br>Next Generation Sequencing (NGS) Technology base Genetic Panel Test<br>주1. 「요양급여비용의 100분의 100미만의 범위에서 본인부담률을 달리 적용하는 항목 및 부담률의 결정 등에 관한 기준」 별표2에 따른 요양급여적용<br>주2. RNA fusion gene을 검사한 경우에는 소정점수에 10%를 가산한다.(산정코드 두 번째자리에 1로 기재) | |
| | CB011,<br>CB012,<br>CB013,<br>CB014,<br>CB015,<br>CB016 | 주3. 식품의약품안전처 「차세대염기서열분석(NGS) 임상검사실」 인증 요양기관에서 식품의약품안전처장의 허가(신고) 받은 시약·장비를 사용하지 않은 경우는 Level Ⅰ 8,873.24점, Level Ⅱ 12,676.06점을 산정한다.<br>(⊙가(1)$^{1)}$, 가(2)$^{2)}$, 나(1)(가)$^{3)}$, 나(1)(나)$^{4)}$, 나(2)(가)$^{5)}$, 나(2)(나)$^{6)}$ | |
| | | 가. 유전성 유전자검사<br>Genetic Tests for Germline Variants | |
| | CB001 | (1) **Level Ⅰ** | 9,859.15 |
| | CB002 | (2) **Level Ⅱ** | 14,084.51 |
| | | 나. 비유전성 유전자검사<br>Genetic Tests for Somatic Variants<br>(1) 고형암 Solid malignant tumor | |
| | CB003 | (가) **Level Ⅰ** | 9,859.15 |
| | CB004 | (나) **Level Ⅱ** | 14,084.51 |
| | | (2)혈액암 Hematologic malignancy | |
| | CB005 | (가) **Level Ⅰ** | 9,859.15 |
| | CB006 | (나) **Level Ⅱ** | 14,084.51 |

## 보건복지부 고시 제2017 – 15호 요양급여의 적용기준 및 방법에 관한 세부사항

차세대 염기서열분석 기반 유전자 패널검사(Next Generation Sequencing(NGS)Technology base Genetic Panel Test) 는 「요양급여비용의 100분의 100 미만의 범위에서 본인부담률을 달리 적용하는 항목 및 부담률의 결정 등에 관한 기준」별첨3에 따라 승인된 요양기관에서 실시한 경우 다음과 같이 인정함.

| | |
|---|---|
| **수가산정 방법 및 인정횟수** | ○ 유전성 유전자 검사 : 질환별로 1회 인정<br>- Level Ⅰ : 유전자수 2~30개 이거나 유전자 길이가 150kb 이하인 경우<br>- Level Ⅱ : 유전자수 31개이상 이거나 유전자 길이가 150kb 초과한 경우로서 유전성 망막색소변성, 유전성 난청, 샤르코마리투스병에 한하여 인정<br>○ 비유전성 유전자 검사 : 진단시 1회 인정을 원칙으로 함.<br>다만, 재발 및 치료불응 시에 한하여 추가 1회를 인정<br>- Level Ⅰ : 유전자수 5~50개 이거나 유전자 길이 150kb 이하인 경우<br>- Level Ⅱ : 유전자수 51개이상 이거나 유전자 길이 150kb 초과한 경우 |

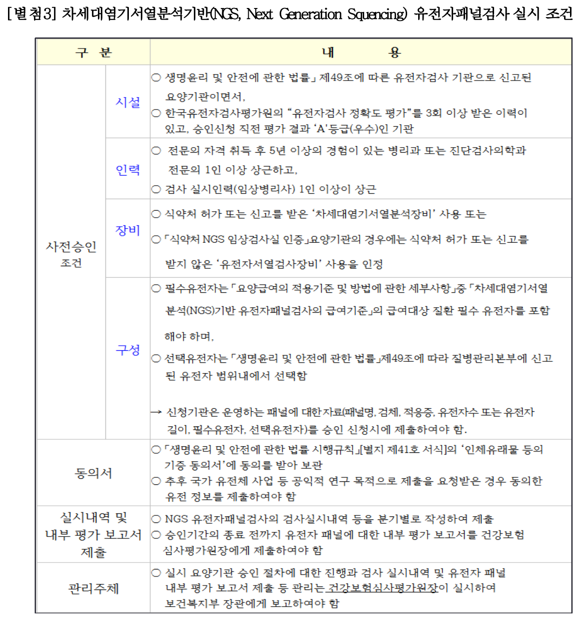
## 가. 급여대상 질환 및 필수유전자

1) 급여 대상 질환은 아래와 같고 필수유전자가 지정된 경우 유전자 패널에 반드시 포함하여 구성하고 실시하여야 함.

| 급여 대상 질환 | 필수유전자 |
|---|---|
| 유전성 망막색소변성* | PRPF31, RHO, RP1, RP2, USH2A, PRPH2, RPGR |
| 유전성 난청* | GJB2, POU3F4, SLC26A4, TECTA |
| 샤르코마리투스병* | GJB1, MFN2, MPZ, PMP22 |
| 상기세가지(*)질환을제외한 유전성 질환 | 없음 |
| **위암, 폐암, 대장암, 유방암, 난소암, 흑색종, 위장관 기질종양, 뇌척수의 악성종양, 소아신경모세포종, 원발불명암** | **HER2, EGFR, ALK, KRAS, NRAS, BRAF, BRCA1, BRCA2, KIT, PDGFRA, IDH1, IDH2, MYC(CYC(C-myc), N-myc (MYCN)** |
| 형질세포종 | NRAS, KRAS, TP53 |
| 급성 골수성 백혈병 | CEBPA, FLT3, JAK2, KIT, NPM1, RUNX1, TP53, DH1, DH2 |
| 급성림프구성 백혈병 | TP53, RB1, JAK2, NRAS, IKZF1 |
| 골수형성이상, 골수증식종양 | ASXL1, CALR, CSF3R, DNMT3A, JAK2, MPL, RUNX1, SETBP1, SF3B1, SRSF2, TET2 |
| 악성림프종 | MYD88, BRAF, TP53 |

2) RNA fusion gene 검사 시는 급성백혈병에서만 필수유전자를 아래와 같이 함.

– ABL1, BCR, CBFB, ETV6, KMT2A, PML, RARA



[별첨3] 차세대염기서열분석기반(NGS, Next Generation Squencing) 유전자패널검사 실시 조건

| 구 분 | | 내 용 |
|---|---|---|
| 사전승인 조건 | 시설 | ○ 생명윤리 및 안전에 관한 법률」제49조에 따른 유전자검사 기관으로 신고된 요양기관이면서,<br>○ 한국유전자검사평가원의 "유전자검사 정확도 평가"를 3회 이상 받은 이력이 있고, 승인신청 직전 평가 결과 'A'등급(우수)인 기관 |
| | 인력 | ○ 전문의 자격 취득 후 5년 이상의 경험이 있는 병리과 또는 진단검사의학과 전문의 1인 이상 상근하고,<br>○ 검사 실시인력(임상병리사) 1인 이상이 상근 |
| | 장비 | ○ 식약처 허가 또는 신고를 받은 '차세대염기서열분석장비' 사용 또는<br>○「식약처 NGS 임상검사실 인증」요양기관의 경우에는 식약처 허가 또는 신고를 받지 않은 '유전자서열검사장비' 사용을 인정 |
| | 구성 | ○ 필수유전자는「요양급여의 적용기준 및 방법에 관한 세부사항」중「차세대염기서열분석(NGS)기반 유전자패널검사의 급여기준」의 급여대상 질환 필수 유전자를 포함해야 하며,<br>○ 선택유전자는「생명윤리 및 안전에 관한 법률」제49조에 따라 질병관리본부에 신고된 유전자 범위내에서 선택함<br>→ 신청기관은 운영하는 패널에 대한자료(패널명, 검체, 적응증, 유전자수 또는 유전자 길이, 필수유전자, 선택유전자)를 승인 신청시에 제출하여야 함. |
| 동의서 | | ○「생명윤리 및 안전에 관한 법률 시행규칙」[별지 제41호 서식]의 '인체유래물 등의 기증 동의서'에 동의를 받아 보관<br>○ 추후 국가 유전체 사업 등 공익적 연구 목적으로 제출을 요청받은 경우 동의한 유전 정보를 제출하여야 함 |
| 실시내역 및 내부 평가 보고서 제출 | | ○ NGS 유전자패널검사의 검사실시내역 등을 분기별로 작성하여 제출<br>○ 승인기간의 종료 전까지 유전자 패널에 대한 내부 평가 보고서를 건강보험심사평가원장에게 제출하여야 함 |
| 관리주체 | | ○ 실시 요양기관 승인 절차에 대한 진행과 검사 실시내역 및 유전자 패널 내부 평가 보고서 제출 등 관리는 건강보험심사평가원장이 실시하여 보건복지부 장관에게 보고하여야 함 |