

Towards high-quality clinical trials and  
the implementation of genomic medicine

# ATLAS Training Program

Course: Cancer Genomic Medicine: Essential Course

Lecture: Next-generation sequencing principles and analysis files

Speaker: Takashi Kubo

# Takashi Kubo, Ph.D.

Department of Laboratory Medicine, National Cancer Center Hospital

## EDUCATION

Kyorin University School of Health Sciences, Japan (1990 - 1994)

Kyorin University Graduated School of Health Sciences, Japan (1994-1999)

## WORK EXPERIENCE

Research resident fellowship, Radiobiology Division, National Cancer Center Research Institute (1999 – 2002)

Postdoctoral fellow, Division of Pharmacology, National Institute of Health Sciences (2002 – 2005)

Fellowship researcher, Cancer Genomics Project, National Cancer Center Research Institute (2006 – 2009)

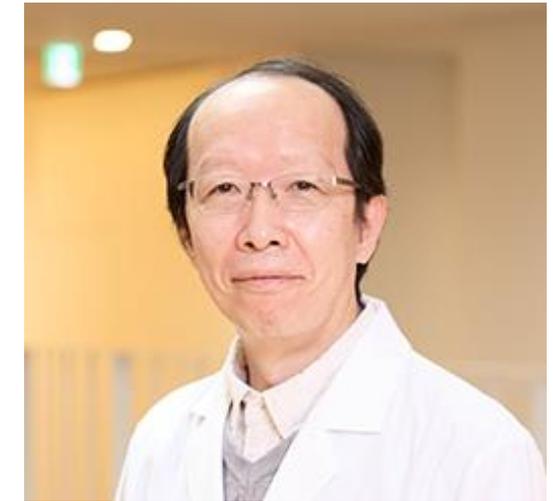
Fellowship researcher, Molecular Oncology Division, National Cancer Center Research Institute (2009 – 2010)

Research assistant, Division of Pharmacology, National Institute of Health Sciences (2010 – 2013)

Staff scientist, Division of Translational Genomics, National Cancer Center EPOC (2014 – 2019)

Staff scientist, Department of Clinical Genomics, National Cancer Center Research Institute (2014 – present)

Medical technologist, Division of Clinical Laboratory, National Cancer Center Hospital (2019 – present)



# Objectives

- Understand the flow of gene panel testing
- Understand the principles of next-generation sequencing (NGS)
- Learn about data formats for analyses

# Overview

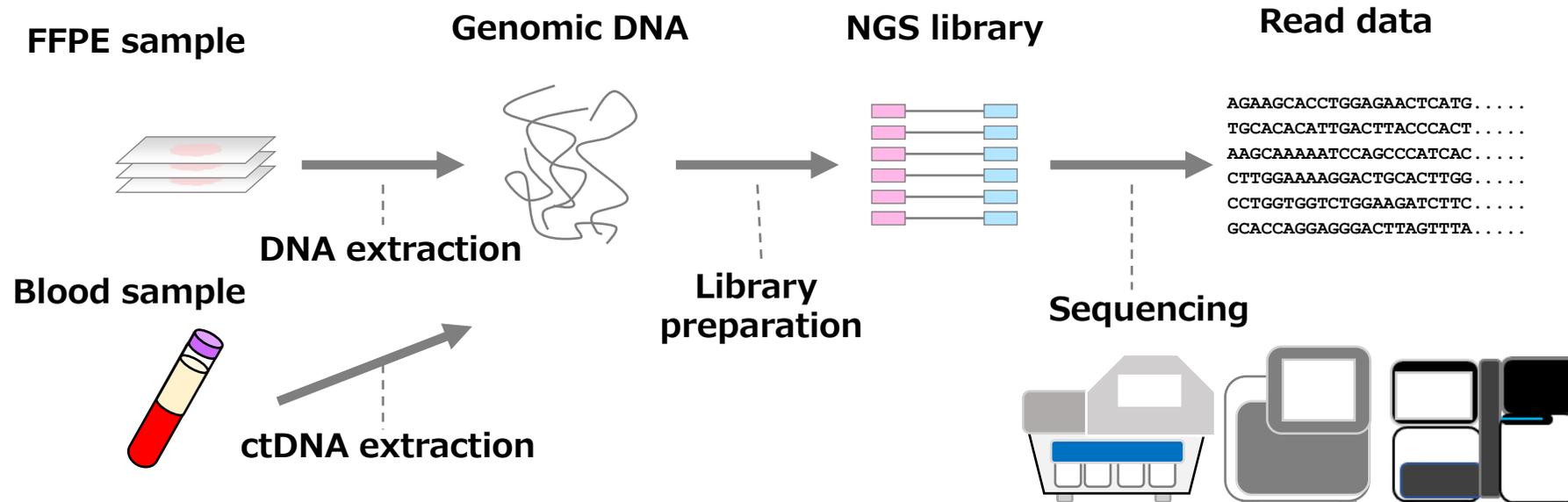
- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- Gene panel test workflow (Dry bench process)
- File format output in NGS analyses

# Overview

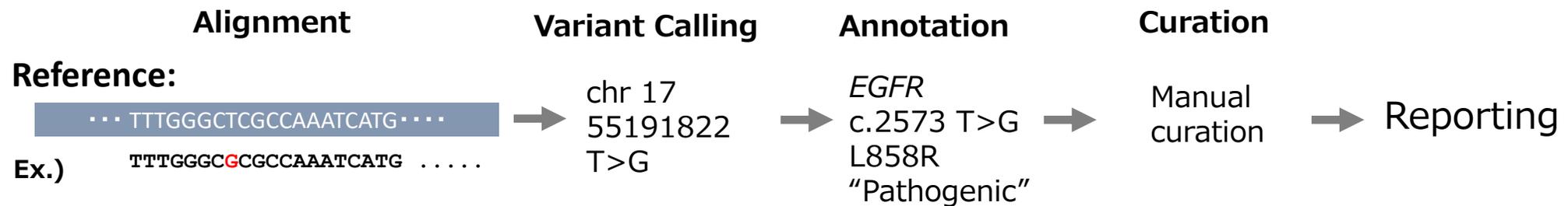
- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- Gene panel test workflow (Dry bench process)
- Data file output by NGS analysis

# Overview of the main steps in the NGS workflow

## Wet bench process

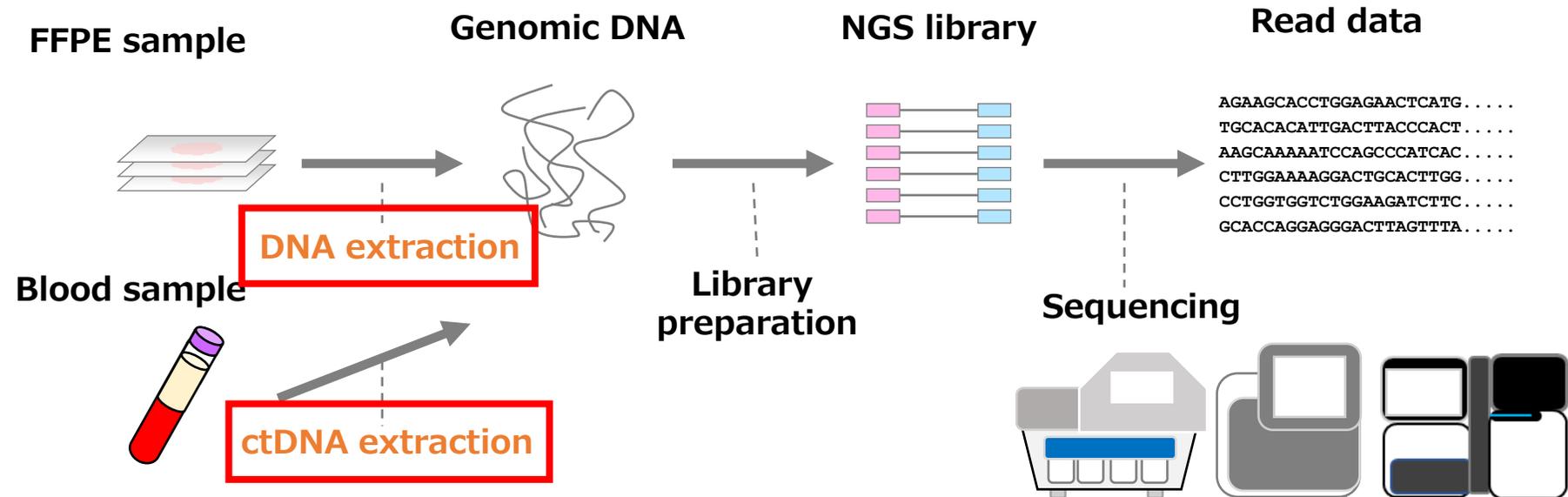


## Dry bench process

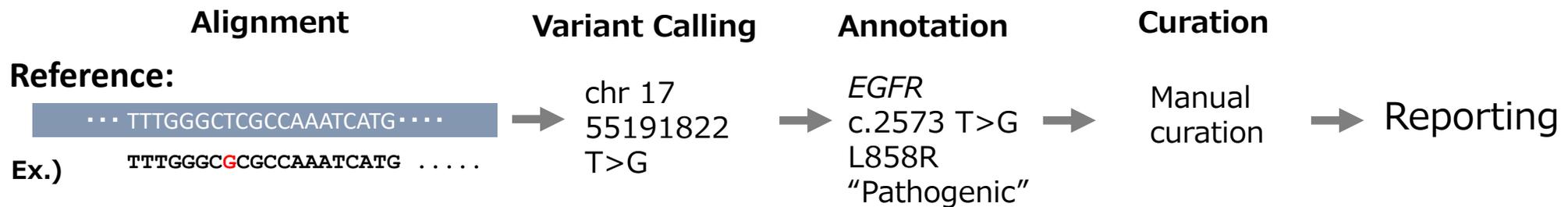


# Overview of the main steps in the NGS workflow

## Wet bench process

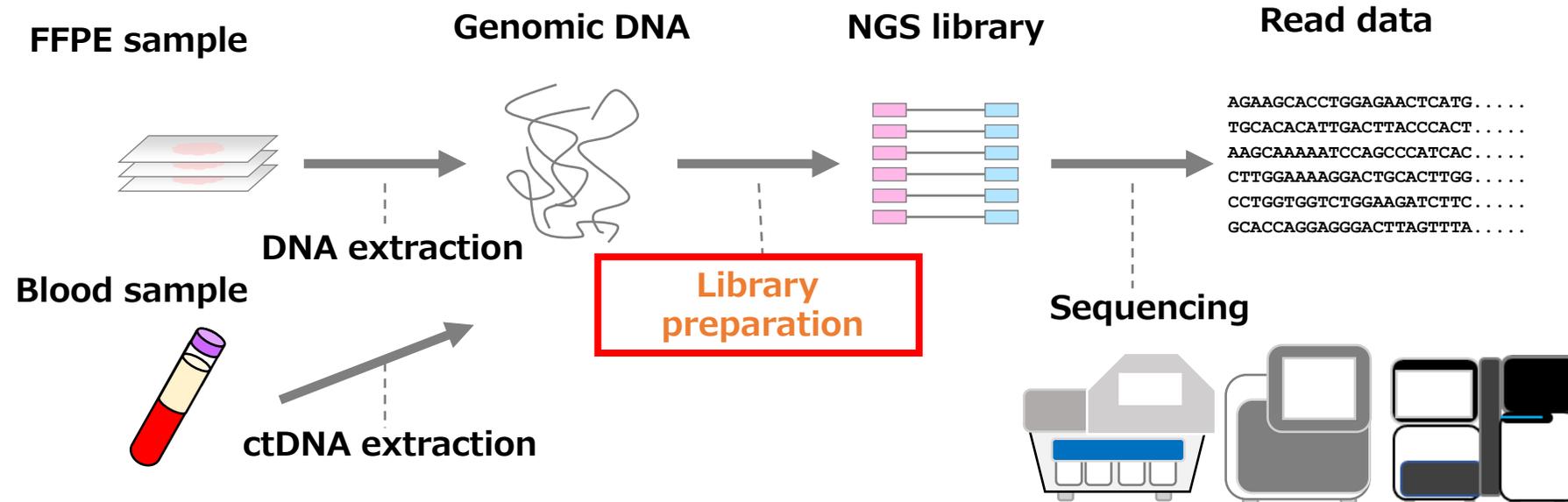


## Dry part

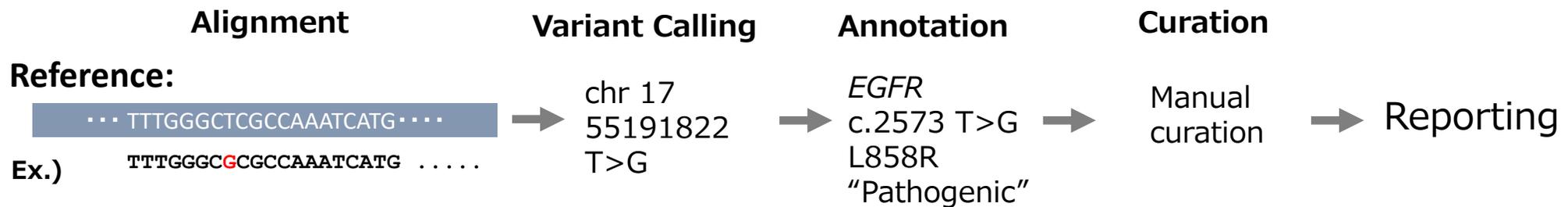


# Overview of the main steps in the NGS workflow

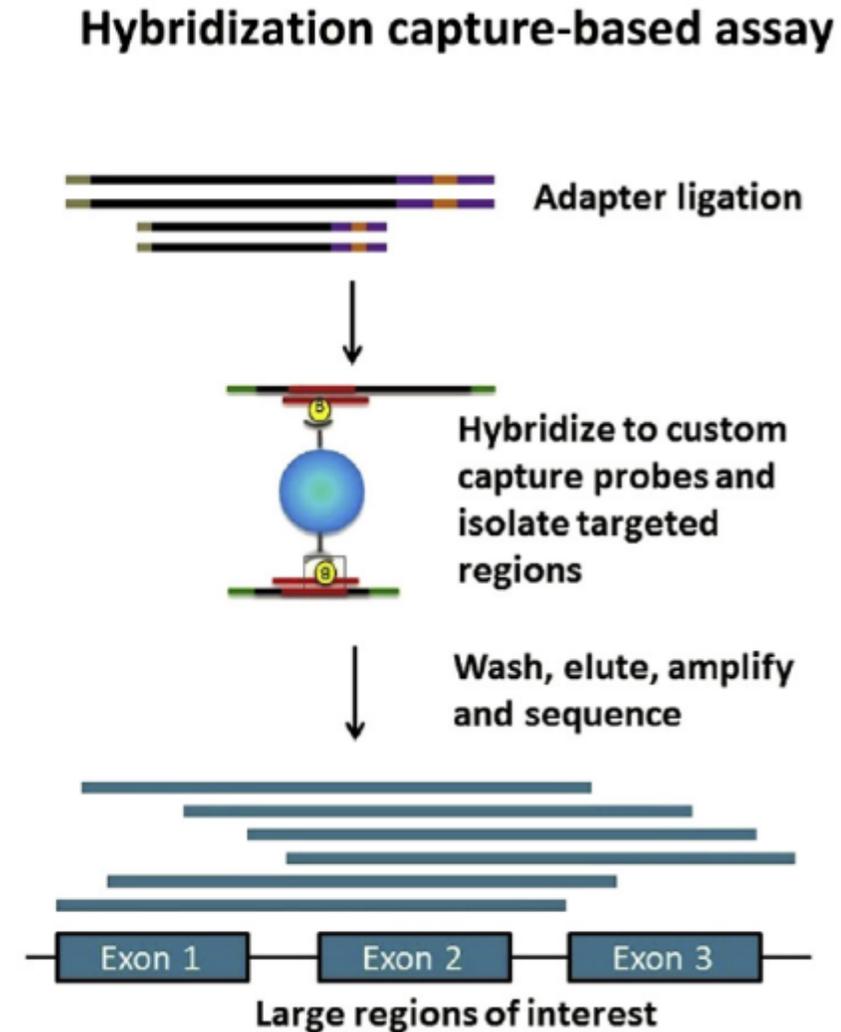
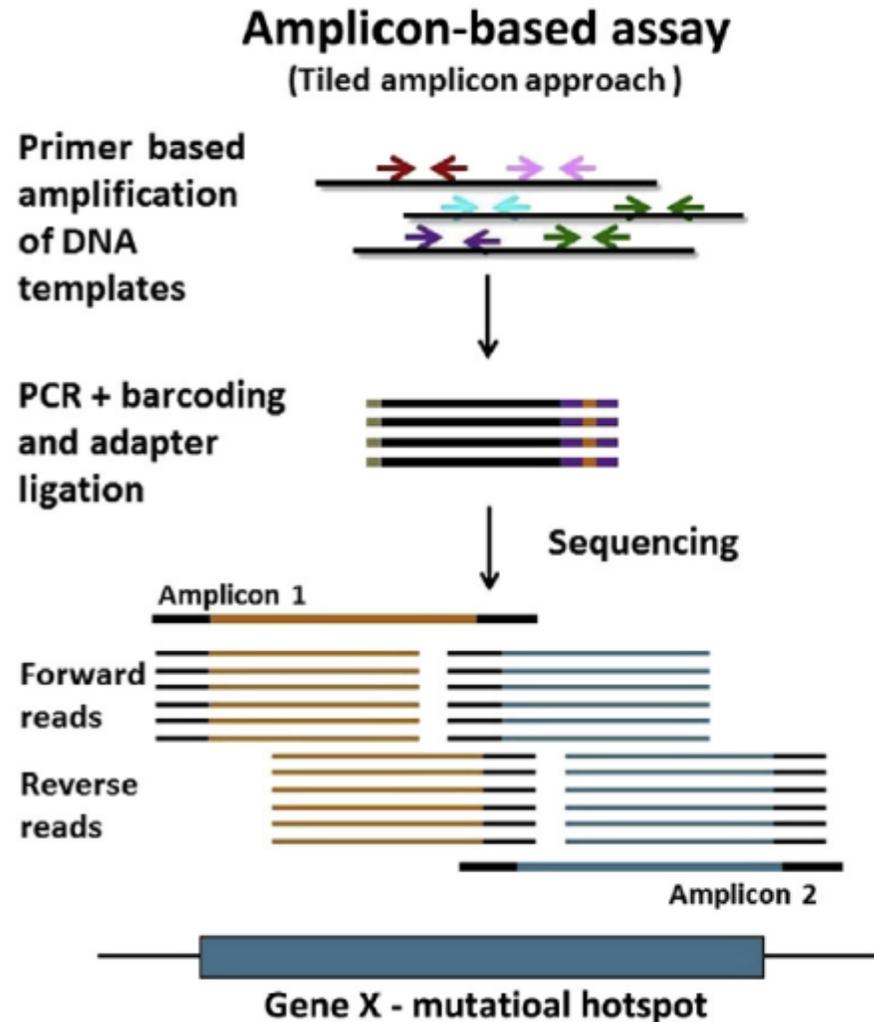
## Wet bench process



## Dry part



# Targeted Sequencing Methods



# Characteristics of each library preparation method

	Amplicon-based assay	Hybridization capture-based assay
Necessary amount of DNA	Can be performed with a small amount	Better with a high amount
Work process/required time	Short	Long
Target sequence	Suitable for small regions	Suitable for wide regions
Uniformity of coverage	Low	High
Target sequence stringency	Important	Flexible, to some extent
Test in use	Oncomine Dx TT	Foundation One CDx Foundation One Liquid CDx OncoGuide NCC Oncopanel
	➡ For companion diagnostic tests	➡ Suitable for genomic profiling tests

➡ For companion diagnostic tests

➡ Suitable for genomic profiling tests

# Basic structure of DNA molecules that make up the library (e.g., for Illumina NGS)

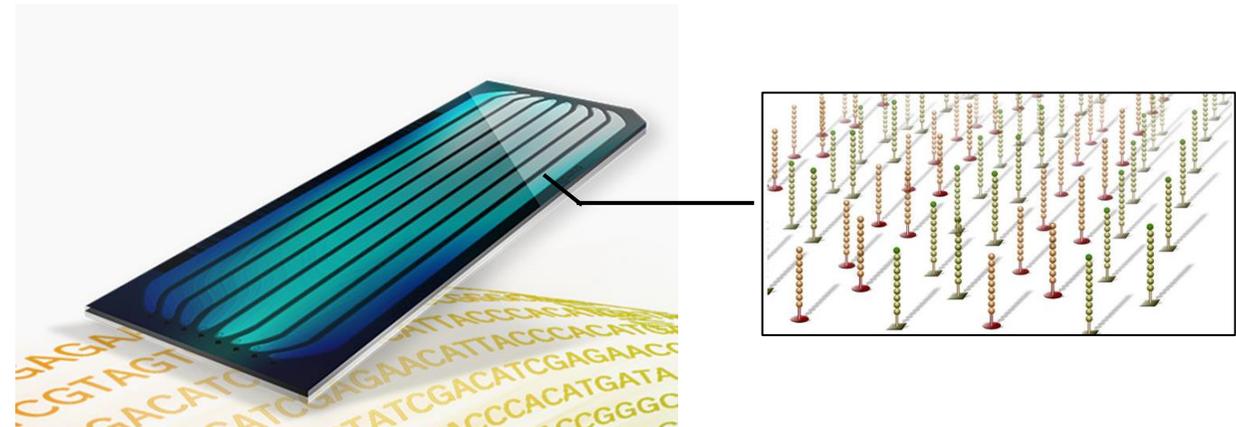


**DNA Insert:** DNA to be analyzed fragmented into hundreds of bp

**P5, P7:** sequences that bind to flow cells

**SP:** Sequencing primer binding sites

**Index:** A sequence for specimen identification



Flow cell (HiSeq 4000)

# Overview

- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- Gene panel test workflow (Dry bench process)
- Data file output by NGS analysis

# Next-generation sequencing (NGS) systems

Illumina Inc.

NGS machine



NextSeq 550Dx



HiSeq 4000

Bridge PCR on fluorescence/flow cells

Sequencing By synthesis (SBS)

120 Gb

1300 to 1500 Gb

Ion Torrent  
(Thermo Fisher Scientific)



Ion PGM Dx

pH change/emulsion PCR

Proton measurement method

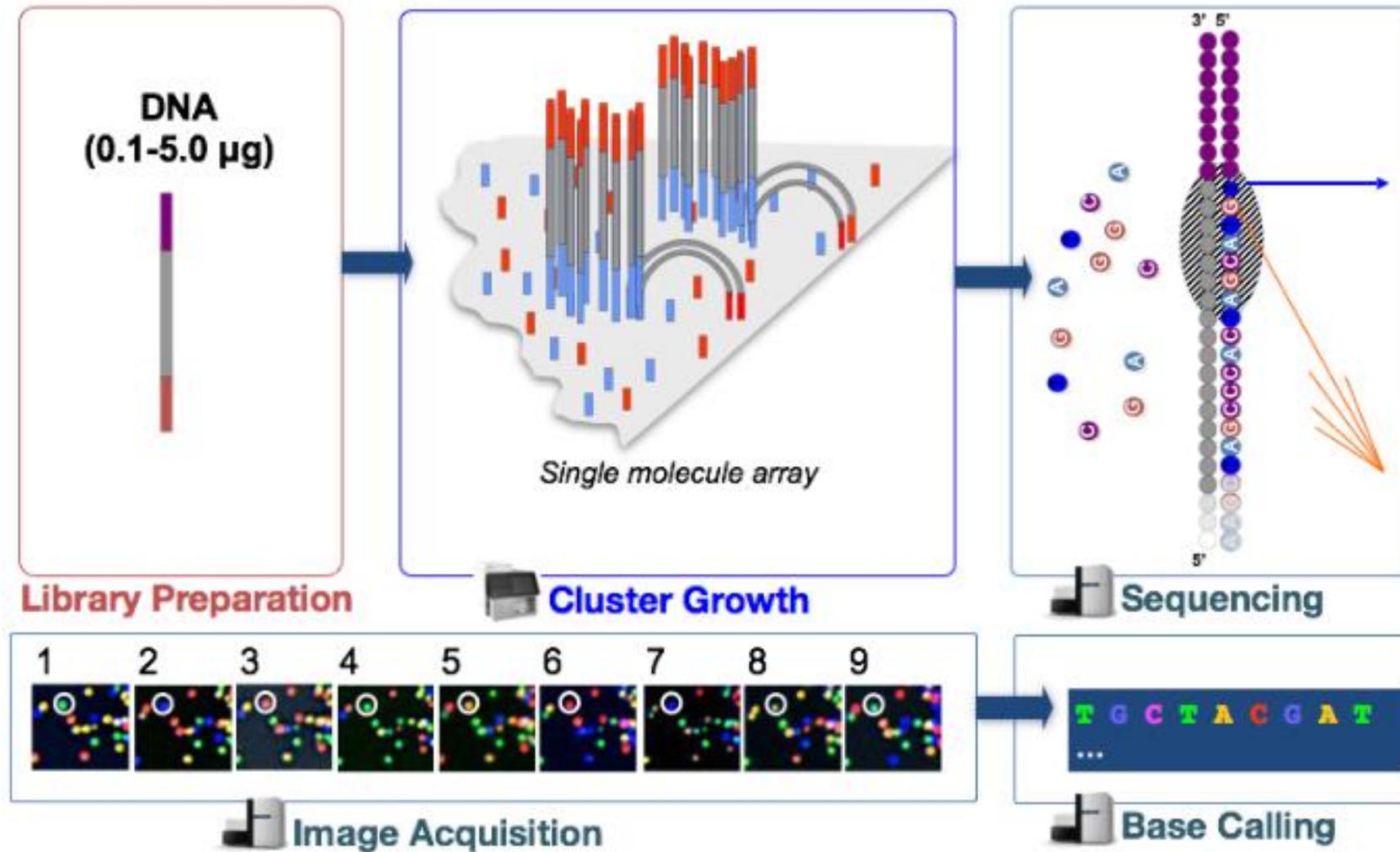
600 Mb to 1 Gb

Detection target/Enhancement method

Measurement principle

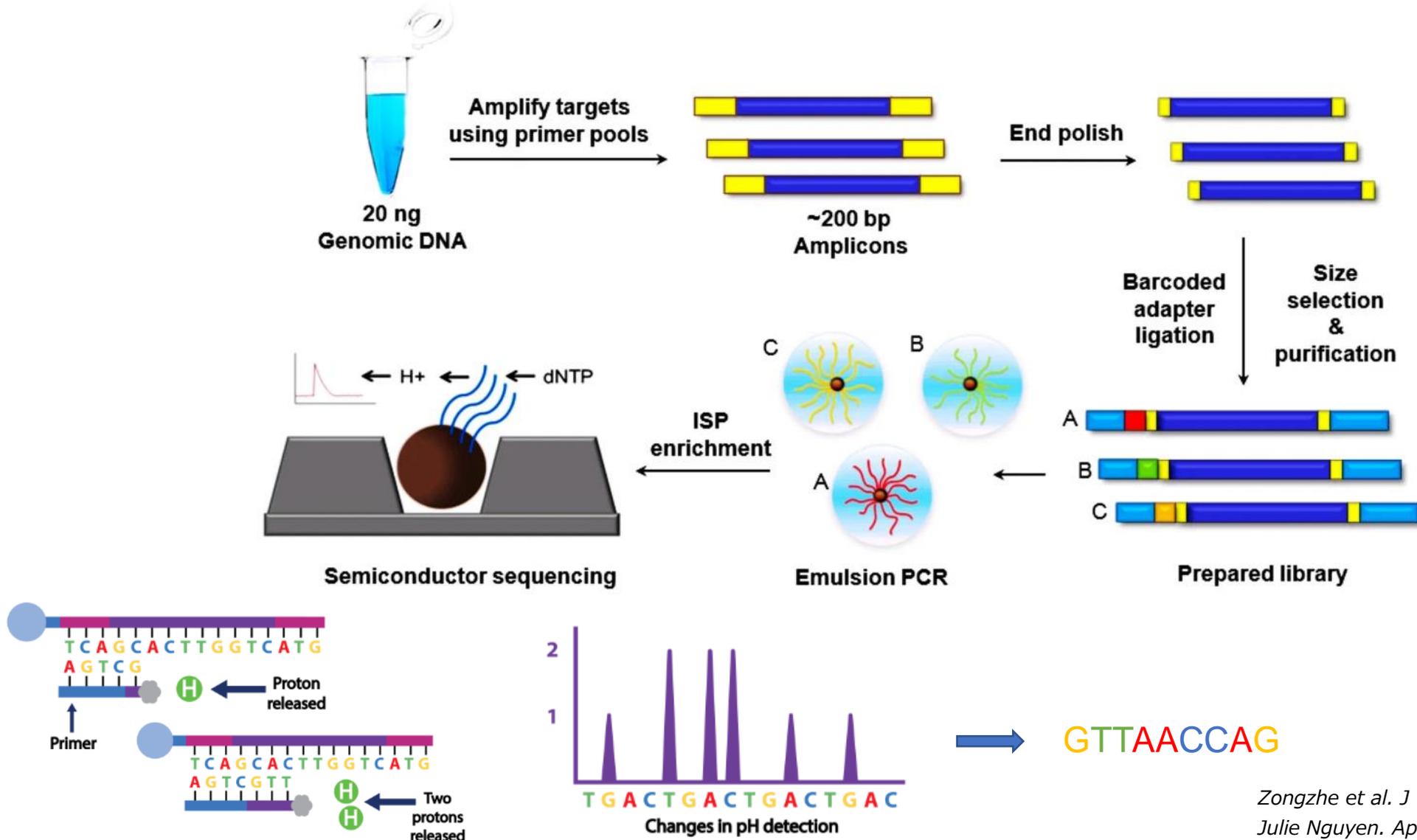
Output data volume/Run

# Sequencing By Illumina NGS



Mary Piper and Radhika Khetani. *Intro-to-ChIPseq in Github, modified*

# Sequencing By Ion Torrent NGS



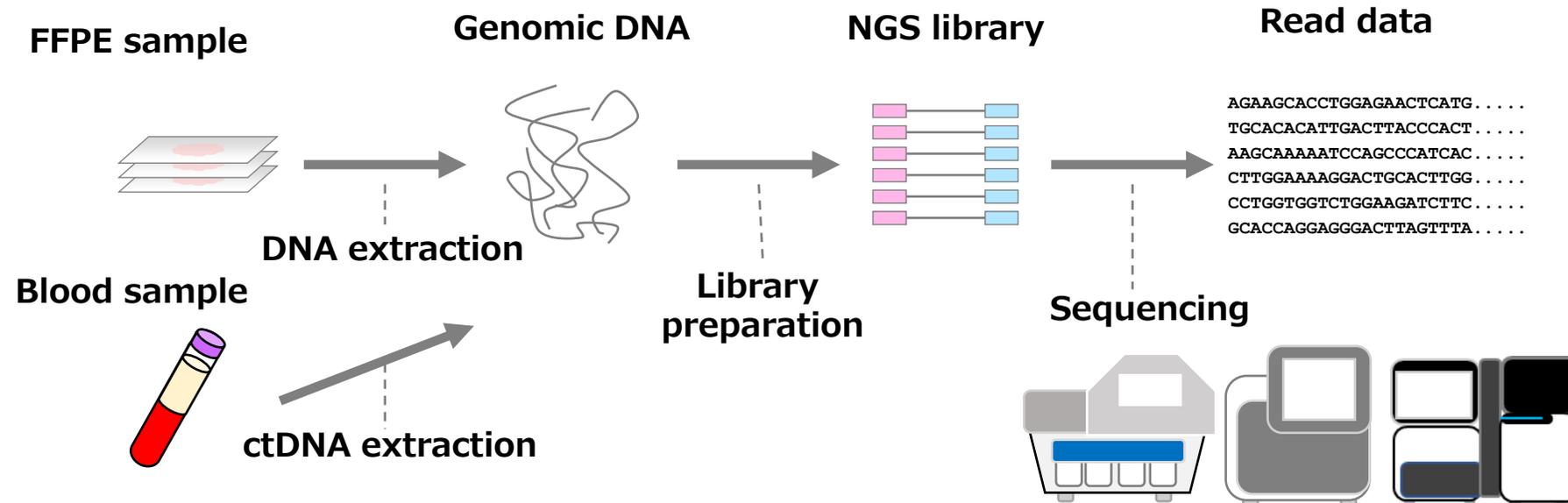
Zongzhe et al. *J Transl. Medicine* 173, 2014, modified  
 Julie Nguyen. *Apollo Institute web site*, modified

# Overview

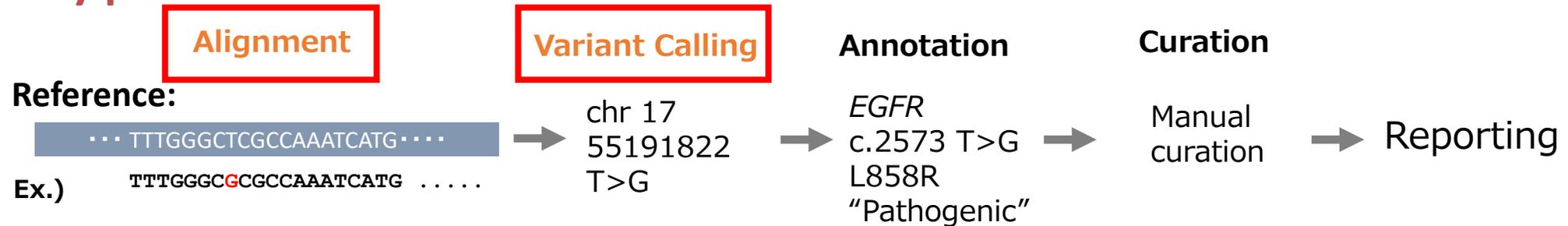
- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- **Gene panel test workflow (Dry bench process)**
- Data file output by NGS analysis

# Overview of the main steps in the NGS workflow

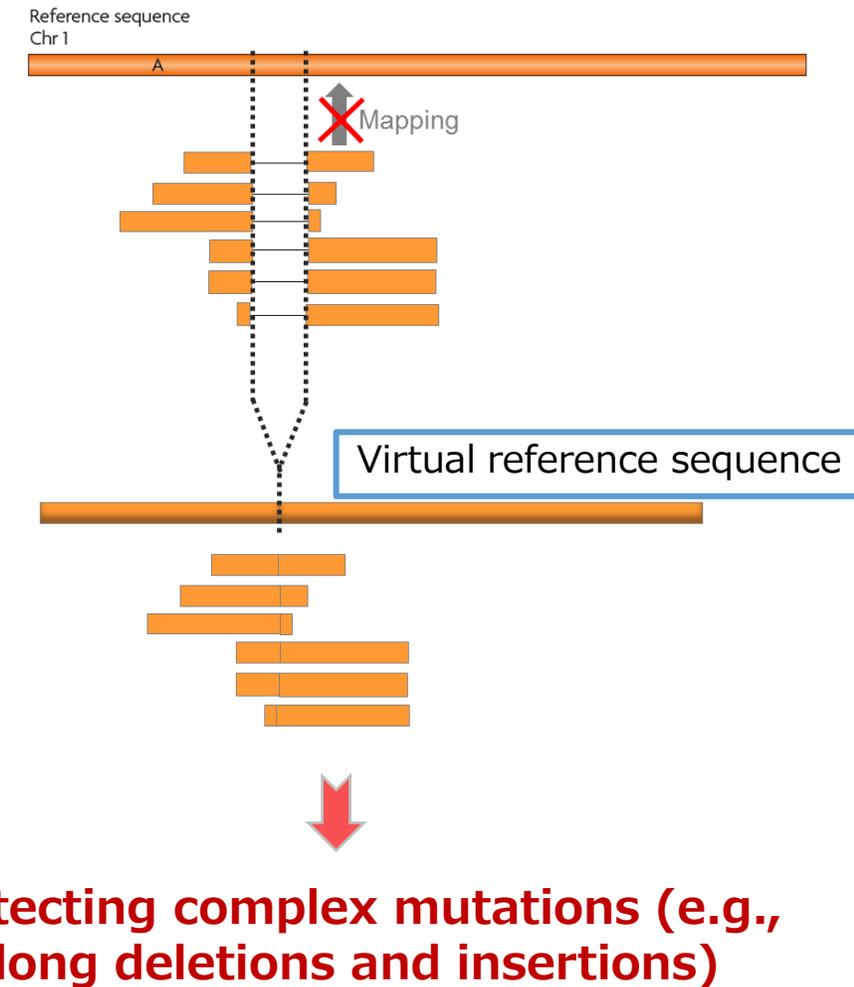
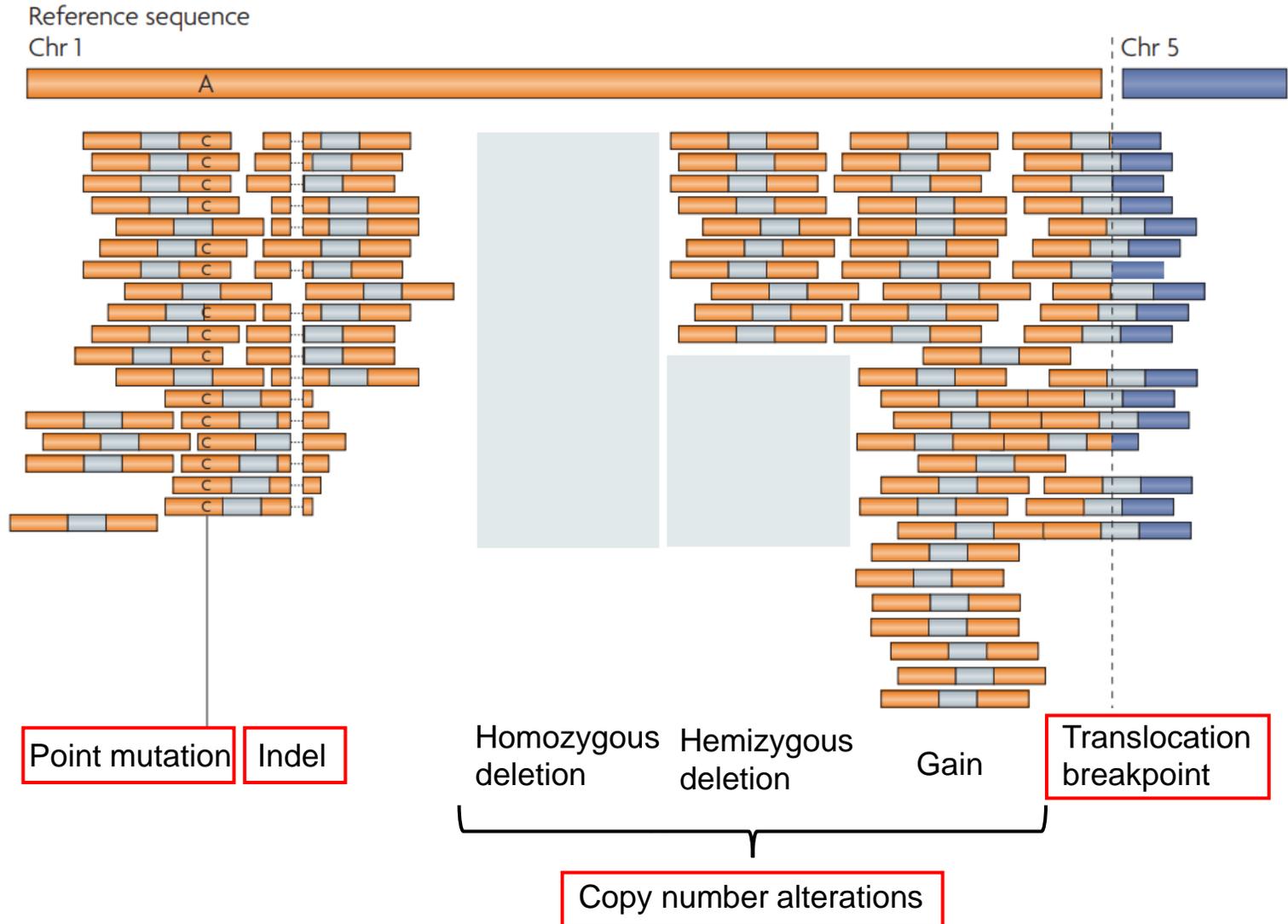
## Wet bench process



## Dry part

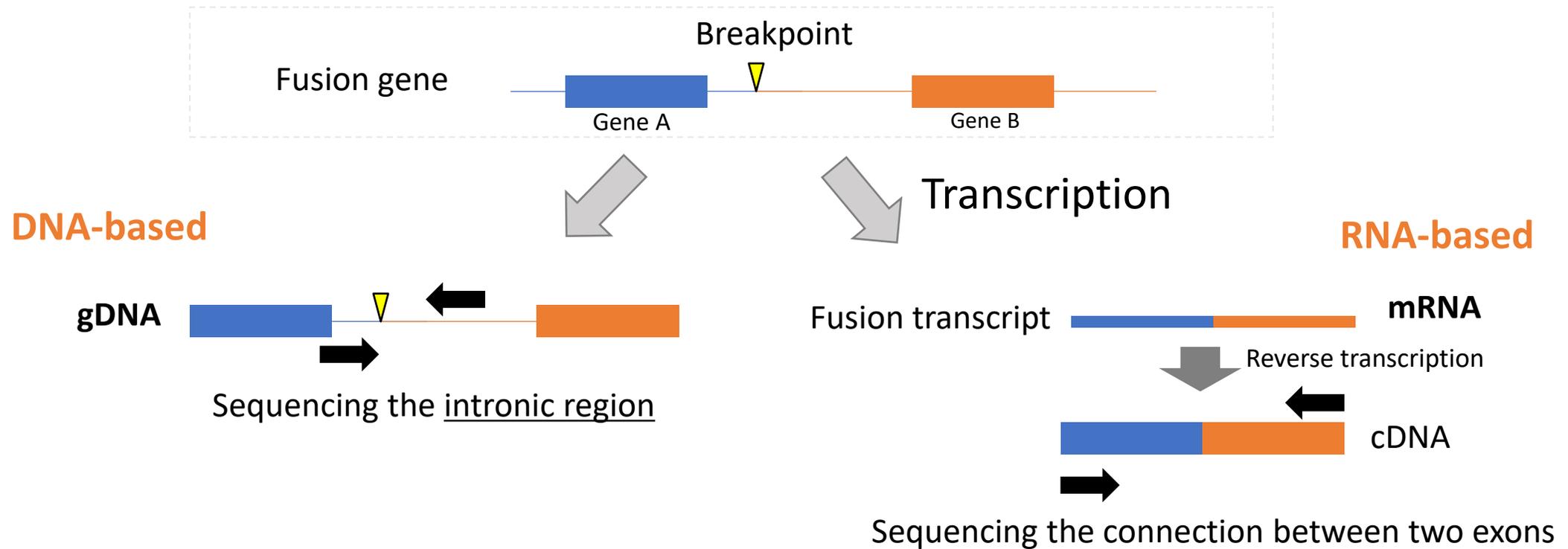


# Detection of gene mutations, amplifications/deletions, and fusions



Meyerson *et al.*, *Nat Rev Genet* **11**, 685 (2010), modified

# DNA- vs. RNA-based tests for detecting fusion genes



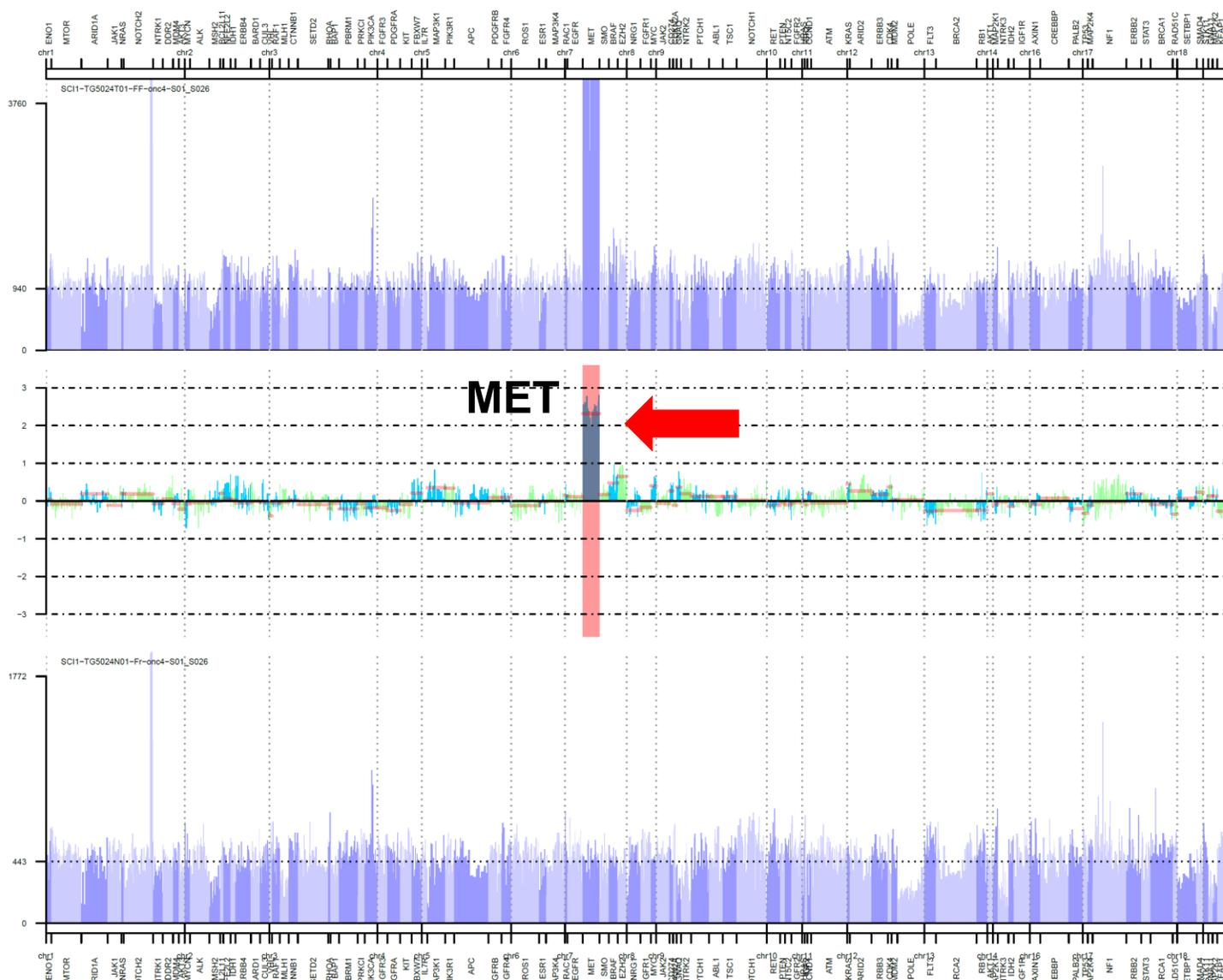
# Amplification/deletion analysis

Copy number ratio

Tumor tissue

(tumor) - (normal)

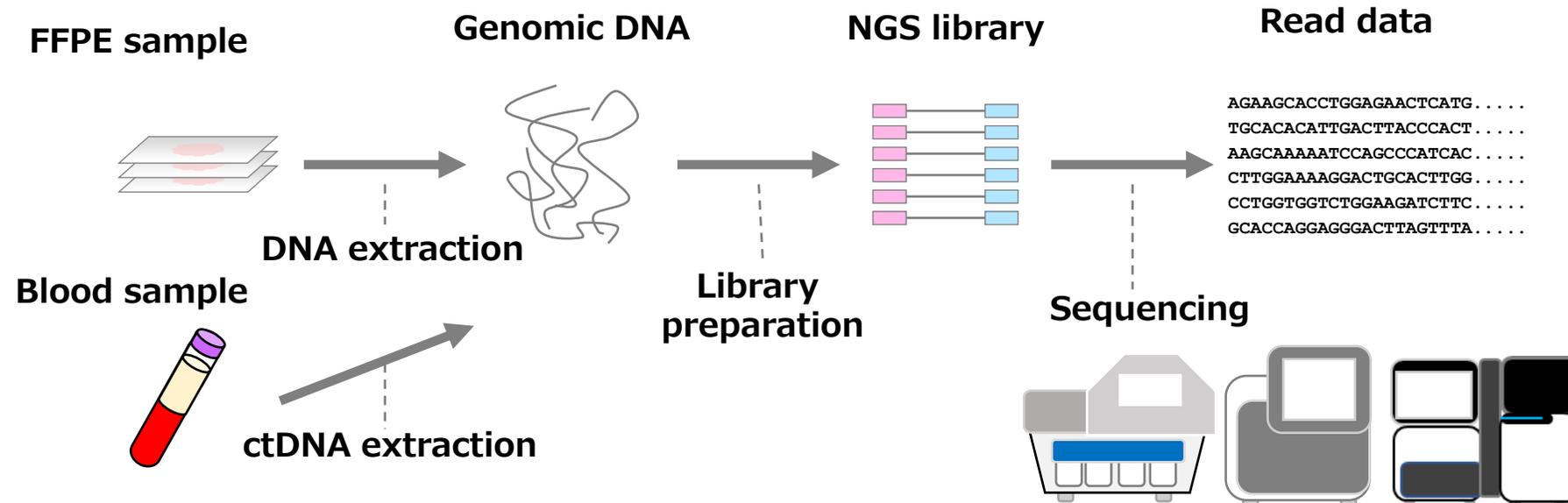
Normal Tissue



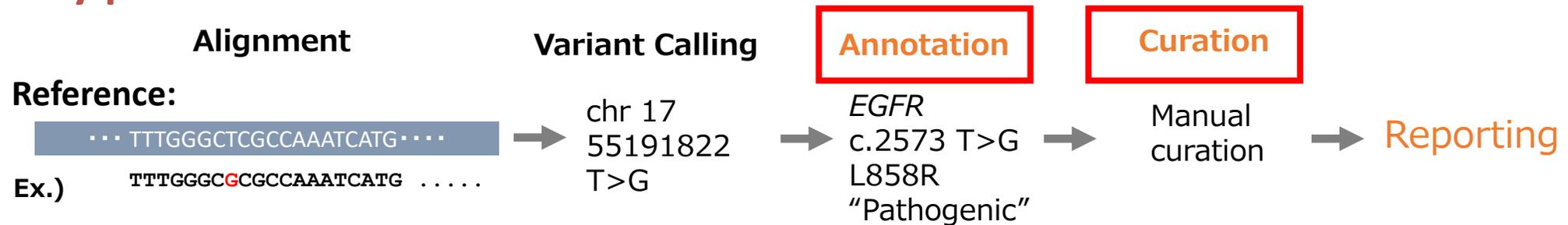
1	2	3	4
Chr	Gene	Matched SCIT-TG?	Matched SCIT-TG?
39	5 PDGFRB	0.049833023	Not Amplified
40	5 FGFR4	0.06378649	Not Amplified
41	6 ROS1	-0.095340778	Not Amplified
42	6 ESR1	-0.032254974	Not Amplified
43	6 MAP3K4	-0.046316759	Not Amplified
44	7 RAC1	0.107825619	Not Amplified
45	7 EGFR	0.041985642	Not Amplified
66	7 MET	2.403834415	Amplified
47	7 SMO	0.169437069	Not Amplified
48	7 BRAF	0.389560012	Not Amplified
49	7 EZH2	0.660616221	Not Amplified
50	8 NRG1	-0.110659849	Not Amplified
51	8 FGFR1	-0.255713441	Not Amplified
52	8 MYC	0.406503583	Not Amplified
53	9 JAK2	0.02944836	Not Amplified
54	9 CD274	0.411647442	Not Amplified
55	9 CDKN2A	-0.11073413	Not Amplified
56	9 GNAQ	0.366262943	Not Amplified
57	9 NTRK2	0.282641559	Not Amplified
58	9 PTCH1	0.120612122	Not Amplified
59	9 ABL1	-0.019033239	Not Amplified
60	9 TSC1	0.107717038	Not Amplified
61	9 NOTCH1	-0.049057647	Not Amplified
62	10 RET	-0.12507721	Not Amplified
63	10 PTEN	-0.069297297	Not Amplified
64	10 NT5C2	0.050348424	Not Amplified
65	10 FGFR2	-0.027340331	Not Amplified
66	11 HRAS	0.074482023	Not Amplified

# Overview of the main steps in the NGS workflow

## Wet bench process



## Dry part



# Reporting (FoundationOne CDx)

## MHLW APPROVED CLAIMS

**FOUNDATIONONE<sup>®</sup>CDx** PATIENT: -2021-03500203\_04 TUMOR TYPE: Colon adenocarcinoma (CRC) REPORT DATE: 17 Nov 2021 ORDERED TEST #: [REDACTED]

**Companion Diagnostic (CDx) Associated Findings**

GENOMIC FINDINGS DETECTED	APPROVED THERAPEUTIC OPTIONS IN JAPAN
<b>KRAS/NRAS</b> wildtype (codons 12, 13, 59, 61, 117, & 146)	Cetuximab (genetical recombination) Panitumumab (genetical recombination)

**OTHER ALTERATIONS & BIOMARKERS IDENTIFIED**  
Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See professional services section for additional information.

**Microsatellite status** MS-Stable<sup>§</sup>  
**Tumor Mutational Burden** 4 Muts/5 Mb<sup>§</sup>  
**ACVR1B** D324fs\*11

**AKT1** E17K  
**BRAF** V600E  
**RET** V804M

*§ Refer to appendix for limitation statements.  
Please refer to appendix for Explanation of Clinical Significance Classification and/or variants of unknown significance (VUS).*

**Intended Use Overview**

GENOMIC ALTERATION	TYPE OF CANCER	CONSEQUENT DRUGS
GNMT1A/ACT11A/AS1818	Non-small-cell lung cancer	Alectinib, osimertinib, hydrochlorothiazide, gliclazide, or vildagliptin
ACT11A/AS1818	Non-small-cell lung cancer	Osimertinib
EGFR exon 20 T790M alterations	Non-small-cell lung cancer	Osimertinib
ALL fusion genes	Non-small-cell lung cancer	Alectinib, hydrochlorothiazide, osimertinib, vildagliptin, or gliclazide
RET exon 14 fusing alterations	Non-small-cell lung cancer	Entrectinib
BRM V502E or V503R alterations	Non-small-cell lung cancer	Cepanotril hydrochloride hydrate
EGFR copy number alterations (EGFR gene amplification pathway)	Colorectal cancer	Trastuzumab, trastuzumab emtansine, or trastuzumab deruxtrcan
BRCA1/BRCA2 wildtype	Colorectal cancer	Cetuximab (genetical recombination) or panitumumab (genetical recombination)
Microsatellite instability-high	Colorectal cancer	Trastuzumab (genetical recombination)
Microsatellite instability-high	Solid tumors	Pembrolizumab (genetical recombination)
KIT/KIF5B fusion genes	Solid tumors	Entrectinib, trametinib, or selpercatinib
BRCA2 alterations	Ovarian cancer	Oligomycin
BRCA1 alterations	Prostate cancer	Oligomycin
ESRRB fusion genes	Kidney metastatic cancer	Everolimus

*\* Absence of mutations in columns 12, 13, 59, 61, 117, & 146*

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 Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141-1124 | CLIA: 2202027531  
 Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141-1124 | CLIA: 2202027531  
 Post-Sequencing Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141-1124 | CLIA: 2202027531  
 Foundation Medicine, Inc.

MHLW APPROVED CLAIMS - PAGE 1 of 1

## PROFESSIONAL SERVICES

**FOUNDATIONONE<sup>®</sup>CDx** TUMOR TYPE: Colon adenocarcinoma (CRC) REPORT DATE: 17 Nov 2021 ORDERED TEST #: ORD-1233312-01

**Interpretive content on this page and subsequent pages is provided as a professional service, and is not reviewed or approved by the FDA.**

**Sensitivity for the detection of copy number alterations is reduced due to sample quality.**

**FOUNDATIONONE<sup>®</sup>CDx** TUMOR TYPE: Colon adenocarcinoma (CRC) REPORT DATE: 17 Nov 2021 ORDERED TEST #: ORD-1233312-01

GENOMIC FINDINGS	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
<b>BRAF</b> = V600E	Encorafenib + Cetuximab [2A] Dabrafenib + Trametinib Panitumumab	Dabrafenib + Trametinib Encorafenib + Binimetinib Selumetinib Trametinib
10 Trials see p. 20		Vemurafenib Lemurafenib + Cobimetinib
<b>KRAS</b> = wildtype	Cetuximab	none
0 Trials	Panitumumab	
<b>NRAS</b> = wildtype	Cetuximab	none
0 Trials	Panitumumab	
<b>AKT1</b> = E17K	none	none
10 Trials see p. 18		
<b>RET</b> = V804M	none	none
10 Trials see p. 22		

**Microsatellite status** MS-Stable<sup>§</sup>  
**Tumor Mutational Burden** 4 Muts/5 Mb<sup>§</sup>

**ACVR1B** D324fs\*11

**AKT1** E17K  
**BRAF** V600E  
**RET** V804M

*§ Refer to appendix for limitation statements.  
Please refer to appendix for Explanation of Clinical Significance Classification and/or variants of unknown significance (VUS).*

**VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING IN SELECT CANCER SUSCEPTIBILITY GENES**

Findings below have been previously reported as pathogenic germline in the ClinVar genomic database and were detected at an allele frequency of >10%. See appendix for details.

**RET - V804M** p. 9

This report does not indicate whether variants listed above are germline or somatic in this patient. In the appropriate clinical context, follow-up germline testing would be needed to determine whether a finding is germline or somatic.

**GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS**

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

**ACVR1B - D324fs\*11** p. 10

**PROFESSIONAL SERVICES - PAGE 2 of 23**

## APPENDIX

**FOUNDATIONONE<sup>®</sup>CDx** TUMOR TYPE: Colon adenocarcinoma (CRC) REPORT DATE: 17 Nov 2021 ORDERED TEST #: ORD-1233312-01

**APPENDIX Variants of Unknown Significance**

**CASP8** P45Q  
**P2RY8** M344I

**EPH1B1** A872V  
**PBRM1** Q1346H

**KIT** A755T

**MAP2K4** H364Y

**NOTE:** One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

**FOUNDATIONONE<sup>®</sup>CDx** TUMOR TYPE: Colon adenocarcinoma (CRC) REPORT DATE: 17 Nov 2021 ORDERED TEST #: ORD-1233312-01

**APPENDIX References Associated with Professional Services Content**

1. Gaultis J, et al. Cancer Epidemiol Biomarkers Prev. 2014; 23(12):2372-79	48. Roth BA, et al. Science. 2015; 349:1087-91	91. Hsu HC, et al. Oncotarget. 2016; 7(26):38987-97
2. Fritzsche-Ober A, et al. Oncotarget. 2015; 6(26):21402-10	49. Johnson BE, et al. Science. 2014; 343:1367-70	92. Sammut MG, et al. Clin. Cancer Res. 2017; 23(12):2372-79
3. Liu N, et al. Oncotarget. 2015; 6(26):21402-10	50. Choi S, et al. Neuro-oncology. 2016; 24(4):247-53	93. Haddadfarid M, et al. Nat. Rev. Cancer. 2014; 14(12):797-99
4. Laif N, et al. Oncotarget. 2015; 6(26):21402-10	51. Chang S, et al. Neuro-oncology. 2016; 24(4):247-53	94. Burchett M, et al. Cancer. 2014; 124(12):3047-57
5. Ayres AT, et al. 2016 ASCO Abstract 2016	52. Braganca A, et al. Pathol. 2013; 2013:2343-70	95. Davies K, et al. Nature. 2002; 415:823-28
6. Carabelli A, et al. 2016 ASCO Abstract 138A-004	53. Braganca A, et al. Pathol. 2013; 2013:2343-70	96. Kuroki K, et al. Nature. 2013; 501:392-95
7. Sirocica PA, et al. Clin. Oncol. 2013; 24(19):39	54. Yager R, et al. Curr. Opin. Genet. Dev. 2014; 24(1):1-7	97. Graweo WO, et al. Mol. Diagn. 2013; 13(2):173-76
8. Gao H, et al. Clin. Cancer Res. 2012; 18(24):7538-45	55. Roberts SA, et al. Nat. Rev. Cancer. 2014; 14(12):797-99	98. Khan CL, et al. Eur. J. Cancer. 2013; 49(12):2277-81
9. Bujdak MM, et al. J. Clin. Oncol. 2009; 27(17):2757-61	56. Carrozzini M, et al. Clin. Cancer Res. 2014; 20(12):3157-65	99. Hsu HC, et al. Clin. Cancer Res. 2014; 20(12):3157-65
10. Van Cutsem E, et al. Clin. Oncol. 2009; 20(12):3157-65	57. Hyman DM, et al. N. Engl. J. Med. 2013; 369(11):2505-12	100. Yager R, et al. Cancer Manag Res. 2013; 2013:204-16
11. Ribic CM, et al. N. Engl. J. Med. 2007; 356(26):2483-92	58. Yager R, et al. Clin. Cancer Res. 2013; 19(26):8623-31	101. Kuan T, et al. Cancer. 2013; 113(12):2273-76
12. Sargent RD, et al. J. Clin. Oncol. 2009; 27(24):3928-35	59. Kageura M, et al. J. Clin. Oncol. 2013; 31(26):3342-47	102. Pooni G, et al. Eur. J. Cancer. 2013; 49(12):2277-81
13. Park B, et al. Clin. Cancer Res. 2003; 9(12):1623-24	60. Kageura M, et al. 2020 ESCO Abstract 50-26	103. Yager R, et al. J. Clin. Oncol. 2013; 31(26):3342-47
14. Gaudreault C, et al. Eur. J. Cancer. 2010; 46(12):2047-55	61. Carrozzini M, et al. 2020 ASCO Abstract 121	104. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
15. Park B, et al. Clin. Cancer Res. 2010; 16(12):3678-85	62. Carrozzini M, et al. Clin. Oncol. 2013; 24(12):3047-57	105. Kuan T, et al. Cancer. 2013; 113(12):2273-76
16. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	63. Akerkar S, et al. ASCO Abstract 1918	106. Pooni G, et al. Clin. Oncol. 2014; 26(12):3157-65
17. Nishio K, et al. J. Clin. Oncol. 2013; 31(26):3342-47	64. Papatrakis F, et al. Eur. J. Cancer. 2013; 49(12):2277-81	107. Graweo WO, et al. Mol. Diagn. 2013; 13(2):173-76
18. Hsing AH, et al. Am. J. Pathol. 2011; 177(4):626-34	65. Kageura M, et al. J. Clin. Oncol. 2013; 31(26):3342-47	108. Hsu HC, et al. Clin. Cancer Res. 2014; 20(12):3157-65
19. Sawyers RT, et al. Cancer Epidemiol Biomarkers Prev. 2014; 23(12):2372-79	66. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	109. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
20. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	67. Nishio K, et al. J. Clin. Oncol. 2013; 31(26):3342-47	110. Kuan T, et al. Cancer. 2013; 113(12):2273-76
21. Braganca A, et al. Clin. Cancer Res. 2014; 20(12):3157-65	68. Nishio K, et al. J. Clin. Oncol. 2013; 31(26):3342-47	111. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
22. Braganca A, et al. Clin. Cancer Res. 2014; 20(12):3157-65	69. Smith CE, et al. Clin. Cancer Res. 2013; 19(26):8623-31	112. Kuan T, et al. Cancer. 2013; 113(12):2273-76
23. Braganca A, et al. Clin. Cancer Res. 2014; 20(12):3157-65	70. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	113. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
24. Gryk R, et al. N. Engl. J. Med. 2000; 343(26):2032-34	71. Papatrakis F, et al. Eur. J. Cancer. 2013; 49(12):2277-81	114. Kuan T, et al. Cancer. 2013; 113(12):2273-76
25. Gryk R, et al. N. Engl. J. Med. 2000; 343(26):2032-34	72. Papatrakis F, et al. Eur. J. Cancer. 2013; 49(12):2277-81	115. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
26. Lagy L, et al. Dig. Dis. 2012; 27(2):256	73. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	116. Kuan T, et al. Cancer. 2013; 113(12):2273-76
27. You R, et al. J. Cancer. 2013; 113(12):2273-76	74. Kocardi M, et al. Gastroenterol. 2011; 141(5):1201-10	117. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
28. Barone M, et al. Methods Mol Biol. 2014; 1040:209-22	75. Nishio K, et al. J. Clin. Oncol. 2013; 31(26):3342-47	118. Kuan T, et al. Cancer. 2013; 113(12):2273-76
29. Boland R, et al. Clin. Cancer Res. 2013; 19(26):8623-31	76. Nishio K, et al. J. Clin. Oncol. 2013; 31(26):3342-47	119. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
30. Boland R, et al. Clin. Cancer Res. 2013; 19(26):8623-31	77. Boland R, et al. N. Engl. J. Med. 2000; 343(26):2032-34	120. Wang X, et al. Clin. Oncol. 2013; 24(12):3047-57
31. Boland R, et al. Clin. Cancer Res. 2013; 19(26):8623-31	78. Frangou D, et al. Clin. Cancer Res. 2013; 19(26):8623-31	121. Guo F, et al. Sci. Rep. 2013; 3(12):2463-37
32. Boland R, et al. Clin. Cancer Res. 2013; 19(26):8623-31	79. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	122. Marmol M, et al. Int. J. Mol. Sci. 2013; 14(12):23904-16
33. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	80. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	123. Kuan T, et al. Cancer. 2013; 113(12):2273-76
34. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	81. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	124. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
35. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	82. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	125. Kuan T, et al. Cancer. 2013; 113(12):2273-76
36. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	83. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	126. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
37. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	84. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	127. Kuan T, et al. Cancer. 2013; 113(12):2273-76
38. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	85. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	128. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
39. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	86. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	129. Kuan T, et al. Cancer. 2013; 113(12):2273-76
40. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	87. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	130. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
41. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	88. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	131. Kuan T, et al. Cancer. 2013; 113(12):2273-76
42. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	89. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	132. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
43. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	90. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	133. Kuan T, et al. Cancer. 2013; 113(12):2273-76
44. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	91. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	134. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
45. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	92. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	135. Kuan T, et al. Cancer. 2013; 113(12):2273-76
46. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	93. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	136. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
47. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	94. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	137. Kuan T, et al. Cancer. 2013; 113(12):2273-76
48. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	95. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	138. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57
49. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	96. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	139. Kuan T, et al. Cancer. 2013; 113(12):2273-76
50. Guo F, et al. Clin. Cancer Res. 2013; 19(26):8623-31	97. Suda K, et al. Int. J. Cancer. 2013; 133(12):2879-83	140. Yager R, et al. Clin. Oncol. 2013; 24(12):3047-57

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PROFESSIONAL SERVICES - PAGE 2 of 23

# Reporting (OncoGuide NCC Oncopanel System)

## RG Summary Report draft

## RG Sequencing Report

## RG QC report

RG サマリーレポート原案

※本レポートは、OncoGuide NCCオンコパネル 解析プログラムVer.1.02-00 により作成された原案をもとに、確認シエンスが作成したものです

### RG サマリーレポート原案

■検査情報

検査名	NCCオンコパネルシステム検査					
システム名	OncoGuide™ NCC オンコパネル システム					

■サンプル情報

匿名符号(患者識別ID等)	[Redacted]					
C-CAT患者識別ID	[Redacted]					

■遺伝子変異

遺伝子名	変異アレル頻度	CDS変化	アミノ酸変化	COSMIC ID (登録数)	
KRAS	62.3(2,143/3,441)	exon2:c.35G>A	G12D	521(10,871)	
TP53	22.6(256/1,127)	exon8:c.814G>A	V272M	10891(121)	

■遺伝子増幅・欠失情報

遺伝子名	遺伝子コピー数(補正リード数比)
-	-

■遺伝子再構成(融合)情報

遺伝子名	物理位置
-	-

\*1リード数が閾値を下回った変異です

■体細胞変異数

領域区分		SNV		InDel		合計	
		変異出現数	変異出現率**	変異出現数	変異出現率**	変異出現数	変異出現率**
エキソン	nonsyn	3	8.4 /Mb	0	0.0 /Mb	3	8.4 /Mb
	syn	0	0.0 /Mb	0	0.0 /Mb	0	0.0 /Mb
非エキソン		1	1.1 /Mb	0	0.0 /Mb	1	1.1 /Mb
	領域全体	4	3.1 /Mb	0	0.0 /Mb	4	3.1 /Mb

\*\* 変異出現率=1Mbpあたりの変異数

■解析レポート

- KRAS:G12D: 既知の活性化変異である。
- TP53:V272M: COSMICデータベースに多数の登録があり、機能欠失変異である可能性が高い。

検査結果に影響を与える可能性のある事項  
なし

報告書原案作成日:2019年09月05日 確認サイン: \_\_\_\_\_

■使用データベースバージョン

EPDB	20180202_v5.2
refGene	20171218
ensGene	20140406
1000人ゲノム	Phase_3(20130502)
ESP6500	V2-SSA137
ExAC	r0.3.1(20160316)
HGVD	v2.10(20170202)
COSMIC	v71(20180226)

1 / 2

RG シーケンシングレポート

※本レポートは、OncoGuide NCCオンコパネル 解析プログラムVer.1.02-00 により作成された原案をもとに、確認シエンスが作成したものです

### RG シーケンシングレポート

報告書作成日: 2019年09月05日

■検査情報

検査名	NCCオンコパネルシステム検査	
システム名	OncoGuide™ NCC オンコパネル システム	

■サンプル情報

匿名符号(患者識別ID等)	[Redacted]	
C-CAT患者識別ID	[Redacted]	
C-CAT登録ID	[Redacted]	
C-CAT中核拠点病院施設コード	[Redacted]	
C-CAT連携病院施設コード	[Redacted]	

■データ解析

インサートサイズ平均値	2,559.8
インサートサイズ中央値	208.0
モジュール	cisCall-7.1.7, cisGermline-1.0.1, cisAnnotate-1.1.4, cisReport-1.0.2
データセット	Dataset-1.00-180411
遺伝子異常選択条件(SNV, InDel)	Exon/Splicing, Syn, SNP(+COSMIC), VAF>=0.05
遺伝子異常選択条件(CNV)	CNR>=4.0
遺伝子異常選択条件(Fusion)	target

■腫瘍組織シーケンス解析情報

パネル

試薬

シーケンサーラン日

リードデータ名

総リード数

リードマッピング率(%)

デュプリケーション率(%)

Discordance率(%)

Mismatch率(%)

Deletion率(%)

Insertion率(%)

COSMIC(ClinVar登録ID

COSMIC(ClinVar登録数

COSMIC Status|ClinVar Significance

SNPデータベース

抽出方法

インサートサイズ平均値

インサートサイズ中央値

■正常組織シーケンス解析情報

パネル

試薬

シーケンサーラン日

リードデータ名

総リード数

リードマッピング率(%)

デュプリケーション率(%)

Discordance率(%)

Mismatch率(%)

Deletion率(%)

Insertion率(%)

読取深度平均値

読取深度中央値

1 / 6

### NCCオンコパネルシステム検査RG QCLレポート

検査名	NCCオンコパネルシステム検査	システム名	OncoGuide™ NCC オンコパネル システム
検査機関	株式会社理研ジェネシス川崎検査所	依頼元	株式会社エスアールエル
匿名符号(患者識別ID等)	[Redacted]	依頼元検査ID	[Redacted]
C-CAT患者識別ID	[Redacted]	レポート作成日	[Redacted]

検体情報

種別	提出	検査使用	RG Sample ID	受付日	採取日/採血日	腫瘍細胞含有率(%)	マイクロセクション
腫瘍	初回	<input type="radio"/>	[Redacted]	2019年08月19日	2019年05月01日	20	必要
正常	初回	<input type="radio"/>	[Redacted]	2019年08月19日	2019年08月14日	-	-

QC結果

種別	Qubit測定 DNA量 (ng)	qPCR測定 ΔΔCq
腫瘍	3,106.40	-1.57
正常	18,944.00	-

種別	ライブラリ ピークサイズ (bp)	ライブラリ量 (ng)	キャプチャーライブラリ ピークサイズ (bp)	キャプチャーライブラリ 濃度(nM)
腫瘍	294	1,174.80	327	16.30
正常	308	2,232.00	355	8.59

種別	シーケンスラン Q30 (%)	リード数	リード数(合計)
腫瘍	87.25	32,751,612	44,582,004
正常	-	11,830,392	-

コメント:  
すべての品質項目について判定基準を満たしている

RG品質基準値及び判定基準

抽出DNA:

- 腫瘍検体(FPE切片)由来のDNA:
  - Qubit測定DNA量 (ng): フルオロメーターにて測定した収量が 200 ng 以上であること
  - qPCR測定 ΔΔCq: リアルタイムPCR測定により算出した分解度が 2 未満であること
- 血液由来のDNA:
  - Qubit測定DNA量 (ng): フルオロメーターにて測定した収量が 200 ng 以上であること

ライブラリ:

- ライブラリピークサイズ (bp): 電気泳動分析のピークトップが 200 ~ 400 bp の範囲内であること
- ライブラリ量 (ng): 収量が 500 ng 以上であること

キャプチャーライブラリ:

- キャプチャーライブラリピークサイズ (bp): 電気泳動分析のピークトップが 250 ~ 450 bp の範囲内であること
- キャプチャーライブラリ濃度 (nM): 濃度が 2 nM 以上であること

シーケンスラン:

- シーケンスランQ30 (%): シーケンスランの Q30 が 80 % 以上であること
- リード数(合計): 腫瘍検体、正常検体の合計リード数が 35,000,000 リード以上であること

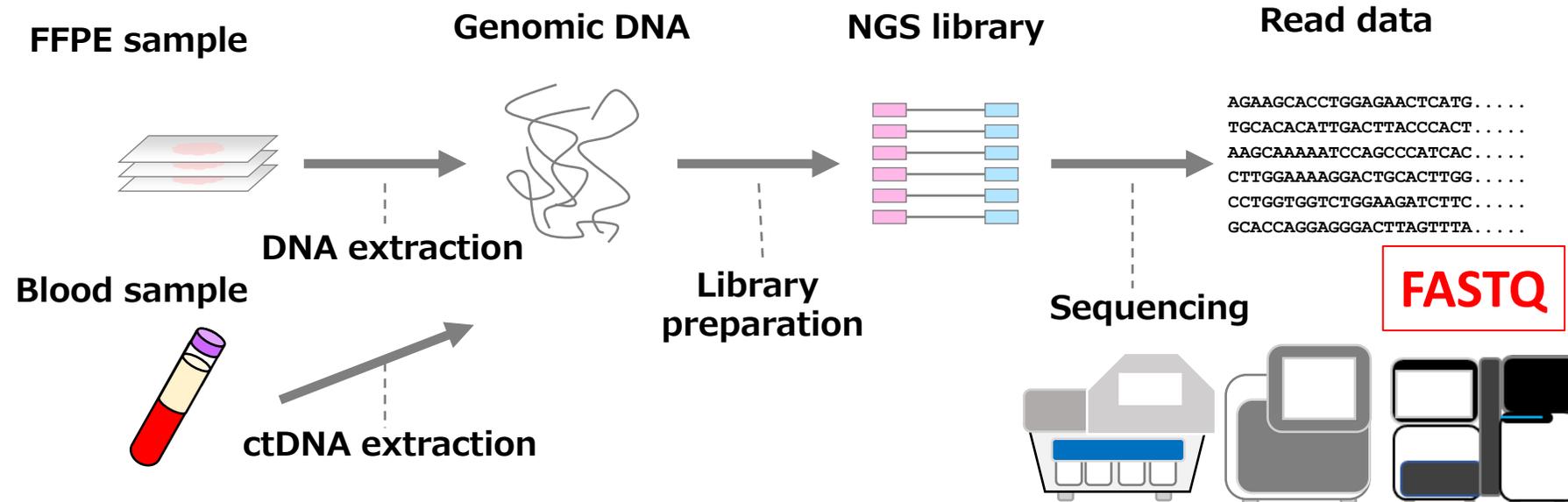
1 / 1

# Overview

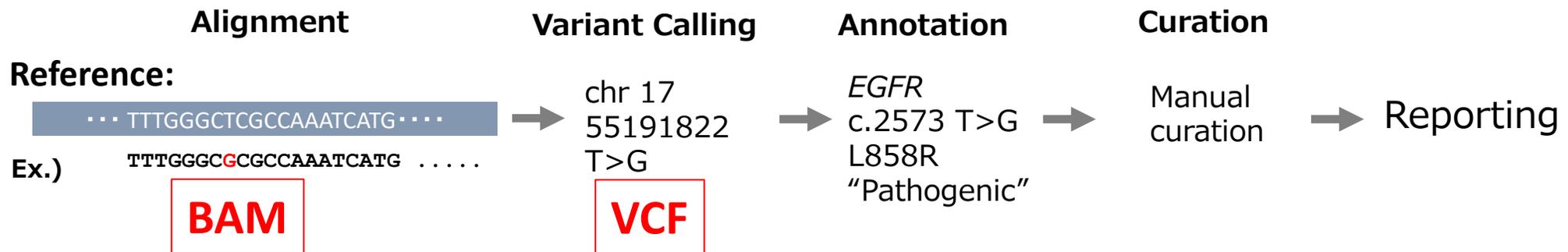
- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- Gene panel test workflow (Dry bench process)
- **Data file output by NGS analysis**

# Overview of the main steps in the NGS workflow

## Wet bench process



## Dry part





# BAM File

- Alignment information: read mapping to the reference genome



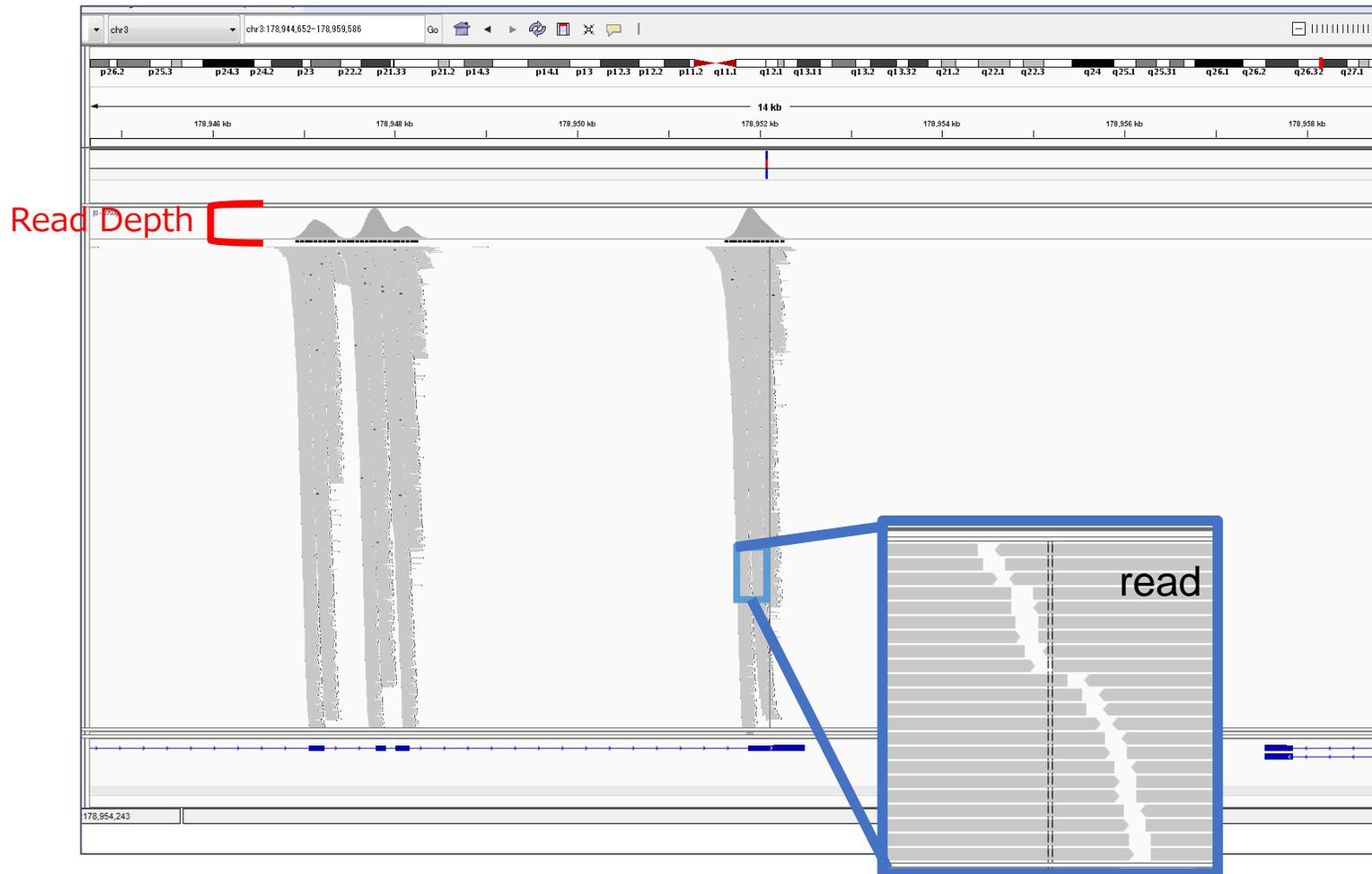
- A single line shows data from one sequence read, containing information about sequences that differ from the reference sequence.

```
NB501521:147:HHVG3AFX:2:11212:17736:6634 19 chr1 13133 0 151M chr9 13153 0
TTCCTACCTGAGGCTGAGGAAGGAGAAGGGGATGCACTGTTGGGAAGGCAGCTGTA ACTCAAAGCCTTAGCCTCTGTGCCACGAAGGCAGGGCCATCAGGCACCAAATGGATTCTGCCAGCATAGTGCTCCTGGACCAGTGATACACC
/AE/A/A//EEEE//EEEE/E6/6///EA//EA/E/A/AA/A/<A/AA<AE/E/6EA///E//E/AEA<//EA//AE/EEEE//</EE<///EA//AE/EEE/////////E/EA///A/E/E/////////E/E/AE//AE//EE////AA
XT:A:R NM:i:3 X0:i:2 X1:i:2 XM:i:3 X0:i:0 XG:i:0 MD:Z:45G32T30G41 XA:Z:chr15:1:102531392,+102517887,151M,3;chr2:1:243199373,+114357733,151M,4;
chr16:1:90354753,-62814,151M,4;
```

- Can be visualized with Integrative Genomics Viewer (IGV), etc.

# IGV: Integrative Genomics Viewer

- A genome browser (freeware) created by the Broad Institute (United States)



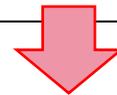
Read information can be visualized by opening the BAM file with IGV

# VCF File

- All information for a single mutation is listed on one line, separated by tabs.
- Includes detected variants, sequencing data, and various annotation information

## 1 variant information

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	NOA
chr17	7577552	_	CAT	C	100	PASS	GENE=T	GT:DP:CN	1/1:1805:0.948004



GENE=TP53;AF=0.5224;DP=1805;VFR=inf;DPP=7577553;DPB=1904;FG=836|10|16|0|943|0;BG=1904|0|0|0|0|0;HL=1;SMAF=0.5224;Func=exonic;ExomeFunc=frame\_shift;FullIAA=TP53;NM\_001126117:exon3:c.331\_332delAT:p.M111fs\*20:7:4;FullIAAEns=ENSG00000141510:ENST00000413465:exon6:c.727\_728delAT:p.M243fs\*43:7:4;Transcript=NM\_001126112;NucleotideChange=c.727\_728delAT;AA=p.M243fs\*20;1000G\_ALL=.;1000G\_AFR=.;1000G\_AMR=.;1000G\_EAR=.;1000G\_EUR=.;1000G\_SAS=.;ExAC\_Freq=.;ExAC\_AFR=.;ExAC\_AMR=.;ExAC\_EAS=.;ExAC\_FIN=.;ExAC\_NFE=.;ExAC\_OTH=.;ExAC\_SAS=.;ESP6500si\_ALL=.;ESP6500si\_AA=.;HGVD\_Qual=.;HGVD\_ALL=.;COSMIC\_ID=.;COSMIC\_STATUS=.;COSMIC\_CNT=.;COSMIC\_DIS=.;ClinVar\_SIG=.;ClinVar\_DIS=.;ClinVar\_STATUS=.;ClinVar\_ID=.;ClinVar\_DB=.;1000G\_VER=Phase\_3(20130502);ExAC\_VER=r0.3.1(20160316);HGVD\_VER=v2.10(20170202);COSMIC\_VER=v71(20180226);ClinVar\_VER=20170905;RefGene\_VER=20171218;RMCNT=0;CURATED=.;ClinVar\_DBID=.;ESP6500si\_EA=.;ESP6500si\_VER=V2-SSA137;ExonNo=7;GSD=.;RMASK=.;SMPLRPT=.

Ex) NCC Oncopanel System: vcf by cisCall contains annotation information, such as:

- Changes in bases and amino acids
- Registration status in the polymorphism database
- Registration status in COSMIC and ClinVar

# Overview

- Gene panel testing workflow (Wet bench process)
- Principles of base sequencing by NGS
- Gene panel test workflow (Dry part)
- Data file output by NGS analysis