Welcome to the Meet the NGS experts – 4 part global webinar series

Next-Generation Sequencing in Clinical Research

Part 1: NGS in the clinic
Dr. Funke will overview the state of clinical NGS and challenges associated with its implementation.

Speaker: Birgit Funke, Ph.D., Harvard Medical School, US
Date: 11:00 a.m.–12:00 p.m. EST; 4:00 p.m. GMT, January 19, 2016

Part 2: Translational genomics and prostate cancer
Dr. Bono will present how analysis of complex molecular landscapes of advanced castration-resistant prostate cancer led to key molecular target discoveries.

Speaker: Johann De Bono, M.D., Ph.D., Royal Marsden NHS Foundation, UK
Date: 11:00 a.m.–12:00 p.m. EST; 4:00 p.m. GMT, January 28, 2016

Part 3: NGS to identify clinically relevant mutations in cfDNA
Dr. Tepper will show how the new targeted NGS assays can be used to detect low frequency mutations in cell-free cfDNA in prostate cancer patients.

Speaker: Clifford Tepper, Ph.D., UC Davis School of Medicine, US
Date: 11:00 a.m.–12:00 p.m. EST; 4:00 p.m. GMT, February 11, 2016

Part 4: Clinical sequencing system to classify meningioma
Dr. Nishihara will discuss how he used targeted amplicon sequencing strategy to develop a rapid sequencing system to categorize meningiomas.

Speaker: Hirosh Nishihara, M.D. Ph.D., Hokkaido University, Japan
Date: 6:00–7:30 p.m. EST; 4:00 p.m. GMT, February 18, 2016
Japan Time: 8 a.m. JST, Feb 19, 2016
Webinar on

Developing a rapid clinical sequencing system to classify meningioma

Hiroshi Nishihara, MD, PhD
Professor, Department of Translational Pathology
Hokkaido University, Japan
Developing a rapid clinical sequencing system to classify meningioma

Hiroshi Nishihara, MD, PhD

Department of Translational Pathology, Hokkaido University, Graduate School of Medicine
Translational Research Laboratory, Hokkaido University Hospital
Sapporo, Japan
Hokkaido University

- Located in Sapporo city
- Initially, Sapporo Agricultural Collage was built by Dr. Clark
- Population
  - Sapporo city: 2,000,000
  - Hokkaido state: 5,000,000
Beautiful Campus Conveniently Located
Self Introduction

Hiroshi Nishihara MD, PhD.
Professor, Dept of Translational Pathology, Sch. of Med
Director, Translation Research Laboratory, Hokkaido Univ. Hosp.

"Active Biobank" strongly support clinical research in Hokkaido

Individualized medicine based on gene profile

北海道大学
Development of genomic medicine promotes *Individualized Medicine.*

Integrate multiple patients’ genomic data

High quality Clinical Information

Clinical Research (ARO)

“Clinical Sequencing”
- Real-time genomic analysis
- Analysis of individual samples
- Clinically relevant outputs

Biostatistics

Development of Clinical Science

Individualized Medicine
Clinical BioBank (Translational Research Laboratory)

- Universities Institutes
  - Biobank Japan National Center....
- Clinical Biobank in Hospital
  - Sampling
  - Rapid and adequate sample processing
  - Mass Analysis
  - High quality biospecimen
- Specific institutes or universities
  - High quality sample linked with clinical information
  - Rapid feedback to physicians
  - Promote specific clinical studies
  - Clinical sequencing in TR Laboratory
  - Drug discovery
  - Research paper

- Government oriented clinical studies
- Pharmaceutical companies
For use, not for storage of biospecimen
【On-Demand type banking and advanced analysis of biospecimen】

Valued biospecimen, especially for clinical studies
【Availability for genomic and molecular analyses】

Physical support for clinical studies
【Core facility for clinical study】

Sampling and storage based on specific protocols
【Project-based repository system】
Tissue sampling

PFPE tissue blocks
PMBC for control

FFPE blocks from pathological materials

Frozen materials

Pathological evaluation
Tumor content rate, viability

Extraction of DNA, RNA

dsDNA (Qubit®)

\( \Delta CT \) (RT-PCR)

RIN (Bioanalyzer®)

Individualized Medicine

Team conference for final diagnosis with physician, bioinformatician

Original pipeline for SNV, CNV analysis

Targeted Amplicon Sequence with GeneRead®

QIA symphony

MiSeq® Illumina

Clinical Sequence System in Hokkaido University Hospital
Meningioma, arising from meningotheelial cells, is the most common primary brain tumor, and accounts for about 25% of all intracranial tumors.

Loss of *neurofibromin 2 (NF2)* has been found in about half of sporadic meningiomas.

Mutations in *TRAF7, KLF4, AKT1* and *SMO* were recently reported in non-NF2 meningiomas by NGS analysis.

We established a clinical sequence system for meningioma to determine the genotype as a routine laboratory examination in addition to concurrent pathological diagnosis.
GeneRead DNAseq Targeted Panel V2

- Multiplex PCR-enabled enrichment of any region, gene, or set of genes in the human genome
  - average amplicon size 150 bp
  - Need just 10 ng of DNA/pool
  - Takes only 3 hours for target enrichment
  - Integrated data analysis and biological interpretation

Multiplex PCR primer sets
## Clinically Relevant Panels

Largest collection of wet-bench verified catalog gene panels

Reference databases:
- The Cancer Genome Atlas
- National Comprehensive Cancer Network
- COSMIC
- Cancer Genome Census
- OMIM®
- ClinVar (NCBI)

<table>
<thead>
<tr>
<th>Type</th>
<th>Panel name</th>
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</thead>
<tbody>
<tr>
<td>Solid tumor</td>
<td>Clinically Relevant Tumor</td>
</tr>
<tr>
<td></td>
<td>Tumor Actionable Mutations</td>
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<tr>
<td>Hematologic malignancies</td>
<td>Myeloid Neoplasms</td>
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<tr>
<td>Tissue-specific</td>
<td>Breast Cancer</td>
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<td></td>
<td>Colorectal Cancer</td>
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<td>Liver Cancer</td>
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<td>Ovarian Cancer</td>
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<td>Gastric Cancer</td>
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<td></td>
<td>Cardiomyopathy</td>
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<td>Comprehensive</td>
<td>Cancer Predisposition</td>
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<td>Comprehensive Cancer</td>
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<td>Carrier Testing</td>
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<tr>
<td>Gene specific</td>
<td>BRCA1/2 Panel</td>
</tr>
</tbody>
</table>

Ingenuity Systems

Sample to Insight
GeneRead DNAseq Custom Panel V2

Gene(s) or genomic region(s) of interest

Online custom panel builder:
- Increase breadth of coverage
- Adjust amplicon length
- Allow denser tiling

Whole exon for…
NF2, TRAF7, KLF4, AKT, SMO

Fully customized panel

Turnaround time: 3 weeks
Simple Protocol

1. Add genomic DNA (10 ng/reaction) and GeneRead DNAseq Panel PCR Kit V2.

2. PCR amplification (3 hours).

3. Pool reactions for each sample and purify (AMPure® bead purification).

4. NGS library preparation.
Standardized and rapid workflow

For 12 samples

Turnaround time: 4 days

Day 1
- FFPE DNA isolation: 3:45
- GeneRead QuantiMIZE Kit: 2:00
- GeneRead DNAseq Panel: 3:00
- AMPure bead purification: 1:00
- GeneRead Library Prep: 2:00
- GeneRead Size Selection: 1:15
- AMPure bead purification: 1:00
- GeneRead amplification: 0:45
- QIAquick PCR Purification: 0:30
- GeneRead Library Quant Kit: 3:00
- Sequencing: 24:00
- CLC Cancer Workbench: 5:00

Day 2
- Day 3
- Day 4

Turnaround time: 4 days

Sample to Insight
Workflow of NGS

Frozen tumor samples (median size in diameter 7 mm, range 2.5–12)

PAXgene-fixed formalin-embedded (PFPE) blocks

Confirmation of histology

GeneRead Mix-n-Match Panel
- NF2, AKT1, SMO, ERBB2, KIT, MET
GeneRead Custom Panel
- TRAF7, KLF4

GeneRead DNAseq Variant Calling Service (QIAGEN)
BioReT System (Amelieff)

Isolate genomic DNA

Perform multiplex PCR-based targeted enrichment

Pool amplicons

Any NGS platform

Detect mutations that matter
MRI and HE staining of representative cases

A  T251 (NF2 loss + NF2 E342X)

B  T430 (NF2 loss)

C  T752 (TRAF7 R641H + KLF4 K409Q)

D  T518 (TRAF7 R641H + AKT1 E17K)

E  T709 (AKT1 E17K)

F  T396 (SMO W535L)
The score $Q$ was used to set a threshold ($Q \geq 50$) to identify clusters with significant copy number changes and score $P$ correlated with the precision of the copy number estimate.

J. T799
- NF2 mutation: No
- FISH: unanalyzable

K. T243
- NF2 mutation: No
- FISH: diploid (0%)

L. T839
- NF2 mutation: p.Gln655*/c.193C>T
- FISH: diploid (0%)

M. T624
- NF2 mutation: No
- FISH: del(22q) (20%)

N. T798
- NF2 mutation: p.Arg198*/c.592C>T
- FISH: diploid (0%)

O. T407
- NF2 mutation: c.364_381delTCTCCCTTGTGCTCCTTT
- FISH: diploid (0%)

P. T771
- NF2 mutation: No
- FISH: monosomy (60%)

Q. T603
- NF2 mutation: p.Cys133_Pro134fs/c.399_400insC
- FISH: monosomy (68%)

R. T690
- NF2 mutation: p.Arg447fs/c.1341_1345delGCAGG
- FISH: monosomy (81%)
A: T524: Diploid (NGS score Q = 48)
B: T750: Diploid (NGS score Q = 58)
C: T369: Diploid (NGS score Q = 144)
D: T407: Diploid (NGS score Q = 205)
E: T624: del(22q) in 20% of cells (NGS score Q = 2)
F: T581: del(22q) in 12% of cells (NGS score Q = 170)
G: T629: Monosomy in 100% of cells (NGS score Q = 124)
H: T693: Monosomy in 68% of cells (NGS score Q = 236)
I: T571: Monosomy in 100% of cells (NGS score Q = 250)
Preoperative diagnosis
Age, sex, tumor location, imaging findings, symptoms, neurological examination

Operation
Appropriate fixation and preservation of tumor specimens

Routine pathological study
H&E staining
Immunohistochemistry
Pathological diagnosis
About 5–7 days after surgery

Clinical sequence analysis
DNA extraction
Targeted amplicon sequencing
Confirmation of genotype
About 10–14 days after surgery

Postoperative management
The determination of early radiation therapy, short follow-up interval, or molecular targeted therapy
Division of Cancer-Genetic Diagnosis

Staff: Doctor, Technical staff

- Sample preparation
- NGS sequencing
- Data analysis

TR Laboratory

Prof. Nishihara

Dept. of Oncology

- Sample preparation
- NGS sequencing
- Data analysis

Genetic Medicine

Gene counseling for hereditary cancer (BRCA1, Rinch-syndrome)

Companion diagnosis for insurance-covered gene (Ras, EGFR, RAF for specific types of cancer)

Dept. of Pathology

- Companion diagnosis for out patients without health insurance
- OncoPrime, MSK-IMPACT (outsourcing)
- In house targeted sequencing
- Team conference for final diagnosis

Out Patient

Individualized medicine based on gene profile

Outsourcing

- OncoPrime
- MSK-IMPACT

MSS (Mitsubishi Space Software)
Clinical BioBank Study Group

1. Handing of high quality biospecimen
   • SOP for tissue sampling
   • SOP for sample storage
   • Linkage of sample and clinical information

2. Analysis of biospecimen for clinical sequence
   • Quality certification of biospecimen
   • In house analysis using desktop type NGS
   • Adequate system for supporting clinical research

3. Networking of clinical biobank in Japan
   • Collaboration with “Mega-Bank” in Japan
   • Sharing the banking system and data base

President Hiroshi Nishihara MD. PhD (Hokkaido University)
Vice president Shinichi Toyooka MD, PhD (Okayama University)
Director Manabu Mutoh MD, PhD (Kyoto University)
Director Kazuyuki Matsushita MD, PhD (Chiba University)
Director Kazuhiro Okano PhD (QIAGEN)
Thank you for your attention!
Thank you!

Contact Technical Support
9 AM – 6 PM Eastern M – F

Telephone: 800-362-7737

Email: brcsupport@qiagen.com

Hiroshi Nishihara, MD, PhD
Professor, Department of Translational Pathology
Hokkaido University, Japan
Thank you for watching!

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