Mutation testing: Current state of the art, FDA approved assays and future directions

WuXiNextCODE

| TWO THOUSAND NINETEEN

OUTLINE

- Mutation testing in tissue
- Mutation testing in plasma
- Future directions

WuXi NextCODE | Purpose-built for Big Genomics

Steeped in a rich history of population genomics and advanced analytics, WXNC became the world's first end-to-end, discovery-focused CGO (Contract Genomics Organization).

deCODE genetics
a subsidiary of AMGEN

Birthplace of scalable population genomics and the **source of WXNC's current genomics platform, architecture, and technology**

NEXTCODE HEALTH

Founded based on deCODE's world-leading technology platform, with evolved clinical and deep learning capabilities

药明康德 WuXi AppTec

World-renowned CRO for drug discovery and development

WuXiNextCODE

Delivers on the promise of genomic big data for life sciences companies, ***enabling next-generation drug discovery and clinical development***

WuxiNextCODE: A Global Contract Genomics Organization

Natively Global, rapid expansion – 700+ employees, raised $260 million (Oct 2017)
Nov 2018: GMI Acquisition and $200 million investment

ICELAND

- Birthplace of population genomics
- Database, Clinical Interpretation, Sequence Analysis development

deCODE genetics
a subsidiary of AMGEN

US

- Global capital of life sciences
- World-leading clinical, deep learning capabilities

NEXTCODE HEALTH

CHINA

- WuXi – the quality leader in Life Sciences with pharma
- CLIA, CAP certified laboratory in China

药明康德 WuXi AppTec

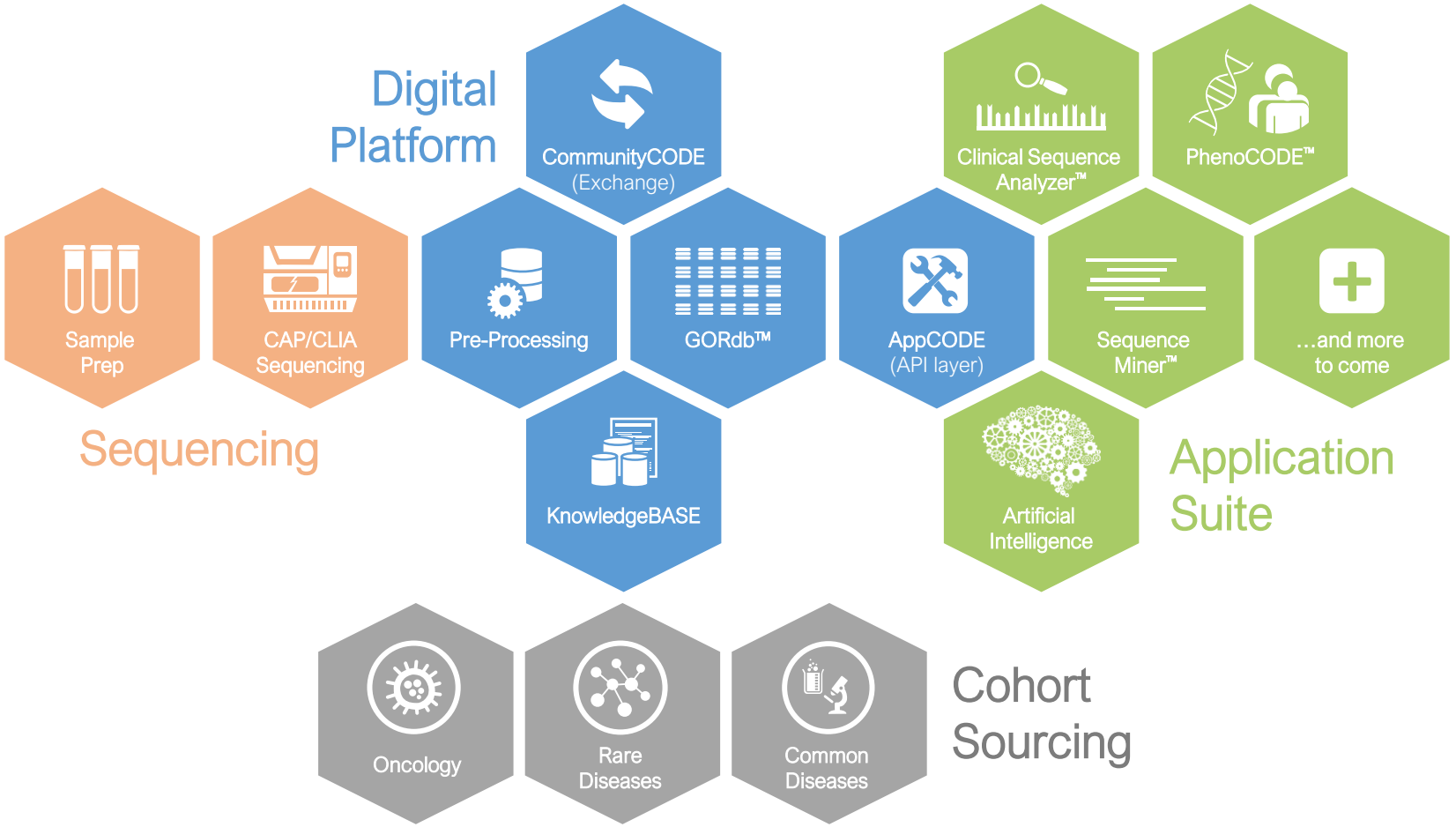
IRELAND

- GMI – now a wholly-owned subsidiary of WXNC
- Recruit & Whole genome sequence (WGS) 400,000 of the Irish population

gmi genomics medicine ireland

From Sample to Insights, WXNC Does It All

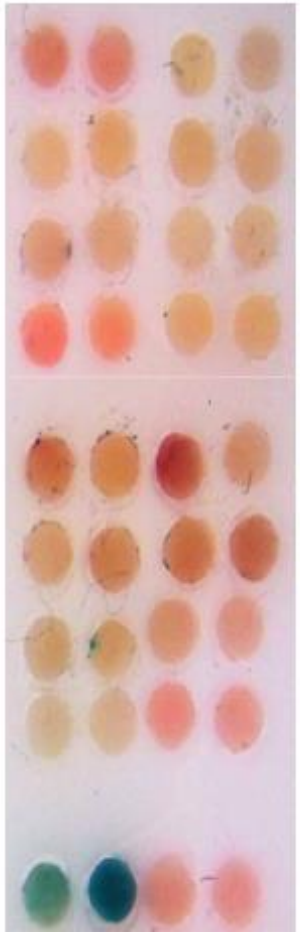
WXNC's CGO model provides end-to-end modular capabilities that can be utilized separately or together for a fully integrated workflow.



*A single digital platform to seamlessly **optimize**, **mine**, and **share** genomic data and insights.*

FFPE Sample Multi-omics & Analysis with WXNC AI

FFPE tumor specimens offer many benefits, but present technical challenges



Benefits of FFPE Specimen Utilization

- **Lower costs** compared to frozen/fresh materials
- Access to **large numbers** (millions) of samples
- **Streamlined logistics:** new patient recruitment not required
- **Clinical data and outcomes** already exist

Challenges of Traditional FFPE Sequencing

- **Extraction** – small fragments, only a few hundred bp in size
- **Bias** – many users report CtoT bias from archival FFPE samples
- **Depth** – users often employ very high depth to compensate, eg >1000x (FMI panel) or >300x (WES)
- **Evenness of Coverage** – WGS often fails due to fragmented DNA and uneven coverage

Table 2. Overview of nucleic acids extracted with the different extraction kits from commercial vendors.

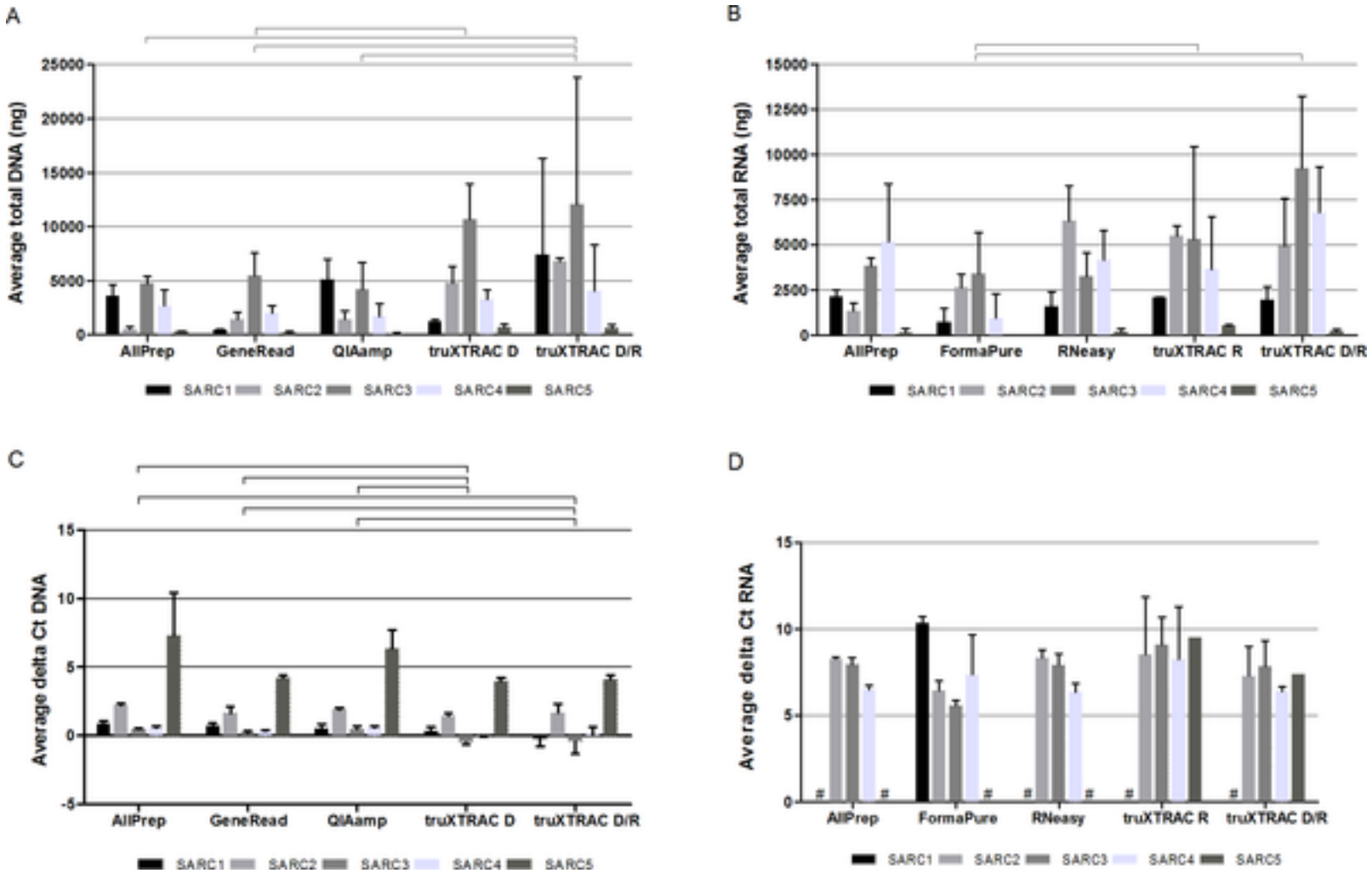
Extraction kit	Nucleic acid extracted	Vendor
AllPrep DNA/RNA FFPE Kit	DNA and RNA	QIAGEN, Inc., Hilden, Germany
GeneRead DNA FFPE Kit	DNA	QIAGEN, Inc., Hilden, Germany
QIAamp DNA FFPE Tissue Kit	DNA	QIAGEN, Inc., Hilden, Germany
truXTRAC FFPE DNA Kit	DNA/DNA and RNA*	Covaris, Inc., Woburn, MA, USA
Agencourt FormaPure Kit	RNA	Beckman Coulter, Inc., Indianapolis, IN, USA
RNeasy FFPE Kit	RNA	QIAGEN, Inc., Hilden, Germany
truXTRAC FFPE RNA Kit	RNA/DNA and RNA*	Covaris, Inc., Woburn, MA, USA

*The simultaneous extraction of DNA and RNA uses both truXTRAC kits

https://doi.org/10.1371/journal.pone.0197456.t002

Kresse SH, Namløs HM, Lorenz S, Berner JM, Myklebost O, et al. (2018) Evaluation of commercial DNA and RNA extraction methods for high-throughput sequencing of FFPE samples. PLOS ONE 13(5): e0197456. https://doi.org/10.1371/journal.pone.0197456
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0197456

Fig 1. Yield and amplifiability of extracted DNA and RNA.



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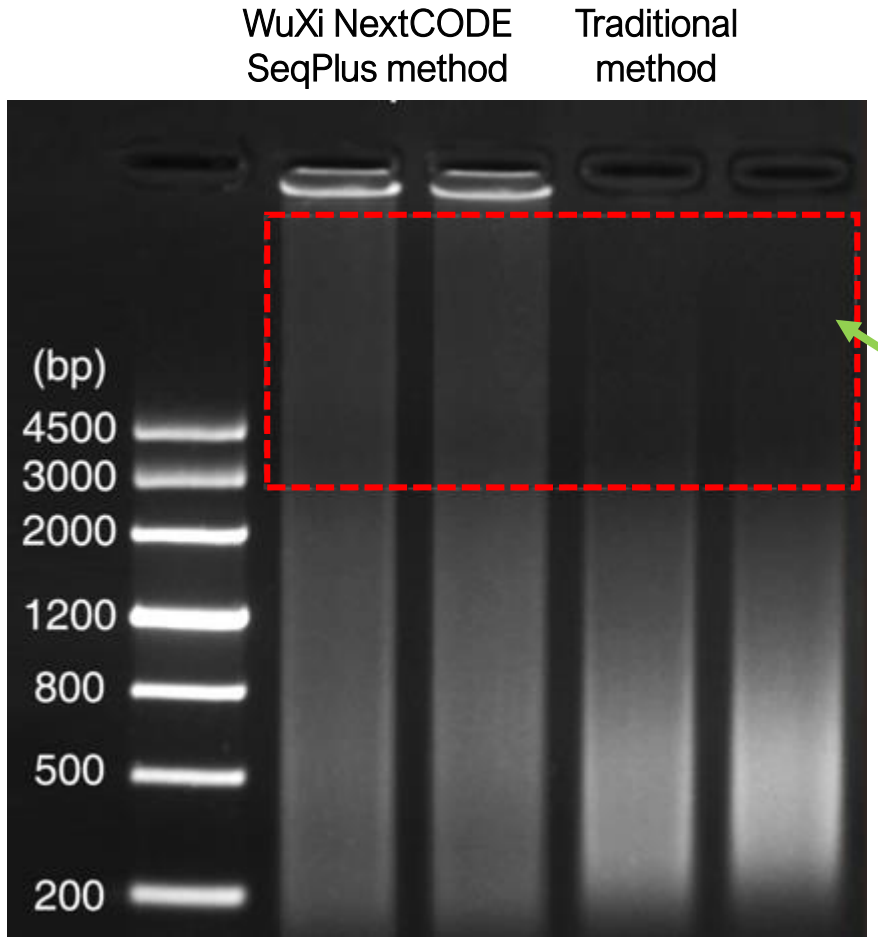
SeqPlus Offers Vastly Improved WGS & WES from FFPE

SeqPlus is our novel, proprietary FFPE extraction methodology coupled with optimized NGS sequencing library preparation to deliver superior WGS and WES data



Higher molecular weight DNA fragments isolated by WuXi NextCODE method are absent in other methods

SeqPlus Delivers

- **Extraction** of much larger fragments of DNA, with median size of several kb
- **Compatibility** with routine FFPE (up to 15+ yrs) and alcohol fixed samples
- **No observed CtoT bias**
- **For WGS** – Success rates routinely >95% to deliver high complexity libraries & coverage of genome at 20X
- **For WES** – More even exome coverage in independent head-to-head comparisons

SeqPlus Enables Very Even Coverage in WGS:

In 515 tumor-normal pairs, where FFPE WGS Tumor was sequenced at 70X,
99% of genome is covered at 10X, 98% of genome is covered at 20X

QC item	Paired Tumor Target 70X Mean values	Paired Normal Target 30X Mean values	Other Fresh samples Target 30X Mean values
Total Reads	1903.73M	852.72M	821.06M
Read Length	150	150	150
Total Throughput	285.6 Gb	127.90 Gb	123.1 Gb
Percentage Mapped	98.8%	98.6%	99.7%
Percentage PCR duplicate	26.9%	21.1%	11.42%
Percentage Properly Paired	96.1%	95.8%	98.2%
Percentage Singletons	0.3%	0.3%	0.18%
Percentage With Mate Mapped To A Different Chr(mapQ>=5)	0.8%	0.8%	0.35%
Coverage ≥ 1x	99.7%	99.6%	99.5%
Coverage ≥ 4x	99.5%	99.2%	99.4%
Coverage ≥ 10x	**99.0%**	98.1%	99.1%
Coverage ≥ 20x	**98.0%**	92.6%	96.8%

Variant Detection in Tumors

- Panel-Based Sequencing
  - FoundationOne CDx
    - First FDA-approved broad companion diagnostic with Medicare coverage for qualifying patients across all solid tumors, including: NSCLC, Colorectal, Breast, Ovarian, and Melanoma
  - MSKCC IMPACT
  - Many, many local LDTs
- Whole exome sequencing – mostly research
- Whole genome sequencing – mostly research

FDA approvals of Somatic Genetic Testing

Tumor tissue

- https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests
  - Removed germline
  - Removed instruments and reagents
  - Removed allogenic testing
- 64 Approved/Cleared Assays for Somatic Mutation Testing
  - ~21 are in situ hybridization assay (FISH, CISH)
  - ~24 are 1-2 gene assays
  - ~6 are multi-gene assays
  - ~1 for circulating tumor DNA
  - ~13 microarray, methylation, misc

Plasma-based mutation testing

WuXiNextCODE

Plasma-based mutation assessment

- Advantages
  - Readily available
  - Likely to represent broad tumor profile
  - Likely to represent present tumor profile
- Challenges
  - Require higher sensitivity assays
  - Ct DNA is ~165bp
  - Real estate of an assay is limiting
  - Cost of assay is limiting

Landscape of plamsa based mutation testing

- Single genes
  - Real-time PCR assay of EGFR, detects 42 mutations
  - Tissue and plasma assay
  - Companion diagnostic for Tarceva and Tagrisso
- Medium panels
  - Resolution Bio ctDx Lung assay
    - 22 genes
    - Resolution Biosciences assay with tiled/short 40bp probes
    - Detects SNV, fusions and CNVs
- Large panels
  - TSO 500 Ct DNA
  - Foundation Medicine FoundationOne Liquid
    - 70 Genes
    - MSI, fusions
  - Guardant

FDA activity in Somatic Genetic Testing

Plasma

- Approval
  - Roche EGFR tumor and plasma assay
- Breakthrough designation
  - Natera's Signatera
  - PGDx Elio Plasma Resolve cell-free DNA assay
  - Resolution HRD™ liquid biopsy assay
  - FoundationOne Liquid

Future directions for tumor testing

- Democratization
  - Testing kits that can be employed by any clinical laboratory
  - LDT labs are making kits
- Indication-based testing
  - Move away from one test for one therapeutic drug
  - Already happening in lung cancer
    - Oncomine Dx test
- Over time will move to WES or WGS testing
- Continued innovation through LDT development