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The use of Genexus and OPA as fast and sensitive NGS solution for solid tumour molecular characterization

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Manchester Pathology, 7th July 2021

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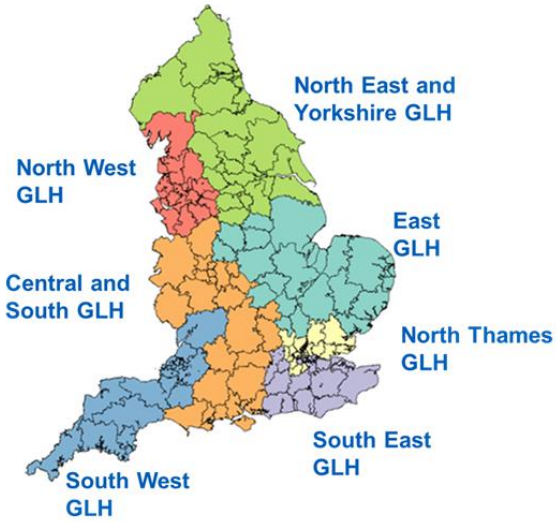
Introduction to NHS East GLH

NHS
East Genomic Laboratory Hub

- One of 7 Genomic Laboratory Hubs (GLHs) set up to deliver consistent and equitable national Genomic Medicine Service
- Covers 9.8m population
- 32 Trusts, 23 hospitals, 19 pathology groups
- Led by CUH, working with labs in UHL and NUH
- Undergoing substantial laboratory and workforce transformation…
- … to implement high-throughput molecular assays needed to deliver National Genomic Test Directory

The Seven Genomic Laboratory Hub regions of the NHS Genomic Medicine Service



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Genomic Medicine Service

Implementation of cancer test directory largely driven by NGS assays

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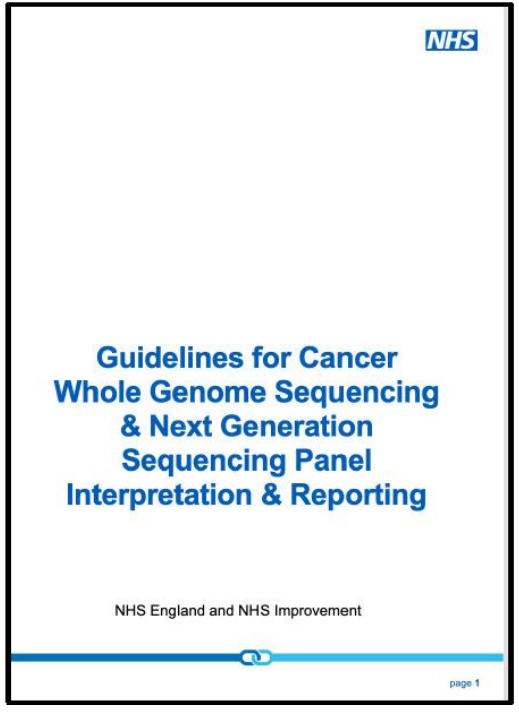

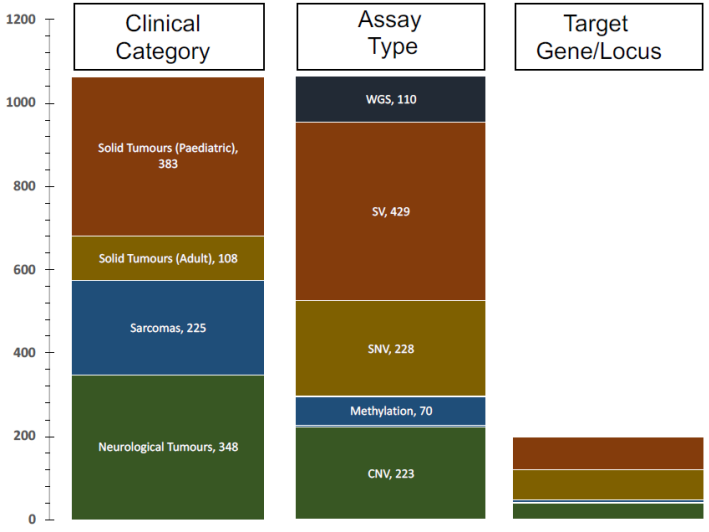

Mutation analysis by NGS for hotspot cancer gene panel or by single gene testing

50 hotspot cancer gene panel (gene list available on request)

Non-small cell lung cancer.....	*EGFR/BRAF*	Melanoma.....	*BRAF/NRAS/KIT*
Gastrointestinal stromal tumour.....	*KIT/PDGFRA/BRAF*	Lynch syndrome...	*BRAF*
Lymphoplasmacytic lymphoma.....	*MYD88*	Glioma.....	*IDH1/IDH2*
Histiocytic and dendritic cell neoplasms	*BRAF*	Colorectal cancer.	*KRAS/NRAS/BRAF*

East GLH solid tumour molecular testing pathway

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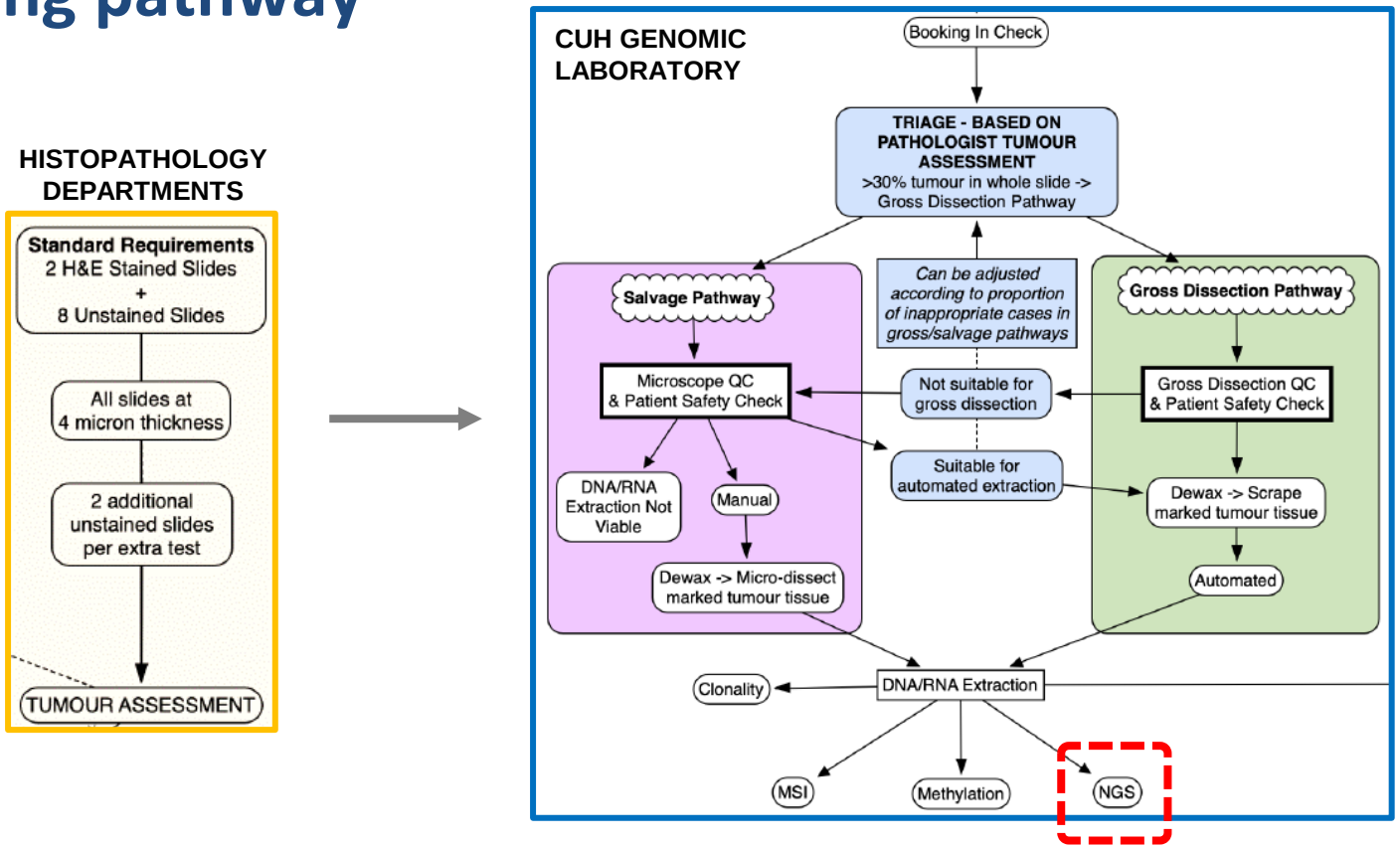

NGS panel for hotspot cancer gene testing – OPA on Genexus

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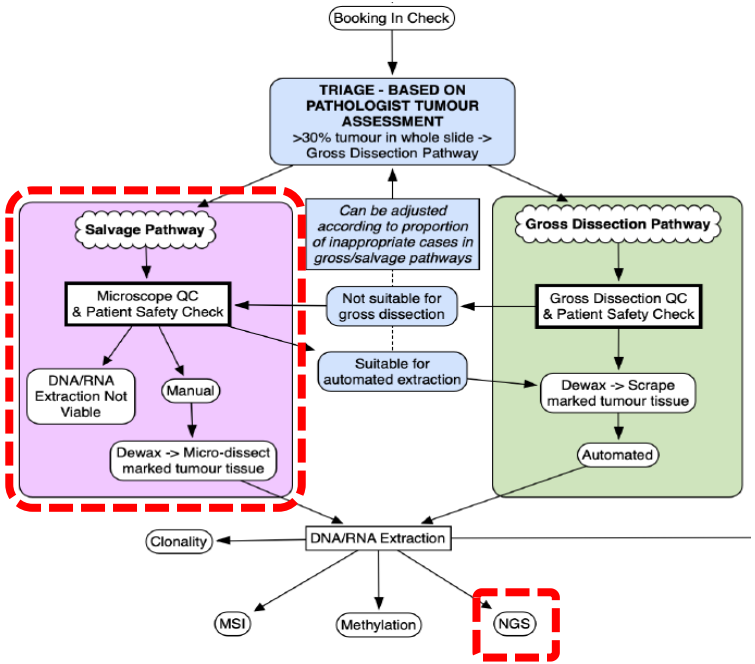

2005

Personal Genome Machine
+
AmpliSeq Cancer Hotspot 50 Gene Panel

2021

Genexus
+
Oncomine Precision Assay

DNA hotspots					CNVs		Inter-genetic fusions		Intra-genetic fusions
AKT1	*CHEK2*	*FGFR3*	*KIT*	*NTRK3*	*ALK*	*FGFR1*	*ALK*	*NTRK1*	*AR*
AKT2	*CTNNB1*	*FGFR4*	*KRAS*	*PDGFRA*	*AR*	*FGFR2*	*BRAF*	*NTRK2*	*EGFR*
AKT3	*EGFR*	*FLT3*	*MAP2K1*	*PIK3CA*	*CD274*	*FGFR3*	*ESR1*	*NTRK3*	*MET*
ALK	*ERBB2*	*GNA11*	*MAP2K2*	*PTEN*	*CDKN2A*	*KRAS*	*FGFR1*	*NUTM1*	
AR	*ERBB3*	*GNAQ*	*MET*	*RAF1*	*EGFR*	*MET*	*FGFR2*	*RET*	
ARAF	*ERBB4*	*GNAS*	*MTOR*	*RET*	*ERBB2*	*PIK3CA*	*FGFR3*	*ROS1*	
BRAF	*ESR1*	*HRAS*	*NRAS*	*ROS1*	*ERBB3*	*PTEN*	*MET*	*RSPO2*	
CDK4	*FGFR1*	*IDH1*	*NTRK1*	*SMO*			*NRG1*	*RSPO3*	
CDKN2A	*FGFR2*	*IDH2*	*NTRK2*	*TP53*					

Figure 2. Oncomine Precision Assay gene list.

OPA features

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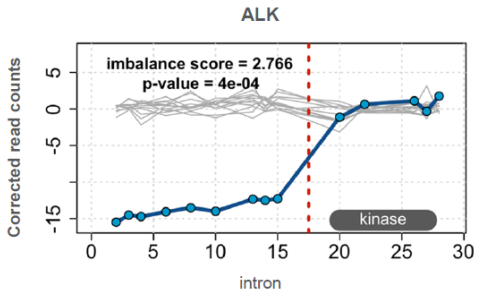
Relevant content	(Super) fusion detection	Molecular tagging	Tissue and plasma	Genexus system
Pan-cancer biomarkers Resistance markers	Increased targeted isoforms Novel fusion detection	Low level detection AmpliSeq HD chemistry	Advantageous for NSCLC clinical research	Ease of use / low TAT Economical at low batches

Novel Fusion Detection Using Exon Tiling Imbalance

Determines if there is an "imbalanced" expression between the 5' and 3' ends of select fusion driver genes—indicates a fusion for that gene (so will detect 'novel' fusions).



What is included?

- **Graphical representation** of an "imbalance" (blue line = positive sample vs. grey lines of normal)
- Estimated **exonic breakpoint** (red dotted line)
- Visual representation of the **kinase domain**
- **Imbalance score**, and **P-value** (statistical confidence)

Ion AmpliSeq HD technology

Our ultrahigh sensitivity solution for translational and clinical researchers who need customization

- Single configuration
  Made-to-Order
- Ultra-high sensitivity - As low as 0.1% LOD[1]
- Low sample input – as little as 1 ng DNA or 1ng FFPE RNA
- (≥2.8 ng/µL).

East GLH OPA Verification Overview

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Verification of OPA for Fusion Detection

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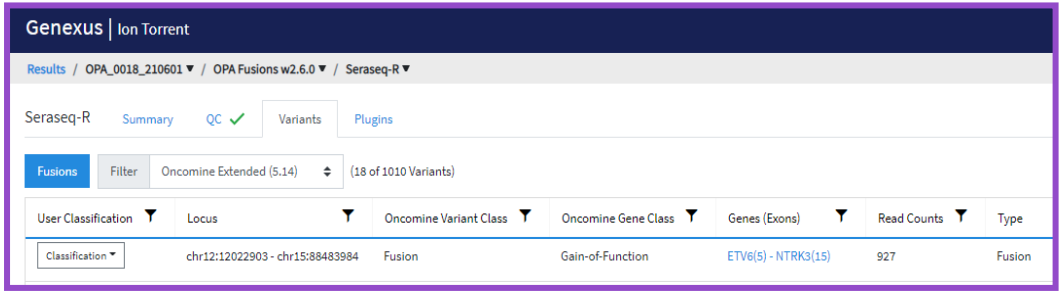
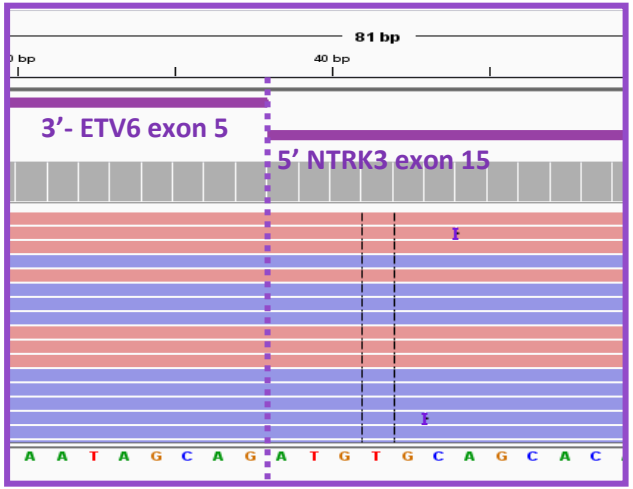
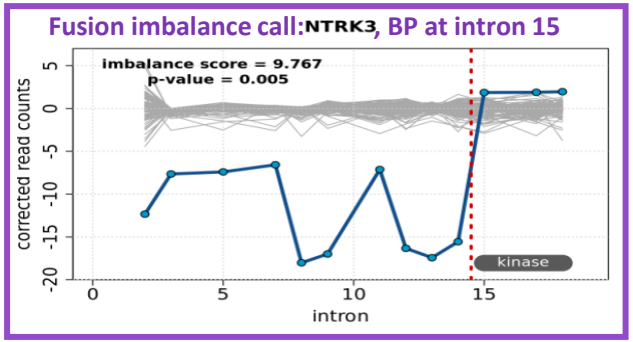
100% sensitivity for 17 OPA- targetable fusions in commercial FFPE controls

Fusion driver gene	Fusion expected	Fusion detected	Concordant	Sample source
ALK	EML4-ALK	EML4(13) - ALK(20)	Yes	SeraCare*
	EML4-ALK	EML4(13) - ALK(20)	Yes	Horizon#
BRAF	SLC45A3-BRAF	SLC45A3(1) - BRAF(8)	Yes	SeraCare
FGFR3	FGFR3-BAIAP2L1	FGFR3(17) - BAIAP2L1(2)	Yes	SeraCare
	FGFR3-TACC3	FGFR3(17) - TACC3(10/11)	Yes	SeraCare
MET	MET ex 14 Skipping	MET(13) - MET(15)	Yes	SeraCare
NTRK1	LMNA-NTRK1	LMNA(2) - NTRK1(11)	Yes	SeraCare
	TFG-NTRK1	TFG(5) - NTRK1(10)	Yes	SeraCare
	TPM3-NTRK1	TPM3(7) - NTRK1(10)	Yes	SeraCare
NTRK3	ETV6-NTRK3	ETV6(5) - NTRK3(15)	Yes	SeraCare
RET	CCDC6-RET	CCDC6(1) - RET(12)	Yes	SeraCare
	CCDC6-RET	CCDC6(1) - RET(12)	Yes	Horizon
	KIF5B-RET	KIF5B(24) - RET(11)	Yes	SeraCare
	NCOA4-RET	NCOA4(7) - RET(12)	Yes	SeraCare
ROS1	D74-ROS1	CD74(6) - ROS1(34)	Yes	SeraCare
	SLC34A2-ROS1	SLC34A2(4) - ROS1(34)	Yes	SeraCare
	SLC34A2-ROS1	SLC34A2(4) - ROS1(32/34)	Yes	Horizon

*. Seraseq® FFPE Tumor Fusion RNA v4; #. Horizon ALK-RET-ROS1 Fusion FFPE RNA Ref Standard







Verification of OPA for Fusion Detection

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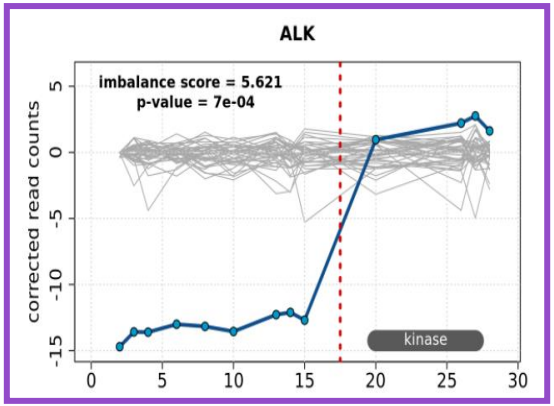
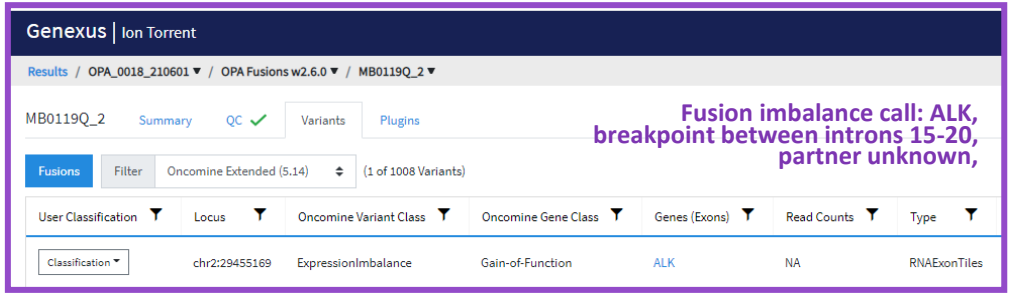
100% sensitivity for fusion detection in FFPE tumour specimens in 20 clinical research cases

Fusion driver gene	Fusion expected	Control method	Fusion detected	Concordant	Tumour diagnosis
ALK	ALK rearranged	FISH	EML4(2) - ALK(20)	Yes	Lung adenocarcinoma
	ALK rearranged	FISH	EML4(2) - ALK(20)	Yes	Lung adenocarcinoma
	ALK rearranged	FISH	NPM1(4) - ALK(20)	Yes	Anaplastic large cell lymphoma
	ALK rearranged	FISH	TPM3(7) - ALK(20)	Yes	Inflammatory myofibroblastic tumour
	ALK rearranged	FISH	TPM3(7) - ALK(20)	Yes	Inflammatory myofibroblastic tumour
	ALK rearranged	FISH	ALK expression imbalance	Yes	Lung adenocarcinoma
	ALK-CCDC88A	Sequencing	CCDC88A(12) - ALK(20)	Yes	Malignant hemispheric glioma
BRAF	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9/10)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(14) - BRAF(9)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(14) - BRAF(9)	Yes	Pliocytic Astrocytoma
MET	MET ex 14 skipping	Sequencing	MET(13)-MET(15)	Yes	Lung adenocarcinoma
NTRK3	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secreotry carcinoma of salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secretory carcinoma of Salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secretory carcinoma of Salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Adenocarcinoma
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Congenital mesoblastic nephroma
	ETV6-NTRK3	Sequencing	ETV6(5) - NTRK3(15)	Yes	Infantile fibrosarcoma
ROS1	ROS1 rearranged	FISH	CD74(6) - ROS1(34)	Yes	Lung adenocarcinoma
	ROS1 rearranged	FISH & IHC	CD74(6) - ROS1(34)	Yes	Lung adenocarcinoma





Verification of OPA for CNV Detection

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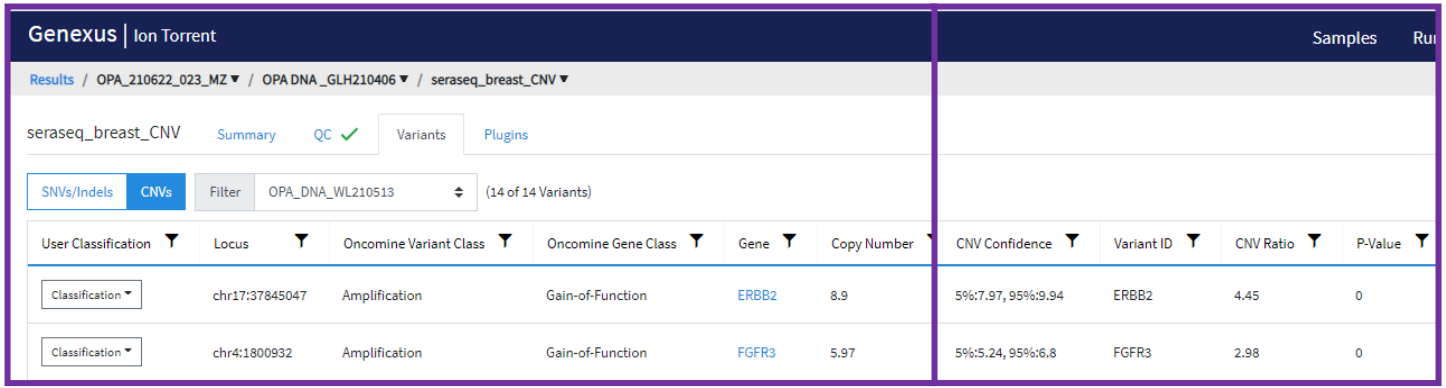
100% sensitivity in 5 OPA- targetable genes for copy number variant detection in commercial and clinical FFPE samples

Gene	Copy No. expected	Control method	Run 1		Run 2		Concordant	Sample type
			Copy No. detected	Copy No. ratio	Copy No. detected	Copy No. ratio		
EGFR	5.6	Commercial standard	5.9	3.0	6.2	3.1	Yes	Seraseq™1 (Cat. 0710-0414)
MET	5.7	Commercial standard	7.3	3.6	7.6	3.8	Yes	Seraseq™1 (Cat. 0710-0414)
AR	1.0	Commercial standard	0.9	0.5	0.9	0.5	Yes	Seraseq™1 (Cat. 0710-0414)
ERBB2	8.5	Commercial standard	8.6	4.3	8.9	4.5	Yes	Seraseq™2 (Cat. 0710-0412)
FGFR3	8.3	Commercial standard	6.4	3.2	6.0	3.0	Yes	Seraseq™2 (Cat. 0710-0412)
ERBB2		IHC 3+	10.0	5.0			Yes	Breast cancer
ERBB2		IHC 3+	9.6	4.8			Yes	Breast cancer
ERBB2		IHC 3+	4.7	2.3			Yes	Breast cancer
ERBB2		IHC 3+	9.6	4.8			Yes	Breast cancer
All targets			CNV not detected	N/A			Yes	Tonsil



Solid Tumour Tissue Pathway

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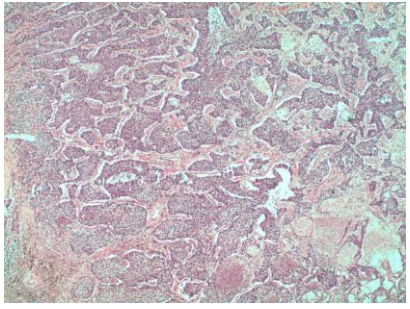
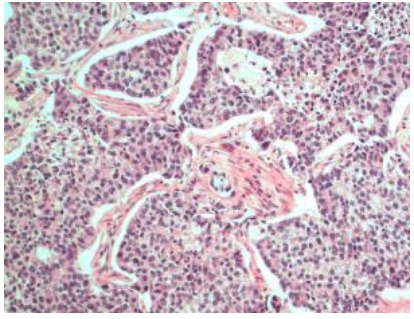
- Clinical research samples are hugely variable.
  - Vast majority are FFPE tissue blocks, some are unstained paraffin sections. Tissue is often cut out and stained slides are the only materials available.
  - Tissues can be surgical resections, needle biopsies or fine needle aspirate (FNA) so size ranges from megablocks, small cores to cell clot with few cells.
  - Tissue can be freshly fixed, fixed following decalcification (if bone) or archival materials stored for years or even decades.
  - Tissue can be necrotic, haemorrhagic and pigmented (melanoma).

All these affect quality and quantity of nucleic acids extracted.

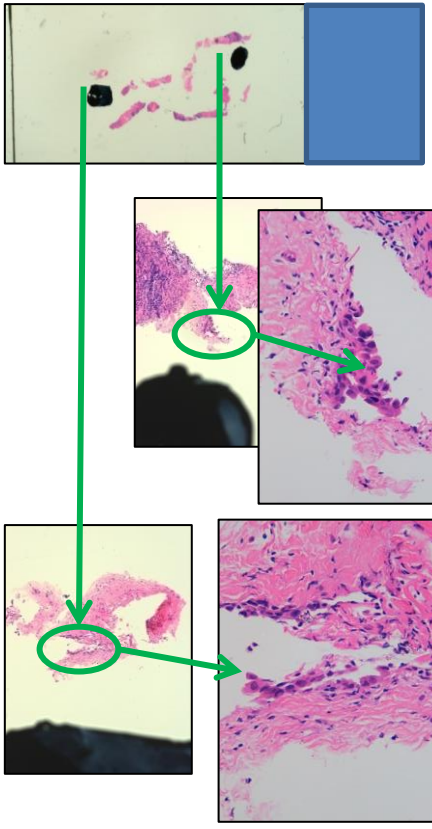
Tissues vary in size and amount of tumour cells

Resection specimen with lung adenocarcinoma

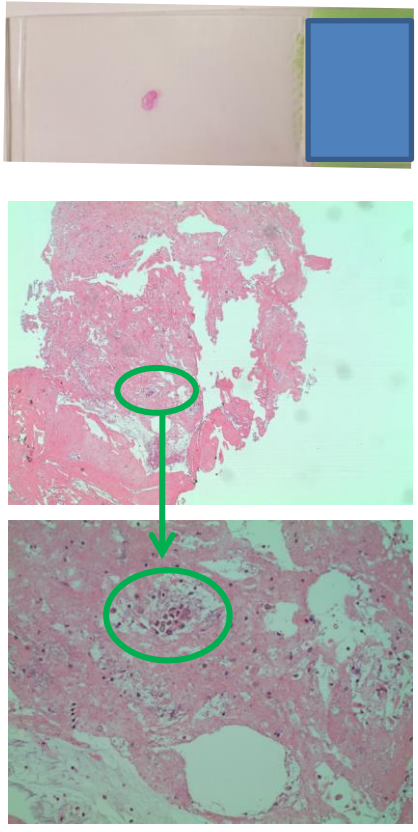




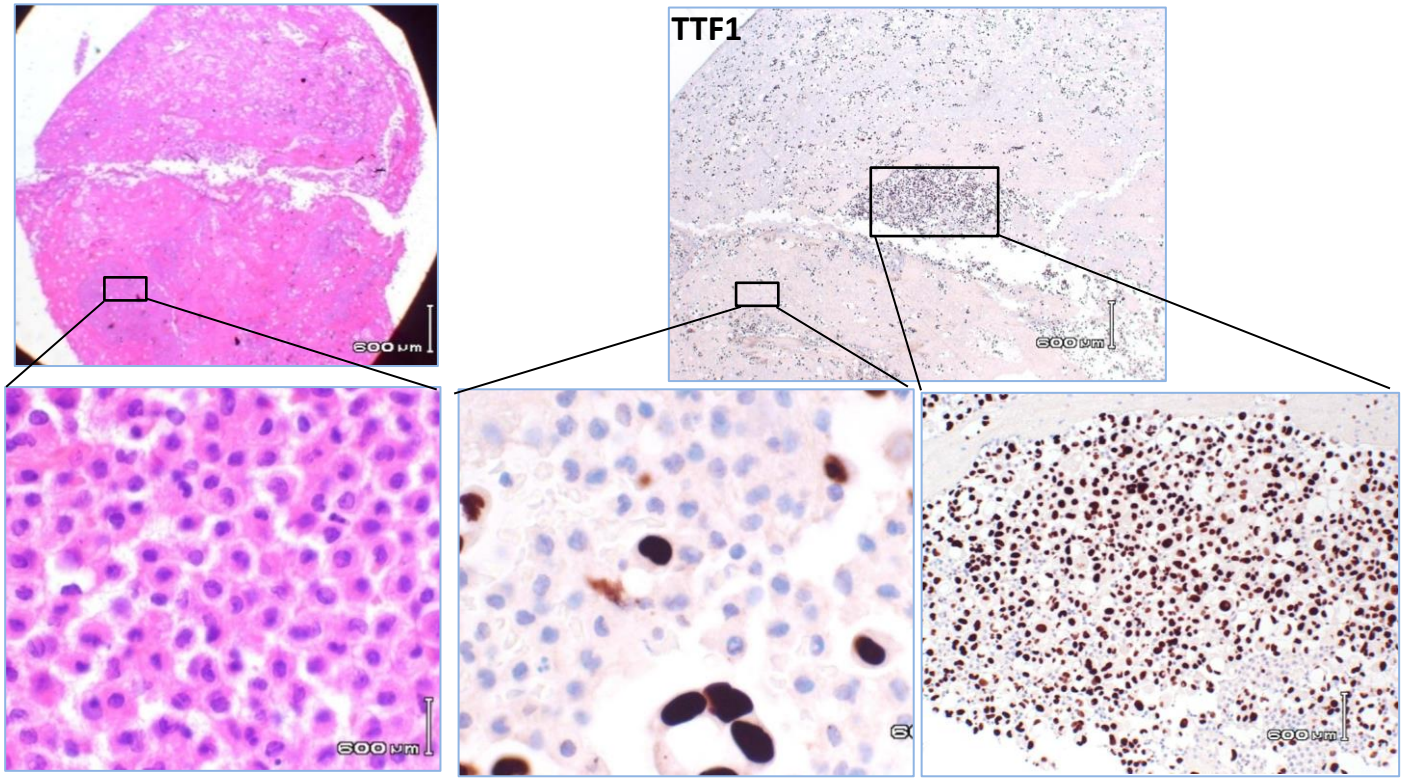
Needle biopsy with lung adenocarcinoma



FNA with lung adenocarcinoma

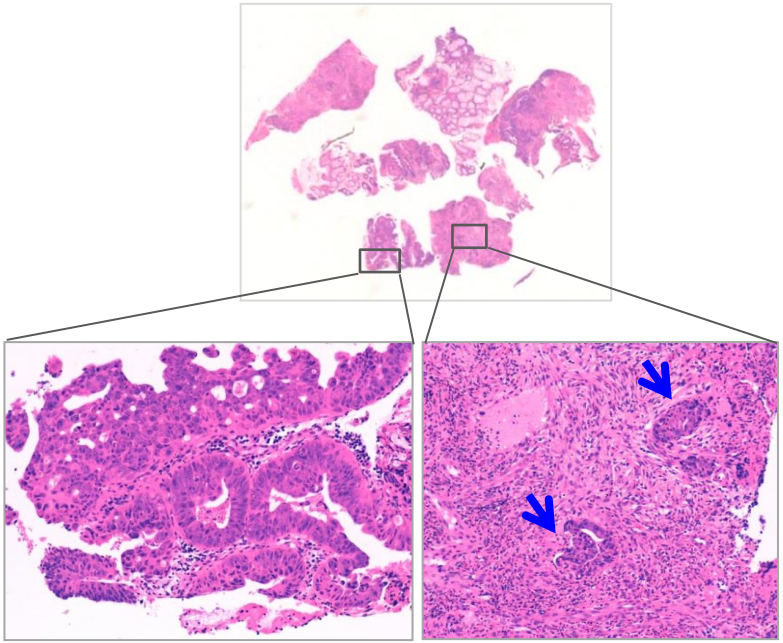


A pleural cytology sample with metastatic NSCLC



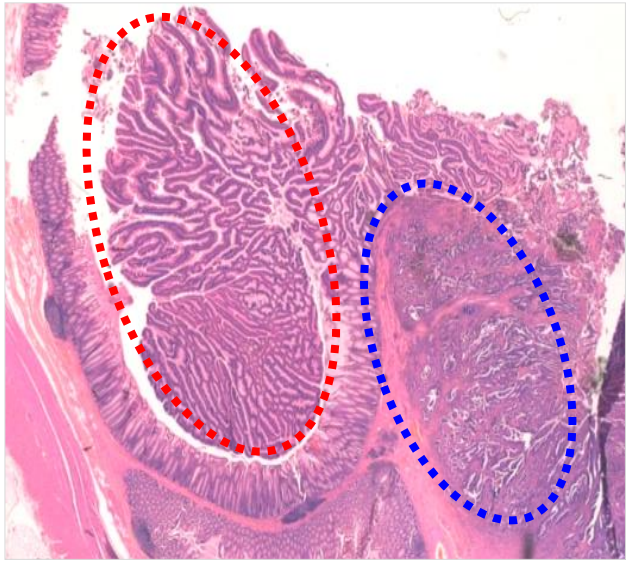
Difficult to distinguish mesothelial cells from tumour cells on morphology

Rectal biopsies with invasive CRC in adenoma background



High grade dysplasia

Invasive carcinoma



Adenoma
BRAF
V600E

Adenocarcinoma
BRAF wt

Identify correct cell population for analysis

Dissect only invasive tumour for testing

Sample processing considerations

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- Sample should always be morphologically assessed prior to testing.
  - Is tumour present? Are there enough tumour cells?
  - Is micro- or macro-dissection required to enrich tumour cells to the minimum level required by the assay?

Section tissue and stain the first and last sections to assess tissue morphology

↓

Mark tumour areas/cells

↓

Dissect the marked areas/cells +/- a microscope estimate tumour cell %

↓

Extract DNA

Need to work closely with pathologists

Audit of 1st Three-Month DNA Sequencing Results

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A total of 196 samples sequenced by 22/06/21 with 2 failed and 194 successful, a success rate of 99%

Sample type (n=194)		
PB/BMA	4	2.1%
FFPE	190	97.9%

Type of FFPE sample (n=190)		
Resection	84	44.2%
Small biopsy	75	39.5%
Cytology	26	13.7%
Unspecified	5	2.6%

FFPE tissue processing (n=190)		
Non-dissection	11	5.8%
Macro-dissection	55	28.9%
Micro-dissection	124	65.3%

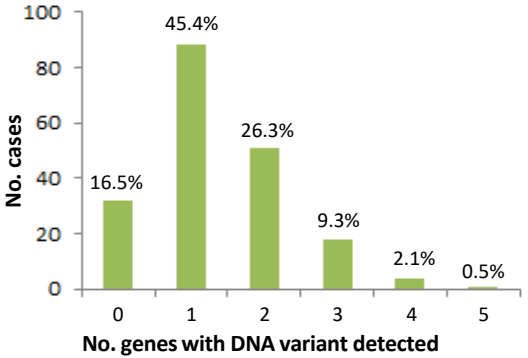
Tumour cell content in dissected sample (n=190)		
>20% but <30%	3	1.6%
>30%%	187	98.4%

Tumour sample type (n=194)		
Lung cancer	89	45.9%
Colorectal cancer	39	20.1%
Melanoma	19	9.8%
Brain tumour	15	7.7%
GIST	11	5.7%
Haematological malignancy	10	5.2%
Sarcoma	6	3.1%
Thyroid cancer	1	0.5%
Hepatocellualr adenoma	1	0.5%
Undifferentiated	3	1.5%

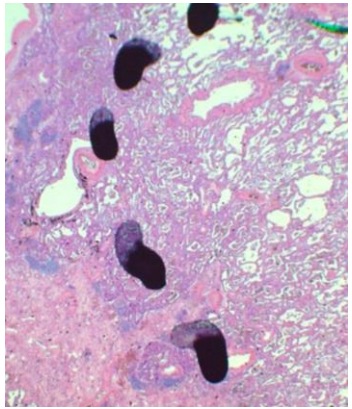
DNA extraction		
Crude proteinase K digest	181	95%
Purified	9	5%

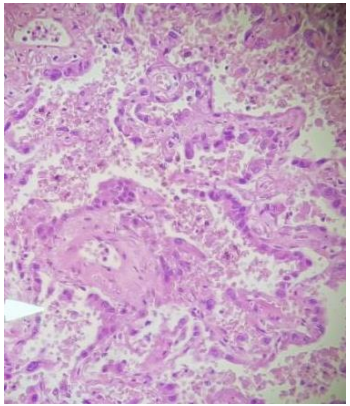
DNA quantity		
Not quantifiable	3	(1.6%)
<10ng	11	(5.8%, 3 < 5ng)
>10ng	176	(92.6%)

Anatomic site of samples (n=190)		
Lung	52	27.4%
Lymph node	35	18.4%
Large bowel	33	17.4%
Brain	14	7.4%
Skin	9	4.7%
Stomach	7	3.7%
Pleural	5	2.6%
Liver	4	2.1%
Bone	4	2.1%
Others	27	14.2%

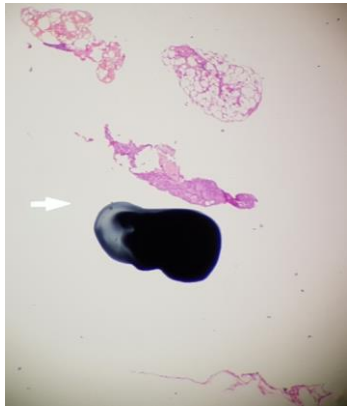


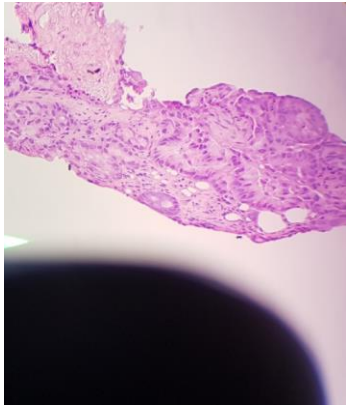
Case example 1



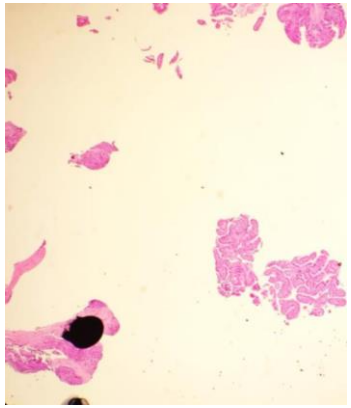


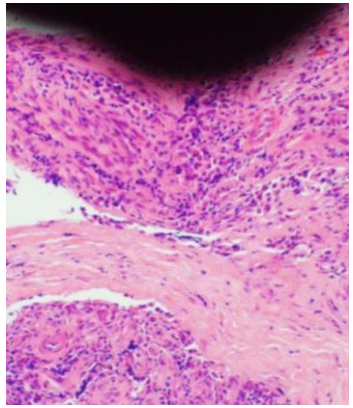
Case example 2





Case example 3





Summary

East Genomic Laboratory Hub

- Our verification results and initial clinical experience showed OPA on Genexus to be highly successful in solid cancer testing for often challenging tissue samples
- Genexus platform is a fully automated system, integrating library preparation, templating, sequencing, data analysis and reporting into a single-day, single-instrument run and on-instrument software.
- OPA covers most relevant targets for SNV, CNV and fusions and enable accurate detection of key biomarkers for prediction of response to targeted therapies, and for disease diagnosis and patient prognostication in multiple cancers
- Works well with FFPE tissue and all types of routine tumour samples, with 99% success rate
- Requires Low DNA input (1-10ng, crude PK digest, H&E or IHC stained)
- Has high sensitivity (5% VAF), >2000X coverage in average
- Is capable of delivering rapid, high-throughput and highly standardised testing service
- Is suitable for testing of suboptimal samples triaged into in the salvage pathway

Acknowledgements

East Genomic Laboratory Hub

All staff in East GLH Solid Cancer Team

- NGS and Technical Team: Dr Wanhua Lu, Dr Saskia Neuert, Jennifer Jones, Maria ZafraPinto, Georgina Corfield
- Sample Reception and Booking in Team
- Dissection Team: Thomas Roberts, Jennifer Jones
- Reporting Scientists and Molecular Pathologists
- East GLH Quality Team and Senior Management

Thermo Fisher Scientific for training, education, and ongoing support for Genexus assays