

Next Generation Sequencing (NGS)

Clonal sequencing using the Illumina MiSeq platform has been introduced as a diagnostic service in the Laboratory in June 2013 replacing conventional Sanger sequencing as required following discussion with referring clinicians. Our validation studies* demonstrated a test sensitivity and specificity at least comparable to, and in some cases surpassing, those for conventional sequencing. The laboratory has now introduced a number of services utilising this technology with more currently in development. In addition, the laboratory will be introducing a number of clonal sequencing services using the LifeTechnologies Ion PGM platform.

* In-house data

Current NGS Services

SureSelect

Glycogen Storage Disorders

(SureSelect Design ID: 0423281).

Liver panel	Muscle panel	Heart Panel	Generalised Panel
AGL	AGL	AGL	GAA
FBP1	GAA	GBE1	EPM2A
G6PC	GBE1	PRKAG2	LAMP2
GBE1	GYS1		NHLRC1
GYS2	PFKM		
PHKA2	PGAM2		
PHKB	PHKA1		
PHKG2	PHKG1		
PYGL	PYGM		
SLC37A4	ALDOA		
GYG2	ENO3		
PFKL	FBP2		
SLC2A2	GYG1		
	LDHA		
	PGK1		

Connective Tissue Disorders

(SureSelect Design ID: 0423291).

EDS		
Vascular	Classic	Kyphoscoliotic
<i>COL3A1</i>	<i>COL5A1;</i> <i>COL5A2</i>	<i>CHST14</i> <i>PLOD1</i> <i>FKBP14</i> <i>RIN2</i> <i>PRDM5</i> <i>ZNF469</i> <i>B4GALT7</i> <i>SLC39A13</i>

Familial Thoracic Aortic Aneurysms
<i>COL3A1</i> <i>FBN1</i> <i>TGFBR1</i> <i>TGFBR2</i> <i>FBN2</i> <i>ACTA2</i> <i>MYH11</i> <i>SLC2A10</i> <i>TGFB2</i> <i>FLNA</i> <i>SMAD3</i>

Osteogenesis Imperfecta		
Dominant	Autosomal Recessive	Complete
<i>COL1A1</i> <i>COL1A2</i> <i>IFITM5</i>	<i>CRTAP</i> <i>LEPRE1</i> <i>PPIB</i> <i>SP7</i> <i>FKBP10</i> <i>SERPINF1</i> <i>SERPINH1</i> <i>PLOD2</i> <i>BMP1</i> <i>TMEM38B</i> <i>WNT1</i>	<i>COL1A1</i> <i>COL1A2</i> <i>IFITM5</i> <i>CRTAP</i> <i>LEPRE1</i> <i>PPIB</i> <i>SP7</i> <i>FKBP10</i> <i>SERPINF1</i> <i>SERPINH1</i> <i>PLOD2</i> <i>BMP1</i> <i>TMEM38B</i> <i>WNT1</i>

Familial Porencephaly
<i>COL4A1</i> <i>COL4A2</i>

TruSight Cancer Panels

(Illumina Catalog No. TG-141-1002).

Breast and Ovarian Cancer	
Primary	Extended
<i>BRCA1</i> <i>BRCA2</i> <i>TP53</i>	<i>ATM</i> <i>BAP1</i> <i>BRCA1</i> <i>BRCA2</i> <i>BRIP1</i> <i>CDH1</i> <i>CHEK2</i> <i>PALB2 (FANCN)</i> <i>RAD51C (FANCO)</i> <i>RAD51D</i> <i>RB1</i> <i>TP53</i> <i>PTEN</i> <i>STK11</i> <i>PPM1D</i> <i>NBN</i>

Colorectal Cancer	
Primary	Extended
<i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS1</i> <i>PMS2</i> <i>APC</i> <i>MUTYH</i>	<i>APC</i> <i>BMPR1A</i> <i>EPCAM</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>MUTYH</i> <i>PMS1</i> <i>PMS2</i> <i>PTEN</i> <i>SMAD4</i> <i>STK11</i>

Endocrine Cancer
<i>MEN1</i> <i>PRKAR1A</i> <i>RET</i> <i>SDHB</i> <i>SDHC</i> <i>SDHD</i> <i>SDHAF2</i>

Renal Cancer
<i>BAP1</i> <i>FH</i> <i>FLCN (BHD)</i> <i>GPC3</i> <i>HNF1A</i> <i>MET</i> <i>MITF</i> <i>TSC1</i> <i>TSC2</i> <i>VHL</i> <i>WT1</i>

Fanconi Anaemia
<i>FANCA</i> <i>FANCB</i> <i>FANCC</i> <i>BRCA2</i> <i>FANCD2</i> <i>FANCE</i> <i>FANCF</i> <i>FANCG</i> <i>FANCI</i> <i>BRIP1</i> <i>FANCL</i> <i>FANCM</i> <i>PALB2</i> <i>RAD51C</i> <i>SLX4</i>

Testing Workflow

Library Preparation

SureSelect

- Shearing of genomic DNA using the Covaris E220 sonicator.
- End repair, A tailing and ligation of adaptors using SureSelectXT library system (Agilent Technologies).
- Enrichment by SureSelect target enrichment (Agilent Technologies) using custom in house designed probes. Samples have barcode tags added following target enrichment.
- Sequencing on the Illumina MiSeq using the MiSeq Reagent Kit v2 performing 2 x 150 bp end paired reads.

TruSight

- Genomic DNA fragmented, barcode tagged and captured using the TruSight™ Rapid Capture (Illumina) protocol.
- Sequencing on the Illumina MiSeq using the MiSeq Reagent Kit v2 performing 2 x 150 bp end paired reads.

Data Analysis

- Based on the open source 'Best Practices' workflow by the Broad Institute (for additional information, see <http://www.broadinstitute.org/gatk/guide/best-practices>).
- BWA alignment of reads to human genome build hg19.
- Generation of depth of coverage reports. Checked using Alamut v 2.2 (Rev1) (Interactive Biosoftware).
- A minimum threshold of 30-fold read depth is set for exonic sequences and intronic sequences upto and including 5 bp from exon. A minimum threshold of 18-fold read depth is set for intronic sequences from 6 bp to 25 bp from exon.
- Identification of variants using HaplotypeCaller. Annotation from dbSNP and COSMIC (currently dbSNP138 and COSMIC v67 but updated with new releases)
- Variants filtered against in-house polymorphism lists and Best Practice Guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics (Association for Clinical Genetic Science).

Post analysis

- Confirmation of all clinically significant sequence variants by Sanger sequencing.
- Filling of gaps with low depth of coverage by Sanger sequencing.
- Creation of a diagnostic report combining clonal and Sanger sequence data.

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