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
AGL
FBP1
G6PC
GBE1
GYS2
PHKA2
PHKB
PHKG2
PYGL
SLC37A4
GYG2
PFKL
SLC2A2

Muscle panel
AGL
GAA
GBE1
GYS1
PFKM
PGAM2
PHKA1
PHKG1
PYGM
ALDOA
ENO3
FBP2
GYG1
LDHA
PGK1

Heart Panel
AGL
GBE1
PRKAG2

Generalised Panel
GAA
EPM2A
LAMP2
NHLRC1

Connective Tissue Disorders

(SureSelect Design ID: 0423291).

EDS		
Vascular	**Classic**	**Kyphoscoliotic**
COL3A1	*COL5A1;*	*CHST14*
	COL5A2	*PLOD1*
		FKBP14
		RIN2
		PRDM5
		ZNF469
		B4GALT7
		SLC39A13

Familial Thoracic Aortic Aneurysms
COL3A1
FBN1
TGFBR1
TGFBR2
FBN2
ACTA2
MYH11
SLC2A10
TGFB2
FLNA
SMAD3

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

Familial Porencephaly
COL4A1
COL4A2

TruSight Cancer Panels

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

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

Colorectal Cancer	
Primary	**Extended**
MLH1	*APC*
MSH2	*BMPR1A*
MSH6	*EPCAM*
PMS1	*MLH1*
PMS2	*MSH2*
	MSH6
APC	*MUTYH*
MUTYH	*PMS1*
	PMS2
	PTEN
	SMAD4
	STK11

Endocrine Cancer
MEN1
PRKAR1A
RET
SDHB
SDHC
SDHD
SDHAF2

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

Fanconi Anaemia
FANCA
FANCB
FANCC
BRCA2
FANCD2
FANCE
FANCF
FANCG
FANCI
BRIP1
FANCL
FANCM
PALB2
RAD51C
SLX4
ERCC4

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.