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 of Glycogen Storage Diseases as required following discussion with referring clinicians. Our validation studies* demonstrated a test sensitivity and specificity comparable to conventional sequencing. The laboratory will be introducing a number of additional services utilising this technology. 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

Glycogen Storage Disorders

(Design ID: 0423281).

Primary liver panel		**Liver panel**	
(available during validation phase)	AGL		AGL
	FBP1		FBP1
	G6PC		G6PC
	GBE1		GBE1
	GYS2		GYS2
	PHKA2		PHKA2
	PHKB		PHKB
	PHKG2		PHKG2
	PYGL		PYGL
	SLC37A4		SLC37A4
			GYG2
			PFKL
			SLC2A2
Primary muscle panel		**Muscle panel**	
(available during validation phase)	AGL		AGL
	GAA		GAA
	GBE1		GBE1
	GYS1		GYS1
	PFKM		PFKM
	PGAM2		PGAM2
	PHKA1		PHKA1
	PHKG1		PHKG1
	PYGM		PYGM
			ALDOA
			ENO3
			FBP2
			GYG1
			LDHA
			PGK1

Testing Workflow

Library Preparation

- 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.
- Quantification of tagged samples by QPCR using KAPA Library quantification kit for Illumina and lightcycler 480.
- Sequencing on the Illumina MiSeq using the MiSeq Reagent Kit v2 performing 2 x 150bp end paired reads.

Data Analysis

- Data analysis using NextGENe v2.3.1 & v2.3.3 (SoftGenetics) software.
- Data aligned to human genome build hg19 (dbSNP135).
- Generation of mutation reports and coverage curves.
- Read depth checked visually using Alamut v 2.2 (Rev1) (Interactive Biosoftware)
- Variants filtered following Best Practice Guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics (Association for Clinical Genetic Science).
- Variant frequency checked using dbSNP build 137.
- A minimum threshold of 30-fold read depth is set for diagnostic sequencing.

Post analysis

- Confirmation of all clinically significant sequence variants by Sanger sequencing.
- Creation of a diagnostic report combining clonal and Sanger sequence data.