The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

NewGene
Next Generation Diagnostics

Newcastle Genetic Diagnostic Services

NewGene Ltd
Northern Genetics Service
Newcastle Mitochondrial NSCT Diagnostic Lab

This service provides centralised access to the genetic diagnostic capability that can be found in Newcastle Upon Tyne, UK, offering comprehensive genetic and molecular testing provision.

NewGene Ltd Established through a partnership between the Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University, NewGene is a pioneer in developing, validating and delivering molecular diagnostics using the latest high throughput sequencing and genotyping technologies.

Northern Genetics Service Specialised genetic services have developed in the United Kingdom as regional centres of expertise, and in the Northern Region these services are co-ordinated through the Northern Genetics Service, which is part of the Institute of Genetic Medicine.

Newcastle Mitochondrial NSCT Diagnostic Laboratory has been providing a comprehensive, diagnostic and clinical management service for patients with suspected mitochondrial disease for over 10 years. Awarded National Specialist Commissioning (NCG) funding from the Department of Health in 2007, the laboratory works with partners provide specialist clinical and diagnostic investigations

Full details on the range of tests available and samples required can be found in this document.

To express interest please contact Dr Angela Silmon at NewGene Limited, Bioscience Building, International Centre for Life, Newcastle upon Tyne, NE1 4EP, UK

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Hereditary Multigene Disorders

Full gene sequencing using massively parallel next generation sequencing techniques.

Disorder	**Genes included**
Hereditary Breast Cancer	*BRCA1* and *BRCA2*
Hereditary Colorectal Cancer: HNPCC	*MLH1, MSH2, MSH6* (excluding exon 1) *PMS2*, 3' UTR of *EPCAM*
Hereditary Colorectal Cancer: FAP	*APC, MUTYH*
Noonan's Syndrome	*PTPN11, BRAF, SOS1, RAF1, KRAS, HRAS, NRAS, SHOC2* (exon 1 only), *CBL, SPRED1, MAP2K1, MAP2K2*
Atypical Haemolytic Uraemia Syndrome	*CFH, CD46, CF1, C3, CFB* In collaboration with NGS

Sample type – 5ml of whole blood in EDTA tube or genomic DNA.
Turn around time – 8 weeks from receipt of sample.

Personalised Medicine

Analysis of specific mutation hot spots associated with disease that can predict the patient response to treatment. Genotype analysis using the Sequenom MassARRAY 4 platform.

Disorder	**Associated genes**
Metastatic Colorectal Cancer	*KRAS, NRAS, BRAF*
Non-small Cell Lung Cancer	*EGFR*
Melanoma	*BRAF*
Gastrointestinal Stromal Tumours	*cKIT, PDGFRA*
*Adverse drug reactions to thiopurines	*TPMT*

Sample type – FFPE blocks or curls, or genomic DNA.
* 5ml of whole blood in EDTA tube or genomic DNA.
Turn around time – 5 days from receipt of sample.

Haemato-oncology Testing

Genotype analysis in blood based cancers and myeloproliferative disorders.

Disorder	**Analysis**
Chronic Myeloid Leukaemia	*BCR-ABL* fusion gene including T315I (c.944C>T)
Myeloproliferative disorders	*JAK2* exon 12 including V617F, *MPL*
Hairy Cell Leukaemia	*BRAF* V600E
Mastocytosis	*cKIT* D816V

Sample type – 5ml of whole blood in EDTA tube or genomic DNA.
Turn around time – 7-10 days from receipt of sample.

Molecular Diagnostics

Disorder	Comment
Alpha-1-antitrypsin deficiency	Diagnostic and carrier testing
Angelman syndrome / Prader Willi syndrome	Diagnostic testing Recurrence risk using linkage markers
Autoimmune polyendocrinopathy type 1	Diagnostic testing and carrier testing Prenatal diagnosis – 1:4 risk
Breast cancer	Testing for known causative mutation Deletion testing (MLPA) - per gene
Cartilage Hair Hypoplasia (RMRP gene)	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Cockayne Syndrome	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Coffin Lowry syndrome	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Chronic Mucocutaneous Candidiasis (STAT1 gene)	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Connexin 32	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Connexin 26-related deafness	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Congenital adrenal hypoplasia	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Cornelia de Lange syndrome	Mutation searching Testing for known causative mutation Prenatal diagnosis (known mutation)
Cystic fibrosis	Mutation testing for 50 mutations in CFTR Testing for known causative mutation Prenatal diagnosis

Disorder	Comment
Dentatorubral-pallidoluysian atrophy (DRPLA)	Diagnostic testing
Duchenne & Becker muscular dystrophy	Deletion/duplication screen (MLPA) Linkage analysis Prenatal diagnosis (known mutation) Prenatal diagnosis (linkage)
Dystonia	Diagnostic testing
Facioscapulohumeral muscular dystrophy (FSHMD)	Diagnostic testing Prenatal diagnosis
Familial Adenomatous Polyposis Coli	Testing for known mutations
FLT3	Testing for 2 known mutations
Fragile X syndrome	Diagnostic testing and carrier testing - PCR + Southern blotting Prenatal diagnosis
Friedreich ataxia	Diagnostic testing and carrier testing Prenatal diagnosis
Haemochromatosis	Diagnostic testing and carrier testing
Haemophilia A	Diagnostic testing and carrier testing Detection of intron 22 and/or intron 1 inversions Mutation searching Linked markers Testing for known causative mutation Prenatal diagnosis
Hemolytic uremic syndrome	In collaboration with NewGene Presymptomatic testing - known causative mutation
Hereditary motor and sensory neuropathy type 1A (HMSN1)	Diagnostic testing
Hereditary neuropathy with liability to pressure palsies (HNPP)	Diagnostic testing
Hereditary non-polyposis colon cancer (HNPCC)	Deletion testing (MLPA) - per gene Presymptomatic testing - known causative mutation
Huntington's disease	Diagnostic and predictive testing Prenatal diagnosis (parent is a known mutation carrier) Prenatal exclusion testing (max 5 samples, 2 markers)

Disorder	Comment
Identity testing	Powerplex 16 assay (per sample) Y-plex assay

Medium chain acyl-CoA dehydrogenase deficiency (MCAD)	Diagnostic and carrier testing Prenatal diagnosis - 1:4 risk
Microsatellite instability	MSI testing
MYH associated polyposis	Testing for 2 common mutations Testing for known mutation in relatives
Myotonic dystrophy types 1 and 2	Diagnostic testing and carrier testing Prenatal diagnosis

Neuroferritinopathy	Diagnostic and predictive testing

RETT syndrome	Mutation searching (MECP2) in patients with clinical symptoms (incl. MLPA) Mutation searching (CDKL5) in patients with clinical symptoms Prenatal diagnosis

Simpson-Golabi-Behmel Syndrome	Mutation searching in patients with clinical symptoms (incl. MLPA) Testing for known causative mutation in relatives Prenatal diagnosis
Spinal Muscular Atrophy (types 1, 2 and 3)	Diagnostic testing (MLPA) Prenatal diagnosis
Spinocerebellar ataxia types 1, 2, 3,6, 7, 17	Diagnostic testing - all SCAs Diagnostic/presymptomatic testing - per SCA gene tested
Spinal and bulbar muscular atrophy (Kennedy's disease)	Diagnostic testing

X inactivation studies	Using CAG in androgen receptor

Zygosity testing	Clinical referrals only (per twin pair)

Sample type – 5ml of whole blood in EDTA tube or genomic DNA.
Turn around time – 8 weeks from receipt of sample.

Limb Girdle Muscular Dystrophy

Gene	Comment
LGMD 1B - Lamin A/C	
LGMD 1C - Caveolin 3	
LGMD 2A - Calpain 3	
LGMD 2B - Dysferlinopathy	
All 4 Sarccoglycan Genes (Alpha / Beta / Gamma/ Delta) are analysed together in one assay	
LGMD 2D - Alpha-sarcoglycan	
LGMD 2E - Beta-sarcoglycan	
LGMD 2C - Gamma-sarcoglycan	
LGMD 2F - Delta-sarcoglycan	
LGMD 2I - FKRP	
LGMD 2L - Anoctamin 5	
DNAJB6	
FHL1	
Myofibrillar Myopathy Genes	Desmin, Myotilin, CRYAB, ZASP
BAG3	
Emerin	
GNE	
VCP	
Titin	Mutation searching in exons 293 & 308 for common mutations in patients with clinical symptoms

Mutation searching in patients with clinical symptoms, deletion testing (MLPA), testing for known causative mutation in relatives (per mutation) and prenatal diagnosis available as appropriate for each gene.

Sample type – 5ml of whole blood in EDTA tube or genomic DNA.
Turn around time – 8 weeks from receipt of sample.

Cytogenetics

Constitutional / Syndromal Investigations	Array CGH in developmental and neuro-cognitive disorders Karyotype FISH QF-PCR Chromosome fragility testing
Prenatal	Amniotic fluid or CVS QF-PCR and Karyotype
Fetal Loss investigation	Tissue culture and analysis by karyotype QF-PCR or array CGH
Cancer	Bone marrow / tumour karyotyping in lekaemias and solid tumours. Fresh / frozen / paraffinised tumour: FISH and/or RT-PCR for detection of translocations / gene fusions ALK rearrangements in lung cancer. 1p/19q deletions in oligodendroglioma. Genetic prognostic screening in chronic lymphocytic leukaemia.
Others	Cell line characterisation by karyotype Fibroblast culture and liqN storage MLPA

Newcastle Mitochondrial NSCT Diagnostic Service

Histological / Histochemical Analyses

- H&E staining of cryostat-cut sections
- Cytochrome c oxidase (COX) activity
- Succinate dehydrogenase (SDH) activity
- Sequential COX-SDH activities
- Modified Gomori trichrome staining

Sample type: unfixed frozen muscle sample, in transverse orientation, measuring approximately 3mm x 3mm x 3mm (25 mg) is the minimum required
Turn Around Time: 2 weeks

Mitochondrial Respiratory Chain Analyses

- Complex I (NADH:ubiquinone oxidoreductase) activity
- Complex II (Succinate:ubiquinone oxidoreductase) activity
- Complex III (Ubiquinol:cytochrome c oxidoreductase) activity
- Complex IV (Cytochrome c oxidase) activity
- Citrate Synthase

Sample type: unfixed, snap-frozen muscle sample is required for these investigations, preferably unmounted and free from OCT and cork. Ideally, between 100-150 mg muscle tissue is preferred.
Turn Around Time: 4-6 weeks

Molecular Genetic Analyses

Genes	**Disorder**	**Type of Test**	**Type of Sample**
m.3243A>G point mutation	MELAS syndrome, MIDD (maternally-inherited diabetes deafness)	Sequencing	Blood DNA Muscle if available
m.8344A>G m.8356T>C m.8363G>A *MTTK* (tRNALys) gene	MERRF syndrome	Sequencing	Blood DNA Muscle if available
m.8993T>G/C m.9176T>G/C mutations *MTATP6* and *MTATP8* genes	NARP MILS (maternally-inherited Leigh Syndrome)	Sequencing	Blood DNA

Genes	Disorder	Type of Test	Type of Sample
m.3460G>A m.11778G>A m.14484T>C	LHON mutations	Sequencing	Blood DNA
	mtDNA rearrangement disorders Kearns-Sayre, Pearsons syndrome	Long-range PCR and/or Southern blotting of affected tissues	Muscle DNA essential Blood DNA for Pearson's Syndrome
MTRNR1 (12S rRNA) *including* m.1555A>G *MTTS1* ($tRNA^{Ser(UCN)}$)	Mitochondrial deafness mutations	Sequencing	Blood DNA
All mitochondrial encoded complex I genes *MTND* gene sequencing	Isolated complex I deficiency LHON patients where common mutations excluded	Sequencing	DNA from affected tissue LHON can be assessed in blood
Mitochondrial encoded cytochrome *b* gene *MTCYB* gene	Isolated complex III deficiency	Sequencing	DNA from affected tissue
Mitochondrial genome sequencing	Screen for rare or novel pathogenic mtDNA mutations	Sequencing	DNA from affected tissue
Quantification and screening of mtDNA rearrangements in individual muscle fibres (COX deficient and COX positive)	Single fibre real-time PCR	real-time PCR	An unfixed frozen muscle biopsy sample, in transverse orientation
18S rRNA and mtDNA *MTND1* genes Assessment of mtDNA copy number	mtDNA depletion syndromes	Taqman real-time PCR assay	DNA from affected tissues essential
POLG1	Phenotypic spectrum ranging from severe encephalopathy and liver failure typical of Alpers syndrome to late-onset PEO, ataxia, myopathy and epilepsy	Sequencing	Blood DNA
PEO1 (Twinkle)	Patients with dominant/recessive PEO and multiple mtDNA deletions	Sequencing	Blood DNA
SLC25A4 (ANT1)	Patients with dominant PEO and multiple mtDNA deletions	Sequencing	Blood DNA
POLG2	Patients with dominant PEO and multiple mtDNA deletions	Sequencing	Blood DNA

Genes	Disorder	Type of Test	Type of Sample
TK2 and *RRM2B*	Patients with dominant/recessive PEO and multiple mtDNA deletions	Sequencing	Blood DNA
TK2 and *RRM2B*	Patients with evidence of mtDNA depletion in skeletal muscle	Sequencing	Blood DNA
DGUOK and *MPV17*	Patients with evidence of mtDNA depletion in liver	Sequencing	Blood DNA
SUCLA2 and *SUCLG1*	Patients with evidence of mtDNA depletion and methylmalonic aciduria	Sequencing	Blood DNA
TRMU	Patients with evidence of acute liver disease and respiratory chain deficiency	Sequencing	Blood DNA
DARS2	Patients with mitochondrial leukoencephalopathy, brain stem and spinal cord involvement, lactic acidosis (LBSL)	Sequencing	Blood DNA
RARS2	Patients with multiple respiratory chain complex deficiencies and pontocerebellar hyoplasia	Sequencing	Blood DNA
AARS2	Patients with multiple respiratory chain complex deficiencies and hypertrophic cardiomyopathy	Sequencing	Blood DNA
NDUFV1, *NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS4,* NDUFS6, *NDUFS7, NDUFS8, NDUFAB1*	Nuclear complex I structural genes Isolated complex I deficiency	Sequencing	Blood DNA
ACAD9 – complex I assembly factor	Patients with isolated complex I deficiency; often associated with cardiac phenotype	Sequencing	Blood DNA
structural genes SDHA, *SDHB, SDHC, SDHD* known assembly factors SDHAF1 and *SDHAF2*	Complex II structural and assembly genes Patients with evidence of isolated complex II deficiency.	Sequencing	Blood DNA
BCS1L	Nuclear complex III genes Isolated complex III deficiency	Sequencing	Blood DNA
TMEM70, ATPAF2 and *ATP5E*	Suspected complex V deficiency	Sequencing	Blood DNA

Genes	Disorder	Type of Test	Type of Sample
AGK	Patients with multiple respiratory chain complex deficiencies and Sengers syndrome(cataracts, HCM and elevated lactate)	Sequencing	Blood DNA
NFU1	Patients with multiple respiratory chain complex deficiencies (complex I, III and III) – FeS cluster scaffold gene defect	Sequencing	Blood DNA
MTO1	Patients with multiple respiratory chain complex Deficiencies, hypertrophic cardiomyopathy and elevated lactate	Sequencing	Blood DNA

Other tests available on request

- Targeted Haloplex panels for Nuclear Complex I structural genes and mtDNA maintenance genes
- CVB and prenatal testing for nuclear and mtDNA mutations
- PGD for specific mtDNA mutations

Sample type: 5ml of whole blood in EDTA tube or genomic DNA

Muscle: If a muscle biopsy is being referred histochemical or biochemical assessment, we are able to extract DNA either directly from cryostat-cut sections or from a residual, nuclear pellet generated during the preparation of an enriched mitochondrial fraction for enzyme studies.

For other tissue types please enquire for requirements

Turn Around Time: 8 – 12 weeks depending upon the genetic analysis.