Massively Parallel Sequencing for Brittle Bone Disorders and Hereditary Rickets

Osteogenesis imperfecta (OI) comprises a group of inherited disorders characterized by bone fragility and increased susceptibility to fractures. The Brittle Bone Disorders panel contains genes known to cause both autosomal dominant and recessive forms of OI, with 90% of OI individuals heterozygous for causative variants in the *COL1A1* and *COL1A2* genes. The Hereditary Rickets panel contain genes known to cause X-linked, autosomal dominant and autosomal recessive forms of rickets, with heritable forms of rickets most commonly caused by mutations in the *PHEX* gene which leads to X-linked hypophosphataemic rickets. All the genes in the panels can be assessed in a single test using massively parallel sequencing (MPS) technology.

Important notice for MPS panel testing: We require a specific **MPS request form** for each sample, in order to assist with results interpretation. Please contact the laboratory at molgenlab.schn@health.nsw.gov.au for a copy. Testing will NOT commence unless this form has been received.

While MPS analysis will be restricted to the genes listed in the panel there remains a small risk of incidental findings and should be discussed with the patient prior to testing. **Written consent for genetic testing** from the patient must be obtained prior to testing and retained with the patient's medical record. A copy should be forwarded to the laboratory along with the request form.

Pricing and Turnaround times

Index case (Proband)

Bone Fragility panel testing using Massively Parallel Sequencing (MPS)		
Panel	**Price AUD$**	**Turnaround time**
Brittle Bone Disorders panel *BMP1, COL1A1, COL1A2, CRTAP, FKBP10, IFITM5, LEPRE1, LRP5, PLOD2, PPIB, SERPINF1, SERPINH1, SP7*	$1,500	16 weeks
Hereditary Rickets panel *PHEX, FGF23, ENPP1, DMP1, VDR, CYP27B1, SLC34A3, CLCN3, ALPL*	$1,200	16 weeks

Service includes DNA extraction (if required), TruSight target enrichment and library preparation, sequencing on Illumina HiSeq, bioinformatics analysis and variant interpretation. Sanger confirmation will be carried out for likely pathogenic mutation(s), such as those previously reported in the literature, mutations in domains associated with a disorder/phenotype with a coding change predicted to be damaging (based on in silico predictions), nonsense or frameshifting mutations, and changes affecting canonical splice sites. Other variants of uncertain significance (VOUS) will only be confirmed by Sanger sequencing upon request (for an additional charge).

Additional services		
Description	**Price AUD$**	**Turnaround time**
Additional Sanger confirmation for VOUS	$250 per variant	4 weeks
Re-analysis of sequencing data for additional genes	Price on inquiry	4 weeks
Additional Sanger "gap-filling" for ***COL1A1*** and ***COL1A2***	$600 per patient	4 weeks
Additional Sanger "gap-filling" for ***PHEX***	$300 per patient	4 weeks
Additional Sanger "gap-filling" for **other genes**	$100 per gap	4 weeks

Cascade testing

Once a family-specific mutation has been identified, cascade testing can be used for at-risk family members.

Cascade testing (known mutation)		
Sample description	**Price AUD$**	**Turnaround time**
Single patient (Sanger sequencing)	$250	4 weeks
Multiple patients (Sanger sequencing) More than one patient tested at the same time for the same familial mutation	$200	4 weeks
Predictive testing 2nd sample (Sanger sequencing) 2nd sample received after 1st sample has been tested	$100	4 weeks
Prenatal testing of familial mutation (Sanger sequencing)	$600	<2 weeks

Methodology

Illumina TruSight technology is used to perform gene targeting and library enrichment. The enrichment kit currently employed is the TruSight One panel (FC-141-1006). The full lists of genes can be found on the Illumina website (www.illumina.com/clinical/translational_genomics/panels.ilmn). Target regions of interest are restricted to coding regions and the canonical splice sites. Library sequencing will be performed using Illumina HiSeq 2500. The average performance for a sample meeting the appropriate quality control parameters (see Specimen) is an average coverage of 200x reads over the entire TruSight panel, equivalent to a yield of >97% of target bases with at least 20x coverage. Performance may vary across different gene sets.

Raw data will be aligned and variants will be called using SoftGenetics NextGene. The variants investigated will be limited to the genes/panel requested to minimize the risk of incidental findings. Raw data will be stored for 5 years and re-analysis can be carried out for additional genes when requested. Variants identified will be annotated using Alamut Batch and potential for pathogenicity will be assessed using Alamut interpretation software. Only mutations deemed likely to be pathogenic will be confirmed by Sanger sequencing. Additional Sanger confirmation of variants will incur extra charges.

A report will be generated to provide information on any putative pathogenic variants and to list any variants of uncertain significance (defined as having an allele frequency of <0.1% for dominant disorders, or <1% for recessive disorders; with a predicted coding consequence). The report will state parameters of sequencing performance, such as the average depth of coverage and the target rate for each of the genes of interest.

Currently, detection of copy number variations (CNV) is not available with our MPS panels. The methodology is undergoing validation. However, we offer CNV detection via multiplex ligation-dependent probe amplification (MLPA) for some of the genes included in the MPS panels. Please contact the laboratory for details.

Our MPS services also include an aortopathy panel and developmental eye panels for congenital cataracts, anterior segment dysgenesis and microphthalmia/anophthalmia. Additional gene panels under development include OXPHOS disorders, renal disorders, Fanconi anaemia, autoinflammatory disorders, and retinal dystrophies . We will also screen for mutations in custom gene panels, when a gene list is provided to us. Please contact the laboratory for enquiries on any of our existing or upcoming panels, or if you would like to build a custom panel to suit your needs.

Brittle Bone Disorders Panel				
Gene	Associated phenotype	OMIM number		Expected target rate (%)**
BMP1	Osteogenesis imperfecta, type XIII	614856	AR	>95%
COL1A1	Osteogenesis imperfecta, type I Osteogenesis imperfecta, type II Osteogenesis imperfecta, type III Osteogenesis imperfecta, type IV Ehlers-Danlos syndrome, type VIIA	166200 166210 259420 166220 130060	AD AD AD AD AD	>93%
COL1A2	Osteogenesis imperfecta, type II Osteogenesis imperfecta, type III Osteogenesis imperfecta, type IV Ehlers-Danlos syndrome, type VIIB	166210 259420 166220 130060	AD AD AD AD	>95%
CRTAP	Osteogenesis imperfecta, type VII	610682	AR	>97%
FKBP10	Osteogenesis imperfecta, type XI	610968	AR	>88%
IFITM5	Osteogenesis imperfecta, type V	610967	AD	>88%
LEPRE1	Osteogenesis imperfecta, type VIII	610915	AR	>99%
PLOD2	Bruck syndrome 2	609220	AR	>99%
PPIB	Osteogenesis imperfecta, type IX	259440	AR	>96%
SERPINF1	Osteogenesis imperfecta, type VI	613982	AR	>91%
SERPINH1	Osteogenesis imperfecta, type X	613848	AR	>97%
SP7	Osteogenesis imperfecta, type XII	613849	AR	>73%
LRP5	Osteoporosis-pseudoglioma syndrome Osteopetrosis, autosomal dominant 1	259770 607634	AR AD	>85%

Hereditary Rickets Panel				
Gene	Associated phenotype	OMIM number	Mode of inheritance*	Expected target rate (%)**
PHEX	Hypophosphatemic rickets, X-linked dominant	307800	XD	>97%
FGF23	Hypophosphatemic rickets, autosomal dominant	193100	AD	>91%
ENPP1	Hypophosphatemic rickets-2	613312	AR	>96%
DMP1	Hypophosphatemic rickets, AR	241520	AR	>98%
VDR	Rickets, vitamin D-resistant, type IIA	2774400	AR	>94%
CYP27B1	Vitamin D-dependent rickets, type I	264700	AR	>98%
SLC34A3	Hypophosphatemic rickets with hypercalciuria	241530	AR	>96%
CLCN5	Dent disease Hypophosphatemic rickets	300009 300554	AR XR	>99%
ALPL	Hypophosphatasia, adult Hypophosphatasia, childhood Hypophosphatasia, infantile	146300 241510 241500	AR/AD AR AR	>97%

*AD, autosomal dominant; AR, autosomal recessive; XD, X-linked dominant; XR, X-linked recessive.
**Expected target rate indicates the percentage of target bases within a particular gene that will be sequenced at a minimum of 20x coverage. Figures provided for illustration purposes only and actual performance may vary between samples.

Specimen

2 to 5mls EDTA whole blood or extracted DNA from blood with at least 2 unique identifiers (Minimum amount of DNA 2 µg, minimum concentration 100 ng/µL, ratio of A260 to A280 greater than 1.8). Stored DNA may be used, but we may request a new sample if the quality is below the parameters listed. DNA from some sources (FFPE, blood spots) or DNA with suboptimal quality may result in test performance below the statistics quoted. In these cases, gap-filling by Sanger sequencing will not be provided. Please contact the laboratory for further information.

Billing

Please indicate who should be invoiced for the testing. The organisation or the individual must have agreed to pay for the testing. Testing will not commence without billing consent.

Clinical and Counselling Services

Within Australia details of the clinical and counselling services available in your area can be obtained from your State Health service or via links from the NSW Genetic Education Program ph [02] 9926 7324; fax [02] 9906 7529; www.genetics.com.au

Transport

Please transport the specimens at room temperature. Referring laboratories will be responsible for arranging and paying for the transportation of specimens to the laboratory. For countries outside of Australia, blood and DNA samples without a known infectious risk, do not need quarantine inspection if external packaging is clearly labeled, i.e. "Contents: Product of human origin, non-hazardous, non-infectious, for diagnostic in-vitro testing only" and "Exempt human specimen. Diagnostic specimens packed in compliance with IATA packaging instructions 650". If package is not correctly labeled and incurs a quarantine charge, this fee will be passed onto the referring laboratory. See http://www.daff.gov.au/biosecurity/import

Address

Postal:

Department of Molecular Genetics, Western Sydney Genetics Program, The Children's Hospital at Westmead, Locked Bag 4001, Westmead NSW 2145 AUSTRALIA

Courier:

Department of Molecular Genetics, Western Sydney Genetics Program, The Children's Hospital at Westmead, Loading dock 5, Redbank Rd, Northmead NSW 2152 AUSTRALIA

Contacts

For laboratory enquiries:

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