Bone Marrow Failure Gene Sequencing Panel

Genes Tested:

ABCB7	*ACD*	*ADA2 (CECR1)*	*AK2*	*AP3B1*
ATM	*ATR*	*BLM*	*BRCA1*	*BRCA2*
BRIP1	*CD40LG*	*CLPB*	*CSF3R*	*CTC1*
CXCR2	*CXCR4*	*DKC1*	*DNAJC21*	*EFL1*
EIF2AK3	*ELANE*	*EPO*	*ERCC4*	*ERCC6L2*
FANCA	*FANCB*	*FANCC*	*FANCD2*	*FANCE*
FANCF	*FANCG*	*FANCI*	*FANCL*	*FANCM*
G6PC3	*GATA1*	*GATA2*	*GFI1*	*HAX1*
HYOU1	*JAGN1*	*LAMTOR2*	*LIG4*	*LYST*
MAD2L2	*MPL*	*MRTFA (MKL1)*	*MYSM1*	*NAF1*
NBN	*NHEJ1*	*NHP2*	*NOP10*	*NSMCE3*
PALB2	*PARN*	*POT1*	*RAB27A*	*RAC2*
RAD51	*RAD51C*	*RBM8A*	*RFWD3*	*RMRP*
RNF168	*RPL11*	*RPL15*	*RPL18*	*RPL26*
RPL27	*RPL31*	*RPL35*	*RPL35A*	*RPL5*
RPL9	*RPS10*	*RPS15*	*RPS15A*	*RPS17*
RPS19	*RPS24*	*RPS26*	*RPS27*	*RPS27a*
RPS28	*RPS29*	*RPS7*	*RTEL1*	*RUNX1*
SBDS	*SLC37A4*	*SLX4*	*SMARCD2*	*SRP54*
SRP72	*STK4*	*STN1*	*TAZ*	*TCIRG1*
TCN2	*TERC*	*TERF2IP*	*TERT*	*TINF2*
TP53	*TSR2*	*UBE2T*	*USB1*	*VPS13B*
VPS45	*WAS*	*WDR1*	*WIPF1*	*WRAP53*
XRCC2				

Description:

This panel is specifically designed to diagnose the most common genetic causes of bone marrow failure including dyskeratosis congenita, Diamond Blackfan anemia, Fanconi anemia, familial bone marrow failure, Schwachman Diamond syndrome, congenital amegakaryocytic thrombocytopenia, and inherited causes of neutropenia. Bone marrow failure syndromes may be inherited as autosomal dominant, autosomal recessive, or X- linked disorders. Malignant transformation is a significant risk for individuals with many of these disorders; thus, accurate and timely diagnosis is crucial for appropriate medical surveillance and management.

This panel also includes sequencing for somatic level variants in *CSF3R, RUNX1,* and *TP53.* Acquired variants in *CSF3R* have been reported in patients with severe congenital neutropenia (SCN), as well as in patients whose SCN has undergone progression to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (Germeshausen et al. 2007; Touw 2015). Acquired variants in *RUNX1* have been reported in patients with MDS/AML who have undergone progression from SCN, including in combination with previously acquired *CSF3R* variants (Skokowa et al. 2014). Acquired variants in *TP53* have been reported in patients with Shwachman-Diamond syndrome (SDS), and may be an early event predisposing SDS patients to transformation to MDS/AML (Xia et al. 2018). Variants in these 3 genes are reported if the variant allele frequency is 5% or higher.

Test Offerings:

Bone marrow failure syndromes 116 gene panel by NGS

Sub-panels are available for specific conditions:

- Chromosome Breakage Disorders
- Dyskeratosis congenita and telomere disorders
- Diamond Blackfan anemia
- Fanconi anemia
- Inherited neutropenia

Cincinnati Children's®
changing the outcome together

Laboratory of Genetics and Genomics
CLIA#: 36D0656333
Phone: (513) 636-4474
Fax: (513) 636-4373
Email: LabGeneticCounselors@cchmc.org
www.cincinnatichildrens.org/genetics

Indications:

Bone Marrow Failure Syndromes Panel by NGS:

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of bone marrow failure or associated syndrome
- Carrier identification or presymptomatic diagnosis in individuals with a family history of bone marrow failure of unknown genetic basis

Gene Specific or Sub-panel Sequencing:

- Confirmation of genetic diagnosis in a patient with bone marrow failure and in whom a specific genetic diagnosis is suspected

Variant Specific Analysis:

- Presymptomatic testing of at-risk siblings and parents for medical management and prior to bone marrow donation
- Carrier identification in individuals in whom specific variant(s) have been identified in the proband with bone marrow failure
- Prenatal diagnosis of an at-risk fetus, after confirmation of variant(s) in the parent(s) and by prior arrangement only.

Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube or saliva in an Oragene saliva kit. Please call 513-636-4474 for a free saliva collection kit.

Note: For post-transplant patients, we accept pre-transplant samples or post-transplant skin fibroblasts ONLY (blood, saliva, and cytobrushes are not accepted). Culturing of skin fibroblasts is done at an additional charge.

Testing Methodology:

Bone Marrow Failure Syndromes Panel by NGS:
This test is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5' and 3'), as well as known pathogenic variants (HGMD 2018.4) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >50X coverage at every target base. Regions with <50X will be filled in by Sanger sequencing.

All pathogenic and likely pathogenic variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing. The limit of detection of somatic variants in *CSF3R, RUNX1,* and *TP53* with this methodology is 5%. Somatic variants with <20% variant allele frequency may not be confirmed by Sanger sequencing. A detailed non-coding variant list is available upon request.

Gene specific sequencing: PCR-based sequencing of the entire coding region and intron/ exon boundaries of the specified gene and selected known pathogenic variants in the promoter and deep intronic regions.

Variant specific analysis: Sanger sequencing following PCR amplification of the targeted variant(s) of the specified gene.

Test Sensitivity:

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Somatic variants in *TP53, RUNX1,* and *CSF3R* are expected to be identifiable when they are present at a variant allele frequency greater than 5%.

Limitations: Variants in the regulatory regions and non-reported variants in the untranslated regions may not be detected by this test. Large deletions/ duplications, large insertions and other complex genetic events will not be identified using sequencing methodology.

Note: Deletion/duplication is available for many of the genes on this panel. For further details, visit: www.cincinnatichildrens.org/deldup.

Turn-Around Time:

- Bone Marrow Failure Syndromes Panel by NGS: Up to 6 weeks
- Single Gene Sequencing: 28 days

Results:

Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for the clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

Genetic Conditions Commonly Associated with Bone Marrow Failure

Gene	Inheritance	Condition
ABCB7	X linked	Sideroblastic anemia with ataxia
ACD	AR and AD	Dyskeratosis congenita
ADA2 (CECR1)	AR	Vasculitis, autoinflammation, immunodeficiency, and hematologic defects syndrome
AK2	AR	Reticular dysgenesis
AP3B1	AR	Hermansky Pudlak type 2
ATM	AR	Ataxia-telangiectasia
ATR	AR	Seckel syndrome
BLM	AR	Bloom syndrome
BRCA1, BRCA2 (FANCD1), BRIP1 (FANCJ), ERCC4 (FANCQ), FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, MAD2L2, PALB2 (FANCN), RAD51, RAD51C (FANCO), RFWD3, SLX4 (FANCP), UBE2T, XRCC2	AR; except: *FANCB* — X linked *RAD51* — AD	Fanconi anemia
CD40LG	X linked	X-linked hyper IgM syndrome
CLPB	AR	3-methylglutaconic aciduria type VII, with cataracts, neurologic involvement and neutropenia
CSF3R	AD, AR and somatic	Severe congenital neutropenia 7 (SCN7) (germline); predisposition to myelodysplastic syndrome (somatic)
CTC1	AR	Coats plus syndrome
CXCR2	AR	Myelokathexis
CXCR4	AD	WHIM syndrome
DKC1	XR	Dyskeratosis congenita or Hoyeraal Hreidarsson syndrome
DNAJC21	AR	Familial bone marrow failure syndrome type 3
EFL1	AR	Shwachman-Diamond syndrome
EIF2AK3	AR	Wolcott-Rallison syndrome
ELANE (ELA2)	AD	SCN1
EPO	AR, AD	Diamond Blackfan anemia; erythrocytosis.
ERCC6L2	AR	Familial bone marrow failure syndrome type 2
G6PC3	AR	SCN4, nonsyndromic SCN, Dursun syndrome
GATA1	X linked	GATA1-related X-linked cytopenia
GATA2	AD	GATA2 deficiency
GFI1	AD	SCN2
HAX1	AR	SCN3, Kostmann syndrome
HYOU1	AR	Immunodeficiency and hypoglycemia
JAGN1	AR	SCN6
LAMTOR2 (ROBLD3)	AR	p14 deficiency
LIG4	AR	LIG4 syndrome
LYST	AR	Chediak Higashi syndrome
MPL	AR	Congenital amegakaryocytic thrombocytopenia

Genetic Conditions Commonly Associated with Bone Marrow Failure, Cont.

Gene	Inheritance	Condition
MRTFA (MKL1)	AR	Neutropenia with combined immune deficiency
MYSM1	AR	Familial bone marrow failure syndrome type 4
NAF1	AD	Pulmonary fibrosis and emphysema
NBN	AR	Nijmegen breakage syndrome
NHEJ1	AR	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation
NHP2 (NOLA2)	AR	Dyskeratosis congenita
NOP10 (NOLA3)	AR	Dyskeratosis congenita
NSMCE3	AR	Lung disease, immunodeficiency and chromosome breakage syndrome
PARN	AD and AR	Dyskeratosis congenita; Pulmonary fibrosis and/or bone marrow failure
POT1	AD	Familial chronic lymphocytic leukemia
RAB27A	AR	Griscelli syndrome type 2
RAC2	AR	Neutrophil immunodeficiency syndrome
RBM8A	AR	Thrombocytopenia-absent radius syndrome
RMRP	AR	Cartilage-hair hypoplasia
RNF168	AR	RIDDLE syndrome
RPL5, RPL9, RPL11, RPL15, RPL18, RPL26, RPL27, RPL31, RPL35, RPL35A, RPS7, RPS10, RPS15, RPS15A, RPS17, RPS19, RPS24, RPS26, RPS27, RPS27A, RPS28, RPS29, TSR2	AD; except: *TSR2* — X linked	Diamond Blackfan anemia
RTEL1	AD and AR	Dyskeratosis congenita
RUNX1	AD and somatic	Familial platelet disorders (germline); acute myeloid leukemia (germline); predisposition to myelodysplastic syndrome/ acute myeloid leukemia (somatic)
SBDS	AR	Shwachman Diamond syndrome (SDS)
SLC37A4	AR	Glycogen storage disease type IB
SMARCD2	AR	Specific granule deficiency 2
SRP54	AD	Congenital neutropenia
SRP72	AD	Familial bone marrow failure syndrome type 1
STK4	AR	STK4 deficiency
STN1	AR	Coats plus syndrome with telomere defects
TAZ	X linked	Barth syndrome
TCIRG1	AR, AD	Osteopetrosis (AR), Congenital neutropenia (AD)
TCN2	AR	Transcobalamin II deficiency
TERC (hTR)	AD	Dyskeratosis congenita
TERF2IP	AD	Familial melanoma
TERT	AD and AR	Dyskeratosis congenita
TINF2	AD	Classic or severe DC, Revesz syndrome, Hoyeraal Hreidarrson syndrome; AD 3

Genetic Conditions Commonly Associated with Bone Marrow Failure, Cont.

Gene	Inheritance	Condition
TP53	AD and somatic	Familial bone marrow failure syndrome 5 (germline); transformation to myelodysplastic syndrome/acute myeloid leukemia in patients with Schwachman Diamond syndrome (somatic)
USB1	AR	Clericuzio-type poikiloderma with neutropenia
VPS13B	AR	Cohen syndrome; congenital neutropenia with retinopathy
VPS45	AR	SCN5
WAS	X linked	Wiskott Aldrich syndrome, X-linked
WDR1	AR	WDR1 deficiency
WIPF1	AR	Wiskott Aldrich syndrome
WRAP53 (TCAB1, WDR79)	AR	Dyskeratosis congenita, Revesz syndrome, Hoyeraal Hreidarrson syndrome

CPT Codes:

- **Bone Marrow Failure NGS Panel:** 81443
- **Single gene sequencing, targeted variant analysis, and deletion/duplication:** call for information.

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

Shipping Instructions:

Please enclose **test requisition** with sample.
All information must be completed before sample can be processed.

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

Chirnomas, S.D. and G.M. Kupfer (2013) The Inherited Bone Marrow Failure Syndromes. Pediatric Clinics of North America 60(6): 1291-310.

Dietz, A.C., S.A. Savage, et al. (2017) Late Effects Screening Guidelines after Hematopoietic Cell Transplantation for Inherited Bone Marrow Failure Syndromes: Consensus Statement From the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects After Pediatric HCT. Biol Blood Marrow Transplant. 23(9):1422-1428.

Dietz, A.C., P.A. Mehta, et al. (2017) Current Knowledge and Priorities for Future Research in Late Effects after Hematopoietic Cell Transplantation for Inherited Bone Marrow Failure Syndromes: Consensus Statement from the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects after Pediatric Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 23(5):726-735.

Germeshausen, M., M. Ballmaier, et al. 2007. Incidence of CSF3R mutations in severe congenital neutropenia and relevance for leukemogenesis: results of a long-term survey. Blood. 109(1):93-9.

Kallen, M.E., A. Dulau-Florea, et al. (2019) Acquired and germline predisposition to bone marrow failure: Diagnostic features and clinical implications. Semin Hematol. 2019 Jan;56(1):69-82.

Skokowa, J., D. Steinemann, et al. 2014. Cooperativity of RUNX1 and CSF3R mutations in severe congenital neutropenia: a unique pathway in myeloid leukemogenesis. Blood. 123(14):2229-37.

Thusberg, J., A. Olatubosun, et al. (2011). Performance of mutation pathogenicity prediction methods on missense variants. Hum Mutat 32(4): 358-368.

Touw, I.P. 2015 Game of clones: the genomic evolution of severe congenital neutropenia. Hematology Am Soc Hematol Educ Program. 2015:1-7.

Townsley, D.M, B. Dumitriu, et al. (2014). Bone marrow failure and the telomeropathies. Blood 124(18): 2775-83.

Wegman-Ostrosky, T. and S.A. Savage (2017) The Genomics of Inherited Bone Marrow Failure: From Mechanism to the Clinic. British Journal of Haematology.

Xia, J., C.A. Miller, et al. 2018. Somatic mutations and clonal hematopoiesis in congenital neutropenia. Blood. 131(4):408-416.