Bone Marrow Failure Gene Sequencing Panel

Genes Tested:

ABCB7, ACD, ADA2 (CECR1), AK2, ALAS2, ANKRD26, AP3B1, ATM, ATR, BLM, BRCA1, BRCA2, BRIP1, C15orf41, CARD11, CBL, CD40LG, CDAN1, CEBPA, CLPB, CSF3R, CTC1, CXCR2, CXCR4, CYCS, DDX41, DKC1, DNAJC21, DNMT3A, DUT, EFL1, EIF2AK3, ELANE, EPO, ERCC4, ERCC6L2, ETV6, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, G6PC3, GATA1, GATA2, GFI1, GLRX5, GP1BA, GP1BB, GP9, GRHL2, HAX1, HOXA11, HYOU1, IKZF1, ITGA2B, ITGB3, JAGN1, JAK2, KIF23, KIT, KLF1, KRAS, LAMTOR2, LIG4, LYST, MAD2L2, MASTL, MBD4, MECOM, MPL, MRTFA (MKL1), MYH9, MYSM1, NAF1, NBN, NHEJ1, NHP2, NOP10, NSMCE3, PALB2, PARN, PAX5, PGM3, POT1, PTPN11, PUS1, RAB27A, RAC2, RAD51, RAD51C, RBM8A, RFWD3, RMRP, RNF168, RPL11, RPL15, RPL18, RPL26, RPL27, RPL31, RPL35, RPL35A, RPL5, RPL9, RPS10, RPS15, RPS15A, RPS19, RPS24, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS7, RTEL1, RUNX1, SALL4, SAMD9, SAMD9L, SBDS, SBF2, SEC23B, SH2B3, SLC19A2, SLC25A38, SLC35C1, SLC37A4, SLX4, SMARCD2, SRP54, SRP72, STIM1, STK4, STN1, TAZ, TCIRG1, TCN2, TERC, TERF2IP, TERT, TET2, THPO, TINF2, TLR8, TP53, TRNT1, TSR2, TUBB1, UBE2T, USB1, VPS13B, VPS45, WAS, WDR1, WIPF1, WRAP53, XRCC2, YARS2, ZCCHC8

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, congenital dyserythropoietic anemia, familial bone marrow failure, Schwachman Diamond syndrome, congenital amegakaryocytic thrombocytopenia/other syndromic thrombocytppenia, 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*, *TLR8* 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). Mosaic gain-of-function variants in *TLR8* have been reported to cause neutropenia, antibody deficiency, lymphoproliferation, and bone marrow failure (Aluri et al. 2021). Variants in these 4 genes are reported if the variant allele frequency is 5% or higher.

Test Offerings:

Bone marrow failure syndromes 165 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

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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 2021.2) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >20X coverage at every target base. Regions with <20X 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, TLR8* 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, TLR8, 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.

Regions of Homology

These gene regions with homology may generate suboptimal data with potential false negative results.

GENE	TRANSCRIPT	EXON	CHROM	EXON_START	EXON_END
RPL15	NM_002948	4	3	23960686	23960992
EFL1	NM_024580	7	15	82530647	82530862
RBM8A	NM_005105	6	1	145509165	145509211
FANCD2	NM_033084	22	3	10106039	10106113
FANCD2	NM_033084	14	3	10085512	10085548
FANCD2	NM_033084	17	3	10091057	10091189

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

Genetic Conditions Commonly Associated with Bone Marrow Failure

Gene	Inheritance	Condition
ABCB7	X linked	Sideroblastic anemia with ataxia
ACD	AD, AR	Dyskeratosis congenita
ADA2 (CECR1)	AR	Vasculitis, autoinflammation, immunodeficiency, and hematologic defects syndrome
AK2	AR	Reticular dysgenesis
ALAS2	X linked	Anemia, sideroblastic, 1; Protoporphyria, erythropoietic, X-linked
ANKRD26	AD	Thrombocytopenia 2
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, MAD2L2, PALB2 (FANCN), RAD51, RAD51C (FANCO), RFWD3, SLX4 (FANCP), UBE2T, XRCC2	AR; except: *FANCB* - X linked, *RAD51* - AD	Fancomi anemia
C15orf41	AR	Dyserythropoietic anemia, congenital, type Ib
CARD11	AD, AR	Immunodeficiency
CBL	AD	Noonan syndrome-like disorder with or without Juvenile Myelomonocytic Leukemia
CD40LG	X linked	X-linked hyper IgM syndrome
CDAN1	AR	Dyserythropoietic anemia, congenital, type Ia
CEBPA	AD	Leukemia, acute myeloid
CLPB	AR	3-methylglutaconic aciduria type VII, with cataracts, neurologic involvement and neutropenia
CSF3R	AD, AR and somatic	Severe congenital neutropenia 7 (SCN7) (AR); Hereditary neutrophilia (AD) predisposition to myelodysplastic syndrome (somatic)
CTC1	AR	Coats plus syndrome
CXCR2	AR	Myelokathexis
CXCR4	AD	WHIM syndrome
CYCS	AD	Thrombocytopenia 4
DDX41	AD	Myeloproliferative/lymphoproliferative Neoplasms, familial (multiple types), susceptibility to
DKC1	X linked	Dyskeratosis congenita or Hoyeraal Hreidarsson syndrome
DNAJC21	AR	Familial bone marrow failure syndrome type 3
DNMT3A	AD	Heyn-Sproul-Jackson syndrome; Tatton-Brown-Rahman syndrome; Acute Myeloid Leukemia
DUT	AR	Bone Marrow failure and Diabetes
EFL1	AR	Shwachman-Diamond syndrome
EIF2AK3	AR	Wolcott-Rallison syndrome
ELANE (ELA2)	AD	SCN1
EPO	AD, AR	Diamond Blackfan anemia (AR); erythrocytosis (AD)
ERCC6L2	AR	Familial bone marrow failure syndrome type 2
ETV6	AD	Thrombocytopenia 5; Acute myeloid leukemia

Genetic Conditions Commonly Associated with Bone Marrow Failure, Cont.

Gene	Inheritance	Condition
G6PC3	AR	SCN4, nonsyndromic SCN, Dursun syndrome
GATA1	X linked	GATA1-related X-linked cytopenia
GATA2	AD	GATA2 deficiency
GFI1	AD	SCN2
GLRX5	AR	Anemia, sideroblastic, 3, pyridoxine-refractory
GP1BA	AD, AR	Bernard-Soulier syndrome, type A1/A2
GP1BB	AR	Bernard-Soulier syndrome, type B; Giant Platelet disorder, isolated
GP9	AR	Bernard-Soulier syndrome, type C
GRHL2	AR	Ectodermal dysplasia/short stature syndrome
HAX1	AR	SCN3, Kostmann syndrome
HOXA11	AD	Radioulnar synostosis with amegakaryocytic thrombocytopenia 1
HYOU1	AR	Immunodeficiency and hypoglycemia
IKZF1	AD	Immunodeficiency, common variable, 13
ITGA2B	AD, AR	Bleeding disorder, Platelet-type, 16, autosomal dominant; Glanzmann thrombasthenia 1, autosomal recessive
ITGB3	AD, AR	Bleeding disorder, Platelet-type, 24, autosomal dominant; Glanzmann thrombasthenia 2, autosomal recessive
JAGN1	AR	SCN6
JAK2	AD	Thrombocythemia 3 (AD, somatic); Erythrocytosis (somatic); Myelofibrosis (somatic), AML (somatic)
KIF23	AD	Anemia, congenital dyserythropoietic, type IIIA
KIT	AD	Mastocytosis, cutaneous; acute myeloid leukemia
KLF1	AD	Dyserythropoietic anemia, congenital, type IV
KRAS	AD	RAS-associated autoimmune leukoproliferative disorder; Cardiofaciocutaneous syndrome 2; Noonan syndrome 3
LAMTOR2 (ROBLD3)	AR	p14 deficiency
LIG4	AR	LIG4 syndrome
LYST	AR	Chediak Higashi syndrome
MASTL	AD	thrombocytopenia
MBD4	AR	Tumor predisposition syndrome 2
MECOM	AD	Radioulnar synostosis with amegakaryocytic thrombocytopenia 2
MPL	AR	Congenital amegakaryocytic thrombocytopenia
MRTFA (MKL1)	AR	Neutropenia with combined immune deficiency
MYH9	AD	Macrothrombocytopenia and Granulocyte inclusions with or without Nephritis or sensorineural Hearing Loss
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

Genetic Conditions Commonly Associated with Bone Marrow Failure, Cont.

Gene	Inheritance	Condition
PARN	AD, AR	Dyskeratosis congenita (AR); Pulmonary fibrosis and/or bone marrow failure (AD)
PAX5	AD	Leukemia, acute lymphoblastic, susceptibility to, 3
PGM3	AR	Immunodeficiency 23
POT1	AD	Familial chronic lymphocytic leukemia
PTPN11	AD	Noonan syndrome 1
PUS1	AR	Myopathy, lactic acidosis, and sideroblastic anemia 1
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, RPS19, RPS24, RPS26, RPS27, RPS27A, RPS28, RPS29, TSR2	AD; except: TSR2 - X linked	Diamond Blackfan anemia
RTEL1	AD, AR	Dyskeratosis congenita
RUNX1	AD and somatic	Familial platelet disorders (germline); acute myeloid leukemia (germline); predisposition to myelodysplastic syndrome/ acute myeloid leukemia (somatic)
SALL4	AD	IVIC syndrome; Duane-radial ray syndrome
SAMD9	AD	MIRAGE syndrome
SAMD9L	AD	Ataxia-pancytopenia syndrome
SBDS	AR	Shwachman Diamond syndrome (SDS)
SBF2	AR	Thrombocytopaenia
SEC23B	AR	Dyserythropoietic anemia, congenital, type II
SH2B3	AD and somatic	Thrombocythemia; Myelofibrosis; Erythrocytosis
SLC19A2	AR	Thiamine-responsive megaloblastic anemia syndrome
SLC25A38	AR	Anemia, sideroblastic, 2, pyridoxine-refractory
SLC35C1	AR	Congenital disorder of glycosylation, type IIc
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
STIM1	AD, AR	Stormorken syndrome (AD); Immunodeficiency 10 (AR)
STK4	AR	STK4 deficiency
STN1	AR	Coats plus syndrome with telomere defects
TAZ	X linked	Barth syndrome
TCIRG1	AD, AR	Osteopetrosis (AR), Congenital neutropenia (AD)
TCN2	AR	Transcobalamin II deficiency
TERC (hTR)	AD	Dyskeratosis congenita
TERF2IP	AD	Familial melanoma
TERT	AD, AR	Dyskeratosis congenita
TET2	AR	Immunodeficiency 75

Genetic Conditions Commonly Associated with Bone Marrow Failure, Cont.

Gene	Inheritance	Condition
THPO	AD	Thrombocythemia 1
TINF2	AD	Classic or severe DC, Revesz syndrome, Hoyeraal Hreidarrson syndrome; AD 3
TLR8	X Linked and somatic	Immunodeficiency 98 with autoinflammation, X-linked
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)
TRNT1	AR	Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay; Retinitis Pigmentosa and erythrocytic microcytosis
TUBB1	AD	Macrothrombocytopenia, isolated, 1, autosomal dominant
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
YARS2	AR	Myopathy, lactic acidosis, and sideroblastic anemia 2
ZCCHC8	AD	Pulmonary fibrosis and/or bone marrow failure, telomere-related, 5

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.

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.

Turn-Around Time:

- Bone Marrow Failure Syndromes Panel by NGS: 28 days
- Single Gene Sequencing: 28 days

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 Saturday.

Ship to:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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