

# Chromosome Breakage Disorders Gene Sequencing Panel

## Genes Tested:

<i>ATM</i>	<i>BLM</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRIP1</i>
<i>ERCC4</i>	<i>FANCA</i>	<i>FANCB</i>	<i>FANCC</i>	<i>FANCD2</i>
<i>FANCE</i>	<i>FANCF</i>	<i>FANCG</i>	<i>FANCI</i>	<i>FANCL</i>
<i>FANCM</i>	<i>LIG4</i>	<i>MAD2L2</i>	<i>MYSM1</i>	<i>NBN</i>
<i>NHEJ1</i>	<i>NSMCE3</i>	<i>PALB2</i>	<i>RAD51</i>	<i>RAD51C</i>
<i>RFWD3</i>	<i>SLX4</i>	<i>UBE2T</i>	<i>XRCC2</i>	

## Description:

**Chromosome breakage disorders** are a group of related diseases which are characterized by spontaneous chromosome breakage, immunodeficiency and predisposition to malignancy. These conditions include Fanconi Anemia, Ataxia-Telangiectasia, Bloom syndrome, LIG4 syndrome, and Nijmegen breakage syndrome.

**Fanconi Anemia (FA)** is a rare, inherited DNA-instability syndrome which manifests with varied clinical manifestations, most commonly progressive bone marrow failure by age 40-45, progressive pancytopenia, and congenital malformations including short stature, radial aplasia, urinary tract abnormalities, hyperpigmentation, and/or developmental delay. Patients with FA also have a predisposition to cancer, particularly acute myeloid leukemia, as well as an increased risk of developing solid tumors in the head, neck, skin, GI tract, and genital tract.

**Ataxia-Telangiectasia (A-T)** is a rare, neurodegenerative disorder, with an estimated incidence of 1 in 40,000-100,000 births. A-T is caused by pathogenic variants in the *ATM* gene and is characterized by immunodeficiency, progressive cerebellar ataxia, telangiectasia of the skin and eyes, and susceptibility to cancer. Approximately one-third of A-T patients develop cancer, typically leukemia or lymphoma in childhood, while approximately one-half of patients have immunodeficiencies, usually characterized by deficiency of naïve T cells and decreased or absent IgA, IgE and IgG2. Malignancy, pneumonia and chronic lung disease, as a result of immunodeficiency, contribute to early deaths.

**Bloom syndrome** is caused by pathogenic variants in the *BLM* gene. Our cytogenetics laboratory offers diagnostic testing via sister chromatid exchange (SCE) analysis, rates of which are elevated in patients with Bloom syndrome. Bloom syndrome is characterized by immune deficiency and predisposition to cancer, severe pre- and postnatal growth deficiency, sparseness of subcutaneous fat tissue, an erythematous, sun-sensitive “butterfly” lesion on the face and impaired fertility. Serum concentrations of immunoglobulins are typically low. Common health complications include life-threatening infections and chronic obstructive lung disease, gastroesophageal reflux, and type 2 diabetes. Patients with Bloom syndrome have a dramatically increased risk of cancer, primarily leukemias and lymphomas in childhood and solid tumors in adulthood, occurring at earlier than normal ages. The most common cancers detected in adults include tumors of the lower enteric tract, integument, esophageal/upper respiratory tract and genital/urinary tract.

**Nijmegen breakage syndrome, LIG4 syndrome and NHEJ1 deficiency**, caused by biallelic mutations in *NBN*, *LIG4* and *NHEJ1* respectively, are similar disorders characterized by microcephaly, growth retardation, combined immune deficiency and sensitivity to ionizing radiation. All are associated with much elevated risks of malignancy in affected individuals.

## Genetics:

These syndromes may be inherited as autosomal dominant, autosomal recessive, or X-linked disorders.

## Indications:

Confirmation of diagnosis in individuals with the following symptoms:

- Unexplained pre- and postnatal growth deficiency, failure to thrive and small stature in association with immune deficiency or cancer
- Characteristic “butterfly” erythematous facial lesion
- Progressive cerebellar ataxia in young children

- Recurrent infections or immunodeficiency in association with microcephaly
- History of leukemia, lymphoma or solid tumor at an earlier than expected age, particularly in association with other features of chromosome breakage disorder
- Increased sister chromatid exchange as detected cytogenetically, chromosomal instability, or increased cellular sensitivity to ionizing radiation
- Unexpected toxicity to chemotherapy or radiation therapy
- Borderline increased chromosome breakage with DEB exposure
- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of Fanconi anemia or suspected Fanconi anemia.

#### Genetic Conditions Commonly Associated with Chromosome Breakage Disorders

Gene	Inheritance	Condition
<i>ATM</i>	AR	Ataxia-telangiectasia
<i>BLM</i>	AR	Bloom syndrome
<i>BRCA1, BRCA2 (FANCD1), BRIP1 (FANCI), ERCC4 (FANCG), FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, MAD2L2, PALB2 (FANCN), RAD51, RAD51C (FANCO), RFW3, SLX4 (FANCP), UBE2T, XRCC2</i>	AR; except:  <i>FANCB</i> — X-linked  <i>RAD51</i> — AD	Fanconi anemia
<i>LIG4</i>	AR	LIG4 syndrome
<i>MYSM1</i>	AR	Familial bone marrow failure syndrome type 4
<i>NBN</i>	AR	Nijmegen breakage syndrome
<i>NHEJ1</i>	AR	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation
<i>NSMCE3</i>	AR	Lung disease, immunodeficiency and chromosome breakage syndrome

#### 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:

**Chromosome Breakage Disorders Panel:** 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. All pathogenic and likely pathogenic variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing. Regions with <50X will be filled in 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.

**Limitations:** Variants in regulatory regions and non-reported variants in 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:** Single gene sequencing and targeted variant analysis is also available for all genes on the Chromosome Breakage Disorders Panel. Deletion/duplication is available for many of the genes on this panel. For further details, visit: [www.cincinnatichildrens.org/deldup](http://www.cincinnatichildrens.org/deldup).

### Turn-Around Time:

- NGS panel: up to 6 weeks
- Single gene sequencing: up to 28 days

### CPT Codes:

- **Chromosome Breakage Disorders by NGS:** 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

### Results:

Results will be reported to the referring physician or health care provider as specified on the test requisition form.

### References:

Arora, H., A.H. Chacon, et al. (2014) Bloom Syndrome. *International Journal of Dermatology* 53(7): 798–802.

Chandrasekharappa SC, et al. Massively parallel sequencing, aCGH, and RNA-Seq technologies provide a comprehensive molecular diagnosis of Fanconi anemia. *Blood*. 2013 May 30;121(22):e138-48. doi:10.1182/blood-2012-12-474585. Epub 2013 Apr 23.

Cheung R.S., T. Taniguchi, (2017) Recent insights into the molecular basis of Fanconi anemia: genes, modifiers, and drivers. *Int J Hematol* 106(3):335-344.

Flynn, E.K., A. Kamat, et al. (2014) Comprehensive Analysis of Pathogenic Deletion Variants in Fanconi Anemia Genes. *Human Mutation* 35(11): 1342-53.

Kee, Y. and A.D. D'Andrea (2012) Molecular Pathogenesis and Clinical Management of Fanconi Anemia. *The Journal of Clinical Investigation* 122(11): 3799-806.

O'Driscoll, M., K. M. et al. (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell* 8(6): 1175-1185.

Schneider, M., K. Chandler, et al. (2014) Fanconi Anaemia: Genetics, Molecular Biology, and Cancer - Implications for Clinical Management in Children and Adults. *Clinical Genetics*.

Tewhey, R., J.B. Warner, et al. (2009). Microdroplet-based PCR enrichment for large-scale targeted sequencing. *Nat Biotechnol* 27(11): 1025-1031.

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

Walsh, M.F., V.Y. Chang, et al. (2017). Recommendations for Childhood Cancer Screening and Surveillance in DNA Repair Disorders. *Clin Cancer Res* 23(11):e23-e31.