THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Fanconi Anemia

Clinical Features:
Fanconi anemia (FA) is a chromosomal instability disorder associated congenital anomalies, progressive bone marrow failure, and cancer predisposition [1]. The most commonly described anomalies include thumb and radial bone abnormalities, short stature and skin hyperpigmentation [1]. Some patients lack these characteristic physical features and first present with bone marrow failure or cancer [2]. Associated cancers include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and solid tumors of the head, neck, skin, gastrointestinal tract and genital tract [1]. The majority of cases of FA are inherited in an autosomal recessive manner, with the exception of *FANCB* (X-linked) and *RAD51/FANCR* (autosomal dominant).

Our Fanconi Anemia Panel includes sequence and deletion/duplication analysis of the 19 genes listed below.

Fanconi Anemia Sequencing Panel					
BRCA1(FANCS)	BRCA2 (FANCD1)	BRIP1 (FANCJ)	ERCC4 (FANCQ)	FANCA	FANCB
FANCC	FANCD2	FANCE	FANCF	FANCG	FANCI
FANCL	PALB2 (FANCN)	RAD51 (FANCR)	RAD51C (FANCO)	SLX4 (FANCP)	UBE2T(FANCT)
XRCC2					

Gene	Clinical Features
BRCA1 (FANCS)	Recently, two cases of individuals harboring biallelic deleterious BRCA1 mutations were reported [3, 4]. Detailed phenotypic and cellular characterization of one patient provided lines of evidence supporting the hypothesis that biallelic BRCA1 mutations cause a new Fanconi anemia subtype associated with increased breast and ovarian cancer susceptibility [3].
BRCA2 (FANCD1)	Homozygous or compound heterozygous mutations in *BRCA2* are associated with FA complementation group D1. *BRCA2* mutations are associated with early-onset leukemia and solid tumors, and a high rate of spontaneous chromosome aberration compared to other types of FA [5, 6]. Heterozygous mutations in *BRCA2* are associated with hereditary breast and ovarian cancer [7].
BRIP1 (FANCJ)	FA complementation group J is associated with biallelic mutations in the *BRIP1* gene [8]. There is some evidence that heterozygous BRIP1 mutations may be associated with increased breast cancer susceptibility [9].
ERCC4 (FANCQ)	FA complementation group Q is associated with biallelic *ERCC4* mutations [10]. *ERCC4* mutations can also be associated with xeroderma pigmentosa [11].
FANCA	Biallelic *FANCA* mutations are associated with FA complementation group A [12]. Patients with mutations associated with no FANCA protein production may have earlier onset anemia and higher risk of leukemia, compared with patients with production of an abnormal FANCA protein [12].
FANCB	Mutations in the X-linked *FANCB* are associated with FA complementation group B. Affected patients typically have multiple malformations, including a ventriculomegaly or hydrocephalus, bilateral radial defects, vertebral defects, and renal agenesis [13]. An estimated 50% of affected males do not survive the perinatal period; heterozygous females are typically unaffected and exhibit skewed X-inactivation [13].
FANCC	FA complementation group C is associated with biallelic mutations in *FANCC*. A founder mutation in *FANCC* exists in the Ashkenazi Jewish population, and has a carrier frequency of 1 in 100 [14].
FANCD2	Biallelic mutations in *FANCD2* are associated with FA complementation group D2, and account for approximately 3-6% of all cases of FA [15]. Patients with *FANCD2* mutations frequently have congenital malformations, and have earlier onset hematological manifestations compared FA cases overall [15].
FANCE	Homozygous mutations in FANCE have previously been identified in 2 Turkish patients and 1 Bangladeshi patient with FA complementation group E [16].
FANCF	FA complementation group F is caused by homozygous or compound heterozygous mutations in the *FANCF* gene [17].
FANCG	Biallelic *FANCG* mutations are associated with FA complementation group G. FANCG mutations are typically associated with more severe cytopenia and a higher risk of leukemia than is observed with cases of FA in

	general [12].
FANCI	FA complementation group I is caused by homozygous or compound heterozygous mutations in the *FANCI* gene [18].
FANCL	A patient with FA complementation group L and compound heterozygous mutations in *FANCL* has previously been described [19].
PALB2 (FANCN)	FA complementation group N has been associated with compound heterozygous mutations in *PALB2*. Heterozygous mutations in *PALB2* have been associated with increased susceptibility to breast cancer [20].
RAD51 (FANCR)	Wang *et al.* (2015) identified a novel heterozygous mutation in *RAD51* in a patient with a FA-like phenotype [21].
RAD51C (FANCO)	A homozygous mutation in *RAD51C* has previously been described in a family with FA complementation group O [22]. Heterozygous mutations in this gene have been associated with breast cancer predisposition [23].
UBE2T (FANCT)	Two unrelated individuals were reported with biallelic *UBE2T* missense mutations that rendered the UBE2T protein unable to interact with FANCL and caused Fanconi anemia [24].
SLX4 (FANCP)	FA complementation group P has been associated with either homozygous or compound heterozygous mutations in the *SLX4* gene [25].

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Fanconi Anemia Panel

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube. NOTE: blood samples are not accepted if patient has a history of MDS or leukemia. Please send 2 T-25 flasks of cultured skin fibroblasts instead.
Cost:	$3000
CPT codes:	81406, 81407
Turn-around time:	6 weeks

Note: We cannot bill insurance for the Fanconi Anemia Sequencing panel

Results:
Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire Fanconi Anemia Sequencing Panel. All abnormal results are reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu. You can also contact us at 773-834-0555 or ucgslabs@genetics.uchicago.edu

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