Episignature Analysis

Description:

Global DNA methylation patterns have been identified in individuals with a variety of conditions including a number of single gene disorders, chromosomal disorders, imprinting disorders, Fragile X syndrome, and certain specific syndromes related to exposures during the fetal period. These identified epigenetic patterns associated with specific syndromes are called episignatures. This test can identify episignatures associated with many single gene disorders including many mendelian disorders of epigenetic machinery, other single gene disorders, certain chromosome conditions, imprinting disorders, multi-locus imprinting disorder, Fragile X syndrome, fetal valproate syndrome, and recurrent constellations of embryonic malformations (including VACTERL and VATER).

Episignature Targeted Analysis can be ordered to evaluate for one specific signature. Episignature Complete Analysis can be ordered to evaluate for many conditions in the same assay. See the tables below for which signatures are included in complete analysis and which are available only through targeted analysis.

Indications:

- Global developmental delay
- Intellectual Disability
- Multiple congenital anomalies with or without growth abnormalities
- Concern for VACTERL or VATER
- Possible valproate exposure during the fetal period and concern for fetal valproate syndrome
- Uniparental disomy suggesting imprinting disorder
- Previous genetic testing and/or evaluation was uncertain for a condition for which episignature analysis is available.
- Unsolved diagnosis after other testing
- Symptoms or testing concerning for more than one imprinting disorder or for multi-locus imprinting disorder
- First line test to guide future genetic evaluation in individuals where multiple conditions on Episign complete are on the differential

Specimen:

At least 3mLs whole blood in a lavender top (EDTA) tube or 1.5ug DNA extracted in a CLIA lab from peripheral blood collected in an EDTA tube

Testing Methodology:

Genomic DNA is isolated from peripheral blood and treated for bisulfite conversion using the EZ DNA methylation kit (Zymo Research). Genome-wide DNA methylation measurements are processed using the Infinium Assay with the Methylation EPIC v2.0 BeadChip platform (Illumina). This chip contains approximately 935K genome-wide CpG markers. The disease-associated episignature profile is assessed using the clinically validated EpiSign™ classifier (version 5) and the EpiSign™ Knowledge Database at the London Health Sciences Center (London, ON, Canada; https://episign.lhsc.on.ca/). The EpiSign™ classifier is designed to select probes that are both highly sensitive and specific for a cohort of interest and uses machine learning-based algorithms to compare patient's blood DNA methylation to reference samples.

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Test Limitations:

The episignature analysis through the EpiSign™ classifier is limited to peripheral blood where large reference databases are available and current knowledge of the disease mechanisms. Sensitivities can be variable for different episignatures. This assay has lower sensitivity for conditions with moderate confidence due to limited signature strength, small cohort size, or types of disease-causing variants tested. The EpiSign™ classifier evolves over time with the increased size of the reference database and functional evidence, so it may lead to different interpretations between classifier versions. This test cannot detect structural variants, and only analyses of the methylation signatures of interest were performed. In addition, low-level mosaic disorders may not be detectable.

The episignature results should be interpreted in correlation with the patient's clinical presentation. Negative results do not rule out the possibility that the patient is affected by genetic conditions. Inconclusive results are possible. If there is strong clinical suspicion of any genetic condition, additional testing is recommended to confirm or rule out a diagnosis. Females with X-linked conditions may not have a detectable episignature. Neonates (under six months of age) could have a general hypomethylated profile that can interfere with the sensitivity and specificity of some signatures; repeating the episignature study after a year of age with a fresh sample is recommended following the initial analysis in such cases.

Genetic Conditions (version 5):

Table 1. Conditions Only Available as Targeted Episignature Analysis by Request

**Reduced sensitivity and/or specificity may be observed*

Conditions	Related regions/ genes
Coffin-Siris syndrome 12*	BICRA*
Desanto-Shinawi syndrome	WAC
Developmental delay with or without dysmorphic facies and autism	TRRAP (only for variants p.960-p.1159)
Diamond-Blackfan anemia 1*	RPS19*
Diamond-Blackfan anemia 5*	RPL35A*
Fetal Valproate syndrome	Not applicable
Hypercholesterolemia, familial, 1 *Sensitivity against other hereditary hypercholesterolemia disorders has not been evaluated. Both monoallelic and biallelic cases are detected.*	LDLR
Hypermethioninemia with deficiency of S-adenosylhomocysteine hydrolase	AHCY
Intellectual developmental disorder with dysmorphic facies and behavioral abnormalities	FBXO11
Intellectual developmental disorder, autosomal dominant 57	TLK2
Intellectual developmental disorder, X-linked 112*	ZMYM3* (male cases only)
Intellectual developmental disorder, X-linked syndromic, Siderius type	PHF8 (male cases only)
KMT2C-related syndrome*	KMT2C*
Neurodevelopmental-craniofacial syndrome with variable renal and cardiac abnormalities	ZMYM2
Neurofibromatosis, type 1*	NF1*

(Table 1. continued on page 3)

Table 1. Conditions Only Available as Targeted Episignature Analysis by Request (CONTINUED)

**Reduced sensitivity and/or specificity may be observed*

Conditions	Related regions/ genes
NOTCH1 associated syndrome*	NOTCH1*
PHF12-related syndrome	PHF12
Recurrent constellations of embryonic malformations *Includes cases with phenotypic presentation of OAV, OAVV, VACTERL and VATER.*	Not applicable
Schuurs-Hoeijmakers syndrome*	PACS1*
SETD1A-related syndrome	SETD1A
Tessadori-Bicknell-van Haaften neurodevelopmental syndrome 1, 3 and 4	H4C3, H4C4, H4C5, H4C9

Table 2. Complete Episignature Analysis I: Fragile X Syndrome and Imprinting Disorders

Conditions	Related regions/ genes
Angelman syndrome (AS) *The SNRPN promoter is sensitive but not specific for AS and only reported with a positive result for SNURF.*	15q11.2-q13 (SNRPN promoter, SNURF)
Prader-Willi syndrome (PWS) *The SNRPN promoter is sensitive but not specific for PWS and only reported with a positive result for SNURF.*	15q11.2-q13 (SNRPN promoter, SNURF)
Beckwith-Wiedemann syndrome	11p15.5 (IC1 and IC2)
Silver Russel syndrome 1 & 2	11p15.5 (IC1 and IC2), 7q32.2
Diabetes mellitus, transient neonatal 1	6q24 (PLAGL1)
Fragile X syndrome	FMR1 promoter
Kagami-Ogata syndrome	14q32 (MEG3 promoter)
Mulchandani-Bhoj-Conlin syndrome	20q11-q13 (GNAS)
Multi-locus imprinting disturbances *Only positive sites will be reported.*	All EpiSign imprinting regions
Pseudohypoparathyroidism IA & IB	20q11-q13 (GNAS)
Temple syndrome	14q32 (MEG3 promoter)

Table 3. Complete Episignature Analysis II: Single Gene and Chromosomal Disorders

**Reduced sensitivity may be observed. All genomic coordinates are provided in accordance with GRCh37/hg19.*

Conditions	Related regions/ genes
Alpha-thalassemia/Impaired intellectual development syndrome, X-linked	ATRX (male cases only)
Arboleda-Tham syndrome*	KAT6A*
ARID1A duplication-related syndrome*	ARID1A dup* (Chr1: 26,964,202-27,099,490)
BAFopathies: Coffin-Siris syndrome (CSS) 1-4 & Nicolaides-Baraitser syndromes	ARID1A, ARID1B, SMARCB1, SMARCA4, SMARCA2
• Coffin-Siris syndrome 1*	ARID1B*
• Coffin-Siris syndrome 2*	ARID1A*
• Coffin-Siris syndrome 3*	SMARCB1*
• Coffin-Siris syndrome 4*	SMARCA4*
• Nicolaides-Baraitser syndrome*	SMARCA2*
Patients with other BAFopathy genes may be detected, but not confirmed. Secondary signatures must also be positive for BAFopathy.	
BAFopathies: Coffin-Siris syndrome 1 & 2 (only for variants near c.6200) *No separate episignature due to small cohort size, however these samples cluster separately from other BAFopathy/ CSS1&2 samples.*	ARID1A, ARID1B (near c.6200 in both genes)
Coffin-Siris syndrome 6	ARID2
Beck-Fahrner syndrome *Healthy carriers and those with incomplete penetrance are detectable. Patients with biallelic variants are distinguishable from those with monoallelic variants.*	TET3
Blepharophimosis-impaired intellectual development syndrome	SMARCA2
Börjeson-Forssman-Lehmann, Chung-Jansen and White Kernohan syndromes (CHU_BFL_WHI)	PHIP, PHF6, DDB1
• Börjeson-Forssman-Lehmann syndrome*	PHF6* (male cases only)
• Chung-Jansen syndrome*	PHIP*
• White-Kernohan syndrome*	DDB1*
Secondary signatures must also be positive for CHU_BFL_WHI.	
Branchial arch abnormalities, choanal atresia, athelia, hearing loss, and hypothyroidism syndrome*	KMT2D* (only for variants within p.3400-p.3700)
Cerebellar ataxia, deafness, and narcolepsy, autosomal dominant*	DNMT1*
CHARGE syndrome	CHD7
Chromosome 1p36 deletion syndrome* *CNVs overlapping or expanding this region may also be detected.*	1p36 del* (Chr1: 1,019,753-2,867,961)

(Table 3. continued on page 5)

Table 3. Complete Episignature Analysis II: Single Gene and Chromosomal Disorders (CONTINUED)
**Reduced sensitivity may be observed. All genomic coordinates are provided in accordance with GRCh37/hg19.*

Conditions	Related regions/ genes
Chromosome 19p13.13 deletion syndrome *CNVs overlapping or expanding this region may also be detected. Only for CNVs. NFIX sequence variants not match the episignature.*	19p13.13p13.2 del (Chr19: 13,201,983-13,213,144)
Chromosome Xp11.22 duplication syndrome* *CNVs overlapping or expanding this region may also be detected. Male cases only.*	Xp11.22 dup* (ChrX: 53,559,057-53,654,518)
Clark-Baraitser syndrome	TRIP12
Congenital heart defects, dysmorphic facial features, and Intellectual DD*	CDK13*, CCNK*
Cornelia de Lange syndromes (CdLs) 1-4	NIPBL, RAD21, SMC3, SMC1A
• Cornelia de Lange syndrome 1*	NIPBL*
• Cornelia de Lange syndrome 2*	SMC1A*
• Cornelia de Lange syndrome 3*	*SMC3**
• Cornelia de Lange syndrome 4*	RAD21*
Male CdLS5 patients (HDAC8 mutations) may be detected, but not confirmed. Secondary signatures must also be positive for CdLs1-4.	
DEGCAGS syndrome *Heterozygotes have been shown to not match the episignature.*	ZNF699
Developmental and epileptic encephalopathy 54	HNRNPU
Developmental and epileptic encephalopathy 94	CHD2
Developmental delay with variable intellectual disability and dysmorphic facies*	JARID2*
Diets-Jongmans syndrome	KDM3B
Down syndrome	Trisomy 21
Dystonia 28, childhood-onset	KMT2B
Fanconi anemia *Heterozygotes have been shown to not match the episignature. Patients with other FANC genes may be detected, but not confirmed.*	FANCA, FANCC, FANCD2, FANCG, FANCI, FANCL
Floating Harbour syndrome	SRCAP
Gabriele-de Vries syndrome*	YY1*
Genitopatellar syndrome (GTPTS)* and Ohdo syndrome, SBBYSS variant* *Since GTPTS and SBBYSS are both caused by variants in KAT6B, it is recommended to request both episignatures for VUS assessment.*	KAT6B*
Hao-Fountain syndrome	USP7
Helsmoortel-van der Aa syndrome _Central episignature	ADNP (only for variants within c.2054-c.2340)

(Table 3. continued on page 6)

Table 3. Complete Episignature Analysis II: Single Gene and Chromosomal Disorders (CONTINUED)
**Reduced sensitivity may be observed. All genomic coordinates are provided in accordance with GRCh37/hg19.*

Conditions	Related regions/ genes
Helsmoortel-van der Aa syndrome_Terminal episignature	ADNP (outside of c.2054-c.2340)
Hunter McAlpine craniosynostosis syndrome *CNVs overlapping or expanding this region may also be detected.*	5q35 dup involving NSD1 (Chr5:175839681-176904798)
Immunodeficiency-centromeric instability-facial anomalies syndrome 1*	DNMT3B*
Immunodeficiency-centromeric instability-facial anomalies syndrome 2-4*	CDCA7*, ZBTB24*, HELLS*
Intellectual developmental disorder (IDD) with autism and macrocephaly*	CHD8*
IDD with dysmorphic facies, speech delay, and T-cell abnormalities*	BCL11B*
IDD with microcephaly and with or without ocular malformations or hypogonadotropic hypogonadism*	SOX11*
Intellectual developmental disorder with seizures and language delay	SETD1B
Intellectual developmental disorder, autosomal dominant 7	*DYRK1A*
Intellectual developmental disorder, autosomal dominant 21	CTCF
IDD, autosomal dominant 23; KBGS syndrome (KBGS_MRD23)	*SETD5, ANKRD11*
• IDD, autosomal dominant 23 (MRD23)*	SETD5*
• KBG syndrome*	ANKRD11*
Secondary signatures must also be positive for KBGS_MRD23.	
Intellectual developmental disorder, autosomal dominant 51* *Healthy carriers and those with incomplete penetrance are detectable.*	KMT5B*
Intellectual developmental disorder, X-linked 93* *Healthy carriers and those with incomplete penetrance are detectable.*	BRWD3*
Intellectual developmental disorder, X-linked 97*	ZNF711*
Intellectual developmental disorder, X-linked syndromic, Nascimento type*	UBE2A* (male cases only)
IDD, X-linked syndromic, Snyder-Robinson type*	SMS*
Intellectual developmental disorder, X-linked, syndromic, Armfield type*	FAM50A* (male cases only)
Intellectual developmental disorder, X-linked, syndromic, Claes-Jensen type *Healthy carriers and those with incomplete penetrance are detectable. Heterozygotes have a distinct profile from hemizygotes.*	KDM5C
Kabuki syndrome 1 & 2	KMT2D, KDM6A
• Kabuki syndrome 1*	KMT2D*
• Kabuki syndrome 2*	KDM6A*
Secondary signatures must also be positive for Kabuki syndrome 1 &2.	
KDM2B-related syndrome	KDM2B

(Table 3. continued on page 7)

Table 3. Complete Episignature Analysis II: Single Gene and Chromosomal Disorders (CONTINUED)
**Reduced sensitivity may be observed. All genomic coordinates are provided in accordance with GRCh37/hg19.*

Conditions	Related regions/ genes
Kleefstra syndrome 1	EHMT1
Klinefelter syndrome *XXX and XYY cases may also be detected.*	ChrX duplication; 47,XXY
Koolen de Vreis syndrome	KANSL1
Luscan-Lumish syndrome	SETD2
Menke-Hennekam syndrome 1 & 2 *Only for domain ID4. MKHK1 & MKHK2 exhibit a shared ID4 domain episignature and therefore cannot be distinguished. Other domains of MKHK1/2 are not available.*	CREBBP, EP300
Mowat-Wilson syndrome	ZEB2
MSL2-related syndrome*	MSL2*
Neurodevelopmental disorder with dysmorphic facies and behavioral abnormalities	*SRSF1*
Neurodevelopmental disorder with hypotonia, stereotypic hand movements, and impaired language	MEF2C
Neuroocular syndrome* *Healthy carriers and those with incomplete penetrance are detectable.*	*PRR12**
NSD2 duplication-related syndrome *CNVs overlapping or expanding this region may also be detected.*	NSD2 dup (Chr4: 1,832,733-1,975,031)
Phelan-McDermid syndrome *CNVs overlapping or expanding this region may also be detected. Only for CNVs. SHANK3 sequence variants do not match the episignature.*	22q13.3 del (Chr22: 49,238,268-50,248,907)
Pitt-Hopkins syndrome	TCF4
Potocki-Lupski syndrome* *CNVs overlapping or expanding this region may also be detected.*	17p11.2 dup* (Chr17:16,779,412-20,231,379)
PRC2 complex disorders (Weaver and Cohen-Gibson syndromes) *Shared episignature between PRC2 complex syndromes WVS & COGIS. Imagawa-Matsumoto syndrome cases with variants in SUZ12 have also been detected.*	EZH2, EED
Rahman syndrome	H1-4
Renpenning syndrome*	PQBP1* (male cases only)
Rubinstein-Taybi syndrome 1 and 2 (RSTS 1&2)	CREBBP, EP300
• Rubinstein-Taybi syndrome 1	CREBBP
• Rubinstein-Taybi syndrome 2	EP300
Secondary signatures must also be positive for RSTS 1 & 2.	
Sifrim-Hitz-Weiss syndrome	CHD4
SLC32A1-related syndrome*	SLC32A1*

(Table 3. continued on page 8)

Table 3. Complete Episignature Analysis II: Single Gene and Chromosomal Disorders (CONTINUED)
**Reduced sensitivity may be observed. All genomic coordinates are provided in accordance with GRCh37/hg19.*

Conditions	Related regions/ genes
Sifrim-Hitz-Weiss syndrome	CHD4
SLC32A1-related syndrome*	SLC32A1*
Smith-Magenis syndrome *CNVs overlapping or expanding this region may also be detected. Only for CNVs. RAI1 sequence variants do not match the episignature.*	17p11.2 del (Chr17: 17,322,913-18,515,769)
Sotos syndrome	NSD1
Tatton-Brown-Rahman syndrome*	DNMT3A*
Turner syndrome	ChrX deletion; 45,X
Velocardiofacial syndrome *CNVs overlapping or expanding these regions may be detected.*	22q11.2 del (Chr22: 19,510,547-20,285,090)
White-Sutton syndrome	POGZ
Wieacker-Wolff syndrome*	ZC4H2* (male cases only)
Wiedemann-Steiner syndrome	KMT2A
Williams-Beuren region duplication syndrome *CNVs overlapping or expanding this region may also be detected.*	7q11.23 dup (Chr7: 73,953,518-74,138,459)
Williams-Beuren syndrome *CNVs overlapping or expanding 7q11.23 may also be detected.*	7q11.23 deletion
Witteveen-Kolk syndrome*	SIN3A*
Wolf-Hirschhorn syndrome & Rauch-Steindl syndrome *CNVs overlapping or expanding this region may also be detected. NSD2 sequence variants have been shown to match the episignature.*	4p16.3 del involving NSD2 (Chr4: 679,715-2,169,001)

Results:

Results will be reported to the ordering provider and/or genetic counselor as specified on the requisition form.

Turn-Around Time:

Episignature Complete Analysis: 42 days
Episignature Targeted Analysis: 42 days

CPT Codes:

Episignature Complete Analysis: **81479**
Episignature Targeted Analysis: **81479**

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 for overnight delivery to arrive Monday through Saturday*. Please call the lab at 513-636-4474 with shipment tracking information when available.

Ship to:

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

*For Saturday deliveries only: Please add "Dock 5" to the address and select the Saturday delivery check box on the shipping label if applicable.

References:

Aref-Eshghi E, et al. (2017) Clinical Validation of a Genome-Wide DNA Methylation Assay for Molecular Diagnosis of Imprinting Disorders. J Mol Diagn. 19(6):848-856

Haghshenas S, et al. (2024). Discovery of DNA methylation signature in the peripheral blood of individuals with history of antenatal exposure to valproic acid. Genet Med. 26(10):101226.

Haghshenas S, et al. (2024) Identification of a DNA methylation episignature for recurrent constellations of embryonic malformations. Am J Hum Genet. 111(8):1643-1655

Husson, T.,. et al. (2024) Episignatures in practice: independent evaluation of published episignatures for the molecular diagnostics of ten neurodevelopmental disorders. Eur J Hum Genet.32(2):190-199.

Kerkhof J, et al. (2024) Diagnostic utility and reporting recommendations for clinical DNA methylation episignature testing in genetically undiagnosed rare diseases. Genet Med. 26(5):101075.

LaFlamme CW, et al. (2024) Diagnostic utility of DNA methylation analysis in genetically unsolved pediatric epilepsies and CHD2 episignature refinement. Nat Commun. 15(1):6524.

Levy MA, et al. (2021) Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders. HGG Adv. 3(1):100075.

Mackay DJG, et al. (2024) Multi-locus imprinting disturbance (MLID): interim joint statement for clinical and molecular diagnosis. Clin Epigenetics. 16(1):99.

Sadikovic B, et al. (2021) Clinical epigenomics: genome-wide DNA methylation analysis for the diagnosis of Mendelian disorders. Genet Med. 23(6):1065-1074.