FirstGene™ Technical Specifications
Myriad Women's Health
Effective: June 2, 2025
TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS

Description of Analysis:

The FirstGene screen is a prenatal cell-free DNA (cfDNA) next generation sequencing assay that offers comprehensive genetic risk assessment for a singleton pregnancy from a single blood sample from the pregnant person alone. The assay employs targeted DNA-based next generation sequencing (NGS) analysis using a custom capture panel designed to target selected chromosomes, *RHD*, and genes associated with autosomal recessive and X-linked diseases. The FirstGene screen evaluates fetal and pregnant person genotypes via a combination of novel molecular and computational strategies that boost and modulate fetal-derived signal while also reducing background noise in the sequencing data.

Description of Method:

The FirstGene screen is a hybridization capture-based deep sequencing assay. Prenatal cfDNA samples extracted from plasma of pregnant persons are prepared into sequencing libraries with unique molecular identifiers (UMIs) to reduce PCR-associated read count biases. Libraries are captured by a custom hybridization panel with optimized reaction conditions and then size-selected prior to sequencing. In addition, the FirstGene screen employs an in-silico size-based analysis that further boosts fetal fraction levels and provides a trajectory—shifts of allele balance or depth as a function of fetal fraction—to disambiguate fetal from genotypes of the pregnant person. Statistical models and trajectory-based calling algorithms have been developed for each of the assay components to predict fetal and pregnant person genomic variations. The screening assay includes four components:

Fetal aneuploidy analysis

The FirstGene screen is designed to detect common trisomies of chromosomes 13, 18, and 21, microdeletion in the 22q11.2 region of chromosome 22, fetal sex (XX, XY), and sex chromosome aneuploidies (X, XXX, XXY, XYY) through analysis of sequencing depth at these targeted regions.

Pregnant person recessive disease carrier status analysis

The pregnant person's carrier status is determined through full-gene sequencing analysis. Their carrier status is predicted based on the number of deleterious single nucleotide variants (SNVs), small insertions and deletions (INDELs), and exon-level copy number variants (CNVs) detected in the sample. We provide analysis of gene-level copy number for two conditions - spinal muscular atrophy (SMA) and Hb Bart syndrome. SMA carrier status is determined by the copy number of exon 7 of the *SMN1* gene. For Hb Bart syndrome, double-cis deletion carrier status is determined by the copy number analysis for common deletions (--SEA, --FIL/THAI, --(alpha)20.5, --BRIT, --MEDI/II) involving the *HBA1* and *HBA2* genes. Pregnant person *FMR1* triplet repeat analysis for fragile X syndrome is completed by evaluating CGG trinucleotide expansions of the *FMR1* promoter measured by PCR amplification and capillary electrophoresis.

Fetal recessive disease status analysis

The FirstGene screen predicts fetal recessive disease status (non-carrier, carrier, and affected) for the same conditions tested for the pregnant person (except for fragile X syndrome). Full-gene sequencing analysis is conducted. Fetal disease status is predicted based on the number of deleterious SNVs or INDELs present. Exon-level CNV detection is not available for the fetus. We provide full gene copy number analysis on *SMN1* and *HBA1/HBA2* in the fetus only when the pregnant person is predicted to be a carrier for the corresponding disease. Fetal SMA status is determined by the copy number of exon 7 of the *SMN1* gene. Fetal Hb Bart status is determined by the copy number analysis for common deletions (--SEA, --FIL/THAI, --(alpha)20.5, --BRIT, --MEDI/II) involving the *HBA1* and *HBA2* genes. Full gene copy number analysis is not performed for other genes.

Rhesus D blood type compatibility analysis

The FirstGene screen analyzes the full gene copy number of *RHD* in the fetus to assess Rhesus D (RhD) compatibility between pregnant person and fetus. RhD compatibility analysis is performed only for pregnancies where the provider indicates on the Test Requisition Form that the pregnant person is RhD negative.

Performance Characteristics:

Analytical Specificity and Sensitivity

The FirstGene screen was validated comparing its performance on 571 samples with corresponding orthogonal assays for each test component, achieving over 98.6% analytical sensitivity and over 99.6% analytical specificity for each aspect of the assay.

Test Reproducibility

Inter- and intra-batch reproducibility were each validated using four unique patient samples replicated three times. The selected samples represented positive results for each test component. The FirstGene screen demonstrated 100% intra- and inter-batch reproducibility on reviewable sequencing results for both the fetus and pregnant person.

Limitations of method

The FirstGene screen is a screening test; therefore, false positive and false negative results can occur. This test is validated for singleton pregnancies with a gestational age of ≥10 weeks. Clinical correlation with ultrasound findings and history is indicated. Fetal results will not be available for conditions for which a pregnant person is known to be affected or identified to have >1 pathogenic variant in a gene. Diagnostic testing is recommended in these scenarios. If definitive diagnosis is desired during pregnancy, chorionic villus sampling or amniocentesis is necessary. Negative results do not fully exclude the presence of any genetic condition, birth defect, or other condition. The result from this screen is only informative for the pregnancy at time of sample collection and has limited utility in determining risks

for future pregnancies. This test is not intended to identify pregnancies at risk for open neural tube defects.

The FirstGene™ screen is designed to detect and report germline (constitutional) alterations. Mosaicism may not be detected, and if it is detected, it may not be reported. If >1 variant is detected in a gene, additional studies may be necessary to determine if those variants reside on the same chromosome or different chromosomes (i.e., phase). This assay is only validated to detect sex chromosome copy number variations in the fetus but not in the pregnant person; however, limited sex chromosome analysis is performed on the pregnant person for quality control purposes. If present, fetal or pregnant person chromosome variations including copy number variations, aneuploidies, rearrangements, or other structural changes may significantly reduce test sensitivity and accuracy of risk estimates. The FirstGene screen reports status for only genes/phenotypes available and specified by the ordering healthcare provider. Other heritable and non-heritable conditions and defects exist that are not addressed by this test. Furthermore, not all forms of genetic variation are detected by this assay (e.g., duplications [except in *CFTR*], chromosomal rearrangements, structural abnormalities, etc.). Additional testing may be appropriate for some individuals. Pseudogenes and other regions of homology may interfere with this analysis. In an unknown number of cases, other genetic variation may interfere with variant detection. Rare carrier states where complementary changes exist between genes or chromosomes may not be detected by the assay.

Other possible sources of error include, but are not limited to, sample mix-up, trace contamination, bone marrow or organ transplantation, blood transfusion, chimerism or mosaicism, pregnant person neoplasm, subchromosomal abnormalities, and technical or analytical errors.

Detection rates are determined using published scientific literature and/or reputable databases, when available, to estimate the fraction of disease alleles, weighted by frequency, that the methodology is predicted to be able or unable to detect. Detection rates are approximate and only account for analytical sensitivity. Certain variants that have been previously described in the literature may not be reported if there is insufficient evidence for pathogenicity. Detection rates do not account for the disease-specific rates of *de novo* variation.

This test was developed, and its performance characteristics determined, by Myriad Women's Health, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's evaluation.

Description of Nomenclature:

Description of Nomenclature: All sequencing mutations and genetic variants are referenced to cDNA positions on their respective primary transcripts and named according to the HGVS convention. The reference sequence used for variant naming is Genome Reference Consortium Human Build 37 [GRCh37]/hg19). Transcript IDs for genes with clinically actionable variants are indicated on patient reports with their associated variants (Table 1). Allele differences have been documented at a limited number of nucleotide locations, based on the major/minor alleles observed upon testing and reference sequences used historically at Myriad Women's Health.

Interpretive Criteria:

Fetal aneuploidy

High Risk: Results are consistent with aneuploidy for chromosome 13, 18 and/or 21, and/or sex chromosome aneuploidy (X, XXX, XXY or XYY). The fetus has a high risk of being affected.

Low Risk: Results are consistent with two copies of chromosomes 13, 18 and 21, as well as two sex chromosomes (XX or XY).

Fetal 22q11.2 deletion syndrome

High Risk: Results are consistent with a deletion in the region of 22q11.2. The fetus has a high risk of being affected with 22q11.2 deletion syndrome.

Low Risk: No microdeletions detected in the region of interest (22q11.2).

Recessive conditions

Pregnant person

Negative: The risk for the individual to be a carrier of any of the conditions tested is low.

Carrier: This individual is a carrier of one (or more) of the conditions tested. Carriers generally do not experience symptoms.

Affected: This individual is at risk of being affected with one (or more) of the conditions tested. Clinical correlation is needed.

Fetus

Low Risk: The risk of the fetus being affected by one of the conditions tested is low but cannot be definitively ruled out.

Carrier: One variant was detected in one (or more) of the genes tested. The fetus is predicted to be a carrier of the associated condition(s). Carriers generally do not experience symptoms. The risk for the fetus to be affected is low, but the presence of a second variant cannot be definitively ruled out with this assay.

High Risk: Two variants were detected in one (or more) of the genes tested. The fetus has a high risk of being affected with the associated condition(s). Diagnostic testing is required to determine precise fetal risks.

RhD incompatibility

Predicted RhD POSITIVE: The fetal RhD status is predicted to be incompatible with the reported RhD status of the pregnant patient. Treatment via injection with Anti-D immune globulin or RhoGAM is indicated.

Predicted RhD NEGATIVE: Results suggest that the fetus has zero copies of the *RHD* gene, consistent with RhD negative status. The fetal RhD status is predicted to be compatible with the reported RhD status of the pregnant patient. The risk for Rh incompatibility between the patient and the fetus is low but cannot be definitively ruled out with this assay.

Change of interpretation and issuance of amended reports

The classification and interpretation of all variants identified in the assay reflects the current state of scientific understanding at the time the report is issued. Variant classification and interpretation may change over time for a variety of reasons, including but not limited to, improvements to classification techniques, availability of additional scientific information, and observation of a variant in additional individuals. If the classification of one or more variants identified in this report changes, an updated report reflecting the new classification generally will not be issued. If a report is updated in an ongoing pregnancy or re-issued for other reasons, the variants reported may change based on their classification at the time of re-issue.

Table 1. Conditions screened by the FirstGene™ screen.

	Content (genomic region)	***Variants reported***
Pregnant person	Cystic fibrosis (*CFTR*)	SNVs, INDELs, and exon-level CNVs (deletions and duplications)
	Spinal muscular atrophy (*SMN1*)	*SMN1* copy number
	Sickle cell disease and beta thalassemia (*HBB*)	SNVs, INDELs, and exon-level CNVs (deletions only)
	Hb Bart syndrome (*HBA1, HBA2*)	*HBA1+HBA2* double cis deletion
	Other autosomal recessive diseases (*HEXA, PMM2, ACADM, ASPA, DHCR7, PAH)*	SNVs, indels, and exon-level CNVs (deletions only)
	Fragile X syndrome (*FMR1*)	CGG trinucleotide expansions of the *FMR1* promoter
Fetus	Cystic fibrosis (*CFTR*)	SNVs and INDELs
	Spinal muscular atrophy (*SMN1*)	*SMN1* copy number if pregnant person is a carrier
	Sickle cell disease and beta thalassemia (*HBB*)	SNVs and INDELs
	Hb Bart syndrome (*HBA1, HBA2*)	*HBA1+HBA2* double cis deletion if pregnant person is a carrier
	Other autosomal recessive diseases (*HEXA, PMM2, ACADM, ASPA, DHCR7, PAH)*	SNVs and INDELs
	Rhesus D (RhD) blood type compatibility (*RHD*)	*RHD* copy number when pregnant person is RhD negative
	Patau Syndrome (Chromosome 13)	Trisomy 13
	Edwards Syndrome (Chromosome 18)	Trisomy 18
	Down Syndrome (Chromosome 21)	Trisomy 21
	22q11.2 deletion syndrome (22q11.2 region on chromosome 22)	22q11.2 deletion
	Fetal sex (chrX, chrY)	XX, XY
	Sex chromosome abnormalities (chrX, chrY)	X, XXX, XXY, XYY

Table 2. Analytical sensitivity and specificity of each test component of the FirstGene screen.

	Test component	***Sensitivity % 95% CI***	***Specificity % 95% CI***	***Calls***	***TP***	***TN***	***FP***	***FN***
Fetal	Aneuploidy	100 (95.91-100)	99.91 (99.68-99.98)	T13, T18, T21	57	1,351	1	0
				22q	6	462	0	0
				X, XXX, XXY, XYY	36	428	1	0
	Recessive disease	98.68 (98.45-98.87)	99.60 (99.55-99.66)	SNV/INDEL	5,212	51,435[§]	38	42
					5,815[*]		166	106
		100 (82.41-100)[±]	100 (70.09-100)[±]	*SMN1* copy	1	0	0	0
					13[*]	9	0	0
				HBA1/HBA2 copy	4[*]	0	0	0
	RHD compatibility	100 (72.25-100)	100 (72.25-100)	*RHD* copy	10	10	0	0
Maternal	Recessive disease	99.92 (99.84-99.94)	>99.99 (99.99-100)	SNV/INDEL	17,746	125,254[§]	4	18
		100 (87.13-100)	100 (99.24-100)	*SMN1* copy	16	248	0	0
				HBA1/HBA2 copy	10	254	0	0
		100 (81.57-100)	100 (98.38-100)	CNV	17	234	0	0

CI: confidence interval, TP: true positive, TN: true negative, FP: false positive, FN: false negative, CNV: copy number variation. [±] When pregnant patient is predicted to be a carrier of the disease. [*]Calls observed in cell-line mixtures. [§]TNs were conservatively defined as the number of homozygous reference calls made at sites for which an alternative variant was correctly identified in at least one sample in the cohort.