

<b>Patient name:</b> Rosalind Franklin	<b>Sample type:</b> Blood	<b>Report date:</b> 25-OCT-2023
<b>DOB:</b> 25-JUL-1920	<b>Sample collection date:</b> NA	<b>Invitae #:</b> RQ3798166
<b>Sex assigned at birth:</b> Female	<b>Sample accession date:</b> NA	<b>Clinical team:</b>
<b>Gender:</b>		
<b>Patient ID (MRN):</b>		

**Reason for testing**

Carrier screening

**Test performed**

Invitae Core Carrier Screen

- Primary Panel (CF, SMA)
- Add-on FMR1 gene


**RESULT: NEGATIVE**

This carrier test evaluated 3 gene(s) for genetic changes (variants) that are associated with an increased risk of having a child with a genetic condition. Knowledge of carrier status for one of these conditions may provide information that can be used to assist with family planning and/or preparation. Carrier screening is not intended for diagnostic purposes. To identify a potential genetic basis for a condition in the individual being tested, diagnostic testing for the gene(s) of interest is recommended.

This test did not identify any genetic changes in the gene(s) analyzed that are currently recognized as clinically significant. This negative result reduces, but does not eliminate, the chance that this individual is a carrier for conditions caused by any of the genes tested. This individual may still be a carrier for a genetic condition that is not evaluated by this test.

## Next steps

- Even for genes that have a negative test result, there is always a small risk that an individual could still be a carrier. This is called “residual risk.” See the Carrier detection rates and residual risks document.
- Discussion with a physician and/or genetic counselor is recommended to further review the implications of this test result and to understand these results in the context of any family history of a genetic condition.
- All patients, regardless of result, may wish to consider additional screening for hemoglobinopathies by complete blood count (CBC) and hemoglobin electrophoresis, if this has not already been completed.
- Individuals can register their tests at <https://www.invitae.com/patients/> to access online results, educational resources, and next steps.



## Results to note

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### FMR1

- Normal triplet repeats observed: 21,35. CCG repeat ranges: normal (<45 CCG repeats), intermediate (45-54 CCG repeats), premutation (55-200 CCG repeats), full mutation (>200 CCG repeats).

### SMN1

- Negative result. SMN1: 2 copies; c.\*3+80T>G not detected.

## Residual risk

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No carrier test can detect 100% of carriers. There still remains a small risk of being a carrier after a negative test (residual risk). Residual risk values assume a negative family history and are inferred from published carrier frequencies and estimated detection rates based on testing technologies used at Invitae. You can view Invitae's complete Carrier detection rates and residual risks document (containing all carrier genes) online at <https://www.invitae.com/carrier-residual-risks/>. Additionally, the order-specific information for this report is available to download in the portal (under this order's documents) or can be requested by contacting Invitae Client Services. The complete Carrier detection rates and residual risks document will not be applicable for any genes with specimen-specific limitations in sequencing and/or deletion/duplication coverage. Please see the final bullet point in the Limitations section of this report to view if this specimen had any gene-specific coverage gaps.



## Genes analyzed

This table represents a complete list of genes analyzed for this individual, including the relevant gene transcript(s). If more than one transcript is listed for a single gene, variants were reported using the first transcript listed unless otherwise indicated in the report. An asterisk (\*) indicates that this gene has a limitation. Please see the Limitations section for details. Results are negative, unless otherwise indicated in the report.

GENE	TRANSCRIPT	GENE	TRANSCRIPT	GENE	TRANSCRIPT
CFTR*	NM_000492.3	FMR1*	NM_002024.5	SMN1*	NM_000344.3

## Methods

- Genomic DNA obtained from the submitted sample is enriched for targeted regions using a hybridization-based protocol, and sequenced using Illumina technology. Unless otherwise indicated, all targeted regions are sequenced with  $\geq 50x$  depth or are supplemented with additional analysis. Reads are aligned to a reference sequence (GRCh37), and sequence changes are identified and interpreted in the context of a single clinically relevant transcript, indicated in the Genes analyzed section. Enrichment and analysis focus on the coding sequence of the indicated transcripts, 20bp of flanking intronic sequence, and other specific genomic regions demonstrated to be causative of disease at the time of assay design. Promoters, untranslated regions, and other non-coding regions are not otherwise interrogated. Exonic deletions and duplications are called using an in-house algorithm that determines copy number at each target by comparing the read depth for each target in the proband sequence with both mean read-depth and read-depth distribution, obtained from a set of clinical samples. Markers across the X and Y chromosomes are analyzed for quality control purposes and may detect deviations from the expected sex chromosome complement. Such deviations may be included in the report in accordance with internal guidelines. Invitae utilizes a classification methodology to identify next-generation sequencing (NGS)-detected variants that require orthogonal confirmation (Lincoln, et al. J Mol Diagn. 2019 Mar;21(2):318-329.). Confirmation of the presence and location of reportable variants is performed based on stringent criteria established by Invitae (1400 16th Street, San Francisco, CA 94103, #05D2040778), as needed, using one of several validated orthogonal approaches (PubMed ID 30610921). The following analyses are performed if relevant to the requisition. For GBA the reference genome has been modified to mask the sites of polymorphic paralog sequence variants (PSVs) in both the gene and pseudogene. For CYP21A2 and GBA, if one or more reportable variants, gene conversion, or fusion event is identified via our NGS pipeline (see Limitations), these variants are confirmed by PacBio sequencing of an amplicon generated by long-range PCR and subsequent short-range PCR. In some cases, it may not be possible to disambiguate between the gene and pseudogene. For GJB2, the reportable range includes large upstream deletions overlapping GJB6. For HBA1/2, the reference genome has been modified to force some sequencing reads derived from HBA1 to align to HBA2, and variant calling algorithms are modified to support an expectation of 4 alleles in these regions. HBA1/2 copy number calling is performed by a custom hypothesis testing algorithm which generates diplotype calls. If sequence data for a sample does not support a unique high confidence match from among hypotheses tested, that sample is flagged for manual review. Copy number variation is only reported for coding sequence of HBA1 and HBA2 and the HS-40 region. This assay does not distinguish among the  $\alpha 3.7$  subtypes, and all  $\alpha 3.7$  variants are called as HBA1 deletions. This assay may not detect overlapping copy gain and copy loss events when the breakpoints of those events are similar. For FMR1, triplet repeats are detected by PCR with fluorescently labeled primers followed by capillary electrophoresis. Reference ranges: Normal: <45 CGG repeats, intermediate: 45-54 CGG repeats, premutation: 55-200 CGG repeats, full mutation: >200 CGG repeats. For alleles with 55-90 triplet repeats, the region surrounding the FMR1 repeat is amplified by PCR. The PCR amplicons are then processed through PacBio SMRTBell library prep and sequenced using PacBio long read technology. The number of AGG interruptions within the 55-90 triplet repeat is read directly from the resulting DNA sequences. Technical component of confirmatory sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778).
- This report only includes variants that have a clinically significant association with the conditions tested as of the report date. Variants of uncertain significance, benign variants, and likely benign variants are not included in this report. However, if additional evidence becomes available to indicate that the clinical significance of a variant has changed, Invitae may update this report and provide notification.
- A PMID is a unique identifier referring to a published, scientific paper. Search by PMID at <http://www.ncbi.nlm.nih.gov/pubmed>. An rsID is a unique identifier referring to a single genomic position, and is used to associate population frequency information with sequence changes at that position. Reported population frequencies are derived from a number of public sites that aggregate data from large-scale population sequencing projects, including ExAC (<http://exac.broadinstitute.org>), gnomAD (<http://gnomad.broadinstitute.org>), and dbSNP (<http://ncbi.nlm.nih.gov/SNP>).

## Disclaimer

DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it is not a diagnosis itself. This test was developed and its performance characteristics determined by Invitae. It has not been cleared or approved by

the FDA. The laboratory is regulated under the Clinical Laboratory Improvement Act (CLIA) as qualified to perform high-complexity clinical tests (CLIA ID: 05D2040778). This test is used for clinical purposes. It should not be regarded as investigational or for research.

## Limitations

- Based on validation study results, this assay achieves >99% analytical sensitivity and specificity for single nucleotide variants, insertions and deletions <15bp in length, and exon-level deletions and duplications. Invitae's methods also detect insertions and deletions larger than 15bp but smaller than a full exon but sensitivity for these may be marginally reduced. Invitae's deletion/duplication analysis determines copy number at a single exon resolution at virtually all targeted exons. However, in rare situations, single-exon copy number events may not be analyzed due to inherent sequence properties or isolated reduction in data quality. Certain types of variants, such as structural rearrangements (e.g. inversions, gene conversion events, translocations, etc.) or variants embedded in sequence with complex architecture (e.g. short tandem repeats or segmental duplications), may not be detected. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity. Unless explicitly guaranteed, sequence changes in the promoter, non-coding exons, and other non-coding regions are not covered by this assay. Please consult the test definition on our website for details regarding regions or types of variants that are covered or excluded for this test. This report reflects the analysis of an extracted genomic DNA sample. While this test is intended to reflect the analysis of extracted genomic DNA from a referred patient, in very rare cases the analyzed DNA may not represent that individual's constitutional genome, such as in the case of a circulating hematolymphoid neoplasm, bone marrow transplant, blood transfusion, chimerism, culture artifact or maternal cell contamination.
- FMR1: Sizing accuracy is expected to be +/-1 for CGG repeat alleles less than or equal to 90 repeat units and +/-3 for CGG repeat alleles greater than 90 repeat units. If the two CGG repeats listed are the same, this may indicate that both alleles are the same size or that one allele is too small to be detected by this analysis. The number of AGG interruptions is only determined for females with triplet repeat sizes of 55-90. SMN1 or SMN2: NM\_000344.3:c.\*3+80T>G variant only. CFTR: Sequencing analysis for exons 7 includes only cds +/- 10 bp. SMN1: Systematic exon numbering is used for all genes, including SMN1, and for this reason the exon typically referred to as exon 7 in the literature (PMID: 8838816) is referred to as exon 8 in this report. This assay unambiguously detects SMN1 exon 8 copy number. The presence of the g.27134T>G variant (also known as c.\*3+80T>G) is reported if SMN1 copy number = 2.

## This report has been reviewed and approved by:



UNSIGNED

This table displays residual risks after a negative result for each of the genes and corresponding disorders. The values provided assume a negative family history and the absence of symptoms for each disorder. For genes associated with both dominant and recessive inheritance, the numbers in this table apply to the recessive condition(s) associated with the gene, unless otherwise noted. Residual risk values are provided for disorders when carrier frequency is greater than 1 in 500. For disorders with carrier frequency equal to, or less than, 1 in 500, residual risk is considered to be reduced substantially. When provided, residual risk values are inferred from published carrier frequencies, and estimated detection rates are based on testing technologies used at Invitae. Residual risks are provided only as a guide for assessing approximate risk given a negative result; values may vary based on the ethnic background(s) of an individual. For any genes marked with an asterisk\*, refer to the Limitations section of the patient report for detailed coverage information. In the case of a sample-specific limitation, "N/A" indicates that a residual risk value could not be calculated. AR = autosomal recessive, XL = X-linked, AD = autosomal dominant.

DISORDER (INHERITANCE)	GENE	ETHNICITY	CARRIER FREQUENCY	DETECTION RATE	RISK TO BE A CARRIER AFTER NEGATIVE RESULT
CFTR-related conditions (AR) NM_000492.3	CFTR *	Pan-ethnic - classic CF	1 in 45	99%	1 in 4400
		Pan-ethnic - classic CF and CFTR-related disorders	1 in 9	99%	1 in 800
FMR1-related conditions including fragile X syndrome (XL) NM_002024.5	FMR1 *	Ashkenazi Jewish	1 in 58	99%	1 in 5700
		Asian	≤1 in 500	99%	Reduced
		Caucasian	1 in 187	99%	1 in 18600
		Hispanic	≤1 in 500	99%	Reduced
		Pan-ethnic	1 in 259	99%	1 in 25800
Spinal muscular atrophy (AR) NM_000344.3 Carrier residual risks listed are for 2 copy SMN1 results. Carrier residual risk for >2 copies are 5- to 10-fold lower.	SMN1 *	African-American	1 in 59	83%	1 in 342
		Ashkenazi Jewish	1 in 62	94%	1 in 1017
		Asian	1 in 50	93%	1 in 701
		Caucasian	1 in 45	95%	1 in 880
		Hispanic	1 in 48	94%	1 in 784
		Pan-ethnic	1 in 49	94%	1 in 800