510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K211499

B. Purpose for Submission:

New device

C. Measurand:

G84E variant in the HOXB13 gene

D. Type of Test:

Qualitiative genetic test for detection of the G84E variant in the HOXB13 gene

E. Applicant:

23andMe, Inc.

F. Proprietary and Established Names:

23andMe Personal Genome Service (PGS) Risk Report for Hereditary Prostate Cancer (HOXB13-Related)

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.6090

2. Classification:
   Class II

3. Product code:
   QAZ

4. Panel:
   Pathology

H. Intended Use:

1. Indication(s) for use:
   The 23andMe Personal Genome Service (PGS) uses qualitative genotyping to detect select clinically relevant variants in genomic DNA isolated from human saliva collected from individuals ≥18 years for the purpose of reporting and interpreting genetic health risks, including the 23andMe PGS Genetic Health Risk Report for Hereditary Prostate Cancer (HOXB13-Related). The 23andMe PGS Genetic Health Risk Report for Hereditary Prostate Cancer (HOXB13- Related) is indicated for reporting of the G84E variant in the HOXB13 gene. The report describes if a person has the G84E variant and if a male is at increased risk for prostate cancer. The variant included in this report is most common in people of European descent. The test report does not describe a person's overall risk of developing any type of cancer, and the absence of a variant tested does not rule out the presence of other variants that may be cancer-related. This test is not a substitute for visits to a healthcare provider for recommended screenings or appropriate follow-up and should not be used for diagnosis, to determine any treatments or medical interventions.

2. Special conditions for use statement(s):
   a. For over-the-counter (OTC) use.
   b. The test does not diagnose cancer or any other health condition and should not be used to make medical decisions. Results should be confirmed in a clinical setting before taking any medical action.
   c. This test is not a substitute for visits to a healthcare provider for recommended screening or appropriate follow-up. It is recommended that users consult with a healthcare provider if there are any questions or concerns about the test results or their current state of health.
   d. The 23andMe PGS Genetic Health Risk Report for Hereditary Prostate Cancer (HOXB13-Related) detects only one variant in the HOXB13 gene and does not detect all genetic variants in this gene associated with increased risk of developing prostate cancer. The absence of the variant tested does not rule out the presence of other genetic variants that may be disease-related.
   e. The test is intended for users ≥ 18 years old.
   f. The laboratory may not be able to process a user's sample. The probability that the laboratory cannot process a sample can be up to 6-33%.
   g. One potentially interfering mutation near G84E was identified and is noted below. Interference due to this mutation was not tested.

Common Name	SNP	Potentially Interfering Mutation
HOXB13	rs138213197 (G84E)	Rs529392210 (G85S) (prevalence in GnomAD: 0.0012% across all included populations, 0.0009% in non-Finnish Europeans, 0% in Finnish Europeans)

   h. A user's race, ethnicity, age, and sex may affect how the genetic test results are interpreted.
   i. It is important for the user to discuss their personal or family history of cancer with a

healthcare professional. If the user has a personal or family history of cancer, or think they may have symptoms of cancer; the user should consult with their healthcare provider about appropriate testing.

j. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.6090.

4. Special instrument requirements:

Tecan Evo, Illumina iScan and GenomeStudio system (qualified by 23andMe)

I. Device Description:

The 23andMe Personal Genome Service (PGS) is an over-the-counter (direct-to-consumer), DNA testing service that provides information and tools for consumers to learn about and explore their DNA.

The 23andMe Personal Genome Service (PGS) is a currently marketed, non-invasive genetic information service that combines qualitative genotyping data covering genetic ancestry, traits, and certain heritable health conditions from a single multiplex assay with descriptive information derived from peer reviewed, published genetic research studies. It is a home use, over-thecounter (direct-to-consumer) DNA testing service intended to provide information and tools for consumers to learn about and explore their DNA.

Customer saliva is self-collected using the Oragene·Dx® Device manufactured by DNA Genotek, Inc. (previously cleared for carrier screening indications under K141410, and the same collection kit used to generate performance data for DEN140044, DEN160026, DEN170046, K182784, DEN180028, and K193492, which consists of a sealable collection tube containing a stabilizing buffer solution. Once the sample is collected, it is shipped to one of the 23andMe Personal Genome Service Clinical Laboratory Improvement Amendments (CLIA) certified laboratories for testing.

DNA is isolated from the saliva and tested in a multiplex assay using a customized genotyping beadchip, and off the shelf reagents and instrumentation manufactured by Illumina. The multiplex assay simultaneously tests for more than 500,000 variants, including those for the previously authorized indications, as well as for the indications proposed herein.

Raw data is generated using Illumina GenomeStudio software, and then sent to 23andMe. The data is then analyzed using 23andMe's proprietary Coregen software, where a genotype is determined for each tested SNP. The results for certain of these SNPs are used to generate personalized reports for the customer that provide information about the detected genotype.

Personalized reports are generated for each user that provide results of the testing performed. These reports tell the user which genetic health risk variant(s) have been detected in their sample and provide information about the disease associated with the variant(s). If no variant was detected, that information is also provided. The personalized reports are designed to present scientific concepts to users in an easy-to-understand format. The reports provide scientifically

valid information about the risks associated with the presence of a particular variant. The reports are designed to help users understand the meaning of their results and any appropriate actions that may be taken based on their results.

The modified components of the Personal Genome Service included in this 510(k) submission are new labeling to include (a) one new variant to be reported, and (b) the qualitative reporting of one's Genetic Health Risk for Hereditary Prostate Cancer (HOXB13-Related).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   23andMe Personal Genome Service (PGS) Risk Report for BRCA1/BRCA2 (Selected Variants)

2. Predicate 510(k) number(s):

   DEN170046

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Technology	Customized Illumina BeadChip	Same platform
Specimen type	Saliva	Same
Collection Device	Oragene·Dx® saliva collection device (OGD-500.001)	Same
Measurand	DNA	Same
Instruments and software	Tecan Evo, Illumina iScan and Genome Studio Coregen	Same

Differences		
Item	Device	Predicate
Variants to be reported	G84E variant in the HOXB13 gene	185delAG and 5382insC in BRCA1 and 6174delT in BRCA 2 gene
Indication	Qualitative reporting of risk for HOXB13-related prostate cancer	Qualitative reporting of risk for Breast and Ovarian Cancer in women, Breast and Prostate Cancer in men

K. Standard/Guidance Document Referenced (if applicable):
Not applicable

L. Test Principle:

The assay uses multiplex microarray technology for the simultaneous detection of variants in human DNA. The BeadChip v5 assay (Illumina Infinium HumanOmniExpress-24 format chip) consists of silicon wafers etched to form wells loaded with silica beads, on which oligonucleotide capture probes are immobilized. DNA from saliva is fragmented and captured on a bead array by hybridization to immobilized SNP-specific primers, followed by extension with hapten-labeled nucleotides. The primers hybridize adjacent to the SNPs and are extended with a single nucleotide corresponding to the variant allele. The incorporated hapten-modified nucleotides are detected by adding fluorescently labeled antibodies in several steps to amplify the signals. The Tecan Evo and Illumina iScan instruments are used for extraction and processing of the DNA, and the BeadChip for scanning and quantification of the results. The genotype content is separated, analyzed, and then integrated into pre-defined report templates specific for each condition associated with each genotype. Genotypes are determined using the GenomeStudio and Coregen software packages. For the 23andMe PGS Genetic Health Risk Report for Hereditary Prostate Cancer (HOXB13-Related), information on one specific variant (G84E) in the HOXB13 gene is integrated into the report.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   23andMe performed a precision study to determine the reproducibility of the 23andMe assay for genotype calls for the G84E variant in the HOXB13 gene. The goal of the study was to evaluate the following precision parameters of the assay: intra-assay, inter-lot, inter-instrument, inter-operator, inter-day, and inter-lab differences. DNA samples were selected based on their confirmed genotypes, and were obtained from the 23andMe biobank. To confirm, each sample was sequenced by bi-directional Sanger sequencing. The same samples were genotyped by the assay in a blinded fashion, with 3 lots of reagents, by multiple operator teams per day, using 3 different serial numbers of each of 2 instruments (Tecan and iScan), over 3 days, at each of 2 laboratory sites. Assay genotypes were compared with sequenced genotypes to determine the rates of correct genotype calls.

   This precision study yielded 100% correct genotype calls for all samples across multiple days, operator teams, instruments, and reagent lots at two independent laboratory sites. Therefore, the study passed the acceptance criteria of at least 99% correct calls at each of the two laboratory sites. There was no variation between any study conditions or any replicates for a given sample. Therefore, the study demonstrated 100% reproducibility and 100% repeatability for a given sample. The results are shown in Table 1.

   Table 1. Precision Study Results Stratified by Site and Genotype

Genotype	Lab	Total Replicates Pass QC	# of Correct Calls	# of Incorrect Calls	# of No Calls	# of FQCs (After Rerun)	% Correct Calls
CC (Homozygous Common)	NGI	81	81	0	0	0	100%
CT (Heterozygous)	NGI	81	81	0	0	0	100%
TT (Homozygous Rare)	*NGI*	*81*	*81*	*0*	*0*	*0*	*100%*
CC (Homozygous Common)	DNA	81	81	0	0	0	100%
CT (Heterozygous)	DNA	81	81	0	0	0	100%
TT (Homozygous Rare)	DNA	81	81	0	0	0	100%

b. *Linearity/assay reportable range:*

Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The assay requires two types of controls: the sample processing control and the reproducibility control. The information provided demonstrates that the sample processing control is stable for up to three months and the reproducibility control is stable for up to 12 months. See DEN140044 for detailed information.

d. *Detection limit:*

The Limit of Detection (LoD) study was performed to assess the sensitivity of the Personal Genome Service (PGS) assay for Hereditary Prostate Cancer (HOXB13-Related). Samples were identified from the 23andMe customer database based on their putative genotype. Each sample was diluted to 3 different concentrations and genotyped by the assay in a blinded fashion using 3 lots of reagents. Genotype results were confirmed using bidirectional Sanger sequencing. The minimum DNA requirement was defined as the lowest concentration at which at least 95% of samples yielded the correct call. To confirm the genotype call, all DNA samples were genotyped using bidirectional Sanger sequencing performed in a qualified laboratory listed in the 23andMe approved supplier list. Genotypes derived from sequencing were considered "truth" and were used to assess the accuracy of PGS assay genotypes. To confirm the genotype call, each PGS assay genotype calls were compared to bidirectional Sanger sequencing results.

The LoD was defined as the lowest DNA concentration at which at least 95% of samples yielded the correct call. This study yielded 100% correct call rates for all samples across all reagent lots at all sample concentrations tested. Therefore, the study passed the acceptance criteria of 95% correct calls at the lowest concentration tested (5 ng/µL). This study demonstrated that the PGS assay is valid for samples with a DNA concentration range of 5 ng/µL DNA to 50 ng/µL DNA.

e. *Analytical specificity:*

Interfering Substances – Endogenous and Exogenous Substances

A series of studies were conducted to assess the effects of endogenous substances, exogenous substances, microbial substances, and smoking on the 23andMe PGS Test. The results of the Endogenous and Exogenous Interference studies can be found in the Decision Summary for DEN140044. No interference was observed with the endogenous substances tested. The study indicated that saliva samples should be collected at least 30 minutes after eating, drinking, chewing gum, using mouthwash.

Interfering Mutations

Analyses were performed to identify potentially interfering mutation within the one HOXB13 variant detected by the test. One potentially interfering mutation near G84E for the variant being tested has been identified. Because of the low frequency of this variant (prevalence in GnomAD: 0.0012% across all included populations), the Company was unable to identify additional user or commercially available samples for these potentially interfering mutations. Therefore, a statement has been added to the limitations section of the package insert stating that the impact of interfering mutations has not been evaluated.

Smoking and Microbial Interference

The effects of smoking before the saliva collection and microbial interference were performed. The studies indicated that saliva samples should be collected at least 30 minnutes after smoking and there is no effect on the accuracy of the test by five microbes that maybe found in human saliva. See DEN140044 for additional information.

f. *Assay cut-off:*

Not applicable.

g. *Specimen Stability*

Saliva samples for testing are collected with the Oragene·Dx collection device. The claimed specimen stability is 12 months at ambient temperature. See K110701 for sample stability information.

f. *Shipping Stability:*

Saliva samples are shipped for testing in the Oragene·Dx collection device. Environmental conditions experienced during shipping were simulated by subjecting samples to freeze-thaw cycles (samples stored at high temperatures that could be experienced during shipping were evaluated in specimen stability. The claimed shipping stability is up to three freeze-thaw cycles. See K110701 for sample shipping stability information.

2. Comparison studies:

   a. *Method comparison with Sanger Bidirectional Sequencing:*
   23andMe performed a method comparison study to assess the accuracy of the 23andMe PGS assay for Hereditary Prostate Cancer (HOXB13-Related). The goal of the study was to show equivalent genotype assignment between the 23andMe PGS assay and bidirectional Sanger sequencing. Samples were identified from the 23andMe customer database based on their predetermined genotype. Genotyping of these samples was performed at a CLIA certified contract laboratory. All chosen samples were then tested using bidirectional Sanger sequencing. Genotyping results were compared between the 23andMe PGS assay and sequencing to calculate positive percent agreement (PPA) and negative percent agreement (NPA), with the sequencing results considered to be "truth". The passing criteria were greater than 99% PPA and greater than 99% NPA for each SNP. This method comparison study yielded 100% agreement. Therefore, the study passed the acceptance criteria of greater than 99% PPA and greater than 99% NPA. The method comparison study showed that the 23andMe assay is comparable to bidirectional Sanger sequencing.

Table 3. Percent Agreement Results for HOXB13 G84E by Genotype

G84E HOXB13 (rs138213197) **Genotype**	**# of Samples Compared**	**Correct***	**Incorrect***	**PPA**	**NPA**	**95% CI †**
CC (Homozygous Common)	20	20	0	100%	100%	86.1 - 100%
CT (Heterozygous)	26	26	0	100%	100%	89.1 - 100%
TT (Homozygous Rare)	22	22	0	100%	100%	87.3 - 100%

   b. *Matrix comparison:*

   Not applicable. This test is for use with human saliva samples only.

3. Clinical studies:

a. Disease Description and Clinical Summary:

The 23andMe PGS® Genetic Health Risk Test for Hereditary Prostate Cancer (HOXB13-Related) is indicated for reporting of the G84E variant in the HOXB13 gene. This variant is associated with an increased risk of developing prostate cancer. Studies suggest that 33–53% of males with the G84E variant develop prostate cancer during their lifetime (Karlsson 2014, PMID 22841674; Hoffmann 2015, PMID 25629170; Nyberg 2019, PMID 30527799), compared to about 12% of males in the general population (SEER Cancer Statistics Review, 1975-2017). Males with this variant who develop prostate cancer also tend to do so at an earlier age (Ewing 2012, PMID 22236224). The HOXB13 G84E variant is estimated to account for up to 5% of hereditary prostate cancer cases in families of European descent (Xu 2013, PMID 23064873), and in some Nordic countries this variant accounts for about 22% (Finland) and 8% (Sweden) of hereditary prostate cancer cases (Xu 2013, PMID 23064873). About 10% of Swedish men with early-onset prostate cancer (age at diagnosis: younger than 55) are carriers of the HOXB13 G84E variant (Karlsson 2014, PMID 22841674). The carrier frequency of the HOXB13 G84E variant in the European population varies by country, and ranges from 0.1%–1.4% (based on frequencies among controls) (Kluźniak 2013, PMID 23334858; Laitinen 2013, PMID 23292082; Karlsson 2014, PMID 22841674; Kote-Jarai 2015, PMID 25595936; Storebjerg 2016, PMID 26779768; Chen 2018, PMID 29181843).

The 23andMe PGS® Genetic Health Risk Report for Hereditary Prostate Cancer (HOXB13-Related) specifically presents the test results only for the G84E variant (obtained from the overall PGS® Test). The report describes if a male is at increased risk of developing prostate cancer and if a female has the tested variant indicating that their male relatives may be at increased risk for prostate cancer. The report also provides information about prostate cancer risk associated with this variant. It does not provide a definitive determination of a person's overall risk of developing prostate cancer or any other types of cancer. Many additional factors, including non-genetic factors and genetic variants not covered by the test, can also affect whether a person develops prostate cancer. This test does not detect other variants in the HOXB13 gene or variants in other genes that can increase the risk for prostate cancer.

Table 4 below listed the frequency of the G84E HOXB13 variant in 23andMe customers and public databases.

Table 4 HOXB13 G84E Allele Frequencies (%) in the 23andMe database and the gnomAD database[1]

[1] [a]Based on approximately 5,552,000 individuals with European ancestry, 303,000 individuals with African American ancestry, 168,000 individuals with Ashkenazi Jewish ancestry, 235,000 individuals with East Asian ancestry, 957,000 individuals with Hispanic/Latino ancestry, 57,000 individuals with South Asian ancestry, and 60,000 individuals with Middle Eastern ancestry. Because of the privacy considerations surrounding the use of customer data (namely, the risk of exposing the identity of individuals in the database), the frequencies provided are rounded to a hundredth of a percent and truncated at a minimum frequency if the number of individuals with a variant is fewer than five.
[b] Data were extracted from the gnomAD database: https://gnomad.broadinstitute.org/ accessed 09May2020.

Ancestry group	23andMe	gnomAD[b]
European	0.18%	0.76%[c] 0.24%[d]
African American	0.04%	0.04%
Ashkenazi Jewish	<0.01%	0.01%
East Asian	0.00%	0.00%
Hispanic/Latino	0.06%	0.003%
South Asian	0.00%	0.00%
Middle Eastern	<0.01%	n/a

Clinical validity of the variants is supported by published data and NCCN and ACG guidelines. Clinical data relating to the pathogenic G84E variant in HOXB13 is summarized in Table 5 below.

Table 5 Numerical risk estimates provided in the 23andMe PGS® Hereditary Prostate Cancer (HOXB13-Related) test reports

Test reports	Risk estimate provided	References
0 variants detected (Male)	General population risk for prostate cancer: About 12% (1 in 8 males)	SEER Cancer Statistics Review, 1975-2017 (Howlader 2019)
1 variant detected (Male)	Risk for prostate cancer in males with one copy of the HOXB13 G84E variant: 33-53% by age 80	Karlsson 2014 (PMID 22841674); Hoffmann 2015 (PMID 25629170); Nyberg 2019 (PMID 30527799)

[c] European (Finnish)
[d] European (Non-Finnish)

2 copies of a variant detected (Male)	Risk for prostate cancer in males with one copy of the HOXB13 G84E variant: 33-53% by age 80 Males with two copies of this variant are expected to have prostate cancer risks at least as high as males with just one variant.	Karlsson 2014 (PMID 22841674); Hoffmann 2015 (PMID 25629170); Nyberg 2019 (PMID 30527799)

The 23andMe PGS® Genetic Health Risk Test for Hereditary Prostate Cancer (HOXB13-Related) is indicated for reporting of the G84E variant in the HOXB13 gene.This variant is associated with an increased risk of developing prostate cancer. Studies suggest that 33–53% of males with the G84E variant develop prostate cancer during their lifetime (by age 80) (Karlsson 2014, PMID 22841674; Hoffmann 2015, PMID 25629170; Nyberg 2019, PMID 30527799), compared to about 12% of males in the general population (SEER Cancer Statistics Review, 1975-2017). Males with this variant who develop prostate cancer also tend to do so at an earlier age (Ewing 2012, PMID 22236224).

b. Other clinical supportive data:

i. User Comprehension Study

User comprehension studies were performed to assess the comprehension of the Genetic Health Risk report. See DEN160026 supportive user comprehension studies.

ii. Frequently Asked Questions Material

The Manufacturer has developed a Frequently Asked Questions (FAQ) section for the 23andMe PGS Genetic Risk Report for Hereditary Prostate Cancer (HOXB13-Related), which is included in the test report and accessible to the user on the Manufacturer's public website. The FAQs are specific to the variants and disease risk associations being reported, where applicable. The FAQ section was created to provide users with information to adequately understand the purpose, limitations and meaning of the results of the test. The FAQ section was developed using methodology consistent with the Manufacturer's labeling design, identification of primary communication messages, and label comprehension. The concepts covered in the FAQ section include: the test results, purpose of the test, limitations of the test, relevance of race and ethnicity on test results, meaning of the result, other risk factors that contribute to disease, appropriate follow-up procedures, how the results of the test may affect the user's family and children, and links to resources that provide additional information. Additionally, the FAQ section provides definitions for terminology found in Genetic Health Risk Reports that is used to describe risks associated with detected variants.

iii. User Opt-In Page

Prior to receiving the test results, a pre-purchase page informs users that there is a choice of whether or not to receive the 23andMe PGS Genetic Risk Report for Hereditary Prostate Cancer (HOXB13-Related). Users have an opportunity to opt into receiving these results after reviewing important information included in an opt-in page. The opt-in page is provided for the 23andMe PGS Genetic Risk Report for Hereditary Prostate Cancer (HOXB13-Related) users due to the nature of the diseases and associated risks for this report and the fact that this test is not designed to inform clinical decision-making. Users will be directed to a page entitled, "Choose your health reports" which provides the option to exclude this report from the users account. The report selection page includes important information to allow the users to make an informed decision. Results of the 23andMe PGS Genetic Risk Report for Hereditary Prostate Cancer (HOXB13-Related) are locked by default, and will never be shown to users unless they have specifically chosen to receive the report at any time, including after results for other reports have been received.

4. Expected values/Reference range:

Not applicable.

N. Instrument Name:

Illumina iScan BeadChip scanner with GenomeStudio software (qualified by the laboratory).

O. System Descriptions:

1. Modes of Operation:

Same as referenced in DEN140044

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X_____ or No _____

Level of Concern:

Moderate

Software Description:

Same as referenced in DEN140044

Revision Level History:

A software revision history record for the 23andMe software system software was acceptable.

Unresolved Anomalies:

There are no known unresolved anomalies associated with the system software.

EMC Testing:

Not applicable.

3. Specimen Identification:

   Same as referenced in DEN140044

4. Specimen Sampling and Handling:

   Same as referenced in DEN140044

5. Calibration:

   Same as referenced in DEN140044

6. Quality Control:

   Same as referenced in DEN140044

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Refer to K141410 for saliva collection device details and study results.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.